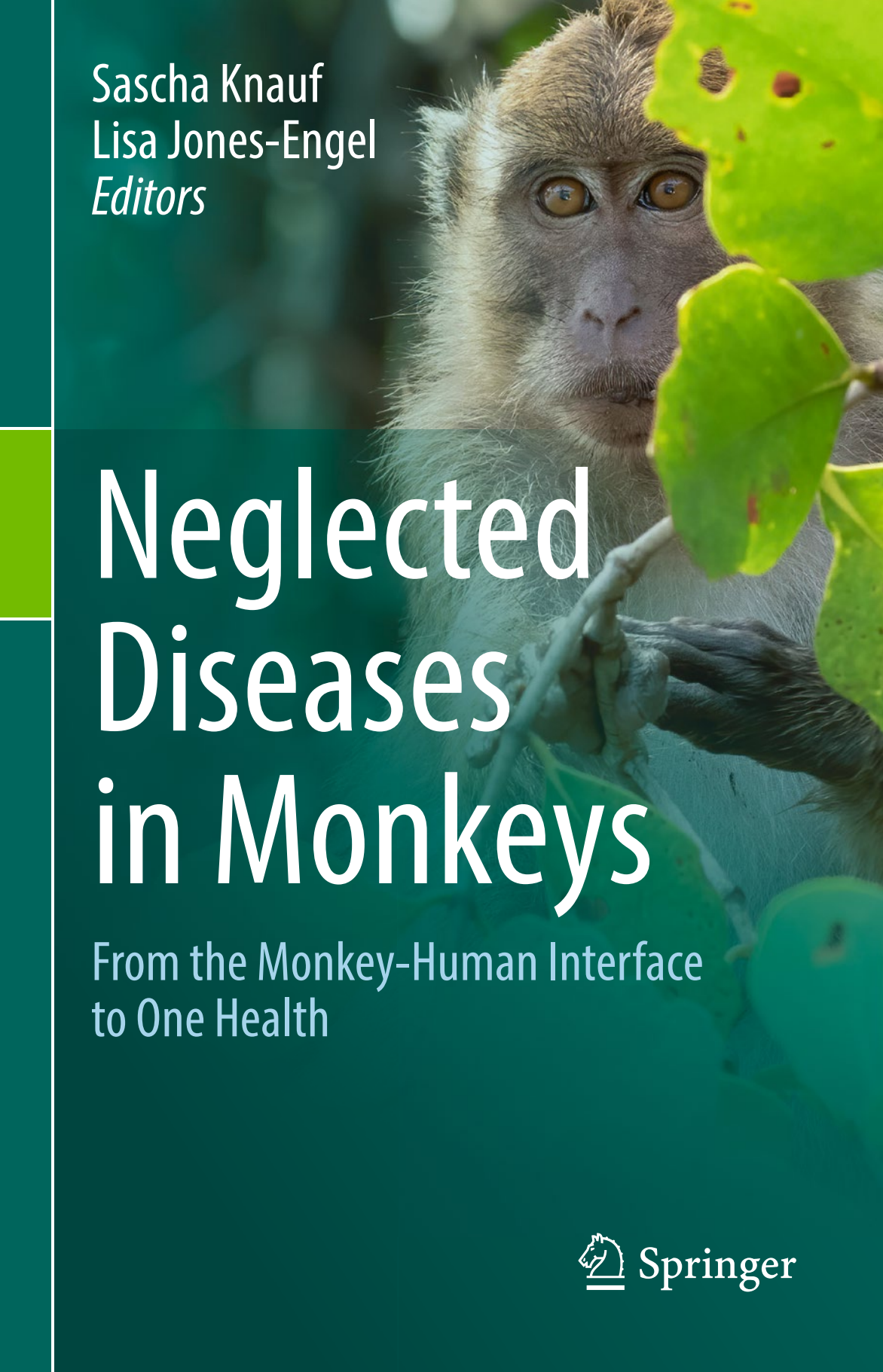
Sascha Knauf
Lisa Jones-Engel
Editors



Neglected Diseases in Monkeys

From the Monkey-Human Interface to One Health

Springer

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Foreword

I'm so honored to write the foreword to this impressive book on health and neglected diseases in monkeys. Having been an avid follower of the work of Dr. Knauf and Dr. Jones-Engel for decades I immediately responded in the affirmative when they invited me to write a brief foreword. Dr. Jones-Engel's groundbreaking work many years ago on non-invasive methods for studying primate health opened the eyes of so many of us conducting infectious disease research in nonhuman primates and provided an elegant, safe, and non-traumatic approach to sampling that is still being used today. Dr. Knauf first came to my aid when I was noticing what appeared to be treponemal infections disfiguring great apes in Africa. He immediately responded to my questions and since that time has continued to provide guidance and sound advice on best practices for the care and health of nonhuman primates. I'm delighted to see the two of them working together and enlisting this illustrious group of authors to share their knowledge and do the hard work of compiling this book to benefit all of us.

The second reason I'm so pleased to be writing this is that it makes me recall the early days of my career. In the first edition of Murray Fowler's *Zoo and Wildlife Medicine* there was a comprehensive chapter on infectious diseases of nonhuman primates written by Dr. Janis Joslin. That chapter in particular provided me guidance for decades and I know that many of you will not only remember it fondly but also appreciate the enormous effort required to compile and share that information with us. This new book will serve the same purpose for the decades to come. It provides in-depth information on neglected diseases that would not be included in most journal articles and puts it in one place for us and our colleagues to refer to whenever we have a pressing question.

Lastly, primatologists and medical scientists all understand the close relationship between human and nonhuman primates. Having coined the term, I cannot think of a better way to describe this relationship than as One Health. As this book comes out, we will all have experienced the ramifications of the COVID-19 pandemic. The complete One Health circle is already being demonstrated with devastating and long-lasting socio-economic consequences and providing the world with another clear

example of an animal origin virus adapting to human to human transmission and then spilling over from humans to animals. Laboratory science has demonstrated the susceptibility of macaques and we know how closely macaques interact with humans in so many countries. Molecular studies suggest that all apes, African and Asian monkeys as well as some lemurs, have the correct receptor binding site used by this virus to infect its host. While we do not yet know the outcomes, it is safe to say that your work with primates will help to answer many of the questions surrounding this pathogen and those still to come. And, as with the editors and all of the authors who so diligently worked to compile and share their knowledge in this volume, I want to applaud their efforts and yours as well in contributing to the health and well-being of both human and nonhuman primates for years to come.

William B. Karesh, D.V.M
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Preface

This 1st Edition of *Neglected Monkey Diseases: From the Monkey-Human Interface to One Health* comes to press as SARS CoV-2 emerges. Once again humans' complex relationship with animals and the environment has expedited a global pandemic. Monkeys may not be implicated in the emergence of *this* pandemic, but under the One Health paradigm where the health of humans and animals are established as linked, they too may become victims.

One Health is a holistic approach that does not prioritize protecting humans from diseases emerging in animals, rather One Health recognizes that stakeholders must work collaboratively to mitigate harm to any living organism on this planet from the rapidly occurring, mostly anthropogenic-driven, changes in ecology. Though they share behavioral, immunological, and physiological characteristics, humans and nonhuman primates have a patchwork of relationships predicated on a number of variables. Infectious agents, can and all too frequently, move across the porous interfaces where humans and nonhuman primates come together. However, not all nonhuman primates have the ecological flexibility and population sizes that allow them to successfully thrive alongside humans. We initiated this volume because our work and collaborations in the field and laboratories made it clear to us that monkeys, rather than the great apes, are the pivotal players at the human-primate interface.

We sought contributors to this volume from multiple disciplines, countries, and perspectives. The decision to solicit and include such a diverse group of authors was intentional and reflected the spirit and practice of One Health. We are indebted to these talented scientists without whom this volume would not have emerged. Finally, we must acknowledge the monkeys themselves, continuing to exist in the forests, savannahs, temples, urban areas, sanctuaries, living rooms, and laboratories.

We thank the people who have allowed us to work so intensely on this 1st edition. At first, these are our families who supported us throughout the years, both in the field and home. Thank you Gregory, Hanna, and Leah Engel and Yvonne and Ella Siv Aina Knauf. Second, Simone Lueert is thanked for her enormous support during routine laboratory work, which had to continue even during peak editorial sessions.

Third, we can only express our deepest gratitude to Springer Nature and the team that supported us throughout the years. Without the help of Silvia Herold and Sivachandran Ravanan and the many people involved in the production of this book, our project would have not been possible.

It is not the strongest of the species that survives,
not the most intelligent. . .
It is the one that is the most adaptable to change (Charles Darwin)

Goettingen, Germany — Sascha Knauf
Seattle, WA, USA — Lisa Jones-Engel

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Chapter 1
An Introduction to One Health and Neglected Diseases in Monkeys

Sascha Knauf and Lisa Jones-Engel

Humans are dramatically and possibly irrevocably altering the global ecosystem, resulting in ecological boundaries between humans and non-human primates (NHPs) that are porous and increasingly blurred. By 2050 it is estimated that 9.6 billion humans will cover the earth (Gerland et al. 2014). In almost all countries where NHPs naturally occur, humans have converted forest habitats into an agriculture-dominated landscape to serve the demand for meat, palm oil or fruits (Estrada et al. 2017). This dramatic shift in landscape ecology has resulted in an ever-growing human–domestic livestock–NHP interface. Estrada et al. (2017) estimated that approximately 60% of all known NHP taxonomic families are threatened with extinction and a further 75% of all NHP species-populations are decreasing. The speed and the extent of these anthropocentric ecological changes are the main drivers for emerging infectious diseases of wildlife (Daszak et al. 2000) and spillovers from wildlife to humans (Karesh et al. 2012).

This volume has emerged out of the recognition that the human–monkey interface far exceeds the one shared between humans and great apes. Millions of monkeys share habitat with more than a billion humans. There are currently 315 recognized monkey species distributed across the planet compared to seven recognized great ape species (Mittermeier 2013; Nater et al. 2017). Studies of synanthropic monkeys, such as macaques (*Macaca* spp.), baboons (*Papio* spp.), vervets (*Cercopithecus* spp.) or capuchins (*Cebus* spp. and *Sepajus* spp.), which thrive in the ecological niches that humans make as they alter the habitat, provide critical insights into the field of One Health (Oberste et al. 2012). Monkeys' behaviour, ecology and health

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are often impacted by their mutual and extensive overlap with humans. Studies have shown that in some species of macaques, population densities and birth rates increase when these behavioural and ecologically flexible monkeys occupy the same environment as humans (see Chap. 2). In contrast, great ape populations are known to suffer when they overlap with human populations (Walsh et al. 2003). Certainly, not all monkeys are as successful as macaques or baboons and the majority of monkey species are critically endangered (Estrada et al. 2017). But there is no denying that the large number of monkey genera and monkeys' ability to maintain very large groups while exploiting human habitats yields a monkey–human interface that far exceeds the one shared between humans and their closest relatives, the great apes. Moreover, large numbers of monkeys are kept as pets and for decades certain species of monkeys have been used for basic and applied research. Taken together, these contexts further extend and intensify the contact rate between humans and monkeys.

The use of monkeys in biomedical research underscores the potential for humans and monkeys to share pathogens. Several examples exist where baboons have become naturally infected with pathogens that are known to infect and cause disease in humans (Nasher 1988; Drewe et al. 2012; Mafuyai et al. 2013; Knauf et al. 2018; Thiele et al. 2018; Imwong et al. 2019) (Chaps. 4 and 5). Macaques, the Darwinian superstars of the NHP-world, known for their ability to co-exist in virtually any environment, are naturally infected with malaria parasites (Imwong et al. 2019), multi-resistant bacteria (Chap. 7), as well as one of the most feared and presumably misunderstood pathogens, the *Macacine herpesvirus* 1 (Chap. 8). However, the role that monkey species play as a natural pathogen source and disease reservoir for human infection is in many cases not well understood (e.g. Chagas and Trypanosomiasis, Chap. 15).

For humans, the term 'Neglected Tropical Diseases' is used to describe diseases that affect the poorest and marginalized populations which have limited access to healthcare (Hotez and Kamath 2009). However, the term doesn't refer to the frequency and/or intensity of research on a given disease. As a consequence, the World Health Organization categorizes well-studied diseases such as rabies (Chap. 11) or soil-transmitted helminths (Chap. 13) as Neglected Tropical Diseases in humans. In this book, and in contrast to the term 'Neglected Tropical Diseases' in humans, we apply the term 'Neglected Diseases' to pathogens in monkeys that, in our view, are truly under-studied.

Providing the framework for all the chapters in this book is the concept of One Health, which recognizes the connections between human, animal and environmental health and is widely accepted in public health. A common misconception of One Health is that its directionality is artifically skewed in favour of human health. As the authors throughout this volume demonstrate, that is certainly not the case. Pathogens can be transmitted in all directions and there are numerous examples where wild NHPs acquired diseases from humans (reviewed in (Dunay et al. 2018)) or share diseases with livestock as documented with Reston ebolavirus (Chap. 12). The current 2019 coronavirus outbreak (Wu et al. 2020), which likely has its origin in wildlife (Andersen et al. 2020), is the most recent reminder that our understanding of diseases in the context of natural ecosystems is key to disease management and elimination. Knowledge on biodiversity and (in this case human) behaviour is as important as the

Table 1.1 List of terms that are used in divergent ways across the different research disciplines and the definition of the term and how it is used in this book

Terminology	Definition	References
Primate	Non-human primates and humans	Mittermeier (2013)
Non-human primate	Non-human primates excluding humans	Mittermeier (2013)
Ape	Great- (gorilla, chimpanzee, bonobo, orangutan) and small-apes (gibbons and siamangs)	Mittermeier (2013)
Bacterium	A unicellular prokaryotic microorganism that has its own metabolism	Quinn et al. (2016)
Virus	A nonliving submicroscopic infectious agent that contains RNA or DNA surrounded by proteins. It depends on a living cell for replication	Quinn et al. (2016)
Parasite	A protozoa, helminth or ectoparasite that lives on or in and at the expenses of a larger organism called the host	Bowman (2009)
Macroparasite	Helminths and all ectoparasites	Quinn (2016)
Microparasite	Parasites that are not seen by the naked eye (e.g. protozoa)	Quinn (2016)
Pathogen	A microbe that is capable of causing host damage	Casadevall and Pirofski (1999)
One Health	Recognizes that the health of humans, animals and ecosystems is connected and involves a coordinated, collaborative, interdisciplinary and cross-sectoral approach to fight infectious diseases. The approach is based on the Manhattan Principles (Cook et al. 2004)	Zinsstag (2012)
Eco(system) Health	Presupposes that human survival depends on healthy and diverse ecosystems. It strives for the health of people, animals and ecosystems by promoting discovery and understanding through transdisciplinary action-research	Zinsstag (2012)
Global Health	Collaborative transnational research and actions for promoting health for all	Beaglehole and Bonita (2010)
Team science	Research collaboration among investigators from different disciplines who work interdependently to share leadership and responsibility	Tebes and Thai (2018)

full molecular characterization of a pathogen. Chapters 2 and 3 discuss these aspects and issue a call to overcome the widespread silo mentality in monkey disease research.

Multidisciplinary teamwork requires a vocabulary that is clear and understandable across all disciplines (Hallmaier-Wacker et al. 2017). This volume includes contributions from researchers representing numerous disciplines including primatology, veterinary and human medicine, microbiology, ecology and epidemiology. Traditionally, different disciplines use the same term in different ways. Finding a common language is, therefore, the first step when multidisciplinary teams are created. In an ecologist's understanding, for example, the term 'parasites' is mostly inclusive of any viruses, bacteria or parasites. In medicine, however, the term 'parasites' is used to describe protozoa, helminths and ectoparasites. In this book, and to overcome translation errors between the disciplines, we applied a single language across all chapters. In general, we followed the definitions used in medical and infectious diseases research. Table 1.1 provides a list of terms and how they are used across all chapters in this book.

Many chapters deal with pathogens that infect wild and captive monkeys alike, such as tapeworms (Chap. 14), morbilliviruses (Chap. 9) and simian foamy viruses (Chap. 10). However, some of the pathogens such as the bacterium *Chlamydia trachomatis* (Chap. 6) are not yet reported as natural infections in free-living NHPs. Infection pathways are complex and they depend on multiple factors such as animal density, animal behaviour, the immune and nutritional status, the ecology and dynamics of the disease or the infectious dose (Plowright et al. 2017).

We sincerely hope that this book will inspire and foster new research collaborations on diseases in monkeys. Compared to the critical situation in great apes, many monkey species have a realistic chance of survival in a human-dominated landscape. This, however, requires monitoring of disease transmission between monkeys and humans while also protecting remaining habitats. Monkey health is a team sport (Chap. 3), and this book should motivate primatologists, conservationists, behaviour scientists, physicians, veterinarians and disease researchers to collaborate. In chap. 16, Wolf and colleagues provide a detailed example of these types of collaborations in action at Gombe National Park in Tanzania.

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Chapter 2
Ethnoprimatology: Assessing How the Interface Between Humans and Monkeys Influences Infectious Agent Transmission

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Abstract In this chapter, I provide an overview of ethnoprimatological approaches as a theoretical and methodological context with the potential to create an opening for One Health to more fully engage with anthropological and primatological complexities at the interface of humans and monkeys. I present overviews of the human–macaque interface at two sites, Padangtegal, Bali, Indonesia and Gibraltar, UK, where the contrasting local cultural contexts and ecological patterns of interaction between humans and macaques demonstrate the importance of an ethnoprimatological and niche-constructive perspective when attempting to assess pathogen risk and management for human–macaque interactions.

Keywords Macaques · Bali · Gibraltar · Monkey forests · Human–primate interface · Padangtegal · Tourists · One Health · Aerosol transmission · Fecal–oral transmission · Pathogens · Sympatry

2.1 Ethnoprimatology and the Multispecies-ness of the Twenty-First Century

Becoming human is a multispecies endeavor. Humans are biological amalgamations – we are the result of melding bodies and genetic legacies; we are holobionts (Gilbert et al. 2012; Roughgarden et al. 2017). Human biological selves are multispecies communities, and that matters in a One Health approach. Whole ecologies of flora and fauna are biologically passed from parent to offspring and the microbiome is a critical component of the human being (as it is with all other organisms). Even in that most mammalian of actions, nursing a child, the mother passes not just sugars and fats to her infant, but a plethora of microbes, bacteria, and

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other microbiota. Ancient bacteria and viruses have even written themselves into human genomes (Roughgarden et al. 2017). This is how we cocreate the multispecies kinship community that we call human. But this multispecies interactivity process goes on at a level above and beyond the individual human. In regard to bodies, ecosystems, and health, humans and other species cocreate the patterns and processes that shape landscapes and interspecies relationships, but our inputs are often not of an equal scale.

Today, in the twenty-first century, humans are changing global and local ecologies as fast as, or faster than, we can study them. This process opens numerous opportunities for infectious agent exchange and countless other ecological factors related to One Health contexts (i.e., health processes and connectivity of humans and all the organisms we interact with and/or encounter). Because of this context recognizing the ecological processes (writ large) of our roles as animals and with other animals, can help us gain a better grasp on methods, theory and practical approaches to better understand and ameliorate challenges to global health. This is especially salient in the case of human interfaces with monkeys (Jones-Engel et al. 2008; Engel and Besnard 2017). Humans and monkeys can be important partners in the construction of social and ecological niches (Fuentes 2010, 2012; Dore et al. 2017). Shared histories from long-term sympatry (geographic overlap) between humans and monkeys can result in co-ecologies. Shared ecological pressures might impact humans and monkeys such that they share similar immunological and physiological adaptations and behavioral, ecological, and even cultural responses to environmental challenges (Fuentes 2010, 2012).

In the particular case of the human–monkey interface employing a revised primatological practice, one that sees humans and monkeys as occupying integrated and shared ecological and social spaces has become a necessary approach. Such an approach is epitomized by the emerging arena of ethnoprimatology (Dore et al. 2017; Fuentes 2012; Fuentes and Hockings 2010; Jones-Engel et al. 2011; Riley 2013).

Ethnoprimatology recognizes the core role of mutual overlap and pathogen exchange across species boundaries and in ecosystems, and problematizes simplistic or reductionist approaches by discarding the view that the human–nonhuman primate (NHP) interface is best viewed under the rubric of conflict and competition for resources. It also takes an explicitly anthropological perspective in rejecting that there are ecosystems on the planet in which humans have no impact and that studying monkeys in minimally impacted "natural" settings gives us higher-quality, and more valuable, information about their ecology, behavior, and health. Ethnoprimatology (see Box 2.1) rejects the idea that humans are separate from natural ecosystems and mandates that relational and systems-based approaches be included in behavioral ecological research on other primates.

Box 2.1 Core assumtions of ethnoprimatology
There are three core assumptions underlying the ethnoprimatological approach (Fuentes 2012):

1. Much in what we consider "normative" behaviors for primates may be stimulated by specific anthropogenic contexts.
2. The assumption that most primate populations have never been influenced by, or been forced to respond to, human activities in their recent or evolutionary histories is incorrect.
3. Physiological, phylogenetic, and behavioral affiliations between humans and the other primates result in the two groups' relationships having a special significance, ecologically, physiologically, behaviorally, and evolutionarily.

To best understand the dynamics of ethnoprimatological interfaces, in regard to their potential implications for human and macaque health, with a view toward managing and reducing potential pathogen transmission risks, we need a methodology and theoretical toolkit that will not only facilitate comparisons across sites but will also allow for the creation and testing of models that will produce reliable predictions. Data relevant to developing such models include macaque group and population structure, demography, rate and pattern of interactions between humans and NHPs, age/sex class, rate of contact and noncontact interactions, rate of aggressive interactions, bite rate, prevalence of simian enzootic infectious agents in populations, nationality and distance traveled by tourists, health status of the humans, clothing patterns of humans, economic and other cultural impacts of primate populations on local human populations, amount of range overlap between humans and primates at the site, feeding and provisioning strategies by locals and tourists, and locations and descriptions of shared resources (Dore et al. 2017; Fuentes 2006a).

In ethnoprimatology, the "ethno" prefix highlights the fact that multiple anthropogenic aspects, including social, economic, and political histories and contexts of human societies, can be seen as core components of inquiry into the lives of other primates and their interfaces with humans. This is specifically different from the use of the "ethno" prefix in "ethnobotany" or "ethnomathematics," in which the "ethno" marks a cultural distinction in the specific way of knowing under study from Western forms of the practice. This approach creates a fruitful venue for integrating subareas of anthropological practice and assessing the mutual ecologies, evolutionary histories, and social lives at the interface of humans and other primates.

2.2 Contemporary Ecology, Niches, and Mutual Co-Ecologies: A Baseline for Assessing Infectious Agent Transmission Between Other Primates and Humans

Human and the entire range of NHP share a number of interconnections, overlaps, and interfaces in what can be termed "zones of sympatry" where humans and NHP have coexisted since the Pleistocene. These areas include much of the continent of Africa, parts of the Middle East, most of South and Southeast Asia, portions of East Asia and, South and Central America (at least since humans moved in during the terminal Pleistocene). This long-term sympatry, the overlap of NHP space and human place, especially when it involves mutual usage of the same habitats can produce a kind of co-ecology, one where a particularly active niche-constructing primate (humans) has an extensive hand in shaping the landscape, and thus the contexts in which the other primates (especially monkeys) live (Fuentes 2007, 2012).

Here I offer that interfaces between humans and monkeys is an interesting place to focus when thinking of contemporary ecological systems in the light of One Health approaches. Ecological pressures impact mammals in particular ways, so mammals that share so many morphological and physiological facets in common, such as monkeys and humans, might be experiencing particularly robust similarities in their relationships with local ecologies and the infectious agents within them; especially if the sympatric species play significant roles in shaping those ecologies via physical and social activities. For much of human evolutionary history, monkeys have been the other primates most often sharing space and ecosystems with humans. Since at least the middle Pleistocene (~ one million years ago) baboons and macaques (the two widest spread monkey genera) have overlapped in range and diet with members of the genus *Homo* (humans). These monkeys, due to their behavioral and ecological plasticity (and adaptability), are much more flexible than apes or prosimian primates and thus have longer and more intensive overlaps with people (Fuentes 2010, 2012; Riley 2013; Dore et al. 2017).

Such similarities act to facilitate patterns of integration or engagement between the humans and other primates that result in particularly complex interweavings of cultural and ecological relationships, potentially setting up particular contexts under which the exchange of pathogens can occur. In areas of overlap humans incorporate other primates into their mythos, their daily lives, and often their diets with regularity (Fuentes et al. 2016). But the variation in these overlaps, in shared human–NHP ecologies, is substantial (Dore et al. 2017).

Humans and monkeys can be important partners in the construction of social and ecological niches (Fuentes 2010, 2012). Usually such overlap is placed along the "wild vs. domesticated continuum" but the actual relationships between humans and monkeys are much more complex. In fact, many primates live "in-between" these categorizations and are participants in an anthropogenic ecology that is itself a 'naturecultural' phenomenon (human-created dynamics are central aspects of the ecosystems) (Fuentes 2007, 2010).

Traditional primatological approaches are rooted in ethology and the socio-ecological model and arose from advances in ecological investigations especially in measuring energetics, the development of optimal feeding and foraging models, or the quantification of habitat use patterns. These methods, and the insights they produced, are couched within the standard evolutionary approach, and contemporarily referred to as "behavioral ecology". In this approach, natural selection, assumptions of optimality striving organisms, and a focus on patterns and processes of adaptation became the central foci for primate studies. The basic assumptions were that obtaining reproductive success (fitness) is the ultimate structuring mechanism for primate behavior such that the distribution of females, affected by food intake (food distribution and density) + predation risk + infanticide risk affected female–female competition patterns which structured male relationships and distribution and affected optimal group size and subsequent social organization in a given environment (Fuentes 2011; Strier 2016).

However, such simplified standard approaches to ecological thinking underplay the flexibility of ecological niches, and the potentially constructive and mutually reciprocal interfaces between monkey and human participants in these niches; especially if we are interested in the potential exchange of infectious agents. Consider the concept of social and ecological niche – the dynamic multidimensional and multispecies space that an organism lives in and simultaneously creates interactively with its social environment/local ecology. And consider, too, the process of the building, modifying, and altering of social and ecological niches and the concomitant pressures that play back on organisms. Niche construction (Odling-Smee et al. 2003) provides an important tool for understanding the relevance of a simultaneous examination of humans and other primates. Niche construction, a process by which organisms simultaneously shape and are shaped by their ecologies, plays a key role in primate evolutionary processes (Fuentes 2017). Niche construction results in the building and destroying of niches by organisms and the mutually mutable and synergistic interactions between organisms and their environments. Niche construction creates feedback within the evolutionary dynamic, with organisms engaged in niche construction modifying the evolutionary pressures acting on them, on their descendants, and on unrelated populations sharing the same landscape. Niche construction reflects a synthesis of ecological, biological, and social processes rather than treating them as discreet spheres.

One can envision some relationships between humans and monkeys where a form of physiological and social niche construction, over long social and biological timespans, occurs between the participants (Fuentes 2010). This has specific implications for infectious agent transmission between those species of monkeys that exhibit broad and long-term overlap with humans.

2.3 Human–Monkey Interfaces and the Contexts for Infectious Agent Transmission

Our understandings of the patterns and contexts of infectious agent and disease transmissions between human and monkeys necessitate situating the investigations in shared pathogen environments, local–mutual ecologies, and elucidating not just transmission mechanism but the ecologies and behavior complexes that facilitate and/or inhibit transmission possibilities (Fuentes 2006a; Jones-Engel et al. 2011).

The authors throughout this volume demonstrate that for humans and monkeys, close contact and/or range overlap introduces the context for the exchange of a variety of parasitic multicellular, bacterial, and viral pathogens. There is substantial variation throughout many primates' ranges as to how much they share spaces with humans, with certain species overlapping much more than others. However, nearly all species have some degree of overlap and this is a trend that looks to expand dramatically during the twenty-first century (Estrada et al. 2017).

Macaques (genus *Macaca*) have been ecological partners, competitors, and companions for humans for much of both of the genera's (*Homo* and *Macaca*) evolutionary history. In more recent times, macaque monkeys have played substantive roles in human diets, cultures, mythos, economies, and in the most recent phases of our history have even been a center piece in our medical research (Fuentes 2013). The pervasiveness of macaques in human spaces and lives, from temples, to pets, to performing animals, to working picking coconuts for humans, to crop raiders, to coresidents in urban contexts, to being used as a favored biomedical model, humans and macaques are probably among the most heavily intertwined primate species (Dore et al. 2017; Fuentes 2012, 2013).

Macaques form a natural locus for investigations into human–other primate pathogen transmission relationships. Thus, identifying, describing, and contextualizing the human–macaque interface is an important facet of the quest to understand pathogen transmission, and disease, patterns between humans and other primates. Here I offer brief overviews of the human–macaque interface at two sites where such work has been conducted.

Comparing the sites of Padangtegal, Bali, Indonesia, and Gibraltar, UK, in the style and pattern of interaction between humans and macaques, in the local cultural context and the local ecologies, demonstrates the importance of an ethnoprimatological and niche-constructive perspective when attempting to assess pathogen risk and management for human–macaque interactions (Engel and Jones-Engel 2012; Fuentes 2006a; Fuentes 2012; Jones-Engel et al. 2008).

2.4 Padangtegal and Gibraltar: The Basal Context Necessary to Engage Human-Monkey Interface Analyses

The site of Padangtegal Wanara Wana (a Hindu temple complex and associated forest) is located in south-central Bali, Indonesia. At this site, a population of over 600 long-tailed macaques (*Macaca fascicularis*) lives in 8 multimale/multifemale social groups (Fuentes et al. 2011; Brotcorne et al. 2011). The macaques have used the site for at least a century and possibly as much as 600 years (Wheatley, 1999). The area around this site averages a human density of greater than 400 people per square kilometer (Fuentes et al. 2005), and consists of dense towns, tourist infrastructure (hotels, restaurants, etc.), rice fields and other agriculture, and large and small paved roads. The macaques at this site range across the temple forest and the surrounding river gorges and rice fields. They frequently venture into the surrounding towns and tourist areas. Local Balinese use the temples at the site for religious ceremonies and move through the forest as a shortcut between towns. Locals using the temples interact with the macaques, offering bits of food or chasing the macaques away from offerings or packages they are carrying. As of 2017, more than 500,000 tourists from around the world visit this site annually both for its famous monkeys and for the temple complex.

The Gibraltar Upper Rock Nature Reserve is home to ~200 Barbary macaques (*Macaca sylvanus*) in six to eight social groups ranging from 20 to 60 individuals. The macaques have free range throughout the reserve, occasionally moving into areas of the neighboring urban zones. The city of Gibraltar has approximately 30,000 inhabitants with residential areas and hotels abutting some of the macaque groups' ranges. Officially designated a Nature Reserve in 1993, the Upper Rock is home to a variety of flora and fauna in addition to the Barbary macaque (Fuentes et al. 2007b). Approximately 800,000 tourists from more than 19 countries visit the upper rock reserve annually, including more than 72,000 a month during peak season June–September (Perez and Bensusan 2005). The majority of tourists entering Gibraltar are from the European Union and on day visits from neighboring Spain (which shares a land border with Gibraltar). Those crossing the border are either ferried up to the Upper Rock Nature Reserve by Gibraltaran taxis/coaches, via the cable car that runs tourists to the top of the Nature Reserve, or by the car they crossed the border in. Approximately 2% of those visiting Gibraltar arrive on cruise ships and yachts, and ~ 1.3% by air (Perez and Bensusan 2005).

At both sites, human–macaque interactions involving contact occur, but a closer analysis demonstrates that this general pattern masks critical differences between the sites. The sites differ in frequencies of aggressive interactions and rates of biting. If one considers the prevalence of simian enzootic infectious agents and the potential for disease transmission, then it is evident that the difference in types, patterns, and contexts of actual contact is very relevant – both for human-to-macaque as well as macaque-to-human transmission of pathogens.

Many of the pathogens that can be transmitted from humans to macaques are aerosol dispersed. The frequency of close contact and thus the relative spatial positioning of the faces (nose and mouth) of the humans and monkeys impacts the risk level of transmission. At Gibraltar there is frequent climbing of macaques onto humans, placing both human and macaque faces in close proximity and thus respiratory zones into close contact. This is also true at Padangtegal; however, the average age of macaques climbing on humans is younger at Padangtegal than at Gibraltar. In addition to placing both species' faces near one another, the climbing also acts to place macaque urine and fecal matter (potentially on their hands, feet and ano-genital areas) in contact with human clothing and skin. The age of the macaque might also increase the relative frequency of mucosal or saliva contacts between macaques and humans, since the younger monkeys often mouth the hair and clothing of the tourists, while the older monkeys are more focused on food rewards. At both sites, the interactions act to create a similar dynamic conducive to potential infectious agent transmission, but with potentially important different macaque age and behavior variables, coupled with human behavior (including patterns of clothing), altering the actual dangers/content of the interactions. In this case, primate physiology and behavior must be placed in the context of human behavior, and human cultural variation, and the local ecology, to effectively understand the risks.

At Padangtegal there is a much higher rate of macaques biting humans than at Gibraltar (Fuentes 2006a; Fuentes et al. 2007a). Due to this biting frequency difference, pathogens that are most easily transmitted via mucosal or saliva contact become more important in assessing the human–macaque interface at Padangtegal. At both sites, adult male macaques are overrepresented in aggressive interactions, and adult females are underrepresented (Fuentes et al. 2007a). Adult male macaques may offer a higher potential risk of transmitting pathogens to humans, and adult females a lower risk. Thus, sex-based difference in behavior and pathogen load (if there are any) comes into play.

At both sites, the overall frequency of contact interactions sets the stage for assessing the risk of transmission of bacterial pathogens found in the hands or feet of the macaques (which frequently have fresh or dry feces and urine on them), of aerosol-dispersed pathogens, and of pathogens (viral, bacterial, and eukaryotic) that are easily transmitted through mucosal contact (Jones-Engel et al. 2005). Because of the differences in bite rates between Padangtegal and Gibraltar, humans may be at greater risk of contracting certain pathogens at Padangtegal. This is also particularly important as a few potentially pathogenic viruses are known to occur in long-tailed macaques (*Macaca fascicularis*) in Bali, but have not been positively identified in the Gibraltar population of the barbary macaque (*Macaca sylvanus*) (Engel et al. 2006, 2008).

At both sites, interactions are officially discouraged by management staff, but occur at a high frequency regardless. However, specific human cultural patterns can affect the details of transmission threat. Many tourists at Padangtegal come from Europe, North America, or Australia and New Zealand, and specific cultural clothing patterns associated with vacation/holiday travel and high ambient temperatures (shorts, short sleeves, minimal shoulder covering, etc.. . .) can result in a high degree

of exposure of human skin to macaque hands, feet, bodies, and mouths. At Padangtegal, tourists from East, Southeast, and South Asia are also common but, on average, have less direct exposure because of a range of cultural styles of dress that act to cover most of the body, especially in women. A similar example of cultural impact can be seen in the small number of Moroccan tourists who visit Gibraltar, as their clothing patterns (at least for adults) also minimize exposed skin.

These variants in dress style combined with a relative lack of familiarity with monkeys on the part of Europeans and North Americans can also result behavioral actions by humans that create higher risks of pathogen transmission in specific cultural groups relative to others. For example, video data from Padangtegal and Gibraltar suggest that "startle" responses (yelling/screaming, waving arms, and running) are more commonly exhibited by European adult females compared to other cultural demographic groups (Fuentes 2006a, b; Fuentes et al. 2007a, b). These behaviors often elicit excitement and aggression in the macaques, leading to more interaction and potential bites. Coupled with the generally high degree of exposed skin on European female tourists, the combination of these factors may indicate an increased risk for this group (especially in Bali).

Another major factor at Padangtegal and Gibraltar is the role played by taxi/coach drivers in Gibraltar and the role of forest wardens/managerial staff at Padangtegal. Since there are approximately 150–200 taxi/coach drivers in Gibraltar working in those roles at any given time, a substantial percentage of interactions occur repeatedly between these specific humans and a subsection of the macaques. The Gibraltar data demonstrate a disproportionate participation in interactions by taxi drivers with the macaques (Fuentes 2006a; Fuentes et al. 2007a, b). The Gibraltarian gendered cultural division of labor (>90% of taxi drivers are male) and strong economic incentives are factors for these aspects of the interaction patterns in Gibraltar. The perception by the taxi drivers is that getting the macaques to climb onto and physically interact with the tourist results in a better financial return for the taxi/coach driver. Interviews with tourists suggest that this may not in itself reflect actual tourist behavior/interests; rather, it may indicate a cultural perception on the part of the taxi drivers (Fuentes 2006a). At Padangtegal, the (also mostly male) forest staff monitor interactions between tourists and macaques, interceding if they escalate and often preventing macaques from climbing on tourists, or removing them when they have already done so, but are not allowed to obtain financial gain (via tips) for their actions. However, while there are numerous signs warning people to avoid physical contact with the macaques at Padangtegal the staff is quite lax about enforcing this edict and thus, while not encouraging physical contact as with the taxi/coach drivers in Gibraltar, they do not strenuously restrict it. Unfortunately, there is occasional use of food to lure the macaques off of the humans, thus connecting a food reward for approaching tourists.

Another important context of assessment of interactions and potential pathogen transmission can be found in the demographic makeup of the tourist populations at each site (beyond the clothing styles and familiarity with monkeys noted above). Because of the great distances traveled by the European and North American tourists in Padangtegal, there is a high likelihood they will have fewer physiological defenses

to local pathogens relative to local humans from Bali or those from Southeast Asia. The short time they have to acclimatize immunologically to the ambient ecology and to the prevalent bacteria and other aspects of the pathogen environment may result in a weaker (or less effective) immune response to pathogens overall, thus including those potentially transmitted via interactions with macaques. This situation is somewhat different for the tourists at Gibraltar who have traveled across shorter distances and to fewer ecological zones to arrive at the site.

2.5 Assessing Dynamic Interfaces

Attempts to assess, model, and ameliorate (and manage) pathogen transmission risks in interactions between humans and macaques at Padangtegal and Gibraltar are greatly assisted by examining the sites through the lens of ethnoprimaotology and considering the mutual interfaces of humans and other macaques in the co-construction of the niches at the sites (Fuentes 2010; Fuentes et al. 2007b). The salient primatological factors between the sites of Gibraltar and Padangtegal include differences in macaque species, age and sex class behavior, varying prevalence of infectious agents, and local ecologies. Salient factors for examination in the humans include demography, gender, geo-cultural point of origin, clothing styles, immunological status, and relative familiarity with primates.

Clearly, analyzing such data requires an ethnoprimatological lens methodologically and theoretically (e.g., Dore et al. 2017; Jones-Engel et al. 2011; Lane-de Graaf et al. 2014; Riley et al. 2010). Finally, if one is to think of these contexts as ecological systems, then the niche construction approach offers a particular toolkit with which to model the feedback processes inherent in the interfaces and enables one to reach beyond standard evolutionary approaches to incorporate models of ecosystem engineering and a synthesis of ecological, biological, and social processes rather than treating them as discrete spheres.

If One Health is to be seen as a strategy for expanding interdisciplinary collaborations and communications in all aspects of health care for humans, animals, and the environment, then highly integrative methods and approach must be undertaken. What I have outlined here are two perspectives (ethnoprimatology and niche construction) and one example (macaque–human interfaces at two sites) in order to illustrate a few of the aspects of the kinds of synergy, data, and contexts that need to be taken into account when thinking through the human–monkey pathogen sharing landscape. One can easily see this is an incomplete overview of the wide range of collaborators one needs to truly and effectively engage these issues. By focusing here on the primatological and the anthropological, I have not discussed in any detail the epidemiological, veterinary, economic, and the myriad of other foci that are also implicated in a One Health understanding of these contexts. However, the other chapters in this volume engage with many of those facets and I leave it to the reader to absorb, connect, and synthesize these various elements. As in any case of a truly

One Health approach, diverse bodies of knowledge must be connected, synthesized, and applied by a team of collaborators, not by single individuals.

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Chapter 3
Monkey Health Is a Team Sport

Tiffany M. Wolf, Jessica R. Deere, Marissa S. Milstein, Christopher A. Shaffer, and Dominic A. Travis

Abstract In today's increasingly complex world, a more robust approach is needed to combat the dynamic nature of emerging and reemerging infectious diseases. This is certainly the case where monkeys and neglected diseases (NDs), defined in this volume as diseases not well studied in monkeys, are concerned. The diversity of monkey species and their behavioral ecology, the pathogens to which they are susceptible, and the number of potential interfaces for transmission, both intra- and interspecies, demands the integration of disparate disciplines to address this "Grand Challenge." Thus, this subject matter provides a case statement for the development of new "team science" approaches. In this chapter, we briefly explore how the diversity of pathogens, monkey hosts, and ecological drivers of disease transmission require the development of diverse research teams. With this need established, we review terminology and basic approaches to the development of multidisciplinary research that, when employed in an ecosystem health context, provides an approach to characterizing and/or optimizing risks associated with diseases in monkeys.

Keywords Interdisciplinary · Team science · Ecosystem health · Infectious disease transmission · Complexity · Pathogen · Multidisciplinary · Zoonotic · Biosentinels · Translational · Transmission routes · Host specificity · Morbidity · Mortality · Epidemic · Diversity · Neglected diseases

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3.1 Introduction

In 2005, Parkes et al. wrote a convincing argument for the increased need for multidisciplinarity in infectious disease research (Parkes et al. 2005). Historically, at the start of the twentieth century, western European thought relied primarily on two disciplines to explain and control infectious diseases: microbiology and epidemiology. They concluded that in today's increasingly complex world, a more robust approach is needed due to the increasing complexity of this issue and proposed a new integrated multidisciplinary model to combat the dynamic nature of emerging and reemerging infectious diseases (Parkes et al. 2005). The diversity of monkey species and their behavioral ecology, the pathogens to which they are susceptible, and the number of potential interfaces for transmission, both intra- and interspecies, demands the integration of disparate disciplines to address this "Grand Challenge." The first step to disentangling this complexity, and suggesting a way forward, is to examine the complex and dynamic role of monkeys in the ecology of infectious disease transmission. Are they reservoirs serving as sources of infection for other species, as hypothesized in regard to the eradication of yaws in humans (Knauf et al. 2015; Zobaníková et al. 2013; see Chap. 5)? Do changes in their morbidity and mortality signal epidemic disease in an ecosystem, as has been the case of *Mycobacterium bovis* spillover in baboons (Keet et al. 2000; Sapolsky and Else 1987; Tarara et al. 1985; Wolf et al. 2014; see Chap. 4)? In such cases where disease spills over into monkey populations from other reservoir hosts, can transmission be maintained in their populations, and if so, under what conditions (see Chaps. 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15)? Or, do we care about diseases in monkeys because of the direct impacts on monkey conservation and population sustainability? The answer is likely yes to each of these, with inherent variability associated with the species of interest, geographical context, and the pathogens present. In this chapter, we briefly explore how the diversity of pathogens, monkey hosts, and ecological drivers of disease transmission require the development of diverse research teams. With this need established, we review terminology and basic approaches to the development of multidisciplinary research that, when employed in an ecosystem health context, provides an approach to characterizing and/or optimizing risks associated with diseases in monkeys.

3.2 Layers of Complexity

Understanding disease transmission and associated impacts on monkey populations is complex due to the diversity of potential pathogens, host physiology and response, and ecological interfaces that influence transmission risk. A traditional approach to studying disease ecology among populations has been to model pathogen transmission as a function of contact between susceptible and infectious individuals and probability of infection with contact (Anderson and May 1979). This general

approach to disease ecology has been extended to consider various types of pathways (e.g., direct, vector-borne, environmental) and hosts (e.g., sex, species) in transmission with increasing complexity, though often models of disease transmission remain limited to the dynamics of a single pathogen (Anderson and May 1979; May and Anderson 1979; Nunn et al. 2007). Thus, these approaches generally remain a simplistic representation of transmission and often do not come close to capturing the complexity of the natural system where a diversity of pathogens, hosts, and interfaces interact at different scales within a shared environment (Fig. 3.1) (Buhnerkempe et al. 2015; Lloyd-smith et al. 2009). As anthropogenic processes continue to impact the environment and animal populations, it is also imperative to understand the influence of these changes on pathogen transmission dynamics, particularly in regard to diseases of monkeys. Thus, in moving toward a more holistic understanding of monkey diseases, we must consider the levels of complexity of these natural systems, the role of monkeys in pathogen transmission, and how we might best integrate system complexity in our approach to infectious disease research. There is not a single discipline of science that maintains the expertise to address the diverse processes that contribute to this complexity; therefore, we consider along the way the various disciplines that might come together to achieve this more holistic research approach.

3.2.1 Pathogen Diversity

The diversity of pathogens that infect nonhuman primates (NHP) and their transmission characteristics is extensive (Calle and Joslin 2015; Nunn et al. 2005). A recent search of the Global Mammal Parasite Database (Nunn and Altizer 2005; www.mammalparasites.org) resulted in the retrieval of 5840 records of 4896 pathogen and parasite species reported from 54 genera of NHP, including 150 identified NHP species (GMPD, 5/24/2018). Among the broad taxonomic groups of pathogens and parasites reported, 2097 (44.3%) were of helminth, 1484 (31.4%) protozoal, 671 (14.2%) viral, 251 (5.3%) bacterial, 221 (4.7%) arthropod, and 9 (0.2%) of fungal nature. In addition to these, PREDICT, a surveillance and virus discovery component of the Emerging Pandemic Threats program initiated by the United States Agency for International Development (USAID) in 2009, has reported the detection of 235 novel and 54 known viruses of primates (USAID PREDICT 2014). The identified viruses represent 17 and 11 viral families or genera, respectively. While these surveillance efforts identified many significant human and NHP pathogens and novel viruses closely related to known pathogens, the propensity for many of the newly discovered viruses to cause disease in NHP or other species remains to be seen. Thus, it is clear that expertise in pathogen discovery has made substantial advances in our knowledge of the occurrence of existing, new, and potentially emerging pathogens in monkeys, but to fully understand (1) the pathogenicity, or ability to cause disease, of these organisms in monkeys and (2) their potential to

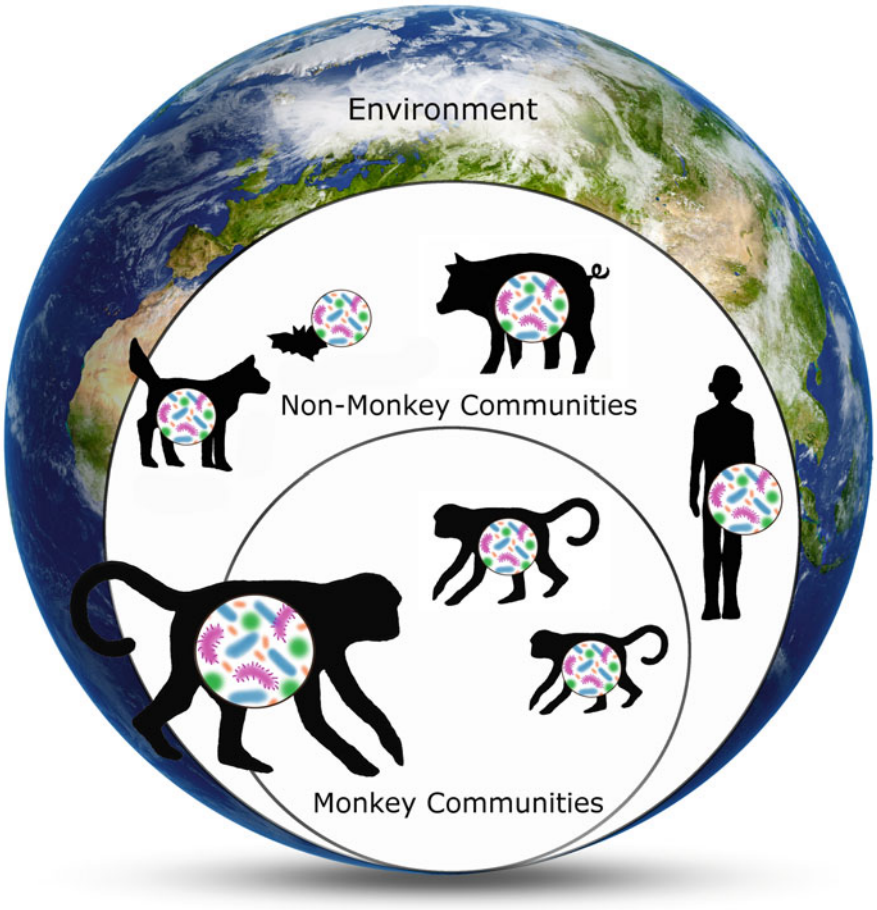

Fig. 3.1 A conceptual diagram of the layers of complexity that influence disease transmission among monkeys. Understanding diseases in monkeys requires a systems-based approach, which considers interactions among pathogens, hosts, and the environment. Such an approach recognizes the many levels of interactions, from those among communities of microorganisms up to communities of different host species, as well as the dynamics (e.g. species behavior, anthropogenic change to the environment) at the various interfaces that influence disease transmission. This figure is a simple representation of these interfaces at the various scales (*each level represented by a different sphere*), between pathogens and other micro and macro-organisms within a host, pathogens and host, and host among hosts, all within the natural environment. From the level of the *Monkey Communities* on up to the *Environment*, each sphere contains numerous smaller spheres representing the numerous individual hosts and their associated pathogens, all interacting across the various interfaces contained within. These multiple levels of interaction should be considered when designing strategies and assembling teams for the detection and measurement of disease in a system. Image of the Earth: © 1xpert / Fotolia, used with permission

Table 3.1 Major routes of pathogen transmission, along with associated primate behaviors and specific disease examples

Transmission pathway	Mechanism	Description	Behaviors associated with transmission	Examples
Direct	**Close contact**	Contact or interaction where transfer of infectious particles in body fluids, excretions, or aerosols may occur	Grooming, biting, scratching, playing, huddling	*Macacine herpesvirus 1*, *Morbillivirus,* pathogenic *Mycobacteria*
	Blood-borne	Exposure to blood of infected individuals	Fighting, primate consumption	Simian immunodeficiency virus, simian foamy viruses
	Sexual	Copulation	Mating behaviors	Simian immunodeficiency virus, simian foamy viruses, herpesviruses
	Vertical	Congenital or during parturition	Reproduction	Simian immunodeficiency virus, simian foamy viruses, Cytomegalovirus
Indirect	**Environmental**	Transmission through environmental sources, such as soil, water, food, fomites	Habitat use, foraging and feeding, drinking from contaminated water sources	*Schistosoma*, *Toxoplasma*, soilborne *Mycobacteria*, soil-transmitted helminths
	Vector-borne	Involves the bite, consumption, or sharing environment with an intermediate host such as arthropods, insects, or snails	Habitat use within vector range	West Nile virus, *Trypanosoma, Malaria,* dengue

impact monkey populations, additional expertise in pathology, veterinary science, epidemiology, and ecology are needed.

Aside from the taxonomic diversity, there are relevant pathogen characteristics, such as transmission routes, host specificity, and pathogen interactions that need to be considered when studying diseases in monkeys. These pathogens move through populations by a variety of mechanisms that fall within two main transmission pathways: direct and indirect (Table 3.1). Transmission may occur via a single pathway, as is the case for the *Plasmodium* parasites causing malaria, which are transmitted by mosquitoes (Bueno et al. 2013; Deane 1992; Liu et al. 2010; Prugnolle et al. 2010; Springer et al. 2015); whereas, some pathways are more complex, as we see with *Schistosoma* spp., where transmission occurs through a freshwater environment with aquatic snails as intermediate hosts (Fenwick 1969;

Müller-Graf et al. 1997; Rudge et al. 2013; Standley et al. 2012). Still others have evolved transmission via multiple pathways; the Herpesviruses are a prime example, being transmitted by direct, sexual, and vertical (i.e., from mother to unborn offspring) pathways (Kilbourn et al. 2003; Lee et al. 2015) (see Chap. 8). The behavioral ecology of different monkey species might enhance transmission via some routes more than others, which may contribute to the variation and diversity of pathogens we observe across monkey species even within the same system.

The risk of disease transmission between and among a set of potential hosts relies upon (or is limited by) certain fundamental characteristics of the pathogen itself. One such characteristic, host specificity, refers to the range of species that a pathogen may infect through any of its life stages (Nunn and Altizer 2006). Pathogens with a more limited host range, specialist or species-specific pathogens, have evolved a genetic framework for invasion, replication, and transmission that targets a particular host or range of closely related host species. Pathogens with a large host range are considered generalist or multihost pathogens and have evolutionarily derived traits that allow host plasticity, such as rapid mutation rates and high genetic variability that enhances *invasion* of new hosts and *evasion* of host immune defenses (Cleaveland et al. 2001; Gupta et al. 1998; Morand et al. 1996; Woolhouse et al. 2001). From an ecological perspective, generalist pathogens have also evolved mechanisms that bring them in contact with a greater range of hosts, such as through biting vectors or long-term survival in the environment (Woolhouse et al. 2001). The degree of host specificity can also vary between the different life stages of pathogens with complex life cycles. For example, some *Schistosoma* spp. have a broad mammalian definitive host range, including NHP (Fenwick 1969; Müller-Graf et al. 1997; Rudge et al. 2013; Standley et al. 2012), but a limited molluscan intermediate host range (Bush et al. 2001; Morgan et al. 2001). While it is important to consider this diversity in host specificity as it pertains to infection risk, we must also recognize that our knowledge of the extent of this among NHP pathogens is likely incomplete due to limitations in sampling and detection across NHP species and understanding of the ecology of pathogen transmission between respective hosts.

Finally, it is becoming increasingly evident that we must decrease our reliance upon individual pathogen models and focus more on the complex reality of multiple or coinfections. Community-level interactions among pathogens and other micro- and macroorganisms can influence host infection and subsequent outcomes (e.g., replication, virulence, transmission). Competition between pathogen strains may select for the emergence of more virulent strains (de Roode et al. 2005) or community-level interactions between taxa may inhibit pathogen replication and transmission (Bian et al. 2010; Moreira et al. 2009). There is a growing body of evidence from gut microbiome studies that commensal microbial communities protect against pathogen infection through direct microbial competition as well as priming of the host immune system (Clayton et al. 2018; Khosravi and Mazmanian 2013). Considering that pathogen coinfections are the norm in free-living primates, these community-level interactions should be considered further to fully understand variations in patterns of disease as well as the impact of ecological processes on these

microbial communities. This creates an exciting opportunity to include experts in metagenomics, bioinformatics, and community ecology in disease research of monkeys. Taken together, each of these characteristics that contribute to the pathogen diversity of monkeys illustrates the need to build research teams that integrate the deep organismal and biological knowledge of parasitology, virology, or microbiology with the system-level knowledge of host ecology and epidemiology to address our many knowledge gaps.

3.2.2 Diversity of Monkey Hosts

In addition to the complexity of pathogens and the host–pathogen relationship highlighted above, primates are characterized by tremendous diversity in their socioecology, exhibiting extensive variation in body size, locomotion and substrate use, social structure and social behavior, ranging behavior, and diet. Understanding how variation in these characteristics (epidemiological "risk factors") across species influences host exposure and susceptibility to pathogens is essential for determining disease transmission risk. There is also increasing evidence that a pathogen's effect on its host(s) has been an important selective pressure in the evolution of primate socioecology (Chapman et al. 2009; Gillespie et al. 2008; McCabe et al. 2014; Nunn and Altizer 2006). While our understanding of the variables that impact host susceptibility has increased considerably in the past two decades, the relationship between many traits and infection risk remains ambiguous or untested empirically. Much of the conflicting data on the relationship between socioecological variables and disease in primates likely results from the complex interactions between behavioral, phylogenetic, ecological, and morphological variables, as well as the challenge of conducting integrated ethological and laboratory research (Gillespie et al. 2008; Nunn and Altizer 2006). Thus, multidisciplinary team approaches that combine behavioral field studies with phylogenetics, morphology, parasitology, microbiology, and virology are critical.

Research that includes the level of complexity discussed in this section is generally in its infancy and does not always point in a clear direction. For example, while large body size and increased terrestriality have long been thought to increase parasite richness (reviewed in Gillespie et al. 2008), neither variable was found to be associated with parasite richness after controlling for phylogenetic similarity (Nunn et al. 2003; Nunn 2002; Vitone et al. 2004). The lack of clear association appears to result from the confounding variables of diet, group size, ranging behavior, and dominance rank (Nunn and Altizer 2006). While terrestriality may not be associated with overall parasite richness, specific pathogens that require terrestrial or aquatic intermediate hosts may be more common in terrestrial primates (Müller-Graf et al. 1997; Munene et al. 1998; Nunn and Altizer 2006).

Another example of complexity occurs when attempting to integrate social and life-history traits into disease risk models. NHP are among the most social of all mammals but exhibit extensive variation in group size, social structure, mating

behavior, and dispersal patterns, all of which may affect pathogen transmission. Because of this diversity and the complex interactions among group size, composition, cohesiveness, and dispersal, finding consistent relationships between specific social variables and disease risk across primates has proven difficult. Many researchers have suggested a positive relationship between group size and disease risk (Côté and Poulinb 1995; Davies et al. 1991; Freeland 1976, 1979; Nunn and Heymann 2005; Tutin 2000), but this prediction has found relatively little empirical support in comparative studies (Nunn and Altizer 2006; Nunn et al. 2003; Nunn 2012), with the exception of neotropical malaria (Davies et al. 1991; Nunn and Heymann 2005).

Using theoretical approaches, several authors have argued that primates in larger groups may mitigate disease risk through subgrouping (Griffin and Nunn 2012; Wilson et al. 2003) and some have suggested that pathogens may have played an underappreciated role in the evolution of fission–fusion dynamics in primates (Nunn and Altizer 2006; Walsh et al. 2009). Therefore, the interaction between group size and group cohesiveness may be more important than group size taken alone in determining disease risk. Nunn et al. (2008) also demonstrated through theoretical disease modeling the potential role of social structure in disease transmission. Their research demonstrated different patterns of pathogen spread among polygynous and multimale–multifemale NHP. Due to pathogen-mediated dispersal (where females disperse after the dominant male in a polygynous group dies from disease), polygyny facilitates increased disease spread across groups. In contrast, multimale-multifemale NHP show larger numbers of individuals affected but decreased spread from one group to another. Despite the insights that theoretical models have revealed on social structure and disease risk, many remain untested in wild populations.

Studies on the relationship between social status and disease risk have also produced conflicting results (MacIntosh et al. 2012; Meade 1984; Müller-Graf et al. 1997; Nunn and Altizer 2006). A positive association between dominance rank and disease risk has been reported in some studies (MacIntosh et al. 2012) while negative associations have been found in others (Cheney et al. 1988). A variety of factors related to social status appear to influence these conflicting results, with decreased dietary quality, increased stress levels, and centrality in the social network, particularly increased contact with conspecifics, that is, grooming and mating opportunities, thought to be positively associated with disease risk.

Ranging behavior is yet another important socioecological variable that may affect exposure to pathogens across NHP, adding to the complexity of disease risk. Day range appears to be positively associated with parasite richness, likely due to increased exposure to habitat diversity (Nunn et al. 2003). D-index (a measure of intensity of home range use) has also been positively associated with parasite richness, possibly reflecting the increased accumulation of parasites in more intensively used home ranges (Nunn and Dokey 2006). In contrast, the prediction that increased home range overlap facilitates the spread of pathogens has found little empirical support (Nunn and Dokey 2006; but see Eilenberger 1997). In some cases, exposure to pathogens is mitigated when NHP naturally adjust their ranging behavior, including alternating sleeping and defecation sites and avoiding reuse of travel

routes during some periods of the year (Hausfater and Meade 1982; Milton 1996; Moore 2002; Nunn and Altizer 2006).

Disease risk may also be influenced by the considerable dietary diversity across NHP species. While NHP as an order are characterized by generalist diets and most species incorporate a wide spectrum of food items, the percentages of different plant and animal food types that different taxa consume varies considerably. As many pathogens are spread through contact with contaminated food, this dietary diversity has important implications for disease risk. For example, folivory of NHP has been positively associated with parasite richness, and it has been suggested that the increased biomass that folivores consume may lead to a higher risk of ingesting infectious stages of parasites (Nguyen et al. 2015; Nunn et al. 2003; Vitone et al. 2004). However, higher levels of secondary compounds like tannins in leaves compared to fruit may reduce the prevalence of some parasites (Rothman et al. 2008). In addition, some primates may ingest plants and soil that primarily function to reduce parasite loads in a form of self-medication (Huffman 1997, 2001; Lozano 1998; Phillips-Conroy 1986). In this area of research alone, there remains much work ahead in untangling the complexity of diet and foraging habits, medicinal properties and use, and disease risk or modulation among NHP.

These few examples illustrate another layer of complexity in disease transmission of monkeys that affords an opportunity for interdisciplinary, and even multiteam collaboration. Research on the role of NHP socioecology on disease risk really focuses on pathogen exposure and susceptibility in the NHP host, but this research rarely intersects with the complexity of pathogen diversity discussed in the previous section. Although the interaction of these two axes may be well-recognized, integrating them in science is an important hurdle that is more easily surmounted through team science.

3.2.3 Ecological Interfaces

The community ecology of monkeys is also characterized by an impressive range of diversity. This diversity makes it inherently challenging to understand what factors influence the spread of pathogens between monkeys and other taxa, requiring multidisciplinary approaches to adequately characterize host–pathogen interactions within this complex of ecological interfaces. In regard to pathogen transmission, relevant ecological interfaces may be largely characterized as interspecific, environmental, and anthropogenic. These interfaces are important for pathogen transmission when they allow opportunities for effective contact or contact that results in pathogen transmission to a susceptible host.

The interspecific interface of monkeys is important to consider where infectious disease transmission is concerned. For example, polyspecific associations, where two or more species come together to travel and forage as a unit, have been reported in many NHP (Chapman and Chapman 2000; Cords 1990; Shaffer et al. 2016). While these associations provide several benefits for the species involved, the close

contact between different taxa potentially increases the risk of directly transmitted diseases (Nunn and Altizer 2006). Further, polyspecific associations inherently increase group size, and the larger size of polyspecific groups may not have the same mitigating effects afforded by subgrouping described for individual species (Altizer et al. 2003; Freeland 1977, 1980; Gillespie et al. 2008). Predator–prey interactions among NHP that prey upon vertebrates, including mammals and even other primate species, can also influence transmission. Chimpanzee hunting of monkeys, including red colobus monkeys and galagos, has been widely documented throughout equatorial Africa (Goodall 1986; Mitani and Watts 1999) and shown to be a route of blood-borne viral disease transmission from monkeys to apes (Sharp and Hahn 2011). Although the interspecific interface may be well-recognized where disease risk for NHP is concerned, more research is needed to better understand the role of host diversity or sympatry with specific hosts (e.g., known reservoirs or maintenance hosts) on disease patterns in monkeys. This is an opportunity where multidisciplinary research that integrates disparate ecological research on different species or taxa with that of epidemiology may provide new insights on disease ecology at this interface.

The environmental interface is most important for the indirect transmission of pathogens that have the ability to survive for long periods outside of the host or a life stage with an environmental component (e.g., Müller-Graf et al. 1997; Parsons et al. 2015; Rwego et al. 2008). Feasible pathways for pathogen transmission at the environmental interface include shared resources. For example, the high consumption of *Ficus* fruits by chimpanzees and *Hypsygnathus monstrosus* bats, a possible reservoir host for the Ebola virus, puts chimpanzees at a greater risk of exposure than less frugivorous primates (Walsh et al. 2009). NHP also frequently share sleeping sites and water sources with a variety of other animals, potentially facilitating transmission of generalist pathogens (Gillespie et al. 2008; Nunn et al. 2003). Researchers targeting this interface often highlight habitat overlap of hosts in their justification for their focus on relevant environmentally transmitted pathogens, but rarely is the examination of environmental or shared resources of overlapping species reported (Parsons et al. 2015). Certainly, the techniques and methods for environmental sampling and pathogen detection are diverse and research teams tackling questions at this interface would benefit from the relevant expertise that environmental microbiology might have to offer.

The final ecological interface on which we focus in our consideration of the complexity of disease transmission is arguably the most profound. The ecological landscape of the NHP–pathogen interface is complicated by increasing anthropogenic change. Human encroachment on wildlife from hunting, deforestation, and climate change can significantly alter the dynamics of NHP at other ecological interfaces (e.g., interspecific and environment) as well as host–pathogen relationships. Alterations in these dynamics can lead to increased infection risk, changes in pathogen and vector geographic distribution, and the emergence of novel pathogens (Chapman et al. 2009). Deforestation, mainly resulting from large-scale agriculture, is the foremost threat to NHP conservation worldwide (Estrada et al. 2017), with the majority of NHP populations living in anthropogenically disturbed habitats. Often

these habitats are fragmented and lead to higher densities of primates in smaller areas, where proximity and human interactions are increased, thereby increasing the disease transmission risk (Chapman et al. 2005; Daszak et al. 2001; Dobson and Foufopoulos 2001). Secondary effects of forest fragmentation from long-term deforestation can also result in intensive resource use by NHP (as a result of higher densities), nutritional stress and suppressed immune function, contact with domestic animals or exposure to domestic animal pathogens, and increased exposure to vector-borne diseases (Chapman et al. 2005; Solomons and Scott 1994). While this is generally not observed as a cascade of events, the multiple risk factors, their interactions, and the combined effects on pathogen exposure, susceptibility, morbidity, and mortality among NHP are likely only partly understood and sometimes recognized. As more NHP populations experience these pressures at the anthropogenic interface, there is an increasing likelihood of observing the synergistic effects of these conditions.

While the anthropogenic interface is generally assumed to increase disease risk, this may not always be the case. For example, the effect of habitat disturbance and biodiversity loss on disease transmission is complex and appears to vary across NHP species (Chapman et al. 2009; Young et al. 2013). In a meta-analysis of 14 studies of habitat disturbance (including fragmentation, logging, agriculture, and hunting) and parasite prevalence, Young et al. (2013) found that six studies showed a negative effect of disturbance on prevalence, seven showed a positive effect, and one showed no effect. Hunting in particular is a well-recognized threat to NHP conservation worldwide (Estrada et al. 2017), and while the hunting, butchery, and consumption of NHP is an important pathway of zoonotic disease risk for humans, it may be less so for human pathogen risk to NHP. However, because population density is one of the most significant predictors of disease transmission, hunting may actually decrease NHP disease risk by decreasing the density of NHP populations (Chapman et al. 2009). Finally, much attention has been focused at these ecological interfaces where spillover of zoonotic disease occurs from NHP (or other wildlife) to humans (Jones et al. 2008; Plowright et al. 2016; USAID PREDICT 2014; Woolhouse and Gowtage-Sequeria 2005), but less in the direction from humans to NHPs (Epstein and Price 2009; Schaumburg et al. 2012; Wolf et al. 2014), and still less in regard to transmission from other species to NHP within the same ecosystem (Rwego et al. 2008). At a time when the dynamics of each of these ecological interfaces may be influenced by human pressure, it is critical to understand their role, interactions, and impacts on disease transmission in monkeys.

3.2.4 Detecting and Measuring Disease

NHP are recognized as important species in which to conduct emerging disease surveillance, as they may either be biosentinels or sources of zoonotic infections to humans; thus, disease surveillance in NHP has been steadily increasing (Calvignac-Spencer et al. 2012; Jones et al. 2008; Wolfe et al. 2007, 2016, 2019a, b; Woolhouse

and Gowtage-Sequeria 2005). It is also critical because of the recognized threat that infectious diseases pose to these populations and their conservation (Epstein and Price 2009). Surveillance of bush meat and confiscated, rehabilitated NHP have provided key insights into the pathogens with which NHP may be infected or were previously exposed (Mugisha et al. 2011; Schaumburg et al. 2012; Whittier 2009; Wolfe et al. 2005). NHP populations that are closely monitored for research and tourism also present an opportunity for disease surveillance (Coscolla et al. 2013; Cranfield 2008; Jones-Engel et al. 2006; Keele et al. 2009; Terio et al. 2011; Wolf et al. 2016, 2019a, b).

Good surveillance requires the use of accurate diagnostic tests, of which there is a significant need for NHP and other wildlife. Although diagnostic tests are available for a variety of diseases to which NHP are susceptible, application is not always appropriate in species for which the tests were not developed. Due to the high cost of diagnostic test development and validation, most commercially available diagnostic tests have been developed for use in humans or domestic animals, for which use is high and funding more readily available. Unfortunately, because many traditional tests, such as those screening for antibodies, utilize species-specific reagents, accurate test performance may be hindered even in closely related species. The advancement of molecular methods for disease detection (e.g., genomics, proteomics, metabolomics) offers a path around such limitations (Gillespie et al. 2008; Leendertz et al. 2006; Standley et al. 2012). While many of the molecular methods still require specialized laboratory equipment and analytical technologies, these are becoming more commonplace, offering new opportunities for disease detection and measurement in wild NHP populations.

The next big challenge in surveillance is the standardization of techniques in sample collection, storage, and testing to ensure comparability across field sites and laboratories, followed by rigorous assessment of surveillance system performance (e.g., Wolf et al. 2019a, b). In 2006, The Max Planck Institute created the Great Ape Health Monitoring Unit (http://pin.primate.wisc.edu/idp/idp/entry/601) to attempt to align the great ape health community in terms of methods and protocols for disease detection and surveillance. The discussion is best reflected in the document "Best Practice Guidelines for Health Monitoring and Disease Control in Great Ape Populations," published by the IUCN Species Survival Commission's Primate Specialist Group (https://portals.iucn.org/library/sites/library/files/documents/ssc-op-056.pdf). Perhaps it is time to reinvigorate this initiative across the NHP community at large. The achievement of this ambitious goal will require not only collaboration of laboratory experts and epidemiologists, but a larger effort of collaboration, transparent communication, methodology, and data sharing across NHP researchers.

3.3 Team Science: A Good Idea, But an Implementation Challenge

The previous sections of this chapter outline the complexity of the issue of neglected monkey diseases and the need for innovative new approaches, one of which is a focus on the development of multidisciplinary, team science approaches. The call for multidisciplinary research and team science as a tool for attacking modern problems of growing scope and complexity is on the rise (Errecaborde et al. 2019). However, team science does not just happen: it takes a much higher degree of planning, coordination, and communication than is required when employing most single-disciplinary approaches. Language (disciplinary in this case) is often recognized by most disciplinary reviews as the first barrier to success. As exemplified by Hallmaier-Wacker et al. (2017) in their discussion of the multiple uses of the phrase "disease reservoir," a lack of standardized terminology among closely related disciplines can complicate successful implementation of a team science approach to something as fundamental as the ecology of an emerging infectious disease (Ebola virus in this case). In fact, in the experience of this authorship team, the issue of disciplinary language has become one of the core barriers to the implementation of the so-called One Health approach in many settings. For instance, in much of the literature, "transdisciplinary" and "interdisciplinary" are used to describe similar concepts or approaches but have technically different meanings. Since a philosophical discussion of the difference between these terms is beyond the scope of this conversation, they will be used synonymously hereafter with complete acknowledgment and recognition that this does not adequately describe the nuances of the subject matter. When discussing terms used in specific publications, we will defer to the language used therein.

The relatively recent emergence of practices such as "team science," "complexity science," and "collaborative research" lends some evidence that multiple disciplines working together helps solve problems when *effectively* employed. One of the largest complaints against the One Health movement is the perceived blind acceptance of team science as *the way* (or so-called silver bullet), without a body of peer-reviewed literature to test and validate the cost–benefit of these methods. Thus, practitioners are increasingly focusing on the evaluation of outcomes in these areas to further define what constitutes "effective." When attempting to derive best practices from the literature, one finds that the rapidly growing breadth of literature on these topics is vast. For instance, a literature search encompassing four databases (Google Scholar, Scopus, PubMed, and Web of Science) from 2005 to 2018 – using two separate inclusion criteria ("multidisciplinary science/research, transdisciplinary science/research, interdisciplinary science/research" and health and "interprofessional research, complexity science, team science, science of team science, collaborative research, interprofessional collaborative research/practice") – resulted in over 60,000 records. In 2015, a systematic review published in Nature on the topic of "interdisciplinary research" examining more than 35 million papers in the Web of Science database showed that the fraction of references (as defined by

incorporation of the word 'interdisciplinary' in the title) has continuously risen in both natural and social sciences since 1980. In addition, the study found that interdisciplinary research tends to increase in impact over time and that "health sciences" generally rank as highly interdisciplinary (relative to many other fields and disciplines) (Van Noorden 2015). The scope of potential collaborations needed to adequately address the topic "monkeys and tropical disease" is incredibly broad, covering at a minimum the fields of [medical] anthropology, primatology, behavioral science, lab animal medicine, human and animal health, epidemiology, infectious disease ecology, conservation, and environmental science. There is no one publication that provides sound methods for engaging in team science among all the required fields for this discussion. However, examination of a number of evaluative models covering multi-/inter-/transdisciplinarity from different disciplinary cultures may provide useful insights for those wishing to engage in evidence-based team science.

3.3.1 Team Science in the Medical Professions

The recognition of these values has become integral to all areas of health science in recent years. For instance, the United States' National Institutes of Health has invested heavily in conceptualizing, implementing, evaluating, and training the principles of "interprofessional collaborative research practice," which is synonymous with "team science" (Bennett and Gadlin 2012). The (US) National Cancer Institute provides access to all publications and tools catalogued in this area, available under this program at: https://www.teamsciencetoolkit.cancer.gov/Public/Home.aspx under the term "Science of Team Science." Hall et al. (2012) conducted a systematic review of this literature and found that scientists are generally trained in unidisciplinary approaches and may have little training in, or exposure to, both the scientific skills and team/leadership processes necessary to collaborate successfully with experts in disparate disciplines and fields. They then created a basic conceptual model of the four-stage process of "Transdisciplinary Research," which incorporates the sequential steps of problem formulation and conceptualization, transdisciplinary study design and implementation, and analysis and evaluation for further iteration (Hall et al. 2012).

3.3.2 Team Science and Environmental Health

As discussed earlier in this chapter, environmental issues are often borne of, or characterized by, interactions between humans and ecosystems. However, researchers have historically addressed the interaction between environmental change and human and animal well-being from within traditional disciplines (e.g., the division in Universities between the natural and social sciences), severely

limiting adequate development of, and practice with, multidisciplinary partnerships in this area. Recently, scientific and grant funding communities have committed a great deal of financial resources to stimulate partnerships via funding mechanisms such as the Coupled Human and Natural Systems research program of the United States National Science Foundation, among many others (Roy et al. 2013). Unfortunately, the opportunity to create teams does not ensure that they will be strategically constructive or effective. Roy et al. (2013) conducted a survey of primarily North American interdisciplinary environmental researchers and found that benefits included "fostering the ability to view issues from differing perspectives, intellectual stimulation, knowledge creation and connection of knowledge bases, positive effects of collaboration including personal satisfaction and [sometimes] promotion." Drawbacks included "primarily communication difficulties, differences in perspective, differences in culture and research methods, time and funding limitations and lack of existing collaborative frameworks." Lack of funding was considered the greatest obstacle, as well as lack of credit given for career advancement. This is especially important in academia where the finding that teams tend to produce fewer short-term outputs, but twice as many over a 10-year span, can be a barrier to promotion (Hall et al. 2012). The more broad sweeping conclusion from the survey conducted by Roy et al. (2013) was agreement that there was a need for training in "how" teams are effectively formulated and function. They also agreed that the undergraduate level is the most important time to begin training individuals in "interdisciplinary" research.

A parallel study in the area of Conservation Science conducted by Pooley et al. examined 50 years of literature indexed in the SCOPUS database under "conservation biology" and related terms (Pooley et al. 2014). They found that barriers to conducting effective interdisciplinary science fell consistently into five main categories: methodological challenges, value judgments, differing theories of knowledge, disciplinary prejudices, and interdisciplinary communication. Based upon these findings, the authors recommend the following points to consider for the development of a successful multidisciplinary project:

- Careful recruitment of collaborators and project staff
- Broad stakeholder inclusion
- Inclusive research question development
- Negotiating team and stakeholder power dynamics upfront
- Understanding disciplinary or cultural conceptual differences
- Joint agreement on methods
- Developing a shared language
- Agreement upon a strong structure of communication
- A pre thought-out plan for data integration and project outcomes/endpoints/success

3.3.3 Team Science and Infectious Disease Research

Multidisciplinarity was not the approach to infectious disease research at the start of the twentieth century, when western European thought relied primarily on microbiology and epidemiology alone to explain and control infectious diseases. Parkes et al. (2005) wrote a convincing argument for the increased need of multidisciplinarity in infectious disease research, concluding that in today's increasingly complex world, a more robust approach is needed. To meet this need, they proposed a new integrated multidisciplinary model to match the complex, dynamic nature of emerging and reemerging infectious diseases (Parkes et al. 2005). This model focuses on linking teams across knowledge perspectives, including community and culture, practitioners and field personnel, disciplines across biological and social sciences, and the inclusion of appropriate units of governance.

With the turn of the millennium, the growth of concepts such as "One Health" and "The Science of Team Science" reinforced the use of collaborative terms such as eco-epidemiology (ecology and epidemiology), disease ecology, and ethnoprimatology. To some degree, these terms were meant to unify very different traditional disciplinary approaches to field and laboratory-based disease investigation and predictive modeling. In 2012, Restif et al. (2012) proposed practical guidelines to help with effective integration among mathematical modeling, field investigation, and diagnosticians**.** The "model-guided fieldwork framework" (MGF) highlights a stepwise approach to disciplinary integration beginning with *a priori* ecological model generation and exploration, which leads to study design for empirical field and laboratory research, which in turn leads to model validation in an iterative cycle of improvement and innovation (Restif et al. 2012). However, the effects of this paradigm in teamwork have rarely been quantitatively described. In 2016, Manlove et al. (2016) systematically surveyed the published literature and used social network analysis to measure multidisciplinarity in One Health studies constructing dynamic pathogen transmission models (Manlove et al. 2016)**.** The number of publications in this area increased by 14.6% per year and clustered into three communities: one used by ecologists, one used by veterinarians, and a third diverse-authorship community used by population biologists, mathematicians, epidemiologists, and experts in human health. Overlap between these communities increased through time in terms of author number, diversity of coauthor affiliations, and diversity of citations. However, communities continue to differ in the systems studied, questions asked, and methods employed. This study shows that infectious disease research may still be more siloed than many may espouse or hope. This is a place where the collective "monkey health" community should actively engage, and represents a great opportunity for collective progress.

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Chapter 4
Mycobacterial Infections in Monkeys

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In loving memory of Dr. Franciso (Paco) Mendoza

Abstract Mycobacteria are a group of acid-fast bacilli that cause a range of clinical manifestations of public health relevance across the globe including tuberculosis, atypical mycobacterial infections, and leprosy. Nonhuman primates are naturally susceptible to infection with mycobacteria, and infections in captive, synanthropic, and free-roaming contexts are documented across most continents. Infection with mycobacterial species capable of causing tuberculosis is more thoroughly described compared to reports of atypical infection and infection with leprosy-causing strains. Monkeys are also used as animal models for biomedical studies of the disease tuberculosis. The full range of the immune response to infection, clinical manifestation of infection, and variations in species-level host susceptibility to all naturally acquired mycobacterial infections are poorly understood in monkeys. Transmission of mycobacteria between nonhuman primates, humans, and other species of domestic and wild animals complicates the current understanding of the epidemiology of these diseases as well as the implementation of effective surveillance and control measures, which is further compounded by a lack of rapid, feasible detection methods outside of the laboratory setting. The range of mycobacterial infections in monkeys, immunology of infection, and control and prevention measures are presented and discussed from a One Health perspective.

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4.1 Introduction

The genus Mycobacterium consists of over 150 species of small, aerobic, acid-fast bacilli that are generally nonmotile and belong to the family Actinobacteria. The genus includes pathogenic, nonpathogenic, and saprophytic species (King et al. 2017; Rastogi et al. 2001). The Greek prefix "myco" means fungus, which was ascribed to the genus because of its mold-like appearance on the surface of liquid media when grown in culture. Today, mycobacteria can be described by their relevance to health and disease using the following three categories: (1) *Mycobacterium tuberculosis* Complex (MTBC) bacteria, which are capable of causing the disease Tuberculosis (TB); (2) Nontuberculous mycobacteria (NTM), a large group of potentially pathogenic mycobacteria, including the *Mycobacteria avium* complex (MAC), which are capable of causing a range of clinical disease in humans and animals; and (3) *Mycobacteria leprae*, which causes the disease leprosy.

Mycobacteria are obligate pathogens with a prolonged history of coevolution with humans and animals over millions of years (Comas et al. 2013). They were also some of the first bacteria recognized to cause disease in humans. The Norwegian physician Gerhard Armauer Hansen described the first known mycobacterium, *M. leprae,* in 1873 (Hansen 1875). Robert Koch, a German physician and microbiologist, then described the causative agent of TB in 1882 (Koch 1884). Koch isolated the bacillus and demonstrated its ability to cause TB by using material from the lungs of apes and brains of humans to inoculate guinea pigs and faithfully reproduce disease. Shortly thereafter followed the discoveries of the causative agents of TB in birds and cattle, *M. avium* and *M. bovis*, respectively (Lehmann and Neumann 1896). Ancient MTBC DNA has since been recovered from Egyptian mummies, ancient Peruvian skeletons, and relics from China and India. The first report of TB in a nonhuman primate (NHP) occurred in 1863 following postmortem evaluation of a deceased chimpanzee at a zoo in London. NTM were initially described in the late 1800s as nonpathogenic saprophytes, but did not gain recognition as disease-causing pathogens in humans until the 1930s (Branch 1931; Kazda et al. 2009; Wagner and Young 2004).

While all three categories are capable of causing a diverse range of serious and devastating diseases, they are unified by their phylogenetic similarity, which imparts similar morphologic, immunologic, and pathogenic characteristics. They are also unified by their demonstrated ability to affect a range of primate species, yet their true burden of disease as well as the evolutionary ecology of these organisms in captive, free-ranging and synanthropic monkeys is significantly understudied (Wachtman et al. 2011). This is compounded by a global gap in robust studies describing the geographic composition and diversity of environmental, human, and animal reservoirs for mycobacteria.

4.2 The MTBC

Tuberculosis can be caused by any member of a group of genetically related mycobacteria collectively referred to as the MTBC (e.g., *M. tuberculosis, M. africanum, M. bovis, M. canetti, M. microti,* and others). This obligate pathogenic group of mycobacteria is capable of infecting a wide range of animal species around the globe, but many MTBC bacteria are associated with a particular host, such as *M. microti* with the meadow vole, *M. bovis* with ruminants, and *M. tuberculosis, M. africanum,* and *M. canetti* with humans (Botha et al. 2013; Kazda et al. 2009; Malone and Gordon 2017).

Around the globe, TB is a catastrophic disease that affects approximately ten million people each year and is the leading cause of death from an infectious agent, killing more people than human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) (WHO 2017).

Due to the burden of TB in human populations, concerted efforts have been made to characterize the disease, to reduce incidence rates, to improve access to and compliance with treatment, and to address the growing threat of drug-resistant TB infections. In NHPs, however, we still lack basic knowledge of the true burden of disease and range of manifestations across species, limiting our ability to make informed clinical, conservation, and public health decisions when managing and studying NHP populations (Wilbur et al. 2012a).

4.2.1 Species Affected

All primates are considered susceptible to MTBC infection, and the development of TB has been observed in several captive monkey species. According to available data on TB occurrence across primate species, Asian species seem more susceptible to illness, followed by African monkeys and great apes (Une and Mori 2007). Neotropical monkeys are thought to be the least prone to developing TB symptoms among NHPs (Une and Mori 2007); however, this may reflect a sampling bias.

Phylogenetic characterization of MTBC strains affecting human patients have demonstrated wide genetic variation in association with geographic origin, as well as relationships between host and mycobacterial genotypes in the development of disease (Brown et al. 2010; Caws et al. 2008). Human infection with MTBC is usually caused by one of seven human-adapted and variably pathogenic phylogenetic lineages that are associated with distinct populations and geographic ranges, with lineage 4 being the most widespread across the globe (Coscolla and Gagneux 2014). Two additional lineages are considered animal-adapted (Brites and Gagneux 2015). While TB in humans was historically thought to have originated from *M. bovis*, more recent genomic analysis shows that animal-associated TB-causing lineages nest within the genetically diverse human-adapted strains (Brosch et al. 2002). For this reason, and because of the history of contact with humans in most reported cases of TB in monkeys, the cases described to date are presumed to be of human origin, and thus the geographic context where these infections occurred must be taken into account to describe the profiles of mycobacterial circulation in monkeys. However, a novel MTBC strain recently isolated from a wild chimpanzee in Africa suggests that natural infection with genetic variants not of human origin may exist in primates (Coscolla et al. 2013). Coevolution between humans and MTBC lineages is supported by the fact that most humans exposed to MTBC do not develop active disease (Brites and Gagneux 2015). Primates may also express genetic resistance to infection with MTBC lineages adapted to their geographical origin but their transport for research, zoos, and trade exposes them to lineages associated with populations outside of their geographic range. More research is needed to demonstrate differential susceptibility of primate species and possible patterns of resistance across species and populations.

Most published reports in monkeys come from laboratory settings and experimental models. Macaques, especially cynomolgus (*Macaca fascicularis*) and rhesus macaques (*Macaca mulatta*), are the preferred primate experimental model. Most laboratory NHPs used in TB studies are immunocompromised and/or coinfected with retroviruses to replicate the full spectrum of immunological and pathological patterns of human TB. Up to 90% and 60% of experimental infections in rhesus macaques and cynomolgus macaques, respectively, develop acute TB in the laboratory setting (Maiello et al. 2018; Peña and Ho 2015). It has been suggested that among macaques, there are species-level differences in susceptibility to infection. Reports of experimental infections document rhesus macaques and Mauritian cynomolgus macaques are more likely to exhibit signs of disease progression compared to Chinese cynomolgus macaques (Maiello et al. 2018; Scanga and Flynn 2014), but these results are not generalizable to situations outside the laboratory such as natural infection. White-tufted-ear marmosets (*Callithrix jacchus*) have recently become an experimental model for TB research (Scanga and Flynn 2014) because of their susceptibility to several tuberculous (Via et al. 2013) and non-tuberculous (Wachtman et al. 2011) mycobacterial strains and the development of clinical disease of diverse severity upon induced (Cadena et al. 2016b) and natural infections (Michel and Huchzermeyer 1998).

Beyond the laboratory setting, screening of captive or synanthropic monkeys has produced reports of natural infection in several contexts in which monkeys are exposed to humans, including temples, sanctuaries, rescue centers, zoos, households, and wet markets. A review of published reports of naturally acquired MTBC infection in monkeys is presented in Table 4.1. Captive Old World monkeys dominate the literature describing symptomatic cases following natural infection, which may reflect the long history of research and experimentation on these species. Active TB has rarely been described in captive Neotropical primates other than the common marmoset (*Callithrix jacchus*) and the common squirrel monkey (*Saimiri sciureus*) (Alfonso et al. 2004), but MTBC has been detected in a range of asymptomatic individuals across numerous species (Alfonso et al. 2004; Barragán and Brieva 2005; Rosenbaum et al. 2015). Circulation of asymptomatic MTBC has also been documented in synanthropic temple macaques in Asia (Wilbur et al. 2012b).

Monkeys likely acquire mycobacterial infections in the wild following exposure to human and domestic animals; however, the only confirmed cases reported to date describe outbreaks in baboons (Keet et al. 2000; Michel et al. 2009; Sapolsky and Else 1987; Singh et al. 2011; Tarara et al. 1985). Baboons exposed to slaughtered cows in Kenya (Sapolsky and Else 1987; Tarara et al. 1985) and buffalo carcasses in South Africa (Keet et al. 2000; Michel et al. 2009) were infected with *M. bovis*, resulting in rapid disease progression and fatalities within months. In the South African outbreak, acid-fast staining revealed high loads of mycobacterial shedding in aerosol, feces, and urine samples that enabled baboon-to-baboon transmission within the same group. There was no evidence of spillover into other baboon groups within the same region that did not have access to the initial exposure, which was likely an infected buffalo carcass (Keet et al. 2000).

Though MTBC infections are more commonly reported in captive monkeys, prevalence estimates are difficult to obtain because of limited surveillance at the population level. Broad surveys are often performed following outbreaks in experimental colonies or zoological facilities (Garcia et al. 2004a; Une and Mori 2007), but few studies have reported the results of multiyear routine screening or surveys with negative results (Navarrete et al. 2014; Wolf et al. 2016). Thus, it is not possible to differentiate between species that are naturally resistant to infection and those that remain unstudied. We notice the infrequent report of cases from countries with higher incidence of human tuberculosis, which may reflect a large reporting bias. Case reports from monkeys in unconventional settings such as pet, trafficked, and confiscated monkeys are even rarer. The observed variations in manifestations of clinical disease versus asymptomatic infection among NHP species both temporally and geographically are likely due to a combination of the hosts' susceptibility to infection, the immune status of the host, and the lineage of MTBC involved in the event. Understanding phylogenetic composition and evolution of MTBC strains circulating among NHPs would assist in identifying the origin of infection as well as the true susceptibility of NHP species (Coscolla and Gagneux 2014).

Table 4.1 Review of naturally acquired MTBC infections in monkeys

Year[a]	Country	Context	(+)	N[b]	Taxa[c]	ID mechanism	Mycobacterial ID	References
>1901–1923	USA	Zoo	18		*Cebidae*	Necropsy	MTBC	Fox (1923)
>1901–1923	USA	Zoo	171		*Cercopithecidae*	Necropsy	MTBC	Fox (1923)
1909	USA	Zoo	1		*Cebidae*	Not reported	Not reported	Moreland (1970)
1928–1932	England	Lab	50	597	*Macaca mulatta*	Histopathology, necropsy	*Mycobacterium*	Fairbrother and Hust (1932)
1931–1939	France	Zoo	2		*Cercocebus*	Necropsy	MTBC	van Bogaert and Innes (1962)
1931–1939	France	Zoo	10		*Cercopithecus*	Necropsy	MTBC	Van Bogaert and Innes (1962)
1931–1939	France	Zoo	130		*Macaca*	Necropsy	MTBC	Van Bogaert and Innes (1962)
1931–1939	France	Zoo	175		*Papio*	Necropsy	MTBC	Van Bogaert and Innes (1962)
1931–1939	France	Zoo	1		*Macaca radiata*	Necropsy	MTBC	Van Bogaert and Innes (1962)
1934–1940	USA	Lab	207	~1860	*Macaca mulatta*	Necropsy	Not reported	Kennard and Willner (1941)
1934–1940	USA	Lab	290	~135	*Cercocebus torquatus*	Necropsy	Not reported	Kennard and Willner (1941)
1934–1940	USA	Lab	18	~165	*Ateles, Cebus,* others	Necropsy	Not reported	Kennard and Willner (1941)
1938	USA	Lab	59	382	*Macaca mulatta, Papio, Cercocebus*	TST, necropsy, AFB	*Mycobacterium*	Kennard et al. (1939)
1940	France	Zoo	1		*Cercocebus*	Necropsy	MTBC	Wilbur et al. (2012b)

1940	France	Zoo	2		*Papio*	Necropsy	MTBC	Wilbur et al. (2012b)
1941–1942	USA	Lab	37	494	*Macaca, Cercopithecus*	Not reported	Not reported	Riordan (1943)
1942–1944	USA	Trade[d]	191	892	*Macaca mulatta*	TST	MTBC	Habel (1947)
1942–1944	USA	Lab	290	665	*Macaca mulatta*	Necropsy	MTBC	Habel (1947)
1948	England	Zoo	3		*Saimiri sciureus*	Necropsy	Not reported	Rewell (1950)
1948	England	Zoo	1		*Cercopithecus mona*	Necropsy	Not reported	Rewell (1950)
1948	England	Zoo	9		*Macaca mulatta*	Necropsy	Not reported	Rewell (1950)
1951–1963	England	Zoo	1	89+	*Callitrichidae*	Necropsy	*M. bovis* (presumed)	Fiennes (1965)
1952	England	Zoo	2	?	*Saimiri sciureus*	Necropsy	*M. tuberculosis* (presumed)	Fiennes (1965)
1953	USA	Lab	106	306	*Macaca mulatta*	TST, necropsy	Not reported	Benson et al. (1955)
1953	England	Zoo	1	?	*Aotus trivirgatus*	Not reported	Not reported	Hill (1954)
1953	England	Zoo	2	?	*Callithrix penicillata*	Not reported	Not reported	Hill (1954)
1953	England	Zoo	58	?	*Macaca mulatta*	Necropsy	Not reported	Hill (1954)
1953	England	Zoo	1	?	*Cercopithecus mona*	Necropsy	Not reported	Hill (1954)
1954	England	Zoo	1	?	*Cercocebus*	Necropsy	Not reported	Hill (1955)
1954	England	Zoo	2	?	*Mandrilus sphinx*	Necropsy	Not reported	Hill (1955)
1954	England	Zoo	2	?	*Macaca silenus*	Necropsy, histopathology	Not reported	Hill (1955)
1954	England	Zoo	1	?	*Cebus apella*	Necropsy	Not reported	Hill (1955)
1954	England	Zoo	25	?	*Catarrhini*	Necropsy	Not reported	Hill (1955)
1954	England	Zoo	4	?	*Platyrrhini*	Necropsy	Not reported	Hill (1955)
1954	England	Zoo	1	?	*Ateles paniscus*	Necropsy	Not reported	Hill (1955)

(continued)

Table 4.1 (continued)

Year[a]	Country	Context	(+)	N[b]	Taxa[c]	ID mechanism	Mycobacterial ID	References
1954	England	Zoo	4	?	*Cebus apella*	Not reported	Not reported	Moreland (1970)
1955	USA	Lab	69		*Macaca*	Necropsy	MTBC	van Bogaert and Innes (1962)
1957	USA	Lab	101		*Macaca*	Necropsy	MTBC	van Bogaert and Innes (1962)
1962	England	Zoo	3	?	*Ateles geoffroyi*	Not reported	Not reported	Moreland (1970)
1964	Various locations	Zoo & Private Collections	16	79	*Semnopithecus*	Not reported	Not reported	Hill (1964)
1967	USA	Lab	1	4	*Saimiri sciureus* (Brazil)	Necropsy, histopathology, AFB, xenodiagnostics	*M. tuberculosis var. hominis*	Hessler and Moreland (1968)
1967	USA	Lab	1	1	*Macaca mulatta*	Necropsy, histopathology, AFB, xenodiagnostics	*M. tuberculosis var. hominis*	Hessler and Moreland (1968)
1968	USA	Trade	1	4	*Saimiri sciureus* (Peru)	Necropsy, histopathology, AFB	Not reported	Chrisp et al. (1968)
1968	USA	Trade	2	100	*Macaca mulatta*	TST, necropsy, histopathology, AFB	*Mycobacterium*	Martin et al. (1968)
1968–1970	USA	Lab	3	?	*Saimiri sciureus*	Necropsy	Not reported	Moreland (1970)
1972–1978	USA	Trade	7	130	*Macaca mulatta*	TST, necropsy, histopathology, AFB, culture	*M. tuberculosis, M. bovis*	Zumpe et al. (1980)
1973	USA	Trade	8	15	*Macaca mulatta*	Necropsy, histopathology	MTBC	Machotka et al. (1975)
1974*	USA	Lab	5	?	*Macaca arctoides*	TST, necropsy, AFB, culture	*M. bovis* phage type A3	Renner and Bartholomew (1974)

1974*	USA	Trade	3	?	*Macaca mulatta*	TST, necropsy, AFB, culture	*M. bovis* phage type A3	Renner and Bartholomew (1974)
1975	USA	Lab	13	44	*Macaca mulatta*	TST, necropsy, histopathology, culture, X-rays	*M. tuberculosis* phage type B	Mayhall et al. (1981)
1975	USA	Lab	5	5	*Saimiri*	TST, necropsy, histopathology, culture	*M. tuberculosis* phage type B	Mayhall et al. (1981)
1976*	England	Trade	16	47	*Papio* (Kenya)	TST, necropsy, erythrocyte sedimentation	Not reported	Tribe and Welburn (1976)
1976*	England	Trade	11	~50	*Papio* (Ethiopia)	TST, necropsy, erythrocyte sedimentation	Not reported	Tribe and Welburn (1976)
1976	USA	Lab	0	3	*Cebus albifrons*	TST, necropsy, histopathology, AFB, culture	None	Leathers and Hamm (1976)
1976	USA	Lab	1	3	*Cebus apella*	TST, necropsy, histopathology, AFB, culture	*M. tuberculosis*	Leathers and Hamm (1976)
1976	USA	Lab	1	5	*Macaca mulatta*	TST, necropsy, histopathology, AFB, culture	*M. tuberculosis*	Leathers and Hamm (1976)
1976	USA	Lab	1	15	*Saimiri sciureus* (Brazil)	TST, necropsy, histopathology, AFB, culture	*M. tuberculosis*	Leathers and Hamm (1976)
1978	USA	Lab	43	45	*Macaca mulatta*	Necropsy, AFB, culture	*M. bovis* (verified only in three individuals)	Zumpe et al. (1980)
1978	USA	Trade	1		*Macaca mulatta* (India)	TST, necropsy, culture	*M. tuberculosis*	Zumpe et al. (1980)
1980*	Gabon	Lab	1		*Cercopithecus*	Necropsy, AFB, culture	*M. africanum*	Thorel (1980)
1981	USA	Zoo	1		*Ateles geoffroyi*	Necropsy, histopathology, culture	*M. bovis*	West et al. (1981)
1981	USA	Lab	1		*Macaca mulatta*	Necropsy, histopathology, culture	*M. tuberculosis*	West et al. (1981)

(continued)

Table 4.1 (continued)

Year[a]	Country	Context	(+)	N[b]	Taxa[c]	ID mechanism	Mycobacterial ID	References
1981	Ireland	Zoo	0	15	*Cebus capucinus*	TST, necropsy, AFB, culture	None	Wilson et al. (1984)
1981	Ireland	Zoo	1	1	*Erythrocebus patas*	TST, necropsy, AFB, culture	*M. bovis*	Wilson et al. (1984)
1981	Ireland	Zoo	1	1	*Macaca silenus*	TST, necropsy, AFB, culture	*M. bovis*	Wilson et al. (1984)
1981	Ireland	Zoo	0	1	*Saimiri sciureus*	TST, necropsy, AFB, culture	None	Wilson et al. (1984)
1982	South Africa	Lab	11	91	*Papio ursinus*	TST, necropsy, culture, X-ray	Not reported	Fourie and Odendaal (1983)
1982	Kenya	Free range	2	7	*Papio cynocephalus*	TST, necropsy, AFB, amino acid uptake	*M. bovis*	Tarara et al. (1985)
1983	Kenya	Free range	5	55	*Papio cynocephalus*	Necropsy, AFB	*M. bovis* (not confirmed)	Sapolsky and Else 1987
1983	Kenya	Free range	4	29	*Papio cynocephalus*	TST	*M. bovis* (not confirmed)	Sapolsky and Else 1987
1987	Peru	Lab	1	?	*Aotus trivirgatus* (Peru)	Necropsy, AFB, culture	*Mycobacterium* (not confirmed)	Gozalo et al. (1994)
1990–1993	USA	Trade	0	712	*Cercopitecus aethiops* (saint Kitts, Barbados, Tanzania)	TST, necropsy, histopathology, AFB	*Mycobacterium*	CDC (1993)
1990–1993	USA	Trade	3	7703	*Macaca fascicularis* (Indonesia)	TST, necropsy, histopathology, AFB	*Mycobacterium*	CDC (1993)
1990–1993	USA	Trade	76	3967	*Macaca fascicularis* (Mauritius)	TST, necropsy, histopathology, AFB	*Mycobacterium*	CDC (1993)
1990–1993	USA	Trade	2	8910	*Macaca fascicularis* (Philippines)	TST, necropsy, histopathology, AFB	*Mycobacterium*	CDC (1993)

1990–1993	USA	Trade	9	1621	*Macaca mulatta* (China, Myanmar, Canada)	TST, necropsy, histopathology, AFB	*Mycobacterium*	CDC (1993)
1993	South Africa	Zoo	1	?	*Papio ursinus*	Culture, X-ray, PCR, RFLP	*M. tuberculosis*	Michel et al. (2003)
1995*	USA	Lab	1	180	*Macaca mulatta*	TST, necropsy, PCR, serology	*M. tuberculosis*	Rock et al. (1995)
1996	South Africa	Free range	14	30	*Papio ursinus*	TST, necropsy, histopathology, culture, PCR, RFLP	*M. bovis*	Keet et al. (2000)
1996	South Africa	Free range	4	9	*Papio*	PGRS RFLP	*M. bovis*	Michel et al. (2009)
1996	South Africa	Pet	1	1	*Callithrix jacchus*	Necropsy, AFB, culture, PCR, RFLP	*M. tuberculosis*	Michel and Huchzermeyer (1998)
1999	South Africa	Zoo	1	?	*Semnopithecus*	PCR, RFLP	*M. tuberculosis*	Michel et al. (2003)
2000	Bali	Temple	23	37	*Macaca fascicularis*	PCR	MTBC	Wilbur et al. (2012b)
2000	Sulawesi	Pet	34	47	*Macaca tonkeana*	PCR	MTBC	Wilbur et al. (2012b)
2000	Sulawesi	Pet	6	7	*Macaca maura*	PCR	MTBC	Wilbur et al. (2012b)
2000	Sulawesi	Pet	1	2	*Macaca nigrescens*	PCR	MTBC	Wilbur et al. (2012b)
2000	Sulawesi	Pet	7	10	*Macaca nigra*	PCR	MTBC	Wilbur et al. (2012b)
2000	Sulawesi	Pet	1	1	*Macaca ochreata*	PCR	MTBC	Wilbur et al. (2012b)
2000	Sulawesi	Pet	2	4	*Macaca fascicularis*	PCR	MTBC	Wilbur et al. (2012b)

(continued)

Table 4.1 (continued)

Year[a]	Country	Context	(+)	N[b]	Taxa[c]	ID mechanism	Mycobacterial ID	References
2000	Sulawesi	Pet	1	1	*Macaca nemestrina*	PCR	MTBC	Wilbur et al. (2012b)
2000	Sulawesi	Pet	2	4	Macaque hybrids	PCR	MTBC	Wilbur et al. (2012b)
2000–2004	Japan	Trade	o	10,462	Several species	TST	None	Une and Mori (2007)
2001	USA	Lab	19	52	*Macaca fascicularis* (Mauritius)	TST, necropsy, histopathology, AFB, spoligotyping, IFN-γ	*M. bovis*	Garcia et al. (2004a)
2001	USA	Lab	8	28		TST, necropsy, histopathology, AFB, spoligotyping, IFN-γ	*M. bovis*	Garcia et al. (2004a)
2002	Colombia	Zoo	0	6	*Cebus albifrons*	AFB, PCR, RFLP, blots	None	Alfonso et al. (2004)
2002	Colombia	Zoo	1	8	*Cebus capucinus*	AFB, PCR, RFLP, blots	MTBC	Alfonso et al. (2004)
2002	Colombia	Zoo	0	2	*Alouatta seniculus*	AFB, PCR, RFLP, blots	None	Alfonso et al. (2004)
2002	Colombia	Zoo	1	2	*Aotus sp.*	AFB, PCR, RFLP, blots	MTBC	Alfonso et al. (2004)
2002	Colombia	Zoo	2	7	*Cebus apella*	AFB, PCR, RFLP, blots	MTBC	Alfonso et al. (2004)
2002	Colombia	Zoo	2	9	*Saimiri sciureus*	AFB, PCR, RFLP, blots	MTBC	Alfonso et al. (2004)
2002	Colombia	Zoo	0	9	*Ateles paniscus*	AFB, PCR, RFLP, blots	None	Alfonso et al. (2004)
2002	Colombia	Zoo	0	6	*Lagothrix sp.*	AFB, PCR, RFLP, blots	None	Alfonso et al. (2004)

2002	Colombia	Zoo	0	2	*Callithrix pygmaea*	AFB, PCR, RFLP, blots	None	Alfonso et al. (2004)
2002	Colombia	Zoo	0	9	*Saguinus oedipus*	AFB, PCR, RFLP, blots	None	Alfonso et al. (2004)
2002	Colombia	Zoo	0	5	*Saguinus fuscicollis*	AFB, PCR, RFLP, blots	None	Alfonso et al. (2004)
2002	Colombia	Zoo	0	1	*Saguinus leucopus*	AFB, PCR, RFLP, blots	None	Alfonso et al. (2004)
2002	Colombia	Zoo	0	2	*Saguinus geoffroyi*	AFB, PCR, RFLP, blots	None	Alfonso et al. (2004)
2002	Thailand	Zoo	5	10	*Macaca fascicularis*	PCR	MTBC	Wilbur et al. (2012b)
2002	Thailand	Zoo	0	4	*Macaca arctoides*	PCR	MTBC	Wilbur et al. (2012b)
2002	Thailand	Zoo	0	2	*Macaca assamensis*	PCR	MTBC	Wilbur et al. (2012b)
2003	Java	Performing	0	22	*Macaca fascicularis*	PCR	MTBC	Wilbur et al. (2012b)
2003	Nepal	Temple	1	39	*Macaca mulatta*	PCR	MTBC	Wilbur et al. (2012b)
2003	Singapore	Free range	1	37	*Macaca fascicularis*	PCR	MTBC	Wilbur et al. (2012b)
2004	Japan	Zoo	8	8	*Cebus apella*	AFB	*M. tuberculosis* Beijing?	Une and Mori (2007)
2004	Japan	Zoo	4	9	*Colobus guereza*	AFB	*M. tuberculosis* Beijing?	Une and Mori (2007)
2004	Japan	Zoo	17	17	*Macaca*	Not reported	Not reported	Une and Mori (2007)
2005	Colombia	Rescue center	1	?	*Saguinus leucopus*	Spoligotyping	*M. africanum*	Barragán and Brieva (2005)

(continued)

Table 4.1 (continued)

Year[a]	Country	Context	(+)	N[b]	Taxa[c]	ID mechanism	Mycobacterial ID	References
2005	Colombia	Rescue center	1	?	*Saimiri sciureus*	Spoligotyping	*M. microti*	Barragán and Brieva (2005)
2005	Gibraltar	Free range	0	36	*Macaca sylvanus*	PCR	MTBC	Wilbur et al. (2012b)
2006*	USA	Zoo	1	15	*Papio cynocephalus*	TST, necropsy, histopathology, AFB, culture, X-ray	*M. tuberculosis*	Martino et al. (2007)
2006	Portugal	Zoo	2	10	*Mandrilus sphinx*	PCR, RFLP, spoligotyping	*M. africanum type II*	Amado et al. (2006)
2006–2010	Tanzania	Free range	2	11	*Chlorocebus pygerythrus*	AFB, culture, PCR, spoligotyping	*M. bovis*	Clifford et al. (2013)
2006–2010	Tanzania	Free range	1	7	*Papio cynocephalus*	AFB, culture, PCR, spoligotyping	*M. bovis*	Clifford et al. (2013)
2006–2010	Tanzania	Free range	1	7	*Papio cynocephalus*	AFB, culture, PCR, spoligotyping	MTBC	Clifford et al. (2013)
2007*	USA	Trade	1		*Macaca mulatta* (China)	TST, necropsy, histopathology, AFB, IFN-γ	*M. tuberculosis*	Shipley et al. (2008)
2007	Brazil	Zoo	1	1	*Ateles paniscus*	Necropsy, AFB, PCR, spoligotyping	*M. tuberculosis*	Rocha et al. (2011)
2007*	Germany	Zoo	3		*Saimiri sciureus*	Necropsy, histopathology, AFB, PCR, spoligotyping	*M. microti*	Henrich et al. (2007)
2008	USA	Trade	33	80	*Macaca fascicularis* (China)	TST, necropsy, histopathology, culture, PCR	*M. bovis* (verified only in three)	Panarella and Bimes (2010)
2008	Bangladesh	Zoo	2	2	*Macaca mulatta*	Necropsy, AFB, culture, PCR	*Mycobacterium orygis*	Rahim et al. (2017)
2009	Thailand	Lab	1	600	*Macaca*	TST, necropsy, histopathology, culture, AFB	*M. tuberculosis*	Payne et al. (2011)

2009	India	Zoo	62		*Macaca assamensis*	Not reported	*Mycobacterium*	Singh et al. (2009)
2009	USA	Lab	1	?	*Macaca nemestrina*	TST, necropsy, histopathology, culture, AFB, PCR	MTBC	Stockinger et al. (2011)
2010	USA	Lab	7	?	*Macaca nemestrina*	TST, necropsy, histopathology, AFB, PCR	MTBC	Engel et al. (2012)
2010–2013	Peru	Pet	2	29	*Cebus apella*	PCR	MTBC	Rosenbaum et al. (2015)
2010–2013	Peru	Rescue center	2	11	*Alouatta seniculus*	PCR	MTBC	Rosenbaum et al. (2015)
2010–2013	Peru	Rescue center	13	58	*Lagothrix lagotricha*	PCR	MTBC	Rosenbaum et al. (2015)
2010–2013	Peru	Rescue center & zoo	6	28	*Ateles chamek*	PCR	MTBC	Rosenbaum et al. (2015)
2010–2013	Peru	Wetmarket	1	1	*Callithrix pygmaea*	PCR	MTBC	Rosenbaum et al. (2015)
2010–2013	Peru	Wetmarket	1	4	*Saimiri boliviensis*	PCR	MTBC	Rosenbaum et al. (2015)
2010–2013	Peru	Wetmarket	4	56	*Saimiri sciureus*	PCR	MTBC	Rosenbaum et al. (2015)
2013	China	Zoo	55	84	*Macaca mulatta*	Necropsy, histopathology, culture, serology, qRT-PCR	*M. tuberculosis*	Gong et al. (2017)
2014	Tanzania	Free range	0	70	*Papio anubis*	PCR	None	Wolf et al. (2016)
2014	Bangladesh	Trade	4	14	*Macaca mulatta*	TST, necropsy, AFB	*Mycobacterium*	Avi et al. (2017)
2015*	Thailand	Trade	1	2	*Cercocebus atys* (Africa)	Necropsy, AFB, PCR, spoligotyping	*M. tuberculosis SIT52*	Kesdangsakonwut et al. (2015)
2015*	Thailand	Trade	0	4	*Cercocebus atys* (Africa)	Not reported	None	Kesdangsakonwut et al. (2015)

(continued)

Table 4.1 (continued)

Year[a]	Country	Context	(+)	N[b]	Taxa[c]	ID mechanism	Mycobacterial ID	References
2015	Panama	Lab	5	~378	*Aotus trivirgatus*	Necropsy, histopathology, AFB, culture	*M. tuberculosis* (isolated only in three individuals)	Obaldia 3rd et al. (2018)

(+) Number of cases, *N* population at risk, *TST* tuberculin skin test, *AFB* acid-fast bacilli, *PCR* polymerase chain reaction, *RFLP* restriction fragment length polymorphism, *PGRS* polymorphic guanine-cytosine-rich sequence, *IFN-γ* interferon gamma stimulation assay, *qRT-PCR* quantitative reverse transcription polymerase chain reaction

[a]Year of occurrence. When unknown, the year of publication is followed by *

[b]Empty cells correspond to isolated cases or cases reported individually (population at risk is unknown or equal to the number of cases). "?" population at risk is larger than the number of cases but it is not mentioned in the paper and cannot be deducted. "~" minimum population at risk is estimated from information provided in the paper

[c]If known, origin is indicated in parenthesis

[d]"Lab" includes breeding and experimental facilities. "Trade" includes animals surveyed or diagnosed during quarantine, inspection after arrival to a lab, or upon confiscation

4.2.2 *Mycobacterial Infections in Captive Neotropical Primates*

Based on work in captive populations, Neotropical primates are considered the least susceptible to Mycobacteria among primates. Reports of symptomatic TB caused by *M. tuberculosis* are documented in the common marmoset (*Callithrix jacchus*), the common squirrel monkey (*Saimiri sciureus*), the tufted capuchin (*Cebus apella),* the white-fronted capuchin (*Cebus albifrons),* the black spider monkey (*Ateles paniscus),* the black-headed spider monkey (*Ateles geoffroyi)*, and the night monkey (*Aotus trivirgatus)*. In addition, MTBC has been detected in gastric lavage and saliva samples from asymptomatic individuals of these and other species (Table 4.1).

Neotropical primates are abundant in captivity and several species are bred in large colonies at experimental facilities. However, mycobacterial detection is most commonly found in case reports rather than robust population-based surveys. Five multispecies surveys performed in apparently healthy monkeys outside of the experimental context in Latin America seem to support low susceptibility of this group to TB. Alfonso et al. (2004) isolated NTM from 54.4% of the primate population of the Cali Zoo in Cali, Colombia (N = 68), but only 7.4% were identified as *M. tuberculosis* by PCR; Barragán and Brieva (2005) detected NTM in 7.2% and MTBC in 2.4% of the primates received at two rescue centers in Colombia (N = 83); Rosenbaum et al. (2015) amplified MTBC DNA in 13% of oral swabs from trafficked primates in different contexts in Peru (N = 220); however, Navarrete et al. (2014) and Estrada-Cely et al. (2011) failed to detect any positive animals through serology and staining for acid-fast bacilli (AFB) at a rescue center in Peru (N = 56) or through TST of pet monkeys in Colombia (N = 20), respectively.

A survey in captive trafficked monkeys in Peru found oral swab samples from members of the family Atelidae (spider, wooly, and howler monkeys) more likely to harbor MTBC DNA than those of Cebidae (capuchins and squirrel monkeys), although this association was nonsignificant when adjusted for context, sex, and age (Rosenbaum et al. 2015). In fact, case reports are more frequent among Cebidae, but it is not clear if this reflects true susceptibility to infection and disease or their popularity in captive settings and experimental research. Wild-caught squirrel monkeys (*Saimiri spp.*) have been diagnosed with TB upon their arrival to biomedical laboratories, following exposure to humans, macaques, and other primate species during their transit through the importer's facilities and quarantines (Hessler and Moreland 1968; Leathers and Hamm 1976; Mayhall et al. 1981). Cebids have contracted TB during multispecies outbreaks in zoos (Fiennes 1965; Hill 1954; Rewell 1950; Une and Mori 2007), although there is at least one case where they were the only taxa not affected (Wilson et al. 1984). In several cases, TB lesions have been detected during necropsy of animals that died from other causes (Moreland 1970). All cases of TB affecting squirrel monkeys and capuchins accessible in the literature exhibited pulmonary TB with compromise of the hilar lymph nodes and hematogenous dissemination evidenced by miliary granulomas in several

organs. The only instances where granulomatous pneumonia was not the predominant lesion correspond to a capuchin monkey who died of uremic toxicity secondary to TB-associated nephritis with limited dissemination to the omentum and spleen (Hill 1955), and a squirrel monkey with granulomas in the spleen that were incidental findings during necropsy (Fiennes 1965). Calcification of the lung lesions has been observed in squirrel monkeys, although they are uncommon in other NHPs (Hessler and Moreland 1968; Moreland 1970).

An intraocular caseous granuloma with histological characteristics typical of TB (i.e., acid-fast bacilli surrounded by polymorphonuclear cells, macrophages, and lymphocytes) was found affecting the anterior and posterior chambers of the eye in the black-headed spider monkey. Upon further investigation, miliary calcified tuberculosis in the lungs and pleura caused by *M. bovis* was identified. The clinical manifestation of this case involved suppurative conjunctivitis, severe uveitis, and corneal edema without other external symptoms resembling TB (West et al. 1981). The only other clinical case detailed for a spider monkey corresponds to a black spider monkey with emaciation, lymphadenopathy, and generalized caseous nodules in the pleura, lung, liver, spleen, and kidney caused by *M. tuberculosis* (Rocha et al. 2011). Sporadic references of TB in spider monkeys show they are susceptible to the disease, but disease prevalence may be extremely low in this genus. MTBC has been detected through IS6110 PCR in apparently healthy spider monkeys at a zoo and a rescue center in Peru (Rosenbaum et al. 2015), but an earlier survey of the same population did not find any seroreactors or evidence of acid-fast bacilli (Navarrete et al. 2014).

MTBC reports in callitrichids (tamarins and marmosets) are also rare. The mortality of these species in captivity is high, but findings suggestive of mycobacterial disease are uncommon (Debyser 1995; Gozalo and Montoya 1992; Leong et al. 2004). There are two clinical cases reported from a zoo in London: a black-tufted marmoset (*Callithrix penicillata*) with abdominal tuberculosis and acute bronchopneumonia, as reported by Hill (1954), and a marmoset with caseous granulomas restricted to the spleen reported by Fiennes (1965). In a third case, TB developed in a common marmoset (*Callithrix jacchus*) kept as a pet by a human patient with pulmonary TB in South Africa (Michel and Huchzermeyer 1998). The only finding in this case was an abscess in a mesenteric lymph node. Subsequent laboratory experiments in this species have demonstrated diverse symptomatology, which has led to their use as an animal model for TB research (Cadena et al. 2016b; Via et al. 2013).

The common marmoset may be the only Neotropical primate in which partial containment of TB progression has been observed. Most experiments with this species resulted in fulminant disease with lethal onset approximately 5 weeks after inoculation, but at least one individual receiving a low dose of *M. tuberculosis* developed subclinical infection with delayed weight loss, limited dissemination, sterile and nonsterile lesions in the lungs, and survival for more than 300 days (Cadena et al. 2016b).

Owl monkeys (Genus: *Aotus*) were previously considered highly resistant to Mycobacterial infection. Cases of spontaneous disease reported in this species

include generalized TB secondary to an abscess in the floor of the mouth of a zoo monkey (Hill 1954), TB in a wild-caught monkey hosted for 7 years in a breeding colony (Gozalo et al. 1994), and infection in a splenectomized monkey that acquired the infection upon inoculation of Vero-cells contaminated with *M. abscessus*. In the latter case, the monkey became emaciated and developed granulomatous pneumonia and hepatitis (Karlson et al. 1970). However, in two of the three aforementioned cases, owl monkeys paired with the infected monkeys in the same cage remained uninfected and healthy. These observations match the findings of an experimental study that failed to infect three out of four owl monkeys upon intra-tracheal inoculation of about 40,000 viable *M. tuberculosis* organisms. The fourth owl monkey died following hematogenous dissemination 42 days after inoculation (Bone and Soave 1970).

A recent outbreak of TB in an experimental facility affected seven captive-bred *Aotus* spp. in a colony of about 300 animals. The cases were all lethal and dispersed across the facility suggesting a human-origin of the infection. At least three animals hosted in the same cage and belonging to the same family died of TB disease at intervals of 6 and 7 weeks, supporting transmission within the family group. As in the cases mentioned above, the infection presented with emaciation and generalized granulomatous disease affecting the lungs, spleen, liver, lymph nodes, and heart. *M. tuberculosis* was isolated from three individuals and *M. kansasii* was isolated from the index case that also developed hemorrhagic enteritis, ascites, and abscesses in the mesenteric lymph nodes (Obaldia 3rd et al. 2018). These data suggest that mycobacterial disease is highly lethal for owl monkeys, despite their apparent resistance to infection. However, the isolation of *M. tuberculosis* and *M. chelonae* in an asymptomatic individual contradicts this hypothesis (Alfonso et al. 2004). Cases of sudden death, emaciation, pneumonia, and/or granulomatous disease in owl monkeys must be carefully examined for the detection of mycobacterial agents. Pneumonias are a common cause of death in Nancy Ma's night owls (*Aotus nancymaae*) and in Spix's night monkeys (*Aotus vociferans*) (Sánchez et al. 2006), and tuberculosis-like lesions have been caused by injections of Freund's adjuvant in the grey-bellied night monkey (*Aotus lemurinus*) (Málaga et al. 2004).

The scattered reports of mycobacterial infections in Neotropical primates suggest that infections are rare but that infected monkeys may be highly susceptible to the progression of disease. The onset of symptoms, if any, may be delayed up to 7 months after the disease has been established, limiting the possibilities for containment within primate colonies if cases remain undetected. The historical avoidance of TB testing in these species, previously considered resistant to mycobacterial infection, has led to the dissemination of cases within captive facilities. Neotropical primates should be routinely screened for TB, as it is recommended for all primate species. Mixed-species housing with Old World monkeys should be avoided and precautions must be taken to prevent dissemination of mycobacteria between these NHP groups.

4.2.3 TB in Captive Populations: Zoos and Biomedical Facilities

Tuberculosis is a well-documented problem in primates housed at zoo and biomedical facilities (Avi et al. 2017; Garcia et al. 2004a; Gong et al. 2017; Parsons et al. 2009; Wilbur et al. 2012b; Wilson et al. 1984), and it has been reported in pet monkeys (Michel and Huchzermeyer 1998). The majority of these occurrences have a human origin. Wild-caught monkeys acquire the infection from humans in captivity, whether at the country of origin, during transportation, or during housing (Kennard and Willner 1941; Montali et al. 2001). Cohousing with infected and diseased monkeys has resulted in outbreaks within captive colonies during and after quarantine (Hessler and Moreland 1968; Leathers and Hamm 1976; Moreland 1970). Screening during quarantine is mandatory for imported animals and has proven to be helpful in detecting the progression of disease following transportation and prior to the incorporation of animals into a captive colony (Avi et al. 2017; CDC 1993). However, the intradermal tuberculin test (TST), the test used for this purpose, has variable results across species and a low overall sensitivity, so many cases remain undetected (Lecu and Ball 2011; Lerche et al. 2008). Unnoticed cases in monkeys have repeatedly caused the contamination of captive pens and zoo enclosures affecting other species of NHPs and nonprimates (Garcia et al. 2004b; Mayhall et al. 1981; Wilson et al. 1984), and reactivation of latent cases have resulted in outbreaks within closed colonies (Fourie and Odendaal 1983; Payne et al. 2011; Zumpe et al. 1980). Transmission in captivity is more common through aerosols and contamination of water and enclosures but can also occur as a result of the use of rectal thermometers, contaminated food and fomites, and handling without adequate protective equipment (Fourie and Odendaal 1983; Michel et al. 2003; Riordan 1943).

Tuberculosis has been reported in zoos since the early nineteenth century. In an early review, Schroeder refers to an annual TB incidence of 10% in primates (Schroeder 1938). Prevalence ranged between 1.7 and 25.8% for primates at European zoos in the late 1970s (Michel et al. 2003). Attack rates as high as 65% and 100% in macaques have been documented at zoos in China and Japan, respectively, (Gong et al. 2017; Une and Mori 2007), and a prevalence as low as 7% was found in a multispecies population of Neotropical monkeys in a Colombian zoo (Alfonso et al. 2004). However, most occurrences at zoos remain unreported, and existing reports often fail to provide information about the entire population at risk, making it difficult to estimate prevalence and attack rates.

Captive macaques have been used for decades as experimental models for TB in biomedical research, and the physiopathology of disease upon experimental inoculation is well documented for these species (Peña and Ho 2015; Scanga and Flynn 2014). Natural occurrence is also common in experimental facilities housing them for studies other than TB. Rhesus macaques are capable of developing progressive, lethal tuberculosis upon very low challenge with *M. tuberculosis* (Scanga and Flynn 2014). The progression of disease can take several weeks before the first symptoms

are noticed. Even with strict quarantine procedures and tuberculin skin test (TST) screening every 2 weeks, early spread can reach a large proportion of the population at risk before the first TST reaction is observed (Tribe and Welburn 1976). A broad survey performed by the CDC in 18 quarantine facilities receiving Old World monkeys found a prevalence of 0.6–80% among 249 shipments of cynomolgus macaques arriving to the United States. Upon necropsy, granulomatous lesions were found in 56% of TST-negative, apparently healthy animals from one shipment (CDC 1993). Similarly, 41% of a group of cynomolgus macaques imported to the United States from China (Panarella and Bimes 2010), and 4 out of 14 rhesus macaques received at a zoo in Bangladesh were TST-reactors. In Bangladesh, only one of the monkeys showed clinical signs, including emaciation and coughing. Necropsy following the monkey's death revealed numerous tubercles in the lungs and other organs as well as the presence of AFB, confirming TB diagnosis (Avi et al. 2017).

Baboons are also susceptible to TB. The spread of TB through a closed colony of chacma baboons resulted in a 12% attack rate and the reactivation of latent TB during pregnancy has been suggested in a yellow baboon after 3 years of negative testing (Fourie and Odendaal 1983; Martino et al. 2007). Chacma baboons have been affected by fatal infections with *M. bovis* in the wild (Keet et al. 2000), and high prevalence have been observed in wild-caught baboons shipped to the United States (Tribe and Welburn 1976).

4.2.4 Signs and Symptoms

Most monkeys infected with MTBC are asymptomatic, either because they are in the early stages of active disease, or because their course of infection is subclinical or latent. Detection usually occurs during quarantine, routine screening, or at necropsy. Clinical cases can start with lethargy, weight loss, and anorexia. Coughing and diarrhea are infrequent and considered poor indicators of TB because they are nonspecific symptoms commonly seen with a variety of diseases (Fourie and Odendaal 1983). Primary lesions depend on the route of transmission, but dissemination usually involves caseous or miliary lesions in lungs, liver, spleen, and lymph nodes in Old World monkeys (Montali et al. 2001). Lymphadenopathy is common in most primates with TB and should raise suspicion for disease if observed during clinical examination (Montali et al. 2001). Compromise of the gastrointestinal tract and serous membranes are not always present but may indicate generalized TB and are probably more common in New World primates (West et al. 1981).

Most mycobacterial infections remain latent and are difficult to detect; however, the extent of the lesions and the histological characteristics of the granulomas differ between active and latent infections and can be assessed during necropsy. Macaques with active disease present with caseous, nonnecrotizing, and suppurative granulomas and can develop tuberculous pneumonia or cavitary lesions. Caseous, mineralized, or fibrotic granulomas can be found in macaques with latent infection (Lin et al. 2009). The cellular characteristics of these granulomas are explained in detail in Lin

et al. 2009. Microgranulomas can be present in apparently nonaffected tissue and should be observed if early active infection is suspected and in species that are more prone to develop miliary tuberculosis (Ferreira Neto et al. 2014; Lin et al. 2009). Atypical manifestations found during necropsy include suppurative skin lesions (Rock et al. 1995) as well as cerebral (Machotka et al. 1975), spinal (Martin et al. 1968), and intraocular (West et al. 1981) TB.

4.2.5 Immunity in Mycobacterial Infections

After mycobacteria enter the respiratory tract through aerosols, the first immune response is led by alveolar macrophages that phagocytose the bacterium and produce the inflammatory cytokines IFN-γ and TNF-α, which recruit macrophages and monocytes to the site of infection (Cambier et al. 2017; Raja 2004). If the microbicidal action of the phagocytes is effective, the mycobacteria is inhibited and killed without progression of the infection (Cadena et al. 2016a). Additional recruitment of effector cells such as neutrophils and dendritic cells follows the secretion of cytokines in parallel with antigen presentation to T-cells to trigger adaptive immunity (Raja 2004). This T-cell reactivity is detected by delayed hypersensitivity tests like the TST and the Interferon gamma (IFN-γ) release assays, which are reactive during the first 2–4 weeks of infection in experimental primate models and are therefore interpreted as a sign of recent exposure and active infection (Lerche et al. 2008). A robust immune response may contain the bacteria at the site of infection and clear it in the course of several months without TST conversion (Cadena et al. 2016a).

Antigen presentation by dendritic cells to naïve lymphocytes at local lymph nodes and the migration of primed lymphocytes to the site of infection determine the onset of adaptive immunity (Cadena et al. 2016a). Early immune evasion mechanisms utilized by mycobacteria involve the avoidance of the bactericidal action of macrophages, recruitment of growth-permissive cells with lower microbicidal action (e.g., CCR2+ monocytes and neutrophils) (Cambier et al. 2017), production of anti-inflammatory cytokines, and impaired or delayed antigen presentation (Raja 2004). These mechanisms favor the dissemination of active mycobacteria to the lymph nodes through infected monocytes and dendritic cells and allow mycobacteria to grow in both lymph nodes and the site of infection (Cadena et al. 2016a). Mycobacteria are then attacked by immune cells that accumulate to contain the infection, creating a characteristic granuloma, the hallmark of tuberculosis (Lin et al. 2008).

Most of the mycobacteria are efficiently contained into sterile lesions and do not disseminate (Lin et al. 2014; Martin et al. 2017). An increase in the size and number of granulomas after 3 weeks of infection has been observed in animals that will progress to develop active infection, while the number and size of lesions remain stable in latency (Coleman et al. 2014; Lin et al. 2014). The immune system's ability to contain mycobacteria determines the pathology of the lesions and the progression to latency or active infection. A hosts' immune system may temporarily contain the

lesion and kill the mycobacteria, which is characterized by abundant neutrophil apoptosis as observed in caseous granulomas. Effective containment in this phase may result in fibrocalcitic granulomas. In contrast, pneumonic disease has been associated with a failure to contain mycobacteria infection and can occur at any time in the infection (Lin et al. 2014). In some cases of latency, mycobacteria can escape the granuloma and trigger a new inflammatory process, reactivating the disease or becoming contained again. In the latter scenario, immune response drops, and the infection remains latent but may be intermittently detected through direct and serological assays (Lin et al. 2009).

A reduced cellular immune response to mycobacterial infection, whether due to immune evasion by the pathogen or host-immune deficiency, is marked by a drop in the production of IFN-γ and is associated with inconsistent reactivity with the IFN-γ assays and intermittent reactivity to the TST assay later in the progression of disease (Welsh et al. 2005). The shift in predominance from cellular immunity toward the humoral pathway has been associated with relapse or maintenance of active TB (Lecu and Ball 2011); therefore, the presence of antibodies is considered indicative of active disease (Welsh et al. 2005). A rise in antibody production marks the progression from latent to active disease in humans (Lin et al. 2008); higher antibody titers correlate with bacterial burden, the severity of lesions, and the potential for dissemination (Davidow et al. 2005). Hence, antibody detection can be used to track disease and identify individuals who may be infectious (Lecu and Ball 2011).

Antigens expressed in different phases of mycobacterial growth will trigger antibody production in response to diverse epitopes (Davidow et al. 2005). Thus, the differentiation/identification of antibodies induced during dormant and active phases can also inform the status of tuberculosis infection through serology (Lin et al. 2008). Up to 35 antigenic mycobacterial proteins have been recognized in humans (Weldingh et al. 2005), as well as their potential to signal the different presentations of TB disease (Abebe et al. 2007; Davidow et al. 2005). A number of these antigens have been tested and used for serodiagnostic purposes in primates (Brusasca et al. 2003; Lyashchenko et al. 2007; Min et al. 2015), and their relevance to the different stages of disease has been explored, identifying ESAT-6 among the most seroreactive proteins and the choice antigen for early detection. However, no single antigenic protein is involved in all stages of TB disease, and a combination of them is preferred for diagnostic procedures (Brusasca et al. 2003).

4.2.6 Old and New Diagnostic Approaches

One of the main challenges when selecting a test for the diagnosis of mycobacterial infections in NHPs is its sensitivity and specificity to detect early and latent infections. Affordability and feasibility of the chosen tests are also restrictive in resource-limited situations and/or field settings. This is compounded by the fact that many gaps exist in our knowledge of mycobacterial colonization, dissemination, and virulence across NHP species, which may have unique species-specific susceptibility

profiles and immune responses. Because of this, initial TB diagnosis in primates has been based on our knowledge of how TB behaves in humans and in NHP animal models, primarily macaques.

Since the 1940s, the intradermal TST has been the standard practice for screening captive NHPs for TB. Screening for TB using the TST is currently required by the Centers for Disease Control (CDC), the United States Department of Agriculture (USDA), the Institute for Laboratory Animal Research (ILAR), and other regulatory organizations for primates in import quarantine (Lerche et al. 2008). The TST has also been recommended as the initial screening test by scientific and professional associations such as the National Institutes of Health (NIH), the American Association of Zoo Veterinarians, the European Primate Veterinary Association, the International Union for the Conservation of Nature (IUCN), and the World Organization for Animal Health (OIE). However, caution is advised in the interpretation of results. The test consists of three intradermal injections of tuberculin toxin in the eyelid or abdomen at 2-week intervals. It induces a delayed type II hypersensitivity reaction in immunocompetent animals, which is observed as edema, erythema, swelling, or ptosis at the site of injection. This reaction is monitored 24, 48, and 72 h postinjection and considered positive if it produces induration >10 mm in the abdomen or scores over 3 in a 0–5 score in the eyelid (Lerche et al. 2008; NIH 2017). TST in NHPs requires the use of 0.1 ml (1500 units) of undiluted mammalian old tuberculin (MOT). Using MOT overcomes the lack of sensitivity observed in NHPs when using purified protein derivatives (PPD), the form of tuberculin used for screening humans for TB (OIE 2017). Despite increased sensitivity using MOT in NHPs, results are variable and context-dependent; the assay has low sensitivity for early infections and can result in intermittently positive or negative results in infected animals. Nonspecific reactions to NTM are also common. For these reasons, additional tests are needed to rule out mycobacterial infection in primates and to determine the etiologic agent (e.g., MTBC vs. NTM vs. MAC) causing the immune reaction (Lerche et al. 2008). Moreover, TST is not recommended as a reliable assay in species with tolerance to mycobacterial toxins or in species for which the test has not been validated (OIE 2017). The TST is also not practical for use in free-ranging populations of NHP.

Enzyme immunoassays (EIAs), designed to detect in vitro activity of IFN-γ, are more specific than TST and have been proposed as reliable confirmatory tests (Garcia et al. 2004a; Lerche et al. 2008; Simsek et al. 2010; Vervenne et al. 2004). The whole-blood Interferon-γ assay (WB IFN-γ, PRIMAGAM®) challenges blood against *Mycobacterium bovis* PPD and *Mycobacterium avium* PPD, and after 24 h of incubation at 37°C determines the levels of IFN-γ by EIA. A higher level of IFN-γ stimulated by the bovine antigen compared to the avian antigen is considered positive, although a second test is recommended if higher levels of avian PPD are detected on the first attempt (Vervenne et al. 2004). The enzyme-linked immunosorbent spot assay (PB IFN-γ, ELISPOT®, T SPOT-TB®) applies the same principle but uses mononuclear cells instead of whole blood and, after incubation, measures the number of cells releasing IFN-γ by counting IFN-γ labeled "spots" previously marked with specific mycobacterial antigens (Simsek et al. 2010). The main

limitation of IFN-γ assays lies in the interpretation of proven exposure in relationship to the course of infection (Lerche et al. 2008), and its use is preferable for surveillance or in a context of expected low prevalence. Better results may be achieved with the combined use of IFN-γ and TST, which had increased detectability up to 100% in at least two TB outbreaks reported in macaques (Garcia et al. 2004b; Lerche et al. 2008).

Alternatively, the PPD can be replaced in the TST by specific mycobacterial antigens (e.g., ESAT-6, CPF-10, TB10.4, TB 7.7) to discriminate TB reactions from NTM and vaccinated individuals (Lerche et al. 2008; van Pinxteren et al. 2000). Higher reactivity and better sensitivity is achieved by immunoassays that test against a cocktail of several antigens, and a better specificity is obtained with the use of recombinant proteins or fusion polyproteins (e.g., CFP10-ESAT-6) (Min et al. 2011). The QuantiFERON-TB®, an IFN-γ test that uses specific antigens, challenges whole blood against an antigen cocktail directly in a sample tube, with the considerable advantage of having minimal requirements for the handling and processing of samples in the field (Parsons et al. 2010; Parsons et al. 2009). Numerous other immunological assays used in NHPs to detect antibodies against specific mycobacterial antigens include: Enzyme-linked immunosorbent assay (ELISA), multiantigen print immunoassay (MAPIA), multiplex microbead immunoassay (MMIA), and the lateral flow assay (Prima TB STAT-PAK®) (Lerche et al. 2008). The accuracy and simplicity of these tests, which are also relatively affordable, are considerable advantages for the screening of NHPs. For instance, the rapid test Prima TB STAT-PAK® requires only 30 μl of serum, plasma, or whole blood to detect positive status in only 20 min, avoiding the necessity of laboratory infrastructure. It has been shown to reach up to 99% specificity in experimentally infected rhesus macaques, cynomolgus macaques, and African greens (Lyashchenko et al. 2007) and had a high agreement with TB-negative status in silvered langurs with nonspecific TST-reactivity (Georoff et al. 2010).

IgG can be detected 4 weeks after infection (Lyashchenko et al. 2007; Min et al. 2011), while TST and IFN-γ reactivity starts within the first 2–4 weeks. However, antibody tests show a sustained reactivity for the entire course of infection while delayed hypersensitivity tested by TST and IFN-γ varies with time (Lerche et al. 2008). When used together, TST and rapid kits to detect tuberculosis infections provide the highest sensitivity for the screening of NHPs (Lyashchenko et al. 2007). Immunoassays and serologic assays are not, however, useful in differentiating genetic lineages of MTBC, a distinction that is important in considering the global epidemiology of MTBC and host-adapted phylogenies.

Bacterial culture from clinical specimens remains the gold standard for the detection of active tuberculosis, although the organisms' slow growth makes it impractical for timely diagnosis. Culture is more commonly used to confirm cases and test for antimicrobial resistance (Lecu and Ball 2011). Acid-fast staining and microscopic examination confirms the presence of bacilli consistent with mycobacteria in clinical specimens, which can be obtained through bronchoalveolar lavage, gastric aspiration, biopsy, necropsy samples, and oral swabbing (Alves da

Silva et al. 2017; Engel et al. 2012; Lerche et al. 2008; Wilbur et al. 2012b; Wolf et al. 2015).

Molecular detection of MTBC is becoming increasingly common and provides the ability to distinguish between mycobacteria species and subspecies. Amplification of the insertion element IS6110 via conventional or real-time PCR is used to detect active and recurrent MTBC infections in humans (Eisenach 1994). It has been used to detect the bacillus in lavages of lesions (Alves da Silva et al. 2017), oral swabs (Rosenbaum et al. 2015; Alicia K. Wilbur et al. 2012b; Engel et al. 2012), and fecal samples from NHPs (Wolf et al. 2015), although the interpretation of PCR-positive results in asymptomatic carriers remains controversial. In particular, the use of oral swabs as an alternative sample type has gained traction as a diagnostic tool (Luabeya et al. 2018; Wood et al. 2015). Recent proof of concept in clinical trials combining oral swabs with amplification of the IS6110 insertion element have demonstrated >90% sensitivity and specificity using this assay in humans. This technique is attractive for use in primates because it overcomes challenges associated with diagnosing TB in primates, including sample acquisition (i.e., serum, lavage) and the need for a competent immune system upon which the serologic assays rely. In addition, the amplification of distinct genomic regions by multiplex PCR allows differentiation between members of the MTBC (Warren et al. 2006). The semi-automated Xpert MTB/RIF rapid molecular assay detects *M. tuberculosis* in parallel with mutations that confer antibiotic resistance in just 2 h (Helb et al. 2010) and has been used to produce the first report of multidrug-resistant mycobacteria in squirrel monkeys (Alves da Silva et al. 2017).

Whole-genome sequencing has been used for deeper phylogenetic analysis of mycobacteria with epidemiological purposes (Lee and Pai 2017). Although the use of this technology in NHP samples has not been reported, next generation sequencing now provides opportunities for full molecular characterization of mycobacteria from clinical and field samples in a relatively short-amount of time (about 7.5 h), avoiding time-consuming culture techniques and DNA amplification, and allowing the identification of resistance genes and the assessment of transmission networks (Votintseva et al. 2017). Molecular typing methods such as spoligotyping, mycobacterial interspersed repetitive units-variable numbers of tandem repeats typing (MIRU-VNTR), and SNP-typing, though time consuming, are also routinely used to determine the molecular epidemiology of MTBC from clinical isolates (Rasoahanitralisoa et al. 2017).

In human medicine, thoracic radiographs accompany most TB diagnostics to look for evidence of pulmonary TB. Thoracic radiographs are of lower significance for TB diagnostics in NHPs because pulmonary granulomas are rare in monkeys with active disease (Capuano 3rd et al. 2003). Positron emission tomography (PET) has been successfully used to track the progression of active and latent pulmonary tuberculosis in experimental primate models (Cadena et al. 2016a, b; Coleman et al. 2014). The detection of biomarkers of *M. tuberculosis* in exhaled breath has been explored in anesthetized macaques and may represent a future option for MTBC diagnosis during routine procedures in captive facilities (Mellors et al. 2017).

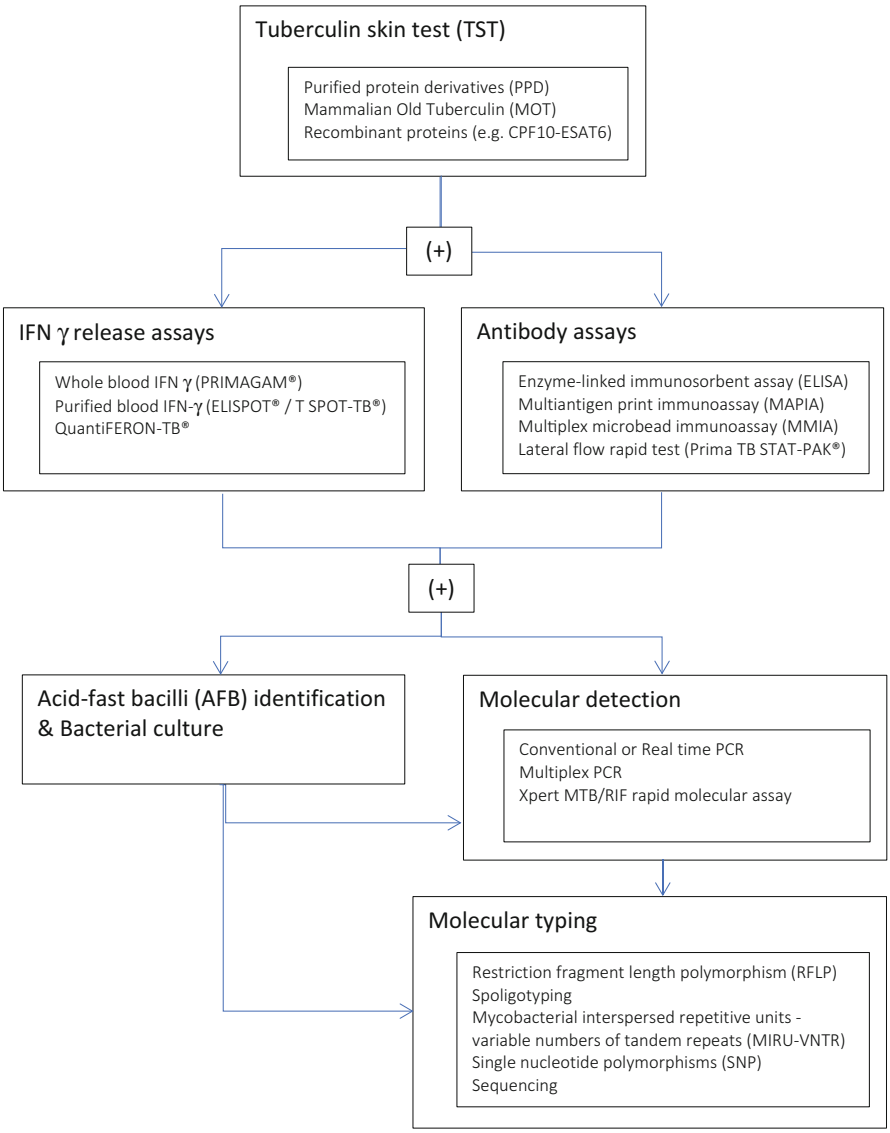

Fig. 4.1 Flowchart of diagnostic tests used for the detection of MTBC infections in monkeys

While the diagnostics discussed here can be combined to deliver accurate MTBC detection and diagnosis in captive monkeys (Fig. 4.1), challenges remain in our ability to detect and diagnose disease in free-ranging primate groups without the use of chemical restraint. Conventional and real-time PCR targeting the IS6110 gene have been used to successfully detect MTBC in fecal samples from experimentally inoculated cynomolgus macaques and rhesus macaques, and while specificity is near

Fig. 4.2 Habituated free-roaming black-faced spider monkeys (*Ateles chamek*) in the Tambopata National Reserve (**a**, **b**) and a red howler monkey (*Alouatta seniculus*) at the Taricaya Rescue Center (**c**) in Madre De Dios, Peru, accept rope swabs tied to a retrieval string used to obtain an oral mucosal sample for infectious disease surveillance

100%, the assay is <50% sensitive (Wolf et al. 2015). This assay was also employed using feces from naturally exposed sanctuary chimpanzees (*Pan troglodytes*) in East Africa, yielding three positives from individuals who exhibited variable TST and serologic status (Wolf et al. 2015). When used to screen fecal samples from chimpanzees and baboons (*Papio anubis*) in Gombe National Park, Tanzania, the same approach did not yield any positive results (Wolf et al. 2016). While fecal collection followed by molecular detection is attractive due to its feasibility, this method is not yet sufficiently sensitive to be used for reliable surveillance of wild groups, nor tested and optimized across a range of primate species. Furthermore, fecal DNA is highly fragmented and may not be suitable to generate high-quality genome data.

The use of oral swabs to recover saliva for the detection of MTBC DNA represents an opportunity for surveillance and has been successfully used in several species of Asian and South American primates (Engel et al. 2012; Rosenbaum et al. 2015; Wilbur et al. 2012b). Development of noninvasive saliva collection techniques using absorbent rope or discarded plant material has been used to recover viral DNA and RNA from partially habituated wild free-roaming Old World primates in Africa and Asia (Evans et al. 2015; Smiley Evans et al. 2016), as well as in captive and free-roaming New World primates in South America (Fig. 4.2; McDermott et al. 2020). The use of noninvasive saliva collection followed by IS6110 PCR was tested by Wilbur et al. (2012b) and holds promise to improve surveillance for mycobacterial infection in wild primates.

4.2.7 Control and Prevention

Control and prevention of mycobacterial infections can be achieved through proper quarantine, appropriate diagnostics, occupational health, and bio-containment. However, it is important to recognize that MTBC infections may take up to 7 months to become symptomatic and at least 3 weeks to show TST conversion in monkeys (Lecu and Ball 2011). In addition, the lack of pathognomonic symptoms associated with TB hinders clinical detection of disease over the entire course of infection. Thus, preventive measures must be exhaustive to limit mycobacterial spread within captive facilities. Quarantine must be applied upon reception of any NHP, independent of their source, and stricter measures must be observed if the origin and history of the animal is unknown (Frost et al. 2014). Housing of different species in the same room or enclosure should be avoided during quarantine (Frost et al. 2014). Quarantine and isolation procedures must be extended not only to the facilities but also to the personnel taking care of the animals: movement of equipment and staff between potentially contaminated areas to other spaces hosting animals must be avoided or follow strict measures to prevent indirect transmission (Shipley et al. 2008). A yearly TB screening is recommended for both monkeys and their caretakers. Extreme precautions must be observed to limit the exposure of humans and other animals during the necropsy and disposal of carcasses of TB-infected individuals (Lecu and Ball 2011).

The treatment of diseased animals is controversial and must be considered with extreme caution on a case-by-case basis when euthanasia is not an option (Frost et al. 2014). Successful treatment may only revert the infection to a latent stage, leaving the possibility of reactivation if treatment is discontinued (Lecu and Ball, 2011). In addition, the possibility of developing mycobacterial resistance or maintaining a source of infection within the facilities threatens the safety of other animals and people.

4.3 Nontuberculous Mycobacteria

The Nontuberculous Mycobacteria (NTM) are a group of emerging opportunistic pathogens, previously referred to as "environmental mycobacteria," "mycobacteria other than tuberculosis" (MOTT), or "atypical mycobacteria" (Wolinsky 1979). The NTM are globally distributed, and over 150 species have been described (Johnson and Odell 2008). Species important to human health include *M. kansasii, M. abscessus, M. chelonae, M. fortuitum, M. scrofulaceum, M. ulcerans,* M. *malmoense*, *M. xenopi*, and *M. marinum*. The *Mycobacterium avium* complex (MAC) is a related group of NTMs most commonly associated with disease in humans and animals and includes *Mycobacterium avium* subsp. *avium, Mycobacterium avium* subsp. *paratuberculosis, Mycobacterium avium* subsp. *hominissuis, Mycobacterium intracellulare,* and others (Cayrou et al. 2010; Griffith et al. 2007).

Many NTM have been identified as free-living saprophytes that thrive in the natural environment, including in aerosols, water, soil, and animal feces (Falkinham 3rd 2009; Falkinham 2002; Kazda et al. 2009). Historically considered environmental contaminants, the NTM became more readily recognized as a source of infection in humans as the incidence of TB declined following global eradication efforts (Covert et al. 1999; Falkinham 3rd 1996; Kazda et al. 2009). When the AIDS epidemic emerged in humans in the early 1980s, NTM incidence rose, disproportionately affecting AIDS patients in the United States and Europe, who were predominantly affected by disseminated MAC (Horsburgh Jr. and Selik 1989; Peters et al. 1989). As chemotherapeutics and advances in AIDS management improved, NTM infections caused by *M. ulcerans*, which causes a chronic necrotizing ulcerative disease termed Buruli ulcer, increased. *M. ulcerans* now follows behind TB and leprosy as the third most common mycobacterial infection in people worldwide (Asiedu and Wansbrough-Jones 2007; Brown-Elliott et al. 2002; Walsh et al. 2010). *M. a. paratuberculosis* is a major pathogen among the veterinary medical profession because it causes Johne's disease, which can cause widespread morbidity and economic loss in cattle and other ruminants, and has more recently been implicated in the etiology of Crohn's disease in humans (Cayrou et al. 2010; Hermon-Taylor et al. 2000; Kuenstner et al. 2017; Pierce 2009).

A broad spectrum of disease is associated with NTM infection and includes: chronic bronchopulmonary disease, lymphadenitis, skin and soft tissue infection, middle ear infection, skeletal infection, and foreign body and surgical-site-related infections (Wagner and Young 2004). The relative abundance of NTM in the environment is balanced by their low virulence, resulting in few infections in the immunocompetent host, often limited to the lungs, cervical lymph nodes, skin, or joints following trauma or corticosteroid administration. In immunosuppressed individuals, NTM is more commonly found in the disseminated form (Falkinham 3rd 1996; Wagner and Young 2004). Diagnosis of NTM infections can be challenging due to misdiagnosis as TB and the organism's ability to hide from detection. Culture-based diagnostic assays and/or direct molecular detection techniques such as nucleic acid amplification and PCR-based genetic sequencing are gaining traction as they enable species-level identification of organisms (CDC 2009; Chemlal and Portaels 2003; Woods 2002).

In contrast to TB, person-to-person transmission of NTM has not been established. Instead, transmission occurs from contact with the natural or man-made environment. Water systems are thought to be a major avenue for disseminating NTM to humans and animals, as the bacterium can incorporate into biofilms, grow in a wide pH range, and withstand common disinfectants such as chlorine (Falkinham 3rd 2009; Schulze-Röbbecke and Fischeder 1989; September et al. 2004). Evidence suggests that NTM in animals are associated with NTM found in their environment (Gcebe et al. 2013).

4.3.1 NTM in Monkeys

The NTM can cause both asymptomatic infections as well as a wide range of disease in nonhuman animals, including protozoa, amoebas, arthropods, reptiles, amphibians, and mammalian species (Bercovier and Vincent 2001; Bowenkamp et al. 2001; Gutter et al. 1987; Malama et al. 2014). Table 4.2 summarizes documented cases of naturally acquired NTM infections in monkeys.

The first report of NTM in a monkey dates back to a 1949 case of avian tuberculosis in a common marmoset (*Callithrix jacchus*) in the context of a zoo (Moreland 1970). Numerous other monkeys infected with NTMs have since been documented, primarily in research settings. *M. avium* complex (MAC) has been identified in multiple distinct colonies of laboratory rhesus macaques (*Macaca mulatta*) (Holmberg et al. 1982; Renner and Bartholomew 1974; Smith et al. 1973). *M. gordonae* has also been isolated from squirrel monkeys following a positive reaction to tuberculin in a laboratory in California; however, no gross lesions were observed and the monkeys were clinically asymptomatic (Soave et al. 1981). Asymptomatic *M. kansasii* infection was also reported in four of five tuberculin-positive squirrel monkeys (*S. sciureus sciureus*) from a laboratory colony in Michigan. The positive monkeys were all tuberculin-negative on their arrival to the colony and were not housed in the same group at the laboratory facility. Notably, the cage mate of each of the positive cases was not tuberculin-positive, suggesting a lack of transmission between cage mates. While the infected monkeys were asymptomatic, both gross and histologic lesions were present in the lymph nodes (enlargement and pyogranulomatous lymphadenitis) and liver (multifocal microscopic granulomas), and diagnosis was confirmed by culture from the bronchial lymph nodes of three of the monkeys. The source of infection was not determined and the affected monkeys were inconsistently TST-positive over time (Brammer et al. 1995).

Another outbreak of *M. kansasii* in rhesus macaque monkeys (*Macaca mulatta*) also lacked evidence of transmission between NHPs. Sixty of 71 tuberculin-reactive animals had positive culture results for *M. kansasii* at a breeding colony in Virginia (Valerio et al. 1978). Though housed in the same quarantine facility, monkeys who arrived following a specific date did not contract the disease despite direct contact with the diseased animals, suggesting that transmission did not occur between the primates and exposure was environmental (Valerio et al. 1978). *M. kansasii* was also isolated from a single laboratory rhesus macaque in a colony of 15 that had received immunosuppressive radiation. The infected macaque developed lesions typical of pulmonary tuberculosis and tested positive to TST. None of the other animals in the colony tested positive (Jackson et al. 1989). As described in Sect. 4.2.2, *M. kansasii* was also recently isolated for the first time during a TB outbreak among owl monkeys, previously considered highly resistant to tuberculoid infection (Obaldia et al. 2018). In the scenarios above involving rhesus macaques, the TST was able to reliably detect infection with *M. kansasii* while this was not the case in the scenario involving the squirrel monkeys, which may be attributable to species-specific variations in immune response or the immunologic competence of the individual

Table 4.2 Review of naturally acquired NTM infections in monkeys

Year[a]	Country	Context[b]	(+)	N[c]	Species	ID mechanism	Mycobacterial ID	References
1949	France	Zoo	1		*Callithrix jacchus*	Not reported	*M. avium* (presumed)	Moreland (1970)
1970–1978	IUSA	Lab	42	752–1098	*Macaca mulatta*	TST, necropsy, histopathology, culture, AFB	*Mycobacterium avium-intracellulare*	Holmberg et al. (1982)
1973*	USA	Lab	60	?	*Macaca mulatta, Macaca nemestrina, Macaca speciosa, Macaca cynomologi*	TST, culture	*M. avium, M. intracellulare*	Smith et al. (1973)
1973*	USA	Lab	3	?	*Macaca mulatta*	TST, culture	*M. avium*	Smith et al. (1973)
1974*	USA	Lab	1	?	*Macaca mulatta*	TST, necropsy, culture, AFB	*Mycobacterium avium* complex	Renner and Bartholomew (1974)
1974–1975	USA	Lab	74	201	*Macaca mulatta*	TST, necropsy, histopathology, culture	*M. kansasii*	Valerio et al. (1978)
1978	USA	Lab	29	38	*Macaca arctoides*	Necropsy, histopathology, culture, AFB, RFLP, ELISA	*M. paratuberculosis*	McClure et al. (1987)
1978	USA	Lab	3	275	*Saimiri sciureus*	TST, necropsy, culture, AFB	*M. gordonae*	Soave et al. (1981)
1979	?	Lab	2	12	*Erythrocebus patas*	TST, culture	*M. scrofulaceum*	Renquist and Potkay (1979)
1988–1994	USA	Lab	23	135	Macaques	Necropsy, histopathology, culture, AFB, DNA:RNA hybridization	*M. avium*	Mansfield et al. (1997)
1989*	USA	Lab	1	15	*Macaca mulatta*	TST, necropsy, histopathology, culture, X-ray	*M. kansasii*	Jackson et al. (1989)

1993	USA	Lab	5	47	*Saimiri sciureus*	TST, necropsy, culture, PCR	*M. kansasii*	Brammer et al. (1995)
1995*	USA	Lab	2	180	*Macaca mulatta*	Serology	*M. chelonae subsp. abscessus*	Rock et al. (1995)
1997	USA	Lab	1	1	*Pithecia pithecia*	TST, necropsy, PCR	*M. avium*	Heard et al. (1997)
1998–2010	South Africa	Free range	1	2	*Chlorocebus pygerythrus*	TST, histopathology, culture, PCR	*M. simiae*	Gcebe and Hlokwe (2017)
1998–2010	South Africa	Free range	1	2	*Chlorocebus pygerythrus*	TST, histopathology, culture, PCR	*M. hecheshornense, M. sydneyiensis, M. xenopi*	Gcebe and Hlokwe (2017)
1999	Germany	Lab	1		*Macaca mulatta*	Necropsy, histopathology, culture, AFB, PCR, IHC, biochemical tests	*M. simiae*	Didier et al. (1999)
2002*	Colombia	Zoo	37	68	*Cebus, Lagothrix, Saguinus, Ateles*	AFB, PCR, RFLP, blots	*M. fortuitum, M. nonchromogenicum, M. triviale, M. terrae, M. chelonae*	Alfonso et al. (2004)
2002*	USA	Zoo	1	1	*Papio sphinx*	Necropsy, histopathology, culture, AFB, PCR	*M. avium subsp. paratuberculosis*	Zwick et al. (2002)
2005	Colombia	Rescue center	12	83	Neotropical primates	Spoligotyping	*Mycobacterium phlei, M. terrae, M. vaccae, M. flavescens, M. szulgai*	Barragán and Brieva (2005)
2006*	USA	Pet	1		*Saguinus midas*	Clinical signs, TST, histopathology, culture, HPLC, 16s rDNA sequencing	*M. asiaticum*	Siegal-Willott et al. (2006)
2008	USA	Trade	1	80	*Macaca fascicularis (Chinese origin)*	TST, necropsy, histopathology, culture, PCR	*M. paraffinicum* (coinfection with *M. bovis*)	Panarella and Bimes (2010)

(continued)

Table 4.2 (continued)

Year[a]	Country	Context[b]	(+)	N[c]	Species	ID mechanism	Mycobacterial ID	References
2008–2009	Germany	Zoo	10	10**	*Saguinus oedipus*	AFB, PCR	*M. avium subsp. paratuberculosis*	Münster et al. (2013)
2008–2009	Germany	Zoo	1	10**	*Theropithecus gelada*	AFB, PCR	*M. avium subsp. paratuberculosis*	Münster et al. (2013)
2008–2009	USA	Lab	10	278	*Callithrix jacchus*	TST, necropsy, histopathology, culture, AFB, PCR, X-ray	*M. gordonae, M. kansasii*	Wachtman et al. (2011)
2010	Germany	Zoo	2	5	*Pygathrix nemaeus*	Necropsy, histopathology, AFB, PCR, X-ray	*M. avium*	Plesker et al. (2010)
2011*	India	Free range	10	25***	*Macaca mulatta*	AFB, PCR	*M. avium subsp. paratuberculosis*	Singh et al. (2011)
2015	Panama	Lab	1	378****	*Aotus trivirgatus*	Necropsy, histopathology, culture, AFB	*M. kansasii*	Obaldia 3rd et al. (2018)
2017*	Germany	Zoo	1	1	*Saguinus oedipus*	Necropsy, histopathology, culture, PCR	*M. avium subsp. paratuberculosis*	Fechner et al. (2017)
2017*	Germany	Lab	1	8	*Callithrix jacchus*	Necropsy, histopathology, culture, PCR	*M. avium subsp. paratuberculosis*	Fechner et al. (2017)

(+) Number of cases, *N* population at risk, *TST* tuberculin skin test, *AFB* acid-fast bacilli, *RFLP* restriction fragment length polymorphism, *ELISA* enzyme-linked immunosorbent assay, *HA* hemagglutination assay, *PCR* polymerase chain reaction, *IHC* immunohistochemistry, *HPLC* high-performance liquid chromatography

[a]Year of occurrence. When unknown, the year of publication is followed by *

[b]"Lab" includes breeding and experimental facilities. "Trade" includes animals surveyed or diagnosed during quarantine, inspection after arrival to a lab, or upon condication

[c]Empty cells correspond to isolated cases or cases reported individually (population at risk is unknown or equal to the number of cases). "?" population at risk is larger than the number of cases but it is not mentioned in the paper and cannot be deducted. ** Aliquots of a pooled sample, *** samples, not individuals, **** Population in 2016

monkeys. This highlights the need for more reliable screening assays for mycobacteria that are both sensitive and specific across a range of NHPs and mycobacterial species.

Monkeys in the laboratory context have also been infected with NTM species beyond *M. kansasii*. Pulmonary TB-like disease, including granulomas in the lungs, liver, and spleen, has been reported in clinically asymptomatic but TST-positive *Erythrocebus patas* monkeys infected with *M. scrofulaceum* (Renquist and Potkay 1979). A serological study identified macaques in a research colony with evidence of M. *chelonae subsp. abscessus* exposure (Rock et al. 1995). Both *M. gordonae* and *M. kansasii* were identified in a closed laboratory colony of common marmosets (*Callithrix jacchus*) that exhibited positive or questionable reactivity to the TST. Intestinal colonization with *M. gordonae* was associated with positive tuberculin reactions, but on necropsy, granulomatous lesions on lymph nodes were culture-positive for *M. kansasii*, which is generally considered more virulent than *M. gordonae* (Wachtman et al. 2011). Notable for its comparison with human AIDS patients, a study of SIV-infected macaques found that 17% of animals that succumbed to the disease were also infected with MAC (Mansfield et al. 1997), while another SIV-infected macaque suffering from chronic diarrhea and weight loss was diagnosed with *M. simiae* infection during necropsy (Didier et al. 1999).

NTM has also been isolated from monkeys housed in zoos and other captive settings. In 2004, an entire colony of 68 young and adult primates at the Cali Zoo in Colombia were tested, and 39 individuals representing 10 species of New World primates (57%) were positive for a range of NTMs. There was no association between NTM status and clinical signs of disease (Alfonso et al. 2004). In Germany, two red-shanked douc langurs (*Pygathrix nemaeus nemaeus*) were diagnosed with *M. avium* infection, though only one showed clinical signs and was PCR- and TST-positive. Though the other monkey was asymptomatic and negative for a TST reaction, and no mycobacterial DNA could be isolated, both monkeys had lung nodules and enlarged lymph nodes on necropsy (Plesker et al. 2010). A TB outbreak in a group of 80 crab-eating macaques (*Macaca fascicularis*) imported to the United States from China included a case of *M. paraffinicum* and a case of MAC infection: both monkeys were asymptomatic but found to have nodules on their lungs during necropsy (Panarella and Bimes 2010).

NTM clinical infections have been reported in New World monkeys. Among callitrichids (tamarins and marmosets), *M. kansasii, M. avium,* and *M. gordonae* have been isolated from *Callithrix jacchus* and *Saguinus oedipus*, often in association with paratuberculosis or compromised abdominal organs and membranes (Alfonso et al. 2004; Fechner et al. 2017; Moreland 1970; Münster et al. 2013; Wachtman et al. 2011). High rates of intestinal colonization by NTM were found in association with TST-seroreactivity in the common marmoset (*Callithrix jacchus)* (Wachtman et al. 2011). To date, there is a single report of mycobacterial infection among the Pitheciidae family (saki, titi, and uakari monkeys). A white-faced saki (*Pithecia pithecia*) imported to the United States from England was TST-reactive and developed hyperglobulinemia and cystic abscesses in mesenteric lymph nodes; he was subsequently found to harbor *M. avium* (Heard et al. 1997).

Similar to its clinical signs in other species, MAC infections have often been associated with gastrointestinal signs in monkeys. Following death after episodes of abdominal bloat, diarrhea, and weight loss, a mandrill (*Papio sphinx*) at the Lincoln Park Zoo in Chicago was diagnosed with *M. avium subsp. paratuberculosis* (Zwick et al. 2002). *M. a. paratuberculosis* has also been implicated in the cause of chronic intestinal inflammation in a colony of stump-tailed macaques (*Macaca arctoides*) and cotton-top tamarins (*Saguinus Oedipus)*, as well as in baboons and gibbons (Hermon-Taylor et al. 2000; McClure et al. 1987). *M. a. intracellulare* has been documented to affect a range of ages and sexes in a colony of captive-born and wild-caught rhesus macaques (*Macaca mulatta*), manifesting as gastrointestinal disease with lesions in the large and small intestines as well as the lymph nodes of the affected animals (Holmberg et al. 1982).

Several cases of NTM infection at rehabilitation centers in South Africa and Colombia and in free-ranging NHPs in India are important to note for their potential effect on disease transmission to wild populations. A study at several wildlife rescue centers in Colombia found a 7.2% prevalence of NTM species among their monkeys. Species identified included *M. phlei*, *M. terrae*, *M. vaccae*, *M. flavescens*, and *M. szulgai* (Barragán and Brieva 2005). In South Africa, two vervet monkeys (*Chlorocebus pygerythrus*) on a game reserve tested TST-positive, and several NTMs were isolated (Gcebe and Hlokwe 2017). Furthermore, a strain of *M. avium paratuberculosis* genetically typified as "Indian bison type," commonly isolated from wild and domestic ruminants in India, was found in free-ranging Indian rhesus macaques, confirming pathogen transfer between primates and livestock (Singh et al. 2011).

It is clear that both captive and free-range monkeys are susceptible to a diverse range of NTM infections, including MAC. Those caring for monkeys in research, zoo, or other captive settings should be especially aware of NTM as a differential for TST-positive NHPs. Without a better understanding of the prevalence of NTM amongst wild monkey populations, it is unclear what effects these NTM species may have beyond the context of captivity.

4.4 *Mycobacterium leprae*

Though worldwide eradication efforts have reduced the prevalence of human leprosy cases in many countries, new cases of leprosy continue to arise. In 2017, over 200,000 new leprosy cases were registered globally. The current distribution in humans is most heavily weighted in India, Brazil, and Indonesia (Reibel et al. 2015; WHO 2018). *Mycobacterium leprae* and *Mycobacterium lepromatosis* are both known to cause leprosy in humans, though *M. leprae* causes the vast majority of cases. *M. lepromatosis* infection is rarer and predominantly occurs in Mexico and the Caribbean (Raghunathan et al. 2005; Vera-Cabrera et al. 2011).

Leprosy has a long incubation period in humans and the disease affects the skin, peripheral nerves, eyes, and upper respiratory tract (Cahill 2011; WHO 2012). Manifestation of leprosy occurs along a spectrum and depends on the host's immune response. Tuberculoid leprosy is characterized by a strong cellular immune response and manifests as one or very few hyposensitive or anesthetic lesions with gradually thickened nerves. Lepromatous, or multibacillary, leprosy has greater malignancy and is characterized by a weak cellular response. It presents as many nonanesthetic skin lesions or papules, called lepromas, and diffuse peripheral nerve and skin thickening and damage. The face, eyes, respiratory tract, larynx, testes, and kidneys can all be affected (Cahill 2011; Reibel et al. 2015; Sugita 1995; Walker and Lockwood 2007). For treatment purposes, the WHO distinguishes tuberculoid leprosy by the presence of fewer than five skin lesions and/or one impaired nerve, and lepromatous leprosy by more than five skin lesions or impaired nerves (Reibel et al. 2015; WHO 2012). There are also several types of borderline leprosy presentations, with clinical signs between tuberculoid and lepromatous: borderline tuberculoid leprosy, borderline leprosy, and borderline lepromatous leprosy. Tuberculoid leprosy and borderline leprosy are collectively called paucibacillary leprosy (Cahill 2011).

With early multidrug therapy (MDT), leprosy can be cured, though resistance is a concern (Reibel et al. 2015; WHO 2012). Left untreated, this chronic infectious disease can cause significant disability in the form of permanent damage to the eyes, skin, limbs, and nerves (Walker and Lockwood 2007). The specific modes of infection of *M. leprae* remain unclear, but transmission appears to be primarily through respiratory secretions and close contact with untreated individuals, though environmental sources of infection have also been implicated (Kazda et al. 2009; WHO 2012). Some evidence also suggests transmission can occur through contact with injured skin (Job et al. 2008). Because the disease can cause disfigurement and disability, stigmatization and isolation of individuals affected by leprosy was common practice in many societies around the world, and leprosy-affected individuals were often forced to live in geographic or socially isolated groups (Cahill 2011; Gelber 1993).

M. leprae is highly infective and slow-growing (Reibel et al. 2015). Like other mycobacterial species, *M. leprae* is an acid-fact bacilli (AFB) and is indistinguishable morphologically from *M. tuberculosis*. However, the presence of AFB nerve infiltration is diagnostic of leprosy based on the anatomic location (Massone et al. 2015). The lepromin test is a prognostic measure of cell-mediated immunity to *M. leprae*. Killed M. *leprae* is injected intradermally, and a granulomatous nodule appears after 4 weeks in positive tests. Serologic tests exist but they lack sensitivity and specificity and should not be used alone (Reibel et al. 2015; Sugita 1995). PCR can also be used to confirm leprosy diagnosis (Reibel et al. 2015). There is no reliable diagnostic test for detection of subclinical *M. leprae* infection (Reibel et al. 2015; Suzuki et al. 2011).

Because *M. leprae* cannot be grown in vitro, considerable effort has been focused on the development of an animal model for the disease. In 1960, *M. leprae* was successfully grown in the foot pads of mice, and this method of culture has been used

to study characteristics of *M. leprae* such as drug resistance (Meyers et al. 1991; Shepherd 1960). The organism prefers growth temperatures below that of the body's core and thrives in the nine-banded armadillo, which is commonly used to study and cultivate *M. leprae* in the laboratory setting (Cahill 2011). The nine-banded armadillo is also thought to serve as a natural reservoir for *M. leprae* in the southwest United States and other parts of the Americas (Hamilton et al. 2008; Sharma et al. 2015; Truman et al. 2011).

4.4.1 Mycobacterium leprae *in Monkeys*

Due to their close phylogenetic relationship to humans, NHPs were naturally an attractive target for studies involving leprosy disease progression, vaccination, and therapeutics (Martin et al. 1984; Meyers et al. 1991). However, the establishment of an appropriate NHP model was difficult and attempts to do so date back to 1882 (Hansen 1882a; b; Martin et al. 1984). The first report of experimental disseminated leprosy in an NHP did not occur until 1958 when two chimpanzees in Liberia were inoculated with material from human leprosy lesions. One of the inoculated chimpanzees developed lesions that were consistent with leprosy (Gunders 1958). About a decade later, two infant chimpanzees inoculated with *M. leprae* from lepromatous patients in 1965 shortly after birth and again 6 months later developed self-limiting borderline and tuberculoid leprosy 1 year after their second inoculation (Martin et al. 1984). In 1978, evidence of leprosy infection in a gibbon (*Hylobates lar*) that had been inoculated 15 years prior was discovered during necropsy, though the gibbon had shown no clinical signs of leprosy while alive. As the primate was taken care of by leprosy patients, natural infection could not be ruled out as source of infection (Waters et al. 1978).

Naturally acquired leprosy has been reported only seven times in NHPs, with four of these cases occurring in chimpanzees. All seven cases occurred in captive NHPs who developed clinical signs prior to diagnosis. The cases occurring in monkeys are summarized in Table 4.3.

Table 4.3 Review of naturally acquired *M. leprae* infections in monkeys

Year	Country	Country of origin	Context	(+)	NHP Taxonomic ID	References
1979	USA	Nigeria	Lab	1	*Cercocebus atys*	Meyers et al. (1985) Meyers et al. (1991)
1986	USA	Nigeria	Lab	1	*Cercocebus atys*	Gormus et al. (1988), Meyers et al. (1991)
1994	USA	The Philippines	Lab	1	*Macaca fascicularis*	Valverde et al. (1998)

(+) Number of cases. Population at risk is not mentioned in these papers

In 1975, a chimpanzee that had been imported from Sierra Leone to the United States for research purposes (Donham and Leininger 1977) began showing clinical signs of leprosy 2 months after inoculation with bovine leukemia virus. Nodular thickenings developed on the ears, and a maculopapular rash developed on the abdomen and medial thighs and spread to the trunk and limbs. Over the next 14 months, nodular lesions developed elsewhere on the chimpanzee's face, carpus, and scrotum (Donham and Leininger 1977; Leininger et al. 1978). The chimpanzee was not treated for leprosy, and his disease advanced from borderline to lepromatous leprosy (Leininger et al. 1978). When the chimpanzee passed away during sedation 33 months after the appearance of lesions, gross observation and histologic findings during necropsy showed nerve invasion by histiocytes, lymphocytes, and AFB, consistent with the diagnosis of leprosy (Leininger et al. 1980; Mitsuda 1936; Powell and Swan 1955), though the chimpanzee did not show the typical gross lesions on the spleen, liver, testes, and adrenal glands found in human leprosy patients. The chimpanzee had also developed interstitial histiocytic pneumonia, which is uncommon in humans with leprosy (Donham and Leininger 1977; Mitsuda 1936; Powell and Swan 1955).

In 1979, a sooty mangabey in the United States imported from West Africa 4 years prior for research purposes was the first monkey diagnosed with naturally acquired leprosy (Meyers et al. 1991; Meyers et al. 1985). Facial lesions were the initial clinical sign of disease, and deformities of the hands and digits were observed after 1 year. The disease was classified as subpolar lepromatous to borderline-lepromatous, and the monkey responded well to therapy: when he passed away 8 years later, no evidence of leprosy infection was found during necropsy (Gormus et al. 1988; Meyers et al. 1991). The *M. leprae* strain from this sooty mangabey monkey was found to be partially resistant to dapsone, suggesting it may have been acquired from a human who had received dapsone therapy, though a later study did not find a mutation in a known dapsone-resistance gene for this strain (Honap et al. 2018; Meyers et al. 1985).

Sooty mangabeys experimentally inoculated with this "mangabey strain" of *M. leprae* were found to develop leprosy more quickly than sooty mangabeys inoculated with a human-origin strain (Wolf et al. 1985; Meyers et al. 1991). In the "mangabey strain" inoculated monkeys, evidence of dissemination was present, and lesions were discovered in sites of the body distant from the initial inoculation. In the monkeys inoculated with the human strain, lesions were only present surrounding the sites of inoculation, though nasal secretions indicated the bacteria had disseminated. The "mangabey strain" might thus be adapted to NHPs, indicative of endemicity in this species in nature. Rhesus macaques and African green monkeys were also inoculated and infected in this experiment, but inoculation did not induce disease in squirrel monkeys (Wolf et al. 1985). Since this time, a range of monkeys have been experimentally infected with leprosy, including rhesus monkeys, mangabeys, and African green monkeys (Gormus et al. 1998; Rojas-Espinosa and Lovik 2001; Wolf et al. 1985), though recent research has suggested limited susceptibility of cynomolgus monkeys (Walsh et al. 2012; Wolf et al. 1985). Experimentally

infected mangabeys with lepromatous leprosy have a similar depressed immune response to that seen in humans (Martin et al. 1985).

While it was clear monkeys were susceptible to leprosy, questions remained as to the transmissibility of the disease until 1986, when a previous cage mate of the first infected sooty mangabey was diagnosed with naturally occurring lepromatous leprosy based on facial and ear lesions and dermal nerve infiltration. Though both sooty mangabeys with naturally acquired leprosy had been imported separately from Nigeria, they had been housed in the same cage for 3 years prior to the first mangabey's development of clinical leprosy signs. The researchers inferred that this represented the first report of monkey-to-monkey leprosy transmission, though it was possible both monkeys were exposed to the same source of infection or two independent sources of leprosy (Gormus et al. 1988).

In 1989, two more chimpanzees were reported to have acquired leprosy in the absence of experimental inoculation. The second captive chimpanzee began self-mutilating his digits at age nine and had several positive tuberculin tests with no evidence of tuberculosis infection. It was not until 9 years later that he developed leprotic lesions on his face, ears, distal penis, and scrotum. Histopathology identified AFB and the presence of foamy histiocytes, and the chimpanzee was diagnosed with borderline leprosy. Despite treatment, the chimpanzee developed a leprae reaction and sustained permanent neurological and musculoskeletal damage (Suzuki et al. 2011). The third chimpanzee developed lesions on his face, ear, and scrotum 23 years after his arrival in the United States. Histopathology revealed AFBs in histiocytes, nerves, and blood vessel walls as well as intracellular AFBs in hepatic histiocytes, and he was diagnosed with subpolar lepromatous to borderline leprosy (Hubbard et al. 1991b; Hubbard et al. 1991a).

Diagnostics utilized in the two chimpanzees that developed leprosy in 1989 represent the first successful utilization of serologic antibody testing using anti-LAM and anti-PGL-1 (a common mycobacterial antigen and *M. leprae*-specific antigen, respectively) for naturally acquired leprosy in NHPs. This technique had gained interest for its use in humans and had been tested in experimentally infected sooty mangabeys (Gormus et al. 1991; Gormus et al. 1990; Hubbard et al. 1991b).

In 1994, a macaque originally from the Philippines was the first recorded Asian primate to develop leprosy naturally, after spending 3 years in a US primate research facility. Lesions were found on the head, feet, and base of the tail. PCR and histopathology revealed the presence of *M. leprae* and an ELISA for IgG anti-PGL-I was positive. Necropsy findings including cutaneous nerve infiltration confirmed diagnosis (Valverde et al. 1998). Phylogenetic analysis of the *M. leprae* strain isolated from this monkey demonstrated it was most closely related to a human strain from New Caledonia (Honap et al. 2018). Notably, this monkey's leprosy was classified as borderline, and she exhibited a stronger antibody response than had been seen in previous cases, broadening the spectrum of leprosy observed in NHPs. With the exception of one chimpanzee and the cynomolgus macaque that had questionable or suspected TST reactions, all other cases of leprosy in NHPs had negative TST tests (Valverde et al. 1998).

The most recent case of leprosy in an NHP occurred in 2009 in a chimpanzee housed at a sanctuary in Japan. The chimpanzee, originally from Sierra Leone, had been used in Hepatitis research. Almost 30 years after she had arrived in Japan, the chimpanzee developed nodular lesions on her eyes, lips, abdomen, forearms, and crus, and was diagnosed with lepromatous leprosy. When the chimpanzee was treated with MDT, her clinical signs diminished (Suzuki et al. 2010). Genetic analysis of the *M. leprae* strain isolated from this chimpanzee strongly suggests she was infected prior to arrival in Japan, and thus had incubated the disease for over 30 years (Suzuki et al. 2010). Retrospective serology showed that she only became positive for anti-PGL-1 antibodies following clinical onset of leprosy signs and became negative again 5 months following treatment initiation, suggesting these antibody levels are indicative of active rather than subclinical infection.

Little is known about the prevalence of *M. leprae* infection or exposure in captive or wild populations of NHPs. In 1983, 26 owned monkeys in two states in India, Andhra Pradesh and Tamilnadu, in which 50% of India's leprosy cases occurred, were evaluated via physical exam and/or ear lobe smear and found to be free of leprosy infection. Six of these monkeys had known daily contact with people with leprosy, and one of these monkeys was found to have a clawed left hand, though ulnar nerve biopsy did not reveal significant lesions (Hagstad 1983). A cross-sectional serological survey of 160 chimpanzees in two research facilities in the United States, both of which had had a confirmed case of a chimpanzee with naturally occurring leprosy, identified seven chimpanzees positive for anti PGL-1 antibodies and five for anti-LAM antibodies. However, whether these results were due to false positives and/or exposure to other mycobacterial species was unclear (Gormus et al. 1991). Acid-fast staining and PCR analysis for *M. leprae* DNA in nasal swabs from 32 chimpanzees in Japan following the most recent case of natural NHP leprosy in 2009 were all negative, and all 13 chimpanzees living with the affected primate were negative for anti-PGL-1 antibodies (Suzuki et al. 2010, 2011).

Several wild populations of primates have also tested negative for *M. leprae* infection. Neither ring-tailed lemurs in the Bezá Mahafaly Special Reserve, Madagascar, nor chimpanzees from Ngogo Kibale National Park, Uganda, had evidence of MTBC or *M. leprae* infection by means of qPCR detection (Honap et al. 2018). The considerably long latent period of infection as well as reduced fitness and shorter life spans for NHPs with leprosy in the wild may contribute to the lack of natural cases observed (Meyers et al. 1992; Suzuki et al. 2011). Furthermore, observational diagnosis of leprosy in NHPs in the wild is difficult without close examination and diagnostics (Suzuki et al. 2011). To date, there are no reports of *M. leprae* prevalence in populations of monkeys from regions of the world in which naturally acquired infections have originated.

The role of NHPs in *M. leprae* transmission is highly speculative and remains unclear. It is possible, though unlikely, that NHPs serve as a reservoir for *M. leprae* in regions in which the disease is endemic (Gormus et al. 1988; Meyers et al. 1992; Walsh et al. 1988). Some researchers have suggested, though no data were provided, that the naturally acquired leprosy infections in chimpanzees occurred due to exposure to other infected chimpanzees in the wild or to infected humans while

they were held in cages awaiting shipment (Donham and Leininger 1977; Gormus et al. 1991). Recent phylogenetic analyses have shown that the *M. leprae* strain from the 2009 chimpanzee case from Sierra Leone and the 1979 sooty mangabey from Nigeria are closely related and situated on the same branch as a human strain found in West Africa and the Caribbean. This suggests that both primates were originally infected prior to leaving West Africa: *M. leprae* may have been introduced into NHP populations from humans and now be transmitted between NHPs, perhaps through contact or predation, in this region (Honap et al. 2018). Furthermore, the close relationship between the *M. leprae* strain found in the macaque from the Philippines and the human strain in New Caledonia suggest that *M. leprae* might likewise be transferred between humans and monkeys in this region of the world (Honap et al. 2018). Whether this transmission occurs through prolonged direct contact with other primates or humans, through contact with the soil, as has been implicated in *M. leprae* transmission from armadillos to humans, or by some other means, remains to be elucidated (Hamilton et al. 2008; Honap et al. 2018). That all cases of naturally acquired leprosy also occurred in animals in captivity also indicates that stress may play a role in the manifestation of this disease (Suzuki et al. 2011). Given the continued prevalence of leprosy in human populations, there is a strong case to be made for further expanding phylogenetic analyses of both human and NHP *M. leprae* strains as well as wild population prevalence testing in regions with persistent human leprosy infection in order to gain a better understanding of the transmission patterns, zoonotic potential, and risks this disease poses to NHP populations.

4.5 Mycobacteria and One Health

One Health approaches to mycobacterial control efforts, particularly for TB, hold potential to reduce the human health and economic impacts of mycobacterial infections while also promoting wildlife conservation and animal health.

Human population expansion and corresponding habitat encroachment drive the convergence of human settlements with monkey habitat, increasing opportunities for disease transmission. NHP exposure to human-associated mycobacteria may occur in a number of settings: because of research and touristic expeditions into monkey habitats, during capture and handling for diverse purposes, through captive management, and in synanthropy. The first outbreaks of TB and paratuberculosis in wild monkeys in Africa and India highlight how important TB containment at the human–wildlife interface is for primate conservation and prevention of spread into other mammalian hosts (Keet et al. 2000; Michel et al. 2009; Michel et al. 2003; Singh et al. 2011; Tarara et al. 1985). That recent phylogenetic research suggests leprosy may also have spread from humans to monkeys lends further support to the need to maintain vigilance about spillover events (Honap et al. 2018).

Habituation of primates for research or tourism carries a risk of human TB spillover. Fortunately, prevention at this interface is highly feasible. TB screening

must become a regular practice among research teams working in any areas where primates can be exposed. The same recommendation must be applied for rangers and tourists in national parks where proximity to monkeys may result in indirect contact and potential transmission.

Potential exposure in captivity provides a more difficult challenge. Mycobacterial infections are frequently found in monkeys that have been captured from the wild and imported for zoos or biomedical facilities. These NHPs are presumably infected following initial exposure to their human captors and handlers. Although screenings are common and often mandatory in these settings, such is not the case for illegally traded primates. Illegal trafficking is a prime opportunity for pathogen transmission, as primates are stressed, malnourished, and may be more susceptible to disease. In addition, they have close and frequent contact with not only humans but also a range of other wild and domestic species. Thus, there is potential for extensive exposure to Mycobacteria of human and animal origin during their transit from the wild to captivity. TB has been reported in trafficked monkeys and MTBC and NTM were detected in captive monkeys that originated in the illegal pet trade (Barragán and Brieva 2005; Kesdangsakonwut et al. 2015; Michel and Huchzermeyer 1998; Rosenbaum et al. 2015).

Monkeys kept as pets or in sanctuaries, living in proximity to both people and other animals, can pose a threat to wild populations through unanticipated escapes, interactions with wild populations in their housing, or release without adequate diagnostics. Escaped or released monkeys that may be infected before reentering the wild or that remain associated with human settlements can become a bridge for mycobacterial introduction into deep-forest areas. Prolonged quarantine and careful screening are advised for monkeys recovered from wildlife trafficking. Keeping monkeys as pets must be discouraged in all instances and forbidden in the proximity of wild populations. Furthermore, monkeys in rehabilitation and release settings must be thoroughly tested and free of disease prior to reintroduction into the wild to avoid TB transmission to other primates or animals.

Synanthropic monkeys, such as macaques and baboons, which thrive in close association with humans, may be exposed to highly contaminated material, such as infected carcasses of domestic animals and fomites created by daily human waste (Stockinger et al. 2011; Tarara et al. 1985). Outbreaks in Africa and India have presumably begun in this way. While naturally circulating MTBC, NTM, and leprosy in wild monkeys has not been confirmed, carriers among synanthropic species extend the risk of disease transmission beyond human boundaries to naïve primate groups. Today, TB infections of humans and cattle are more prevalent in countries where practices such as pastoralism, communal farming, on-site slaughtering and carcass disposal, and poor management of human waste make it difficult to limit mycobacterial spread (Cosivi et al. 1998; Kaneene et al. 2014). These practices reduce the distance between the domestic reservoir and wildlife and also evade epidemiological control programs when in place. For example, culling of infected animals or confiscation of contaminated carcasses is often rejected by subsistence farmers, leading to the existence of informal abattoirs and higher risk of infection for wild animals, including monkeys (Cosivi et al. 1998). In order to

reduce this risk, programs for the control and eradication of bovine TB must reach herds in rural areas adjacent to forests and national parks where NHP exposure can occur. Eradication of TB in cattle is also impaired by the presence of wildlife reservoirs such as buffalo and kudu in Africa (Hlokwe et al. 2016; Kaneene et al. 2014; Renwick et al. 2007). Strategies to limit dissemination to wild ungulates in Africa have been largely discussed and implemented, but the inclusion of monkey troops in prevention efforts has thus far not been prioritized despite the presence of contexts where monkey exposure to cattle is becoming frequent. From a One Health perspective, effective control programs should take into account the diversity of species affected by TB and the landscape for habitat overlap in the affected region (Kaneene et al. 2014). They must also address the necessity to work cooperatively with local communities and farmers for surveillance and early detection in order to achieve TB eradication as a common goal.

Recognizing the ways in which monkeys may contribute to and be affected by mycobacterial zoonoses is important in future control and prevention efforts. Partnerships between animal and public health agencies, wildlife researchers, conservationists, and local communities provide good opportunities for increasing awareness and opportune interventions, both fundamental for a One Health approach to controlling TB (Kaneene et al. 2014).

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Chapter 5
Pathogenic Spirochetes in Monkeys: Stealthy Pathogens of Global Importance

Idrissa S. Chuma, Lena Abel, Luisa K. Hallmaier–Wacker, David Šmajs, and Sascha Knauf

Abstract Spirochetes are helical-shaped gram-negative bacteria that are important for the health of both nonhuman primates (NHPs) and humans. However, little is known about the spirochetes that naturally infect NHPs. Lyme disease and relapsing fever are caused by bacteria of the genus *Borrelia*, obligate parasites transmitted by arthropod vectors. Due to the close phylogenetic relationship of humans and NHPs and the importance of *Borrelia* infections in humans, translational NHP models have been developed. Leptospirosis, caused by different pathogenic bacteria of the genus *Leptospira*, affects both humans and NHPs. Naturally acquired and clinically apparent leptospirosis is rare in NHPs. However, clinically healthy animals tested positive for antibodies against the spirochete, indicating that NHPs might function as a disease reservoir for humans. Syphilis, yaws, and bejel represent infections caused by bacteria of the genus *Treponema*. Naturally occurring *Treponema* infection in NHPs, as well as the continual use of NHPs as experimental models for human treponematoses, have been documented. This chapter discusses three groups of spirochetes that cause considerable diseases in NHPs in the context of naturally and artificially acquired infection: *Borrelia*, *Leptospira* and *Treponema*. Essential is the One Health concept that addresses the connection and spread of diseases between humans and NHPs.

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5.1 Introduction to Spirochetes

The order Spirochaetales includes non-pathogenic and pathogenic bacteria (Paster 2010). Some of these bacteria are free-living saprophytes, whereas others have kept their ancestral ability to survive in the environment, but have developed the ability to infect a broad range of animals and humans. On the other extreme, the group of spirochetes includes bacteria that are so specialized in their biology that they are unable to survive in the environment, as is the case for the syphilis-causing bacterium *Treponema pallidum* (*TP*). Spirochetes are an ancient and deeply branching phylum of gram-negative bacteria and one of the few bacterial orders where phylogeny mostly reflects the organisms' cell morphology (Caro-Quintero et al. 2012; Paster et al. 1984). Almost all members of the phylum are helical-shaped and possess periplasmatic flagella (Charon and Goldstein 2002; Paster and Dewhirst 2000), which mediate motility. Taxonomically, the phylum Spirochaetes consists of a single class, Spirochaetia, which contains spirochetes in one order, Spirochaetales. Subsequently, the order Spirochaetales comprises the four families Brachyspiraceae, Brevinemataceae, Leptospiraceae, and Spirochaetaceae. The family Brachyspiraceae includes the genus *Brachyspira* and the family Brevinemataceae includes the genus *Brevinema*. Leptospiraceae includes the genera *Leptonema* and *Leptospira*, and the family Spirochaetaceae includes the genera *Spirochaeta*, *Borrelia*, *Cristispira*, and *Treponema* (Paster 2015). Figure 5.1 illustrates the phylogenetic relationship of selected spirochetes, including those that are important pathogens for nonhuman primates (NHPs). The construction is based on GenBank published sequence data of the 16S rRNA gene.

Historically, little is known about the spirochetes that infect NHP, although information has begun to accumulate using modern genetics. Three groups of spirochetes, namely, *Borrelia*, *Leptospira* and *Treponema*, are important for human health. However, not all of these pathogens naturally infect NHPs. While *Borrelia,* for example, is accountable for a major disease complex for human health, causing Lyme disease and relapsing fever, bacteria of this genus do not naturally infect monkeys. The second important disease complex is leptospirosis, which is caused by different pathogenic *Leptospira* organisms. In particular, rodents and bats have coevolved with this pathogen and function as a disease reservoir for the infection in both, humans and NHPs. While infection in humans is of significant importance, natural infection in NHPs is mostly acquired during captivity and does

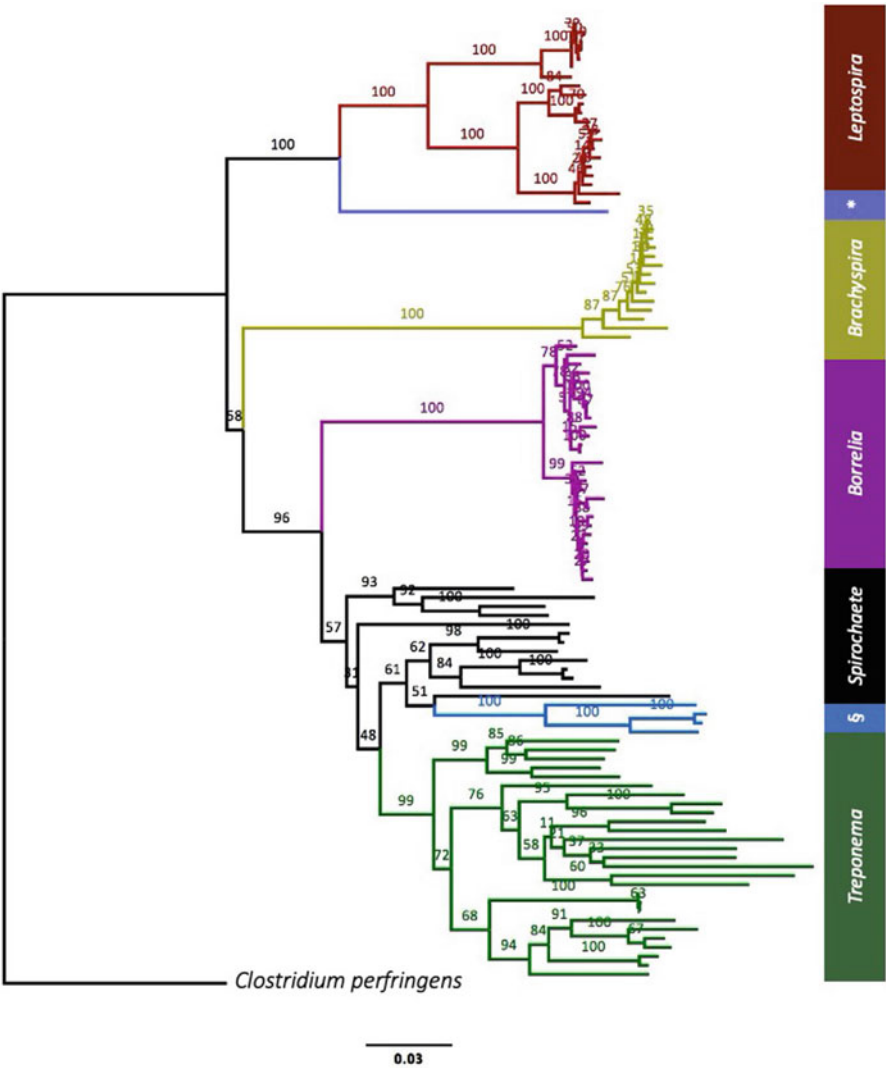

Fig. 5.1 Bio-Neighbor-Joining consensus tree of selected spirochetes. The tree is based on the V2-V8 region of the 16Sr RNA gene with 1,101 sites. The Jukes–Cantor substitution model was chosen and 1,000 bootstrap replicates were performed. Bootstrap values are displayed at respective branches. The bar refers to substitutions per site. *Clostridium perfringens* is used as an outgroup. **Leptonema illini*, §*Sphaerochaeta*

not seem to play a major role in wild monkeys. The last complex contains diseases caused by *Treponema* and is of great importance for human and NHP health. Natural infection with *TP* is common in humans and in wild NHPs, which underlines the need for One Health investigations. In the following text, these three major

pathogens, *Borrelia*, *Leptospira* and *Treponema*, will be discussed in the context of naturally and artificially acquired infection in NHPs.

5.1.1 Borrelia

The genus *Borrelia* belongs to the ancient phylum of spirochete bacteria and includes important human and animal pathogens (Brisson et al. 2012). The genome of the Borreliae is composed of a linear chromosome in conjunction with linear and circular plasmids (Wang and Schwartz 2015). Most genes located on the chromosome are likewise found in other bacteria (Fraser et al. 1997) whereas genes located on the plasmids are generally unique for the genus (Casjens et al. 2000; Fraser et al. 1997). Members of the Borreliae are pathogens transmitted by arthropod vectors (Cutler et al. 2017). This distinguishes them from other spirochetes such as *Treponema* and *Leptospira*. Based on DNA sequence analysis, two major phylogroups within the *Borrelia* genus can be distinguished (Wang and Schwartz 2015). One group contains three pathogenic species (*B. afzelii*, *B. garinii*, and *B. burgdorferi*) as well as seven minimally pathogenic to nonpathogenic *Borrelia* spp. (Wang and Schwartz 2015). The most notable pathogen in this phylogroup is *B. burgdorferi*, which causes Lyme borreliosis and was first isolated from the tick species *Ixodes scapularis* (Burgdorfer et al. 1982). The phylogroup is generally named the *B. burgdorferi* sensu lato (sl) complex and referred to as Lyme borreliosis spirochetes (LBS) (Ytrehus and Vikøren 2012). A common feature of this group is that transmission requires hard-bodied ixodic tick species (*Ixodes ricinus* complex). The second phylogroup consists of a larger number of *Borrelia* spp. (more than 20), which are associated with relapsing fever. Members of this group are either louse-borne (*B. recurrentis*) or soft tick transmitted (Wang and Schwartz 2015), with the exception of *B. theileri*, *B. miyamotoi* and *B. lonestari*, which are transmitted by hard ticks (Cutler et al. 2017). The epidemiology of *Borrelia* infection is predictably associated with the geographic range of the respective arthropod-vector (Fig. 5.2). In addition, the distinct grooming behavior of wild NHPs makes it difficult for arthropod vectors to infect monkeys. Yet, the geographic ranges of hard ticks and NHPs do not significantly overlap, and Lyme borreliosis is not a relevant disease in monkeys. Reports on natural infection are absent (Pritzker and Kessler 2012). *B. harveyi*, however, has been considered to be naturally associated with a monkey reservoir (Wang and Schwartz 2015; Ytrehus and Vikøren 2012). Generally, rodents account for most of the known host and reservoir species, but there are also *Borrelia* species that infect birds and reptiles (Ytrehus and Vikøren, 2012).

In humans, infection with *B. burgdorferi* develops in three stages. After infection, a pathognomonic skin rash, the erythema migrans, develops in addition to fatigue or flu-like symptoms. The latter does not involve the respiratory tract. Left untreated, the disease enters its secondary stage within weeks where neurological signs such as meningopolyneuritis and myo-peri-pancarditis become present. The final stage occurs several months after infection and is associated with severe and painful

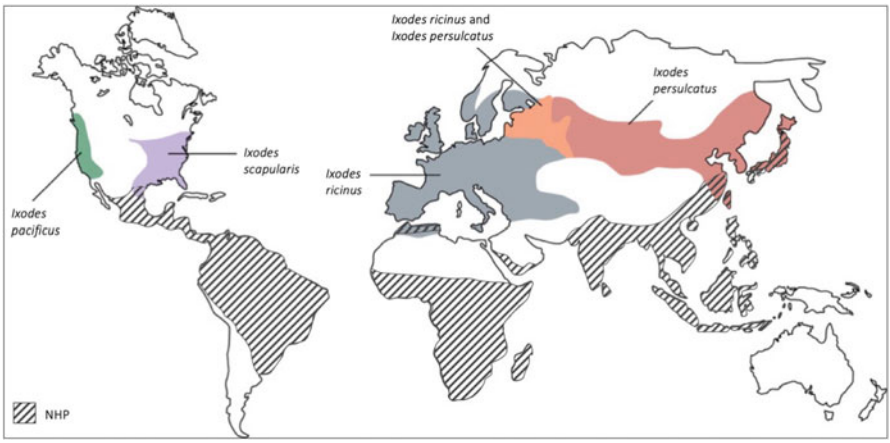

Fig. 5.2 The geographic range of Lyme borreliosis and NHP distribution have little overlap, which is predictive for the absence of naturally occurring Lyme disease in monkeys. Map source: Stanek et al. 2012; copyright © 2012 Elsevier Ltd. Modification: overlay of NHP distribution)

polyarthritis of the major joints and chronic encephalomyelitis. Further details on clinical manifestations and definitions can be found in Stanek et al. (2012). Due to the importance of *Borrelia* infection in humans and the close phylogenetic relationship of humans and NHPs, translational NHP models have been developed (Crossland et al. 2017; Embers et al. 2012; Pachner et al. 1998). While animal models in rats, mice, hamsters, guinea-pigs, gerbils and rabbits develop arthritis only if an individual is immunocompromised (as reviewed in Philipp and Johnson (1994), rhesus macaques (*Macaca mulatta*) when inoculated with *B. burgdorferi*, consistently develop the full range of human Lyme disease symptoms (Embers et al. 2012; Roberts et al. 1995) including neuroborreliosis (Pachner et al. 2001).

5.1.2 Leptospira

Leptospira has been detected in almost all mammalian species that have been investigated (Adler et al. 2011). Various wild and domesticated animals function as the disease reservoir (Andersen-Ranberg et al. 2016), yet rodents are the primary maintenance species for human infection (Ko et al. 2009). The pathogen has a broad global distribution and can be found on all continents except Antarctica (Adler et al. 2011). Despite its adaptability, *Leptospira* favors tropical conditions that are conducive to its transmission cycle (Bharti et al. 2003). Based on 16S rRNA sequence data, three different clades can be distinguished (Lehmann et al. 2014). *Leptospira* evolved from a free-living noninfectious environmental organism (Lehmann et al. 2014), which is reflected in the basal positioning of the nonpathogenic saprophyte-containing clade (Fig. 5.3). The two pathogen-containing groups include 14 species.

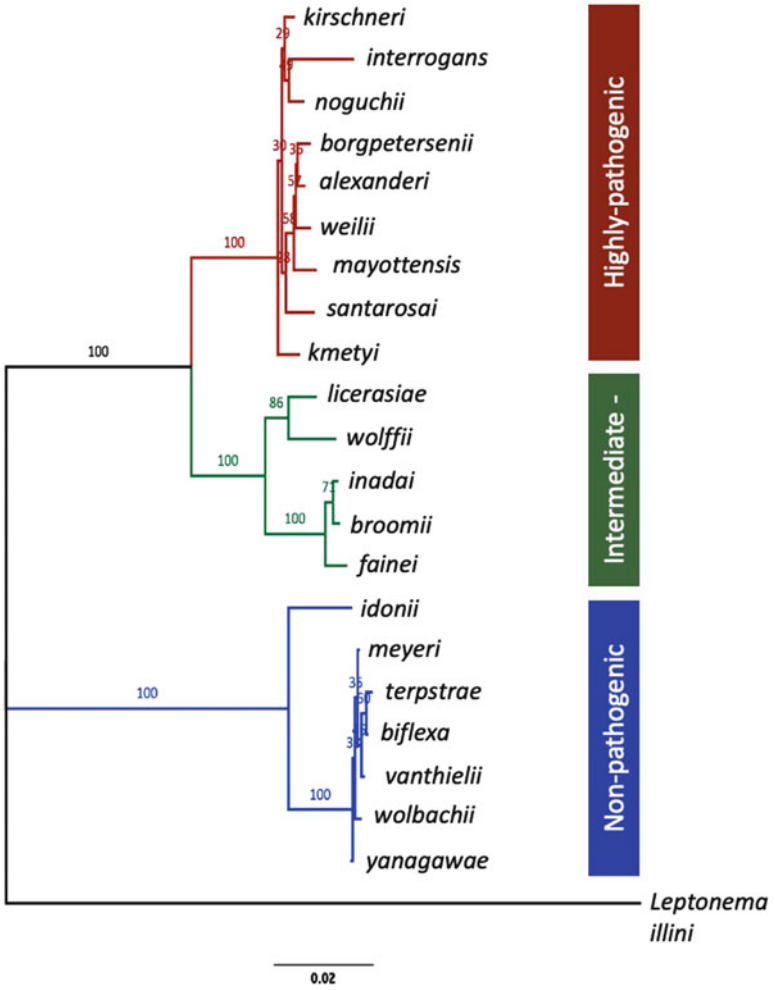

Fig. 5.3 Bio-Neighbor-Joining consensus tree of selected *Leptospira* spp. The tree is based on the V2-V8 region of the 16Sr RNA gene with 1,189 sites. The Jukes–Cantor substitution model was chosen and 1,000 bootstrap replicates were performed. Bootstrap values are displayed at respective branches. The bar refers to substitutions per site. *Leptonema illini* is used as an outgroup. For the interested reader, a more detailed phylogeny can be found in Thibeaux et al. (2018)

Leptospira species have the largest genome size among the spirochetes (>3.9–4.6 Mb) (Picardeau 2015), which is about four times the size of the *TP* genome (1.1 Mb) (Fraser et al. 1998). The *Leptospira* genome is circular and comprises at least two replicons (Zuerner 1991). A third circular replicon (*p47*) has been identified in the nonpathogenic *L. biflexa*. Classification of *Leptospira* spp. into serovars and serogroups is based on their agglutinating antigenic composition, mediated by surface exposed lipopolysaccharide. The serovar classification is based

on isolate-specific antigenic cross-matching, whereas serogroups are identified using microscopic agglutination tests (MATs). Pathogens of the *L. interrogans*-containing clade are subdivided into more than 250 serovars, some of which are associated with renal carriage by a specific mammalian maintenance host species, while others colonize a broader range of mammals (Ellis 2015; Lehmann et al. 2014). This association is not absolute, as a single animal species can carry different serovars in geographically distinct populations (Alexander et al. 1963; Everard et al. 1976, 1980; Tomich 1979). In contrast to the serovar classification, serogroups have no official taxonomic status (Balamurugan et al. 2013). Within one serogroup as well as within one *Leptospira* sp. there can be several serovars. However, multi-locus sequence typing (MLST) has been shown to accurately classify *Leptospira* (Ahmed et al. 2006), but whole genome sequencing is now considered the standard (Levett and Picardeau 2018). Nevertheless, serotyping remains a key tool for epidemiological investigations (Balamurugan et al. 2013; Cerqueira and Picardeau 2009).

Pathogenic *Leptospira* spp. have coevolved with their respective maintenance host, for example, bats (Lei and Olival 2014) where the pathogens cause little to no clinical symptoms (Gomes-Solecki et al. 2017). In the chronically infected maintenance host, the pathogen colonizes the proximal renal tubules from where it is shed to the environment in the urine (Gomes-Solecki et al. 2017; Ratet et al. 2014). Transmission requires continuous enzootic circulation of the pathogen among the maintenance host population (Ko et al. 2009). Key to the transmission cycle of *Leptospira* is its ability to survive in the environment. Under ideal conditions, *L. interrogans*, for example, can survive for up to 28 days in freshwater (Casanovas-Massana et al. 2018). In the laboratory, viable leptospires have been recovered after storage for several years. Susceptible hosts are infected when the highly motile bacterium penetrates abraded skin or mucous membranes. What follows is a rapid systemic infection (Cinco 2010), which leads to the elimination of the bacterium from the blood stream and the chronic bacterial colonization of the proximal tubules of the kidney (Ratet et al. 2014).

In the susceptible nonmaintenance host, tissue damage in multiple organs can be observed (Ko et al. 2009). The initial hallmark of leptospirosis in humans is a nonspecific febrile illness (McBride et al. 2005). In 5–15% of all cases, leptospirosis can end in the severest disease forms known as the hepato-renale syndrome (Weil's disease) and the severe pulmonary hemorrhage syndrome (SPHS). With case fatalities >10% in Weil's disease and > 50% in SPHS, these diseases are a major health burden for humans (McBride et al. 2005). Despite their enormous importance as human pathogens, and although NHPs and humans in the tropics often share the same contaminated habitats, little is reported about naturally acquired infections in NHPs.

Table 5.1 provides a summary of the reported cases of naturally acquired leptospirosis in NHPs. The early perception that naturally acquired and clinically apparent leptospirosis is rare in NHP (Lapin 1962) is still valid today (Simmons and Gibson 2012). While New World monkey (NWM) species and Old World monkeys (OWM) can be experimentally infected with *Leptospira*, there is some indication that

Table 5.1 Summary of the reported cases of naturally acquired *Leptospira* infection in captive and wild NHPs

Geographic region of NHP species origin	Species common name	Scientific name	n animals diseased (total tested)	Captive (C) or wild (W)	Clinical symptoms	References
South America	Common marmoset	*Callithrix jacchus*	16 (28)	C	NA	Pinna et al. (2012)
	Weid's marmoset	*Callithrix kuhlii*	2 (2)	C	Jaundice, anemia, renal failure, death (n = 2)	Baitchman et al. (2006)
	Black-pencilled marmoset	*Callithrix pennicilata*	4 (8)	C	NA	Pinna et al. (2012)
	Cotton-top tamarin	*Saguinus oedipus*	1 (28)	C	Not described	Minette (1966)
	White-lipped tamarin	*Saguinus labiatus*	1 (1)	C	Lethargy and icterus, hemorrhage from the mouth, death	Reid et al. (1993)
	Capuchin monkey	*Cebus capuchinus*	5 (8)	C	NA	Pinna et al. (2012)
			Unknown (15[b])	C	Not described	Johnson and Morter (1969)
	Common squirrel monkey	*Saimiri sciureus*	25	C	Mostly clinically healthy, 11 with jaundice, hemorrhagic syndrome, deaths (n = 10)	Perolat et al. (1992)
			Unknown (15[b])	C	Not described	Johnson and Morter (1969)
			2 (15[b])	C	Depression, lethargy, respiratory distress, icterus, death (n = 2)	Johnson and Morter (1969)
Asia	Macaques	*Macaca* sp. (species not determined)	4 (100)	W-caught	Not described	Füzi and Csóka (1963)

	Rhesus macaque	*Macaca mulatta*	3 (157) and 1 (47)	C and W	Not described	Minette (1966)
			1 (2)	W	Not described	Hemme et al. (2016)
			18 (59)	C	Not described	Ibáñez-Contreras et al. (2010)
	Barbary macaque	*Macaca sylvanus*	1 (2)	C	Pericarditis, death	Urbain et al. (1954)
			4 (104[a])	C	NA	Jaffe et al. (2007)
			3 (26)	C	Jaundice, death (n = 3)	Shive et al. (1969)
	Stump-tailed macaque	*Macaca arctoides*	2 (10)	C	Vomitus, depression, renal failure, death (n = 1)	Tschirch (1989)
			3 (22)	C	Not described	Minette (1966)
	Bonnet macaque	*Macaca radiata*	9 (188)	W	Not described	Minette (1966)
	Hanuman langur	*Presbytis entellus*	8 (216)	W	Not described	Minette (1966)
	Lar gibbon	*Hylobates lar*	1 (13)	C	Not described	Minette (1966)
Africa	Baboon	*Papio* sp. (species not determined)	170 (383)	C	Diarrhea, still births, some few deaths	Fear et al. (1968)
	Hamadryas baboon	*Papio hamadryas*	2 (104[a])	C	NA	Jaffe et al. (2007)
	Guinea baboon	*Papio papio*	8 (31)	C	Not described	Minette (1966)

(continued)

Table 5.1 (continued)

Geographic region of NHP species origin	Species common name	Scientific name	n animals diseased (total tested)	Captive (C) or wild (W)	Clinical symptoms	References
	Yellow baboon	*Papio cynocephalus*	11 (63)	C	Not described	Minette (1966)
	Patas monkey	*Erythrocebus patas*	2 (15[b])	C	Not described	Johnson and Morter (1969)
			6 (103)	C	Not described	Minette (1966)
			9 (22)	W	Not described	Hemme et al. (2016)
	Vervet monkey	*Chlorocebus sabeus*	139 (162)	C (n = 81)/ W (n = 81)	NA	Rajeev et al. (2017)
	Grivet	*Cercopithecus aethiops*	6 (63) and 0 (8)	C	Not described	Minette (1966)
	Tana River crested mangabey	*Cercocebus galeritus*	1 (2)	C	Not described	Minette (1966)
			49 (328)	C	Not described	Minette (1966)
			24 (24)	W-caught	Jaundice, depression, hemorrhagic syndrome, death (n = 23)	Wilbert and Delorme (1927, 1928)

[a]Total number of barbary macaques plus hamadryas baboons; [b]The authors make no comment on the species composition. NA not applicable (clinically healthy)

naturally acquired infection is only found in wild OWMs (Minette 1966; Simmons and Gibson 2012). This discrepancy could be an effect of the arboreal lifestyle of most of the NWMs (Minette 1966), as the contact with pathogenic *Leptospira* in the soil is minimized. This inference is further supported by a study in arboreal living Galagos (*Galago senegalensis*) in Africa, which tested negative for *Leptospira* antibodies (Minette 1966). However, only a small sample of Galagos was tested in this study and there are currently no published studies that tested wild NWMs. Additionally, there is some indication that wild-caught monkeys frequently acquired infection during their time in captivity (Minette 1966).

According to Minette (1966), natural infections of macaques (*Macaca* sp.) including a long-tailed macaque (*Macaca fascicularis*), Guinea (*Papio papio*) and hamadryas baboons (*Papio hamadyras*), as well as a chimpanzee (*Pan troglodytes*) have previously been observed by Sanderson (1957). However, we were not able to confirm this information while reviewing the original work of Sanderson (1957).

Naturally acquired infection, summarized in Table 5.1, should be considered the best proxy for the pathogenesis of *Leptospira* in NHPs. Depression, respiratory distress, jaundice, and vomiting are among the most reported clinical manifestations. Death was not uncommon. However, many animals that tested positive for antibodies against the spirochete were described to be clinically healthy, indicating that NHP infection can progress subclinically.

Inoculation experiments with *Leptospira* are frequently described for diagnostic purposes in the pre-genomic era or for the development of translational animal models for human leptospirosis. The infectious doses or application routes used in these experiments do not necessarily reflect what can be expected under natural conditions. This means that the results can neither be directly translated into disease progression nor per se reflect the pathology in naturally infected NHPs. Nevertheless, these inoculation experiments, which have been conducted under standardized conditions, contribute to our understanding of leptospirosis in NHPs. While the clinical manifestations in naturally infected animals were more or less consistent across different primate species (Table 5.1), artificial infection results in some interspecies differences. Based on the historical data of the early 20th century, and although these data must be interpreted with caution in terms of accuracy of study design, infectious dose and the description of pathological results, Asiatic macaques are reported to be less impacted by the pathogen, with only febrile illness and nausea (Babudieri 1939; Badudieri and Bianchi 1940; Erber and Michaut 1932; Huebner and Reiter 1915, 1916; Pettit and Martin 1920; Uhlenhuth and Fromme 1916). The benign course of infection was also described for experiments where white-fronted capuchins (*Cebus albifrons*) (Noguchi et al. 1924), baboons (*Papio* sp.) (Noguchi et al. 1924), and black spider-monkeys (*Ateles chamek*) (Noguchi et al. 1924) were artificially infected. In contrast to this, two marmoset species (*Saguinus oedipus* and *Saguinus geoffroyi*) and large-headed capuchins (*Sapajus macrocephalus*) could be infected with fatal consequences (Noguchi 1919). There is, however, a lack of consistency across the different studies and species. Stefanopoulo (1921), for example, was able to induce a fulminant leptospirosis with fever and icterus over a course of eleven days in one toque macaque (*Macaca sinica*), which is an OWM species

(Stefanopoulo 1921). The animals were inoculated with a high dose of *Leptospira* from guinea pig tissue. The same was demonstrated in patas monkeys (*Erythrocebus patas*), which were also infected with *Leptospira* from tissue of diseased rats (Noc 1920). A baboon (*Papio* sp.) that was infected by the same investigators, most probably using the same protocol and infectious dose, developed no abnormalities (Noc 1920). The susceptibility of baboons to *Leptospira* infection was, however, demonstrated by intracranial injection, which led to meningitis and fever (Troisier 1932). No icterus was present in these animals and both animals recovered. In another study, where three baboons were inoculated with a *Leptospira* isolate from a fatal chimpanzee infection, at least one baboon developed fatal infection (Wilbert and Delorme 1927). Such inconsistencies across studies and species often make the interpretation of the inoculation experiments difficult; in addition, undiagnosed coinfections in the NHP models in these early experiments could have impacted the clinical outcome (Marshall et al. 1980).

In recent years with the introduction of modern genetic techniques, NHP infection with *Leptospira* was no longer conducted for diagnostic purposes. Rather, monkeys are artificially infected with pathogenic *Leptospira* for the development of translational animal models for basic and applied research. These models have been shown to mimic the pathogenesis in human infection. The marmoset (*Callithrix jacchus*) as an established laboratory NHP has been used to mimic the severe pulmonary form of leptospirosis (Pereira et al. 2005).

5.1.3 Treponema

Treponemes are gram-negative motile bacteria of 6–15 μm length and 0.1–0.2 μm diameter. Unlike most of the *Borrelia* and *Leptospira* species, treponemes contain a greater number of noncultivable species (Šmajs et al. 2018). The *Treponema* family furthermore contains pathogenic and nonpathogenic species, a classification that is based on the bacterium's ability to cause disease in humans or animals. Among the most important diseases in humans are: syphilis caused by the *TP* subspecies *pallidum*, yaws caused by the subsp. *pertenue* (*TPE*), and bejel caused by the subsp. *endemicum*. While syphilis is distributed globally, the other two subspecies are causing endemic diseases of which yaws is currently subject to global eradicating efforts (Asiedu et al. 2014). *Treponema carateum*, a pathogen that was formerly classified as a *TP* subspecies, is the most benign of the endemic treponematoses and affects only the skin (Giacani and Lukehart 2014). The geographic distribution of human endemic treponematoses is shown in Fig. 5.4. The phased disease progression with three subsequent stages is common for syphilis and the endemic treponematoses (Giacani and Lukehart 2014; Radolf et al. 2016). The initial lesion appears at the site where the bacterium enters the skin. The developing ulcers disappear spontaneously within a few weeks. In the meantime, the bacterium has disseminated in its host, causing variable systemic illness and often a mucosal and skin rash. This is pathognomonic for the secondary stage of the disease, which again disappears

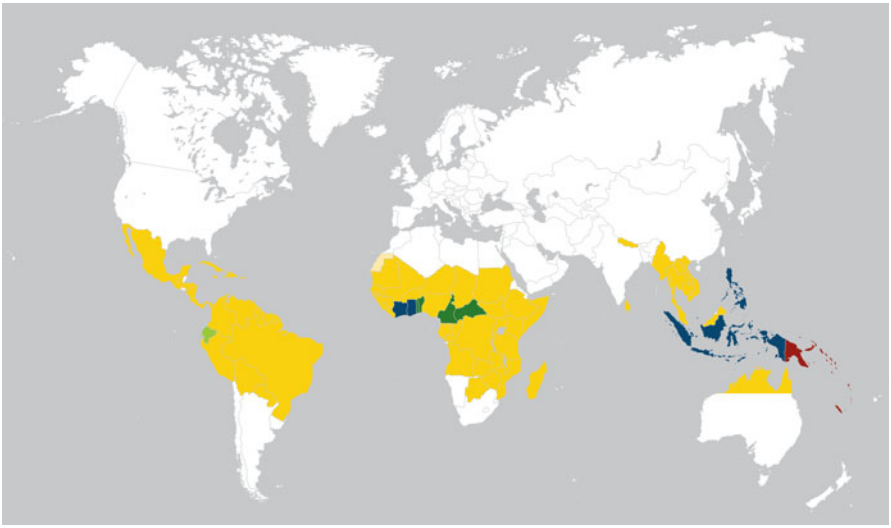

Fig. 5.4 Global distribution of endemic treponematoses. White = nonendemic, yellow = historically endemic (current status unknown), dark green = <1,000 cases, blue = 1,000–9,999 cases, red ≥10,000 cases in 2012. © Bernhard-Nocht-Institute for Tropical Medicine (Knauf 2018)

spontaneously after some weeks or months. In humans, tertiary stage infection is developed one to 20 years after acute infection and can lead to severe disabling conditions caused by cardiovascular and neurological sequelae and cartilage and bone deformation.

NHPs have been frequently used as a translational model for human treponematoses. Early experiments used chimpanzees (Metchnikoff and Roux 1903, 1904, 1905), toque monkeys (Castellani 1907), cynomolgous monkeys (Ashbury and Craig 1907), and rhesus macaques (Nichols 1910). These early experiments spurred the continued use of NHPs as experimental animals for human treponematoses, though with limited success (Clark and Yobs 1968; Elsas et al. 1968; Marra et al. 1998; Sepetjian et al. 1969, 1972; Tansey et al. 2017; Turner and Hollander 1957). Naturally occurring *Treponema* infection has been documented in NHPs (Hanson 1970) and the pathogenic *TP* has been reported in wild NHPs in Africa (Knauf et al. 2013). One of the first reports of an infected NHP came from a Guinea baboon (*Papio papio*) in West Africa in the 1960s (Fribourg-Blanc and Mollaret 1969). The isolated bacterium was identified as *TP* strain Fribourg-Blanc and accounts for the first *TP* whole genome that was sequenced from an NHP (Zobaníková et al. 2013). The strain is genetically highly similar to human yaws-causing strains, which led to the reclassification of the simian strain as subsp. *pertenue* (Zobaníková et al. 2013). The finding supported the *pertenue*-like classification of earlier and current studies on *TP* in Tanzanian NHPs (Chuma et al. 2018; Harper et al. 2012; Knauf et al. 2011) and remains supported by a growing number of published whole genome sequences of simian strains from West- and East Africa (Knauf et al. 2018) (Fig. 5.5). Ongoing

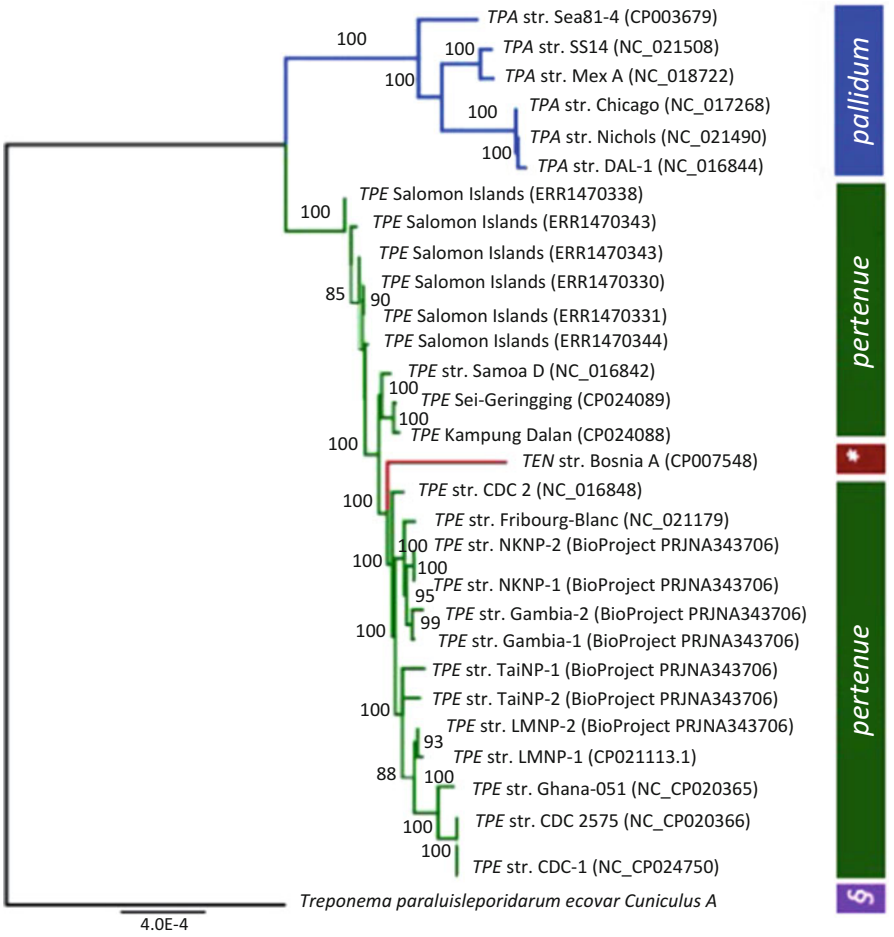

Fig. 5.5 Bio-Neighbor-Joining consensus tree constructed from published *Treponema pallidum* (*TP*) whole genome sequences of human and nonhuman primate origin. The Jukes–Cantor substitution model was chosen and 1,000 bootstrap replicates were performed. *Treponema paraluisleporidarum* ecovar *Cuniculus A* is used as an outgroup. Sequence accession numbers are provided in parentheses, *TPA* = *TP* susp. *pallidum*, *TPE* = *TP* subsp. *pertenue*, numbers indicate percent bootstrap support. * TEN = *TP* subsp. *endemicum*

fieldwork continues to demonstrate that a vast number of NHP host species can be infected (Table 5.2).

A large number of naturally infected NHPs with antibodies against *TP* showed no signs of infection. This has been described in the majority of the published reports where wild NHPs were screened for infection (Baylet et al. 1971a) and is also a key feature of human treponematoses is the latent stage (Marks et al. 2014). In this stage, infected individuals appear clinically healthy, but have both seroconverted and harbor the viable pathogen (Marks et al. 2015). Assuming that the *TPE* strains of

Table 5.2 Summary of African NHP host species naturally infected with *Treponema pallidum* (*TP*). Infection was demonstrated by the presence of antibodies against *TP* and/or PCR

Species' common name	Scientific name	n animals diseased (total tested)	References
Guinea baboon	*Papio papio*	64 (248)	Baylet et al. (1971a)
		18 (20)	Knauf et al. (2015)
Olive baboon	*Papio anubis*	Unknown	Wallis and Lee (1999)
		43 (57)	Knauf et al. (2011)
		86 (137)	Chuma et al. (2018)
		28 (52)	Harper et al. (2012)
Yellow baboon	*Papio cynocephalus*	33 (75)	Chuma et al. (2018)
		253 (835)	Fribourg-Blanc and Mollaret (1969)
Chlorocebus	*Chlorocebus* spp. (species not determined)	3 (15)	Fribourg-Blanc and Mollaret (1969)
		28 (45)	Baylet et al. (1971a)
Green monkey	*Chlorocebus sabaeus*	8 (8)	Knauf et al. (2018)
Vervet monkey	*Chlorocebus pygerythrus*	35 (45)	Chuma et al. (2018)
Chimpanzee	*Pan troglodytes*	3 (9)	Fribourg-Blanc and Mollaret (1969)
		Unknown	Kuhn (1970)
Colobus monkey	*Colobus sp*. (species not determined)	1 (1)	Fribourg-Blanc and Mollaret (1969)
Sooty mangabey	*Cercocebus atys*	5 (5)	Knauf et al. (2018)
Patas monkey	*Erythrocebus patas*	7 (44)	Fribourg-Blanc and Mollaret (1969)
		1 (23)	Felsenfeld and Wolf (1971)
		2 (42)	Baylet et al. (1971a)
Blue monkey	*Cercopithecus mitis*	2 (15)	Chuma et al. (2018)

humans and NHPs share the same biology, clinically healthy but seroconverted NHPs are likely in the latent stage of the disease. Clinical manifestations of variable severity and extent have been described. These range from mild keratotic lesions and ulcers around the muzzle, eyelids, and armpits in a baboon (Baylet et al. 1971b) to more severe anogenital and facial ulcerative skin lesions in a number of different NHP species (Chuma et al. 2018; Knauf et al. 2011, 2018; Levrero et al. 2007; Wallis and Lee 1999) (Fig. 5.6).

It is currently unknown why simian *TPE* strains cause genital lesions, but it underlines the capability of *TP* subspecies to cause atypical lesions, something that has also been described for *endemicum* strains infecting humans (Grange et al. 2016; Noda et al. 2018). However, the genital ulcerative disease in Tanzanian NHPs suggests a sexual transmission mode, which is further supported by the observation that predominantly sexually mature animals present with ulcerative skin lesions (Knauf et al. 2011; Mlengeya 2004; Wallis and Lee 1999). The epidemiological

Fig. 5.6 Skin lesions seen in a *Treponema pallidum* subsp. *pertenue* infected female olive baboon at Gombe National Park (left) and in a male vervet monkey at Mikumi National Park, Tanzania

context of NHP-to-NHP or NHP-to-human infection and vice versa with *TPE* is unclear and subject to ongoing research. The fact that NHP infecting *TPE* strains fall paraphyletic with human yaws-causing strains and the existence of *TPE* in a number of different NHP species indicates that interspecies transmission must have occurred at least at some evolutionary stage. This, however, does not provide evidence for ongoing transmission across the different primate taxa including humans. The bacterium has a significant lack of metabolic activity, which limits its survival in the environment (Lafond and Lukehart 2006; Willcox and Guthe 1966). Therefore, infection must occur mainly through skin-to-skin or mucous membrane contact (Richard et al. 2017). A possible example for a conceivable interspecies transmission pathway was recently described through inverted intergeneric introgression in two different NHP species in Tanzania (Zinner et al. 2018). Proving an epidemiological connection (Fig. 5.7) is complicated by the small number of high-quality but also draft *TPE* genomes from African humans and NHPs. Suitable tools for genetic typing such as MLST are available (Godornes et al. 2017; Katz et al. 2018; Pillay et al. 1998; Marra et al. 2010), but require intensified sampling at locations where both NHPs and humans are infected.

An alternative transmission pathway that would support interspecies transmission was described by Knauf et al. (2016) who demonstrated that *T. pallidum* DNA was present on wild captured flies that were trapped in close proximity to infected olive baboons in their natural habitat at Lake Manyara National Park and Tarangire National Park, Tanzania. This study was able to show that flies have regular contact with the pathogen, thereby providing a potential epidemiological link between humans and NHPs. However, these data did not demonstrate the viability of the pathogen, which is an important prerequirement for a viable transmission route (Hallmaier-Wacker et al. 2017). Under experimental conditions, fly transmission has been demonstrated (Satchell and Harrison 1953; Thomson and Lamborn 1934), although further genetic characterization of the treponemes that were used for these experiments was not conducted.

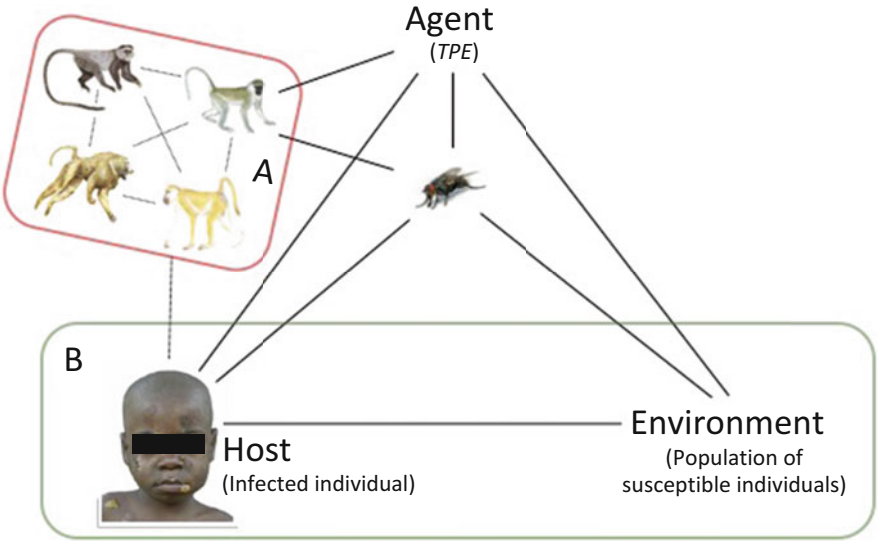

Fig. 5.7 Triad of factors involved in the epidemiology of *Treponema pallidum* subsp. *pertenue* (bold lines). In epidemiology, the classical triad is made of the agent, the host, and the environment: (**a**) describes the possible reservoir system whereas (**b**) defines the target group. Thin lines describe published relationships, whereas dashed lines describe speculated connectivity. Connections are bidirectional. Figure sources: Human = https://www.spaceshipearth.org.uk/yaws.html (last accessed 05.06.2018; modified), NHP figures = (Kingdon 2003) (modified), fly image source = https://www.oldskoolman.de/bilder/plog-content/images/freigestellte-bilder/natur-tiere/fliege-mit-ruessel.jpg (modified)

5.2 Other Treponematoses in NHPs

While chimpanzees that were experimentally infected with pinta developed human-like lesions (Chandler et al. 1972; Kuhn 1970; Varela 1969), there are no reports of naturally occurring *T. carateum* infection in any of the NHP species from the New World, an area where pinta is circulating in human populations. Compared to *TP* infection, little is known about other pathogenic and nonpathogenic treponemes that infect NHPs. Some studies report *Treponema* involvement in naturally occurring periodontitis in rhesus macaques (Colombo et al. 2017) and experimentally induced periodontitis in cynomolgous monkeys (Sela et al. 1987). Furthermore, an association with cardiac disease and treponemes of the gastrointestinal tract in gorillas has been reported (*Gorilla gorilla gorilla*) (Krynak et al. 2017). Unfortunately, the majority of these studies were unable to specify the *Treponema* species. Apart from these descriptions of pathogenic treponemes, spirochetes, and in particular the genus *Treponema*, are found abundantly in gut microbiomes of humans and NHPs without further knowledge on their pathogenicity (Bittar et al. 2014; Schnorr et al. 2014). With reference to the symbiont role that treponemes play in the termite gut system (Breznak 2002), it seems likely that treponemes in the gastrointestinal

tract of herbivorous NHPs such as gorillas (Hicks et al. 2018) play a similar role in the digestion of plant fibers (Schnorr et al. 2014). In termites, treponemes contribute to carbon, nitrogen, and energy requirements that help the host digest otherwise inaccessible plant materials (Warnecke et al. 2007). Other studies report spirochetes (Stumpf et al. 2013) and *Treponema* spp. as part of the vaginal microbiome in healthy baboons (Rivera et al. 2011; Yildirim et al. 2014).

5.3 One Health

Although *Leptospira* and *Treponema* infections are frequently reported in NHPs, there is ongoing debate about the possible reservoir function that NHPs play for human infection. This is in particular the case for *TP*, where it is clear that African NHPs are infected with the yaws-causing subsp. *pertenue* (Knauf et al. 2018). The first yaws eradication campaign between 1952 and 1964 was successful in terms of reducing the global yaws prevalence by 95% (Asiedu et al. 2014). However, decades later, yaws has reemerged in West Africa, Southern Asia, and the Pacific region, which made it necessary to launch a second eradication campaign (Asiedu et al. 2014). While initial trials to treat yaws with a single dose of azithromycin were successful in Papua New Guinea, eradication efforts are impacted by a number of different variables such as a rapidly developing macrolide resistance (Mitjà et al. 2018). The essential underlying question for eradication is whether or not there is ongoing interspecies transmission. Unfortunately, a number of unanswered questions prevent us from making a definite response (Hallmaier-Wacker et al. 2017). In contrast to the possible transmission between NHPs and humans in Africa (Knauf et al. 2013, 2018), it is unclear why infection with *TPE* is absent in wild but not pet macaques in Asia (Felsenfeld and Wolf 1971; Klegarth et al. 2017), despite the fact that human yaws is still endemic in parts of the continent (Kazadi et al. 2014). If NHP-to-human infection exists, it is probably not the main driver for reemergence of yaws. However, eradiation of yaws requires an infinite zero-case scenario and even sporadic and seldomly occurring transmission between an existing nonhuman reservoir and humans would hinder the major achievement of yaws eradication. To achieve lasting eradication of yaws infection in humans and to prepare for the worst-case scenario, it is therefore important to consider the role that NHP may play in the maintenance of yaws in nonhuman populations.

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Chapter 6
Chlamydia Infections in Nonhuman Primates

Luisa K. Hallmaier–Wacker and Sascha Knauf

Abstract Reports of natural infections with the gram-negative obligatory intracellular bacterium *Chlamydia* are rare in nonhuman primates (NHPs). This is surprising since all classes of vertebrates are exposed to this highly adaptive bacterial genus. NHPs are susceptible to inoculation with human strains of *Chlamydia* and have been used as translational models to study *C. trachomatis* and *C. pneumoniae*. Especially, genital and ocular *C. trachomatis* infection remains a significant global health burden in humans and NHPs continue to be used as translational animal models. For this chapter, we will discuss the different species of *Chlamydia* that infect humans and animals. We will focus on NHPs as a translational animal model for human *C. trachomatis* infection and discuss our current knowledge of naturally occurring NHP infection with *Chlamydia*.

Keywords Gram-negative bacteria · Trachoma · Urogenital · One Health · Animal models · Macaque · Sexually transmitted diseases

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6.1 Introduction

Despite the wide host range of *Chlamydia*, there are only a few reports of natural infection in nonhuman primates (NHPs). Animal experiments have demonstrated that NHPs are highly susceptible to *Chlamydia*, which affirms the possibility of further undiagnosed *Chlamydia* infections in wild and captive NHPs. Naturally occurring *Chlamydia* infection in monkeys, if present, may provide an interesting perspective on the biology and history of this bacterial genus. In this chapter, as part of an effort to foster further research in the field, we will use a One Health approach to summarize the current knowledge on the biology of *Chlamydia*, the historical use of NHPs as a translational animal model and our understanding of naturally occurring infections in NHP populations.

6.2 Biology of *Chlamydia*

The family of Chlamydiaceae has been historically subdivided into the two genera, the *Chlamydia* and *Chlamydophila* (Everett et al. 1999). This subdivision is currently controversial, and it has been proposed to amend the classification into a single genus *Chlamydia* (Sachse et al. 2015; Stephens et al. 2009). Based on the 16SrRNA gene sequences as well as the full Chlamydiaceae genomes, the two genera are not consistently separated (Fig. 6.1) (Sachse et al. 2015) and there is no phenotypic differentiator between *Chlamydia* and *Chlamydophila* (Horn 2008). In line with the current discussion, we, therefore, refer to all *Chlamydophila* species as *Chlamydia* in this chapter.

Chlamydia shares characteristics with both, virus particles and bacteria (Moulder 1966). Some of the viral features include the small cell size (approx. 0.3 μm), a reduced metabolic capability (lack of a complete tricarboxylic acid cycle (TCA) or aerobic respiration), the ability to use adenosine triphosphate (ATP) from the host cell cytosol, the formation of cytoplasmic inclusions, and the inability to grow in synthetic media (Omsland et al. 2014; Choroszy-Król et al. 2012). Since 1966, *Chlamydia* has been classified a bacterium as the genus shares typical characteristics with bacteria such as a cell wall similar to gram-negative bacteria, the ability to produce energy independently, and many biomolecules (DNA, RNA and some organelles). Members of the genus *Chlamydia* are obligatory intracellular parasites and infect primarily the mucosa where the bacterium survives and multiplies within the epithelial cells using a unique multistep developmental cycle (Fig. 6.2). *Chlamydia* interchange between two distinct morphological forms, the elementary body (EB) and the reticulate body (RB) (Moulder 1991). The environmentally stable EBs are the infectious form that can attach to and invade susceptible host cells (Matsumoto 1973, 1988). Due to the intracellular nature of *Chlamydia*, EBs use elaborate mechanisms for host cell attachment and entry to be internalized in membrane-bound vacuoles (termed an inclusion; reviewed in Elwell et al. 2016)

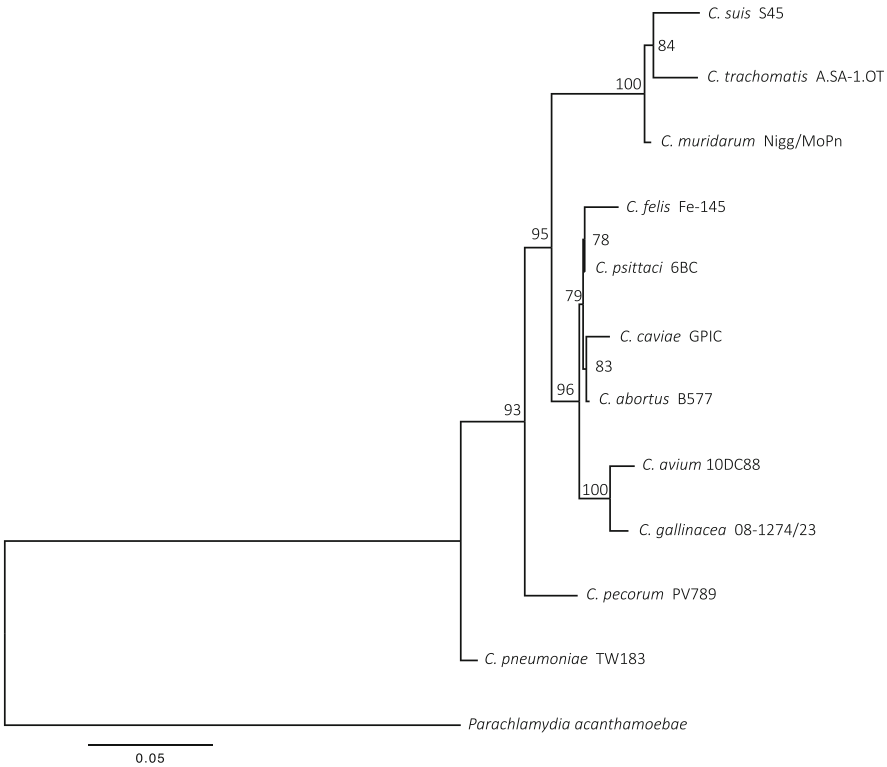

Fig. 6.1 Phylogenetic reconstruction of *Chlamydia* sequence data is based on 1441 positions in the 16S rRNA gene and were constructed using IQ-Tree 1.6.12 (Kalyaanamoorthy et al. 2017) including ModelFinder (Nguyen et al. 2015). All sequence data were retrieved from NCBI GenBank (NR_026527, AB001785, NR_036864, NR_111993, NR_074946, GQ398031, NR_121781, AB001777, D88316, NR_074982, NR_029196, and NR_026357). Bootstrap values indicated at each node are calculated using the Shimodaira-Hasegawa [SH]-aLRT algorithm for likelihood-based measures of branch support and are based on 1000 replicates. *Parachlamydia acanthamoebae* was used as an outgroup

(Fig. 6.2). Upon inclusion, EBs differentiate into the metabolic active and replicative form known as RBs (AbdelRahman and Belland 2005). The RBs divide multiple times via binary fission and differentiate back into EBs (Todd and Caldwell 1985; Abdelrahman et al. 2016). The EBs are then released to infect neighboring cells through either lysis of the host cells or the extrusion of the inclusion (Hybiske and Stephens 2007). Depending on the *Chlamydia* species, EBs exit the host cell 40–72 h postinfection (Fig. 6.2) (Ferrell and Fields 2016). During cellular stress, for example induced by antibiotic treatment, the developmental cycle is disturbed and the RBs transform into a large, atypical, nondividing form (reviewed in AbdelRahman and Belland 2005). The persistent form of RBs is one of the proposed strategies that *Chlamydia* uses to evade the immune response of the host (Elwell et al. 2016).

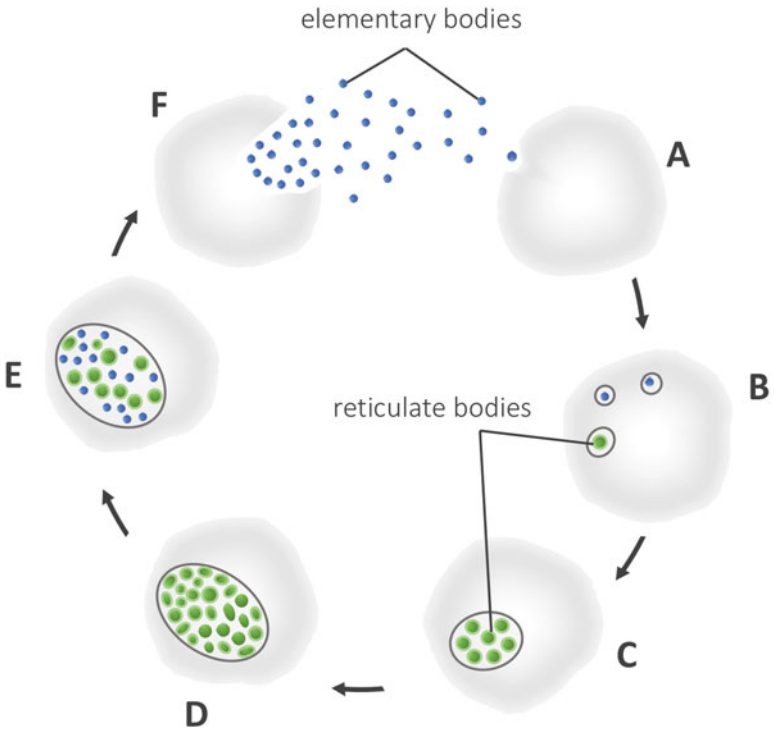

Fig. 6.2 The life cycle of *Chlamydia trachomatis*. (**a**) The elementary body (EB) binds and invades the host cell. (**b**) After endocytosis, the EB is in a membrane-bound compartment, known as the inclusion. (**c**) Bacterial protein synthesis begins and *Chlamydia* converts to the reticulate body (RB) form. (**d**) The RBs divide multiple times via binary fission and (**e**) differentiate back into EBs. (**f**) The EBs exit the host through lysis or extrusion to infect neighboring cells

Once the bacterium has overcome the physical mucosal barrier of the host (Redgrove and McLaughlin 2014), it enters the host cell, where intracellular chlamydial growth activates cell surface receptors, endosomal receptors, and cytosolic innate immune sensors (Elwell et al. 2016). This leads to an innate immune response against the bacterium, which includes the recruitment of neutrophils and natural killer cells to the site of infection (Elwell et al. 2016). Recruited pro-inflammatory cytokines and chemokines play a crucial role in inducing the protective T-helper cell response (Rasmussen et al. 1997; Johnson 2004). In addition to the initial cytokine response, toll-like receptors are activated by the bacterial infections and play their role in the upregulation of transcription factors against chlamydial infections (Prebeck et al. 2001; Iwasaki and Medzhitov 2004). Dendritic cells activate T-cells through MHC class I/II presentation and secrete Thelper 1 cytokines (Marks et al. 2010; Kapsenberg 2003; Matyszak et al. 2002; Ojcius et al. 1998). Overall, T-cells have a crucial role in resolving chlamydial infections (Brunham and Rey-Ladino 2005; Rank et al. 1985; Vasilevsky et al. 2014). The *Chlamydia*-pathogen has developed multiple mechanisms to evade the immune response of

the host, which includes inhibition of pro-inflammatory response as well as subsequent evasion of the adaptive immunity through its intracellular lifestyle (reviewed in Brunham and Rey-Ladino 2005), Fig. 6.2). This can lead to inadequate recognition of the *Chlamydia* and persistence within the cell, which can worsen the clinical course of infection (e.g., increasing the risk of sequelae) (Elwell et al. 2016; Horvat et al. 2010).

6.3 *Chlamydia trachomatis*

C. trachomatis is a human pathogen with the ability to cause both an ocular and a genital infection (Table 6.1) (Nunes and Gomes 2014). Not only is this species of *Chlamydia* the leading cause of preventable blindness in the world, but also the most common bacterial cause of sexually transmitted infections. Both infections often remain asymptomatic but can lead to an inflammation response with various disease outcomes (Table 6.1). Based on the major outer membrane protein (*ompA*), *C. trachomatis* strains are currently classified into 19 serotypes (Yuan et al. 1989). While the ocular strains are associated with serovar A-C, the urogenital strains are associated with D-K and L1-L3 (Table 6.1). However, differences between serotypes neither predict pathobiological differences nor variations in the rest of the genome of different *C. trachomatis* strains (Harris et al. 2012; Brunelle and Sensabaugh 2006). Multi-locus strain typing systems using six to eight housekeeping genes have been used to differentiate *C. trachomatis* strains (Klint et al. 2007; Pannekoek et al. 2008) and are an important tool for epidemiological investigations. Compared to serovars, the differentiated genotypes provide robust and higher resolved data sets that can be used to understand the spread of *Chlamydia* infections within a given community (Klint et al. 2007).

Transmission of *C. trachomatis* occurs through direct mucosal contact (e.g., during birth), indirect contact with mucosal secretions, or indirect passive transmission by eye-seeking flies (ocular strains) (Lanjouw et al. 2016; Gambhir et al. 2007).

Table 6.1 *Chlamydia* pathogens of humans and the relevant translational animal models

Species	Serotype	Serovar[a]	Disease	Animal Model
Trachomatis	Ocular	A, B, Ba, C	Conjunctivitis; trachoma	NHP*, Guinea pigs
	Genital	D, Da, E, F, G, Ga, H, I, Ia, J, K	Urethritis; epididymitis; cervicitis; endometritis; salpingitis; tubal factor infertility; infantile pneumonia	NHP*, rodent*, hamster, pig, rabbit
	LGV	L1, L2, L2b, L3	Lymphogranuloma venereum	NHP*, rodents*
Pneumonia	Human	N/A	Bronchitis; pneumonia; pharyngitis; atherosclerosis	NHP*, rodent*, rabbit*, pigs*

[a]predominately associated; *susceptible to strains of human origin

Screening is usually required to identify an infection, as the genital and ocular infection is often asymptomatic (Malhotra et al. 2013). Nucleic acid amplification tests (NAATs) are the recommended diagnostic tool due to their superior sensitivity, specificity, and practicality (Lanjouw et al. 2016). NAATs can be used to identify genital *Chlamydia* in first-void urine (recommended specimen in males) and vulva-vaginal swabs (recommended specimen in females) (Cook et al. 2005). Serology is not recommended for screening as antibody levels do not provide reliable information for routine diagnostic purposes (Lanjouw et al. 2016). Current treatment is straightforward as there is no evidence of any stable resistance against therapeutic antimicrobial treatment (Wang et al. 2005).

6.3.1 Trachoma

Trachoma is a chronic keratoconjuctivitis caused by a recurring infection with serovar A, B, Ba, and C of *C. trachomatis* (Table 6.1) (Burton and Mabey 2009). Trachoma is the leading infectious cause for blindness and remains endemic in poor and rural areas of Africa, Central and South America, Asia, Australia, as well as the Middle East (Burton and Mabey 2009). Primary ocular infections result in a self-limiting inflammation of the conjunctiva (Gambhir et al. 2007). Repeated reinfection leads to prolonged inflammation-inducing conjunctival scarring, which can result in blinding sequelae (Gambhir et al. 2007). Blindness from trachoma is irreversible and is thus a substantial burden on affected communities. Genital associated serovars D-K can also infect the conjunctiva and cause clinical infection. This often occurs in infants that are delivered through an infected birth canal (Darville 2005). However, these infections seem to be isolated events in adults and do not result in blindness (Burton and Mabey 2009).

6.3.2 Urogenital Infection

Genital associated serovars are subdivided into two groups: serovar D-K, which can be found only in the urogenital tract, and serovar L1-L3, which can disseminate to locoregional lymph nodes (Bébéar and De Barbeyrac 2009). The serotypes D-K are associated with cervicitis in women and urogenital infections in men (for a full list of disease outcomes see Table 6.1) (Bébéar and De Barbeyrac 2009) (Menon et al. 2015). In women, repeated *C. trachomatis* infections can result in tubal factor infertility, ectopic pregnancy, or chronic pelvic pain and thus represents a major health burden (Malhotra et al. 2013; Weström et al. 1992). Different serovars are associated with different pathogenicity (e.g., reinfection, duration of infection or immune response) and serovars E and F predominate in most countries. Tissue damage and the subsequent recruitment of immune cells in the mucosal epithelium during genital *Chlamydia* infection with any serovar can facilitate the spread of other

sexually transmitted diseases (STIs) such as human immunodeficiency virus (HIV) (Turner et al. 2013).

6.4 NHPs as Translational Models

The use of NHPs to model human diseases is associated with ethical concerns. Considerable efforts should be made to replace, refine, and reduce (3Rs) the use of NHP as laboratory animals. Table 6.1 provides a list of alternative models that have been used with varying success to replace NHPs. However, due to their physiological and anatomical similarity to humans, NHPs are commonly used to study various STIs (Miyairi et al. 2010). Genital *C. trachomatis* infections have been modeled in marmosets (*Callithrix jacchus*), grivets (*Chlorocebus aethiops*), yellow baboons (*Papio cynocephalus*), olive baboons (*Papio anubis*) and pig-tail macaques (*Macaca nemestrina*) (see Table 6.2). In particular, the pig-tailed macaque has been used to model genital associated *Chlamydia* infection in the female reproductive tract. Patton et al. developed an in situ model to study the pathogenesis and treatment of *Chlamydia*-associated pelvic inflammatory disease (PID) (De Clercq et al. 2013). In this model, pig-tailed macaques were infected with *C. trachomatis* by cervical or intratubal inoculation. A single inoculation into the fallopian tubes led to a self-limited (28–35 days) tubal infection without evidence of disease sequelae (Patton et al. 1987a). Repeated inoculation, however, led to clinical symptoms similar to that in women with *Chlamydia*–induced PID. Chronic salpingitis with extensive tubal scaring and distal tubal obstruction has been described (Patton et al. 1987a, 1990). Recently, baboons have been proposed as a translational model for studying *C. trachomatis* genital infections (Thygeson and Mengert 1936). A single inoculation led to an infection of the upper reproductive tract in wild-caught baboons and caused a variety of disease outcomes from mild cervicitis to PID (Bell et al. 2010). Collectively, experiments in the laboratory have shown that wild- and captive-female NHPs are highly susceptible to different serovars of *C. trachomatis*.

Until recently, there was a lack of animal models for the study of male-associated symptoms (Mackern-Oberti et al. 2013) although the disease prevalence of *C. trachomatis* infection is similarily high in men (Mackern-Oberti et al. 2011). Inoculation studies have been performed on long-tailed macaques (*Macaca fascicularis*), yellow baboons and grivet monkeys (Bannantine and Rockey 1999; Digiacomo et al. 1975; Møller and Mårdh 1980) (Table 6.2). Infection was either achieved through inoculation into the urethra using a catheter or directly injected into the spermatic cord (Bannantine and Rockey 1999; Digiacomo et al. 1975). Although the studies and animal numbers are limited, it appears that male NHPs are also susceptible to inoculation with *C. trachomatis* and can shed the organism from the urethra for up to three months postinfection (Digiacomo et al. 1975).

NHPs have been used to study rectal *C. trachomatis* infections (Henning et al. 2017; Annan et al. 2009). In particular, long-tailed macaque and rhesus macaque (*Macaca mulatta*) models have been used to investigate the effect of rectal

Table 6.2 Summary of studies using NHPs as model organisms to study genital *C. trachomatis* infections

	Serovar	Primate Species (n)	Sex	Repeated Infection[a]	Tested Medication	References
Genital	L2	*Macaca fasicularis* (2)	M	Yes	No	Bannantine and Rockey (1999)
	D, I	*Papio cynocephalus* (2)	M	No	No	Digiacomo et al. (1975)
	E	*Papio anubis* (10)	F	No	No	Bell et al. (2011)
	D	*Macaca nemestrina* (7)	F	Yes	No	Wolner-Hanssen et al. (1991)
	F, D, J	*Macaca nemestrina* (4)	F	Yes	No	Patton et al. (1987a)
	D, E	*Callithrix jacchus* (8)	F	No	No	Johnson et al. (1980)
	D, E, H	*Callithrix jacchus* (11)	F	Yes	No	Johnson et al. (1981)
	K, I	*Chlorocebus aethiops* (3)	F	No	No	Ripa et al. (1979)
	E, F	*Macaca nemestrina* (4)	F	No	No	Patton et al. (1983)
	K	*Chlorocebus aethiops* (2)	M	No	No	Møller and Mårdh (1980)
	E, F	*Macaca nemestrina* (4)	F	No	No	Patton (1985)
	D	*Macaca nemestrina* (44)	F	Yes	No	Lichtenwalner et al. (1997)
	D, F	*Macaca nemestrina* (11)	F	Yes	No	Patton et al. (1990)
	D	*Macaca nemestrina* (40)	F	Yes	Yes	Patton et al. (1997)
	E	*Macaca nemestrina* (45)	F	Yes	Yes	Patton et al. (2005)
	E	*Macaca nemestrina* (3)	F	Yes	No	Henning et al. (2011)
	D	*Macaca nemestrina* (25)	F	Yes	Yes	Patton et al. (2014)
	E	*Macaca nemestrina* (12)	F	No	Yes	Patton et al. (2006)
	D	*Macaca nemestrina* (40)	F	Yes	Yes	Peeling et al. (1999)
	E	*Macaca nemestrina* (11)	F	Yes	Yes	Patton et al. (1996)
	D	*Macaca nemestrina* (8)	F	No	No	Patton et al. (1993)

(continued)

Table 6.2 (continued)

	Serovar	Primate Species (n)	Sex	Repeated Infection[a]	Tested Medication	References
	K	*Chlorocebus aethiops* (7)	F	No	No	Møller et al. (1980)
	D	*Macaca nemestrina* (9)	F	Yes	No	Henning et al. (2014)
	K	*Chlorocebus aethiops* (6)	F	No	No	Møller and Märdh (1980)
Subcutaneous pocket	E	*Macaca mulatta* (11), *Macaca nemestrina* (1)	F	Yes	No	Patton and Kuo (1989)
	E	*Macaca fasicularis* (2), *Macaca mulatta* (4)	F	No	No	Patton et al. (1987b)
	E	*Macaca nemestrina* (18)	F	Yes	No	Van Voorhis et al. (1997)
	E	*Macaca nemestrina* (12)	F	No	No	Van Voorhis et al. (1996)
	E, B, C	*Macaca nemestrina* (11), *Macaca mulatta* (3)	F	No	No	Patton et al. (1989)
	E	*Macaca nemestrina* (4)	F	No	No	Lichtenwalner et al. (2004)
	E	*Macaca nemestrina* (6)	F	No	Yes	Patton et al. (1994a)
	E	*Macaca nemestrina* (4)	F	No	No	Patton et al. (1994b)

[a]Reinoculation ≥ two inoculation, M = male, F = female

C. trachomatis infection (Henning et al. 2017; Quinn et al. 1986; Zeitz et al. 1988; Vishwanathan et al. 2017). Rectal infection with an LGV strain caused a variety of symptoms from mild proctitis to severe hemorrhagic ulcerative proctitis (Quinn et al. 1986; Zeitz et al. 1988), mimicking the clinical symptoms, histopathology, and immune response of the acute rectal infection in humans (Quinn et al. 1986; Zeitz et al. 1988). A macaque model was furthermore used to examine the effect of rectal *C. trachomatis* infections (serovar E and LGV-L2) on simian-human immunodeficiency virus (SHIV) acquisition (Henning et al. 2017; Vishwanathan et al. 2017). Overall, the study found no effect of rectal *Chlamydia* coinfection on SHIV shedding or risk of acquisition (Vishwanathan et al. 2017). These findings contradict the results of a vaginal coinfection study in pig-tail macaques where there was a 2.5-fold risk increase of intravaginal SHIV acquisition in presence of *C. trachomatis* (serovar D) and *Trichomonas vaginalis* (Henning et al. 2014). These differences in acquisition risk may reflect *C. trachomatis* strain differences, physiological differences between infection site (vaginal vs. rectal), or some other as yet unidentified variable.

Both coinfection studies provide insight into the ongoing discussion on the potential effect of coinfections in the acquisition risk of STIs, especially HIV (Turner et al. 2013; Rotchford et al. 2000; Johnson and Lewis 2008; Ward and Rönn 2010).

The different strains of *C. trachomatis* appear to have a high level of genomic synteny, suggesting that little genetic variation between strains determines the observable pathogen-specific characteristics (Carlson et al. 2004). Therefore, inferences can be made between studies of the genital and ocular *C. trachomatis* infections in NHP models. Many of the commonly used laboratory animals show resistance to ocular infection with serovars of *C. trachomatis (*Taylor 1985*)*. Yet, various species of NHP (Table 6.3) are susceptible to the ocular infection and have been experimentally inoculated (Taylor 1985). The long-tailed macaque is the most commonly used NHP species to study the pathogenicity of trachoma strains (Kari et al. 2008, 2011). Early studies of ocular infection with *C. trachomatis* used a single inoculation of the infectious agent (Taylor 1985; Kuo et al. 1986; Patton and Taylor 1986), which is reported to cause an acute folliculate conjunctivitis but does not result in the pathognomonic trachoma-associated sequelae such as conjunctival scarring and cornea pannus (Kuo et al. 1986; Patton and Taylor 1986). In New and Old World monkeys, the infection resolves spontaneously within weeks (e.g., owl monkeys (*Aotus trivirgatus*) (Bell and Fraser 1969)) to months (e.g., cynomolgus monkeys (Taylor et al. 1982)). To model the chronic trachoma observed in endemic areas, NHPs need to be repeatedly reinfected (Patton and Taylor 1986; Taylor et al. 1981, 1982). Symptoms persist as long as reinfection is performed and conjunctive scarring develops over time (Taylor et al. 1981). Overall, studying the ocular infection in the NHP model has led to a better understanding of the disease progression and thus supports current disease prevention activities in endemic regions (Wright and Taylor 2005).

6.5 One Health and *Chlamydia*

Chlamydiosis in wild and domestic animals is well described and ranges from asymptomatic infections to severe diseases depending on the affected host and the involved chlamydial species (Borel et al. 2018). Various molecular approaches have shown that all classes of vertebrates are exposed to chlamydial infections (Table 6.4) (Horn 2008; Longbottom and Coulter 2003). *Chlamydia pneumoniae*, for example, infects both warm and cold-blooded animals such as horses, Australian marsupials, amphibians, and reptiles (Bodetti et al. 2002). In humans, this pathogen is a widespread respiratory infection that has also been associated with several chronic diseases (e.g., asthma (Sutherland and Martin 2007)) (Saikku 1992). Sequence data suggests that, *C. pneumoniae* in humans is of zoonotic origin (Myers et al. 2009). Other known zoonotic species of *Chlamydia* are *C. psittaci*, *C. abortus*, and *C. felis* (Longbottom and Coulter 2003) (Table 6.4). *C. psittaci* also known as avian chlamydiosis causes diarrhea, anorexia, respiratory distress, and conjunctivitis in wild and domesticated birds and has been isolated from other species (Table 6.4)

Table 6.3 Summary of studies using NHPs as model organisms to study ocular *C. trachomatis* infections

Scrovar	Primate species (n)	Repeated infection[a]	Vaccine study	Tested medication	References
A, B, C	*Macaca fascicularis* (6)	No	No	No	Kari et al. (2008)
C, E	*Macaca nemestrina* (5)	Yes	No	No	Cosgrove et al. (1989)
B, C	*Macaca nemestrina* (10), *Macaca mulatta* (6)	No	No	No	Patton et al. (1987c)
B	*Macaca fascicularis* (10)	Yes	No	No	Patton and Taylor (1986)
B	*Macaca fascicularis* (3)	No	No	Yes	Zhang et al. (1987)
B	*Macaca fascicularis* (3)	No	No	No	Caldwell et al. (1987)
A	*Macaca fascicularis* (6)	Yes	Yes	No	Kari et al. (2011)
A, E	*Macaca fascicularis* (11)	Yes	No	No	Taylor et al. (1982)
E	*Macaca mulatta* (4), *Macaca fascicularis* (4)	Yes	No	No	Taylor et al. (1981)
B, L2	*Macaca fascicularis* (11)	Yes	No	Yes	Taylor et al. (1987a)
E	*Macaca fascicularis* (9)	Yes	No	Yes	Taylor et al. (1983)
Unknown	*Macaca cyclopsis* (5)	Yes	Yes	No	S-p et al. (1967)
A, B	*Aotus trivirgatus* (35)	Yes	No	No	Fraser et al. (1975)
A	*Aotus trivirgatus* (16)	No	No	No	Nichols et al. (1973)
A, B, C	*Aotus trivirgatus* (10)	Yes	No	No	Orenstein et al. (1973)
B	*Aotus trivirgatus* (9)	Yes	No	No	Sacks et al. (1978)
B, L2	*Macaca fascicularis* (15)	No	Yes	No	Taylor et al. (1987b)
B	*Macaca fascicularis* (10)	Yes	No	No	Taylor et al. (1984)
Unknown	*Papio cynocephalus* (2)	No	No	No	Collier (1962)

[a]Reinoculation $\geq$ two inoculations

(Harkinezhad et al. 2009). It is classified as a category B bioterrorism agent as it is easily disseminated and associated with a high mortality rate (CDC/NIH 2009). Infections in humans can vary in severity but usually cause respiratory disease (Vanrompay et al. 1995). Human-to-human transmission is rare but does occur (Wallensten et al. 2014). Transmission of zoonotic *Chlamydia* from animals-to-

Table 6.4 The zoonotic potential of *Chlamydia* infections in wild and domestic animals as summarized by [1](Horn 2008) and by [2](Borel et al. 2018)

Species	Host[1]	Zoonotic Potential[2]
Abortus	Swine, cattle, sheep, goats, turtle, snake	X
Avium	Wild and domesticated birds	NP
Caviae	Guinea pigs	NP
Felis	Cats, iguana	(X)
Gallinacea	Wild and domesticated birds	(X)
Muridarum	Mouse, hamster	NP
Pecorum	Sheep, cattle, swine, goat, koala	NP
Pneumoniae	Horse, marsupials, amphibians, reptiles	NP
Psittaci	Wild and domesticated birds	X
Suis	Swine	(X)

Abbreviations: NP = no current evidence, X = evidence, (X) = partial evidence

humans is often associated with close contact with livestock (Longbottom and Livingstone 2006). For example, exposure to *C. abortus* infected animals through direct contact with products of abortion or infectious aerosols can cause serious flu-like disease in pregnant women, which can lead to preterm stillbirth and preterm labor (Hyde and Benirschke 1997). The zoonotic potential of other species of *Chlamydia*, such as *C. pecorum, C. suis*, and *C. muridarum*, is currently unknown (Table 6.4) (Nunes and Gomes 2014). Noteworthy is the serious health threat that *C. pecorum* infections pose to free-living koalas (*Phascolarctos cinereus*) (Polkinghorne et al. 2013).

Despite the susceptibility of NHPs to inoculations with *Chlamydia* (Table 6.1), there is currently only a single published report of natural *Chlamydia* infection in monkeys. Two long-tailed macaques were reported to have developed a spontaneous *Chlamydia* disease similar to *C. psittaci* infections (Morita et al. 1971). Cytoplasmic inclusion bodies were detected in histological examination of the oral cavity of infected monkeys, electron microscopy showed both EB and RB in the epithelial cells of tongue lesions and seven tested monkeys had anti-*Chlamydia* antibodies in the collected serum (Morita et al. 1971). Lack of genetic identification impedes the characterization of the *Chlamydia* species; however, the case demonstrates that NHP are susceptible to a natural infection of *Chlamydia*. The lack of further reports may be either due to a low incidence of infection or a lack of screening efforts. As the ocular and the genital infection is often asymptomatic, it is likely that natural occurring *Chlamydia* infections remain undiagnosed in wild and captive NHPs. Rushmore et al. screened urine samples from wild chimpanzees for various STIs including *Chlamydia* and found only *Trichomonads* in the population (Rushmore et al. 2015). Further screening of wild and captive NHPs is necessary to examine the prevalence of *Chlamydia* infections in different populations. The detection of a *Chlamydia*-like species in urogenital swabs would not be surprising as novel 16S rRNA gene sequences have been identified in various species in recent years and may all represent novel members of the genus *Chlamydia* (Bodetti et al. 2003;

Soldati et al. 2004). Further analyses based on whole genome sequences are warranted to classify any novel *Chlamydia*-like species of NHP origin.

Considering the general zoonotic potential of *Chlamydia*, a One Health approach should be considered when discussing the eradication of blinding trachoma since animals and humans often share the same habitat. Several control programs have been introduced in endemic countries with limited success (Burton and Mabey 2009). The World Health Assemble aims to resolve blinding trachoma by 2020 (Burton and Mabey 2009). In order to reduce the prevalence of the disease, mass drug administration (MDA) of entire endemic communities has been proposed (Burton and Mabey 2009). In addition to MDA, environmental and hygiene practice improvements can be important aspects of prolonged long-term treatment of endemic communities (Sumamo et al. 2007). Poor facial hygiene, close contact with children, crowded living conditions, and contact with eye-seeking flies have therefore been identified as some of the risk factors for infection (reviewed in Hu et al. 2010 and Emerson et al. 2000). Considering the recent findings suggesting the possible involvement of a nonhuman reservoir in both the yaws (see Chap. 5) and the guinea worm eradication campaigns (Eberhard et al. 2014), as well as the usefulness of NHPs as a suitable animal model for trachoma, further investigation of *Chlamydia* species in wild NHPs is warranted. Additionally, the involvement of eye-seeking flies is of particular interest as a possible interspecies transmission route.

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Chapter 7
Antimicrobial Stewardship in Captive Monkeys

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Abstract Antimicrobial resistance (AMR) is a growing threat in veterinary medicine, and although there is an expanding body of literature of the AMR prevalence in food animal and companion animal populations, little data on AMR exists in primate veterinary medicine. The monkey-human interface is extensive, especially in biomedical research and zoos, and these monkeys are frequently infected with bacteria that can be transmitted between monkeys and humans. The prevalence of antimicrobial resistant zoonotic bacteria has the potential to impact animal, human, and environmental health worldwide. Primate veterinarians frequently treat bidirectional zoonotic infections with antimicrobials classified as "critically important" by the World Health Organization, and such antimicrobial use (AMU) can influence AMR in humans and the environment. Consequently, medical primatologists have a public health obligation to use antimicrobials judiciously. Antimicrobial stewardship is especially important because the selective pressure of repeated AMU can promote the spread of AMR within and between monkeys and to humans with zoonotic transmission. Effective antimicrobial stewardship programs utilize a multi-modal approach involving a coordinated team with protocols for appropriate antimicrobial selection and practice and ongoing monitoring strategies for antimicrobial use,

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AMR, and patient outcomes. This multi-modal tactic is necessary to contest AMR on a One Health scale. Veterinarians are in a position to create comprehensive antimicrobial stewardship programs and reduce the threats of AMR on monkey and human health.

Keywords Antimicrobial resistance · Antimicrobial use · *Escherichia coli* · *Salmonella* · *Mycobacterium* · *Shigella* · *Campylobacter* · Methicillin-resistant *Staphylococcus aureus* (*MRSA*) · *Streptococcus* · *Yersinia* · One Health · Zoonotic · Anthropozoonotic

7.1 The Growing Threat of Antimicrobial Resistance

Scientists, health professionals, and laypeople are increasingly aware of the interconnectedness of animals, humans, and the environment, collectively known as One Health. Without question, all three health fields are inextricably linked. For instance, the health of our food animals affects the environment through methane emissions and food safety through contamination prevention. The rapidly changing health of our globe impacts the geographic ranges of vectors of infectious diseases that impact human and animal health, such as tick-borne Lyme disease (see Chap. 5). As the threat of antimicrobial resistance (AMR) grows, a more interdisciplinary collaborative approach to improve health is urgently needed.

Antimicrobial resistance (AMR) in particular has become a vitally important issue, due to its widespread threat and its complex relationships within One Health. The frequency of resistance to last-line antimicrobials has grown substantially, and the recovery of genetically indistinguishable resistance genes and bacterial strains between humans and animals demonstrates that the problem cannot be approached exclusively from either the veterinary or human medicine perspective (La Plante et al. 2017). Antimicrobial use (AMU) in all professions and in all parts of the globe can contribute to the problem of AMR. Thus, a One Health approach requires a greater partnership across the health and science fields to combat AMR. Although there are extensive literature on AMR and its comparative human impacts in food animal medicine and a growing body of literature in companion animal medicine, there is little investigation in other veterinary fields, including medical primatology. We define medical primatology as the study of the medical management and care of nonhuman primates with the purpose to maximize their well-being, conserve their species, and research comparative knowledge for human pathology and biology. And AMU and AMR are critical components of medical primatology because they can significantly influence all aspects of its definition. This is especially true among captive monkeys in biomedical research and zoos, where they are more frequently exposed to antimicrobials compared to their wild counterparts. As a result, AMU in medical primatology has the potential to impact human and animal health. This was seen with the use of chloramphenicol to treat *Shigella flexneri*, which led to resistance among ten monkey species (Good et al. 1969). The development and

epidemiology of AMR certainly does not discriminate between animal species, and similar to other animals and humans, AMR has and continues to threaten captive monkey health.

However formidable the threat of AMR, we, as veterinarians, physicians, and scientists, have the power to mitigate AMR. Using antimicrobial stewardship programs with coordinated teams, we can reduce the emerging spread of AMR and improve patient outcomes. In order to improve patient outcomes in any veterinary field, including medical primatology, we must weigh the short-term necessity of antimicrobial treatments against the long-term consequences of AMU and enhance the stewardship of antimicrobial resources to reduce AMR.

7.2 The One Health Interface

Monkeys used in biomedical research are typically obtained from domestic monkey breeding colonies or from the importation of purpose-bred monkeys, primarily from Asia, in order to meet research needs or to supplement domestic breeding programs; however, the number of monkeys used in biomedical research has been declining (Lankau et al. 2014; Miller-Spiegel 2011; Roberts and Andrews 2008). Additionally, as a result of the spread of infectious agents of public health interest, including those that have developed antibiotic resistance in imported nonhuman primates (NHP), quarantine procedures were developed to reduce the spread of zoonotic pathogens, which have also improved health outcomes for monkeys used in biomedical research in the United States (Department of Health and Human Services 2013; Roberts and Andrews 2008). Interest in retirement of monkeys is also growing, and its potential to perpetuate AMR should be considered (McAndrew and Helms Tillery 2016; Seelig and Truitt 1999). Furthermore, monkeys in zoos continue to flourish because of veterinarians' roles to protect and conserve animal populations. As collectively illustrated, the monkey-human interface is complex and significant. And despite the major improvements made in biosafety, importation, and medical management, as well as significant reductions of occupational and public health hazards, the monkey-human interface remains an important facet of One Health.

Among all pathogens, monkeys are most commonly infected by bacteria, most of which are zoonotic (Fox et al. 2015). Given the current close interaction with captive monkeys, and the genetic/physiologic similarities between monkeys and humans, zoonotic bacteria pose significant threats to human and animal health. Humans interact closely with captive monkeys in biomedical research laboratories and zoos, and the environmental survivability of zoonotic bacteria increases the likelihood of human exposure. Additionally, monkeys in these settings are more regularly overseen by veterinarians due to federally mandated regulations. As a result, bacteria colonizing or infecting these monkeys are exposed to a larger amount of antimicrobial selective pressure relative to pet or wild monkeys. In other words, the prevalence of AMR is most likely highest in zoo and biomedical monkey populations due to their antimicrobial exposure, and the risk of zoonotic transmission of AMR bacteria

and genes is larger. Additionally, a cross-sectional study evaluated occupational hazards and risks and demonstrated that most of the participating personnel experienced high-risk exposures (needle stick, scratch, bite, mucosal splash) from NHP in a laboratory or zoo setting ($n = 78$, 69.2% and 15.4%, respectively) (Engel and Jones-Engel 2012). Therefore, we have focused this chapter on AMR zoonotic bacteria among monkeys in biomedical research and zoos.

In addition to the impacts on human and animal health, the environment plays a critical role in the growing global threat of AMR. The environmental antimicrobial resistome is a complex framework impacted by antimicrobials and their metabolites in human and animal waste, natural AMR present in the environment, changing landscapes by anthropogenic forces, and more. In 2006, the first comprehensive study investigating AMR in the environment revealed that among their 480 strains of bacteria isolated from soil and 21 tested antimicrobials, all isolates were multi-drug resistant (D'Costa et al. 2006). Wildlife habitats closer to populations of livestock are also more likely to harbor AMR bacteria. Wild mice, voles, and shrews, which are not normally exposed to antimicrobials, were found to be five times more likely to carry tetracycline-resistant *E. coli* if near swine farms (Kozak et al. 2009). Additionally, AMR genes were shown to have been aerially disseminated miles away from cattle feedlots (McEachran et al. 2015). Given the knowledge from other animal populations, the risk of environmental dissemination of AMR bacteria in zoos and biomedical institutions, including the large NHP breeding and importation facilities, should be an important consideration.

7.3 Important Antimicrobials in One Health

Because of the One Health importance of antimicrobials, it is essential that clinicians consider the World Health Organization (WHO) classifications for antimicrobials, which are labeled as "important," "highly important," or "critically important" based on their impact on human health (World Health Organization 2011). This classification is necessary to discern the public health risks for different types of AMU. A large number and variety of antimicrobials have been developed to treat bacterial infections, and the frequency of resistance is not evenly distributed among the range of antimicrobial classes. Resistance in some antimicrobial classes such as fluoroquinolones (e.g., enrofloxacin) has a greater impact on human health, given the limited availability of alternatives for clinicians if AMR is observed. The WHO labels antimicrobial classes with few alternatives as "highly important" (World Health Organization 2011). There are also antimicrobials used to treat bacterial species that have the potential for zoonotic transmission and antimicrobials used to treat bacterial species that have the ability to acquire AMR genes; these antimicrobials are also labeled as "highly important" and include amphenicols, lincosamides, and first-generation cephalosporins (World Health Organization 2011). But antimicrobials that fit into both criteria are labeled as "critically important" (World Health Organization 2011). These classifications are regularly updated by the WHO, which

publishes new editions of *Critically Important Antimicrobials for Human Medicine* to highlight antimicrobials that have the most significant impact on One Health (World Health Organization 2011).

In addition to the WHO, the World Organization for Animal Health (OIE) similarly categorizes antimicrobials as "highly important" or "critically important." The OIE criteria for classification require at least 50% of the participating OIE member countries to identify an antimicrobial class as veterinary critically important (OIE (World Organization for Animal Health) 2007). The second criterion is met when an antimicrobial class is used to treat serious animal disease and has limited availability of alternative antimicrobials (OIE (World Organization for Animal Health) 2007). OIE "highly important" antimicrobials meet one of the above criteria, but "critically important" antimicrobials meet all criteria (OIE (World Organization for Animal Health) 2007). Both the WHO and OIE publications are key references for veterinarians and physicians, illustrating AMU practices that pose the greatest threats to animal and public health.

Moreover, after a literature search, we found antimicrobials reported as effective therapies for diseases affecting monkeys. Table 7.1 illustrates some of these antimicrobials and their associated WHO and OIE classifications. It is important to note, however, that Table 7.1 is not all-inclusive. It is reasonable to assume that some bacterial outbreaks and case reports have not been published, along with their antimicrobial treatment histories. The WHO and OIE prioritizations of antimicrobial classes are useful to inform hazard analyses, risk management strategies, and antimicrobial stewardship programs.

7.4 Antimicrobial Use in Monkeys

The order Primate is a diverse taxonomic group consisting of pygmy marmosets (*Cebuella pygmaea*) weighing less than 200 g to gorillas weighing more than 160 kg (Fox et al. 2015). Several pathogens can be naturally transmitted from monkeys to humans and vice versa (National Research Council of the National Academies 2003). For this reason, treatment and elimination of zoonotic bacterial diseases of monkeys is very important. Appropriate antimicrobial selection is based on the monkey species, size, route and frequency of dose administration, volume, and potential impacts on research data. In practice, off-label AMU is commonplace in medical primatology (Raabe et al. 2011). However, off-label antimicrobials should be prescribed with caution. Dosing antimicrobials off-label should not be assumed to be effective or safe, even when extrapolating data from one monkey species to another. It is also important to consider administration route (e.g., enteral, intramuscular, and intravenous), frequency, and volume when selecting appropriate antimicrobials for monkeys. Daily administration to a monkey can present unique challenges in the captive setting, such as patient compliance with oral administrations, or the potential soft tissue injury related to repeated injections. In addition, the potential subsequent patient stress during the restraint required to provide therapy

Table 7.1 List of antimicrobials used as effective therapies for monkey zoonotic bacterial diseases and their associated categorization assigned by the World Health Organization (WHO) and World Organization for Animal Health (OIE) based on their public health and veterinary importance, respectively

Antimicrobial class	Antimicrobial	WHO categorization	OIE categorization	Reference
Aminoglycoside	Gentamicin	Critically important	Critically important	Cooper and Needham (1976), Kim et al. (2017a), Ward et al. (1985) and Weller (1994)
	Streptomycin			
	Neomycin			
Ansamycin	Rifampin	Critically important	Highly important	Wolf et al. (1988)
Antimycobacterial	Isoniazid	Critically important	–	Ward et al. (1985) and Wolf et al. (1988)
	Ethambutol			
Fluoroquinolone, second generation	Enrofloxacin	Critically important	Critically important	Kim et al. (2017a) and Kolappaswamy et al. (2014)
Macrolide	Tylosin	Critically important	Critically important	Kim et al. (2017a)
	Azithromycin			
	Erythromycin			
Penicillin	Amoxicillin	Critically important	Critically important	Weller (1994) and Wolfensohn (1998)
	Ampicillin			
Amphenicol	Chloramphenicol	Highly important	Critically important	Rosenberg et al. (1980)
Pseudomonic acid	Mupirocin	Highly important	–	Kim et al. (2017b)
Sulfonamide	Trimethoprim-sulfamethoxazole	Highly important	Critically important	Kolappaswamy et al. (2014); Olson et al. (1986) and Weller (1994)
	Trimethoprim-sulfadiazine			
Tetracycline	Tetracycline	Highly important	Critically important	Fox et al. (2015) and Olson et al. (1986)
Nitroimidazole	Metronidazole	Important	–	Kolappaswamy et al. (2014)

can in turn increase occupational hazard and exposure to potentially zoonotic bacteria. Studies investigating long-acting antimicrobials for use in monkeys have had variable results (Papp et al. 2010; Raabe et al. 2011; Salyards et al. 2015); these investigations highlight the need for novel antimicrobials, dosing strategies, and assurance of compliance to overcome these dosing challenges that likely contribute to AMR in monkeys (Angulo et al. 2004).

Although extrapolating AMU from other veterinary and human fields is not recommended, it is commonly practiced because specific recommendations of AMU in monkeys are poorly documented. Currently, the most comprehensive literature of AMU in medical primatology can be found in *Laboratory Animal*

Medicine and *Nonhuman Primates in Biomedical Research: Diseases* (Abee et al. 2012; Fox et al. 2015). Antimicrobial therapy should be promptly administered because some bacterial species such as *Shigella flexneri*, *Streptococcus pneumoniae*, *Yersinia enterocolitica*, and *Y. pseudotuberculosis* can cause severe illness and acute death in monkeys. But once diagnostic test results are returned, targeted AMU based on known antimicrobial susceptibility will maximize patient outcomes and minimize selective pressures influencing AMR. Published data demonstrates that primate veterinarians request susceptibility tests about half to three-quarters of the time when common zoonotic bacteria are cultured, such as *Campylobacter*, *Shigella*, and *Yersinia* (Kim et al. 2017a). However, even with susceptibility test results, careful antimicrobial selection with the consideration of WHO categorizations is important due to their greater public health impacts. One study illustrated that primate veterinarians frequently use macrolides (e.g., tylosin and azithromycin) to treat campylobacteriosis and fluoroquinolones (e.g., enrofloxacin) to treat shigellosis and yersiniosis, demonstrating that WHO classified "critically important antimicrobials" are frequently used (Kim et al. 2017a). Overall, the lack of literature to guide AMU in medical primatology highlights the need for greater investigation to improve antimicrobial stewardship.

7.5 Epidemiology and Ecology of Antimicrobial Resistance

7.5.1 Cellular Mechanisms of Antimicrobial Resistance

Bacteria have developed multiple mechanisms to resist the effects of antimicrobials, creating increasingly challenging clinical cases for veterinarians and physicians. Genetic acquisitions or alterations enable bacteria to inhibit antimicrobials from entering the cells via reduced membrane permeability, active efflux of antimicrobials, altered antimicrobial target sites, and replacement or bypass of target sites (Blair et al. 2014; Munita and Arias 2016). These AMR genes can spread between two bacteria of the same or even different species through transformation, transduction, or conjugation, resulting in acquired resistance in a previously susceptible cell. This horizontal gene transmission complicates the ecology of AMR and has critical implications for the transmission of AMR between animal and human populations. Bacteria can also directly alter antimicrobials via inactivation by hydrolysis or steric hindrance (Blair et al. 2014). Additionally, point mutations in specific genes, such as those encoding for the binding site for fluoroquinolones, lead to AMR. Accumulation of these point mutations in the binding sites results in increasing levels of resistance.

7.5.2 Evolution of Antimicrobial Resistance via Selective Pressure at the Bacterial Level

AMR bacteria, or those bacteria possessing the genetic elements encoding resistance to antimicrobials, exist within most environments at low levels. In the presence of antimicrobials, these bacteria have a large competitive advantage over susceptible populations. Antimicrobials select for resistant populations by killing or inhibiting susceptible bacteria, allowing the remaining resistant bacteria to exploit the available resources, and expand within the environment (e.g., the gastrointestinal tract). Short periods of antimicrobial exposure within an animal followed by withdrawal of the drug can result in a re-establishment of susceptible bacteria and a return to the baseline bacterial populations (Lhermie et al. 2017). However, consistent AMU and selective pressure increases the abundance of AMR bacteria in the environment and increases the likelihood of zoonotic transmission, animal-animal transmission, and dissemination through the environment. And since veterinarians often resort to the same primary antimicrobials to empirically treat common clinical signs such as diarrhea prior to microbial test results (Kim et al. 2017a), the wide and consistent antimicrobial selective pressure in medical primatology increases the probability of AMR bacteria maintenance within monkey populations.

7.5.3 Introduction of Antimicrobial Resistance

The presence of bacteria with resistance to the administered drug is a prerequisite for selective pressure from antimicrobial application. AMR bacteria may initially arise through spontaneous mutations of existing bacterial isolates. More likely, however, AMR bacteria are introduced to a population of monkeys through outside sources. Resistant bacterial strains could be introduced to an established monkey group or colony with the introduction of new monkeys, human movement, wildlife, or contaminated food, water, or fomites. Biomedical research and zoo facilities usually source appropriately treated monkey chow and water, but untreated foods such as fresh fruits and vegetables are also commonly distributed for enrichment. Just as enteric disease outbreaks are frequently foodborne among humans, such outbreaks with resistant bacteria are also possible among monkeys. Monkeys can frequently be asymptomatically colonized with AMR bacteria, and their introduction to naïve groups could result in the dissemination of resistant bacteria, even after appropriate quarantine. Monkey movement between established groups within an institutional colony may also result in the spread of AMR bacteria or the genetic elements of resistance. Group histories of AMR should be considered since monkeys from groups with historically high prevalences of AMR might impact the AMR status of another group. And finally, we cannot eliminate the possibility of captive monkey-wildlife interaction, especially in outdoor colonies. Wild birds and rodents

are commonly infected with AMR strains of *E. coli*, *Salmonella*, and *Yersinia* and can be sources of infection for our monkeys.

7.5.4 Dissemination of Antimicrobial Resistance

Once introduced, resistant bacteria can spread rapidly within a monkey colony. The facility design and maintenance in part determines the ability of AMR bacteria to spread within the environment, between monkeys, and between monkeys and humans. Animal density can promote the spread of AMR bacteria, with greater densities, larger bacterial loads, and more frequent animal-human contact fostering an environment for greater transmission. For instance, livestock operations with larger numbers and/or density of animals typically have higher prevalences of *Salmonella.* Environmental management can help reduce the spread of AMR bacteria with appropriate disinfection, facility design, room order, and general biosafety. This includes appropriate personal protective equipment (PPE) for personnel to minimize zoonotic and anthropozoonotic transmission, as well as transmission of AMR bacteria between monkey groups via fomites and human movement. However, PPE alone is insufficient; good biosafety via PPE requires training of veterinary, husbandry, and laboratory staff and ongoing compliance on best PPE practices.

7.6 Detecting Antimicrobial Resistance

The veterinarian's decision of when to test for AMR, which test method to utilize, and which antimicrobials to test for resistance all can critically affect the monkey's welfare, persons in contact with the monkey, and environment via dissemination of AMR genes. With the varying advantages and disadvantages between tests, susceptibility testing depends on (1) the bacterial species, (2) the range of antimicrobials to be tested, (3) test availability, (4) cost, and (5) time until results. Susceptibility testing is performed on a case-by-case basis, and there is no formula to specify a veterinarians' decision of when to request susceptibility tests. But these tests take time, and delaying such tests can negatively impact the timely initiation of effective therapy. Therefore, it is prudent to collect a culture for susceptibility testing prior to the onset of antimicrobial therapy.

For veterinarians, several effective testing methods exist. First, the broth dilution test leads to reproducible, convenient, and quantifiable results (i.e., mean inhibitory concentration or MIC) using liquid growth medium containing multiple-folds of antimicrobial concentrations (Jorgensen and Ferraro 2009). Second, the antimicrobial gradient method uses thin plastic strips imbedded with a gradient of antimicrobials (Jorgensen and Ferraro 2009). After incubation, an MIC, which is the lowest concentration of antimicrobial that prevents bacterial growth, is determined via visual inspection of the growth inhibition area with indicators on the test strip

(Jorgensen and Ferraro 2009). The Clinical and Laboratory Standards Institute provides breakpoints for interpretation and categorization of MICs (i.e., susceptible, intermediate, or resistant), but the breakpoints often are not veterinary-specific. Nonetheless, isolates classified as intermediate or resistant typically contain the genetic elements for resistance. The MICs also allow primate veterinarians to determine if the MIC is likely to be achieved at the sight of infection and in light of the reported pharmacokinetics of the administered antimicrobial. Third, the disk diffusion test also has standardized breakpoints for resistance created by the Clinical and Laboratory Standards Institute and utilizes paper antimicrobial disks placed on top of agar plates, producing a qualitative result (e.g., susceptible, intermediate, or resistant) determined via visual inspection of a zone of growth inhibition (Jorgensen and Ferraro 2009). Finally, multiple automated instrument systems are also available that can generate rapid susceptibility test results (Jorgensen and Ferraro 2009).

However, next-generation sequencing and genotypic identification of AMR are now available and may eventually become the primary diagnostic techniques to detect AMR. This is largely due to the increasing catalogue of known resistance genes, the widespread availability of high-throughput techniques for gene sequencing, and the rapidly decreasing costs for whole-genome sequencing. This is especially helpful to circumvent the problem of non-culturable or poorly cultured bacteria (Crofts et al. 2017). Furthermore, the development of user-friendly, web browser-based tools for genome assembly and annotation has made the technology approachable for clinicians. For many foodborne and zoonotic pathogens, such as *Campylobacter, Escherichia coli,* and *Salmonella,* whole-genome sequencing can accurately predict the AMR phenotype (McDermott et al. 2016; Tyson et al. 2015; Zhao et al. 2015). But, these next-generation techniques may occasionally provide false predictors that inaccurately predict AMR. For instance, the alteration of antimicrobial targets by bacterial species such as *Mycobacterium tuberculosis* may not be identified in functional metagenomic screens. But the growing AMR gene databases that provide biochemical, phenotypic, and functional validation, combined with advances in probabilistic annotation algorithms, will increase these methods' accuracies and reliabilities. Even with some of the limitations currently observed, these next-generation approaches have led to the discovery of novel AMR genes, advanced AMR detection by target modification, and are likely to become primary tools for clinicians and researchers to identify AMR determinants in the near future (Crofts et al. 2017).

7.7 Comparative Impacts and Prevalence of Antimicrobial Resistance

It is recommended to treat human and animal patients via targeted antimicrobial therapy based on susceptibility test results, but empiric therapy, prior to microbial test results, is often necessary. In such cases, it is appropriate to initiate antimicrobial

therapy based on a known institutional prevalence of AMR, but such information is often insufficient due to infrequent microbial isolation or susceptibility testing. Thus, AMR prevalence data published in literature offers reference points for empiric antimicrobial therapy. Below are published AMR data of zoonotic bacteria frequently infecting monkeys in biomedical research institutions and zoological gardens.

7.7.1 Campylobacter

Campylobacter jejuni and *C. coli* are some of the most frequent causes of illness among monkeys. Campylobacteriosis usually presents as watery diarrhea, but mucohemorrhagic diarrhea can also occur (Fox et al. 2015; Paul et al. 2014). Asymptomatic carriers and reinfection are common (Fox et al. 2015; Russell et al. 1987), which likely leads to the observed increases in prevalences with time in enzootic colonies (Fox et al. 2015). This increasing prevalence poses a great challenge for veterinarians with not only a potential increasing incidence of campylobacteriosis, but also an increased prevalence can create a more suitable environment for the dissemination of AMR.

A review of AMR of zoonotic bacteria in biomedical research monkeys showed that AMR was highest against nalidixic acid, tetracycline, and cephalothin (Kim et al. 2018). Since macrolides appear to be popular choices for antimicrobial therapy, azithromycin, erythromycin, and tylosin are recommended first-line antimicrobials for treating campylobacteriosis (Kim et al. 2017a), which are all WHO critically important antimicrobials. Although macrolides may be appropriate for empiric therapy, AMR has been noted (Tribe and Fleming 1983), and cross-resistance within the antimicrobial class does occur (Carattoli 2001; Klein et al. 2008). Little documentation of AMR in zoo monkeys has been published, but Stirling et al. reported that *C. jejuni*, *C. coli*, and *C. lari* were isolated from their monkeys (Stirling et al. 2008). All of the *C. jejuni* and *C. coli* isolates were resistant to penicillin and cephalexin (100%, 10/10), and the *C. lari* isolate was resistant to ciprofloxacin and nalidixic acid (100%, 1/1) (Stirling et al. 2008). And although most of the *Campylobacter* isolates were cultured from monkeys, the authors did not specify the animal origins of the resistant isolates (Stirling et al. 2008).

Campylobacter threatens humans similar to monkeys. *Campylobacter* is considered to be the world's greatest cause of human gastroenteritis (World Health Organization 2016). Although most infections are foodborne, exposure can also occur via horizontal transmission, albeit rare (CDC 2014), and direct contact with animals (WHO 2012). Like monkeys in biomedical research, high percentages of *C. jejuni* ($n = 1251$) and *C. coli* ($n = 146$) isolates within the United States were resistant to nalidixic acid (26.5%–35.6%, respectively) and tetracycline (48.6%–50.0%, respectively), in addition to ciprofloxacin (26.7%–35.6%, respectively) (Centers for Disease Control and Prevention 2016). This is similar globally with

rising AMR to tetracycline and fluoroquinolones (WHO 2012), ciprofloxacin being the primary antimicrobial to treat human campylobacteriosis (CDC 2014).

7.7.2 Escherichia coli

Escherichia coli is a common commensal inhabitant of the gastrointestinal tract of monkeys and humans (Bailey and Mansfield 2010; Clayton et al. 2014; Kaper et al. 2004), contributing to the gastrointestinal tract health via competitive exclusion and preventing the establishment of pathogenic organisms (Clayton et al. 2014; Kaper et al 2004). *E. coli* infections in monkeys can cause urinary tract infections, sepsis, and enteric disease (Kaper et al. 2004; Nataro and Kaper 1998). Enteric pathotypes of *E. coli* include Shiga toxin-producing *E. coli*, Enterotoxigenic *E. coli*, Enterohemorrhagic *E. coli*, Enteropathogenic *E. coli*, Enteroaggregative *E. coli*, Enteroinvasive *E. coli*, and Diffusely Adherent *E. coli* (Bailey and Mansfield 2010; Kaper et al. 2004; Kolappaswamy et al. 2014; Torres et al. 2005). Among these pathotypes, Enteropathogenic *E. coli* is the most frequent cause of diarrhea in monkeys (Carvalho et al. 2003; Mansfield et al. 2001).

Monkeys with *E. coli* infection have been described, but specific reports of AMR are minimal. Among zoo monkeys, a study was conducted at the Como Zoo in 2009 where the investigators characterized *E. coli* isolates in eight healthy monkeys (Clayton et al. 2014). *E. coli* isolates from the De Brazza's monkeys (*Cercopithecus neglectus*) had a 33.3% prevalence of ampicillin resistance, which was the highest prevalence of the eight monkey species tested. *E. coli* recovered from emperor tamarins (*Saguinus imperator*) had a 41.9% prevalence of tetracycline resistance (Clayton et al. 2014). Wild howler (*Alouatta palliata*) and spider (*Ateles geoffroyi*) monkeys have also been reported with AMR *E. coli* infections, but the authors only assessed AMR to "generic" *E. coli* (Cristóbal-Azkarate et al. 2014), which serves as an indicator organism to measure overall AMR within populations.

AMR *E. coli* also significantly affects human health and as a result is considered a serious threat by the CDC (Centers for Disease Control and Prevention 2013). A 2011 study analyzed the prevalence of AMR in *E. coli* from asymptomatic humans and those hospitalized for diarrhea, although the pathotype was not specified (Cho et al. 2011). This study found less than 10% ($n = 428$) resistance to imipenem, cefotetan, aztreonam, cefepime, cefoxitin, amikacin, and netilmicin (Cho et al. 2011). More than 65% of *E. coli* isolates were resistant to tetracycline and ampicillin (Cho et al. 2011). This study also determined that fecal isolates from healthy (84%, 181/216) and clinically affected (78.8%, 167/212) were multi-resistant to two or more antimicrobial agents (Cho et al. 2011). Comparative to Clayton et al.'s study of AMR in monkeys at the Como Zoo, certain species of monkeys also exhibited a higher prevalence of ampicillin and tetracycline resistance. Another study analyzed 170 fecal samples from children less than 5 years old presenting with diarrhea (Uma et al. 2009). *E. coli* strains ($n = 105$) were isolated, and all isolates were resistant to ampicillin, imipenem, and co-trimoxazole (Uma et al. 2009). Interestingly however,

E. coli AMR to cephalosporin (e.g., ceftriaxone) is the biggest concern among humans and food animals (Centers for Disease Control and Prevention 2013; US Food and Drug Administration 2015).

7.7.3 Mycobacterium

Mycobacterium tuberculosis complex (MTC) consists of *M. tuberculosis* famously causing tuberculosis (TB) in humans, in addition to *M. bovis*, *M. africanum*, *M. canetti*, and *M. microti* (Chege et al. 2008; Coscolla et al. 2013; Rosenbaum et al. 2015; Wachtman et al. 2011; Wilbur et al. 2012). *Mycobacterium tuberculosis* and *M. bovis* are the most concerning species in monkeys (see Chap. 4), with *M. bovis* being the most frequent cause of TB (Bailey and Mansfield 2010; Chege et al. 2008; Choi et al. 2016; Panarella and Bimes 2010; Wachtman et al. 2011). Other atypical species have also been reported in NHP including *M. avium*, *M. intracellulare*, *M. paraffinicum*, and *M. kansasii* (Bailey and Mansfield 2010; Chege et al. 2008; Panarella and Bimes 2010; Parsons et al. 2010). Although TB has been reported in monkeys, there are no reports of isolated *Mycobacterium* that have been tested for AMR. Nonetheless, TB is an important topic to discuss due to its significant comparative impacts across monkey and human health (see Chap. 4).

The first documented case of *M. paraffinicum* in a cynomolgus monkey was reported in 2010 (Panarella and Bimes 2010). Payne et al. reported a unique case of *M. tuberculosis* in a rhesus monkey (*Macaca mulatta*) from a closed colony, after testing negative for 17 years while at the facility, although the source of introduction was unknown. In a separate study, Renner et al. detected *M. bovis* from post-mortem tissue analysis in a single chimpanzee (*Pan troglodytes*) and several rhesus and stump-tailed macaques (*Macaca arctoides*) and further isolated *M. avium-intracellulare* from a single rhesus macaque (Renner and Bartholomew 1974). The detection of *Mycobacterium* has also been reported in wild NHP. Coscolla et al. diagnosed *M. tuberculosis* complex from hepatic and splenic nodules of a wild chimpanzee found dead in Africa (Coscolla et al. 2013). *M. genavense* was recently diagnosed in a 25-year-old Diana monkey (*Cercopithecus diana*) (Kelly et al. 2015). Moreover, Chege et al. diagnosed *M. tuberculosis* and *M. intracellulare* in wild-caught chacma baboons (*Papio ursinus*) (Chege et al. 2008). A *M. tuberculosis* outbreak was also reported in 11 of 91 (12.1%) chacma baboons (Chege et al. 2008; Fourie and Odendaal 1983).

A study conducted by Rosenbaum et al. detected MTC in several species of New World monkeys from markets, pets, sanctuaries, and zoos in Peru (Rosenbaum et al. 2015). Separate cases of TB were reported in squirrel monkeys (*Saimiri sciureus*) (Kaufmann et al. 1975; Leathers and Hamm 1976) and an owl monkey (*Aotus trivirgatus*) (Snyder et al. 1970). Wachtman et al. diagnosed *M. gordonae* infection via fecal PCR in a common marmoset. *M. kansasii* was also cultured from granulomatous lesions from this animal (Wachtman et al. 2011).

Mycobacterium has historically, and still is, one of the greatest causes of human morbidity and mortality worldwide (Centers for Disease Control and Prevention 2013), but out of all bacterial species infecting humans, *M. bovis* has been reported to cause the highest number of human deaths (O'Toole 2010). Among the mycobacterial species, multi-drug-resistant *M. tuberculosis* (MDR-TB), defined as resistance to at least rifampin and isoniazid, poses the greatest public health threat. The WHO estimates 480,000 infections MDR-TB globally, 123,000 confirmed cases, and of those 111,000 cases requiring second-line antimicrobials (World Health Organization n.d.). MDR-TB can be secondary to (1) acquired resistance such as mutation or gene transfer, (2) intrinsic resistance such as passive or specialized resistance, and (3) epigenetic drug tolerance that can be seen with latent or relapsed TB cases (Gillespie 2002; Smith et al. 2013; Zhang and Yew 2015). TB has been a significant challenge because 20.5% of previously treated patients and 3.5% of new patients were diagnosed with MDR-TB (World Health Organization Global Tuberculosis Report 2014). With that said, one of the strongest global initiatives combatting AMR is against MDR-TB, with the Global Project on Anti-TB Drug Resistance Surveillance being one of the oldest and largest surveillance systems used to monitor AMR (World Health Organization 2017). This surveillance system now has data on more than 95% of all TB cases worldwide (World Health Organization 2017).

7.7.4 Salmonella

Salmonella nomenclature is extensive and beyond the scope of this chapter, but the most common subspecies that infects both monkeys and humans is *S. enterica* serotypes Typhimurium and Choleraesuis. While *Salmonella* is a naturally occurring bacterial pathogen in many monkey species (Fox 1975; McClure 1986), recent reports within established monkey colonies are rare (Abee et al. 2012). Colonization in any NHP is rarely associated with disease (Abee et al. 2012; Good et al. 1969; McClure 1986) but has been described to range from asymptomatic infections to watery to mucoid or hemorrhagic diarrhea, pyrexia, and extraintestinal infections including neonatal septicemia, abortion, osteomyelitis, pyelonephritis, and gluteal abscess (Abee et al. 2012; Good et al. 1969; McClure 1986). A single report exists of zoonotic transmission of both multi-drug-resistant and multi-drug-susceptible serotypes of *Salmonella* spp. from an asymptomatic pet spider monkey to its owner, resulting in serious illness, suggesting that monkeys and humans alike may be colonized by serotypes with differing AMR patterns (Fox 1975). While there is a paucity of reports of AMR *Salmonella* occurring in monkeys, the potential exists, and treatment is generally reserved for severe clinical cases so as to avoid the development of a carrier state or additional AMR; alternatively, given the zoonotic potential, humane euthanasia may be elected (Abee et al. 2012).

The widespread emergence of *S. enterica* serotype Typhimurium DT104 (resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline) has led to one of the most significant zoonotic threats to both human and animal

health worldwide (Leekitcharoenphon et al. 2016; Mather et al. 2013). For other strains of *Salmonella*, the therapeutic and nontherapeutic use of antimicrobials in food animals has been linked to the emergence of AMR (Cohen and Tauxe 1986; Glynn et al. 1998; Levy et al. 1976; O'Brien et al. 1982). Many *S. enterica* strains disseminate AMR via DNA conjugation in the form of plasmid exchange (Abee et al. 2012; Carattoli 2003).

7.7.5 Shigella

Shigella is similar to *Campylobacter* in that it is one of the greatest causes of illness among monkeys (Fox et al. 2015). Many species, including *S. sonnei, S. boydii, S. schmitz,* and *S. dysenteriae,* have been reported to infect monkeys, but *S. flexneri* is the most common species, including serotypes 1a, 2a, 3, 4, 5, 6, and 16 (Russell and DeTolla 1993). However, *Shigella* is distinct from other zoonotic bacteria discussed in this chapter because its primary reservoir is the human (Bowen 2015; Fox et al. 2015; Hale and Keusch 1996). Monkey exposure to *Shigella* from humans is possible. This is supported by a study that followed 587 wild-caught Malaysian cynomolgus macaques, which all tested negative for *Shigella* upon capture, 13.2% testing positive later by the importer, and eventually 20.1% testing positive upon entering the United States (Mulder 1971). *Shigella* can spread further within colonies with asymptomatic carriers (Banish et al. 1993; Russell and DeTolla 1993), transmitting via the fecal-oral route with frequent reinfection. Shigellosis presents in many forms including bacillary dysentery and mucoid or bloody diarrhea, but a distinguishing sign is gingivitis (McClure et al. 1976).

Enrofloxacin, a 2nd-generation fluoroquinolone, is a frequent first-line antimicrobial for treating shigellosis in monkeys (Kim et al. 2017a). Keeping in mind that enrofloxacin is a WHO critically important antimicrobial, *Shigella* resistance to enrofloxacin, or its metabolite ciprofloxacin, has not been reported among monkeys, at least at the time of this book's publication. Nonetheless, AMR has frequently been reported to other antimicrobials, with the highest prevalences among *S. flexneri* seen to chloramphenicol, tetracyclines (tetracycline, chlortetracycline, oxytetracycline, doxycycline), aminoglycosides (dihydrostreptomycin, streptomycin, kanamycin, gentamicin), penicillins (ampicillin, amoxicillin), macrolides (erythromycin), sulfonamides (sulfonamide-trimethoprim), and nitrofurans (furazolidone) (Kim et al. 2017a). In addition to *S. flexneri*, similar AMR reports of *S. sonnei* have been published. High prevalence of AMR among *S. sonnei* isolates was observed to tetracyclines (tetracycline, chlortetracycline, oxytetracycline), aminoglycosides (streptomycin), penicillins (penicillin, ampicillin), and macrolides (erythromycin, tylosin) (Cooper and Needham 1976).

It is reasonable to assume that zoological populations of monkeys are equally susceptible to AMR *Shigella*, even though little literature has been published. But the occupational risks of *Shigella* are evident in both zoo and biomedical research settings. During an outbreak of *S. flexneri* among barbary macaques (*Macaca*

sylvanus) and orangutans (*Pongo pygmaeus*), a Vienna Zoo staff member was exposed and became ill in 2004, with laboratory confirmation of identical strains (Lederer et al. 2005). AMR was evident against ampicillin, co-amoxiclav, chloramphenicol, streptomycin, sulfonamides, tetracycline, trimethoprim, and co-trimoxazole (Lederer et al. 2005). Moreover, three employees became ill after laboratory confirmed exposure to *S. flexneri* from research cynomolgus macaques, with two additional employee illnesses from suspected exposure (Kennedy et al. 1993). Clearly, the occupational risks of *Shigella* circulating among monkeys should not be ignored.

AMR *Shigella* among humans is also a significant concern, causing 27,000 drug-resistant infections and 40 deaths per year in the United States alone, but the threat of *Shigella* is not the same worldwide. *S. flexneri* is a greater problem in developing countries, while *S. sonnei* is a greater threat in developed countries. Like many bacteria, the evolution of AMR of *Shigella* varied geographically. In the United States, sulfonamides were first used for therapy in 1945 (Hardy 1945), with tetracycline becoming equally common by 1965. But by 1967, AMR was highly prevalent, and ampicillin became the primary antimicrobial of choice, but ampicillin AMR jumped from 5% to 95% from 1964 to 1971 in Washington, D.C. (Ross et al. 1972). The same trend appeared with trimethoprim-sulfamethoxazole. Clearly, high levels of AMR have been evident in both humans and monkeys and to similar antimicrobials. The primary concern of this similarity is that if zoonotic transmission occurs, many of the antimicrobials commonly used to treat human shigellosis may not be effective. The AMR similarities also encourage veterinarians to inform physicians that immediate culture and susceptibility tests are recommended in the event of zoonotic exposure.

7.7.6 Staphylococcus

Staphylococcus species are ubiquitous in the environment and are common inhabitants of monkey and human upper respiratory tract and skin microbiome. They have been hypothesized to aid in maintaining normal skin pH and host defense by reducing colonization of pathogenic bacteria (Abee et al. 2012; Omenn 2010). But in addition, these bacterial species have caused opportunistic infections, with one of the more commonly involved species being *Staphylococcus aureus*, both historically and as a current public health concern.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is now infamously a public health concern, but MRSA is not exclusive to humans and has also been reported in monkeys. Initial surveys documented the prevalence of MRSA in captive monkey research colonies and described the potential and in some cases real zoonotic and anthropozoonotic risk of MRSA transmission and infection. A survey of chimpanzees used in biomedical research in the United States showed a MRSA prevalence of 69%, demonstrating that the prevalence in this colony was similar to a high-risk human population, such as hospitalized patients in long-term care facilities (Hanley

et al. 2015). Paradoxically however, the strain most commonly isolated (USA300/ST8) was most consistent with community-acquired MRSA (Hanley et al. 2015). In a chimpanzee sanctuary in Africa, MRSA was identified in both the human caretakers (33%, 10/30) and chimpanzees (58%, 36/62); among the isolates cultured from chimpanzees, 45% (16/36) were genetically identical to known human isolates (Schaumburg et al. 2012). This suggests that anthropozoonotic transmission secondary to husbandry practices creates a risk of MRSA transmission for wild chimpanzees through re-introduction (Schaumburg et al. 2012).

Macaque monkeys, in particular rhesus macaques, are one of the most commonly used monkey species in biomedical research. Recently, it has been shown that rhesus macaques harbor a novel, rhesus-specific *S. aureus* in their nasal cavity, indicating it as an important reservoir and possible model for MRSA investigation (van den Berg et al. 2011). Despite this data, relatively few MRSA reports exist in the NHP literature. In a single survey, one case of clinical MRSA was incidentally identified, and subsequently, the carrier prevalence was investigated to be 28% (82/292); the origin of MRSA isolates was determined by characterizing two predominant sequence types (ST188 and a novel genotype resembling ST2817) that were not of human origin (Breed et al. 2016). This study revealed that rhesus macaques may have a high carrier prevalence and low infection rate similar to those of humans (Breed et al. 2016). Additionally, there is a reportedly high incidence of MRSA infection associated with implanted devices, such as catheter tract infections (Abee et al. 2012; Taylor and Grady 1998). Adding on to this, one case report exists in the literature of methicillin-resistant non-*Staphylococcus aureus* acute, which described generalized dermatitis in an immunocompromised rhesus macaque (Abee et al. 2012; Kolappaswamy et al. 2008). A different case report describes the isolation of MRSA in a carrier state from the vaginal cavity of a squirrel monkey and highlights the need for biosafety in monkey colonies even in the face of scarce reports (Donato et al. 2017). Lastly, a large survey in three species of macaques (*Macaca mulatta, M. fascicularis,* and *M. nemestrina*) at a US National Primate Research Center showed a 17.6% (105/596) prevalence of MRSA among the monkeys, a 2.5% (2/79) prevalence among human caretakers, and a 3.6% (3/56) prevalence within the facility on composite cultures; these isolates were monkey in origin, showing ST188 and a novel ST3268 strain, which suggests that these strains were unlikely from human-to-monkey transmission, but highlights the risk to personnel (Soge et al. 2016). Despite the relative scarcity of literature regarding MRSA in captive monkeys used for biomedical research, it is likely that the prevalence of MRSA is underreported, possibly due to a reluctance to report infections given the sensitive nature of biomedical research and the high profile of MRSA (Weese 2010). A recent investigation of MRSA eradication in cynomolgus macaques showed successful treatment outcomes using mupirocin (Kim et al. 2017b).

There have been a number of surveys documenting the prevalence of MRSA in humans. It has been estimated that the human colonization rate of *S. aureus* is 37.2% and that approximately 1% of that is MRSA (Nastaly et al. 2010). One cross-sectional study of MRSA colonization in attendees of a veterinary surgeon conference showed a 17.3% (59/341; 53/308 [17%] veterinarians and 6/33 [18%]

technicians) prevalence, suggesting a much higher MRSA colonization rate in veterinary personnel than the general public and that MRSA in a carrier state is likely an occupational risk for veterinary personnel (Burstiner et al. 2010). However, transmission is bidirectional in that veterinary patients may be MRSA carriers of strains from their owners prior to admission in the veterinary clinic, but the prevalence of MRSA in veterinary settings remains to be fully elucidated (Morgan 2008). Coupled with the likelihood of underreported MRSA colonization and infection in monkeys, as well as the known bidirectional transmission of MRSA between humans and animals, these surveys illustrate the zoonotic concern for individuals in close contact with animals, both as pets and in occupational settings such as veterinary hospitals, laboratories, and zoos, especially those that may be or have family members who are immunocompromised.

7.7.7 Streptococcus

While there are a number of primary and opportunistically pathogenic *Streptococcus* spp., the most commonly isolated species in monkeys is *S. pneumoniae* (Bourne 1975; Abee et al. 2012; Fortman et al. 2018; Fox et al. 2015). Although it is not considered normal flora, this organism has been cultured in the nasal cavities and throats of healthy monkeys and humans. It is, however, an important agent of community-acquired pneumonia infections in humans, which can in turn serve as a potential reservoir for infection of many monkey species, the most notable disease presentation being bacterial meningitis (Abee et al. 2012; Good and May 1971). Clinical disease in monkeys can be initiated by stress secondary to shipping, capture, and quarantine, concurrent viral infections, and other causes of immunosuppression, which can complicate the clinical presentation (Abee et al. 2012). Streptococcal disease can vary from upper and lower respiratory disease to septicemia, peritonitis, and/or pleuritis (Abee et al. 2012). Bacterial meningitis is characterized by low morbidity but high mortality and is usually rapidly progressive and can be fatal without prior clinical signs (Abee et al. 2012). For this reason, prompt diagnosis and initiation of treatment is critical, but success is limited likely due to the fulminant nature of the disease. With that said, no AMR has been reported in captive monkeys, in either the research or zoo setting. But the lack of literature does not indicate a lack of AMR in *S. pneumoniae* infecting monkeys, and AMR should warrant caution when handling any monkey infected with a zoonotic pathogen such as *S. pneumoniae*.

AMR of *S. pneumoniae* to optochin was first documented in the laboratory setting in 1912, which may represent the first documented report of resistance to any antimicrobial, and AMR was later observed in humans in 1917 (Klugman 1990). Treatment of *S. pneumoniae* with penicillin was the standard of care, but in humans, risk factors such as prior AMU, young age, and day care attendance further led to the emergence of AMR, which led to the investigation for newer therapies for bacterial meningitis caused by *S. pneumoniae* (Kaplan and Mason 1998). It has been

hypothesized that *S. pneumoniae* AMR was acquired by natural transformation or the direct incorporation and remodeling of DNA, from closely related oral commensal bacteria secondary to the widespread and inappropriate AMU (Mandell et al. 2002). However, it has been demonstrated that *S. pneumoniae* penicillin resistance can be reduced with the restricted use of penicillin in antimicrobial therapy.

With the development and successful campaign of the pneumococcal conjugate vaccine in 2000, the rate of penicillin-resistant *S. pneumoniae* strains in young children and the elderly dropped by 57% from 1999 to 2004 (Kyaw et al. 2006). For organisms that asymptomatically colonize humans and monkeys, such as *S. pneumoniae*, vaccines may reduce the density of microbial populations and, thereby, the opportunities for genetic exchange of AMR genes (Lipsitch and Siber 2016). While AMR of *S. pneumoniae* has not been documented in monkeys, the use of polyvalent vaccinations has reportedly had variable success in outbreaks of *S. pneumoniae* in NHP (Abee et al. 2012). Vaccination may be a useful tool to combat the possibility of emerging AMR in monkeys.

7.7.8 Yersinia

Yersinia is one of the most famous zoonotic pathogens in human history. *Y. pestis* caused the plague, or Black Death, leading to the unfortunate demise of millions of people, but it is no longer as prevalent. Today, *Y. enterocolitica* and *Y. pseudotuberculosis* are more frequent causes of yersiniosis, in both humans and captive monkeys. However, relative to the other zoonotic bacteria discussed in this chapter, *Yersinia* is not isolated as frequently from monkeys. Even though the incidence is not high, it is an important pathogen because yersiniosis can cause acute death in monkeys (Buhles et al. 1981; MacArthur and Wood 1983; Nakamura et al. 2010; Soto et al. 2013), and infection control of *Yersinia* can be quite challenging due to its wildlife reservoirs (birds and rodents) (Mair 1973). *Yersinia* should be suspected when acute death is observed. Because *Yersinia* control is challenging, combating AMR is also challenging. Thus, it is recommended that veterinarians monitor for *Y. enterocolitica* and *Y. pseudotuberculosis* and their potential resistance within captive monkey colonies. However, due to the low morbidity and high mortality, little literature exists investigating its AMR prevalence.

Among monkeys used in biomedical research, AMR *Yersinia* has been reported against sulfonamides (sulfisoxazole), penicillins (penicillin, cloxacillin, amoxicillin-clavulanic acid, ampicillin, oxacillin, amoxicillin), cephalosporins (cefazolin, ceftazidime, cefotaxime, cefepime), nitrofurans (furazolidone), polypeptides (polymyxin B), and aminocoumarins (novobiocin) (Brack and Hosefelder 1992; Bronson et al. 1972; Kim et al. 2017a; Soto et al. 2013; Taffs and Dunn 1983; Zhao et al. 2016). With that said, the sample sizes, time of isolation, and geographic locations are highly variable, making it difficult for any meaningful interpretation of the prevalence of AMR among *Yersinia* in research monkeys. Even less literature of

AMR exists among zoo monkeys, although concern of *Yersinia* risks to monkey health and occupational personnel is equally evident (Iwata et al. 2008; Iwata et al. 2005; Iwata and Hayashidani 2011; Kumar et al. 2013). Aminoglycosides and trimethoprim-sulfamethoxazole are first-line antimicrobials for therapy (Fox et al. 2015), but fluoroquinolones, cephalosporins, and tetracyclines have also been reported to be effective (Fox et al. 2015; Kim et al. 2017a). Furthermore, there does not appear to be significant surveillance of AMR *Yersinia* among humans. However, the One Health impact potential of *Yersinia* is significant, since it is prevalent in wildlife and humans.

7.8 Antimicrobial Stewardship

Antimicrobial misuse and overuse are two principal contributors to the emergence and spread of AMR. Although any use of antimicrobials can impact AMR, antimicrobials are still necessary to improve patient outcomes. Therefore, veterinarians must be good stewards of antimicrobial resources. Antimicrobial stewardship is a coordinated program to promote the appropriate use of antimicrobials, reduce the prevalence of AMR, and ultimately improve patient outcomes. It includes maximizing the effectiveness of therapy by choosing the best antimicrobial, dose, route, and duration and minimizing the unnecessary application of antimicrobials. This requires excellent leadership, accountability, drug expertise, surveillance, reporting, education, and diligent and proactive action (CDC 2017).

An important category of stewardship is appropriate antimicrobial selection, which is a multistep process requiring several considerations. Factors to consider include the monkey species, bacterial etiology, pharmacodynamics, pharmacokinetics, principles of treatment, and cost (Antimicrobial Therapy in Veterinary Medicine- Fourth Edition). Pharmacodynamics such as the mechanism of action (concentration-dependent killing, time-dependent killing, a combination of the two, and bacteriostatic agents) of specific antimicrobials must be considered (Antimicrobial Therapy in Veterinary Medicine- Fourth Edition). It is also important to consider physiological processes such as absorption, distribution, metabolism, and elimination of an antimicrobial because these govern the time course of drug fate following administration and vary by route (Vet Pharmacology and Therapeutics-Riviere 9th edition). Furthermore, mechanisms of cellular membrane transport such as diffusion, filtration, active transport, and pinocytosis can determine the absorption and fate of antimicrobials. Diagnostic assays such as a gram stain, culture, and/or antimicrobial susceptibility testing can be used to inform targeted therapy (HSU-Handbook of Veterinary Pharmacology). All the above inform a veterinarian's decisions on AMU and can improve targeted therapy.

Components of antimicrobial stewardship plans have been reviewed in detail in other texts (Weese et al. 2013). This chapter will briefly review antimicrobial stewardship plans in the context of monkey populations. AMU is an ongoing process because of the complex interplay between antimicrobial and etiologic bacteria, as

well as the selective pressures on the patient commensal microbiome. This requires the clinician to monitor, tailor, and amend therapy via stewardship with continued diagnostics and adjustment of treatment when outcomes are unsuccessful. A recent study revealed that primate veterinarians frequently use WHO "critically important" antimicrobials (enrofloxacin for treating *Shigella* and *Yersinia*; azithromycin, erythromycin, and tylosin for treating *Campylobacter*) (Kim et al. 2017a). While recent data shows low AMR among these bacteria to their respective antimicrobials, primate veterinarians must carefully consider the risk of selective pressures from the use of "critically important" antimicrobials. Clinicians must continue and improve on diligent and planned antimicrobial therapy, in order to maximally combat AMR via antimicrobial stewardship, keeping in mind that AMU is the single most important contributor to the emergence of AMR, in both the community and hospital settings (Leekha et al. 2011).

Although individual veterinarians should practice good antimicrobial stewardship, a coordinated team with a stewardship leader is the best way to combat AMR. This leader can appropriately assign and delegate action, whether that is AMU monitoring, surveillance of resistance, or environmental management. The team should include support from other clinicians and department heads, epidemiologists, laboratory staff, and technicians (CDC 2017). Antimicrobial stewardship in the veterinary profession and within primate facilities may require extra effort from individuals because a single veterinarian may take on multiple roles (e.g., clinician, diagnostician, and pharmacist). Regardless, the foundation of antimicrobial stewardship programs is identical in biomedical research, zoos, and the community, necessitating monitoring antimicrobial prescription, periodic evaluation of AMU and reporting, surveillance of AMR, and monitoring associated health outcomes. The latter is one of the most important measurements because it provides a metric of success of the antimicrobial stewardship program.

Antimicrobial stewardship programs should be tailored to your institution. In contrast to human hospitals (La Plante et al. 2017), little conclusive evidence has been published regarding the impacts of antimicrobial stewardship programs in veterinary medicine. Although more advances have been reported in human medicine, antimicrobial stewardship in veterinary medicine, and medical primatology, is challenging due to the wide species range and off-label AMU. Nevertheless, multifaceted antimicrobial stewardship programs are advancing and worth adhering to. In fact, some have recommended that antimicrobial stewardship programs should be mandatory in human healthcare (Shea and Shaw 2012), and as Weese, Page, and Prescott stated, it is difficult to justify any less in veterinary medicine. And finally, veterinarians have an ethical obligation to participate in some form of antimicrobial stewardship. Veterinarians throughout the world have taken oaths to ensure animal health and protect the public and environment, which are key principals of antimicrobial stewardship.

Even though antimicrobial stewardship focuses on AMU and resistance, it is not independent of environmental influence and anthropozoonoses. Good animal husbandry, disinfection, room order, personal protective equipment, personal hygiene, and engineering controls can greatly reduce the dissemination of AMR bacteria and

genes, minimize transmission and illness, and ultimately reduce the need for antimicrobials and their selective pressures. The importance of hand hygiene cannot be emphasized enough since it dramatically reduces the risk of transmission (Boyce et al. 2002; Hirschmann et al. 2001), even though hand hygiene compliance is ironically poor in some veterinary fields (Shea and Shaw 2012). Good biosafety protocols and infection control are practical and realistic approaches to improve antimicrobial stewardship.

7.9 Conclusions and Recommendations

Antimicrobial resistance is not a topic that has been discussed extensively in medical primatology, but as the threat grows among humans, food animals, and companion animals, the implementation of antimicrobial stewardship programs is important to mitigate the AMR threat to monkeys. It is clear that AMU impacts AMR on a One Health scale, within individual patients, monkey or human, and others exposed to their AMR residues. AMR has hindered monkey and human patient outcomes, and considering the high prevalences of AMR that have been observed among common zoonotic bacteria in monkeys and humans, the AMR crisis cannot be ignored. It is essential that veterinarians make thoroughly informed and thoughtful decisions when prescribing antimicrobials to monkeys, understanding that antimicrobial stewardship is prudent to maximize patient outcomes and minimize potential AMR spread that can negatively impact future patients. And although antimicrobial stewardship is complex, the prevalence of AMR also illustrates that antimicrobial stewardship must become a prioritized program incorporated into all fields of veterinary medicine.

Because antimicrobial stewardship programs have only recently been implemented into veterinary hospitals, there is little literature detailing the success of veterinary antimicrobial stewardship programs and no evidence in medical primatology. However, the reports detailed above of AMR among monkeys to WHO "critically important" and "highly important" antimicrobials illustrate that antimicrobial stewardship programs are absolutely recommended in medical primatology. No two stewardship programs are identical, but the principals remain the same. Successful reports from human medicine and Weese et al.'s *Antimicrobial Stewardship in Animals* can be translated into medical primatology to inform and monitor AMU, track AMR, and ultimately improve patient outcomes. Primate veterinarians have a great responsibility to monkey and public health, and through antimicrobial stewardship programs, veterinarians can simultaneously meet their patients' needs and combat antimicrobial resistance on a global One Health platform.

Glossary

Anthropozoonosis transmissibility of infectious pathogens from human to vertebrate animal. Also known as "reverse zoonosis"

Antimicrobial resistance also known as antibiotic resistance or drug resistance, it is the ability of microbes to resist the effects of drugs

Antimicrobial susceptibility test a diagnostic test measuring the degree of bacterial resistance to antimicrobials

Antimicrobial selective pressure an influence exerted by the presence of an antimicrobial, killing susceptible bacteria and promoting the growth and spread of bacteria resistant to the antimicrobial present

Commensal organism a microbe that benefits from the presence of another organism, which is unaffected by the microbe

Competitive exclusion the elimination of an organism by another that shares identical resources

Empiric antimicrobial therapy initiation of antimicrobial treatment prior to microbial test results

Fomite object or material that can carry infectious pathogens

Horizontal transmission spread of an infectious agent among individuals of the same generation

Mean inhibitory concentration lowest concentration of antimicrobial that prevents bacterial growth

Medical primatology the study of the medical management and care of nonhuman primates with the purpose to maximize their well-being, conserve their species, and research comparative knowledge for human pathology and biology

Nosocomial infection or disease contracted from a hospital setting

Reverse zoonosis see anthropozoonosis

Resistome the collection of all antimicrobial resistance genes and the precursors

Shedding the act of expelling pathogens from the body

Zoonosis transmissibility of infectious pathogens from vertebrate animal to human

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Chapter 8
Low Incidence, High Lethality or Higher Incidence, Lower Lethality: What We Know and Don't Know About Zoonotic *Macacine alphaherpesvirus* 1 (Monkey B Virus)

R. Eberle and Lisa Jones-Engel

Abstract There is a long history of co-evolution between herpesviruses and their natural hosts; however, when interspecific transmission occurs there can be significant morbidity and/or mortality. NHP and humans are infected with their own complement of typically asymptomatic herpesviruses. However, macaques are notorious for one of their herpesviruses (*Macacine alphaherpesvirus* 1; *Cercopithecine herpesvirus* 1; monkey B virus or; BV) which has caused a small number of human deaths. Since it was first identified, only 28 confirmed cases have been described with approximately 75% causing significant neuropathology and/or death. All documented cases of zoonotic BV infection have occurred in the context of exposures to laboratory macaques in either North America or in one case, the UK. Consequently, BV is the most serious zoonotic concern for research, veterinary, and animal care personnel working with macaques. In contrast, there have been no confirmed reports of zoonotic BV infections resulting from contact with wild macaques. The restriction of zoonotic BV infections to persons having contact with captive research monkeys as opposed to wild macaques raises interesting questions regarding the mechanisms underlying zoonotic BV infections. This review summarizes what is currently known and not known about BV and zoonotic infections and possible factors affecting their occurrence.

Keywords Herpes B · *Cercopithecine herpesvirus 1* · *Macacine alphaherpesvirus 1* · BV · Monkey B virus · Alpha-herpesviruses · Macaques · Synanthropy · Zoonotic

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8.1 Herpesviruses of Nonhuman Primates

Herpesviruses are relatively large (150–200 nm) ubiquitous viruses found in a wide variety of species ranging from humans and great apes to reptiles and fish. A number of herpesviruses of both primates and non-primates readily cross the species barrier to infect species they do not naturally infect, often resulting in severe or lethal infections. Humans host eight different herpesviruses classified into three subgroups. The human alpha-herpesviruses include herpes simplex virus types 1 (causing cold sores and encephalitis) and 2 (causing genital herpes and neonatal infections) and varicella-zoster virus (causing chickenpox and shingles). The human beta-herpesviruses include cytomegalovirus (CMV; causing birth defects following in utero infection and serious infections in severely immunosuppressed individuals) and human herpesviruses 6 and 7. The human gamma-herpesviruses include Epstein-Barr virus (causing infectious mononucleosis and associated with Burkett's lymphoma and nasopharyngeal carcinoma) and human herpesvirus 8 (causing Kaposi's sarcoma). Analogues of most of these human viruses have either been isolated from or detected in other primate species, including both apes and monkeys. Several reviews on simian herpesviruses and the diseases they cause are available. (Barry and William Chang 2007; Haberthur and Messaoudi 2013; Muhe and Wang 2015; Eberle and Jones-Engel 2017; Estep et al. 2010).

The potential for zoonotic infections exists for all the herpesviruses of nonhuman primates (NHPs) since these viruses and their hosts are genetically closely related. It is known that NHP CMVs (beta-herpesviruses) and lymphocryptoviruses (gamma-herpesviruses) can infect human cells in vitro (Lilja and Shenk 2008; Swinkels et al. 1984). In one case, the CMV isolate from a brain biopsy of a pediatric encephalopathy patient was found to be more similar to simian CMV than to human CMV (Huang et al. 1978). However, the only actual confirmed case of zoonotic NHP beta-herpesvirus infection is that of a patient that received a baboon liver transplant (Michaels et al. 2001). Infectious baboon CMV was isolated from the patient a few months after receiving the baboon liver. Although there are no reports of zoonotic simian gamma-herpesvirus infections, simian lymphocryptoviruses have been shown to transform human cells in vitro (Ablashi et al. 1979; Rabin et al. 1978), raising the possibility of zoonotic infections.

In stark contrast to the beta- and gamma-herpesviruses, many alpha-herpesviruses readily cross species barriers and produce disease that is frequently severe or even fatal. The alpha-herpesviruses are members of family *Herpesviridae*, subfamily *Alphaherpesvirinae,* but are further divided into genus *Varicellovirus* (the simian & human varicella viruses) and genus *Simplexvirus* (herpes simplex virus and related simian viruses) (see Table 8.1; (https://talk.ictvonline.org)). The alpha-herpesviruses of primates are all closely related to one another, the degree of relatedness between two viruses generally reflecting the phylogenetic relatedness of their host species and thus supporting host-pathogen co-evolution (Davison 2010; McGeoch et al. 2006). As summarized in Table 8.1, alpha-herpesviruses have been isolated from chimpanzees (Luebcke et al. 2006), baboons (Eberle et al. 1995, 1997; Levin et al. 1988;

Table 8.1 Alpha-herpesviruses of human and nonhuman primates

Host species	Commonly used designations	Official ICTV name
Family Herpesviridae, Subfamily Alphaherpesvirinae, Genus *Simplexvirus*		
Human	HSV 1, HSV2	*Human alphaherpesvirus* 1, 2
Chimpanzee	ChHV	*Panine alphaherpesvirus* 3
Macaque	Herpes B, BV	*Macacine alphaherpesvirus* 1
Baboon	HVP2	*Papiine alphaherpesvirus* 2
Vervet	SA8	*Cercopithecine alphaherpesvirus* 2
Langur	HVL	*Langur herpesvirus*[a]
Squirrel monkey	HVS1, SaHV1	*Saimirine alphaherpesvirus* 1
Spider monkey	HVA1, AtHV1	*Ateline alphaherpesvirus* 1
Family Herpesviridae, Subfamily Alphaherpesvirinae, Genus *Varicellovirus*		
Human	VZV	*Human alphaherpesvirus 3*
Macaque	SVV	*Cercopithecine alphaherpesvirus 9*

[a]Not yet officially classified by the International Committee on Taxonomy of Viruses (Anonymous 2011)

Malherbe and Strickland-Cholmley 1969), macaques (Boulter 1975; Carlson et al. 1997; Eberle et al. 2017; Keeble et al. 1958; Melnick and Banker 1954), vervets (Malherbe and Harwin 1958), langurs (Katz et al. 2002b), and several species of South American monkeys (Holmes et al. 1964; Hull 1973; Leib et al. 1987; Melnick et al. 1964). Serological and PCR survey studies suggest the existence of related α-herpesviruses in other NHP species as well (Eberle 1992; Eberle and Hilliard 1989; Emmons et al. 1968; Henkel et al. 2002; Katz et al. 2002a; Kalter and Heberling 1971; Warren et al. 1998; Sakulwira et al. 2002). If herpesviruses have co-evolved with their host species as phylogenetic analyses suggest, all NHP species are likely to have their own unique alpha-herpesviruses that are probably related to some degree to known NHP herpesviruses, both genetically and antigenically.

While herpesviruses usually do not cause serious infections in healthy members of their natural host, cross-species infections can be very different. Transmission of a virus to a non-adapted host that does not result in disease is likely abortive infections where the virus fails to replicate in the host. Such infections are difficult if not impossible to detect and probably represent the vast majority of cross-species virus transmission events. However, some herpesviruses produce serious, even lethal, infections when transmitted to a nonnatural host species. Monkeys and their herpesviruses require important consideration in this respect owing in part to their taxonomic diversity, sheer population numbers, and frequency of contact with humans across of a number of ecological contexts as well as within biomedical and zoological facilities.

8.2 Monkeys and One Health

Free-ranging monkeys occupy a broad range of ecological niches across the globe, from vanishing habitats remote from centers of human habitation to some of the most densely populated urban areas (Jones-Engel et al. 2006). Synanthropic monkeys (those able to thrive in the niches that are created when humans modify the environment) are key although often overlooked players in One Health. The shared evolutionary history between NHPs and humans makes synanthropic monkeys an especially likely source of zoonotic outbreaks. A study by McFarlane et al. (2012) demonstrated that synanthropic species (including primates as well as a number of other mammals) in Asia are 15 times more likely than other wildlife to be the source of an emerging infectious disease. The authors stress that their findings do not imply that the microbial fauna of synanthropic species are more pathogenic but rather that the likelihood for zoonotic transmission corresponds with the increased opportunity for contact and exposure.

Synanthropy requires that monkeys demonstrate behavioral, ecological, and dietary flexibility as they cope with expanding and contracting resources in human-altered environments. Within the Order *Primates* several members of the genera *Macaca*, *Papio*, and *Chlorocebus* are recognized as highly synanthropic. Among these three genera, *Macaca* in particular stands out with the ubiquitous *M. mulatta* (rhesus), *M. fascicularis* (long tailed), *M. radiata* (toque), and *M. fuscata* (Japanese) species being widely distributed across an expansive range in Asia as well as thriving introduced populations of rhesus macaques in North America.

Across their home range hundreds of thousands of wild macaques as well as macaques kept as pets or performing animals come into contact with humans in a number of contexts including in densely populated urban areas like Delhi, India, Hong Kong, Singapore, and Lopburi, Thailand. The most intriguing context for the synanthropic macaques from a disease transmission perspective is the thousands of sacred sites throughout Asia where monkeys exploit the human-altered environment, taking advantage of protected forests, religious food offerings, and visitors who come to absorb the cultural experience as well as to interact with the monkeys. Within this noteworthy context, macaques and tourists from around the world interact with the monkeys and exposure incidents (bites, scratches, and mucosal splashes) are common. Studies have found that between 6% and 40% of visitors to a monkey temple will be bitten. (see Chap. 2 for further discussion and description of the human-monkey interface).

The widespread distribution of synanthropic macaques has certainly played a major part in one other intense human-macaque interface: their use in biomedical research. Since the 1930s millions of macaques, predominately rhesus and long-tailed macaques, have been trapped from the wild or raised in captive breeding facilities in Asia, Europe, and the Americas for use in research as translational models for human diseases. In the USA alone, nearly 75,000 primates (predominately rhesus and long-tailed macaques) were used in research in 2017 (https://www.aphis.usda.gov/animal_welfare/downloads/reports/Annual-Report-Animal-Usage-

by-FY2017.pdf). The most recent data available on importations indicate that over 22,000 long-tailed and rhesus macaques were imported from habitat countries into the USA in 2014 (http://www.ippl.org/gibbon/u-s-primate-import-statistics-2014/). Furthermore, there has since been a steady increase in the number of macaques imported and used annually in biomedical research in the USA (https://www.sciencemag.org/news/2018/11/record-number-monkeys-being-used-us-research).

All macaques can become naturally infected with the enzootic herpesvirus *Macacine alphaherpesvirus* 1 (Herpes B, monkey B virus; BV). However, the two species of macaques that are synanthropic and which are generally used for biomedical research (rhesus and long-tailed macaques) are notorious for the BV they carry. Although the number of documented cases is very small, there are a handful of occupational exposures in North America and the United Kingdom that have resulted in severe and often fatal infections. Misconceptions and misinformation about BV have often resulted in irrational, non-evidence-based working policies resulting in draconian measures taken against populations of macaques around the world. (http://news.bbc.co.uk/2/hi/uk_news/665686.stm).

8.3 Biology of BV in Macaques

The majority of what we know about BV in macaques stems from work done on captive rhesus and long-tailed macaques housed in biomedical research facilities. In its natural macaque host, BV behaves very similar to the herpes simplex viruses in humans. BV is normally transmitted horizontally via direct contact and exchange of bodily secretions (Huff and Barry 2003; Weigler 1992; Keeble 1960; Keeble et al. 1958; Zwartouw et al. 1984). The prevalence of BV infections in macaque populations is generally related to age, with prevalence increasing progressively from infant to juvenile, adolescent, young adult, and mature adult (Andrade et al. 2003; Weigler et al. 1990; Jensen et al. 2004; Kessler and Hilliard 1990; Lee et al. 2007; Lin et al. 2012; Di Giacomo and Shah 1972). Monkeys under the age of 1 year can be infected following intimate contact with infected adults or other infected infants in the troop, usually via the oral route. There is a marked increase in exposure via genital infections as animals become socially and reproductively mature in their prepubescent and pubertal period (2–4 years of age). The prevalence of BV infections in both wild populations and conventional captive breeding colonies ranges from 70% to nearly 100% in adults (Jones-Engel et al. 2006; Andrade et al. 2003; Weigler et al. 1990; Jensen et al. 2004; Kessler and Hilliard 1990; Lee et al. 2007; Lin et al. 2012; Di Giacomo and Shah 1972).

Primary BV infections are usually asymptomatic, although oral or genital lesions are sometimes visible (Huff and Barry 2003; Keeble 1960; Keeble et al. 1958; Weigler 1992; Anderson et al. 1994). As with HSV, such lesions usually resolve spontaneously (Melnick and Banker 1954; Vizoso 1975). However, in rare instances primary infections in infants may become systemic with a fatal outcome (Anderson et al. 1994; Carlson et al. 1997; Daniel et al. 1975; Simon et al. 1993). Following

initial replication in epithelial tissue, BV invades sensory nerve endings and establishes, through intraaxonal centripedal migration to the cell nucleus, a latent infection in sensory nerve cells that serve the site of the primary infection. Latent infections are characterized by a lack of detectable infectious virus, although viral DNA can be detected by PCR in sensory ganglia harboring latent virus.

Latent BV can reactivate from the latent state in response to various stimuli such as stress. Reactivation results in intraaxonal centrifugal transport of virus where it again infects and replicates in epithelial tissue with shedding of infectious virus that can then be transmitted to a susceptible host. Most recurrences are asymptomatic and only few individuals develop recurrent lesions. Healthy macaques without any outward signs of infection can therefore shed BV. The frequency of BV shedding is quite low (2–3%) in captive macaques under typical husbandry conditions (Huff et al. 2003; Weigler et al. 1993; Weir et al. 1993). Stress related to social challenges, transportation, immunosuppression, or a new housing environment have all been associated with reactivation of latent BV (Chellman et al. 1992; Mitsunaga et al. 2007; Zwartouw and Boulter 1984). In seasonal breeding macaque species like rhesus, reactivation, shedding, and transmission of BV occurs primarily during the breeding season (Huff et al. 2003; Weigler et al. 1993; Zwartouw and Boulter 1984; Zwartouw et al. 1984).

8.4 Molecular Biology of BV

Based on the severity of documented zoonotic infections, BV is classified as a Risk Group 4 pathogen, and Biosafety Level 4 facilities are generally required for work with the virus. As a result of these restrictions, comparatively little molecular research has been done on BV. BV is however very closely related to the human HSV2 virus (and somewhat less to HSV1), so a significant amount of what is "known" about BV structure, protein functions, and viral replication has been inferred from what is known about HSV.

BV has the typical virion structure of alpha-herpesviruses with a linear DNA genome of ~155 kbp enclosed within an icosahedral protein capsid that is embedded in an amorphous protein tegument and surrounded by a lipid/protein membrane envelope (Roizman and Pellett 2001; Whitely and Hilliard 2001). BV has a wide host range in vitro, productively infecting most cell lines. The lytic replication cycle of BV is rapid, with extracellular progeny virus appearing about 6–8 h post-infection (Hilliard et al. 1987). BV gene expression follows the immediate early, early, and late (IE, E, and L) HSV gene expression paradigm.

The BV genome has a very high G + C content (~75%) and is similar to that of HSV2 in its genetic organization, with long and short unique regions (U_L & U_S) flanked by long and short repeat regions (R_L & R_S), respectively. An "a" repeat sequence is also present at each end of the genome and in the R_L/R_S junction. This allows the long and short regions to invert relative to one another, resulting in four genomic isomers. Homologues of every HSV gene are present in the same order and

orientation in the BV genome with one exception: BV lacks a homologue of the RL1 (γ34.5) gene. Given the apparent neurovirulence of BV in humans, it is interesting that in HSV, the RL1 gene has been shown to serve several important roles in HSV replication including neurovirulence in mice (Bolovan et al. 1994; Chou and Roizman 1992).

Earlier studies comparing a limited region of the genome from various BV isolates concluded that different macaque species harbor species-specific BV genotypes (Ohsawa et al. 2002 Ohsawa et al. 2014; Smith et al. 1998; Thompson et al. 2000). These genotypes have been recently confirmed based on complete genome sequences of 19 BV isolates from different macaque host species (Eberle et al. 2017).

Genome sequence comparison of BV isolates indicates that both protein coding sequences and miRNAs are highly conserved (Eberle et al. 2017). Among BV isolates from rhesus macaques, DNA sequence identity of coding regions is >99%, while between BV genotypes coding sequence identity drops to ~89%. The most prominent differences are located in the areas of the R_L and R_S repeat regions that are not known to encode either proteins or miRNAs. There are several areas within R_L and R_S where reiterated sequences occur in all BV isolates. Both the repeated sequence and the number of sequence iterations in these areas vary among individual BV isolates and are greater between different genotypes.

In general, molecular mechanisms of BV replication follow that of HSV with homologous genes performing the same function in BV and HSV, although details may vary (Black et al. 2014; Katz et al. 2017; Patrusheva et al. 2016; Perelygina et al. 2002a, 2015). The infection process initiates when the viral envelope contacts a host cell membrane, allowing viral glycoproteins to bind receptors on the cell surface. Attachment is followed by penetration wherein fusion of the viral envelope and the host cell plasma membrane occurs, resulting in the virion nucleocapsid and surrounding tegument entering the cell cytoplasm, and the nucleocapsid then being transported to the cell nucleus. Some viral proteins forming the tegument play a role in immediately altering cellular functions to favor viral replication. This includes such things as degrading cellular mRNAs and preventing innate antiviral responses.

As for HSV, BV has five genes that encode immediate early (IE) proteins. These IE genes are expressed as soon as the viral DNA enters that host cell nucleus and are involved in both modifying the host cell to make it more amenable to viral replication and upregulating expression of viral early (E) genes. Given the important role played by the IE proteins in initiating and driving viral replication, it is interesting that several of the IE genes are among the genes least conserved among different BV genotypes.

Many of the viral early (E) genes are involved in replication of the viral genome. Herpesviruses encode not only multiple subunits of a DNA polymerase but also several other enzymes involved in modification of cellular dNTP pools and DNA replication. Some E genes function to upregulate expression of late (L) genes and/or downregulate IE gene expression. Following replication of the genome, L genes (encoding many of the virion structural proteins) are expressed. While details are not

known, it appears that BV virions are assembled and released from infected cells by the same mechanisms as for HSV (Crump 2018).

Given the conserved structure and function of many BV and HSV proteins, it is not surprising that most BV proteins share antigenic determinants with their HSV counterparts (Eberle et al. 1989; Hilliard et al. 1989; Hilliard et al. 1987; Katz et al. 1986). This cross-reactive antigenicity is important as it complicates the diagnosis of zoonotic BV infection. While the antigenic cross-reactivity of BV and related primate viruses is extensive, there is also antigenic virus-specificity (Katz et al. 2002a; Hilliard et al. 1989; Eberle et al. 1989; Blewett et al. 1996; Cropper et al. 1992; Katz et al. 2017). Some BV glycoproteins are less conserved and are therefore more virus-specific in their antigenicity. While both the gG and gC glycoproteins are largely BV-specific antigens with respect to HSV, they still exhibit antigenic cross-reactivity with the homologous glycoproteins of HVP2 and SA8 (Perelygina et al. 2003a, Perelygina et al. 2005; Slomka et al. 1995; Huemer et al. 2003).

8.5 Zoonotic BV Infections

All of the documented cases of zoonotic BV where the macaque species involved can be determined have resulted following contact with rhesus or long-tailed macaques used in research (Smith et al. 1998). BV was first discovered in 1932 when Dr. W.B. Brebner, a young physician performing poliovirus research, was bitten on the finger by a rhesus macaque that had been imported from India (Gay and Holden 1933; Sabin and Wright 1934; Pimentel 2008). Dr. Brebner developed herpetic lesions on the finger, but rather than remaining localized like herpetic whitlows caused by HSV, the infection progressed to involve the central nervous system (CNS), and he died from an acute ascending myeloencephalitis. A virus initially identified as HSV was isolated, but it was subsequently found to be distinct from HSV and was designated as "the B(rebner) virus" or more commonly herpes/monkey B virus (Gay and Holden 1933; Sabin and Wright 1934).

Twenty-seven additional confirmed cases of human BV infection have occurred sporadically over the ensuing years. The available information is summarized in Table 8.2. Details from these cases are often obscured and/or contradictory in the published literature (Huff and Barry 2003; Palmer 1987; Weigler 1992; Anonymous 1998a; Davidson and Hummeler 1960). During the three decades following the first BV case when hundreds of thousands of macaques were used in developing a polio vaccine, only 18 additional cases of pathogenic BV were reported. Between 1973 and 1986 no cases of pathogenic BV were reported. However, in the 1980s when there was an upsurge in the importation and use of macaques for research (this time to study retrovirus infections), eight cases of zoonotic BV infection were reported. The most recent case of pathogenic BV infection occurred in 1997 at a US primate research facility. Table 8.3 provides information on the handful of large-scale epidemiological studies that have been completed in North America. Since then there have been no other documented cases of pathogenic zoonotic BV infection.

Table 8.2 Confirmed cases of BV infections in humans

Case number	Infection date or earliest publication date	Exposure geography	Exposure context	Exposure source	Exposure type	Exposure body location	Incubation period	Treatment	Survival	Virus isolation location	Virus isolation technique	Virus identification results	Citations
1	1932	New York, USA	Experimental work with poliomyelitis	Healthy rhesus macaque	Bite	Ungloved left ring and little finger	3 days OR 1 day	Iodine, alcohol, prophylactic injection of tetanus antitoxin	15 OR 17 OR 18 days	Blood, lesions, spinal fluid, brain, medulla, spinal cord, spleen, and regional lymph node of patient	Virus cultures, inoculation into animals, cross-neutralization tests	CSF cultures sterile. Successfully introduced symptoms into some animals. Showed characteristic reaction for herpes virus. Cross-neutralization test positive	Sabin and Wright (1934), Gay and Holden (1933), and Davidson and Hummeler (1960)
2	1949	Cincinnati, Ohio	Experimental work with monkeys	Rhesus macaque	Contamination of fresh superficial wound by monkey saliva from a stomach tube	Right index finger	A few days	N/A	Death	Right auxiliary lymph node, CNS, spinal fluid, liver, spleen	Inoculation into animals. Cross-neutralization test	Inoculation of right auxiliary lymph node and CNS successful. No virus found in CSF, liver, spleen. Cross-neutralization positive	Davidson and Hummeler (1960) and Sabin (1949)
3	January 1956	N/A	Animal attendant	Monkey	Scratch	N/A	2 days	N/A	Recovered gradually over 3 years	Serum	Cross-neutralization test	Before illness; high titer against HSV but not BV. After illness: sera neutralized BV	Davidson and Hummeler (1960)
4	March 1957	Philadelphia, Pennsylvania	Animal attendant at a pharmaceutical and biological manufacturer	Monkey	Scratches and bites on many occasions from October 1956–February 1957	Neck, thumb, palm	N/A	Cortisone	30 days	Stool, serum, axillary lymph node, spleen, brain, brain stem	Antibody titers. Cross-neutralization tests	Rise in antibody titer for HSV. Diagnosis with HSV ruled out through cross-neutralization	Davidson and Hummeler (1960) and Hummeler et al. (1959)

(continued)

Table 8.2 (continued)

Case number	Infection date or earliest publication date	Exposure geography	Exposure context	Exposure source	Exposure type	Exposure body location	Incubation period	Treatment	Survival	Virus isolation location	Virus isolation technique	Virus identification results	Citations
5	March 1957	Ottawa, Canada	Veterinarian engaged in control of poliomyelitis vaccines, primarily by inoculation and autopsies of rhesus monkeys	Rhesus macaque	No history of monkey bite or injury	N/A	N/A	N/A	7 days	Brain, cord, CSF, serum, lung, liver, spleen, kidney	Inoculation into animals. Cross-neutralization tests	Inoculation into some animals successful. Cross-neutralization test positive, as it was not neutralized by patients HSV antisera	Davidson and Hummeler (1960) and Nagler and Klotz (1958)
6	April 1957	N/A	Chemist	Skull of rhesus monkey	No apparent/ history of injury	N/A	N/A	N/A	Few days	Brain	Cross-neutralization test	Positive	Davidson and Hummeler (1960)
7	July 1957	N/A	Animal attendant	Rhesus monkey	Bite	Left index finger	< 5 days	Gamma globulin intramuscularly	26 days	Brain	N/A	Positive	Davidson and Hummeler (1960)
8	October 1957	Isleworth, London	Animal attendant	Cynomolgus monkey and rhesus monkey	2 bites	Right hand OR left index finger, third left finger	14 days OR 21 days	Injury made to bleed and washed under running tap, tincture of iodine, gamma-globulin, cortisone injection intravenously and intramuscularly	Recovered, indication that he would be able to resume normal life	Feces, serum	Cross-neutralization test	Rise in titer to BV over course of illness	Davidson and Hummeler (1960), Breen et al. (1958), and Willox (1958)

9	November 1957	Greenfield, Indiana, USA	Animal attendant and assistant in animal tests for diseases and vaccines at Greenfield Laboratories of the Biological Testing Department of Eli Lilly & Co.	Normal monkeys	7 monkey bites, numerous scratches	N/A	> 4 months	N/A	2–3 days	Tissues from various organs, body secretions, brain, spinal cord, CSF	Cultures. Inoculation into animals. Cross-neutralization tests	Cultures positive for brain, spinal cord, and CSF. The cytopathology highly suggests BV. HSV antiserum failed to inhibit agent, whereas BV antiserum successfully inhibited agent at dilutions of 1 in 64	Davidson and Hummeler (1960) and Pierce et al. (1958)
10	January 1958	Allentown, Pennsylvania, USA	Handled monkey renal cell tissue cultures for poliomyelitis vaccine at a tissue culture laboratory	Broken glass bottles containing tissue cultures of monkey renal cells	2 occasions of lacerations, once in November 1957 and once in January 1958	Hands, arms	2 days–2 months	Gamma globulin intramuscularly, poliomyelitis plasma intravenously, penicillin intramuscularly, oral penicillin, sulfa therapy, medication for his cough	11 days	CSF, right hemisphere, brain stem, cord, spleen, axillary lymph node	Inoculation into animals. Cross-neutralization tests	Inoculation into animals and tissues produced BV symptoms. Cross-neutralization tests exclude all but herpes group viruses. Neutralization results highly suggest BV	Davidson and Hummeler (1960) and Hummeler et al. (1959)
11	February 1958	N/A	Animal attendant	Needle used to inject monkeys	Puncture	N/A	5 days	N/A	~1 week	CNS	Cross-neutralization test	Positive	Davidson and Hummeler (1960)
12	March 1958	N/A	Animal attendant	Needle used to inject monkeys, monkey	Scratch by needle and bite by monkey	Scratch on hand. Unknown location for bite.	5 weeks for needle scratch, 4 weeks for monkey bite	N/A	3 days	Medulla, cord, serum	Cross-neutralization test	Before illness: neutralized HSV but not BV. After illness: neutralized BV	Davidson and Hummeler (1960)

(continued)

Table 8.2 (continued)

Case number	Infection date or earliest publication date	Exposure geography	Exposure context	Exposure source	Exposure type	Exposure body location	Incubation period	Treatment	Survival	Virus isolation location	Virus isolation technique	Virus identification results	Citations
13	1958	Sandwich, England	Animal attendant working in the vaccine unit at Pfizer Limited, Sandwich	Rhesus macaque cage	Scratch	Right hand	3 weeks	Inactivated polio vaccine	6 days	CNS, liver, spleen, suprarenal gland, feces	Inoculation into animals and tissues. Cross-neutralization tests	Virus of herpes group was isolated from tissue cultures. Successful introduction of virus into animals Serological tests confirmed identity of BV	Stones (1966)
14	1959	USA	Worked with monkeys	Rhesus macaque	Unclear, possible scratches and/or oral/nasal entry	Possibly on the forearm	N/A	Penicillin, chloramphenicol	38 days	Cord, medulla, brain, serum, blood clot	Inoculation into animals. Cross-neutralization tests	Inoculation into animals produced B symptoms. Cord and medulla positive for BV. Brain, CSF, serum, blood clot negative for BV	Love et al. (1962)
15	1963	N/A	Cared for monkeys	Rhesus macaque and African green monkeys (predominantly the latter)	N/A	N/A	< 50 months (no neutralizing antibody 50 months before symptoms)	N/A	3 years and 4 months	Fluids from vesicle on shoulder, CSF, serum	Culture. Inoculation into animals. Cross-neutralization tests	Herpes-like virus when inoculated into animals. Cross-neutralization tests inconclusive Patient had no neutralizing anti-body 50 months prior to incident	Hull (1973)

16	1965	Bristol, England	Research worker handled escaped monkey	Vervet monkey or Sykes monkeys. Monkeys may have been exposed to BV in Africa	Minor bites	N/A	~ 2 months	N/A	27 days	Serum and throat swabs from vervet and patient. Cerebrum, cerebellum, medulla midbrain, cord, CSF, from patient	Inoculation into animal and tissues.	Vervet had high neutralizing titer for both HSV and BV The patient showed no neutralization of HSV, but high titer for BV, which increased in concentration as the disease progressed No virus from cerebrum, cerebellum, medulla, midbrain, cord, CSF, or throat swabs (both human and monkey)	Sumner-Smith (1966)
17	1970	USA	Helped establish monkey colonies	Monkeys	N/A	N/A	> 10 years	Prednisone therapy, corticosteroids, intravenous gamma globulin, cytosine arabinoside	Remained in institution unable to walk independently or express himself clearly at the time of publication	Skin scrapings from rash in the thoracic region	Inoculation into monkey and human tissues. Cross-neutralization tests	Inoculations produced cytopathic effect suggesting BV. Cross-neutralization tests suggest BV	Fierer and Bazeley (1973)
18	1970	N/A	Research activities without the use of monkeys or monkey tissue culture	No known contact between monkeys housed at his institute or monkey kidney cultures used at his institute (found to be free of herpesvirus)	Unknown, despite numerous investigations	Unknown, despite numerous investigations	Unknown, despite numerous investigations	N/A	2 years after onset, he is alive and residing in a nursing home. Extent of sequelae unknown	Fluid from vesicular lesion on the chest, pre-infection serum, post-infection serum	Inoculation into tissues and animals. Cross-neutralization tests	Inoculation into animals and tissues produced BV symptoms. No antibody titer for BV in pre-infected sera. Rise in titer against BV from 1:32 in onset serum to 1:256 in sample obtained 28 days later	Hull (1973)

(continued)

Table 8.2 (continued)

Case number	Infection date or earliest publication date	Exposure geography	Exposure context	Exposure source	Exposure type	Exposure body location	Incubation period	Treatment	Survival	Virus isolation location	Virus isolation technique	Virus identification results	Citations
19	1973	California, USA	Worked at the California Primate Center at UC Davis	Rhesus macaque	Multiple bites, contact with monkey vomit	Hands, arms	N/A	N/A	Recovered, with left visual impairment	Serum, CSF	Cross-neutralization tests	Positive	Bryan et al. (1975)
20	1987	Pensacola, Florida, USA	Worked at the Navel Aerospace Medical Research Laboratory	Healthy monkey (Monkey Y)	Scratch from wire cage	N/A	17 days	Oral acyclovir, intravenous acyclovir	Recovered, asymptotic while continuing oral acyclovir	Punch biopsy from exposure site	Virus culture. Restriction endonuclease.	Positive culture. Restriction endonuclease identical to Monkey Y	Holmes et al. (1990)
21	1987	Pensacola, Florida, USA	Exposed at home, but worked at the Navel Aerospace Medical Research Laboratory	Human	Applying BV contaminated zinc oxide cream/hydrocortisone cream to an open area of contact dermatitis on her left ring finger and her own lesions	Left ring finger	N/A	Oral acyclovir, intravenous acyclovir	Recovered, asymptotic while continuing oral acyclovir	Punch biopsy from exposure site	Virus culture. Restriction endonuclease	Positive culture. Restriction endonuclease identical to Monkey X	Holmes et al. (1990)
22	1987	Pensacola, Florida, USA	Worked at the Navel Aerospace Medical Research Laboratory	Monkey suffering from severe bilateral conjunctivitis and diarrhea (Monkey X)	Bite	Left thumb	5 days	Intravenous acyclovir, intravenous ganciclovir	6 months	CSF	Virus culture. Restriction endonuclease	Positive culture. Restriction endonuclease identical to Monkey X	Holmes et al. (1990)
23	1987	Pensacola, Florida, USA	Worked at the Navel Aerospace Medical Research Laboratory	Monkey suffering from severe bilateral conjunctivitis and diarrhea (Monkey X)	Bite	Left forearm	5 days	Intravenous acyclovir, intravenous ganciclovir	49 days	Skin biopsy	Virus culture. Restriction endonuclease	Positive culture. Restriction endonuclease identical to Monkey X	Holmes et al. (1990)

24	1989	Michigan, USA	Worked at an animal research facility Same facility as case 25 and 27	Rhesus macaque	Scratches, bite	Right thumb	15 days	Intravenous acyclovir, ganciclovir, oral acyclovir	Recovered, asymptotic	CSV, conjunctival, pharyngeal specimens, skin biopsy from sites of prior monkey bites	Virus culture. Restriction endonuclease. ELISA. Western blot	Cultures positive for skin biopsy and numerous postmortem specimens Serological evidence for BV. Serologic tests for HSV-1, HSV-2 and SA8 were negative	Davenport et al. (1994)
25	1989	Michigan, USA	Worked at an animal research facility Same facility as case 24 and 27	Rhesus macaque	Bites, scratches (many times)	Upper chest (most recent)	1 month	Intravenous acyclovir	12 days	CSV, conjunctival, pharyngeal specimens, skin biopsy from sites of prior monkey bites, multiple additional tissue specimens	Virus culture. Restriction endonuclease. ELISA. Western blot	Serological evidence for BV Serological evidence for BV. Serologic tests for HSV-1, HSV-2 and SA8 were negative	Davenport et al. (1994)
26	1990	USA	Veterinary technician	Rhesus macaque, known to be seronegative for BV 1 month before injury	Needlestick	Finger	N/A	Oral acyclovir, intravenous acyclovir	Survived	Biopsy from injury site, swab specimens of patient conjunctival and buccal mucosa	Culture. Restriction endonuclease	Initial biopsy positive. Second biopsy positive despite acyclovir. Third biopsy negative	Artenstein et al. (1991)
27	1994	Michigan, USA	Worked at an animal research facility Same facility as case 24 and 25	Rhesus macaque	Multiple bites and scratches	N/A	N/A	Intravenous acyclovir, oral acyclovir	Headache resolved in 11 days without treatment. Recovered from ADHD while continuing to take acyclovir	CSF, conjunctival, pharyngeal specimens	Virus culture. Restriction endonuclease. ELISA. Western blot	Serological evidence for BV Serological evidence of HSV-1	Davenport et al. (1994)
28	1998	USA	Worked at a primate center	Rhesus macaque	Mucosal splash	Eye	11 days	Sulfonamide eye drops, intravenous acyclovir, intravenous ganciclovir, plasmapheresis, steroids, foscarnet	42 days	CSF, serum	Cultures. Western blot	Cultures were negative. The 2 WB tests were indeterminant and positive, confirming BV infection	Center for Disease Control and Prevention (CDC) (1998)

Table 8.3 Epidemiological studies of possible BV exposures in North America

Study number	Year of study	Number of people included in study	Exposure geography	Exposure context	Exposure source	Number of subjects reporting types of exposures	Duration of exposure to primates	Virus isolation location	Virus identification technique	Virus identification result	Citations
1	1990	21	Pensacola, Florida, USA	NAMRL, NASP	Monkeys	Bites: 9 subjects (60%) Scratches: 13 subjects (60%)	N/A	Mean: 6.5 years. Median: 3 years. Range: 0–24 years	Virus culture. ELISA using cross absorption against HSV 1 and BV	Subjects Positive: 0	Holmes et al. (1990)
2	1990	132	Pensacola, Florida, USA	Home, public event, NAMRL, hospital	Infected case patients	N/A	N/A	N/A	Virus culture. ELISA using cross absorption against HSV 1 and BV	Subjects Culture positive: 1* Antibody positive: 1* *This patient is case number 21	Holmes et al. (1990)
3	1990	84	Montreal area (9 labs), Quebec City (2 labs)	Biomedical facilities 30 (36%) from 1 lab worked with monkeys positive for BV. 32 (38%) from 7 labs worked with monkeys negative for BV. 22 (26%) worked with monkeys with unknown BV status.	Cynomolgus macaque (366), rhesus macaque (72), Japanese macaque (50), pig-tailed macaque (19), African green monkey (12), squirrel monkey (79)	N/A	N/A	Sera	N/A	Monkeys Positive: 264 out of 598 (5%)	Nguyen and Lalonde (1990)

4	1994	73	Michigan, USA	Animal research facility	Rhesus macaque	Wounds from monkeys and contamination from monkey secretions: 22 subjects (65%)	N/A	Mouths, eyes, serum from 116 employees. Serum from 205 monkeys	Western blot of serum and CSF	Subjects Indeterminant: 13 Monkeys Positive: 132 (64%)	Davenport et al. (1994)
5	1995	321	USA	40 veterinarians (13%), 148 research scientists (46%), 17 animal caretakers (5%), 116 research technicians (36%)	224 cynomolgus macaques or other macaques (70%). 97 other primates (30%).	Total exposures: 166 subjects (52%). Bites: 102 subjects (32%). Scratches: 124 subjects (39%). Mucosal splashes: 45 subjects (14%). Needle sticks: 46 subjects (14%)	Mean: 8.6 years. Median: 5.0 years. Range: < 1–47 years	N/A	ELISA, Western blot	Subjects Positive: 0. Indeterminant: 5 Negative: 475 (99%) HSV positive 205 (63%)	Freifeld et al. (1995)
6	2002	25	Bayamon, Puerto Rico	Emergency personnel responding to a car accident	Rhesus macaque injured by car	External exposure to monkey fluids	N/A	Serum	ELISA, Western blot	Subjects: Positive: 0 Negative: 25 (100%) Monkeys: Positive: 1 (100%)	Jensen et al. (2004)
7	2019	176	Canada	Laboratory workers	Macaques	176 workers reporting 251 exposures	N/A	N/A	N/A	Subjects: Positive: 0 Negative: 176 (100%) Monkeys: N/D	Barkati et al (2019)

Table 8.4 Clinical reports of unconfirmed BV exposures/infections

Year of incident	Exposure geography	Exposure context	Exposure source	Exposure type	Exposure location	Treatment	Virus isolation location	Virus identification technique	Virus identification result	Comments	
2009	Thailand	Tourism at sanctuary where monkeys roam freely and there is frequent contact with humans	Rhesus macaque	Bite	Head	Rabies immunoglobin, human diploid cell rabies vaccine, acyclovir	Serum	ELISA	Negative	N/A	Ritz et al. (2009)
2011	Louisiana, USA (?)	Pet money in backyard	Bonnet macaque	Scratches, puncture wound	Face, scalp, hands	Sodium hypochlorite, saline solution, rabies immunoglobulin, rabies vaccine, tetanus vaccine, tetanus immunoglobulin, intravenous clindamycin, intravenous acyclovir, trimethoprim sulfamethoxazole	Patient wounds, monkey buccal mucosa, monkey eyes, monkey genitalia	Unclear	Negative	N/A	Tregle et al. (2011)
2015	Bali, Indonesia	Vacation	Macaque	Scratches, bites	Shoulder	Rabies vaccine, oral acyclovir	N/A	N/A	N/A	N/A	Johnston et al. (2015)

2019	Monkey forest, Bali, Indonesia	Vacation	Macaque	Penetrating bite	Right shoulder	Oral acyclovir, rabies postexposure immunization (without rabies immunoglobulin), intravenous acyclovir	CSF, blood, lesion swab, saliva	PCR	Negative	Patient showed possible symptoms of BV infection. He recovered from these symptoms 2 months later, still with genital herpes	Kennedy et al. (2019)
2019	Monkey Island, Vietnam	Vacation	Macaque	Penetrating bite	Upper right arm	Rabies vaccine, acyclovir	Blood samples stored	N/A	N/A	N/A	Kennedy et al. (2019)
2019	Barbodo	Vacation	Vervet monkey	Penetrating bite	Upper arm	Tetanus vaccine, oral antibiotics, valaciclovir	Serum, saliva	PCR	Negative	N/A	Kennedy et al. (2019)

There have however been a handful of unconfirmed reports of BV exposures in the clinical literature where tourists have been treated for presumptive exposures but where there was no definitive evidence of actual BV infection (Table 8.4).

Humans most commonly acquire BV by direct contact with infected macaques (bites or scratches) or contaminated materials (e.g., cuts or scratches on caging). Although aerosol infection has been demonstrated in the experimental laboratory setting (Benda and Polomik 1969; Chappell 1960), there is no evidence that zoonoses occur via aerosol transmission of BV. The majority of human BV infections have been associated with bites or scratches from macaques. However, additional modes of transmission have been implicated including splashing of macaque urine into the eye (Anonymous 1998b), injury with a contaminated needle (Artenstein et al. 1991), and contamination of cuts with material from primary macaque cells in the laboratory (Hummeler et al. 1959). There are also documented cases of BV infection that cannot be traced to any previous exposure incident. This may reflect patient recall bias and/or another mechanism at work. It is noteworthy that the documented cases of persons infected with BV have with only one exception been primate veterinarians, laboratory researchers working with macaques, or animal care personnel. In a single case the spouse of a patient contracted the virus by contact with their spouse's lesions (Anonymous 1987; Holmes et al. 1990).

The clinical course of BV infection in humans can vary (Davidson and Hummeler 1960; Whitely and Hilliard 2001). Initial symptoms usually develop within 1–3 weeks of an exposure incident. The nature of initial clinical symptoms can vary and may include nonspecific flu-like symptoms, vesicular herpetic lesions at the site of inoculation, and/or symptoms indicative of involvement of the peripheral and/or central nervous systems. As the infection progresses, clinical symptoms can also vary among individual patients. BV spreads along sensory nerves into the spinal cord and ultimately the brainstem, resulting in a fulminant encephalomyelitis, respiratory failure, and death. Untreated BV infections in humans have a fatality rate of ~75% with many survivors having significant neurologic sequelae. Since zoonotic BV infection involves sensory neurons, the potential for latent BV infection exists in humans (though never specifically documented).

Cases of zoonotic BV infection that occur without any identifiable previous exposure incident shortly before onset and with confirmed BV sero-positivity not only support the notion that reactivation of latent BV in humans occurs but also imply that zoonotic BV infections can be asymptomatic. The only reported serological testing of several hundred persons working in the US primate facilities failed to detect any evidence of asymptomatic BV infections (Freifeld et al. 1995). However, serological testing of persons working in monkey temples of SE Asia where long-tailed macaques abound found that some individuals had higher levels of antibodies directed against HSV-NHP herpesvirus cross-reactive antigens relative to that normally present in HSV infected persons (Eberle and Jones-Engel 2018). This suggests that these individuals at some point were infected with a virus that (like BV) is antigenically related to but distinct from HSV. If so, this would imply that asymptomatic zoonotic BV infections do occur.

To date only a single case of human-to-human BV infection has been documented (Holmes et al. 1990), where a spouse had repeated direct contact with lesions present on a BV patient during the acute disease phase of infection. However, testing of more than 130 other persons who had contact with the four patients in this report (including co-workers and healthcare personnel) failed to detect any additional BV infections, suggesting that the risk of human-to-human transmission of BV in the absence of direct contact is negligible.

The potential for zoonotic BV exposures exists on many fronts. Although specific pathogen-free (SPF) macaque colonies have been derived to make BV-free macaques available for use in biomedical research (Morton et al. 2008; Ward and Hilliard 2002), non-SPF macaques continue to be widely used in research. Many macaques in zoos are not tested to determine their BV status. Monkeys taken as pets may have a lower likelihood than wild monkeys of being infected with BV (since they are often taken in infancy before becoming infected), but many pet macaques are nonetheless BV positive (Greenwood 2002; Ostrowski et al. 1998; Schillaci et al. 2005). The increasing popularity of visiting monkey temples in SE Asia places tourists in direct contact with wild macaques known to be infected with BV, raising concerns regarding potential BV infections in tourists (Engel et al. 2002; Jones-Engel et al. 2006; Ritz et al. 2009; Sha et al. 2009).

8.6 Other Cross-Species BV Infections

BV infections have been reported in a number of non-macaque monkey species. While most of these cases have been lethal infections, some monkeys do survive BV infection. Lethal infections have been reported in DeBrazza's monkeys (*Cercopithecus neglectus*), a patas monkey (*Erythrocebus patas*), and a black and white colobus monkey (*Colobus* spp.) (Loomis et al. 1981; Thompson et al. 2000; Wilson et al. 1990), all of which were housed in zoos. In the case of the DeBrazza's monkeys, the origin of the infection appears to have been lion-tailed macaques (*M. silenus*) housed in an adjacent cage and sharing a common clinical treatment area (Thompson et al. 2000). While BV was detected in seven out of eight DeBrazza's monkeys, only three died. BV has also been reported in brown capuchin monkeys (*Cebus apella*) housed in the same room as BV positive macaques (*M. mulatta, M. arctoides*) (Coulibaly et al. 2004). What makes this case particularly noteworthy is that while five of seven monkeys were serologically positive for BV and all tested PCR positive, none of the monkeys ever showed clinical symptoms of infection. Interestingly, in experimental studies in mice, only BV isolates from rhesus and long-tailed macaques produced lethal infections; BV isolates from pigtail and lion-tailed macaques were able to infect mice and replicate in the nervous system but were not lethal (Eberle et al. 2017). BV infection in non-macaque species does not necessarily match the highly pathogenic and usually fatal reputation of BV in humans.

8.7 Diagnosis of BV Infections

Various test methods have been used over the years to diagnose BV infections in macaques, including virus isolation, virus neutralization, ELISA, and PCR. While PCR is very sensitive and specific, and a number of PCR assays for detection and quantitation of BV have been described (Black and Eberle 1997; Hirano et al. 2002; Hirano et al. 2000; Perelygina et al. 2003b; Scinicariello et al. 1993a; Slomka et al. 1993; Oya et al. 2004), PCR has only limited suitability for in vivo diagnosis of infected monkeys. Using throat swabs or saliva for testing, only macaques actually shedding virus at the time of testing will test positive; latently infected animals not shedding virus will not test positive. PCR can however be used to detect latent BV in sensory ganglia of deceased animals. Consequently, serological testing, by ELISA or fluorescent bead assays, is routinely used to identify infected monkeys (Katz et al. 2012; Yee et al. 2016). However, positive results of serological testing do not indicate if a monkey is shedding the virus, only that the monkey was previously infected with BV or an antigenically related virus.

While serological assays based on BV viral or infected cell antigens are most desirable, production of BV antigen involves significant biohazard concerns. Taking advantage of the close antigenic relationship between BV and other related simian herpesviruses, several assays utilizing HVP2 or SA8 antigen have been developed (Katz et al. 2002b; Ohsawa et al. 1999; Tanaka et al. 2004; Yamamoto et al. 2005; Takano et al. 2001). While HSV1 has also been used as an alternative antigen, it is clear that the sensitivity of HSV1-based assays is not as great as assays using BV or HVP2 antigen (Ohsawa et al. 1999; Katz et al. 2012; Katz et al. 2002b). Western blot is not as amenable to testing numerous sera as other assays but is used for confirmatory testing of positive or suspect serum samples (Ward and Hilliard 2002; Ward et al. 2000; Katz et al. 2012). It should be noted that none of these serological tests (including ELISAs using BV antigen) specifically identifies what virus a positive monkey is actually infected with; it is implicitly assumed that any positive macaques are infected with the macaque virus (BV).

Diagnosis of BV infections in humans is a much more difficult problem (Kalter et al. 1982; Eberle and Black 1999). Serological testing is possible, but these assays rely on detection of antiviral antibodies that do not develop until at least 7–10 days after infection. Antigenic cross-reactivity between BV and HSV represents another major challenge for diagnosis of zoonotic BV infections. Most adult humans are infected with HSV1 and/or HSV2, and anti-HSV antibodies will react with BV antigen to give a positive test result. When BV infects an HSV-immune person, an anamnestic response to shared antigenic determinants occurs, resulting higher levels of antibodies directed against cross-reactive or shared antigens making detection of BV-specific antibodies even more difficult (Eberle and Black 1999; Kalter et al. 1982). Furthermore, potential zoonotic BV patients are often treated with antiviral drugs immediately after a suspected exposure incident. This can impede BV replication, thereby preventing or lessening the intensity of the immune response to BV (Bernstein et al. 1984). Given all these challenges, serologic assays for detection of

zoonotic BV infections must be both sensitive and virus-specific to detect antibodies specifically directed against BV.

Development of serologic assays that can reliably differentiate BV from HSV infections is not a simple problem. One approach used is to pre-adsorb human sera with HSV antigen to remove antibodies that react with HSV antigen prior to testing for anti-BV antibodies (Katz et al. 1986). Although this approach is labor intensive and reduces assay sensitivity, it has been successfully used for many years to diagnose zoonotic BV infections. A more recent approach is utilization of recombinant DNA technology to produce BV antigens (Perelygina et al. 2002a, b, 2005; Tanabayashi et al. 2001; Fujima et al. 2008; Hondo et al. 2005; Katze et al. 2012). Recombinant antigens are safe and economical to produce and can easily be standardized. Since most BV proteins possess at least some cross-reactive epitopes, expression of only part of a BV protein can produce an antigen that is BV-specific. The combined use of several such recombinant BV proteins (glycoproteins gB, gC, gD, and gG) has provided improved sensitivity and specificity for detection of antibodies to BV (Katze et al. 2012; Perelygina et al. 2005; Slomka et al. 1995).

Monoclonal antibodies (mAbs) to BV have been developed with the aim of using them for serological testing (Blewett et al. 1996; Cropper et al. 1992; Katz et al. 2017). The majority of anti-BV mAbs recognize epitopes common to BV and HSV. Although BV-specific mAbs have been isolated, the epitopes they are directed against have not proven to be consistently recognized by all infected macaques, thus limiting their usefulness in diagnostic assays (Blewett et al. 1999; Norcott and Brown 1993; Katz et al. 2017). In one case, a BV-specific mAb to the gB glycoprotein (which is consistently recognized in infected animals) was isolated, but the mAb was not diagnostically useful because its binding was inhibited by cross-reactive antibodies presumably directed against a nearby epitope, resulting in poor binding of the BV-specific mAb and thus false-negative results (Blewett et al. 1999). Other BV mAb have been shown to detect only a few BV genotypes which limits their usefulness for diagnostic testing (Katz et al. 2017). While mAb-based assays do hold promise, more work will be necessary to produce BV-specific mAbs directed against epitopes that are common to all BV strains and which are consistently recognized by infected humans.

PCR testing is very rapid and sensitive and, with the availability of a number of BV genomic sequences, can be made virus-specific. Unlike serological tests, PCR detects the virus itself, obviating the need to wait for development of a host immune response to the virus. Thus, swabs from a bite or scratch wound site can be tested by PCR to detect the presence of BV. In the case of bites or scratches, the monkey responsible for the injury can also be tested to determine if BV is being shed, thereby providing some measure of the likelihood of BV being transmitted to the patient. Many BV PCR assays have been described, and several have been used to diagnose human infections due to BV (Perelygina et al. 2003b; Scinicariello et al. 1993b; Hirano et al. 2000, 2002).

8.8 Perceptions and Misperceptions of Risk

Over the past 85 years, BV has maintained its reputation as having extreme neurovirulence based on 28 published cases involving individuals who have had contact with captive macaques. In these cases the fatality rate is ~75% with most survivors experiencing neurological sequelae (Weigler 1992; Palmer 1987; Davidson and Hummeler 1960; Whitely and Hilliard 2001). But is this reputation really deserved? Consider that HSV causes ~500 cases of encephalitis each year in the USA, and without treatment such infections are also ~70% fatal with only ~5% of patients fully recovering. From this perspective BV seems to be about as neurovirulent as HSV. However, this must be viewed in the context of a much larger pool of humans that are latently infected with HSV and thousands more acquiring their primary HSV infections each year that do not develop encephalitis; it is very unlikely that an equivalent number of latent or primary zoonotic BV cases occur each year.

Interestingly, while only 28 cases of BV have been documented in the literature (several lacking conclusive isolation of BV, and most early cases relying on notoriously nonspecific serologic assays), the number of cases of zoonotic BV is routinely reported in scientific journals and the lay press as 40, 50, or even 60. During the 1940s–1960s, unpublished cases were often cited as "personal communications" with no evidence of virus isolation or serology being given (see Table 8.2). Rather, it seems that if person was working with macaques or macaque tissues (or in some cases just monkeys or monkey tissues) and developed encephalitis, the presumption was that it was due to BV rather than HSV or some other neurotropic virus (Breen et al. 1958). In two cases patients only had contact with vervets (*Cercopithecus aethiops*) yet were reported as BV despite lacking any exposure to macaques (Nsabimana et al. 2008; Palmer 1987).

In 2002 the BV working group reiterated the recommendations of earlier BV working panels that management of persons potentially exposed to BV (e.g., from a bite, scratch, or mucosal splash) includes laboratory testing of their specimens (Cohen et al. 2002). There are three facilities, two in the USA and one in the UK, that are authorized to receive and screen human samples for BV. Unfortunately, statistics on the annual number of exposure incidents investigated or the number of samples screened is not available, nor are the results of the tests performed. Since 1973, there have been only seven documented cases of zoonotic BV infection reported in the scientific literature, while over these 45 years several million macaques have been used in biomedical research in the USA alone. And while requirements for the use of personal protective equipment when working with macaques have grown more stringent over the last four decades, exposures are still routine at research and breeding facilities where macaques are used. Recently Barkati and colleagues published a decision tool they developed for use when evaluating the need for antiviral prophylaxis after a macaque-related injury in research laboratory workers (Barkati et al. 2019).

Virtually every facility in North America and Europe using macaques has a BV infection prevention policy that requires rigorous adherence to institutional standards for safely handling macaques, wearing proper personal protective equipment (e.g., gloves, long sleeves, face and eye protection), and immediate and thorough cleansing of any site exposed to macaque secretions or tissues. Despite the millions of humans who come into contact with synanthropic macaques and their bodily fluids daily throughout Asia, there are no documented cases of zoonotic BV in these contexts. The GeoSentinel Surveillance Network maintains a global network of clinics that report zoonotic exposures. While local inhabitants rarely seek medical treatment following monkey bites, scratches, or mucosal splashes (Jones-Engel personal observation), these clinics are generally used by tourists. In a 2015 survey of 2697 patients in Southeast Asia who sought treatment following an animal bite, 66% reported that their exposures came from monkeys (Gautret et al. 2015). This likely represents only a fraction of the exposures that occur on a daily basis. Chapter 2 provides a detailed discussion of the types of exposures that humans and macaques have when they share the environment, and Chap. 10 characterizes zoonotic transmission of simian foamy virus, another enzootic macaque virus also transmitted by bites and scratches. From a One Health perspective, the rarity of fatal BV infection in humans, its conflicting epidemiological patterns, and the possible lack of transmission in Asia vs. North America and Europe are not supportive for the weight that BV receives in the work safety policies in biomedical research.

8.9 Information Gaps and Why They Matter for One Health

For almost 60 years scientists have pointed out that we do not know enough about BV to draw firm conclusions regarding particulars of zoonotic transmission or disease (Breen et al. 1958). The exact mode(s) of monkey-to-monkey transmission in the wild are not known, and the rate of shedding in captive colonies vs. free-ranging populations is speculative at best. There are very little data on the levels or frequency of infectious virus or concentration of BV genome copies in monkey saliva or genital secretions for captive or free-ranging macaques. Data on interspecific genetic variation among BV strains from captive macaques are limited (i.e., are BV strains that cause zoonotic infections genetically different from most other BV strains?), and there are no data on strain variation among BV isolates from free-ranging vs. captive macaques.

While most zoonotic cases apparently occur following parenteral or mucosal exposures, a significant number of reported BV cases do not have a confirmed route of exposure. Why some individuals get clinically infected and others don't, even though they were exposed and are presumably susceptible, is unknown. In a 1989 case, 21 of the victim's co-workers who reported a bite or scratch by the facility's macaques all tested negative for BV (Anonymous 1989). Could there be

some host-factor(s) that possibly unite the handful of pathogenic BV cases? There is no information on the molecular epidemiology of BV strains that have been associated with zoonotic pathogenicity, nor have there been any systematic molecular comparisons of BV isolates from fatal vs. nonfatal zoonotic cases or BV isolates from monkeys (not human infections). There are also no data regarding BV strains that naturally circulate among free-ranging macaques, making comparison with BV isolates from captive macaques impossible. And perhaps most remarkably, there is only a single study addressing the possibility of subclinical zoonotic BV infections, and that study only tested workers in US primate facilities (Freifeld et al. 1995); no studies of individuals in Asia, even those subject to multiple serious exposure incidents, have been undertaken.

These gaps in our scientific knowledge continue to have a profound impact on macaques (and humans). The fear of BV infection based on its reputation as being extremely neurovirulent has led to the occasional and, one might argue, irrational culling of macaques. For example, in March 2000, shortly after the last known case of pathogenic BV was reported in the scientific literature, rhesus macaques at the Woburn Safari Park in the UK were screened for antibodies to BV (Anon 2000). Not surprisingly, BV reactive antibodies were detected, and managers of the park, acting on recommendations of the Health and Safety Executive and the zoo licensing authority, authorized the extermination of the entire colony of more than 200 monkeys. Similarly, in August 2008, a colony of Tonkean macaques (*M. tonkeana*) at the Louis Pasteur University in Strasbourg, France (known to be BV seropositive and which had been studied without incidents for more than 25 years), was euthanized out of concern that laboratory workers could be infected with BV (Abbott 2008). A recent study funded by PREDICT (USAID Emerging Pandemic Threat Program) screened 392 free-ranging macaques in Malaysia for BV antibody (Lee et al. 2015). The results of this study were used to justify the culling of more than 100,000 long-tailed macaques in Malaysia (https://www.ctvnews.ca/sci-tech/malaysia-s-mass-kill-of-nearly-100-000-macaques-a-year-raises-ire-1.1211405). A recent paper describing BV seroprevalence and shedding in a population of free-ranging macaques that were introduced nearly 70 years ago into Florida concluded that humans are at risk for exposure to this potentially fatal pathogen (Wisely et al. 2018). Fear mongering in the local press quickly followed with articles proclaiming "Killer Herpes from Florida Monkeys Could Pass to Humans Scientists Warn" (Pirani 2018). Obviously, the extreme neurovirulence reputation of BV, whether actually deserved or not, can have immense and costly implications.

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Chapter 9
Morbillivirus Infections in Non-human Primates: From Humans to Monkeys and Back Again

Rory D. de Vries, Thijs Kuiken, and Rik L. de Swart

Abstract Non-human primates (NHP) have played a crucial rule in the history of morbillivirus research. Although NHPs are naturally susceptible to morbillivirus infections, outbreaks are rare among monkeys and apes living in their natural habitat. However, introduction of a highly contagious morbillivirus in a high-density population or captive colony seronegative for morbilliviruses can easily lead to an efficient transmission chain in which all animals become infected. Secondary infections due to morbillivirus-induced immune suppression can subsequently yield complications, leading to outbreaks with high morbidity and mortality. In this chapter, we provide an overview of morbillivirus outbreaks that have occurred in different monkey species, and we discuss morbillivirus epidemiology in different target species. Furthermore, differences in infection course and severity in various species are discussed. In addition to discussing natural infection of NHP with morbilliviruses, this chapter provides an overview of experimental infections of NHP with wildtype or genetically engineered morbilliviruses. These studies have contributed significantly to a more complete understanding of measles pathogenesis.

Keywords Measles virus · Canine distemper virus · Macaques · NHPs

9.1 Introduction

In 1911 measles virus (MV) was identified as the causative agent of measles when inoculation of macaques with filtered respiratory tract secretions from measles patients caused measles-like symptoms in these animals (Goldberger and Anderson 1911). Interestingly, this was well before isolation of the virus in 1954 (Enders and Peebles 1954). Since then, early measles studies in monkeys were performed and reported variable susceptibility of different NHP species to MV infection,

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complicating the initial measles pathogenesis, pathology, and vaccination studies and questioning the utility of NHPs as a model for measles research. Retrospectively, the low susceptibility of some animals to experimental MV infection was likely due to prior exposure to MV-infected humans, resulting in the development of MV-specific antibodies and rendering them immune.

Peebles et al. were the first to demonstrate that healthy laboratory macaques frequently possessed MV-specific antibodies (Peebles et al. 1957). At that time, it was postulated that measles in NHP and humans was the same disease caused by the same agent and that NHPs living in captivity potentially contracted measles by direct contact with MV-infected humans (Peebles et al. 1957). This was suggested by the fact that monkeys captured in the wild rarely had MV-specific antibodies, whereas these were frequently demonstrated in macaques living in captivity (Meyer et al. 1962). It was shown that many NHP species are naturally susceptible to infection with MV as well as other morbilliviruses such as canine distemper virus (CDV) and many outbreaks among captive NHPs have been reported in literature. Several species have since been used to elucidate measles pathogenesis by experimental infections; however, macaques have been studied most extensively and display a similar pathogenesis as observed in measles in humans.

In this chapter, we provide a background on MV and other morbilliviruses that can naturally cause disease in NHPs, discuss morbillivirus epidemiology in different host species, and report on morbillivirus outbreaks that occurred in captive NHPs. Furthermore, we discuss how experimental infections of NHPs helped elucidate mechanisms of MV entry, pathogenesis, transmission, and immune suppression and can be used to evaluate the efficacy and safety of novel generations of measles vaccines. Finally, we offer a discussion on infections of NHPs as an incentive for continued measles vaccination in the future.

9.2 Morbilliviruses

Morbilliviruses are enveloped viruses with a non-segmented negative-sense RNA genome and belong to the family *Paramyxoviridae*. Morbilliviruses are highly infectious, are spread via the respiratory route, cause a profound immune suppression, and have the propensity to cause large disease outbreaks in previously unexposed populations. Secondary opportunistic infections resulting from immune suppression, but also infection of the central nervous system (CNS), can lead to high morbidity and mortality. The genus *Morbillivirus* contains multiple viruses, including MV, rinderpest virus (RPV), peste des petits ruminants virus (PPRV), canine distemper virus (CDV), phocine distemper virus (PDV), and cetacean morbillivirus (CeMV). Each morbillivirus targets a different host species: MV is regarded the prototype morbillivirus and the only virus that normally infects (non-human) primates.

Measles, caused by MV, is a significant cause of childhood morbidity and mortality in humans and is characterized by fever, rash, cough, conjunctivitis, and

a generalized immune suppression (Griffin 2013; Rota et al. 2016). Availability of safe and effective live-attenuated vaccines has led to a substantial reduction in measles morbidity and mortality (Durrheim et al. 2014), with the number of measles fatalities currently at an all-time low (below 100,000 deaths per year, (WHO 2017)). However, reduced vaccine acceptance in the industrialized world and budget deficits to maintain vaccination coverage in developing countries threaten the success of ongoing measles control programs (Strebel et al. 2011; Durrheim and Crowcroft 2017). Distemper, caused by CDV, is mainly described as an infectious disease of dogs and free-ranging carnivores like raccoons, foxes, wolves, and mustelids but has the capacity to infect a wide range of mammalian hosts. CDV infections are associated with high morbidity and mortality (Beineke et al. 2009), since the virus can virtually obliterate all lymphocytes within a host and easily invades the CNS.

Normally, morbilliviruses are restricted to natural infection of their respective host species. This is due to the fact that infection with a morbillivirus is initiated by a specific interaction between the hemagglutinin (H) protein and at least one of two proteinaceous cellular receptors: CD150 (Tatsuo et al. 2000), mainly expressed on subsets of immune cells, and nectin-4 (Noyce et al. 2011; Muhlebach et al. 2011), mainly expressed on the basolateral side (within the adherens junction) of epithelial cells. Phylogenetically, evolution of morbilliviruses largely parallels that of their host species (Visser et al. 1993; Barrett 1999). The different morbilliviruses most likely evolved from a common ancestral virus that adapted to a specific host, proving that morbilliviruses intrinsically have the ability to adapt to novel host species and measles has a zoonotic origin. For example, it has been postulated that RPV or a closely related virus crossed the species barrier from cattle into humans thousands of years ago (Barrett 1999), leading to the emergence of MV. Currently, although CDV is mainly regarded an infectious agent of carnivores, recent spread of CDV into NHPs suggests that this virus has zoonotic potential and could be devastating for humans (Qiu et al. 2011; Sakai et al. 2013a). Receptor adaptation is a crucial step for morbilliviruses like CDV to be able to cross the species barrier and infect a "non-natural" host species.

9.3 Morbillivirus Epidemiology

In general, seronegative NHPs and humans are equally susceptible to morbillivirus infections. However, since morbilliviruses are highly infectious and infections are short-lived, endemic circulation can only be sustained in a target species that lives in high-density populations of sufficient size to allow for a continuous chain of virus transmission. This explains why measles outbreaks occur frequently among morbillivirus-seronegative human populations but are rare among NHPs living in their natural habitat (Meyer et al. 1962). Therefore, while NHPs can readily be infected with a morbillivirus upon exposure to infected humans, and the infection can rapidly spread through a previously unexposed high-density colony, population sizes of NHP species are generally too small for the infection to be maintained.

9.3.1 Measles Outbreaks in Captive NHP Colonies

An initial study performed in the 1960s proved that wild NHPs in their natural habitat were indeed predominantly MV-seronegative. In this study, most NHPs showed clinical signs related to measles rapidly after trapping, and 84% of the animals seroconverted within 14 weeks after capture and transport (Meyer et al. 1962). Measles was globally endemic among humans in the 1960s, probably facilitating rapid virus transmission to monkeys. However, this study demonstrated the natural susceptibility of monkeys to MV infection and the risk of bidirectional virus transmission at the human-monkey interface. Although outbreaks were frequently reported among NHP species thereafter, it was possible, through appropriate biosecurity measures, to keep several closed populations of NHPs free of MV for decades (Andrade et al. 2003).

9.3.1.1 Measles Outbreaks in Rhesus Macaques (*Macaca mulatta*)

An epidemic of measles in rhesus macaques occurred in a shipment of 100 animals from India to the USA in 1966 and was meticulously described. Fifty-eight cases, including one fatal case, were reported in this study (Potkay et al. 1966). A smaller outbreak was reported in 21 rhesus macaques transported from India to the UK, including 5 lethal cases (Remfry 1976), followed by a larger outbreak in 72 imported rhesus macaques with 7 fatalities in 1979 (MacArthur et al. 1979). Diagnosis of measles in these animals was based on clinical signs (appearance of rash [illustrated in Fig. 9.1a, b], loss of appetite, conjunctivitis, respiratory symptoms) combined with the appearance of MV-specific antibodies. In addition, in some cases necropsies were performed and led to the observation of characteristic large multinucleated giant cells (illustrated in Fig. 9.2h) in the lungs (Warthin 1931; Finkeldey 1931), with or without inclusion bodies. The source of MV was not identified in either of these studies. At that time and since then, many other outbreaks of measles in free-ranging and captive rhesus macaques have been reported (Shishido 1966; Hall et al. 1971; Kessler et al. 1989; Willy et al. 1999; Jones-Engel et al. 2006).

9.3.1.2 Measles Outbreaks in Other Macaque Species

In addition to rhesus macaques, measles outbreaks have also been reported in colonies of other macaque species, including cynomolgus macaques (*Macaca fascicularis*) (Willy et al. 1999; Welshman 1989), pig-tailed macaques (*Macaca nemestrina*) (Willy et al. 1999), and Japanese macaques (*Macaca fuscata*) (Choi et al. 1999).

Welshman et al. reported an outbreak of respiratory symptoms in 65 cynomolgus macaques in a colony of 87 animals. Interestingly, only seven of these animals developed a skin rash (illustrated in Fig. 9.1c1, d1). This is in contrast to rhesus

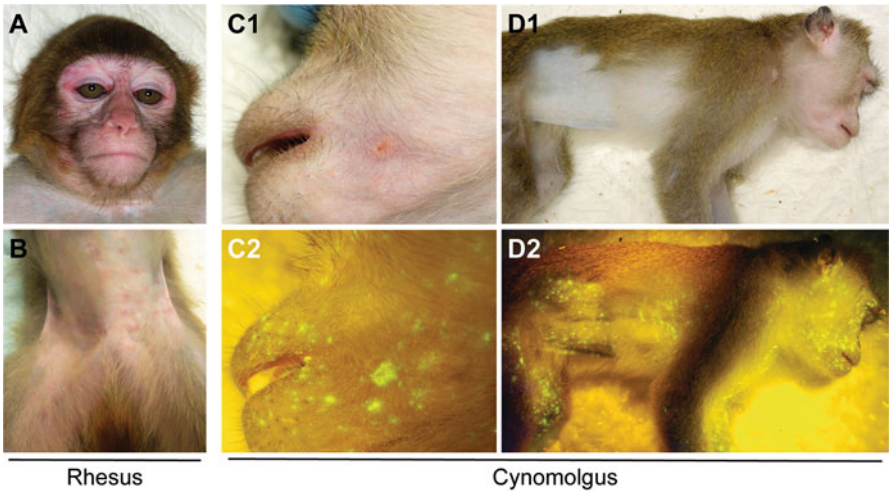

Fig. 9.1 Macroscopic images from macaques experimentally infected with (**a, b**) wild-type MV or a (**c, d**) recombinant MV expressing a fluorescent reporter protein. Figure illustrates rash on the (**a**) face and (**b**) trunk of a rhesus macaque, and less obvious on the (**c1**) face and (**d1**) trunk of a cynomolgus macaque. (**c2** and **d2**) Anatomical locations of MV replication were visualized with a UV lamp when recombinant MV strains expressing a fluorescent reporter protein were used, and these co-localized with the appearance of rash. Panels **a** and **b** are reprinted from El Mubarak et al., J Gen Virol 2007

macaques, which often develop a characteristic rash upon symptomatic MV infection (Auwaerter et al. 1999; El Mubarak et al. 2007). Conjunctivitis was not observed in this outbreak, but all animals in the colony seroconverted to MV (Welshman 1989). In general, clinical signs commonly observed in human measles patients are reproduced more accurately in rhesus macaques, compared to cynomolgus macaques (El Mubarak et al. 2007). In addition to outbreaks in cynomolgus macaque colonies, MV-seropositivity was also shown in so-called performing cynomolgus macaques living at markets in Indonesia, indicating that MV can directly spread from humans to cynomolgus macaques (Schillaci et al. 2006).

There is a particularly well-described measles outbreak among Japanese macaques; it concerns an unvaccinated colony, which lived for 20 years in outdoor exhibits of a Korean zoo before suffering an outbreak with 21% mortality. Although the source of MV was not accurately determined in this outbreak, no new monkeys had been brought into the colony and the colony had no contact with other NHP species. The authors speculate about a MV transmission event from an infected visitor to the colony while the colony was in the outdoor exhibit. Mortality in these macaques was mainly due to complicating secondary infections, in accordance with the immune suppression associated with measles. Diagnosis of measles was made on basis of histopathology, immunohistochemistry, in situ hybridization and electron microscopy, and observation of measles-associated clinical signs (Choi et al. 1999).

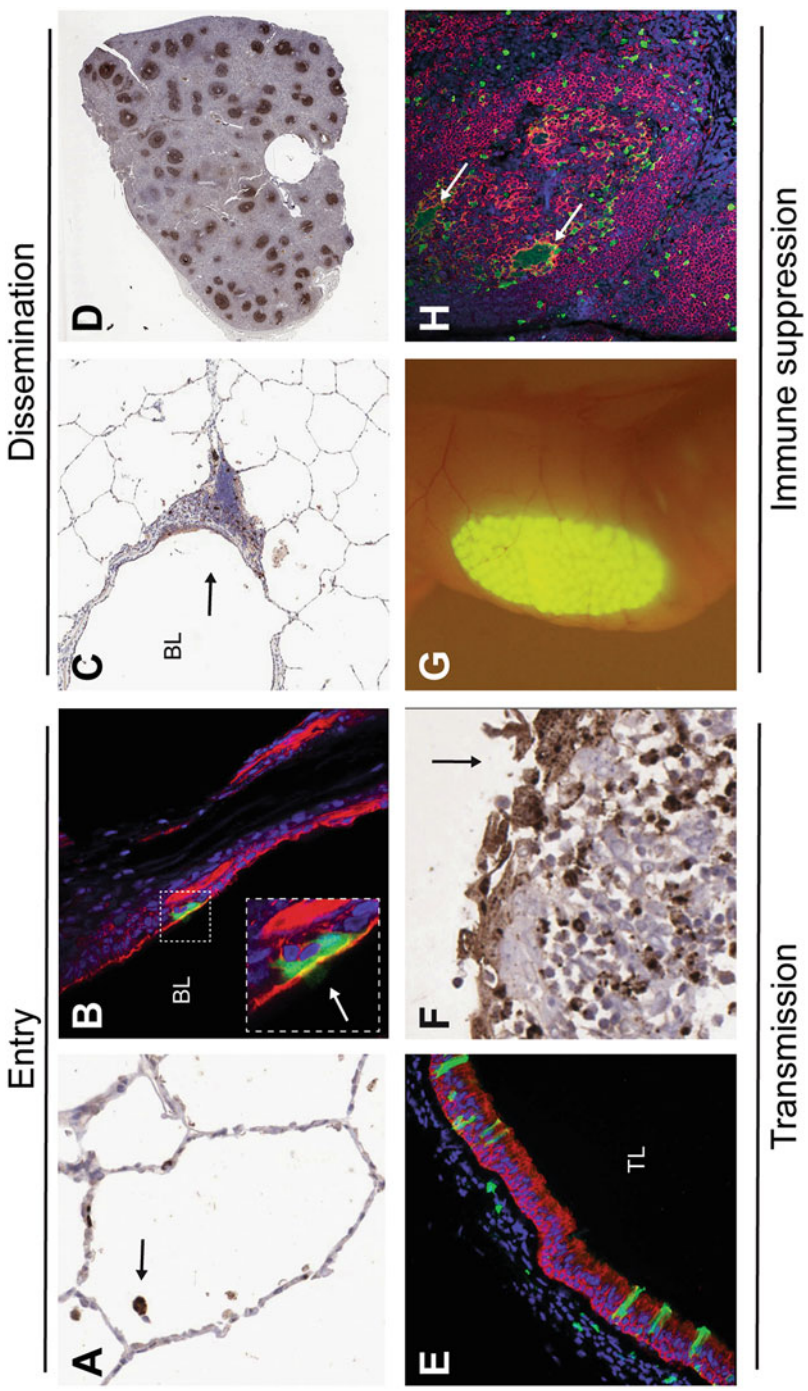

Fig. 9.2 Images collected from experimentally infected NHPs, illustrating mechanisms underlying MV entry (**a**, **b**), dissemination (**c**, **d**), transmission (**e**, **f**), and immune suppression (**g**, **h**). MV-infected cells were detected by immunohistochemical staining (**a**, **c**, **d**, **f**), by immunofluorescent double-staining (**b**, **e**, **h**) or

macroscopic detection (**g**). (**a**) infection of a single cell (arrow, presumably an alveolar macrophage) in the alveolar lumen at 3 days post inoculation (d.p.i.); (**b**) infection of epithelial cells in the trachea at 5 d.p.i. (arrow in insert points at green cilia), green = MV, red = epithelial cells, blue = nuclei; (**c**) infection of myeloid and lymphoid cells in bronchus-associated lymphoid tissue (arrow) at 4 d.p.i., BL = bronchial lumen; (**d**) low-magnification image of a lymph node at 9 d.p.i., with many B-cell follicles containing large concentrations of MV-infected lymphocytes; (**e**) MV-infected epithelial cells in the trachea at 9 d.p.i. (green = MV, red = epithelial cells, blue = nuclei, TL = tracheal lumen); (**f**) Disruption of the epithelium (arrow) of tonsillar tissue containing many MV-infected lymphocytes at 9 d.p.i.; (**g**) MV infection of Peyer's patches in the small intestine at 9 d.p.i.; (**h**) MV-infected B-lymphocytes (including Warthin-Finkeldey syncytia, arrows) in a B-cell follicle at 9 d.p.i. (green = MV, red = B-lymphocytes, blue = nuclei). Panels **a**–**f** are reprinted from Laksono et al., Viruses 8: 2010 (2016). https://doi.org/10.3390/v8080210. Panel **g** is reprinted from De Vries et al., PLoS Pathog 2012

9.3.1.3 Measles Outbreaks in Other NHP Species

Epidemic infections in NHPs during captivity have frequently been reported in macaques but are not restricted to these species. Outbreaks of MV have also been reported in other captive Old World monkeys (colobus monkeys [*Colobus guereza*] and talapoins [*Cercopithecus talapoin*]). Captive New World monkeys (spider monkeys [*Ateles* spp.], marmosets and tamarins [*Callithrix jacchus*, *Saguinus oedipus*, and *Saguinus fuscicollis*]), and apes (chimpanzees [*Pan troglodytes*]) also proved to be susceptible. Outbreaks have been confirmed by the sudden appearance of MV-specific antibodies in serum and presence of multinucleated giant cells in the lungs of several fatal cases (MacArthur et al. 1979; Levy and Mirkovic 1971; van Binnendijk et al. 1995; Drewe et al. 2012).

A small measles outbreak was described among colobus monkeys; 11 animals were caught in Tanzania and transported to the UK. Within days to weeks after arrival, monkeys started to display characteristic clinical symptoms of measles (respiratory symptoms and conjunctivitis), except for the appearance of rash. Mortality was unusually high in this outbreak (100%) and a probable diagnosis of measles was based on microscopic examination of respiratory and lymphoid tissues, and the appearance of large multinucleated giant cells (illustrated in Fig. 9.2h). Measles diagnosis was confirmed by isolation of MV from one of the MV-infected colobus monkeys (Hime et al. 1975; Scott and Keymer 1975).

A natural outbreak among multiple captive marmoset and tamarin species (*Callithrix jacchus*, *Saguinus oedipus*, and *Saguinus fuscicollis*) was reported in 1971. These animals were housed in separate cages, spread out over separate rooms. In this outbreak, an initial fatality due to pneumonia with presence of large multinucleated giant cells was rapidly followed by the death of 57 other marmosets in the same month and another 268 fatalities in the subsequent months. Measles was identified as the probable cause on basis of histology, and MV was isolated from a moribund marmoset. Interestingly, death occurred rapidly, within 8–18 h of the initial appearance of clinical signs, significantly faster than the clinical course of measles observed in other monkey species and humans. Pneumonia was regarded as the main cause of death (Levy and Mirkovic 1971). A similar outbreak with an unidentified morbillivirus in marmosets was reported in 1978 (Fraser et al. 1978). In general, pathogenesis and disease severity in New World monkeys seems to differ from Old World monkeys, with high morbidity and mortality and rapid progression from initial clinical signs to death (Delpeut et al. 2017). CNS involvement seems to be reported more frequently in New World monkeys, confirmed by experimental intracerebral infections of these animals (Albrecht et al. 1981).

An experimental transmission study was performed with two species of tamarins (*Saguinus mystax* and *Saguinus labiatus*). In this study, tamarins were infected with two different strains of MV and housed (in separate cages) together with MV-seronegative and MV-seropositive tamarins. MV rapidly spread to the separately housed MV-seronegative tamarins (probably through airborne transmission, also the main mechanism of MV transmission in humans), confirming the natural

susceptibility of these New World monkeys to MV infection. In contrast to the MV-seronegative animals, MV-seropositive animals (with the exception of 1) survived the outbreak (Lorenz and Albrecht 1980).

9.3.1.4 Measles Severity in NHPs

Although several outbreaks of MV in different species of NHPs have been well documented, it seems that mortality rates are considerably different among different outbreaks. Furthermore, in non-lethal cases, MV causes disease with variable severity in NHPs, which actually accurately parallels measles in humans. In general, New World monkeys seem more at risk of severe disease and have higher mortality rates due to measles compared to Old World monkeys (Delpeut et al. 2017), which more accurately reflect the natural course of measles in humans. Although it is likely that there are inherent species-specific differences in susceptibility to disease from MV, disease severity is probably also dependent on co-factors like the condition of monkeys during housing, or during and shortly following transport, and whether bacterial, viral, or fungal co-infections are present.

9.3.2 Ecotourism Endangering Wild NHPs

As an alternative to mass tourism, tourists increasingly enjoy visiting relatively undisturbed natural and rural areas while maintaining and conserving the natural environment. This form of tourism, known as ecotourism, provides necessary funds for local ecological conservation. However, at the same time, ecotourism frequently leads to interaction between tourists and animals naturally living in that environment, i.e., a close animal-human interface. Anthropozoonotic transmission of viruses at this interface poses a significant threat to wildlife, especially threatening endangered species like the great apes (Muehlenbein et al. 2008; Kondgen et al. 2008). In addition to ecotourists, researchers or poachers form an additional source of viruses. Mainly in areas where anthropozoonotic transmission is likely to occur, the necessary resources to identify outbreaks and their causative agents are often absent (Epstein and Price 2009), limiting the possibility for interrupting outbreaks. Since potential MV transmission from human to NHP could lead to a large measles outbreak with high mortality rates in endangered ape species, vaccination against measles of free-ranging NHP populations in areas frequently visited by ecotourists could be warranted (Epstein and Price 2009).

Besides this being a hypothetical threat, actual anthropozoonotic transmission of MV from humans to mountain gorillas (*Gorilla beringei beringei*) and subsequent mortality were observed in a national park in Rwanda, in 1988 (Byers and Hastings 1991; Hastings et al. 1991; Spelman et al. 2013). Initially, respiratory symptoms were observed in gorillas leading to six fatal cases. Upon necropsy, multinucleated giant cells were observed in the lungs and spleens of these fatalities. Seroconversion

to measles was only observed in a single animal; however the authors speculate that this was due to early sampling during clinical signs, probably prior to seroconversion. More recently, Kaur et al. speculate on measles being the potential causative agent responsible for acute and fatal respiratory illness in wild chimpanzees (*Pan troglodytes*) in Tanzania (Kaur et al. 2008). In some of the outbreaks among chimpanzees in Tanzania, a paramyxovirus was identified as the causative agent. Although the paramyxovirus was not identified, the authors speculate that it is human in origin. Similar observations were made in outbreak situations in Côte d'Ivoire (Kondgen et al. 2008).

9.3.3 Distemper Outbreaks in Captive Monkey Colonies

Although CDV has originally been described as a morbillivirus of dogs, it is regarded the most species-promiscuous morbillivirus that naturally infects a wide range of carnivores and has a relatively high propensity to cross the species barrier. Disease caused by CDV infection has been reported in members of the families of *Ailuridae* (e.g., *red pandas*), *Felidae* (e.g., *lions*), *Hyenidae* (*hyenas*), *Mustelidae* (e.g., *weasels and martens*), *Procyonidae* (e.g., *raccoons*), *Ursidae* (*black bears*), *Viverridae* (*civet cats*), *Megalonychidae* (*sloths*), and *Phocidae* (*seals*) (Ludlow et al. 2014; Sheldon et al. 2017). In addition, CDV has been reported to infect javelinas (Appel et al. 1991) and was detected in rodents (Origgi et al. 2013). Although it was known that monkeys were susceptible to CDV after experimental intracerebral inoculation since the 1970s (Yamanouchi et al. 1977; Matsubara et al. 1985; Nagata et al. 1990), several natural outbreaks with CDV in NHPs have also been reported recently.

9.3.3.1 Distemper Outbreaks in Macaques

An outbreak with CDV infection in NHPs was initially reported in 1989 when a single Japanese macaque died of encephalitis. Upon necropsy, multinucleated giant cells with inclusion bodies were observed in lesions in the brain that stained positive with a CDV-specific monoclonal antibody. This subsequently led to serological testing of all the macaques in the same group. Seroconversion of all 22 animals to CDV, in the absence of antibodies to MV, was observed and proved that a CDV outbreak had occurred in these macaques (Yoshikawa et al. 1989).

More recently, larger outbreaks with higher mortality rates were reported in breeding colonies of both rhesus and cynomolgus macaques in China and Japan. Sun et al. reported a small CDV outbreak in 20 rhesus macaques in China, with 12 lethal cases (Sun et al. 2010). CDV diagnosis was made on basis of clinical signs (respiratory symptoms, anorexia, fever, thickened footpads, and rash), accompanied by electron microscopy and sequencing. From the 12 lethal cases, 11 macaques died of severe pneumonia, and a single macaque showed neurological symptoms.

Whether pneumonia was caused by CDV infection itself or caused by secondary infections was not investigated. Since the sequence isolated from these macaques was identical to CDV circulating in dogs, foxes, and raccoon dogs in several regions in China, the authors speculate on spread from stray animals to macaques. Subsequently, in 2011, a full-blown epidemic was reported in a breeding farm from which the 20 animals described above originated. In this outbreak, approximately 10,000 rhesus macaques were infected with CDV; 5–30% mortality rates were reported (Qiu et al. 2011). Diagnosis was made on basis of measles-like symptoms, seroconversion, CDV isolation, and sequencing. Again, the CDV sequence isolated from these macaques was unique, and the authors speculate on contact between colony monkeys and wild monkeys or stray dogs as cause of initial introduction into the colony. The epidemic was controlled by vaccination.

Following the two outbreaks in China, a large CDV outbreak occurred in cynomolgus macaques in Japan in 2008. This outbreak occurred in macaques imported from China and caused 46 fatalities with severe pneumonia as the main cause of death (Sakai et al. 2013a). Virus isolation, sequencing, and phylogeny indicated that the CDV strain isolated was very closely related to CDV strains associated with the outbreaks in China (Qiu et al. 2011; Sun et al. 2010). Distemper in these macaques was diagnosed on basis of clinical signs, CDV staining, and sequence analysis. Interestingly, the authors performed additional sequence analysis on macaque variants of the morbillivirus receptors and found homology between macaque CD150 and human CD150, and between human, macaque, and dog nectin-4. Notably, the CDV isolated from a moribund monkey efficiently used macaque and dog variants of receptors, but not the human variants (Sakai et al. 2013a). Adaptation of CDV to human CD150, however, appeared relatively easy (Sakai et al. 2013b).

9.3.3.2 Distemper Mortality in Macaques

CDV in its natural host is considered to be neurotropic as well as lymphotropic, whereas MV in its natural host rarely causes CNS complications. However, neurotropism was rarely observed in the few reports on CDV macaque outbreaks. Rather, the main cause of death in these outbreaks was pneumonia, and only a few animals displayed neurological signs. Frequent detection of alternative pathogens suggests that the high case fatality rates were probably related to opportunistic infections resulting from CDV-induced immune suppression and not CDV spreading into the CNS. This fits with observations made in cynomolgus macaques experimentally inoculated with CDV, rapid infection of lymphocytes and lymphopenia, while CDV was not detected in the CNS (de Vries et al. 2014).

9.4 Macaque Model for Measles Pathogenesis

Studies in NHPs have been crucial for our understanding of measles pathology and pathogenesis in humans. After initial macaque studies with filtered respiratory secretions from measles patients in the beginning of the twentieth century identified MV as the causative agent of measles (Goldberger and Anderson 1911), studies in the 1960s with measles virus isolates passaged in vitro provided the basis for currently used live-attenuated measles vaccines. More recently, measles pathogenesis was elucidated by performing experimental infections of different NHP species – mainly cynomolgus and rhesus macaques – with recombinant MV expressing a fluorescent reporter protein. These viruses allow for sensitive detection of morbillivirus-infected cells, which can be visualized macroscopically (Figs. 9.1c, d and 14.2g) and microscopically (Figs. 9.2 and 14.3). These experiments have provided critical contributions to our understanding of MV entry of a host, dissemination throughout a host, and transmission to a subsequent host.

9.4.1 Alternative Measles Animal Models

A variety of animal species has been considered in models of MV infection. Although small laboratory animals seem most attractive, these do not recapitulate the complex pathogenesis of measles as seen in NHPs and humans. Therefore, two options for animal models remain: experimental infections with animal morbilliviruses in their natural host species (e.g., CDV infection of ferrets (von Messling et al. 2003; Ludlow et al. 2012; de Vries et al. 2017)) or experimental infection of NHPs with MV (de Swart 2009; 2017).

9.4.2 Measles Pathogenesis

9.4.2.1 MV Receptors In Vivo

MV is regarded one of the most infectious human pathogens that efficiently spreads through airborne transmission (Herfst et al. 2017). Although initial infection with MV indeed occurs in the respiratory tract, it is not epithelial cells that are initially targeted rather, after host entry measles becomes a systemic disease involving infection of various cell types. Infection of the various cell types is mainly governed by expression of one of the two cellular entry receptors identified to play a role in wild-type MV infections, CD150 (Tatsuo et al. 2000), and nectin-4 (Noyce et al. 2011; Muhlebach et al. 2011). The development of recombinant MV strains that express fluorescent reporter proteins, combined with the large spectrum of

antibodies available for specific phenotyping of NHP cell types, have confirmed the use of these receptors in vivo.

De Swart et al. inoculated rhesus and cynomolgus macaques with recombinant MV expressing enhanced green fluorescent protein (EGFP) via the intra-tracheal route and followed infected animals in time. Blood samples were taken regularly and analyzed by flow cytometry. Co-staining of the MV-infected (or $EGFP^+$) cells with CD150 proved that $CD150^+$ lymphocytes and dendritic cells (DC) were predominantly infected during MV infection of macaques (de Swart et al. 2007a). The importance of interactions between MV and CD150 was subsequently confirmed by the generation of so-called "receptor-blind" viruses, viruses containing a single point mutation in the H protein and therefore poorly able to bind to CD150. When rhesus macaques were infected with a CD150-blind virus via the intranasal route, clinical symptoms were only observed in 1/6 animals. Surprisingly, all animals seroconverted so were apparently infected at a low level (Leonard et al. 2010), proving that entry occurred, albeit inefficiently.

Whereas CD150 is critical for entry and dissemination, the receptor nectin-4 plays an important role in host exit and transmission to the subsequent host. MV only spreads to the respiratory tract epithelium relatively late in infection (Ludlow et al. 2013a, b), through interactions between MV-infected lymphoid or myeloid cells with the basolateral side of epithelial cells. When cynomolgus macaques were infected with a nectin-4-blind virus, MV was cleared more rapidly from the host and could not be detected in secretions from the throat or nose. By performing immunohistochemistry, it was shown that the nectin-4-blind virus did not infect epithelial cells in the trachea, whereas wild-type MV did (Frenzke et al. 2013). Similar observations were made when a wild-type and nectin-4-blind MV were compared side-by-side in a New World monkey model, namely, squirrel monkeys (*Saimiri sciureus*) (Delpeut et al. 2017).

9.4.2.2 MV Entry of the Host

Although the role of the different receptors was accurately elucidated in monkeys as described above, formal proof of the initial target cells of MV in vivo came from a study in which macaques were allowed to inhale recombinant MV expressing EGFP as an aerosol. Since most MV studies in NHPs were performed by inoculating animals with virus via the intra-tracheal or intra-nasal route, not per se reflecting the natural situation, de Vries et al. invested in setting up an aerosol inhalation model for NHPs (de Vries et al. 2010; Lemon et al. 2011; MacLoughlin et al. 2016). In a study specifically designed to identify the initial target cells of MV, which were classically believed to be epithelial cells in the respiratory tract, cynomolgus macaques were exposed to a high dose MV administered as an aerosol and sacrificed at early time points post inoculation. Interestingly, alveolar macrophages (AM) and DC in the lower respiratory tract were identified as initial target cells (Fig. 9.2a), contradictory to the literature at that time that stated that measles starts with MV infection of epithelial cells in the respiratory tract (Lemon et al. 2011). An early time

course of measles pathogenesis in NHPs was established in this study, starting with MV infection of AM and DC in the lower respiratory tract, followed by spread to bronchus-associated lymphoid tissues (BALT) (Fig. 9.2c) and draining lymph nodes and local amplification, before the virus spread systemically and could be detected in the blood and all lymphoid organs (Fig. 9.2d) (Lemon et al. 2011). This time course fits completely with the presence of the different receptors on target cells.

9.4.2.3 MV Clearance from the Host

After systemic spread of MV throughout the host, a peak of virus replication in macaques is usually observed between 7 and 10 days post inoculation, followed by a rapid decline in infected cells in the blood. This rapid decline is mediated by the virus-specific immune response of the host. Although virus neutralizing antibodies are considered the main correlate of protection against MV infection (Chen et al. 1990), viral clearance is predominantly mediated by cellular immune responses. This was originally observed in "experiments of nature," in hypogammaglobulinemic children (lacking proper antibody formation) who recover normally from MV infection (Good and Zak 1956; Nahmias et al. 1967), whereas children with deficits in cellular immune responses develop severe disease and display prolonged viral shedding (Burnet 1968; Permar et al. 2001). These observational studies were confirmed in NHPs. Permar et al. experimentally inoculated NHPs and determined the immunological drivers of viral clearance by depleting these monkeys of either $CD8^+$ T-lymphocytes or B-lymphocytes. Similar to the observations made in children, macaques depleted of B-lymphocytes were able to normally clear virus (Permar et al. 2004), whereas $CD8^+$ T-lymphocyte-depleted macaques presented with a more extensive rash, higher viral loads, and a longer duration of viremia (Permar et al. 2003).

9.4.2.4 MV Transmission to the Subsequent Host

MV is regarded one of the most contagious viruses infecting humans – each infected person can transmit the virus to an average of 15–20 susceptible individuals. Macaque studies have been crucial in the understanding as to how this high rate of transmission is achieved. MV is spread from host-to-host via airborne transmission. Respiratory droplets filled with virus particles are produced by sneezing and coughing and enter the respiratory tract of a susceptible host. As mentioned above, MV uses two different cellular receptors to enter different cell types, and CD150 is regarded as the important "host entry" receptor for MV. Nectin-4 is actually regarded as the "host exit" receptor for MV that facilitates host-to-host transmission. Expression of nectin-4 was shown to be widespread in both the macaque upper and lower respiratory tract (Muhlebach et al. 2011; Ludlow et al. 2013b). When macaques were experimentally infected with MV expressing a fluorescent reporter, the virus was abundantly present in the nasal cavity during the late stage of the infection (around

onset of rash) (Ludlow et al. 2013b), and in experimental infections of NHPs with wild-type viruses, cell-free and cell-associated MV can readily be isolated from nose and throat swabs (Ludlow et al. 2013a).

Based on these macaque studies, and studies that were performed with a nectin-4-blind strain of MV, an "exit strategy" for MV was postulated. MV initially enters the host via DC and AM and is amplified locally before systemic spread. After systemic spread, infected cells (probably lymphocytes) return to the respiratory tract and transfer MV to epithelial cells, where it binds nectin-4 on the basolateral side of the epithelium (Fig. 9.2e). Novel virus particles are subsequently produced and released at the apical side of the epithelium, directly into the airway. Here, aerosol is produced due to coughing and sneezing, facilitating host exit and transmission (Racaniello 2011). In addition to the release of cell-free MV into the respiratory tract, there is also evidence for cell-associated spread of MV to the subsequent host. Disrupted epithelium and cell debris was frequently observed in the respiratory tract of MV-infected macaques, in addition to disruption of tonsillar epithelium (Fig. 9.2f). This epithelial disruption would lead to induction of coughing and sneezing responses, leading to expulsion of cell-free virus produced by lymphocytes, and cell debris exuding from the respiratory submucosa or tonsillar tissues (Ludlow et al. 2013a). Additionally, cell-free virus in the airways encounters relatively little receptor-expressing cells and therefore can remain present in the mucus of the respiratory tract.

9.4.2.5 Measles-Associated Immune Suppression in NHPs

One of the most important clinical sequelae of measles is immune suppression, which is the main cause of measles morbidity and mortality. Interestingly, measles normally causes lymphopenia early after inoculation of NHPs and reduces host resistance to other infections (de Vries et al. 2012) while simultaneously inducing a strong immune response to itself that results in life-long protection from measles. The mechanism behind this "measles paradox," simultaneous immune suppression and immune activation, was elucidated by performing a comprehensive study of virological, immunological, hematological, and histopathological observations made in NHPs euthanized after MV infection via various infection routes. In NHPs, it was observed that MV preferentially infects certain subsets of T- and B-lymphocytes, namely, memory T- and follicular B-lymphocytes. Depletion of these cells was predominantly observed in the lymphoid tissues of these animals (exemplified by heavy infection of Peyer's patches in experimentally infected animals in Fig. 9.2g) (de Vries et al. 2012). Multinucleated giant cells are abundant in all lymphoid tissues (Fig. 9.2h), and immunofluorescence staining indicated that these were of a B-lymphocyte origin (CD20-positive, while CD11c- and CD3-negative) (Fig. 9.3).

In a follow-up publication, the authors described the rapid expansion of MV-specific and bystander lymphocytes following MV clearance as the cause of resolution of lymphopenia. However, the immunological repertoire at that time is still

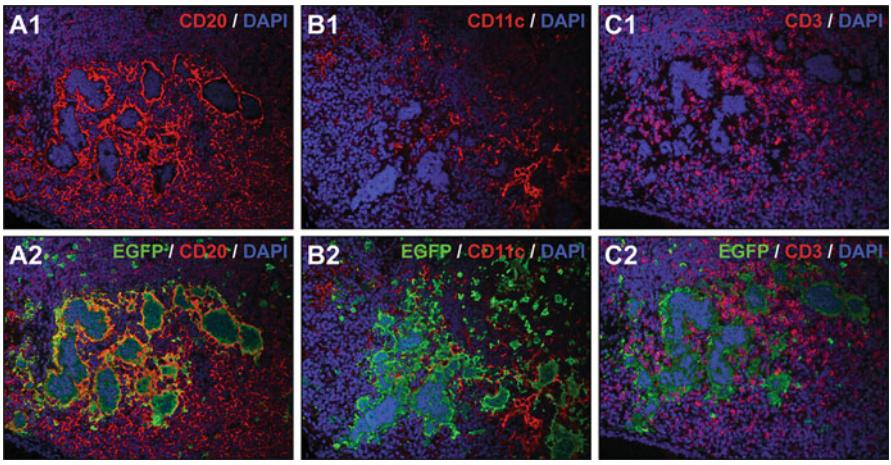

Fig. 9.3 Images collected from lymphoid tissues obtained from experimentally infected NHPs at 7 days post inoculation (d.p.i.), illustrating that multinucleated giant cells are mainly from a B-lymphocyte origin. MV-infected cells were detected by immunofluorescent double staining. (**a–c**) Large numbers of syncytia were observed in the B-lymphocyte follicles and were stained for EGFP (green) as a marker of MV infection. Double stains were performed with a B-lymphocyte marker (CD20, red, **a1** and **a2**), a macrophage/dendritic cell marker (CD11c, red, **b1** and **b2**), or a T cell marker (CD3, red, **c1** and **c2**), and DAPI was used to counterstain the nuclei (blue). Top panels only show the red and blue channels; bottom panels show the combined red, blue and green channels. Multi-nucleated giant cells were mainly of B-lymphocyte origin (panel **a**), and the infection was associated with significant cytopathic effects in lymphoid tissues. All panels reprinted from supplementary data of De Vries et al., PLoS Pathog 2012

severely limited, causing what the authors termed a temporary "immunological amnesia" (de Vries and de Swart 2014). This model explains that measles immune suppression can last for several weeks to months after recovery from measles, whereas lymphopenia is normally rapidly resolved (Mina et al. 2015). Preferential infection of $CD150^+$ lymphocyte subsets as observed in NHPs was recently confirmed in humans (Laksono et al. 2018).

9.5 Macaque Model for Vaccine Evaluation

In addition to the crucial role NHPs played in studying MV tropism and pathogenesis, these animals have also been used to evaluate new generation MV vaccines and novel routes of MV vaccination (van Binnendijk et al. 1997; Zhu et al. 1997; Polack et al. 2000; Combredet et al. 2003). Live-attenuated measles vaccines are safe and effective and have successfully interrupted endemic MV transmission in large geographical areas. Although currently used live-attenuated MV vaccines have already been in successful use for over 50 years, surprisingly little was known about the target cells that sustain vaccine virus replication in vivo, and the molecular

basis for attenuation has remained elusive. It has been known for some time that vaccine viruses have the ability to use an additional cellular entry receptor in vitro, namely, CD46 (Dorig et al. 1993; Naniche et al. 1993; Buckland and Wild 1997), which is expressed on virtually all nucleated cells (Liszewski and Atkinson 1992). However, neither the extent of use of CD46 in vivo nor the tropism of vaccine viruses was known. Macaque studies have shown that after aerosol, intra-tracheal, and intra-muscular administration, vaccine viruses still predominantly replicate in CD150-expressing cells (de Vries et al. 2010; Rennick et al. 2015; de Swart et al. 2017). In the respiratory tract, $CD11c^+$ or $CD68^+$ myeloid cells (CD150-positive) were predominantly infected by live-attenuated MV (de Vries et al. 2010; de Swart et al. 2017), whereas DC and macrophages (CD150-positive) were identified as the predominant target cells of live-attenuated MV after intra-muscular administration (Rennick et al. 2015).

9.5.1 Alternative Measles Vaccines

Despite their success, live-attenuated MV vaccines have several limitations. These include dependency on maintaining the cold chain, requirement for trained health-care workers for administration, and the need for hypodermic needles and safe waste disposal. To address some of these issues, different vaccination platforms for MV have been investigated. Initially, vectored approaches have been evaluated in NHPs, in which macaques were vaccinated with poxviruses expressing the MV surface fusion (F) and H glycoproteins (Stittelaar et al. 2000; Zhu et al. 2000). Vaccine efficacy was demonstrated, but the macaque model was of added value in these studies, as vaccine efficacy could also be addressed in the presence of passively transferred antibodies (simulating the presence of maternal antibodies in infants) (Stittelaar et al. 2000) or in immune-suppressed macaques to evaluate safety in immunocompromised (Stittelaar et al. 2001). Furthermore, alphavirus replicons generated to express both the MV-F and MV-H protein also showed efficacy in macaques.

In addition to vectored vaccine approaches, direct DNA vaccination was evaluated as potential novel MV vaccine in macaques. In these studies, macaques could be protected from wildtype MV challenge by prior vaccination with DNA expressing the F and/or H gene (Polack et al. 2000; Lin et al. 2013). In other studies priming of cellular immune responses was observed (Stittelaar et al. 2002). Again, these novel vaccines could also be evaluated for efficacy in the presence of passively acquired antibodies in the macaque model (Premenko-Lanier et al. 2003).

9.5.2 Alternative Measles Vaccine Administration Routes

In addition to the generation of novel measles vaccines, different routes of administration were also investigated. The focus of this research was predominantly on the generation of needle-free MV vaccination regimens. Vaccine delivery via aerosol inhalation has been considered as a promising possibility (Griffin 2014). Clinical trials have already extensively demonstrated the feasibility of this administration route (Sabin et al. 1984; Dilraj et al. 2000, 2007; Low et al. 2008, 2015).

To support licensing of novel vaccine administration routes, both pre-clinical and clinical studies are required. Inhalation administration routes were extensively investigated in NHPs, comparing administration of the MV vaccine via aerosol inhalation and dry powder inhalation directly with injection (de Swart et al. 2006, 2007b; Lin et al. 2011). In general, aerosol inhalation induced similar immune responses as detected in the injection group and protected macaques from wild-type MV challenge. Variable results were obtained with dry powder. A more recent large-scale study in macaques investigated both the tropism of recombinant vaccine viruses expressing fluorescent reporter proteins and whether vaccination should target the upper or lower respiratory tract to be immunogenic. In this study, four administration routes were compared: intra-tracheal inoculation, intra-nasal instillation, aerosol inhalation, and intra-muscular injection. This study showed that delivery of vaccine virus to the lower respiratory tract is crucial in order to induce optimal immune responses and protection from challenge (de Swart et al. 2017).

In addition to needle-free vaccine administration via the respiratory route, another promising alternative for the use of hypodermic needles is to deliver live-attenuated MV via microneedle patches. Microneedle patches are micron-scale dissolvable polymeric needles that were designed to encapsidate the standard live-attenuated MV. These patches can directly be applied to the skin for intra-dermal vaccination, without the requirement of vaccine reconstitution. NHPs have again been critical to provide the proof of principle of this approach (Edens et al. 2013, 2015).

9.6 Crossing the Species Barrier

9.6.1 Measles Eradication

After the eradication of smallpox, the potential for measles eradication was first proposed in the 1980s. In this period, interruption of endemic MV circulation had not yet been achieved, and eradication of MV was considered to be premature (Henderson 1982). However, in 2001 a formal initiative was established, known as the Measles and Rubella Initiative (https://measlesrubellainitiative.org), which aimed at reduction of global measles mortality (in addition to reducing the number of congenital rubella cases) by initiating mass vaccination campaigns and two-dose vaccination regimens. An impressive reduction in measles mortality has been

obtained since then, with the current measles mortality at an all-time low (WHO 2017). Eradication of MV from the globe is considered feasible, as MV is a monotypic virus that exclusively circulates in humans, and an effective live-attenuated vaccine is available (Moss and Strebel 2011). Furthermore, in 2011 rinderpest was officially declared eradicated from the globe. Since RPV is a close relative of MV, targeted eradication of MV should also be feasible (Morens et al. 2011; Roeder 2011; de Swart et al. 2012). However, there are pitfalls like waning vaccine immunity and declining vaccination coverage, as was clearly demonstrated by the by the difficult endgame of poliovirus eradication (Cochi and Linkins 2012). Furthermore, recent spread of animal morbilliviruses into NHPs illustrates that we should be aware of zoonotic morbillivirus infections in a measles post-eradication era (Qiu et al. 2011; Sakai et al. 2013a; Yoshikawa et al. 1989; Sun et al. 2010).

9.6.2 Post-measles Eradication Era and Implications for One Health

While measles eradication would save many lives, it is also likely to result in reduced compliance to MV vaccination. As a result, many children will grow up without MV-specific immunity, similar to the scenario as observed with smallpox. In the case of smallpox, vaccination was discontinued, thus creating a niche for closely related orthopoxviruses of other mammals to cross the species barrier into humans (Essbauer et al. 2010; Reynolds et al. 2012; Reynolds and Damon 2012). Since we already know that morbillivirus infections induce at least partial cross-protection from other morbilliviruses (Strating 1975), cessation of MV vaccination after eradication might also facilitate cross-species infection and subsequent adaptation of animal morbilliviruses to humans (Cosby 2012). Historically, CDV infections in humans have been described, and some humans have serological evidence for CDV infection (Nicolle 1931; Adams 1952; DeLay et al. 1965). More compelling evidence for the capacity of CDV to adapt to primates comes from outbreaks in monkey colonies, as described above (Qiu et al. 2011; Sakai et al. 2013a; Yoshikawa et al. 1989; Sun et al. 2010). These outbreaks had high mortality rates, indicating that an outbreak of an adapted CDV in MV-naive humans could have catastrophic consequences. An additional concern is that CDV is known to be neurotropic and was shown to have replicate efficiently in experimentally infected NHPs (de Vries et al. 2014).

Therefore, the potential of non-MV morbilliviruses, especially CDV, to adapt to humans should be held in mind in measles eradication scenarios. Currently, immunity in the human population due to MV infection or MV vaccination results in immunity against other morbilliviruses and restricts the possibility of animal morbilliviruses adapting to humans. However, if the envisaged measles eradication leads to a significant drop in vaccination coverage, such adaptation cannot be excluded. For this reason, MV vaccination and serological and virological

surveillance of morbillivirus infections in the human population should be maintained, even in a measles post-eradication era.

9.7 Conclusions

NHPs have played a crucial rule in MV research since the beginning of the twentieth century and continue to do so. MV was initially discovered to be the causative agent of measles by transfer of filtered respiratory tract secretions from measles patients to macaques. Since then, research in NHPs with laboratory-adapted, live-attenuated, and wild-type viruses, sometimes engineered to express fluorescent proteins or to be incapable of binding cellular receptors, have further elucidated measles pathogenesis, from entry into the host to dissemination throughout the host and transmission to the subsequent host and the induction of immune suppression. Additionally, although free-ranging NHP populations are thought not to support endemic MV circulation, they are highly susceptible to natural MV infection (or infection with other morbilliviruses, like CDV). Demographic changes, including the increasing human population size, urbanization, and deforestation, may lead to increased interactions between humans and NHPs (Gortazar et al. 2014). Introduction of a highly contagious MV in a high-density population or captive colony seronegative for MV can easily lead to an efficient transmission chain in which all animals become infected. Secondary infections due to morbillivirus-induced immune suppression can subsequently give complications, leading to outbreaks with high morbidity and mortality. Therefore, MV-seronegative captive colonies should be protected from MV introduction by vaccination or preventing contact with MV-infected individuals. Furthermore, a close interface between humans and free-living NHPs, mainly due to ecotourism and growth of the human population, increases the potential for spread of MV into susceptible monkey and ape populations and vice versa.

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Chapter 10
Simian Foamy Viruses: Infections in Human and Nonhuman Primate Hosts

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Abstract Foamy viruses are ancient and ubiquitous retroviruses that infect a variety of mammalian hosts. In this chapter, we focus on foamy viruses that infect nonhuman primates (NHP), called simian foamy viruses or SFV. Natural SFV infection in monkeys and apes leads to life-long, persistent infections with no associated pathogenicity. Although SFV have coevolved with their natural hosts and show strong cospeciation, there are also many examples of cross-species transmission events. SFV are transmitted primarily via saliva, and humans who come into contact with NHP saliva can become zoonotically infected with SFV. To date, SFV from a variety of NHP species have been transmitted to humans and, as seen in natural infections, there is no pathogenicity associated with these zoonotic infections. However, as in the case of other retroviruses, such as lentiviruses, it is possible that an SFV viral variant could emerge as a human pathogen. The molecular features of SFV, the situations that lead to SFV zoonotic infections, and the implications of these infections are discussed in the global context of the monkey–human interface.

Keywords Zoonotic transmission · Retrovirus · Foamy virus · Recombination · Gene therapy vectors

10.1 Introduction to Virology and Retrovirology

Viruses are the most abundant biological entities on Earth (Edwards and Rohwer 2005). They have been found to infect all known life forms, including bacteria, fungi, plants, and animals. Viruses are entirely dependent on host cells for their

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replication and, as such, are considered obligate intracellular particles. Viruses are also significantly smaller than the cells they infect. For example, human viruses range in size from 20 to 260 nm in diameter. In fact, electron microscopy is required to visualize such small particles and has provided much of our understanding of virus structure.

All viruses are comprised of protein structures called capsids that contain and protect the viral genetic information (genome). Capsids are comprised of repeating viral protein subunits that form highly ordered structures, often with complex geometries. Some viruses may also have a lipid bilayer membrane, called an envelope, which surrounds the capsid and is partially derived from the host cell. Specialized proteins on the surface of virus particles bind to specific molecules, called virus receptors, in host cell plasma membranes. This binding facilitates viral entry into host cells. Binding of the virus particle (virion) to viral receptors determines which host cells viruses can infect. For example, human immunodeficiency virus 1 (HIV-1) uses the host CD4 molecule as its primary virus receptor and, as a consequence, it replicates primarily in human T-cells that express the CD4 protein on their surface. This specific interaction can also influence the pathogenic effects associated with a virus infection, as is the case for HIV-1 where infection and killing of CD4+ T cells leads to immunodeficiency in the infected host.

Viral genomes can be comprised of DNA or RNA and can encode as few as three genes or as many as hundreds of genes. To copy their genomes (viral replication), some viruses co-opt host polymerase enzymes while others use viral-encoded polymerases. Virus replication can often result in the killing of host cells, but not always. There are some viruses that remain within the host cell without injuring the cell. These types of infections, called persistent infections, continue for the lifetime of the cell.

Retroviruses are a family of viruses that can cause persistent infections. Retroviral particles are approximately 100 nm in diameter and are enveloped. They generally have RNA genomes and encode an unusual enzyme called reverse transcriptase (RT) that is essential for viral replication and converts the RNA genome into double-stranded DNA. Another viral enzyme, called Integrase (IN), integrates the reverse-transcribed DNA genome into the host cell chromosome, where it remains permanently. The outcome of lifelong, persistent retroviral infections can range from highly deleterious to the host, as in the case of HIV-1, to apparently benign, as in the case of foamy viruses (FV).

10.2 Foamy Viruses (FV)

Foamy viruses, also called Spumaretroviruses (Khan et al. 2018), are unusual retroviruses in that they have DNA genomes. The virus capsids contain RNA when they are first assembled in the host cell but the reverse transcriptase enzyme generates a double-stranded DNA genome before the virus particles exit the host cell

(Yu et al. 1999). This is in contrast to all other retroviruses, which do not undergo reverse transcription until infection of a new host cell.

Retroviruses are classified as either simple or complex, based on their genome structure. Simple retroviruses encode three genes, *gag*, *pol*, and *env*, in that order from the 5'end (or left end) of the genome. The *gag* gene encodes proteins required for capsid formation and genome incorporation into the capsids (packaging). Interestingly, the Gag polyprotein, which is further cleaved into the viral proteins matrix (MA), capsid (CA), and nucleocapsid (NC) in all other retroviruses, remains a large polyprotein in FV. The *pol* gene encodes proteins required for reverse transcription and integration and undergoes a single cleavage event releasing the mature reverse transcriptase (RT) and integrase (IN) protein. The *env* gene encodes proteins required for viral receptor binding and entry.

In addition to Gag, Pol, and Env, complex retroviruses encode accessory proteins that are not found in the virus particles (virions). FV are complex retroviruses that encode two nonstructural proteins, Tas and Bet. Tas is a transcriptional activator and binds to two different FV viral promoter regions. The primary FV promoter is found near the 5′ end of the viral genome in a region called the 5′ long terminal repeat (LTR). There is a second FV promoter, called the internal promoter (IP) located just upstream of the accessory genes. Basal expression of Tas from the IP is known to be important for establishing a productive FV infection and Tas is absolutely required for viral transcription. In contrast, the function of Bet is poorly understood. A diagram of a foamy virus particle and genome is shown in Fig. 10.1.

A prototype foamy virus (PFV) was originally isolated from a human nasopharyngeal tumor and was thought to be a human foamy virus. In fact, PFV was originally called HFV for human foamy virus. However, sequence comparisons showed that PFV was actually a chimpanzee SFV and that the African from whom the cell line was derived had been zoonotically infected (Herchenroder et al. 1994). PFV can infect and replicate in all vertebrate-derived tissue culture cells and cell lines that have been examined, including cells of many different vertebrate species and tissue origins. In many of these cell lines, PFV replication is robust, leading to cell lysis and high titers of extracellular virus. However, in some cell lines, such as those derived from human hematopoietic cells, PFV replication occurs without affecting cell viability (Yu et al. 1996).

10.3 Foamy Virus (FV) Replication

10.3.1 FV Replication In Vitro

While most work studying FV assembly and replication has used PFV, the findings are also relevant for simian foamy virus (SFV) species. Much of our knowledge comes from in vitro studies using cloned FV viral isolates and established cell lines that support active FV replication leading to cell death (cytopathic infection). However, it is important to note that, in vivo, FV replication is rarely cytopathic.

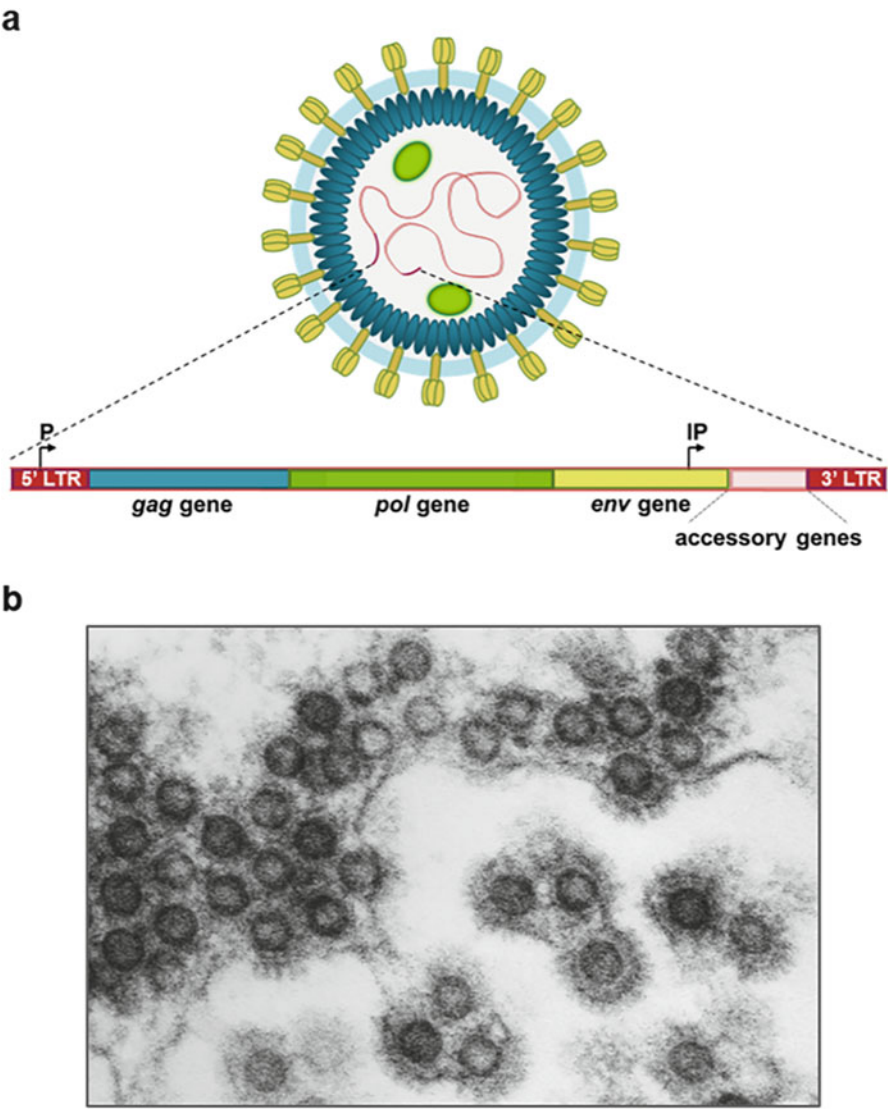

Fig. 10.1 A Schematic diagram of foamy virus (FV) particle and genome organization. The double-stranded DNA genome contains long terminal repeat regions (LTR) at both the 5′ and 3′ ends of the genome. Arrows indicate the location of the two viral promoters: the 5'LTR promoter (P) and the internal promoter (IP). B Electron micrograph of newly assembled particles in prototype foamy virus (PFV)-infected tissue culture cells

This discrepancy is not well understood but suggests that hosts have mechanisms that can modulate FV replication to alter the outcome of infection.

The specific host cell receptor used by any FV for cell entry is yet unknown. To date, vertebrate cell lines resistant to SFV infection have not been identified,

suggesting that the virus receptor molecule is ubiquitously expressed. Often, to identify virus receptors, cellular cDNAs from permissive cells are transfected into resistant cells. Those cells that become permissive after transfection can be analyzed to identify the gene(s) responsible for allowing virus entry. Since there are no vertebrate cells resistant to SFV infection, this approach has not been possible. Heparan sulfate, a cell surface proteoglycan, has been shown to enhance FV attachment to cells (reviewed in (Berka et al. 2013)), but other receptor(s) remain to be identified.

Binding of virus particles to the cell surface is followed by entry into the host cell. Fusion of the viral and cellular membranes results in FV capsid release into the host cytoplasm (reviewed in (Berka et al. 2013)). As is the case of some other animal viruses, FV capsids travel along the cellular microtubule network toward the nucleus (reviewed in (Radtke and Döhner 2006; Berka et al. 2013)).

Capsid disassembly leads to formation of a structure known as the preintegration complex (PIC) (Bieniasz et al. 1995). The PIC, including the viral DNA genome and integrase (IN) proteins, gains access to the nucleus during mitosis, and the viral genomic DNA is permanently integrated into a host chromosome (Nowrouzi et al. 2006). Unlike the case of other retroviruses, FV genome integration has never been found to lead to tumor formation.

Once the FV DNA genome is successfully integrated into the host cell DNA, it is called a provirus. The FV provirus is used as a template to make viral RNA and proteins for the production of new viruses. The transcription (RNA synthesis) and translation (protein synthesis) of the FV genome relies entirely on host cell machinery, such as the host RNA polymerase II enzyme and host ribosomes. In fact, at this point, one can consider the FV provirus to be equivalent to a cellular gene, containing its own promoters that are recognized by host factors. As mentioned above, FV have two promoters (Fig. 10.1). The first (primary) promoter is located at the 5′ end of the provirus in the LTR region. The LTR regions, present at both ends of the provirus, are not found within the RNA transcript of the virus genome but are created during the process of reverse transcription. The second FV promoter, the internal promoter (IP), specifically drives the expression of the accessory proteins Tas and Bet. Efficient transcription from the LTR promoter, resulting in a productive infection, requires high concentrations of Tas. Latent infections arise when levels of Tas are insufficient to activate the LTR promoter (Meiering and Linial 2002). Thus, sufficient activation of the IP within a host cell is essential for active replication and generation of new viruses.

Production of viral RNA transcripts and proteins leads to the cytoplasmic assembly of capsids containing an RNA precursor of the viral genome. The assembled capsids interact with the viral envelope (Env) surface glycoprotein at the cellular membrane, allowing the release of infectious virus particles. Unlike all other retroviruses, which do not begin reverse transcription until entering a new host cell, FV reverse transcription is initiated during capsid assembly and budding. The newly formed virus particles exit the cell through existing host pathways, and the result is the release of infectious FV particles containing DNA genomes. In certain cell types studied in tissue culture, this cycle of FV replication leads to cell lysis, while in other

cell types, new virus particles are released without killing the host cell (Yu et al. 1996).

10.3.2 FV Replication In Vivo

In natural settings, FV can infect many different hosts, including cats (feline foamy virus or FFV), cows (bovine foamy virus or BFV), and all nonhuman primates examined to date (SFV). Despite the ability of PFV and other SFV to replicate in a large variety of cell types in vitro, the situation in vivo is different. The sites of foamy virus replication in vivo are quite limited and in healthy macaques (*Macaca mulatta*), SFV replication has only been detected in the superficial epithelial cells of the oral mucosa (Murray et al. 2008). These cells are naturally sloughed into saliva, whether or not they are infected by SFV. If these cells are SFV-infected, the virus is also shed into saliva from where it can be transmitted to new hosts. It is not known whether SFV are cytopathic in these epithelial cells since they are already destined to die. In other cell types, including peripheral blood mononuclear cells (PBMC), SFV establish latent infections. In the absence of replication in these latently infected cells, they remain healthy (Fig. 10.2).

10.4 Detection of Foamy Virus (FV) Infections

Traditionally, FV-infected individuals have been identified by the presence of anti-FV antibodies in the host. Serum, which contains antibodies, is obtained from blood samples and used in either a Western blot or enzyme-linked immunosorbent assay (ELISA). In both assays, FV proteins are immobilized on a surface and the serum antibodies are added to allow for specific protein–antibody binding. If binding is detected, this indicates that the individual is anti-FV antibody positive and it means that the individual has been exposed to the virus. However, not all exposed individuals are persistently infected. To measure persistent infection, DNA extracted from peripheral blood mononuclear cells (PBMC) is amplified by PCR (Polymerase Chain Reaction) using DNA primers specific to FV. The amplified DNA is often sequenced to specifically identify the origin of the FV that has integrated into the host chromosomes. Thus, animals or humans whose PBMC are FV PCR + are considered to be persistently infected.

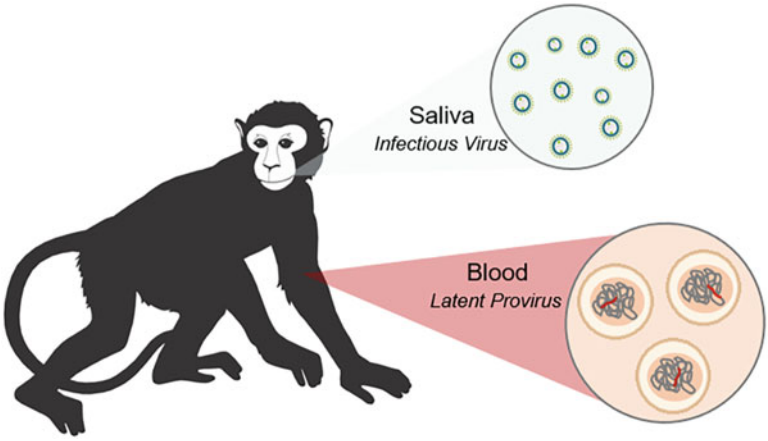

Fig. 10.2 Schematic diagram of SFV within an infected monkey host. Infectious SFV particles are shed into the saliva from infected cells in the oral mucosa. Latent SFV proviral genomes are found integrated into the genome of host peripheral blood mononuclear cells (PBMCs)

10.5 Simian Foamy Virus (SFV) Evolution

SFV have coevolved with their primate hosts for at least 30 million years, making them the oldest known vertebrate RNA viruses (Switzer et al. 2005). Genetic similarities between biological entities can be diagrammed using phylogenetic trees to reveal their evolutionary histories. Comparison of NHP and SFV gene sequences (cytochrome oxidase (COII) gene for NHP and *pol* gene for SFV) showed that the two phylogenetic trees are nearly identical in both branching order and divergence times, indicating that the accumulation of genetic changes occurred over a similar period of time for both NHP and SFV (Switzer et al. 2005) (Fig. 10.3). While highlighting the strong cospeciation of SFV and their NHP hosts, this work also provides evidence of SFV cross-species transmission in the wild (Fig. 10.3, B). For example, a Douc langur SFV sequence (*Pygathrix nemaeus*, Pne500057) clustered with those from macaques, instead of that from the more closely related Francois' langur (*Trachypithecus francoisi*, Tfr083616). Some well-documented examples of cross-species transmission of SFV have also been found in Africa, including chimpanzees infected with SFV from red colobus monkeys (Leendertz et al. 2008) and also from *Cercopithecus* sp. monkeys (Liu et al. 2008). These examples demonstrate that an NHP can be infected by both its intrinsic, highly evolved SFV species as well as an SFV transmitted from a different NHP species.

Viral replication errors and recombination events are two important mechanisms that contribute to viral evolution. For retroviruses, both mechanisms often lead to high levels of genetic change, which can have a significant impact on the outcome of the infection and viral transmission. For example, high levels of genetic variation allow HIV-1 to adapt quickly to selective pressures, such as the ability to escape antiviral drug monotherapy and the ability to switch from macrophage to T-cell

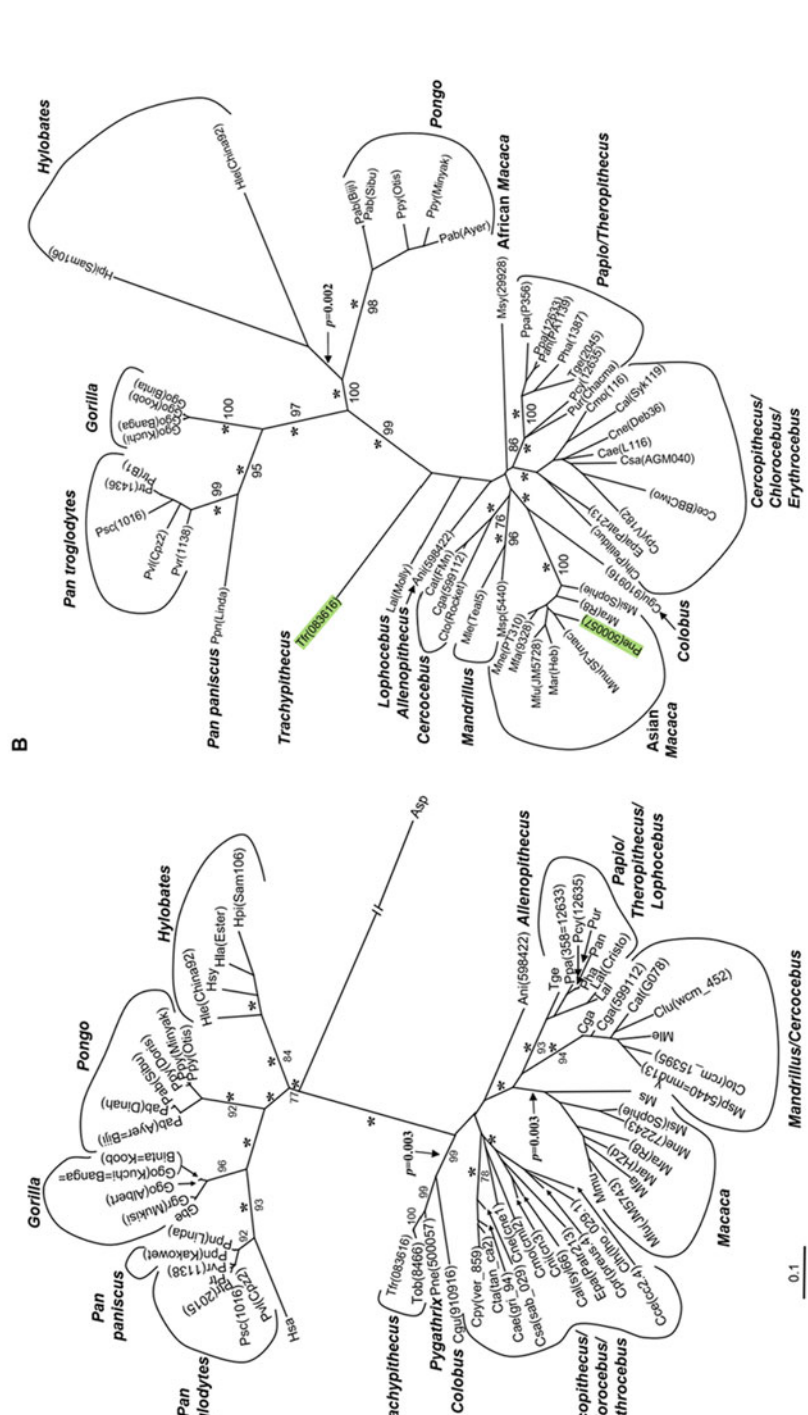

Fig. 10.3 Phylogenetic relationships of NHP cytochrome oxidase II (COII) and SFV *pol* sequences. Primate COII sequences (500 base pairs) (**a**) and SFV *pol* sequences (425 base pairs) (**b**) were compared using maximum-likelihood analysis. Numbers on branches indicate bootstrap support based on 1000 replicates; asterisks indicate significance of $P < 0.001$; branch lengths are drawn to scale; the bar indicates 0.1 nucleotide substitutions per site. Highlighted sequences indicate an SFV from a douc langur (Pne(500057)) that does not cluster with its closest relative, the Francois langur (Tfr(083616)), but with the more distantly related Asian macaques. (Figure kindly provided by W.M. Switzer of the Center for Disease Control (CDC) (Switzer et al. 2005))

tropism within a host (Bieniasz and Cullen 1998). Most retroviruses have a high mutation rate that is attributed to the highly error-prone RT enzyme. Interestingly, unlike most retroviruses, SFV genomes appear to be highly conserved over time (Schweizer et al. 1999). Although in vitro analysis of PFV RT found a mutation rate similar to that of HIV-1 RT, in vivo analysis found that the PFV RT has a higher fidelity than HIV-1 RT (Boyer et al. 2004, 2007; Gartner et al. 2009). These findings suggest that the PFV RT enzyme itself has the potential for high levels of mutation, but in vivo the PFV mutation rates may be lower than those seen in other retroviruses. However, PFV showed frequent recombination in cell culture assays (Gartner et al. 2009). Like all retroviruses, FV RT uses two copies of its single-stranded RNA genome to produce a double-stranded DNA copy. PFV RT was found to jump between the two RNA genome templates during reverse transcription, a process called template switching, more frequently than HIV-1 RT (Gartner et al. 2009). This finding is important because it suggests that recombination events could occur between SFV from two different NHP species in a single coinfected animal. Such cross-species transmission and recombination events can yield viruses with novel properties and are of particular concern given their roles in the emergence of HIV-1 as a human retroviral pathogen (Gao et al. 1999). Cross-species transmission of viruses can be limited by the expression of the appropriate viral receptor in different host species. However, given the apparently ubiquitous expression of the SFV receptor (as yet unknown), it is likely that SFV may be easily transferred between different NHP species, raising further concerns for the emergence of an FV human pathogen.

10.6 Foamy Virus Transmission

Transmission from host to host is a key aspect of the viral life cycle. Many viruses need to alter the host biology to aid in transmission, and these changes can result in pathology. For example, respiratory viruses induce coughing and sneezing to spread virus particles efficiently via aerosols to new hosts. Retroviruses are generally transmitted through body fluids and FV primarily use saliva. This can include saliva-to-saliva transmission as well as saliva-to-blood transmission (see Sect. 10.8). Natural foamy virus hosts share saliva as part of their lifestyle through common behaviors such as grooming and biting, as seen in cats and NHP. Other FV natural hosts, such as cows and horses, share food. Through food sharing, it is also possible that FV are directly transferred from the saliva of one animal to the mouth of another animal. By utilizing common host behaviors involving saliva, foamy virus infections do not require any biological changes in the host in order to ensure efficient transmission. In fact, to date no pathological consequences of foamy virus infections have been reported in natural hosts.

Foamy virus transmission is very efficient in natural hosts. At least 70% of adult NHP in natural habitats as well as in zoos and research settings are infected by SFV (reviewed in (Pinto-Santini et al. 2017)). It is not surprising that transmission of SFV

through saliva is rampant, as SFV are known to replicate primarily in oral mucosa epithelial cells that are sloughed into saliva (Murray et al. 2008). Through grooming, licking wounds, biting, and other such behaviors, infected saliva enters the blood of uninfected animals, which then become infected.

10.7 SFV Infection of Natural Hosts

There are two main complex retroviruses known to infect nonhuman primates (NHP) in their natural habitats, lentiviruses, and foamy viruses. Lentiviruses are found in African Old World Monkeys (OWM) and apes. Foamy viruses are found in all NHP examined to date, including New World Monkeys (NWM), OWM, and apes. Interestingly, neither lentiviruses nor foamy viruses are highly pathogenic in their natural hosts, although sometimes lentiviral infections have minor consequences for their natural NHP hosts (Sharp and Hahn 2010).

To date, there is no evidence of infant NHP SFV infection acquired at birth or transmitted through breastmilk, nor is there evidence for sexual transmission of SFV. Studies in baboons (*Papio cynocephalus anubis*) (Broussard et al. 1997) as well as macaques (*Macaca tonkeana* and *Macaca fascicularis*) (Calattini et al. 2006; Hood et al. 2013) suggest that SFV are most often transmitted through grooming and/or bites. SFV infections are thought to occur in young NHP, as early as 6 months old, via horizontal transmission (Hood et al. 2013).

Once infected with SFV, NHP harbor latent proviruses for the duration of their lives. No impact on the host, including any pathogenicity, has been attributed to these persistent SFV infections. There has been a recent interest in studying the impact that the host microbiome may have on a variety of health states in individuals, including fungal, bacterial, and viral contributions. Given the lifelong presence of SFV in infected individuals, it remains to be explored what effect, if any, SFV have on their natural hosts.

Coinfection of lentiviruses and foamy viruses has not been well studied in natural NHP populations. Since most natural NHP populations are SFV-infected, it is a challenge to determine whether there is any consequence of SIV/SFV coinfections in the wild. Some chimpanzees that were infected with SIVcpz and not SFVcpz have been found, but the health status of these animals was not described compared to those that were coinfected [14]. However, in research settings, there are interesting data about SFV and lentiviral coinfections. When researchers injected macaques (which are not naturally infected with lentiviruses) with a lentivirus that was selected to be pathogenic in macaques (SIVmac239), it was found that the site of SFV replication changed. In SFV+/SIVmac239 + macaques, SFV replication was found in the small intestine (jejunum) where SIV induces T-cell depletion (Murray et al. 2006). Another study examined SIVmac239 pathogenesis in either SFV-negative or SFV-positive animals (Choudhary et al. 2013). In SFV + macaques, SIVmac239 infection was more pathogenic, with higher levels of SIV virions and increased death rates. Thus, in this experimental situation, SFV exacerbated pathogenesis by a

lentivirus. This finding highlights the importance of studying SFV–SIV coinfections in natural NHP populations, particularly in Africa.

10.8 Human–NHP Interactions and SFV Zoonotic Transmissions

SFV zoonotic transmissions have been reported in the Americas, Africa, and Asia. In some regions, interactions with monkeys are a part of daily life (see Chap. 2). Globally, the types of human and monkey interactions that are observed are diverse. In many countries across Africa, NHP represent an important source of bushmeat and they are valued for both food and income. Recent estimates indicate that over 330 million pounds of NHP bushmeat is killed each year in Central and West Africa alone (Switzer et al. 2016). Thus, in certain parts of Africa, where people depend on NHP for their livelihood and subsistence, human interactions often involve hunting and butchering NHP. People across Africa, as well as in Central and South America and Asia, can also be exposed to NHP in other natural settings including villagers who cohabit with NHP, people who keep monkeys as pets, and ecotourists visiting natural NHP habitats. Occupational exposures also occur in in these regions, such as individuals who work at primate centers, laboratory workers who handle NHP blood, and zoo and sanctuary workers who care for captive NHP. Asia, where the majority of interactions are nonoccupational, has some additional human–NHP interactions. For example, in some Asian cultures and religions, monkeys are revered. As a result, a large number of human–NHP interactions occur in temples and religious sites, where monkeys are free ranging. Many urban centers in Asia also have free-range monkeys that often enter homes, either as pets or while scavenging for food. Exposures to NHP in natural settings, such as these, are of particular concern because they represent situations in which SFV zoonotic transmission can occur. In contrast, North Americans and Europeans are typically limited to occupational interactions with NHP in research, zoo, and lab settings, while less frequently as pets. It should be noted that Western tourists make up a significant proportion of visitors to monkey temples in Asia and bites and scratches are common in this population (Engel et al. 2006).

The natural range of NHP determines which types of NHP humans encounter in a given area. For example, in Africa, gorillas, chimpanzees, and *Cercopithecidae* are the most commonly documented types of great apes and OWM that interact with humans. In contrast, in many Asian countries (including Thailand, Indonesia, Nepal, India, Cambodia, Vietnam, China, Japan, Philippines, and Bangladesh), monkeys, specifically macaques and langurs which inhabit the temples, villages, and cities, are in frequent contact with humans. Although apes such as gibbons and orangutans are found in Asia, their contacts with humans are much more limited. Central and South America are home exclusively to NWM, and here again contact between humans and the monkeys is frequent, especially in the context of pet ownership and

ecotourism. Since the late 1930s, an introduced population of rhesus macaques has grown in some areas of the state of Florida in North America. The population is currently estimated at 1000+ monkeys (Wisely et al. 2018). In addition to this free-ranging population, primate pet ownership (e.g., macaques, NWM, vervets, and pattas monkeys) is also common in North America.

Humans have not been found to be persistently infected with FFV or BFV. However, a number of people are known to be persistently infected with SFV. Since the major SFV transmission route is saliva-to-blood, humans who closely interact with NHP and may be bitten and/or scratched are at high risk for SFV infections. Several groups have studied human populations who interact with NHP and the results are summarized in Table 10.1.

Individuals who directly interact with NHP through occupational exposures in research laboratories or hunting/butchering activities have a ca. 2–5% risk of becoming persistently infected with SFV (Table 10.1). Finding SFV-infected individuals in this group is not unexpected, as these people often encounter NHP saliva and/or blood. While SFV transmission from an infected NHP to a human via blood has not been directly documented, it is known that SFV can be transferred from

Table 10.1 Examples of simian foamy virus zoonotic infections

Location	Risk factor (s)	No. of individuals sampled	% persistently infected	SFV source	Reference
North America (US and Canada)	Occupational exposure in zoos, research labs, breeding facilities, etc.	597	2.3	Baboon, chimpanzee, macaque, *Cercopithecus* sp.[1]	Heneine et al. (1998), Sandstrom et al. (2000), Brooks et al. (2002) and Switzer et al. (2004)
Africa (Cameroon and Gabon)	Occupational exposure (hunters, butchers)	1460	4.4	Mandrill, chimpanzee, gorilla, *Cercopithecus* sp.	Wolfe et al. (2004), Calattini et al. (2007), Betsem et al. (2011) and Mouinga-Ondeme et al. (2012)
	Residing near NHP[a] populations	2485	0.24	Gorilla, chimpanzee, *Cercopithecus* sp.	Calattini et al. (2007) and Betsem et al. (2011)
Asia (Bangladesh Thailand, Indonesia, and Nepal)	Residing near NHP populations	514	2.9	Macaques	Jones-Engel et al. (2005, 2008) and Engel et al. (2013)

[a]Nonhuman primates
[b]This genus is comprised of at least 26 species of Old World monkeys

infected to uninfected macaques through blood transfusion (Khan and Kumar 2006; Brooks et al. 2007). Thus, it is an open question as to whether humans can be zoonotically infected with SFV directly via exposure to infected blood and whether SFV positive humans could transfer SFV to other humans via blood transfusion.

Interestingly, people who are not occupationally exposed to SFV but rather reside in areas naturally cohabited by NHP also have a high risk of SFV persistent infections, ranging from ca. 0.2–3% (Table 10.1). These rates of zoonotic infections suggest that SFV could potentially impact a significant number of people worldwide.

Investigators have studied people in Central Africa as well as in Asia who cohabit areas with NHP. As seen in Table 10.1, the percentage of Asian individuals persistently infected with SFV was about 10 times higher than the percentage of persistently infected Africans. This could result from differences in the human populations, the NHP they encounter, the SFV strains transmitted, or the types of human–NHP encounters that occur. In Asia, most interactions which resulted in exposure to SFV followed contact with macaques (Engel et al. 2013; Jones-Engel et al. 2008), while in Africa people mostly interacted with monkeys, such as *Cercopithecus* sp. as well as great apes (Wolfe et al. 2004; Calattini et al. 2007; Betsem et al. 2011; Mouinga-Ondeme et al. 2012). Of note, more Africans were infected with ape SFV (primarily gorilla) than monkey SFV despite documented interactions with both (Betsem et al. 2011). It should be noted that bites from gorillas and chimpanzees are likely associated with more tissue damage than bites from monkeys. In contrast, in Asia SFV zoonotic transmission from monkeys is higher than what would be expected from the African data. Genetic differences in innate immunity or acquired immunity in these populations may contribute to this continental differential susceptibility or these differences could be attributed to the lower density of apes in Asia. An interesting finding is that there is a seminomadic, ethnically homogenous population of humans in South Asia who appear not to be infected by macaque SFV, as measured by both antibodies and PCR (Craig et al. 2015). This group, called the Bedey in Bangladesh, has an intense and long-term association with macaques. A recent study of the Bedey has shown that about 90% of those interviewed about their exposures reported severe, scarring bites following lifelong exposure to the macaques, yet none of the individuals showed persistent infection with SFV (Pinto-Santini et al. 2017). It is not known why the Bedey appear resistant to SFV infection but this warrants further study. This population may give insights into why some humans are persistently infected while others are not, despite similar interactions with NHP.

Table 10.1 does not include any cases of humans persistently infected with NWM SFV. Two research groups examined humans who were occupationally exposed to SFV-infected NWM, either in captivity in North America or in the fields in Central and South America (Stenbak et al. 2014; Muniz et al. 2017). It was found that ca. 12–18% of these people had antibodies to NWM SFV, indicating exposure to the virus. However, of the 18 seropositive individuals examined, none were SFV PCR-positive. Thus, to date there is no published evidence for persistent infection of humans by NWM SFV. The possibility that the humans are able to clear NWM SFV infections before persistence can be established is intriguing but will require further study with larger groups of exposed individuals.

To date, person-to-person transmission of SFV infections has not been documented. Family members of SFV persistently infected humans have been examined, in some cases over several decades, and none have been shown to be infected (Calattini et al. 2007; Betsem et al. 2011). In fact, studies on SFV persistently infected humans have failed to detect viral replication in oral mucosal tissues using sensitive assays for detection of viral RNA synthesis (Rua et al. 2013; Soliven et al. 2013; Engel et al. 2013), which is consistent with the lack of human-to-human transmission. However, there is indirect evidence that a low level of SFV replication occurs in humans (reviewed in (Pinto-Santini et al. 2017)). First, in SFV-infected humans there is approximately 1 provirus in 10^4 PBMC (Stenbak et al. 2014). This level of provirus cannot be explained by the amount of virus transferred directly from an NHP bite. Second, in some SFV-infected humans, the proviral strains varied over time, suggesting that some strains replicate better in humans than others (Engel et al. 2013). Third, the innate immune factor APOBEC was shown to modify SFV proviral DNA in humans (Matsen et al. 2014). Since APOBEC modification occurs only during reverse transcription, this suggests that some reverse transcription and thus viral replication occurs in humans. It is not clear where within the host this viral replication is occurring, or at what frequency, but this information would help define the risk of human-to-human SFV transmission and identify potential preventative measures.

Co-infection with SFV from more than one NWM host species (as measured by SFV *pol* gene sequences) has been documented in NHP (Leendertz et al. 2008; Liu et al. 2008). However, there is no evidence to date of humans infected with a recombinant SFV derived from such a coinfected animal. Humans in Asia have been shown to be coinfected with viral *gag* gene variants of SFV isolated from a single NHP host species, suggesting infection by more than one SFV strain in these people (Feeroz et al. 2013; Engel et al. 2013). Given the ability of SFV to recombine during replication and the documented cross-species and zoonotic SFV infections, it remains possible that a recombinant SFV could be introduced or be generated in a human host and events similar to those that led to the emergence of the retroviral pathogen HIV (Gao et al. 1999) could occur for SFV.

Given the many reports of SFV zoonoses, it is of interest to determine whether SFV infections place humans at a higher risk for pathogenic effects of other viruses. As discussed in Sect. 10.7, SFV coinfection with lentiviruses can increase lentivirus pathogenesis in macaques. The question arises whether this is also true in humans. There are two reports of humans in Africa coinfected with SFV and HIV but no information is available about the consequences of such coinfections (Switzer et al. 2008; Switzer et al. 2016).

10.9 Goals for Current and Future SFV Research

FV are complex retroviruses with large genomes and a broad host range. Additionally, FV are not known to be pathogenic. These characteristics make them attractive for the development of human gene therapy vectors and such work is underway

(reviewed in (Trobridge 2009)). In one report, foamy virus vectors were used to treat dogs with a genetic disorder (Bauer et al. 2007) and monitoring of these dogs for several years after gene therapy treatment with SFV vectors found no unintended pathogenic outcomes (Bauer et al. 2013). The positive results from this work, and others, encourage development of FV vectors to treat human genetic diseases.

Reverse transcription, which is error-prone and can lead to viral mutation, is an integral step in the replication of retroviruses, including foamy viruses. It has also been shown that viral recombination often occurs during retroviral replication. These genetic changes associated with FV RT contradict the documented high levels of genetic stability and strong coevolution of SFV seen in natural hosts and represents a conundrum that remains to be resolved. Further understanding of SFV replication in natural and zoonotic hosts may shed light on this point.

Foamy virus mutation and/or recombination raise the possibility of the emergence of viruses with different, potentially pathogenic properties. Such viruses could become pathogenic in natural NHP hosts and/or zoonotically infected humans. It is clear that in the case of lentiviruses, recombination between minimally pathogenic OWM viruses in chimpanzee hosts eventually led to the emergence of the human pathogen HIV-1 (Sharp and Hahn 2010). It is thus important to continue monitoring SFV-infected NHP and humans for possible viral-induced pathology. In the event that a human FV pathogen does emerge, it is worth noting that many of the antiretroviral drugs designed to treat HIV are not as effective against PFV (Yvon-Groussin et al. 2001). However, the RT inhibitor AZT was found to be efficacious against PFV in cell culture and may have potential as a treatment for human infections (Yu et al. 1999; Rosenblum et al. 2001) although this remains to be explored.

Finally, because foamy virus coinfections appear to increase pathogenicity of lentiviruses in NHP, it is important to monitor SFV-infected humans who are also infected by other viruses. Many humans are persistently infected with viruses such as cytomegaloviruses (CMV) and herpesviruses (HSV-1/2), (see Chap. 8) and/or experience transient infections by viruses such as rhinoviruses (RV A/B) and adenoviruses (AdV). These infections can range from nonpathogenic to highly pathogenic. However, the effect of SFV infection in the context of other viral infections is unknown and should be monitored for potential enhancement of pathogenicity.

10.10 One Health and SFV, the most Commonly Zoonotically Transmitted Retrovirus

FV infect many different vertebrate genera but only NHP foamy viruses have been shown to infect humans. SFV phylogenetics reveals genome conservation and strong cospeciation with their NHP hosts (Fig. 10.3). Far more species of monkeys exist than apes and, as a result, SFV from monkeys are more numerous than SFV from

apes. Given the large number of monkey species, some of which are sympatric, opportunities for SFV cross-species transmission, coinfection, and potential recombination exist in natural settings. While only a few such events have been documented to date, the possibility remains for a rare coinfection and recombination event to be transmitted to humans and ultimately lead to development of a human pathogen This scenario, while unlikely, has already been documented in the emergence of the human retroviral pathogen HIV.

It is important to note that SFV are the retroviruses most frequently zoonotically transmitted to humans from NHP. As a retrovirus, genetic changes caused by mutations occur during SFV replication, providing a mechanism for potential rapid adaptation of SFV to a human host. Taken together, these factors indicate that SFV have the potential to bridge the human–NHP interface and spill over into the human population as a novel pathogen (Engel et al. 2013). It is thus important to monitor humans and NHP with which they interact to detect potentially pathogenic variants that might emerge.

A variety of highly efficacious drugs against HIV that target the RT and protease (PR) enzymes exist, but the majority of these drugs do not appear to inhibit PFV replication in vitro (Yu et al. 1999; Yvon-Groussin et al. 2001; Hartl et al. 2010). Consequently, the current treatments that have helped manage HIV epidemics are not likely to help control a potential FV outbreak. Rapid development of efficacious drugs specific to SFV may be required to prevent a pandemic from arising.

Continued study of foamy viruses in both NHP and humans is necessary for our ability to detect and prevent the emergence of a potential human pathogen. Among the most compelling reasons to warrant such studies are the frequent transmission of SFV to humans, the NHP origins of the retroviral human pathogen HIV, and evidence that SFV coinfection with lentiviruses increases lentiviral pathogenicity in NHP. Given the number and variety of NHP–human interactions that occur around the world, global monitoring of both NHP and humans for FV infections and development of anti-FV treatments are encouraged.

Glossary

Acquired immunity: The development of specific antibodies upon exposure to a pathogen or foreign agent, through vaccination or infection. After infection with a virus, individuals often produce antibodies to the virus that did not exist before infection and are adapted to better respond to subsequent infections with the same virus.

Antibodies: Proteins produced by host B cells that can bind to and inactivate (neutralize) pathogens such as viruses or bacteria.

Bet: A foamy virus accessory protein of unknown function that is not found in virus particles. Foamy viruses lacking Bet can replicate but not as well as the wild type.

Buccal swabs: A noninvasive technique used to collect samples from an individual's inner cheek, which will include host cell DNA as well as foamy viruses.

Complex retrovirus: A retrovirus that encodes the highly conserved Gag, Pol, and Env protein products, as well as additional accessory proteins with various functions.

Env: A retroviral-encoded protein found in the outer layer of a virus particle. It is used to bind viral receptors present on the host cell surface.

Foamy virus: A complex retrovirus whose virion contains prominent spikes on the surface and a central but uncondensed core. Although RNA is packaged, reverse transcription occurs during virus assembly, leading to infectious virions containing DNA genomes.

Gag: A retroviral-encoded protein that forms the viral capsid.

Innate immunity: Nonspecific defense mechanisms present in host organisms prior to infection by viruses or other pathogens. These responses develop soon after infection and do not adapt to the pathogen over time.

Internal promoter (IP): A foamy virus-specific sequence located at the 3′ end of the envelope gene required for transcription of the accessory genes *tas* and *bet*. All other retroviral genomes only contain one promoter located in the 5′ LTR.

Latent infection: This occurs when a viral genome is maintained within a host cell but it is dormant and does not produce new viruses.

Lentivirus: A complex retrovirus with cylindrical or conical virion cores. Their RNA genomes express *gag, pro, pol*, *and env* genes, as well as accessory genes. Some lentiviruses are highly pathogenic, such as HIV-1.

LTR: A long terminal repeat sequence, found at both ends of an integrated retroviral genome. The 5'LTR contains DNA promoter sequences required for transcription of the viral genome while the 3'LTR contains the termination and the poly-adenylation signals.

NHP: Nonhuman primate

NWM: New World monkeys, found in Central and South America.

Orthoretrovirus: A subfamily of the Retrovirus family that include most retroviruses. These viruses all have RNA genomes.

OWM: Old World monkeys, found in Asia and Africa.

PBMC: Peripheral blood mononuclear cells; these are nucleated cells found in blood and include lymphocytic cells such as T and B cells and monocytic cells, such as macrophages.

PCR: Polymerase chain reaction is a technique that allows a researcher to detect, amplify, and sequence DNA. This method amplifies target DNA sequences at least a thousand times to allow easy manipulation.

Persistent infection: An infection that lasts for a long period of time and is not cleared by host antibodies.

Pol: A retroviral-encoded polymerase that has reverse transcriptase activity to synthesize DNA from RNA.

Productive infection: An infection by a virus, or other microorganism, that allows the virus to replicate and produce infectious progeny. Viral productive infections are often pathogenic.

Promoter: A DNA sequence that allows RNA polymerase to initiate transcription.

Retroviruses: A family of viruses that generally contain a plus-strand viral RNA genome in the virus particles. A retrovirus also encodes an enzyme called reverse transcriptase that converts the viral genomic RNA into double-stranded DNA. Using another viral enzyme, called integrase, the resulting DNA is integrated permanently into a host cell chromosome. Retroviruses are divided into two subfamilies: Spumaretrovirus and Orthoretrovirus.

Reverse transcriptase: An enzyme, encoded by retroviruses, that uses an RNA molecule to synthesize DNA.

SIV: Simian immunodeficiency virus is a lentivirus highly related to HIV that naturally infects some African monkeys. It is not found in Asian OWM or NWM.

Spumaretrovirus: A subfamily of the Retrovirus family that include viruses that contain DNA genomes. The only well-characterized Spumaretrovirus is the foamy virus.

Tas: Trans activator protein produced by foamy viruses to increase RNA transcription from the viral promoters. The Tas protein is an accessory protein not found in foamy virus particles.

Transient infection: An infection that only lasts for a limited time, usually the host immune response clears the virus.

Viral recombination: The production of a viral genome containing information from more than one parental virus. In a population of viruses, individual virus particles can differ in their genomic sequences (viral variants). If a cell is infected by more than one viral variant, interactions between variants can occur so that new variants are produced that contain genetic information from both parental viruses.

Virus Receptor: A host cell molecule, usually a cell membrane-associated protein, which viruses use in order to bind to and enter host cells.

Western blot: A technique that allows a researcher to determine the presence and size of a protein(s) that react with a specific antibody. Samples containing proteins are separated by size, immobilized on a membrane, and exposed to antibodies for detection.

Zoonotic infection: A human infection produced by a microbe that naturally infects nonhuman hosts.

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Chapter 11
Rabies in Nonhuman Primates and Potential Risks for Humans

Philippe Gautret

Abstract New World and Old World nonhuman primates (NHPs) are experimentally susceptible to rabies infection as expected for every mammal. The number of rabies cases reported in free-ranging NHPs is rare compared with humans. Marmosets have been demonstrated to act as a reservoir for maintaining a rabies virus variant in Brazil but no other NHPs are known to be a reservoir for maintaining a rabies virus variant in the wild in other regions. Documented cases and subsequent transmission to humans have been reported in South America, Africa, and Asia following contact with infected pet or free-ranging NHPs. International travelers often unfamiliar with NHP behavior have reported NHP-related injuries following contact with monkeys. Little is currently known of the pathobiology of rabies virus shedding in NHPs, which implies that rabies post-exposure prophylaxis and administration of rabies immunoglobulin should be considered in patients with a possible exposure. Large-scale studies aiming at surveying rabies circulation in NHP populations are needed.

Keywords Animal model · natural infection · zoonosis · bites · rabies post-exposure prophylaxis · RABV · New World primates · Old World primates

11.1 Introduction

Neglected tropical diseases represent a diverse group of communicable diseases that prevail in tropical and subtropical areas affecting more than one billion human beings (http://www.who.int/neglected_diseases/diseases/en/). Rabies is among these diseases, the one which causes the most human deaths with an estimated

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60,000 human deaths per year, with the vast majority occurring in Africa and Asia (World Health Organization 2013). Rabies is inevitably lethal in humans once clinical symptoms occur, but 100% preventable during its incubation period with post-exposure prophylaxis (PEP). The latter involves administration of rabies immunoglobulin (RIG) and a multi-dose course of rabies vaccination. Dogs, the principal disease reservoir for rabies in humans, are responsible for >99% of human cases (World Health Organization 2013). Mass vaccination of animal reservoirs reduces the risk of human exposure and can ultimately result in rabies virus elimination. Therefore, the prevention of human rabies deaths is an example of the value of One Health interventions (Cleaveland et al. 2017). All mammals are susceptible to infection, but few are capable of acting as reservoirs for the disease because spillover infections normally result in dead-end infections with no further spread to other hosts despite clinical disease. The *Lyssavirus* genus currently comprises 14 different rabies virus species, with the classical rabies virus (RABV) extensively present in terrestrial mesocarnivores across the globe and bat species in the Americas, and both are responsible for most cases in humans and animals (Fooks et al. 2014). Other rabies viruses are detected mainly in Old World bat species and rarely in non-flying species (World Health Organization 2013; Fooks et al. 2014). Humans may be exposed to nonhuman primate (NHP)-related injuries that are potentially at risk of rabies transmission. This chapter discusses data on experimental and naturally occurring rabies in NHPs and its possible transmission from NHPs to humans.

11.2 Diagnosis of Rabies

In humans, on average, two to three months following a bite from an infected animal excreting the virus in its saliva, the patient will usually present with symptoms of acute progressive encephalitis (classic furious form) including fever, fatigue, myalgia, headaches, and insomnia (World Health Organization 2013). Episodes of psychiatric disorders including confusion, anxiety, agitation, hallucination, and hyperactivity alternate with lucidity. Some patients also present with sore throat, anorexia, nausea, vomiting, and diarrhea. Some symptoms including persistent pain, paresthesia, or pruritus at the site of infection, hydrophobia with hypersalivation, and aerophobia should lead to a high degree of suspicion of rabies. The patients then suffer gradual paralysis, with coma and respiratory arrest. Seizure may occur. The paralytic form of the disease affecting around 20–30% of patients differs from the furious form. It is characterized by paralysis with an early development and slow progression toward coma and death.

A comprehensive clinical description of natural rabies in NHPs is lacking, although the furious form and paralytic form have been mentioned in early reports.

A confirmed diagnosis of rabies in NHPs can be made post-mortem through the detection of RABV from the brain. Usually, both brain stem and cerebellum biopsies are performed. Direct fluorescent antibody testing (Fig. 11.1), immune-histochemistry methods, detection of Negri bodies by histological examination or electron

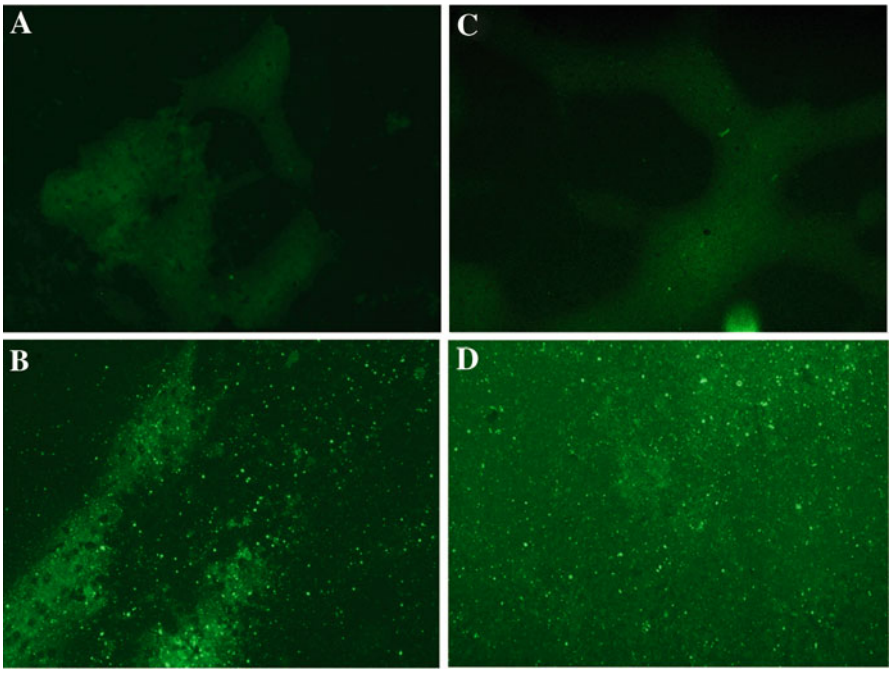

Fig. 11.1 Fluorescence antibody test (FAT). Sections of brain impressions, stained with polyclonal anti-rabies fluorescein isothiocyanate conjugate. (**a**) negative control; (**b**) positive control; (**c**) negative brain from dog; (**d**) infected brain from dog (Courtesy of Hervé Bourhy and Florence Larrous, Pasteur Institute, Paris, France)

microscopy, amplification of RABV RNA, or virus isolation in cell cultures (Fig. 11.2) can be performed.

11.3 Susceptibility of NHPs to Experimental Infection with RABV

NHP rabies models have been rarely used because well-developed and more economical small animal models were available. The majority of experimental rabies infections in NHPs have been conducted in *Macaca* species for the purpose of vaccine experiments (Baer 1988). Different species of macaques have been used as experimental models since the 1970s. *Macaca mulatta* (rhesus macaque) were imported from India to the United States (Sikes et al. 1971; Lavender 1973; Baer et al. 1977; Baer et al. 1979) and *M. fascicularis* (long-tailed macaques) from Malaysia to Germany (Weinmann et al. 1979; Hilfenhaus et al. 1975; Hilfenhaus et al. 1977). These animals showed high susceptibility to RABV experimental infection with high rates of mortality, although some unvaccinated animals survived the infection.

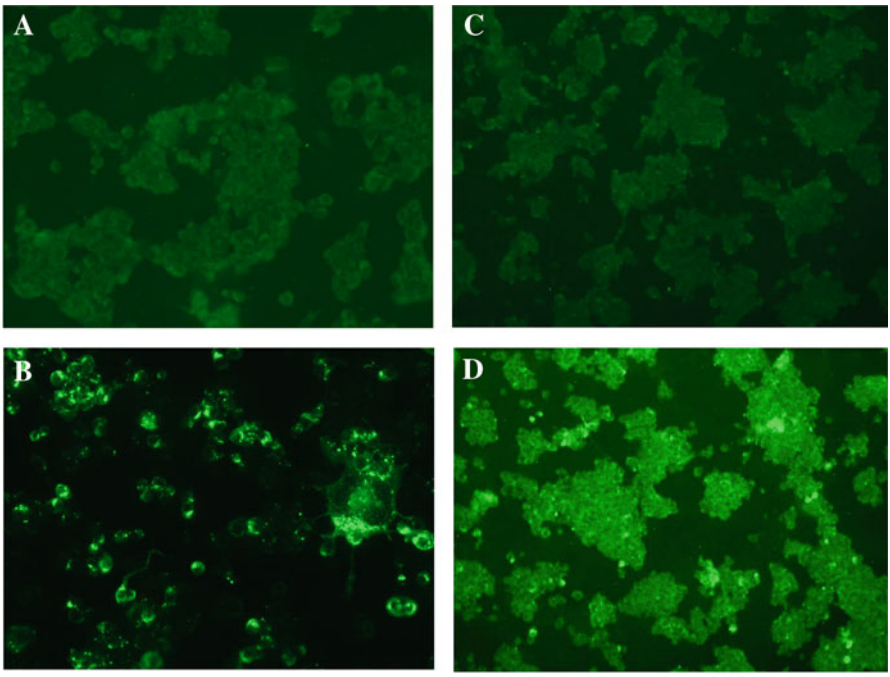

Fig. 11.2 Rapid tissue culture infection test (RTCIT). Brain suspension homogenate supernatants incubated with neuroblastoma cell layer, stained with polyclonal anti-rabies fluorescein isothiocyanate conjugate. (**a**) negative control; (**b**) positive control; (**c**) negative brain from dog; (**d**) infected brain from dog (Courtesy of Hervé Bourhy and Florence Larrous, Pasteur Institute, Paris, France)

For example, among 24 infected rhesus macaques, there were 4 survivors in one experiment (Sikes et al. 1971) while none survived among 23 infected rhesus macaques in another (Baer et al. 1979) and among 50 infected long-tailed macaques, 4 survived over a 4-month follow-up (Weinmann et al. 1979; Hilfenhaus et al. 1975). Such macaque models are still in use for the purpose of testing experimental rabies DNA vaccine (Lodmell et al. 1998; Lodmell et al. 2001; Lodmell et al. 2002) or adenovirus vector vaccine expressing RABV glycoprotein (Xiang et al. 2014) and for studies of circuitry by researchers in the field of neuroscience where rabies virus is used because of its properties as a neuronal tracer (Miyachi et al. 2005; Rathelot and Strick 2009; Dum and Strick 2013). Other macaque species, including South Indian *M. radiata* (bonnet macaque), infected with RABV have also been used for testing DNA vaccine (Biswas et al. 2001).

More recently, New World monkey models of rabies have been developed. *Callithrix* species (marmosets) infected with RABV were used for vaccine studies in Brazil (Andrade et al. 1999). A model using *Cebus apella* (tufted capuchin monkey) infected with RABV was used by US researchers for the purpose of studying neuronal connections (Kelly and Strick 2000). Finally, *Aotus nancymaae* (night owl monkey) infected with RABV was proposed as a new model for the

assessment of post-infection immune response by US and Peruvian researchers (Reaves et al. 2012). In this experiment, only two out of eight infected monkeys developed rabies over 134 days of follow-up.

These models demonstrate that New World and Old World NHPs are at least experimentally susceptible to rabies infection, as expected for every mammal. NHPs in these experiments were infected by injection of RABV suspension generally into cervical or masseter muscles, which is consistent with natural transmission where the virus is usually inoculated into the muscles through a bite. The dose of viruses used in these experiments was calculated as the 10^5–10^7 mouse intra-cerebral 50% percent lethal dose and may be higher than the viral inoculum in natural transmission which is unknown. Although some NHPs will stay asymptomatic and survive, most will develop clinical rabies with high mortality rates and salivary excretion of viruses, so that they may theoretically transmit the disease through bites, based on the observation that mice injected with salivary gland preparation from infected monkeys develop rabies.

11.4 Reports of Natural Infection in NHPs (Figs. 11.3, 11.4, and 11.5)

Cases of zoonotic rabies typically occur following intentional or inadvertent human contact between humans and rabies-infected dogs and other domestic animals. Though humans often come into contact with free-ranging NHP populations in habitat countries (see Chap. 2 Fuentes) and bites and scratches are not uncommon, there are very few confirmed reports of free-ranging NHP-to-human transmission of rabies. There is no large-scale study addressing the seroprevalence of rabies in NHP populations.

11.4.1 South America, Central America, and the Caribbean

The Regional Information System for Epidemiological Surveillance of Rabies (SIRVERA) is a database that started reporting occurrences of rabies in humans and animals in 1969 (available at http://sirvera.panaftosa.org.sbr/login, accessed 4 April, 2019). Until 1998, species of rabid wild animals were not included. From 1999 to 2016, 66 cases of rabies were reported in NHPs, according to SIRVERA database. No detailed information is available about the reason why these NHPs were screened for rabies, but it is likely because they were responsible for biting humans. Cases were reported from Cuba, the Dominican Republic, Columbia, Ecuador, Brazil, Peru, Bolivia, Paraguay, and Argentina. No information is available about the NHP species and no information is provided about the diagnostic criteria that were used. Despite its limitation, data from SIRVERA suggest that rabies has

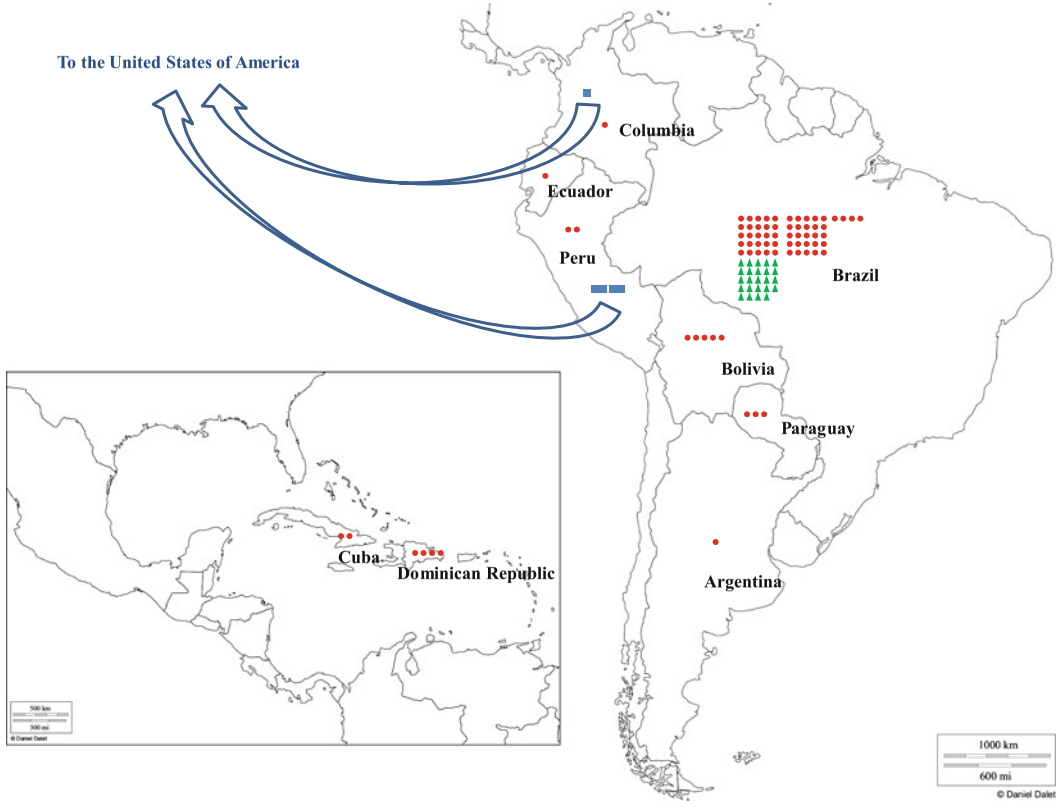

Fig. 11.3 Distribution of natural rabies infection cases in NHPs in Latin America (dots), imported cases of rabid NHPs from Latin America (squares), and rabies cases in local human populations attributed to NHP exposure in Latin America (triangles) (Base map is available free of charge at http://www.histgeo.ac-aix-marseille.fr/ancien_site/carto/)

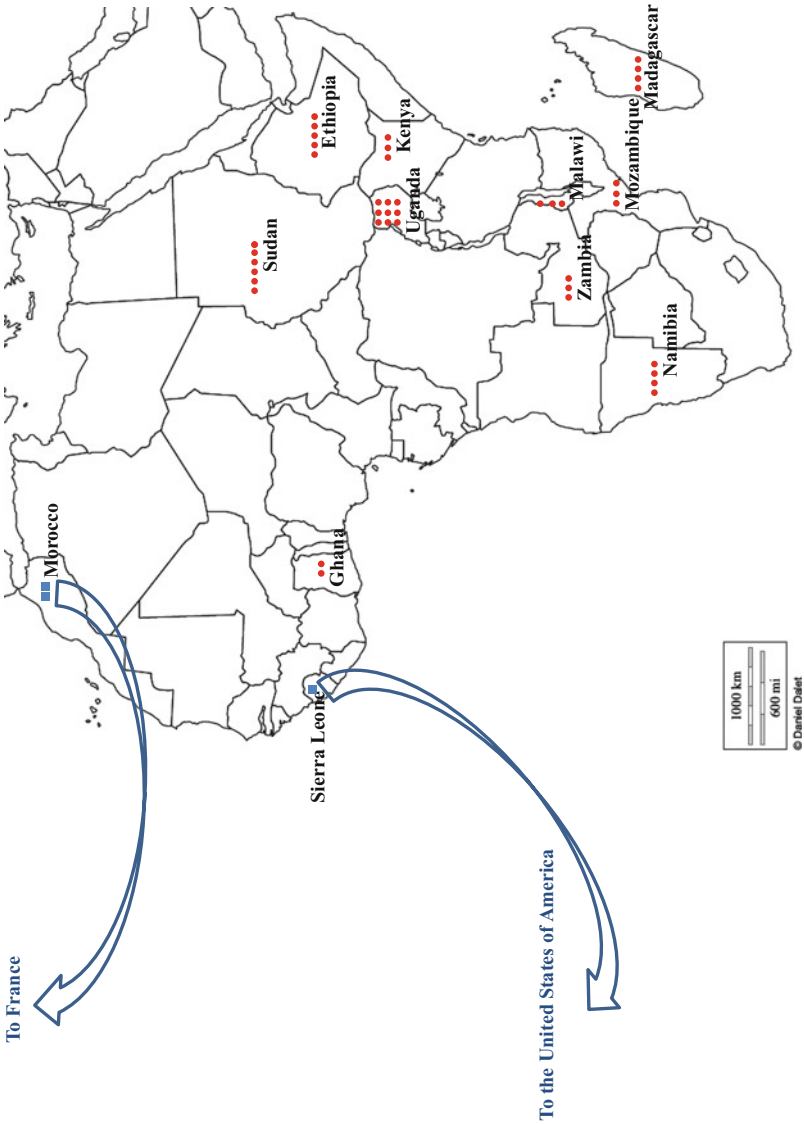

Fig. 11.4 Distribution of natural rabies infection cases in NHPs in Africa (dots) and imported cases of rabid NHPs from Africa (squares) (Base map is available free of charge at http://www.histgeo.ac-aix-marseille.fr/ancien_site/carto/)

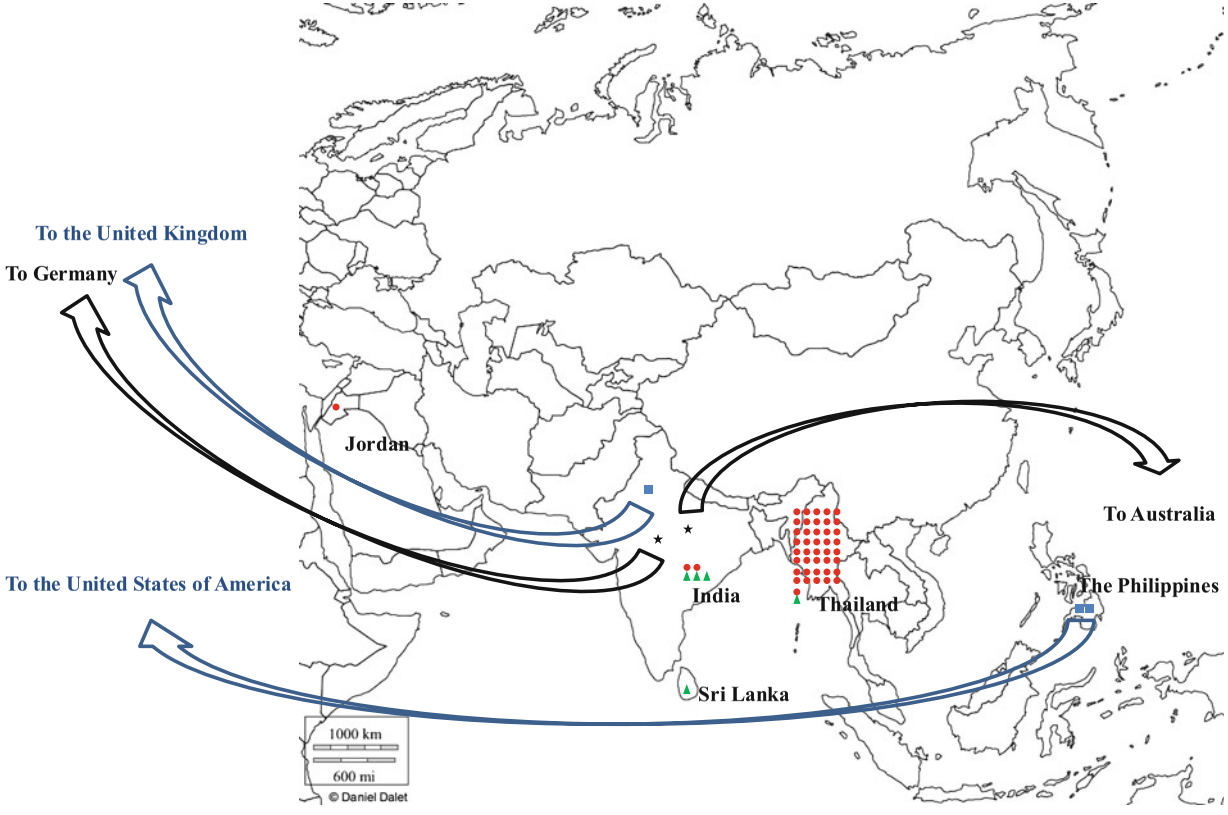

Fig. 11.5 Distribution of natural rabies infection cases in NHPs in Asia and the Middle East (dots), imported cases of rabid NHPs from Asia (squares), rabies cases in humans attributed to NHP exposure (triangles), rabies cases in local human populations attributed to NHP exposure in Asia (triangles), and imported cases of rabies in international travelers attributed to exposure to NHPs in Asia (stars) (Base map is available free of charge at http://www.histgeo.ac-aix-marseille.fr/ancien_site/carto/)

been occasionally reported from most countries on the continent. It should be noted that 72.7% of cases in the database were reported from Ceará, a state, in north-eastern Brazil, where eight cases of rabies in humans following exposure to *Callithrix jacchus* (white-tufted-ear marmoset) were reported from 1991 to 1998 (Favoretto et al. 2001). In this cluster of eight cases, the exposure occurred during attempts to capture the animal in six cases while one animal approached the house and attacked the owner and one animal was raised as a pet. A new RABV variant was isolated in 1998, from two rabid patients bitten by marmosets and from a rabid marmoset. Comparative phylogenetic analyses showed that the Ceará viruses segregated in a group which included RABV genetic variants circulating in bats in the Americas and formed an independent clade (Favoretto et al. 2001; Batista-Morais et al. 2000). The new RABV variant was further isolated from a rabid marmoset in the state of Ceará in 2001 (Favoretto et al. 2006; Aguiar et al. 2011) and from a rabid patient exposed to a marmoset in the neighboring Piaui state in 2001 (Favoretto et al. 2013). In the technical records of the National Program for Rabies Control and Prophylaxis and Pasteur Institute reference laboratory in Sao Paulo, Rocha et al. report 52 cases of confirmed rabies in NHPs from 2002 to 2012. These cases included 51 cases in marmosets from Ceará and one in a *Cebus* species from Mato Grosso with no detailed information about the status of these NHPs (pets, sanctuary, or free-ranging animals) (Rocha et al. 2017). No data are available post-2012. These data strongly suggest that the marmoset RABV virus variant represents a unique, independent rabies endemic and sylvatic cycle. A study conducted in the urban population of Fortaleza, Ceará, evidenced close relations between humans and their pet marmosets and minimal knowledge regarding rabies. Additionally, three out of 11 pet marmosets from a single house were found positive by direct antibody fluorescent testing in brain samples (Aguiar et al. 2011). A RABV variant has been isolated from one specimen of *C. apella* (tufted capuchin monkeys) captured in Mato Grosso, forming a lineage distant from that of marmoset RABV within the bat-related rabies virus cluster (Kobayashi et al. 2013). Overall, from 1990 through 2012, 54 confirmed cases of rabies were observed in NHPs, and 24 cases of NHPs to human transmission of rabies were reported in Brazil with no epidemiological link between cases.

Additionally, a sero-survey conducted in 291 marmosets in Ceará from 2003 to 2013 evidenced 19.2% antibody prevalence with no information about the status of these NHPs (pets, sanctuary, or free-ranging animals) (Cordeiro et al. 2016). Antibodies against rabies have also been found in four out of 36 free-ranging *C. apella* (tufted capuchin monkeys) in south-eastern Brazil in the state of Sao Paulo (Machado et al. 2012). It should be noted, however, that antibody production may have possibly resulted from infection with a Lyssavirus spp. different from marmoset RABV variant that can only be differentiated from bat variants by sequencing.

Historically, one case of rabies was documented in a *Cebus* species monkey imported from Colombia to the United States in 1947 by Negri body on microscopic examination of brain and rabies induced in mice inoculated with brain tissue (Richardson and Humphrey 1971). Three rabies cases were documented in *Saimiri sciureus* (squirrel monkeys) imported from Peru to the United States in the early

1960s by fluorescent rabies antibody testing of brain samples and rabies induced in mice inoculated with brain tissue in all cases, and Negri body on microscopic examination of brain in one case (Richardson and Humphrey 1971). Finally one case was observed in 1974, in a *Saguinus nigricollis* (black mantled tamarin) imported from Peru to the United States by fluorescent rabies antibody testing of brain samples and rabies induced in mice inoculated with brain tissue. The infection in the latter case was very likely vaccine induced given the rabies characteristics in inoculated mice (Aaron et al. 1975). None of these cases resulted in human infections.

11.4.2 Africa

The data in the medical literature about rabies in African NHPs are very limited and often incomplete with little information on the context (pet, free-ranging, captive, laboratory, etc.) or species. Overall, confirmed rabies was reported in 19 NHPs by fluorescent rabies antibody testing of brain samples and/or rabies induced in mice inoculated with brain tissue. Rabies was confirmed in a *Chlorocebus aethiops* monkey (vervet monkey), a lemur *Otolemur crassicaudatus* (bush baby), and one NHP of unknown species in Zambia (Röttcher and Sawchuk 1978). Rabies was diagnosed in three monkeys (unknown species) in Ethiopia (Fekadu 1982), two monkeys (unknown species) in Ghana (Addy 1985), seven monkeys (unknown species) in Sudan (Ali et al. 2006), and two monkeys (unknown species) and two baboons in Namibia (Magwedere et al. 2012). Meeting reports of the Southern and Eastern African Rabies Group (SEARG) (available at https://www.mediterranee-infection.com/acces-ressources/donnees-pour-articles/searg-reports/, accessed 04 April 2019) provide evidence for 25 additional cases of rabies in NHPs over the years 1986–2013, in a number of African countries, including Ethiopia, Kenya, Madagascar, Malawi, Mozambique, Uganda, and Zambia. However, wildlife animal testing is not performed on a regular basis in a number of countries; the type of rabid wildlife animal is often not reported in these reports and the primate species is generally not documented except in a few instances.

In addition, one case was reported in a chimpanzee imported from Sierra Leone to the United States in 1972 with confirmed diagnosis by fluorescent rabies antibody testing of brain samples and rabies induced in mice inoculated with brain tissue (Miot et al. 1973) and two cases were confirmed by PCR on brain material in *M. sylvanus* (Barbary macaques) imported from Morocco to France in 1989 with a probable vaccine-induced infection (Gautret et al. 2014).

Unfortunately, no detailed information about the status of these NHPs (pets, sanctuary or free-ranging animals) is available in these reports.

11.4.3 Asia and the Middle East

In a surveillance survey conducted in Thailand from 1993 to 1996, 41 NHPs out of 511 tested were positive for rabies, including 33 monkeys and eight gibbons (Panichabhongse 2001). However, it is unclear if these NHPs were pets or free-ranging animals, and in this surveillance report, it is not clear if the data represent active or passive surveillance. Furthermore, the diagnosis method is not provided in this report. It, therefore, cannot be excluded that these NHPs might have been positive by serology because they were vaccinated pets tested following injuries caused to humans. Recently, two cases of rabid NHPs were identified in Shimla municipality in India, one in a *M. mulatta* (rhesus macaque) and one in a *Semnopithecus entellus* (Gray langur) (Bharti et al. 2015; Bharti 2016). It should be noted, however, that the diagnosis method is not mentioned in these reports. A rabid *M. mulatta* (rhesus macaque) was imported to London in 1965 for laboratory experiments (Boulger 1966). Unfortunately, no detailed information about the status of these NHPs (pets, sanctuary, or free-ranging animals) is available in these reports. Two rabid animals were imported from the Philippines to the United States; one in 1914 in an undocumented monkey species (Schmitter 1914) and in 1955 in a *M. fascicularis* (long-tailed macaque) (Richardson and Humphrey 1971). One case was documented in a pet monkey in Jordan (Al-Qudah et al. 1997).

Overall, more than 150 cases of natural rabies infection have been reported in NHPs in America, Asia, and Africa (Gautret et al. 2014).

11.5 Rabies in Humans Following Exposure to NHPs (Figs. 11.3, 11.4, and 11.5)

From 1980 to 2016, 24 human rabies cases were reported following NHP-related injuries in Brazil (23, 25, 28, 30, and http://sirvera.panaftosa.org.br/login, accessed 6 March, 2019). Rare human rabies cases following wild monkey bites have been reported in local populations in Thailand, India, and Sri Lanka, based on clinical diagnosis (Panichabhongse 2001; Wilson et al. 1975; Singh et al. 2001; Chhabra et al. 2004). Recently, a laboratory confirmed case of rabies was reported in an Indian patient with a history of monkey bite while climbing a palm tree (Mani et al. 2016). In local populations from Asia, it cannot be excluded that rabies cases reported in patients that sustained monkey bites also sustained unreported previous injuries from dogs.

Rabies was also reported in two travelers returning from India to Australia in 1987 and to Germany in 2004, based on histopathology in the first case and direct immunofluorescence and virus isolation in the second case (Centers for Disease Control 1988; Summer et al. 2004). Of note, the German case had also contacts with dogs although he did not report dog bites.

11.6 NHP-Related Injuries in Humans and Risk of Rabies

Given that rabies infection in humans is always fatal, the WHO recommends that any individual sustaining an injury caused by a mammal in countries endemic for rabies receive rabies PEP. In recent studies conducted among local populations, exposure to NHPs accounted for only a small proportion of individuals receiving rabies PEP: 5.1 percent in India, 1.1 percent in Thailand, 3.6 percent in Chad, 1.3 percent in Côte d'Ivoire, and 0.4% in Brazil, while the majority was exposed to dogs (Sahu et al. 2015; Riesland and Wilde 2015; Frey et al. 2013; Tiembré 2011; Dantas-Torres and Oliveira-Filho 2007). By contrast, studies conducted among international travelers show that an estimated 31% of animal-related injuries leading to rabies PEP are caused by NHPs (Table 11.1) (Shlim et al. 1991; Pandey et al. 2002; Boggild et al. 2007; Menachem et al. 2008; Gautret et al. 2007; Gautret et al. 2008; Shaw et al. 2009; Gautret et al. 2010; Gautret et al. 2011; Wijaya et al. 2011; Mills et al. 2011; Piyaphanee et al. 2012; Mease and Baker 2012; Carroll et al. 2012; Kardamanidis et al. 2013; Park et al. 2014; Shaw et al. 2015; Wieten et al. 2015; Gautret et al. 2015). In Bali, Indonesia, where human–NHP interactions are frequent (see Chap. 2), 69% of international tourists who received PEP were injured by NHPs (Gautret et al. 2011). In a recent GeoSentinel survey involving 2697 travelers requiring rabies PEP, NHPs accounted for 66% of animal bites occurring in Southeast Asia and 92% of NHP-related injuries occurred in tourists (Gautret et al. 2015). In a study conducted among French travelers injured by NHPs and consulting for rabies PEP, two major tourist destinations in Thailand (Monkey Beach on Koh Phi Phi Island and the Lopburi Monkey Temple) and one in Indonesia (Padangtegal, Bali, Indonesia) were identified as locations where NHPs were most likely to bite tourists, reflecting the lack of education about how to act around NHPs and the regional concentration of NHPs and tourist populations (Blaise et al. 2015). Studies have repeatedly shown that visitors to monkey forests are significantly more likely to be bitten or scratched if they feed the animals. In a study conducted at the CIWEC clinic in Kathmandu, Nepal, NHP bites were 13 times more likely to occur in tourists than long-term expatriates, suggesting that tourists pursue activities with greater potential for human–monkey interaction when visiting popular temples.

11.7 Rabies, One Health, and Monkeys

The reports collated so far support the view that confirmed rabies cases in NHPs are rarely observed compared with rabies cases in humans. Several explanations for this finding are possible. Firstly, with the exception of the cluster of marmosets in Ceará, Brazil, NHPs are not known to be a reservoir for maintaining a rabies virus variant in the wild. There is an intensive diversity of the *Lyssavirus* genus. Most Lyssavirus spp. are maintained by chiropteran hosts while the classical RABV is maintained by dogs, foxes, raccoon dogs, raccoons, mongooses, and skunks, but also by bat species in the Americas and kudu in Namibia (Fooks et al. 2017). Secondly, dogs are a

Table 11.1 Proportion of injuries caused by nonhuman primates among international travelers who received rabies post-exposure prophylaxis following an animal-related injury

Study period	Place of exposure	Population	Design of the study	Total number of injured travelers (all animal species)	Proportion of injuries in travelers caused by nonhuman primates	References
Feb 1987–Jan 1989	Nepal	Non-Indian expatriates and tourists presenting at the Katmandu CIWEC clinic (main clinic for foreigners in Nepal)	Observational survey	51	19.2%	Shlim et al. (1991)
Jan 1996–Dec 1998	Nepal	Non-Indian tourist presenting at the Katmandu CIWEC clinic (main clinic for foreigners in Nepal).	Observational survey	56	43.0%	Pandey et al. (2002)
Jul 1998–Mar 2005	Nepal	Expatriates and travelers presenting at the Katmandu CIWEC clinic (main clinic for foreigners in Nepal)	Retrospective survey	544	27.9%	Boggild et al. (2007)
Aug–Dec 2004	Mainly Asia	Israeli travelers (traveling for at least one month)	Cohort survey (815 individuals)	13	30.8%	Menachem et al. (2008)
June 1998–May 2005	Mainly Asia, Latin America, and Africa	Travelers seen after travel at GeoSentinel sites	Multicentric international retrospective survey	321	21.2%	Gautret et al. (2007)
May 1997–May 2005	Mainly Africa and Southeast Asia	Injured travelers returning to Marseille (France), Melbourne (Australia), and Auckland (New Zealand)	Retrospective survey	261	17.3%	Gautret et al. (2008)
Oct 1998–Feb 2006	Mainly Southeast Asia	Injured travelers returning to Auckland and Hamilton (New Zealand)	Retrospective survey	54	18.5%	Shaw et al. (2009)
Jan 1994–Dec 2007	Mainly North Africa and Asia	Injured travelers returning to Marseille (France)	Retrospective study	424	19.6%	Gautret et al. (2010)

(continued)

Table 11.1 (continued)

Study period	Place of exposure	Population	Design of the study	Total number of injured travelers (all animal species)	Proportion of injuries in travelers caused by nonhuman primates	References
Nov 2008–Mar 2010	Bali, Indonesia	Injured travelers returning to Marseille (France), Melbourne (Australia), Singapore, and Auckland (New Zealand)	Retrospective survey	45	68.9%	Gautret et al. (2011)
Jan 2000 – Jul 2009	Mainly Asia and Turkey	Injured travelers returning to Liverpool (United Kingdom)	Retrospective survey	139	16.5%	Wijaya et al. (2011)
Apr 2009–Jul 2010	Mainly Indonesia and Thailand	Injured travelers returning to 3 clinics in Queensland and 1 in Perth (Australia)	Prospective study	65	44.6%	Mills et al. (2011)
Jun 2010–Feb 2011	Mainly Thailand and other Southeast Asian countries	International travelers leaving Bangkok (Thailand)	Cross-sectional survey	36 with animal species documented (out of 219)	38.9%	Piyaphanee et al. (2012)
Sep–Dec 2011	Afghanistan	US military	Retrospective survey	126	7.9%	Mease and Baker (2012)
Jan 2008–April 2012	Mainly Indonesia, Thailand, India and China	Potential rabies exposure incidents reported to public health units in the South Brisbane region of Queensland (Australia)	Prospective study	136	55.8%	Carroll et al. (2012)
Jan 2007–Dec 2011	Mainly Southeast Asia	Injured travelers returning to New South Wales (Australia)	Retrospective survey	780	49.4%	Kardamanidis et al. (2013)
July 2006–Dec 2012	Mainly Southeast Asia	Injured travelers returning to Seoul (Korea)	Retrospective survey	106	24.5%	Park et al. (2014)
Oct 1998–Nov 2012	Mainly Southeast Asia	Injured travelers returning to Auckland (New Zealand)	Retrospective survey	363	28.7%	Shaw et al. (2015)
Jan 2009–Feb 2014	Mainly Southeast Asia	Injured travelers returning to Amsterdam (the Netherlands)	Retrospective survey	173	29.8%	Wieten et al. (2015)
Jan 1997–Dec 2012	Mainly Asia, Latin America, and Africa	Travelers seen after travel at GeoSentinel sites	Multicentric international retrospective survey	2697	23.7%	Gautret et al. (2015)

domesticated species with a high degree of interaction with humans while NHP–dog interactions are likely to be much less frequent. However, NHPs are frequently kept as pets and can be close to dogs and humans in some regions, notably in urban settings in Asia. Finally, underreporting of rabies in NHPs is likely to be significant for two reasons: (i) rabies cases in NHPs are not notifiable in many countries and as such are not recorded in official statistics and (ii) submission of animal specimens for rabies diagnosis and reporting to national authorities in some setting may only cover the few species considered to be economically important or those most important in terms of public health. More complete and precise information pertaining to rabies in NHPs is needed. This information could be obtained not only through field surveys but also through a better coordination between medical doctors and veterinary doctors in reporting rabies PEP after exposure to NHPs and results of rabies diagnosis in submitted animal specimens. Accessibility of information regarding the incidence of rabies in NHPs and its geographic distribution would provide a basis for improving and sustaining the public health debate around the risk evaluation of rabies after human exposure to these species.

The occurrence of documented transmission of rabies from NHPs to humans, although rare, implies that rabies PEP is indicated in patients injured by NHPs in rabies-enzootic countries. Given the catastrophic nature of the disease with a nearly 100% mortality rate and despite the low probability of the disease, rabies vaccine and RIG should be applied in previously unvaccinated people exposed to potentially rabid NHPs. Since a large number of international travelers sustain NHP-related injuries during their trips, reinforced information about the risks posed by exposure to NHPs in enzootic countries, especially in India and Southeast Asia, should be provided at pre-travel consultation to minimize these injuries and the subsequent need for rabies PEP. Travelers should be informed about the usefulness of avoiding contact with animals, avoiding feeding them, avoiding smiling at them (showing teeth is a sign of aggression), avoiding dropping something that a monkey has grabbed, and avoiding showing fear.

It is likely that a significant proportion of tourists receiving rabies PEP following injuries caused by NHPs are actually bitten by uninfected animals. The apparent lack of clinical rabies among temple monkeys in Indonesia does not support intermittent transmission of rabies from dogs to monkeys and from monkey to monkey. To confirm this figure, large-scale studies aimed at surveying rabies circulation in NHP populations in major tourist sites in Asia would be of great help to the medical community in order to provide reliable information on which to base risk assessment and decisions for rabies PEP.

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Chapter 12
Reston Ebolavirus in Macaques

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Abstract Prior to the discovery of the Reston ebolavirus (RESTV) in 1989, filoviruses were thought to be present only in Africa. The virus was discovered in a quarantine facility in Reston, Virginia, USA, following the deaths of imported cynomolgus macaques (*Macaca fascicularis*) from the Philippines displaying severe haemorrhagic disease. It was thought that aerosol and fomite transmission of RESTV occurred between the macaques and humans during this outbreak. In addition to RESTV, the macaques were found to be infected with the Arterivirus, Simian haemorrhagic fever virus, which naturally occurs in African monkeys. An epizootic event involving the cynomolgus macaques occurred again in 1992 in Siena, Italy and in 1996 in Alice, Texas, USA. All of these infections were traced to monkeys exported from a single primate facility located south of metropolitan Manila, on the island of Luzon in the Philippines. This facility was subsequently closed down by the government in 1997 due to non-compliance issues relating to environmental regulations. RESTV has also emerged in pigs in the Philippines in 2008 and China in 2011 where in both cases coinfection with porcine reproductive and respiratory syndrome virus (PRRSV) occurred. It was hypothesized that the source of these outbreaks were from exposure to bats.

More recently, in 2015, RESTV re-emerged in a primate facility located in the province of Batangas, Luzon, Philippines and six macaque deaths were reported.

Despite infection with RESTV being highly pathogenic in these laboratory macaques, humans displayed no apparent symptoms when infected. Due to genetic similarities with other ebolaviruses, there is concern that RESTV could mutate to

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become pathogenic in humans and therefore, the virus remains classified as a Biosafety Level 4 pathogen.

Keywords Reston ebolavirus · Emerging disease · Cynomolgus macaques · Monkeys · Haemorrhagic · Epizootic · Bundibugyo ebolavirus · BEBOV · Sudan ebolavirus · SEBOV · Tai Forest ebolavirus · TFEBOV · Zaire ebolavirus · ZEBOV · Southeast Asia · Africa · Wild-caught · Bushmeat · Philippines · SHFV · Filovirus · Coinfection · Reservoir · Pigs · Bats

12.1 Virus

12.1.1 RESTV Classification

The genus Ebolavirus (order *Mononegavirales*, family *Filoviridae*) consists of four species that are pathogenic in humans: Bundibugyo ebolavirus (BEBOV), Sudan ebolavirus (SEBOV), Tai Forest ebolavirus (TFEBOV) and Zaire ebolavirus (ZEBOV) and one human non-pathogenic species, Reston ebolavirus (RESTV) (Kuhn 2017). Recently, a sixth member of the genus ebolavirus, named Bombali ebolavirus, was discovered in bats from Sierra Leone and is thought to be capable of infecting humans (Goldstein et al. 2018). BEBOV, SEBOV, TFEBOV, ZEBOV and Bombali ebolavirus all occur naturally in Africa, while RESTV is the only non-African ebolavirus described to date.

12.1.2 Virus Genome and Proteins

The *Ebolavirus* genome consists of a non-segmented negative sense RNA strand of approximately 19 kb in length and encodes proteins in the following order: 3-′-nucleoprotein (NP), VP35, VP40, glycoprotein (GP), soluble GP (sGP), small soluble GP (ssGP), VP30, VP24 and RNA-dependent RNA polymerase (L)-5′ (Fig. 12.1) (Groseth et al. 2002). The GP, sGP and ssGP proteins are produced by transcriptional RNA editing of the GP gene (Mehedi et al. 2011). The GP is involved in the attachment to cells and entry via endocytosis. The virus replicates in the cytoplasm of cells and buds from the plasma membrane to be released (Sanchez et al. 2007). The viral particles are pleomorphic with an average length of 950 nM and diameter of 80 nM (Geisbert and Jahrling 1990).

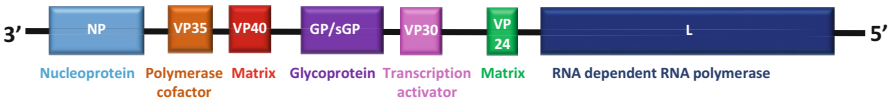

Fig. 12.1 Genome organization of RESTV

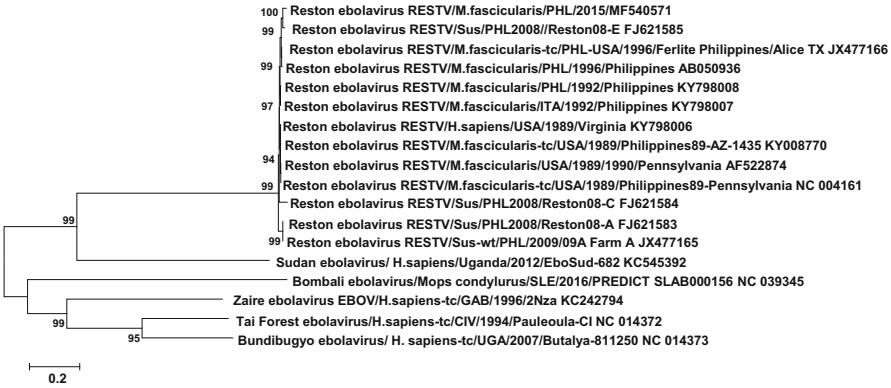

Fig. 12.2 Phylogenetic relationships of the complete ebolavirus genomes (Neighbour joining with bootstrap values) conducted in MEGA6

RESTV has an intergenic region between the GP and VP30 genes, which differs from the other ebolaviruses where these genes overlap in their genome (Sanchez 2001). Replication and transcription of RESTV were found to be less efficient when compared with ZEBOV (Boehmann et al. 2005). It has been suggested that these reduced levels may be due to these differences in their genome organization (Cantoni et al. 2016).

The rate of molecular evolution of RESTV was determined to be 8.21 $X10^{-4}$ nucleotide substitutions/site/year which was similar to ZEBOV (7.06 $X10^{-4}$) but much higher than SEBOV (0.46 $X10^{-4}$) (Carroll et al. 2013).

Phylogenetically, RESTV is most closely related to SEBOV (Fig. 12.2) with the latter exhibiting high mortality in humans. The ebolaviruses appear to share similar transcription and replication strategies and their viral proteins are highly conserved. However, there is a lack of understanding as to why RESTV is non-pathogenic in humans whereas other members of this genus are highly pathogenic in humans.

A comparison of the RESTV genome with the other *Ebolavirus* species reveals an absence of a single change in the viral proteins that would explain differences in pathogenicity (Albariño et al. 2017). However, it has been proposed that the VP24 protein would most likely be responsible for differences in pathogenicity, and that multiple mutations in the VP24 could increase the pathogenicity of RESTV (Pappalardo et al. 2016).

Due to similarities with other ebolaviruses, there is concern that RESTV could mutate to become pathogenic in humans and therefore, the virus remains classified as a BSL4 pathogen.

12.2 Epidemiology of RESTV

12.2.1 Epidemiology of RESTV in Monkeys

The Philippines had been capturing, breeding and exporting cynomolgus macaques (*Macaca fascicularis*) to the United States, Japan and Europe for preclinical research, drug development, biological testing and experimental infections since the early 1980s. Macaques live on the islands of Balabec, Basilan, Cagayan Sulu, Culion, Jolo, Leyte, Luzon, Mindanao, Mindoro, Palawan and Samarin in the Philippines (Ong and Richardson 2008). The monkeys were usually collected in southern areas of the Philippines from the wild for breeding and then transferred to the breeding facilities in Manila. The number of monkeys that were exported to these countries were estimated to be greater than 5000 annually during the 1980s and 1990s (Miranda and Miranda 2011).

RESTV infection was first discovered in cynomolgus macaques in a quarantine facility in Reston, Virginia, USA in 1989 (Jahrling et al. 1990) (Table 12.1). The shipment of 100 macaques from the Philippines to the USA occurred on October 4, 1989 (Dalgard et al. 1992). Necropsies of the dead and dying monkeys revealed haemorrhages in organs, splenomegaly, enlarged kidneys and raised liver enzymes resulting in an initial diagnosis of Simian haemorrhagic fever virus (SHFV) (Jahrling et al. 1990). RESTV was detected in five of the monkeys and SHFV was detected in three monkeys, one of which was also reported to be coinfected with both viruses. The significance of coinfection with RESTV and SHFV is unknown but may have a role in disease severity. Three workers in the export facility in the Philippines were infected with RESTV but did not display any symptoms of infection (Miranda et al. 1991). Based on epidemiological findings, it was proposed that aerosol and fomite transmission occurred between the macaques and humans during the first outbreak of RESTV (Dalgard et al. 1992; Jahrling et al. 1996).

Table 12.1 Outbreaks of Reston ebolavirus in monkeys, humans and pigs

Year	Location	Animal
1989–1990	Philippines	Cynomolgus macaque (*Macaca fascicularis)*, humans
1989–1990	United States (Virginia, Pennsylvania)	Cynomolgus macaque
1989–1990	United States (Virginia, Texas)	Cynomolgus macaque, humans
1992	Italy (Siena)	Cynomolgus macaque
1996	United States (Texas)	Cynomolgus macaque
1996	Philippines	Cynomolgus macaque, humans
2008	Philippines	Pig (*Sus scrofa domesticus*), humans
2011	China	Pig (*Sus scrofa domesticus*)
2015	Philippines	Cynomolgus macaque

Shortly after, outbreaks of RESTV were detected in sick monkeys in quarantine facilities in Virginia, Pennsylvania and Texas, USA from subsequent shipments from the same facility in the Philippines (Centres for Disease Control 1990b). SHFV and RESTV were isolated from monkeys at the facilities in Virginia and Texas (Dalgard et al. 1992). Four workers in the facility in Texas tested positive for RESTV antibodies despite showing no symptoms of infection, one of whom had cut himself during a necropsy of a sick macaque (Centres for Disease Control 1990a, 1990b; Miranda et al. 1991; World Health Organization 2009).

Following an investigation of the export facility in the Philippines, RESTV was detected in 85 of 161 macaques (52.8%) in the Philippines facility that had died over a 2.5 month period, representing a case facility rate of 82.4%. Signs of infection included diarrhoea (35.1%), respiratory illness (36.6%) and haemorrhage (1.2%). It was unknown whether SHFV was present in any of the monkeys in the facilities in the Philippines (Hayes et al. 1992).

RESTV was detected in cynomolgus monkeys imported from the same export facility in the Philippines to Siena, Italy in 1992 when an anorexic monkey died and three more macaques died shortly afterwards. Necropsies of the macaques revealed splenomegaly and small haemorrhages in multiple tissues (World Health Organization 1992).

In March 1996, in a quarantine facility in Alice, Texas, USA, two macaques showing signs of lethargy and anorexia tested positive to RESTV (Pearson et al. 1996; Rollin et al. 1999). An investigation of the macaque breeding and export facilities in the Philippines identified a single facility as the source. Wild caught monkeys were sourced from the southern island of Mindanao. Facility A, located at Calamba, Laguna, on the island of Luzon in the Philippines, had a mortality rate of 13.9% in monkeys in a 5-month period from May to September 1996, which was 1.9% higher than in other facilities. A total of 818 monkeys at Facility A were tested for RESTV. Detectable levels of RESTV in the blood or liver, or both were present in 131 (16%) of the 818 macaques tested. RESTV was identified as a significant cause of death in 113 of the 353 (32%) dead or moribund monkeys tested of which 287 samples were from healthy animals. Eighteen healthy monkeys were found to be infected with RESTV (Miranda et al. 1999).

Wild caught monkeys were implicated as being susceptible to RESTV infection. Out of the 127 monkeys that were positive for RESTV, 114 were wild caught and seven were bred within the facility (there was no record for six). However, it was reported that most of the monkeys were living within the facility for 1–2 years. It was hypothesized that the virus was introduced into the facility via a wild-caught infected monkey (Miranda et al. 2002). Spread of the infection within the facility was attributed to poor husbandry and infection control practices (including the reuse of needles), where most of the RESTV-positive macaques were housed in gang cages. IgG antibodies to RESTV were detected in a serosurvey in three (two wild-caught and one born in the facility) of the 301 healthy macaques and a single worker at the facility. Following this outbreak, the monkey facility was shut down in February 1997 (Miranda et al. 1999, 2002).

The capture of monkeys is no longer permitted, and all monkeys for export are required to be bred within the facilities. Monkeys that are exported are required to undergo screening for RESTV (Demetria, personal communication).

The emergence of RESTV in a monkey facility in the Philippines was detected in August of 2015, when six monkeys died in quarantine and were tested as part of routine testing implemented for sick and dead monkeys (Demetria et al. 2018). As generalized rashes were associated with the deaths, measles virus (MV) testing was included in the differential diagnosis as was Herpes simplex virus and Simian Varicella virus (Demetria, personal communication). The serological and molecular investigation detected both RESTV and MV in the macaques in the affected facility (See Chap. 9 on MV in NHP). Among the 174 serum samples from macaques, 10 (5.7%) and 8 (4.6%) were positive for RESTV IgG and MV IgM, respectively. One (0.6%) macaque was positive for both RESTV IgG and MV IgM. RESTV genome was detected in tissue samples (liver, spleen, or lymph nodes) from deceased macaques. The lymph node from one macaque tested positive for both RESTV and MV by PCR, indicating that a dual infection occurred in this macaque. This macaque also had detectable measles IgM antibodies in its serum. The RESTV sequences obtained from macaques in the 2015 outbreak were most similar to Reston-08-E from pigs in the 2008 Philippines, indicating that RESTV had been circulating in the Philippines after the outbreak in the pig farms in 2008–2009 (Demetria et al. 2018).

The detection of Measles virus (MV) in macaques in the Philippines indicates an anthropozoonotic event. MV is known to infect macaques and can cause signs similar to that in humans including a maculopapular rash (Auwaerter et al. 1999) (see also Chap. 9). It was noteworthy that an outbreak of MV in humans had occurred throughout the country from 2014 to 2015. According to the Department of Health Epidemiology Bureau, in the first 6 months of 2015, there were more than 2200 reported human cases. Phylogenetic analysis of the L gene sequence obtained from sequencing the MV PCR product indicated that MV belonged to genotype B3, which was the same cluster of MV that was circulating in the human population in the Philippines in 2014. However, it is unknown whether the MV infection caused an increase in RESTV pathogenesis in some of the macaques in this outbreak (Demetria et al. 2018).

12.2.2 Role of Pigs in RESTV Epidemiology

In 2008, 115 of 130 tissues samples (88.4%) from pigs displaying severe respiratory signs and reproductive losses in the provinces of Pangasinan, Bulacan, Nueva Ecija and Batangas on the island of Luzon in the Philippines submitted to the Bureau of Animal Industry tested positive for porcine reproductive and respiratory syndrome virus (PRRSV). High pig morbidity and mortality prompted the Philippines' Department of Agriculture to seek assistance from the United States to confirm if the outbreak was caused by atypical or typical PRRSV and whether other pathogens

were present, which might have contributed to the deaths. The presence of PRRSV was confirmed along with a coinfection of porcine circovirus 2 (PCV2). Panviral microarray results revealed the presence of the RESTV L gene, prompting the laboratory to send the samples to the Special Pathogens Branch of the CDC in Atlanta, Georgia, USA, which confirmed the presence of RESTV (Barrette et al. 2009). Of the 28 tissue samples sent to USA, six samples were positive for RESTV (21.4%). As a consequence, two pig farms located in the provinces of Bulacan and Pangasinan in the Philippines were quarantined. In addition, a total of 70 pigs from Pangasinan and 71 pigs from Bulacan were randomly selected and euthanized for serological and molecular assays. Serological testing showed 7.67% of the pigs sampled from Pangasinan and 22.7% from Bulacan were IgG positive. Molecular assay results also revealed that 19 out of 71 tissue samples (26.7%) from the Bulacan farm were positive for RESTV, prompting the Philippine government to depopulate the pig farm containing approximately 6000 pigs. Six people that had either worked on farms or at the abattoir had detectable antibodies against RESTV; however, none of the workers exhibited signs of infection (Barrette et al. 2009).

Similarly, RESTV infection was detected in domestic pigs on three pig farms in Shanghai, China in 2011 during an outbreak of PRRSV, further extending RESTV's known geographic range. A total of 137 spleen samples were collected from the months of February to September, 2011 from pigs that died of PRRSV. Results of the investigation showed that 4 out of 137 (2.92%) of the samples were PCR positive for RESTV. The sequence of the China RESTV was 96.1–98.9% identical to the Philippine RESTV strain (Pan et al. 2014).

Pigs experimentally infected with RESTV alone exhibited shedding from the nasopharynx with no clinical signs suggesting the possibility of aerosol transmission. It was suggested that coinfection with respiratory agents like PRRSV could increase the replication of RESTV, thereby increasing its transmission (Marsh et al. 2011).

12.2.3 Coinfections

Coinfections with RESTV appear to be a common finding. From the first recognized outbreak of RESTV in macaques where coinfections with SHFV were detected (Jahrling et al. 1990), to domestic pigs infected with PRRSV and PCV2 in the Philippines in 2008 (Barrette et al. 2009) and pigs in China in 2011 infected with PRRSV (Pan et al. 2014), to MV detection in one of the macaques that died in the Philippines in 2015. In some of these scenarios, the coinfections could be promoting the transmission of RESTV.

12.3 Pathology of RESTV Infections in Monkeys

Macaques naturally infected with RESTV and coinfected with SHFV in the first outbreak of RESTV displayed splenomegaly, enlarged kidneys, nasal discharge, occasional haemorrhages in organs and the skin and fever. Highly elevated serum lactic dehydrogenase levels (15000–42,000 U/L) were a feature of the infection (Jahrling et al. 1990).

Viral particles were observed by electron microscopy in the plasma, alveoli of the lungs and in the tubular lumina of the kidneys of infected macaques. Viral inclusions, indicative of viral replication were observed in circulating monocytes and macrophages and to a lesser degree in eosinophils and plasma cells. Macrophages in tissues were also observed to be sites of viral replication as were interstitial fibroblasts. High levels of virus were found in urine and in nasopharyngeal washes of naturally and experimentally infected macaques (Geisbert et al. 1992).

Although RESTV causes severe disease in cynomolgus macaques, some of these macaques have been found to be asymptomatic, with antibodies against the virus detected in the absence of any apparent disease (Demetria, personal communication). Experimentally infected African green monkeys (*Chlorocebus aethiops*) were found to be more resistant to RESTV infection (Fisher-Hoch et al. 1992).

RESTV was verified to be free of SHFV prior to conducting experimental infections in cynomolgus macaques. The infectious dose administered (50,000 plaque forming units) in these experimental infections (Jahrling et al. 1996) was substantially higher compared with the ten or less virus particles estimated to be required for natural infection in humans for other Ebola viruses (Franz et al. 1997). Experimental infection with RESTV induced a lethal disease in the macaques similar to Ebolavirus disease in humans. Although the length of the disease was slightly longer for macaques infected with RESTV, the disease presentation was similar to that observed in cynomolgus macaques infected with ZEBOV and SEBOV and in Rhesus macaques (*Macaca mulatta*) experimentally infected with ZEBOV. The onset of disease was rapid, occurring 4–5 days after inoculation and was characterized by splenomegaly, anorexia with weight loss and a fever with death occurring 8–14 days after inoculation. Renal failure was a contributory cause to death with elevated blood urea nitrogen levels. Viremias were detected in all of the macaques with the titre of virus prior to death being greater than 10^7/mL of virus. Elevated serum chemistries were observed for lactic dehydrogenase, alkaline phosphatase, aspartate amino transferase and creatinine phosphokinase. Disseminated intravascular coagulation was evident with increased levels of fibrin degradation product and reduced platelets. Abundant virus was visualized between the alveoli and in the interstitial cells of the lungs of naturally and experimentally infected macaques suggestive of aerosol transmission (Jahrling et al. 1996).

12.4 Laboratory Diagnosis of RESTV

12.4.1 Virus Detection Assays

Virus detection assays involve methods such as virus isolation, electron microscopy, antigen capture enzyme-linked immunosorbent assays (ELISA) (Niikura et al. 2001; Ikegami et al. 2003a) and antigen detection by immunostaining in formalin-fixed tissues (Jahrling et al. 1990; Rollin et al. 1999) and molecular methods (conventional reverse transcriptase polymerase chain reaction (RT-PCR) and real-time quantitative PCR) (Sanchez et al. 1999; Ogawa et al. 2011; Lu et al. 2015; Oloniniyi et al. 2017).

RESTV has been successfully cultured in CV7, MA104, Vero E6 and Vero C1008 cells in BSL4 laboratories (Jahrling et al. 1990; Albariño et al. 2017; Demetria et al. 2018). Virus from cell culture or from clinical samples such as plasma and tissues was visualized using an electron microscope (Jahrling et al. 1990). Isolation of virus allowed for the genome of the virus to be readily sequenced, so that genomes could be compared to study diversity and evolutionary trends and to develop a better understanding of the virus (Carroll et al. 2013; Albariño et al. 2017).

12.4.2 Serological Assays for the Diagnosis of RESTV Infection

Serological tests are useful if the pathogen is no longer detectable, specifically when the host has started to mount an immune response. Serological assays such as indirect fluorescence assay (IFA) (Centres for Disease Control 1990c; Miranda et al. 1991; Hayes et al. 1992; Ikegami et al. 2002; Taniguchi et al. 2011) and enzyme immunoassays (ELISA) can detect the presence of IgM and IgG antibodies in the serum of humans and animals (Ksiazek et al. 1999).

Generally, viral antigens for serological diagnosis of RESTV infection are prepared in a BSL4 laboratory and inactivated by gamma irradiation (Hayes et al. 1992; Ksiazek et al. 1999) so that the assays can be performed in laboratories at lower containment. Recombinant viral proteins offer a useful alternative as antigens for the development of safe diagnostic reagents (Ikegami et al. 2003b).

More recently, Luminex bead-based assays utilizing recombinant RESTV nucleoprotein were used in the detection of antibodies to RESTV in macaques (Demetria et al. 2018). This technology has the advantage of being able to simultaneously detect the presence of antibodies to multiple antigens in an assay and so offers the ability to detect antibodies to multiple infections.

12.5 Reservoir of RESTV

RESTV are known to naturally infect macaques, pigs and humans. Although the reservoir has not been identified, bats are most commonly suspected as the reservoir hosts of filoviruses (Feldmann and Geisbert 2011); however, further research is required for confirmation (Hallmaier-Wacker et al. 2017). Serological screening of bats for RESTV was conducted in the Philippines, with antibodies to RESTV detected in one bat species, *Rousettus amplexicaudatus,* among the 16 bats surveyed, suggesting that *R. amplexicaudatus* is the natural reservoir of RESTV. This bat species is genetically similar to *Rousettus aegyptiacus*, the reservoir of Marburgviruses and Ebolaviruses in Africa (Taniguchi et al. 2011). As the geographical range of *R. amplexicaudatus* bats spans South East Asia including Myanmar, Thailand, Laos, Vietnam, Cambodia, New Guinea and Indonesia, there is a high likelihood that RESTV-like virus or unknown filoviruses might be circulating in the areas other than Africa and the Philippines. In addition, RESTV as well as EBOV antibodies have been found in the related bat species, *Rousettus leschenaultia*, in both Bangladesh and China (Yuan et al. 2012; Olival et al. 2013). Three species of bats from Singapore, *Eonycteris spelaea, Cynopterus brachyotis* and *Penthetor lucasi* were reported to display serological reactivity to the filoviruses Bundibugyo virus (BDBV), Sudan virus (SUDV) and ZEBOV rather than to RESTV (Laing et al. 2018).

While live virus has not been detected, molecular evidence of RESTV was found in oropharyngeal swabs from three insectivorous *Miniopterus schreibersii* bats, and 'potential' serological evidence in three *Acerodon jubatus* and a single *Pteropus vampyrus* bat indicating that ebolavirus infections may be widespread in bat populations in the Philippines (Jayme et al. 2015). However, with low prevalence and very low viral load in bats, further surveillance is needed to better understand the natural reservoir and life cycle of RESTV.

12.6 One Health and Risk Factors to Humans

RESTV is the only known naturally occurring ebolavirus outside of Africa and is endemic in the Philippines and China. Serological evidence of filovirus infection in bats has been reported in the Philippines (Taniguchi et al. 2011), China (Yuan et al. 2012), Bangladesh (Olival et al. 2013) and Singapore (Laing et al. 2018). Infections are most likely occurring naturally in surrounding countries, spilling over into other hosts and going undetected due to a lack of disease surveillance. Despite the growing evidence that bats harbour RESTV, the route of transmission from the natural host of RESTV to monkeys and pigs is unknown; however, it is most likely to be through contaminated food and/or excretions (Fig. 12.3).

Humans have been infected with RESTV through direct or indirect contact with infected macaques or pigs during outbreaks in the Philippines and the USA (Miranda

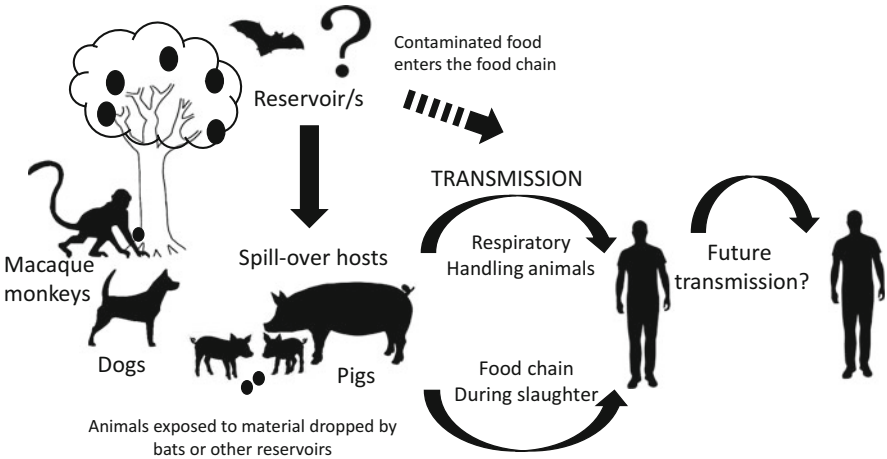

Fig. 12.3 Transmission cycle of RESTV

et al. 1999; Barrette et al. 2009; Miranda and Miranda 2011). Strong evidence suggests that RESTV can be transmitted by aerosols (Jahrling et al. 1990, 1996; Dalgard et al. 1992; Marsh et al. 2011). However, infection by RESTV is considered to be non-pathogenic in humans based on the absence of overt signs of infection and the detection of anti-RESTV antibodies in clinically healthy individuals. Even though the numbers of RESTV recognized in infected humans are low, the possibility that this virus is capable of causing disease and death cannot be ruled out. There have been no reports indicating human-to-human transmission of RESTV. However, asymptomatic disease in humans could mean that human-to-human transmission of the virus could readily occur via contact with infected bodily fluids, which would pose a significant human health risk.

Despite religious constraints, the demand for protein for human consumption places pigs as the preferred source due to their fast growth rate, early return of investment and number of offspring. Global consumption of pork has tripled over the years and this trend is likely to increase in the future. Importantly, infected pigs may enter the food chain (Barrette et al. 2009). The absence of signs of disease in some monkeys and pigs infected with RESTV (Demetria, personal communication, Fisher-Hoch et al. 1992; Marsh et al. 2011) poses a health risk to workers in monkey breeding facilities and piggeries, respectively, abattoir workers and veterinarians. However, the risk of the transmission of the virus between pigs and to humans in pig farming is poorly understood (Atherstone et al. 2014). Transmission of RESTV at the monkey–human interface in the wild has not been reported; however, there is a risk of transmission due to hunting for bushmeat (Scheffers et al. 2012) and if the monkeys are kept as pets.

Coinfections with respiratory pathogen/s could result in increased viral titres and allow for the virus to be more readily transmitted. In addition, coinfections may mask RESTV infection allowing the virus to spread undetected thus posing a

significant threat to public health. This may occur if the other infection/s were diagnosed first and no further testing occurred, which could then allow RESTV to spread. This highlights the importance of a thorough disease investigation.

There is great concern that RESTV may mutate to become more virulent in humans and more readily transmissible in livestock (Cantoni et al. 2016). These circumstances highlight the importance of an extensive diagnostic investigation to identify the causative agents so as to reduce the impact of transmission of RESTV to other animals and humans.

12.7 Concluding Remarks

RESTV causes a fatal haemorrhagic disease in cynomolgus macaques (*Macaca fascicularis*); however, some infections are asymptomatic. The ability of RESTV to be transmitted unnoticed, coupled with the likelihood that the virus can be transmitted via aerosols, and infect a wide range of animals (monkeys, pigs and humans) increases the possibility that the virus may mutate to become more pathogenic and/or more readily transmissible. The risk of the virus mutating into a more pathogenic strain in monkeys, livestock and humans is unknown and therefore the importance of having a panel of assays that will detect this virus in the diagnostic repertoire is important. Regular screening of monkey colonies for the presence of RESTV and other filoviruses is important to reduce the risk of the virus circulating and causing deaths in the colonies and in the workers. Continued surveillance is required to reduce the risk of RESTV posing health consequences for monkeys and humans.

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Chapter 13
Global Diversity and Distribution of Soil-Transmitted Helminths in Monkeys

Liesbeth Frias and Andrew J. J. MacIntosh

Abstract Soil-transmitted helminths (STH) remain neglected tropical parasites, despite infecting millions of people worldwide and being among the most common parasites regulating wildlife populations. Although typically reported in coproscopic surveys of nonhuman primates (NHP), little is known about factors regulating STH diversity and distribution, or how they might affect their hosts. Here, we investigate STH diversity (species richness) and distribution (infection prevalence) in NHP using an extensive database on primate–parasite associations coupled with sociodemographic and environmental data for each host species. We used principal components analysis (PCA) to reduce a large number of correlated explanatory variables to a smaller number of uncorrelated variables explaining their variance. We then applied a Bayesian phylogenetic mixed-effects modeling framework and provide evidence that environmental, including anthropogenic features, and host-related traits influence parasite diversity and distribution. STH diversity was negatively associated with latitude and human population density in each species' geographic range, while STH prevalence was positively associated with both latitude and human population density, as well as primate group size. Understanding how anthropogenic change might alter the intimate ecological relationships formed by primates and their oft-neglected parasites should be a priority in the Anthropocene as human–nonhuman primate interfaces continue to expand at unprecedented rates.

Keywords Host–parasite ecology · Neglected tropical disease · Parasite species richness · Parasite prevalence · Anthropocene · Phylogenetic comparative methods · *Ascaris lumbricoides* · Ascarididae · Whipworm · *Trichuris trichiura* · Trichuridae ·

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Hookworm · *Necator americanus* · *Ancylostoma duodenale* · Ancylostomatidae · Threadworm · *Strongyloides stercoralis* · Rhabditidae · *Oesophagostomum stephanostomum*

13.1 Introduction

13.1.1 Getting Down and Dirty with Soil-Transmitted Helminths

Parasites are ubiquitous in nature, infecting all manner of hosts with all manner of potential outcomes. Their effects can range from the seemingly benign, as seen with gastrointestinal nematodes in the pinworm family (Oxyuridae), to the downright catastrophic, as seen with the epidemic of Ebola hemorrhagic fever that ravaged central African great apes (Leroy et al. 2004; Walsh et al. 2003). In most cases, effects of parasites on hosts can be difficult to pin down, and this is particularly true with endemic parasites that persist in host populations. Such chronic parasitism tends to get overlooked because infections seem apparently asymptomatic and rarely associated with mortality. Furthermore, endemic parasitism is often assumed to be the product of close host-parasite coevolution, a process that conventional wisdom holds should lead to high degrees of host specificity, reduced parasite virulence, and increased host tolerance (Price 1980). However, theoretical and empirical evidence shows that coevolution does not preclude moderate or even high virulence (Anderson and May 1982; May and Anderson 1990), and chronic parasites can have profound impacts on host health, fitness, and abundance (Hudson and Dobson 1995).

In humans, soil-transmitted helminths (hereafter, STH) are exemplary chronic parasites with high endemicity and apparently uncharismatic (though far from insignificant) impacts on the host population, remaining neglected agents of disease despite infecting upwards of 1.5 billion people worldwide (predominantly in the tropics) and contributing substantially to the global burden of disease (World Health Organization 2017). These parasites inhabit the intestines of their hosts, reproducing therein and shedding ova (or immature juvenile worms) via feces into the external environment. Ova then develop to species-typical degrees before infective stages are subsequently acquired by new hosts, making STH dependent on the external environment and their host's interactions with it to maintain their transmission cycles.

The most common STH in humans, collectively referred to as the "Unholy Trinity," include roundworm (*Ascaris lumbricoides*, Ascarididae), whipworm (*Trichuris trichiura*, Trichuridae), and hookworm (*Necator americanus* and *Ancylostoma duodenale*, Ancylostomatidae), followed by threadworm (*Strongyloides stercoralis,* Rhabditidae) (Hotez 2013; Jourdan et al. 2017). And while low-intensity infections may have little influence, more intense infections with these parasites can have myriad clinical effects, including intestinal manifestations

such as diarrhea, abdominal pain and dysentery, chronic malnutrition, general malaise and weakness, as well as impaired physical and cognitive development (Cooper and Bundy 1988; Hotez et al. 2008; Stephenson et al. 2000). As a result, STH contribute to the establishment and maintenance of a vicious cycle of infection, poverty, and stagnant socioeconomic development that disproportionately affects the impoverished and developing regions of the world (Hotez 2013).

Their sheer prevalence in the human population makes it hard not to pause and reflect upon the remarkable adaptation of these helminths to a human–parasitic lifestyle (Bethony et al. 2006; de Silva et al. 2003). However, parasitic nematodes more broadly are among the most diverse groups of parasites infecting mammalian hosts (Dobson et al. 2008; Poulin and Morand 2000), including nonhuman primates (NHP) (Nunn and Altizer 2006). Nematode parasites are also among the best studied of all the helminths (Poulin 2002), and a growing body of literature has begun to document their impacts on host populations. Among mammals, for example, intestinal helminths have been demonstrated to influence host population dynamics, either directly or indirectly through impacts on host nutrition or susceptibility to predation, in Soay sheep (Gulland 1992), Svalbard reindeer (Albon et al. 2002; Stien et al. 2002), white-footed mice (Vandegrift et al. 2008), African ground squirrels (Hillegass et al. 2010), and snowshoe hares (Murray et al. 1997). Still, most of our knowledge comes from a select group of well-characterized model systems, so the full extent to which such parasites regulate host populations more generally, as well as the mechanisms by which they do so, remains to be determined.

13.1.2 The Study of Soil-Transmitted Helminths Is No "Monkey Business"

Despite their ubiquity and potential to regulate host populations, we still have little direct information about how STH may impact NHP under natural conditions. In a recent study at Gombe (Tanzania), it was observed that both chimpanzees and baboons exhibit the characteristic pathophysiology associated with oesophagostomiasis, caused by the intestinal nematode and STH *Oesophagostomum stephanostomum* (Strongylidae) (Terio et al. 2016). In the absence of direct evidence of infection, we are forced to rely on indirect evidence such as behavioral observations of infected individuals to speculate that infection with STH may not be without cost. For example, chimpanzees may swallow whole leaves to purge intestinal worms (Huffman and Caton 2001; Huffman et al. 1996), Japanese macaques may use a suite of hygienic behaviors to minimize their acquisition (Sarabian and MacIntosh 2015), while baboons (*Papio cynocephalus*), mangabeys (*Cercocebus albigena*), and mandrills (*Mandrillus sphinx*) may avoid reusing areas of their range in which parasites have accumulated (Freeland 1980; Hausfater and Meade 1982; Poirotte et al. 2017). Other studies have found evidence for altered behavioral patterns in heavily infected monkeys, perhaps indicative of subtle sickness behaviors

only observable through detailed investigation and analysis (Burgunder et al. 2017; Ghai et al. 2015; MacIntosh et al. 2011). But the question of whether STH are significant contributors to variation in primate health and fitness remains an open one.

Even more fundamentally, we remain in need of further investigation into the diversity and distribution of STH in primates, as well as the factors that regulate them. Quantifying parasitism in wild populations is generally challenging without destructive (lethal) sampling, but STH are to some extent a notable exception because of the available noninvasive sampling techniques. Unfortunately, coprological investigations are weak at best (Gillespie 2006), and molecular characterizations continue to demonstrate a degree of cryptic diversity in these parasites – e.g., when observable morphological traits do not differ across samples but genetic traits suggest divergent populations (Frias et al. 2018; Gasser et al. 2006; Ghai et al. 2014a, b). Species accumulation curves for primate helminths, and for all types of parasites for that matter, have yet to level off (Cooper and Nunn 2013), perhaps in part because studies are largely driven by screening for higher-profile target parasites and in higher-profile hosts such as great apes, or because they are limited to regions of long-term primate research (see Hopkins and Nunn 2007). Nonetheless, there exists a substantial repository of information published about primate parasites reflecting decades of research (see the Global Mammal Parasite Database (GMPD): Nunn and Altizer 2005; Stephens et al. 2017), which can provide a good starting point for assessing general patterns in STH infection.

13.1.3 Worming into the Anthropocene

As parasites comprise most of the species found on Earth, identifying the factors influencing parasite diversity and distribution is fundamental to understanding ecological principles behind biodiversity. Moreover, there are several practical considerations regarding parasite biodiversity that should encourage further research into STH in primates. Given the large-scale anthropogenic change occurring in all of Earth's ecosystems, the human-wildlife interface in all its manifestations has expanded to a previously unseen extent, with significant implications for host–parasite associations and disease emergence (Graham et al. 2008; Hassell et al. 2017; Karesh et al. 2005; Patz et al. 2004; Wolfe et al. 2005). Primates are at once among the most diverse and the most threatened of mammalian taxa, with major threats including habitat destruction and fragmentation, bushmeat hunting and illegal trade, and now climate change and infectious disease (Estrada et al. 2017). Primates are susceptible to many of the same infectious organisms found in humans and domesticated animals. Our close phylogenetic relationship with primates, coupled with the tendency of growing human populations to live at high densities in areas of geographic overlap with them, increases the chances of cross-species transmission (Chapman et al. 2005; Wolfe et al. 1998). The potential for bidirectional exchange of parasites and pathogens thus creates a double hazard to human health and wildlife

conservation, making primates a focal point in research investigating the ecology of infectious diseases and the influence of anthropogenic change thereon (Chapman et al. 2005; Gillespie et al. 2008; Wolfe et al. 1998).

The majority of research in this area tends to focus more on generalist microparasites (viruses, bacteria, and protists), which constitute the major source of emerging infectious diseases of current conservation and public health concern. However, there is some evidence that macroparasites such as STH can also move between human and nonhuman primates. For example, great apes and humans seem to share some of the same nodular worm (*Oesophagostomum* spp.), whipworm (*Trichuris* spp.), and hookworm (*Necator* spp.) variants (Ghai et al. 2014a; Ghai et al. 2014b; Hasegawa et al. 2014; Hasegawa et al. 2010; Hasegawa et al. 2017; Kalousová et al. 2016), with many of these same STH also found in various monkey species in the same regions (Ghai et al. 2014a, b; Ota et al. 2015). While phylogenetic arguments might point to great apes as the most relevant targets for close monitoring, the extent to which humans and great apes overlap is far exceeded by that of humans and monkeys. Furthermore, the behaviors of some monkeys, namely certain macaques, baboons, and langurs, allow them a remarkable ability to thrive in anthropogenic landscapes (Lee and Priston 2005; Richard et al. 1989). These more resilient primates have the potential to act as links between wildlife and humans, and disease amplifiers to more threatened primates in the community. Taking a "One Health" approach (Zinstagg et al. 2015), wherein humans, domestic animals and wildlife, and the environment are all considered integral components of a larger system, holds promise both to identify such risks and find ways to mitigate them. As such research remains biased toward African great apes (e.g., Travis et al. 2018), however, much more work is therefore needed to address parasite diversity and distribution in monkeys, as well as how anthropogenic change might influence patterns of infection therein (e.g., Altizer et al. 2007; Frias and MacIntosh 2019).

13.1.4 Monkeying Around with Soil-Transmitted Helminths

In this chapter, we focus on the factors that may influence STH infection in monkeys, exploring the relationship between host attributes, biogeographical and environmental factors, including some anthropogenic features, and two indices of parasite infection: *prevalence*, which indicates the number of infected individuals given a sample of hosts, and *species richness*, which indicates the number of unique parasite species infecting a given sample of hosts. Studies have shown that parasite diversity is strongly shaped by host exposure to parasites, which is driven by both biotic and abiotic environmental factors, as well as host-related factors like local density, behavior, sociality, morphology, and life history traits (for a review, see Morand 2015). For example, comparative analyses of primates have shown that latitudinal gradients, geographic range size, group size, social network structure, ranging behavior, and body mass can all influence parasite species richness, though results are not always clear or consistent across parasite types or host contexts, and

methodological considerations further impair robust generalization (Griffin and Nunn 2012; Nunn et al. 2003, 2005; Nunn and Dokey 2006; Nunn and Heymann 2005; Rifkin et al. 2012; Vitone et al. 2004).

Here, we use a similar approach to that used by the above studies, taking advantage of the recently updated version 2 of the GMPD (Stephens et al. 2017), a repository of published information about parasites in mammals, with a specific focus on and continued updating of the primate literature (Nunn and Altizer 2005). We were interested in the relationships between numerous factors and parasite richness and prevalence in selected primate species. Among biogeographical/environmental factors, we included (1) *latitudinal gradient*, (2) *geographic range area*, and (3) *precipitation.* We also examined several host-related factors, including (4) *home range*, (5) *host body mass*, (6) *population density*, (7) *group size*, and (8) *terrestriality*. We further included (9) *mean human population density* within the geographic range of each primate species as a general proxy for anthropogenic activity. Finally, we controlled for (10) *sampling effort* in our richness models, as it has been repeatedly shown that the number of parasite species increases with the number of hosts sampled (Nunn et al. 2003; Walther et al. 1995), and sample size varies substantially across host species.

We hope this contribution serves to consolidate what is currently known about STH in monkeys, provides new information about STH diversity and distribution across monkey species, and encourages future work into the dynamics of endemic parasitism in monkey populations and communities worldwide.

13.2 Methods

13.2.1 Data Collection

The data used in this study were derived from multiple repositories. Parasite prevalence and parasite richness counts were obtained from the recently updated version 2.0 of the GMPD (Stephens et al. 2017), an online database of all types of parasites reported in select wild animal taxa (Nunn and Altizer 2005). For our purposes, we extracted data concerning STH for all monkeys examined and merged them with host trait data retrieved from two additional data sources: PanTHERIA (Jones et al. 2009) and the EDGE of Existence program (Redding et al. 2010), which contain life history, ecological, and geographical traits for multiple animal taxa, including measures of human population densities. Here, we included information about each primate's mean adult body mass (g), group size, home range size (km^2), population density (individuals/km^2), geographic range size (km^2), geographic range latitudinal midpoint (decimal degrees), whether the species is largely arboreal or terrestrial, and the mean human population density within the geographic range of each species. Numerical data in each database correlated well, but where data overlapped, we defaulted to the PanTHERIA database as it contained more records for primates than did the EDGE database. Where data were used from each database,

we transformed the values when necessary to ensure all data were in the same unit of measurement, again defaulting to the format of the PanTHERIA database. Details concerning how these data were compiled can be found in the metadata attached to Jones et al. (2009; http://esapubs.org/archive/ecol/e090/184/metadata.htm) and the supplementary materials accompanying Redding et al. (2010). Finally, we controlled for phylogenetic dependency among hosts using the consensus phylogeny found in version 3 of the 10 k Trees database (Arnold et al. 2010), following the primate taxonomy provided by Wilson and Reeder (2005).

13.2.2 Principal Components Analysis

Because we were interested in a large number of explanatory variables but had a limited number of data points (for parasite species richness in particular), and many of these variables were correlated with one another (e.g., latitudinal midpoint and human population density), we first reduced the dimensionality of the data set using principal component analysis (PCA) before performing statistical analyses. All numerical variables listed in Sect. 13.2.1 (kept in the units in which they appear in the source databases but scaled and centered for this analysis) were included in the PCA, which was run using the package *FactoMineR* (Le et al. 2008) in R statistical software version 3.4.1 (R Core Team 2017). To decide on the number of principal components (PC) to retain in our analyses, we used two different methods: Horn's parallel analysis (Horn 1965) and Velicer's MAP (Velicer 1976). These methods have been shown to perform well in different contexts (Cangelosi and Goriely 2007; Zwick and Velicer 1986). The parallel analysis and Velicer's MAP were conducted using the R packages *paran* (Dinno 2012) and *psych* (Revelle 2015), respectively. Where the two differed in the number of components to retain, we included the maximum number of components retained by either method for use our statistical models. We used two criteria to determine which factors (variables) contributed meaningfully to each PC: (1) they explained more of the variance in a given PC than would be expected by chance, which was computed using the simple formula 100/8, because there were 8 explanatory variables and the total must equate to 100%; and (2) their factor loadings were > 0.4, i.e., at least 40% of their total variance was contained in a given PC.

13.2.3 Statistical Models

We used a Bayesian phylogenetic generalized linear mixed-effects modeling framework to analyze our data. All models were run with the package MCMCglmm (Hadfield 2010) in R. Because we wanted the data themselves to influence the results rather than any a priori assumptions about them, we used the weakly informative inverse-gamma prior with shape and scale parameters set to 0.002 in our statistical

models (Hadfield 2010). We ran 5,000,000 MCMC simulations with a burn-in of 10,000 and a thinning interval of 500 to mitigate autocorrelation. Effective sample sizes for our model parameters were therefore ~10,000. We ensured model convergence, full mixing of Markov chains and that there were no issues with either multicollinearity in the fixed-effect structure or MCMC sampling autocorrelation.

To explore variation in STH diversity and distribution in NHP, we set STH richness and prevalence as response variables in each model. Prevalence is given in the GMPD as the percentage of hosts (or samples) found positive for a given parasite at a given site in a given study. We instead used the number of infected individuals (or samples) given the total number of individuals (or samples) examined in the study and modeled prevalence as a count variable with a binomial error distribution. Note that it is not always clear whether studies present individual prevalence or sample prevalence, and we accept that as a limitation of our study. We arbitrarily omitted records of prevalence ($N = 13$) that were based on fewer than 10 fecal samples to increase accuracy in the data set. Parasite richness counts were derived from this prevalence data set as the sum of all unique parasites infecting a given host. We modeled richness as a count response variable with a zero-truncated Poisson distribution, because zero-prevalence parasites are not included in richness counts. Because parasite richness counts depend greatly on sampling effort, and there is considerable bias in sampling effort for parasites across primate species, we controlled for this variable by setting the number of published studies in the GMPD for each primate species as a fixed effect in our statistical models of parasite richness. The main predictor variables of interest in each model included each of the principal components retained in our PCA analyses, as well as whether or not a given primate host is terrestrial (binary variables cannot be incorporated into PCA).

In both models, the primate phylogeny described above was used in the random effect structure to account for any effect of shared ancestry on STH prevalence and richness across primates. In addition, we included continental origin as a random effect in each model to account for a broad, spatial component in the data, characterizing host species as being from Asia, Africa, and the Americas. Finally, for the prevalence model only, additional random effects included host species identity to control for pseudoreplication, citation identity to control for variation in methods used to estimate prevalence, and a term to account for shared parasite ancestry, which nested parasite species within their respective genera, families, and orders.

While interpretation of Bayesian statistics does not typically involve p-values, we include them here to help readers interpret results rapidly. However, we encourage readers to focus on each parameter's posterior mean and associated credible intervals (CI) instead.

13.3 Results

13.3.1 Richness of STH in Monkeys

Overall, we extracted 571 records from the GMPD, which were derived from 103 source studies. These data comprised a total of 41 STH species of 12 genera from 14 families, infecting a total of 60 primate species of 22 genera from 6 families (Table 13.1). However, the fact that just over half of all records (307 of 571 records, 54%) did not identify parasites to the species level suggests that there may be greater diversity in most parasite families than is presently known. This is particularly true for the families of STH most commonly found in the data set, including the Ascarididae (*Ascaris* spp.), Cloacinidae (*Oesophagostomum* spp.), Trichostrongylidae (e.g., *Trichostrongylus* spp.), Strongyloididae (*Strongyloides* spp.) and Trichuridae (*Trichuris* spp.).

Figure 13.1 displays the distribution of STH species across monkey genera, illustrating an uneven distribution across host species. The overall median and interquartile range (IQR) for STH richness across hosts was 3 (IQR = 1.75 ~ 5). Since attribute data were not available for all host species, the statistical model for STH richness was run with only 38 of the original 60 host species. Our statistical model shows that STH richness is strongly influenced by sampling effort (Table 13.2), so it is unsurprising that STH richness is biased toward well-studied host genera. At the same time, parasite richness is known to correlate with primate phylogenetic diversity (Nunn et al. 2004), and some of the more speciose monkey genera are also among those with the largest STH richness here.

Statistical models also indicate that, in addition to sampling effort having a significant positive effect, the first principal component showed a marginally significant negative correlation with STH species richness (Table 13.2). Our PCA retention criteria defined by both Horn's parallel analysis and Velicer's MAP identified only one principal component to retain, despite that PC1 only explained 23.6% of the total variance in the set of explanatory variables. Given such low explanatory power, these results should be interpreted with caution. Nonetheless, the factors that contributed the most to PC1 and loaded meaningfully into it included latitudinal midpoint (contribution to PC1 = 31%; factor loading = 0.58) and human population density (25%; 0.46) (Figure 13.2a). Each of these factors loaded positively into PC1, suggesting that each has a negative relationship with STH species richness (Fig. 13.3). While group size and adult body mass both contributed somewhat more than expected by chance to PC1 (17% and 16%, respectively), factor loadings were below our cutoff of 0.4 (0.33 and 0.31, respectively). All other factors contributed to PC1 less than would be expected by chance (<12.5%) and were therefore ignored. Terrestriality was also unrelated to variation in STH richness in our statistical model.

Some variation in STH richness across host families is evident in Figure 13.4a, and indeed the median phylogenetic signal – i.e., the amount of variance attributed to the phylogenetic tree we used in our parasite richness model (represented by the

Table 13.1 List of soil-transmitted helminths reported in monkeys and compiled in the Global Mammal Parasite Database, version 2 (Stephens et al. 2017)

Parasite	Primate host
Ancylostomatidae	
Ancylostoma sp.	*Macaca silenus, Papio cynocephalus, Rhinopithecus bieti*
A. duodenale	*Macaca fascicularis*
A. quadridentata	*Alouatta caraya*
Necator sp.	*Chlorocebus sabaeus, Erythrocebus patas, Papio papio*
N. americanus	*Ateles fusciceps, Callithrix jacchus, Cercocebus atys, Cercopithecus campbelli, Colobus polykomos, Mandrillus sphinx, Papio anubis, P. cynocephalus, P. ursinus, Piliocolobus badius*
Ascaridiidae	
Ascaridia sp.	*Cercocebus galeritus, Piliocolobus rufomitratus*
A. galli	*Cercocebus galeritus, Piliocolobus rufomitratus*
Ascarididae	
Ascaris sp.	*Alouatta pigra, A. seniculus, Aotus vociferans, Ateles geoffroyi, Cebus apella, Cercocebus galeritus, Colobus guereza, C. vellerosus, Papio anubis, P. hamadryas, P. papio, P. ursinus, Piliocolobus tephrosceles, P. rufomitratus, Rhinopithecus bieti,*
A. elongata	*Alouatta belzebul*
A. lumbricoides	*Alouatta caraya, A. palliata, A. seniculus, Ateles fusciceps*
Cloacinidae	
Oesophagostomum sp.	*Chlorocebus aethiops, C. pygerythrus, Cercocebus galeritus, Cercopithecus albogularis, C. ascanius, C. lhoesti, C. mitis, Colobus guereza, Macaca fascicularis, M. fuscata, Papio anubis, P. cynocephalus, P. papio, P. ursinus, Piliocolobus badius, P. rufomitratus, P. tephrosceles, Rhinopithecus bieti, Trachypithecus cristatus*
O. aculeatum	*Macaca fascicularis, M. fuscata*
O. apiostomum	*Chlorocebus pygerythrus, Macaca mulatta, Trachypithecus cristatus*
O. bifurcum	*Cercopithecus albogularis, Cercopithecus mona, Erythrocebus patas, Papio anubis, P. cynocephalus, P. ursinus*
O. brumpti	*Mandrillus sphinx, Papio hamadryas*
O. maurum	*Papio papio*
O. pachycephalum	*Papio papio*
Heligmosomidae	
Longistriata dubia	*Alouatta caraya, Saimiri sciureus*
Molinidae	
Molineus sp.	*Papio anubis, Saimiri sciureus*
M. elegans	*Saimiri boliviensis, S. sciureus, Saguinus fuscicollis*
M. midas	*Saguinus midas*
M. torulosus	*Cebus apella, C. olivaceus, Saimiri sciureus*
M. vexillarius	*Callithrix jacchus, Saguinus mystax, S. oedipus, Saimiri sciureus*
Trichostrongylidae	
Nochtia nochti	*Macaca fascicularis, M. mulatta*

(continued)

Table 13.1 (continued)

Parasite	Primate host
Trichostrongylus sp.	*Alouatta pigra, A. seniculus, Cercocebus galeritus, Cercopithecus mitis, Macaca fascicularis, Mandrillus sphinx, Papio anubis, P. cynocephalus, P. ursinus, Piliocolobus rufomitratus*
T. axei	*Cercopithecus albogularis*
T. colubriformis	*Papio hamadryas, P. ursinus*
T. falculatus	*Papio ursinus*
T. subtilis	*Papio hamadryas*
Strongyloididae	
Strongyloides sp.	*Alouatta palliata, A. pigra, A. seniculus, Aotus vociferans, Ateles geoffroyi, A. paniscus, Cebus albifrons, C. capucinus, C. paella, Cercocebus galeritus, Cercopithecus ascanius, C. campbelli, C. diana, Chlorocebus aethiops, C. pygerythrus, C. sabaeus, Colobus angolensis, C. guereza, C. polykomos, Erythrocebus patas, Papio anubis, P. cynocephalus, P. papio, Piliocolobus badius, P. rufomitratus, P. tephrosceles, Macaca fuscata, M. sinica, Mandrillus sphinx*
S. cebus	*Brachyteles arachnoides*
S. fuelleborni	*Ateles geoffroyi, Cercocebus galeritus, Cercopithecus albogularis, C. ascanius, C. lhoesti, C. mitis, Chlorocebus aethiops, Colobus angolensis, C. guereza, Macaca fascicularis, M. fuscata, M. hecki, Papio anubis, P. papio, P. ursinus, Piliocolobus rufomitratus, P. tephrosceles, Trachypithecus cristatus*
S. stercoralis	*Papio ursinus, Piliocolobus rufomitratus, P. tephrosceles*
Trichuridae	
Trichuris sp.	*Alouatta pigra, Colobus angolensis C. guereza, C. ascanius, C. lhoesti, C. mitis, Cercocebus galeritus, Chlorocebus aethiops, C. pygerythrus, Colobus angolensis, C. vellerosus, Erythrocebus patas, Lophocebus albigena, Papio anubis, P. cynocephalus, P. hamadryas, P. papio, P. ursinus, Piliocolobus tephrosceles, P. rufomitratus, Macaca fascicularis, M. fuscata, M. sinica, Mandrillus sphinx, Rhinopithecus bieti, Saimiri sciureus, Trachypithecus vetulus*
T. dispar	*Alouatta guariba, A. seniculus*
T. trichiura	*Alouatta seniculus, Cercocebus galeritus, Cercopithecus albogularis, C. campbelli, C. diana, C. lhoesti, C. mitis, Macaca hecki, M. fascicularis, M. fuscata, Colobus polykomos, Papio papio, P. ursinus, Piliocolobus badius, P. rufomitratus, Saguinus fuscicollis, Trachypithecus cristatus*

parameter $\hat{\lambda}$) – was moderate ($\hat{\lambda} \cong 0.30$, IQR = 0.09 ~ 0.64). In other words, relationships between primates have a moderate impact on variation in STH species richness among them. We also observed that the median estimate from the posterior distribution for variation explained by geographic region was 0.59 (IQR = 0.19 ~ 0.90), suggesting that geographic origin explains considerable variation in STH richness across primates (Figure 13.5a).

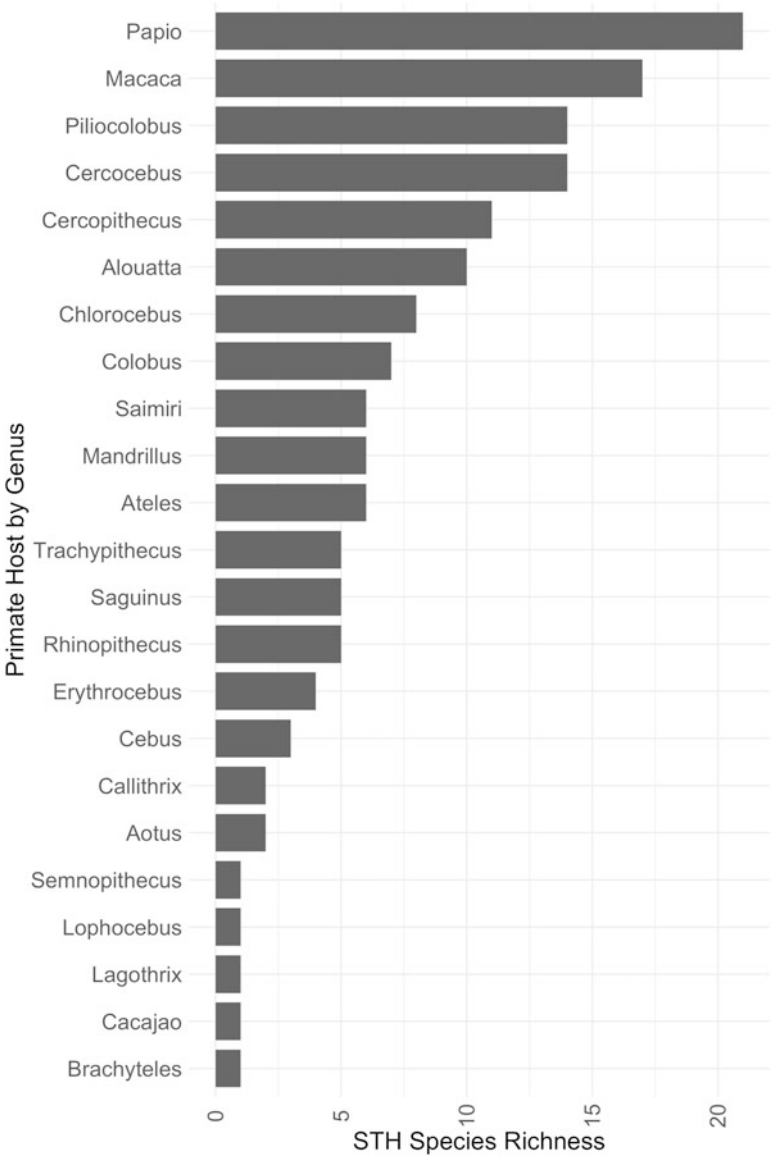

Fig. 13.1 STH species richness across primate genera. Black bars indicate the number of unique STH species found in each host genus

Table 13.2 MCMCglmm model output for variation in soil-transmitted helminth species richness in monkeys. Parameter estimates with 95% credible intervals (CI) that did not overlap zero are emboldened and denoted with (*), while those with 90% CI not overlapping zero are italicized and denoted with (•)

Model term	Posterior mean	Lower 95%CI	Upper 95%CI	Effective sample size	pMCMC
(intercept)	0.459	−0.908	1.641	9694	0.396
Scaled sampling effort	**0.347**	**0.126**	**0.588**	**9100**	**0.004***
Scaled principal component 1	*−0.318*	*−0.686*	*0.020*	*9662*	*0.065•*
Terrestriality (terrest. v arb.)	0.256	−0.486	1.000	9998	0.483

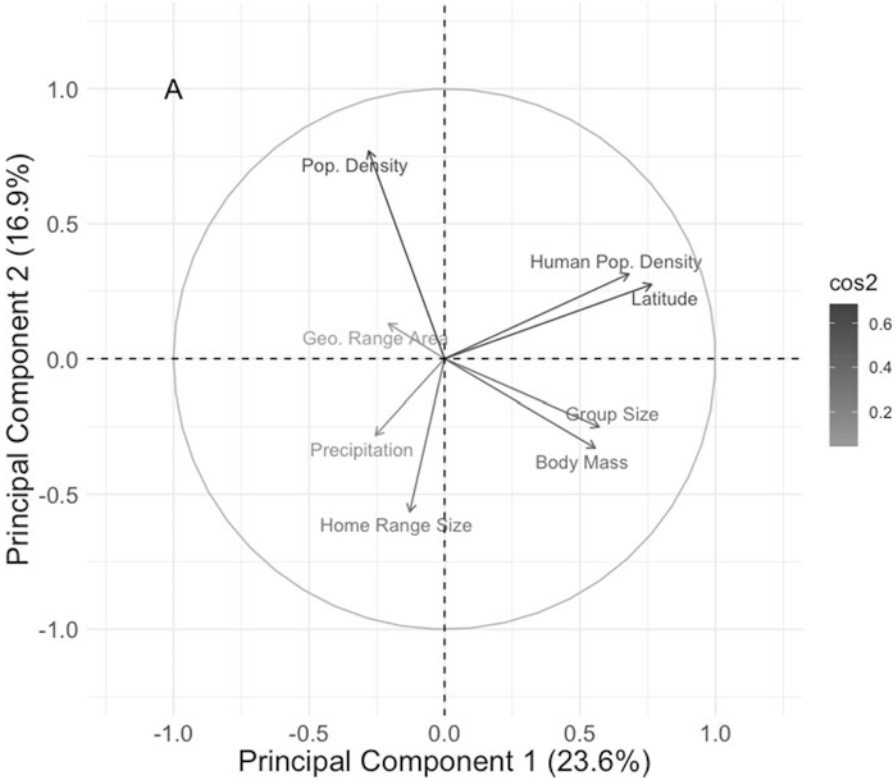

Fig. 13.2 Biplots from principal component analysis (PCA) showing factor loadings for STH species richness (**a**) and STH prevalence (**b**). Arrows indicate the direction of factor loadings with respect to the two principal components shown, with lengths and color gradients indicating the magnitude of loading into either component

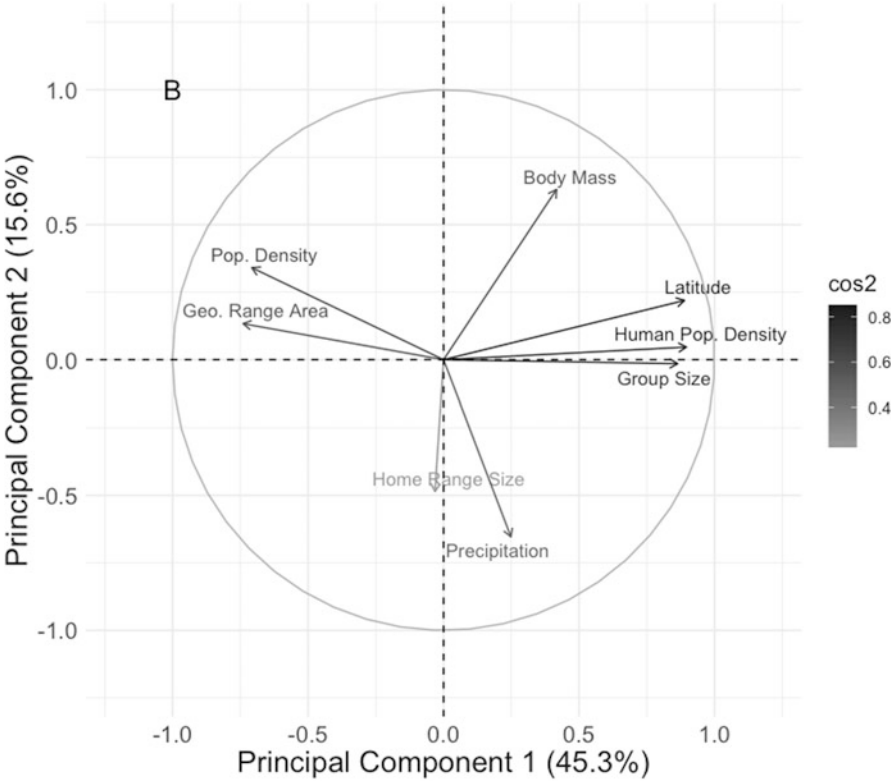

Fig. 13.2 (continued)

13.3.2 Prevalence of STH in Monkeys

The overall median and IQR for STH prevalence across primates included in this study was 0.20 (IQR = 0.07 ~ 0.48), meaning that approximately 20% of monkeys are infected with a given STH. Figure 13.6 shows the average prevalence of each STH genus across host genera. Since prevalence was not reported for all of the 571 records of STH in monkeys appearing in the GMPD, and attribute data were also available only for a subset of hosts, the statistical model for prevalence was conducted using 225 records, including 19 STH species of 9 genera from 8 families, infecting 28 monkey species of 14 genera from 3 families.

The statistical model for prevalence suggests a marginally significant positive relationship between the first principal component and STH prevalence (Table 13.3). Our PCA retention criteria identified three principal components to retain, explaining 45.3%, 15.6%, and 13.8% of the total variance in the set of explanatory variables, respectively, and 74.7% overall. As for STH richness, the variables human population density (contribution = 24%; factor loading = 0.80) and latitudinal midpoint

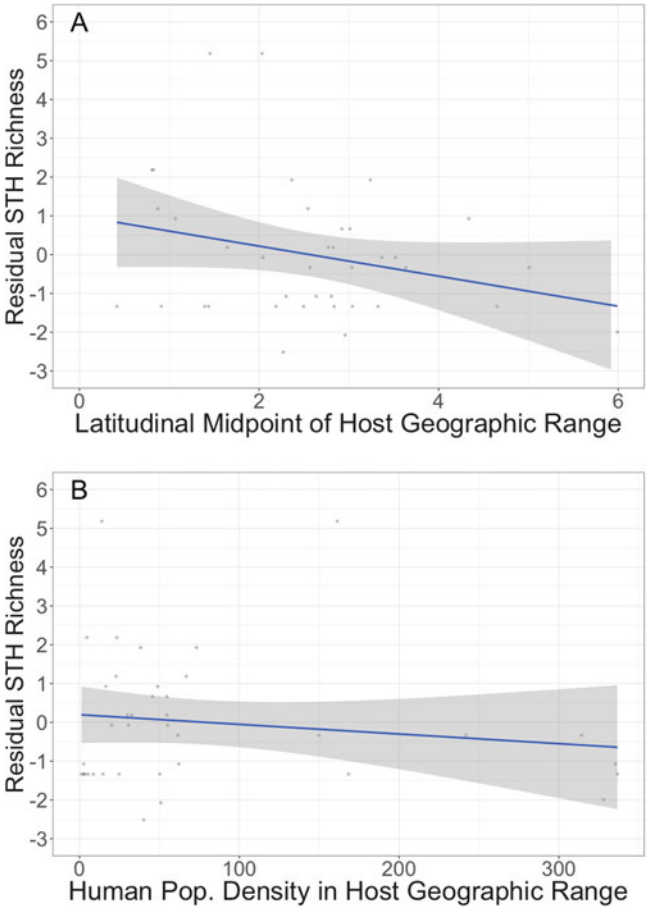

Fig. 13.3 STH species richness as a function of latitudinal midpoint (**a**) shown as log-midpoint values from the geographic range of each primate species and human population density (**b**) shown as the mean number/km^2 (human population estimates from 1995: Jones et al. 2009). Note that, for graphing purposes only, y-axes show residual STH richness after linear regression against the number of citations available per host species, which largely biases richness counts. Blue lines reflect linear trends and shaded areas their respective 95% confidence intervals. Data points are "jittered" to reduce overlap and improve readability

(contribution = 23%; factor loading = 0.79) each loaded positively into PC1 (Figure 13.2b). Thus, in contrast to what we observed for STH diversity, both variables appear to be positively associated with STH prevalence (Fig. 13.7a, b). Primate group size also loaded positively into PC1 (contribution = 21%; factor loading = 0.75), suggesting that STH prevalence is also higher in larger groups (Figure 13.7c). Lastly, although primate geographic range size (contribution = 15%; factor loading = 0.55) and population density (contribution = 13%; factor loading = 0.50) each loaded negatively into PC1, they did not contribute to it

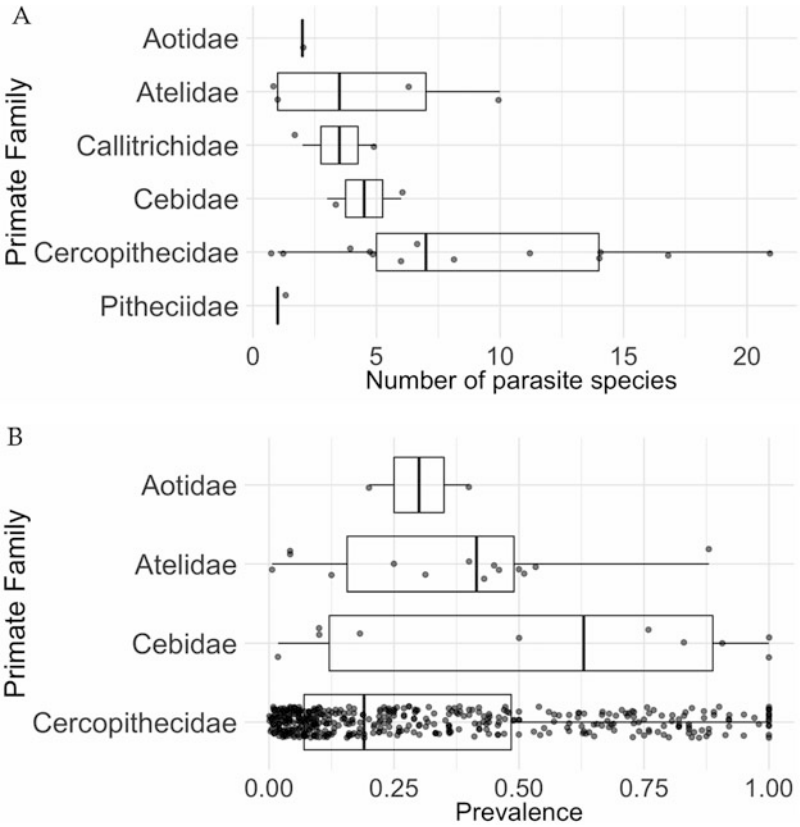

Fig. 13.4 STH species richness (**a**) and prevalence (**b**) in monkeys according to host family. Boxes reflect median values with their respective 25% and 75% quartiles, while whiskers reflect the 5% and 95% quartiles

significantly more than expected by chance (12.5%) and were therefore not considered here. All other factors contributed to PC1 less than would be expected by chance and were therefore ignored. Neither PC2 nor PC3 were associated with variation in STH prevalence in the model and are therefore not discussed here either. Lastly, terrestriality was also unrelated to variation in STH prevalence in our statistical model.

Unlike that from the richness model, we found that the posterior median phylogenetic signal in STH prevalence was close to zero ($\hat{\lambda} \cong 0.005$, IQR = 0.002 ~ 0.012), indicating that relationships between host species cannot explain the variance observed in STH prevalence; indeed, little consistent variation across host families was observed (Figure 13.3b). Similarly, the posterior median estimate for geographic region was low at 0.02 (IQR = 0.004 ~ 0.10), suggesting that geographic origin also has little to no effect on STH prevalence (Figure 13.5c). However, the posterior median estimates for variation explained by parasite taxonomy and citation

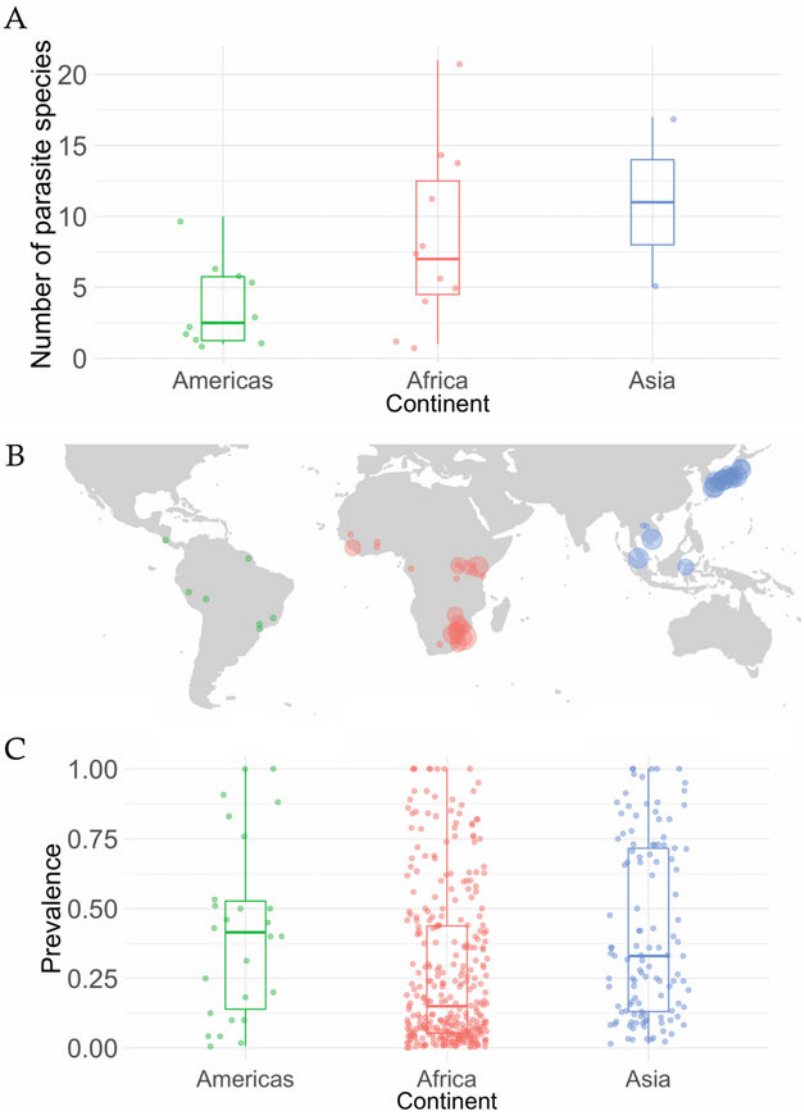

Fig. 13.5 Soil-transmitted helminth species richness (**a**) and prevalence (**c**) in monkeys according to geographic region. The world map (**b**) illustrates the sampling locations for 55 (of 141) studies found in the GMPD with sampling coordinates. The sizes of the circles (**b**) reflect STH species richness at that site (range: 1 ~ 13). The colors of boxes, whiskers, and points reflect geographic origin. Boxes in A and C reflect median values with their respective 25% and 75% quartiles, while whiskers reflect the 5% and 95% limits

identity were 0.47 (IQR = 0.31 ~ 0.62) and 0.46 (IQR = 0.37 ~ 0.53), respectively. Some variance in prevalence therefore depends on the type of STH in question, meaning that some STH species can attain higher prevalences in the host population

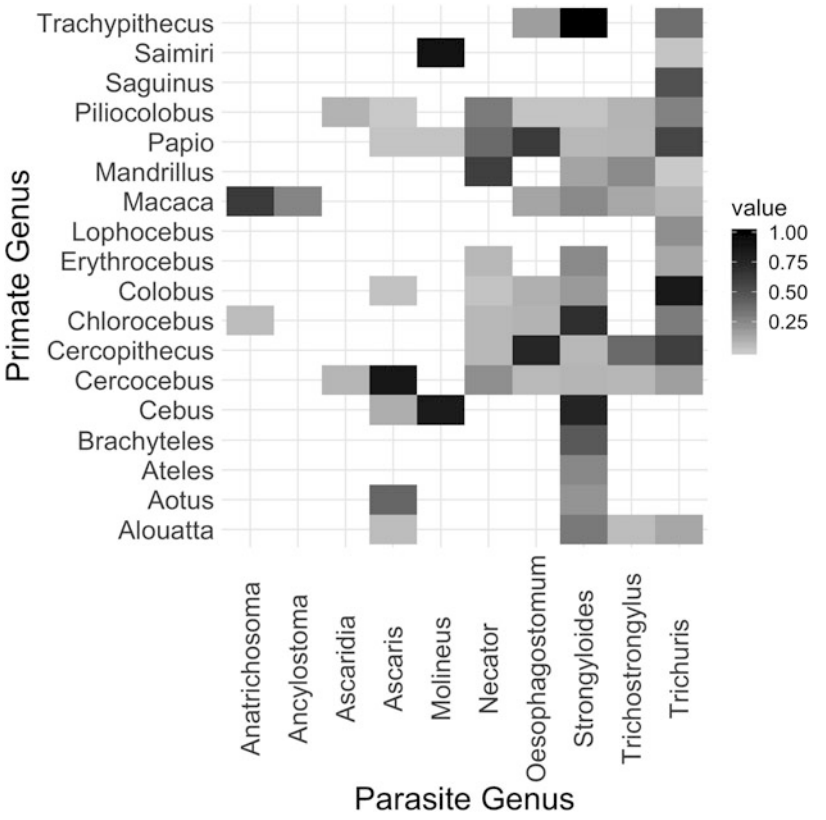

Fig. 13.6 STH prevalence according to primate and parasite genus. Prevalence is expressed as the percentage of hosts infected with a given parasite genus in the sample. Where multiple species within a given genus appear in our data set, prevalence data were pooled and averaged

Table 13.3 MCMCglmm model output for variation in soil-transmitted helminth prevalence in primates

Model term	Posterior mean	Lower 95%CI	Upper 95%CI	Effective sample size	pMCMC
(intercept)	−1.159	−2.978	0.797	9980	0.197
Scaled principal component 1	*0.728*	*−0.117*	*1.567*	*9670*	*0.079•*
Scaled principal component 2	−0.347	−0.807	0.078	10,333	0.113
Scaled principal component 3	0.265	−0.136	0.661	9980	0.193
Terrestriality (terrest. v arb.)	0.213	−1.383	1.629	9980	0.781

Parameter estimates with 95% credible intervals (CI) that did not overlap zero are emboldened and denoted with (*), while those with 90% CI not overlapping zero are italicized and denoted with (•)

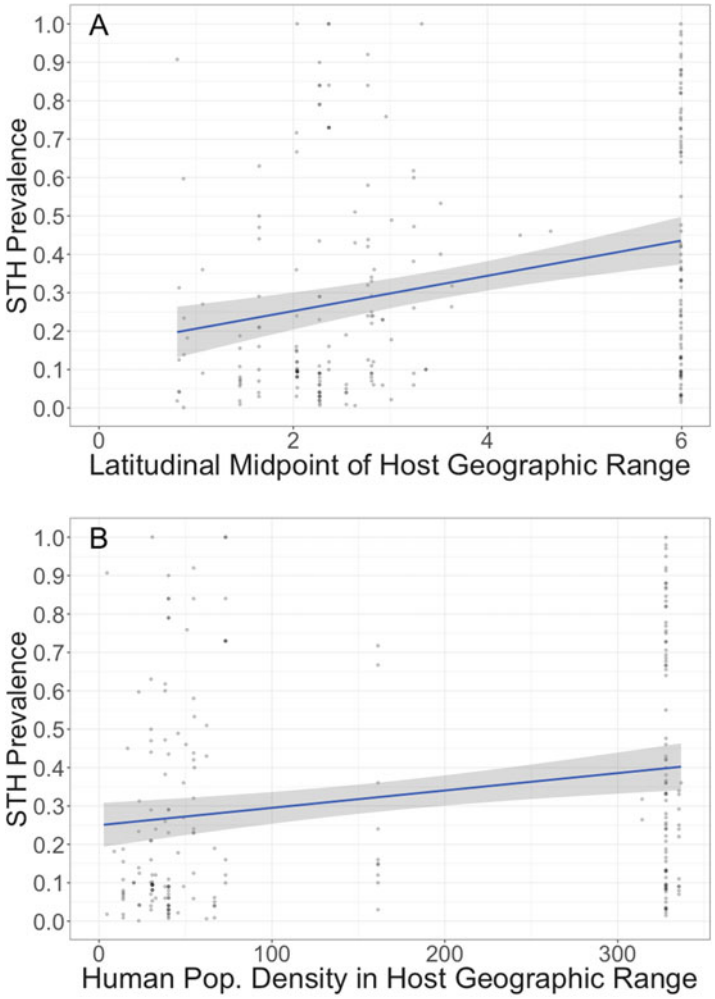

Fig. 13.7 STH prevalence as a function of latitudinal midpoint (**a**) shown as log-midpoint values from the geographic range of each primate species, human population density (**b**) shown as the mean number/km^2 (human population estimates from 1995: Jones et al. 2009), and mean group size (**c**). Blue lines reflect linear trends and shaded areas their respective 95% confidence intervals. Data points are "jittered" to reduce overlap and improve readability

than others. Lastly, concerning citation identity, these results suggest that some publications consistently report higher or lower values of prevalence when multiple host-parasite relationships are investigated in those given studies, illustrating the need to control for such variation in comparative studies.

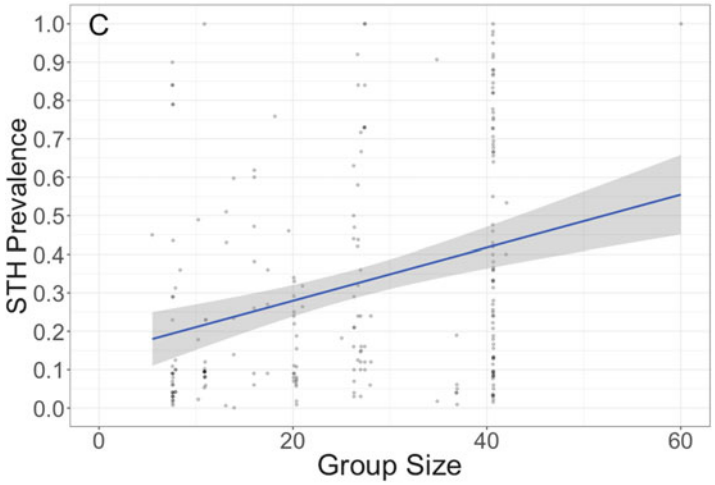

Fig. 13.7 (continued)

13.4 Discussion

In this chapter, we used a phylogenetic comparative framework to explore factors that might cause variation in STH diversity (species richness) and distribution (prevalence) across NHP. Numerous studies have now investigated broad patterns of parasite species richness using the GMPD (e.g., Nunn et al. 2003; Nunn et al. 2005; Nunn and Dokey 2006; Rifkin et al. 2012; Vitone et al. 2004), but to our knowledge, none have previously targeted STH. After reducing a large set of variables that we predicted might influence variation in STH diversity and distribution to a set of principal components explaining their variance, we found that latitude and human population density were the variables found to be associated most with variance in STH diversity, while the same variables plus primate group size were associated with variance in STH prevalence. Furthermore, the primate phylogeny explained moderate variation in STH richness, as has been found before for helminths more generally (Nunn et al. 2003; but see Frias and MacIntosh 2019), but was unrelated to variation in STH prevalence. Richness was further influenced by geographic region, whereas prevalence was influenced by parasite taxonomy and the source of the data, i.e., study identity.

Before discussing these results, however, it is pertinent to note that there are numerous limitations to these results that need to be kept in mind. For example, the host and environmental traits extracted from our data sources are crude and may not reflect the diversity observed within species across their geographic ranges. Moreover, these predictor variables were transformed using PCA prior to analysis, meaning that we cannot distinguish between the effects of variables that co-occur along the same principal components, e.g., latitude, human population density, and group size. Even the available parasitological data vary considerably in both quantity

and quality across sampled hosts. Nonetheless, we feel that such results can help us better understand macroecological and evolutionary relationships between STH and NHP, and guide us in our predictions about future trends, which will become critical as the Anthropocene surges on with its unprecedented unhinging of ecological relationships.

13.4.1 Biogeographical and Environmental Determinants of STH Infection

In line with general expectations of biodiversity (Gaston 2000; Rohde 1992), we observed that the number of STH species infecting primates decreases with distance from the equator. While there are numerous hypotheses that might explain this general pattern in free-living species, temperature and rainfall, which jointly predict primary productivity, are probably the most influential variables associated with latitudinal gradients in species richness (Turner 2013). For parasitic organisms like STH, which do have an environmental component in their life cycles, these environmental variables are likely relevant as well, but the distribution and diversity of hosts in a given location also plays a pivotal role. At least in Africa and the Americas, primate species richness is highest near the equator (Cowlishaw and Hacker 1997; Eeley and Lawes 1999; Peres and Janson 1999), and this may in part explain the pattern of STH diversity observed here.

However, patterns of parasite species richness in relation to latitudinal gradients have shown little consistency (Bordes and Morand 2009; Bordes et al. 2010; Poulin and Leung 2011; Rohde and Heap 1998), and a previous study using the GMPD found that helminth species richness could not be explained by distance from the equator, although richness in parasitic protists and vector-borne parasites did fit this pattern (Nunn et al. 2005). Another comparative analysis failed to find any latitudinal gradients in helminth species richness across vertebrate taxa, but did show that nematode parasites comprised significantly larger proportions of the helminth community nearing the equator in all hosts examined (Poulin and Leung 2011). This might explain the pattern observed in our study because all of our STH were in fact nematodes. This also suggests that incorporating other indices of parasite community structure can shed light on biogeographic patterns of parasitism.

From this perspective, our finding that STH prevalence appears to increase in NHP populations living further away from the equator adds another dimension to this story. Because we expect that local parasite abundance, which is intricately linked with distribution extent (measured as prevalence in parasitic organisms) (Barger and Esch 2002; Morand et al. 2000; Poulin 1999), depends in large part upon the same environmental factors that influence patterns of species diversity, we expected STH prevalence to increase nearing the equator as well. However, it is possible that aspects of both host and parasite diversity contribute to the opposite pattern observed. For example, within-host competitive interactions among parasites

may constrain parasite population establishment and/or growth when the overall parasite infracommunity in the host population is large, i.e., more diverse (Bashey 2015; Chappell 1969; Dobson 1985). Alternatively, there is growing evidence that host diversity dilutes infection risk to any given member of the host community (Civitello et al. 2015; Keesing et al. 2010; Schmidt and Ostfeld 2001). While host diversity is often invoked to explain variation in parasite diversity across host communities, the same principle may apply to parasite distribution and abundance: the presence of sources and sinks in parasite transmission can up- or downregulate parasite population trends, respectively.

These hypotheses are not mutually exclusive, of course, as within-host parasite competition may contribute to the "competence" of each host in a community to both harbor and transmit a given STH. Conversely, host competence may differentially influence each parasite's competitive ability, e.g., through immune-mediated (apparent) parasite competition (Bashey 2015; Johnson and Buller 2011; Ulrich and Schmid-Hempel 2012). Host and parasite diversity may thus interact to determine infectious disease risk in complex ecological communities (Johnson et al. 2013), and in the present case may be contributing to the reduced STH prevalence observed nearer the equator where STH (and allegedly primate) communities are richer. It should be noted also that we previously reported that generalist and specialist parasites may respond differently to shrinking host populations and communities, as generalist parasites appear to achieve significantly higher prevalence than do specialists in primates threatened with extinction (Frias and MacIntosh 2019).

13.4.2 Human Dimensions in STH Infection

Human influence on ecosystems is often measured through geographic proxies, such as population density, land transformation, and proximity to settlements and roads. Although arguably one of the crudest indicators, human population density (HPD) is among the most commonly used in macroecological and biogeographic studies. One widespread phenomenon in human biogeography is that HPD hotspots also tend to co-occur with biodiversity hotspots (Luck 2007): in 1995, nearly 20% of the world population was living within biodiversity hotspots (Cincotta et al. 2000). This is of course tragic given that HPD is still cited as one of the primary causes of species declines worldwide (Cincotta et al. 2000). On the flipside of the coin, however, human infectious diseases were also demonstrated to co-occur with mammalian biodiversity hotspots (Murray et al. 2015), and human parasitic and infectious diseases are most species rich near the equator (Guernier et al. 2004), again where biodiversity tends to be at its peak.

Although HPD has not been causally linked to patterns of parasitism and/or emerging infectious diseases (EID), in contrast to the many links demonstrated between anthropogenic changes to the landscape and disease risk (Patz et al. 2004; Wolfe et al. 2007), it nonetheless constitutes a major predictor of EID events (Jones et al. 2008) as a proxy for anthropogenic activities (McKee et al. 2004). Here, we

found that HPD was associated with both STH species richness and prevalence, with higher HPD being associated with lower parasite species richness but higher prevalence. If we consider the latitudinal gradient in parasitism discussed above, and we must because latitude and HPD co-occurred along the same principal components for both richness and prevalence, then we notice that HPD was in fact negatively correlated with latitude in our study. This makes it exceedingly difficult to determine to what extent each contributed to the observed patterns of infection. At the very least, however, we can say that STH species richness was higher nearer the equator with lower HPD, while STH prevalence increased away from the equator where HPD tended to be higher. Note that these results need not reflect general patterns of biodiversity and HPD, because the data are restricted to the geographic ranges of a limited number of monkey species. Future studies would do well to more precisely link HPD or more explicit indicators of anthropogenic change to patterns of parasite diversity and distribution.

In the PanTHERIA data set, HPD was calculated based on data from 1995 using the Gridded Population of the World (GPW) (CIESIN and SIAT 2005). There was a recent update to this database that projects populations to 2020, and it is certain that HPD has changed significantly in the intervening decades, but such changes are not reflected in our analyses. While we accept this as a limitation of the nature of our study, note that parasitological records in the GMPD date back to 1927 (Stephens et al. 2017) and that host traits were extracted from a wide range of studies conducted over many decades of research (Jones et al. 2009; Redding et al. 2010). It is therefore difficult to assess to what extent mismatches in the corresponding data used for each primate species may have influenced the associations presented here, but this remains a non-trivial problem that adds a cautionary note to the interpretation of such results. Despite these limitations, HPD may serve as a useful proxy of anthropogenic impacts in broad comparative studies such as ours.

While some primate populations experience significant declines in anthropogenic habitats, other species within certain primate groups (e.g., some macaques and colobines) can even thrive in human-modified habitats. These "bridge" species, sometimes referred to as "weed" species, are epidemiologically relevant in that they can potentially connect host communities linked to specific habitats (i.e., natural forests, agricultural lands, urban habitats) through parasite transmission. These usually wide-ranging generalist species derive a survival/fitness advantage from coexisting in anthropogenic habitats, which tends to extend in time if continuous provisioning is available (Becker et al. 2018; Gompper and Wright 2005). Additionally, many of these species can carry zoonotic parasites or act as potential reservoir hosts, contributing to the factors impacting both wildlife conservation and public health.

13.4.3 Host-Related Factors in STH Infection

The search for general patterns in parasite diversity has led numerous studies to address this question in the literature (e.g., Arneberg 2002; Ezenwa et al. 2006; Nunn et al. 2003; Poulin 2004; Poulin and Morand 2000). General findings are that hosts with larger bodies, higher population densities, and wider geographic ranges often exhibit more diverse parasite communities. For STH communities in NHP, however, none of the host traits we tested were associated with parasite richness, and only group size was associated with at least marginal variation in parasite prevalence, which tended to increase in larger groups. Again, given our analytical paradigm, any effect of group size on parasite prevalence cannot be separated from those of latitude or HPD, as all three co-varied along the same principal component; i.e., group size seems to increase away from the equator and in areas with higher HPD. Thus, whether changing climatic regimes, increased human presence and/or larger primate groups, or indeed some subset of the three are responsible for this trend remains to be seen.

It is plausible, however, that group size might play a role in patterns of parasitism, and this relationship has garnered considerable attention in the literature, largely because infectious disease is thought to impose a classical fitness trade-off in the evolution of sociality (Alexander 1974; Moller et al. 1993). However, meta-analyses and large comparative studies have produced contrasting results concerning this relationship (Chapman et al. 2012; Cote and Poulin 1995; Ezenwa et al. 2006; Nunn et al. 2003; Rifkin et al. 2012; Vitone et al. 2004). At least for directly transmitted helminths in vertebrate hosts, Cote and Poulin (1995) did demonstrate a strong positive relationship between group size and both prevalence and intensity of infection. For STH, which cannot pass directly from host-to-host, group size and gregariousness should be seen as proxies for local density and shared space use, which mediates transmission through the quantity of infective stages in the environment and subsequently parasite–host encounters. If larger groups increase opportunities for STH transmission, leading to increased prevalence and local abundance in areas with increased human population densities, such data suggest a recipe for bidirectional exchange of these parasites at interface areas and the need for greater attention to be paid thereat.

13.4.4 Connecting the Dots Between STH and Their Primate Hosts

Although we still have very little information concerning the role of STH in primate health and fitness, macroparasites such as STH do influence their hosts at different levels of infection intensities. Though we could not examine STH abundance or infection intensity in this study, parasites with wide distributions (i.e., high prevalence) are typically locally abundant as well (Barger and Esch 2002; Morand et al.

2000; Poulin 1999). As such, the factors that influenced STH prevalence here also likely influence STH abundance and infection intensity, and by extension, the health risks associated with them. For example, the observed relationship between STH prevalence and primate group size suggests that, where primate populations and group sizes are locally enhanced, e.g., via access to human-derived food resources, the prevalence of certain STH may naturally increase as well. This can create problems if those same STH are generalist parasites that can be shared among primates and humans in the landscape. How this might translate into variation in disease risk is a question currently facing primate conservationists (Chapman et al. 2005).

Complicating matters is the fact that, while parasitic nematodes can negatively influence host populations (Albon et al. 2002; Gulland 1992; Hillegass et al. 2010; Stien et al. 2002; Vandegrift et al. 2008), their loss can also lead to unexpected increases in the prevalence of other, potentially more deleterious parasites (Ezenwa and Jolles 2015; Frias and MacIntosh 2019; Jolles et al. 2008; Pedersen and Greives 2008). Monitoring STH infection dynamics, and changes therein, should therefore be a priority for future research at the human–primate interface.

Another key question regarding this interface revolves around the potential for cross-species transmission of parasites, or spillover events. Rapidly evolving generalist pathogens that can infect a wide range of host species are the main cause of concern in terms of both wildlife conservation and public health. For example, the spread of tuberculosis (*Mycobacterium bovis*) from livestock to chacma baboons (*Papio ursinus*), followed by rapid progression of disease, illustrates the dramatic effects of transmission from a reservoir host to more vulnerable wildlife populations (Keet et al. 2000). Synanthropic macaques in Asia have also been shown to host *Mycobacterium tuberculosis* complex in countries with high incidences of tuberculosis, suggesting that NHP should be considered in screening for such public health threats (see Chap. 4). Similarly, olive baboons (*Papio anubis*) are now recognized as potential reservoirs for *Treponema pallidum pertenue*, the causative agent of human yaws, and exhibit the same characteristic pathology from the disease (Knauf et al. 2017; Knauf et al. 2013) (see Chap. 5). These examples illustrate a need to pay closer attention to neglected tropical diseases and to the primates that may be involved in their spread.

While the extent to which STH are candidates for spillover events in anthropogenic contexts remains largely unknown, studies are beginning to highlight unanticipated degrees of cryptic diversity and host sharing. Moreover, although their impacts are expected to be less dramatic and thus more likely to be overlooked, there exists ample evidence from humans and other wildlife that STH contribute significantly to host morbidity and population dynamics. Among the most common of the neglected tropical diseases, STH should therefore be a target for increased attention, in both human and NHP, particularly in areas in which they overlap most extensively. This research provided a brief overview of factors responsible for their diversity and distribution, but only focused attention can help uncover the intimate relationships between primates and their soil-transmitted helminths.

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Chapter 14
Larval Tapeworm Infections in Primates: Coenurosis, Cysticercosis, and Echinococcosis

India Schneider-Crease

Abstract As globally distributed parasites of humans, livestock, and wildlife, taeniid parasites exploit predator–prey relationships across mammalian systems. Infections with the larval taeniid stage cause symptoms ranging from the neurological (e.g., paralysis, seizures) to the ocular (e.g., blindness) and muscular (e.g., atrophy), result in massive economic losses in livestock, and threaten wildlife populations. While taeniids were once considered to be relatively host-specific in their larval stage, reports of taeniid emergence in nontraditional hosts are increasing in frequency. In this chapter, I take a One-Health approach to examining cases of larval taeniid infections in primates, focusing on the infection of wild geladas (*Theropithecus gelada*) with the larval stage of *Taenia serialis*. By understanding how taeniid species emerge in nontraditional hosts, we can build useful frameworks for predicting and disrupting transmission and thereby protecting captive and wild NHP, domestic animals, and humans in a world with a broadening human–wildlife interface.

Keywords Cestodes · *Echinococcus* · Geladas · Metacestodes · Parasites · Predators · *Taenia* · Trophic transmission

14.1 Introduction

Tapeworms in the Taeniidae family (Eucestoda: Cyclophyllidea) are geographically and phylogenetically widespread, infecting a staggering number of mammals and causing widespread mortality, morbidity, and economic losses on nearly every continent (Craig and Pawłowski 2002; Hoberg 2002; Thompson 2017). Within Taeniidae, species in the *Taenia* and *Echinococcus* genera are particularly relevant

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to primate (human and nonhuman) and livestock health. Like all Cyclophyllidean tapeworms, *Taenia* and *Echinococcus* species require two host species to complete a single life cycle: a definitive host for the sexually reproducing adult stage and an intermediate host for the nonsexually reproducing or asexually reproducing larval stage (also known as the "metacestode" stage) (Abuladze 1964; Loos-Frank 2000; Hoberg 2002; Romig et al. 2017). Unlike most other species within Cyclophyllidea, *Taenia* and *Echinococcus* species infect mammalian species in both their larval and adult stages (Hoberg 2002; Romig et al. 2017). Species of both genera exploit predator–prey relationships, a phenomenon known as "predator-mediated transmission" (Robar et al. 2010) or "parasite-increased trophic transmission" (Lafferty 1999), with the definitive-stage infecting carnivorous predators and the intermediate-stage infecting herbivorous intermediate hosts (Hoberg 2002; Romig et al. 2017).

For such parasites, the completion of the life cycle hinges on the consumption of infected tissue (e.g., liver, musculature) from the intermediate host by the definitive host. Accordingly, parasites employing predator-mediated transmission frequently optimize their fitness by manipulating the behavioral, morphological, or physiological phenotypes of their intermediate hosts (Combes 1991; Moore 2002; Lafferty et al. 2000; Lefèvre et al. 2009; Poulin 2010; Parker et al. 2015). In these systems, increasing host mortality and predation risk in intermediate hosts is likely adaptive for the parasite (Ewald 1995; Poulin et al. 2005; Poulin 2007; Parker et al. 2015), and parasites with parasite-mediate transmission are more likely to cause fitness consequences in their hosts than parasites with direct life cycles (Robar et al. 2010).

Taeniid species infect a wide array of domestic and wildlife species, including humans. Humans are the definitive host for *T. solium* and *T. saginata*, becoming infected upon consuming infected and undercooked pork (*T. solium*) or beef (*T. saginata*). Other predators, including canids and big cats, are the definitive hosts for a number of other taeniid species. Accordingly, the rodent, lagomorph, and ungulate prey species of these predators are the intermediate hosts for the corresponding larval taeniid stages. While infection with the definitive stage (*taeniasis*) carries generally mild symptoms including abdominal pain, diarrhea, and nausea, infection with the larval stage (e.g., *cysticercosis*, *neurocysticercosis*, *coenurosis*, and *echinococcosis*) can result in a suite of severe symptoms ranging from blindness to seizures. Neurocysticercosis, the infection of the human central nervous system by the larval stage of *T. solium*, is the leading cause of adult-onset epilepsy worldwide (Garcia et al. 2014). Such a diversion from the traditional life cycle is relatively rare, but reports of "non-zoonotic" larval tapeworms in humans are increasingly frequent. Host-switching, in which parasites that are regarded as host-specific expand their host repertoire, is likely to become more frequently as climate change and rapid human population growth alter species compositions and trophic relationships. Understanding how taeniid species emerge in new hosts can thus offer important insights into building One-Health frameworks to protect the health of wildlife, domestic animals, and humans.

Investigating cases of larval taeniid infections in nonhuman primates (NHP) holds particular promise for understanding how and when taeniids emerge in new hosts. NHP occupy a broad range of niches across the globe and are closely related to humans, and thus examining the emergence of new taeniid species in these systems can contribute to the understanding of when novel transmissions occur and when zoonotic transmission is expected. Coenurosis, the condition characterizing infection with *T. serialis* and *T. multiceps*, occurs regularly in wild geladas (*Theropithecus gelada*) (Schneider-Crease et al. 2013; Nguyen et al. 2015; Schneider-Crease et al. 2017a), while echinococcosis, the condition characterizing infection with *Echinococcus* species, is frequently reported in captive NHP (e.g., Rogan et al. 1993; Shahar et al. 1995; Taniyama 1996; Brack et al. 1997; Rehmann et al. 2003; Bacciarini et al. 2004; Sato et al. 2005; Tappe et al. 2007). These infections are associated with high mortality in both captive and natural settings. Elucidating the epidemiology and evolution of larval infections with *Taenia* and *Echinococcus* species in NHP sheds light on how emerging parasites can shape health risks for wild and captive NHP and when novel transmission is expected.

14.2 Evolutionary History and Classification

Unlike other parasites such as pinworms (Brooks and Glen 1982; Hugot 1999), taeniids do not co-speciate with their hosts (Hoberg et al. 2000, 2001; Hoberg 2002). Rather, shifts between host species that are not closely related occur when such species share ecological guilds and utilize common resources (Hoberg 2002). For example, humans are the definitive host for at least three taeniid species (*Taenia solium, T. saginata,* and *T. asiatica*) (Craig and Pawłowski 2002; Hoberg 2002), joining classic carnivorous definitive hosts in the canid, felid, hyaenid, mustelid, and viverrid families (Leiby and Dyer 1971; Loos-Frank 2000; Hoberg 2002). This transition is postulated to have emerged when ancestral hominids shifted in diet from herbivory to omnivory, positioning them to join the carnivorous guild feeding on prey species infected with taeniid larval stages (Hoberg et al. 2000; Hoberg et al. 2001; Hoberg 2002).

While taeniids are among the best studied of all tapeworm parasites, disagreement and controversy have surrounded the elucidation of their taxonomic statuses and phylogenetic relationships (Abuladze 1964; Loos-Frank 2000; Hoberg et al. 2000; Hoberg 2002; Nakao et al. 2013; Lymbery 2017). Early authors assigned adult and larval stages to different genera and species (e.g., *Taenia saginata* and *Cysticercus bovis* (now both *T. saginata*), *Hydatigera taeniaeformis* and *Strobilocercus fasciolaris* (now both *T. taeniaeformis*), reviewed in Hoberg et al. 2000; Hoberg 2002, or *Taenia echinococcus cysticus* (now *E. granulosus*), reviewed in Eckert and Thompson 2017). Some studies assigned species and genera names based on larval morphology, while others assigned species and genera names based on host predilection or geographic distribution. These approaches have been rejected in recent years as insufficient for species or genus identification because of the morphological

and behavioral plasticity exhibited by many taeniids (Combes 2001; Nakao et al. 2013; Lymbery 2017) and have been increasingly replaced with genetic tools (Padgett et al. 2005; McManus 2006; Zhang et al. 2007; Jeon et al. 2009; Jia et al. 2010; Avcioglu et al. 2011). In the words of Combes (2001), "molecular techniques have reduced the abusive synonymizing of species distinguished according only to hosts or other uncertain characters" (pg. 54). While the entire range of potential taeniid host species may historically have been obfuscated by erroneous identification, the application of molecular tools in recent, current, and future studies will illuminate the true host breadth of taeniids.

14.3 Life Cycle

In carnivorous definitive host species, the adult taeniid tapeworm is attached to the intestinal tract by means of the hooks and suckers of its scolex (anterior end) (Flisser 1991; Thompson 2017). The scolex produces proglottids, which are the asexually reproducing segments that comprise the tapeworm body. Proximal proglottids mature as they progress distally, and the most distal proglottids drop off as they become gravid. Gravid proglottids migrate to the anus and are expelled in feces during excretion (Flisser 1991; Thompson 2017). As proglottids disintegrate outside of the host body, they release eggs in numbers that can range from 50,000 to 100,000 per proglottid for species within the *Taenia* genus (Gregory 1976; Flisser 1991; Lescano and Zunt 2013) and from 100 to 1500 per proglottid for species within the *Echinococcus* genus (Thompson 2017). These microscopic eggs are dispersed across the landscape and within the soil profile by environmental elements such as rain, wind, and mechanical vectors (Gemmell et al. 1987; Lawson and Gemmell 1990; Torgerson et al. 1992, 1995; Craig and Macpherson 2000; Lescano and Zunt 2013). Eggs are enclosed in adhesive proteinaceous shells (Conn and Swiderski 2008; Jabbar et al. 2010; Thompson 2017) that permit them to stick on vegetation, which is crucial for their transmission to the next host. The second stage of the taeniid commences when intermediate hosts, which include artiodactyl, rodent, and lagomorph species, ingest eggs during foraging (Loos-Frank 2000; Hoberg 2002; Thompson 2017).

As the eggs pass through the stomach of the intermediate host, gastric juices wear away the protective layers of the taeniid egg (Conn and Swiderski 2008). The erosion of these layers, which include the keratin shell, embryophore layers, and embryonic envelopes, releases precursor to the taeniid larval form, the hooked hexacanth oncosphere (Heath 1971; Conn and Swiderski 2008; Jabbar et al. 2010). The oncosphere protects the hooked hexacanth embryo as it invades the villi of the intestinal wall, using vigorous thrusts of its musculature to invade the intestinal lining with the help of secretory products from oncospheral penetration glands (Jabbar et al. 2010; Thompson 2017). After burrowing through the intestinal wall, the hexacanth is picked up by the circulatory systems (Heath 1971; Marty and Neafie 2000; Jabbar et al. 2010). Depending on the species, hexacanths can settle in a

number of places around the body, including somatic tissue and the central nervous system, to begin the larval, asexually budding stage of their development (the metacestode) (Lescano and Zunt 2013; Thompson 2017).

The metacestode stage characteristic of the *Taenia* genus manifests as coenuri (sing., coenurus) or cysticerci (sing., cysticercus) depending on the species (Lescano and Zunt 2013). Coenuri and cysticerci commonly develop in multiple organs and tissues, including the subcutis, the brain, and the eye. In coenurosis, the metacestode (i.e., larval) stage of *T. serialis* and *T. multiceps*, hexacanth embryos develop into fluid-filled exogenously budding capsules containing multiple protoscolices (the immature form of the scolex, the proximal end of the adult tapeworm in the definitive host) (Bowman 2009; Lescano and Zunt 2013). Metacestode development occurs as embryos asexually bud through the branching and invagination of endogenous daughter cysts. By contrast, the cysticerci characteristic of the larval form of *T. saginata* and *T. solium* each contain a single protoscolex within a translucent, fluid-filled capsule (although certain morphotypes of *T. solium* lack a scolex, Rabiela et al. 1989).

The larval stage of *Echinococcus* species is generally classified as either cystic or alveolar echinococcosis. In alveolar echinococcosis (AE), the metacestode stage characteristic of *E. multilocularis*, embryos develop into multivesiculated and multiloculated solid larval masses in the host stroma (Thompson 2017). Cellular proliferation via the lymph or circulatory systems can lead to metastatic infections throughout the body (Thompson 2017). As in *Taenia* spp., asexual reproduction permits exponential growth of the metacestode through evagination and invagination (Thompson 2017). In cystic echinococcosis (CE), the metacestode stage characteristic of *E. granulosus* and *E. equinus*, embryos develop into unilocular larval masses (Rehmann et al. 2003).

The larval form of both genera can only achieve adulthood when larvae are ingested by the appropriate definitive host. Upon predation of an infected intermediate host by a definitive host, each of the scolices in the intermediate host tissue can develop into adult tapeworms in the gastrointestinal tract of the definitive host. The immature scolices attach to the intestinal wall and begin developing proglottids that, as they mature, produce the infectious eggs that are shed into the environment to be infected by herbivorous intermediate hosts. Without the consumption of infected intermediate host tissue by the definitive host, the parasite larvae will never mature and the life cycle will remain incomplete. The most efficient intermediate hosts for taeniid parasites are thus herbivores that are common prey items for carnivorous definitive hosts. Among the most common taeniid definitive hosts are canids (e.g., jackals, foxes, domestic dogs, wild dogs) and feliforms (e.g., lions, tigers, bobcats, domestic cats, hyenas) (Loos-Frank 2000). Taeniid intermediate hosts are generally species that are exposed to eggs during foraging and form the basis of the definitive host diet (e.g., rabbits, rodents, ungulates) (Hoberg 2002). Papionin primates, while not as herbivorous as other intermediate hosts, can also be exposed to taeniid eggs during foraging and can be preyed upon by both canid and feliform definitive hosts (Cowlishaw 1994; Iwamoto et al. 1996). Indeed, taeniid infections have been reported in both captive and wild papionin NHP populations over the past century.

14.4 Taeniids in Primates

Within Taeniidae, *Echinococcus* species exhibit a greater breadth of NHP intermediate hosts than *Taenia* species. Coenurosis and cysticercosis (*Taenia* metacestodes) appear almost exclusively in cercopithecine NHP (with the exception of two published cases in ring-tailed lemurs), while alveolar, cystic, and polycystic echinococcosis appear in cercopithecines, apes, lemurs, and one atelid (Table 14.1). True to their status as parasites of the liver, *Echinococcus* cysts were observed in the liver in nearly every recorded case of alveolar echinococcosis (AE) or cystic echinococcosis (CE) in NHP, with additional cysts reported in other viscera and somatic tissue. Coenurosis and cysticercosis, on the other hand, showed no obvious site predilection, with cysts appearing across organs and tissues (Table 14.1).

Most cases of coenurosis and cysticercosis in NHP appeared in captive, wild-caught individuals, and infection was suspected to have originated in the primate home country. By contrast, both CE and AE in NHP have primarily been reported in captive-born colonies and zoos across Europe, Asia, and the United States. The geographic concentration of CE and AE reporting in the Northern hemisphere may result from a combination of factors. First, the primary definitive host of *E. multilocularis* is the red fox (*Vulpes vulpes*), which is distributed exclusively across Europe, Asia, and North America (Romig et al. 2006; McManus et al. 2003). Thus, cases of AE in NHP are only expected in fox-endemic areas. Indeed, high prevalence rates in foxes are accompanied by high rates of AE in humans (Romig et al. 2006, 2017; Torgerson et al. 2010), which generally correspond with the occurrence of AE in captive NHP housed in red-fox endemic areas.

By contrast, the primary definitive host of *Echinococcus* species that cause CE is the globally distributed domestic dog, and CE is considered to be endemic to every continent except Antarctica (Deplazes et al. 2017). While CE should thus be expected to impact NHP worldwide and in their natural habitats, cases have only been reported at zoos in Europe, the United States, and Israel, with one case of polycystic echinococcosis reported in a red-shanked douc languar living in a wildlife rehabilitation center in Vietnam (Table 14.1). While the lack of reported CE cases in NHP origin countries is surprising, it may be due, at least in part, to undertesting in zoos and in wild populations. Further research is necessary to determine whether lack of testing, exposure, or susceptibility shape patterns of echinococcosis in NHP.

Although echinococcosis reports in NHP are more numerous than coenurosis or cysticercosis reports, coenurosis is the sole taeniid infection that is reported to consistently infect wild NHP populations. Geladas (*Theropithecus gelada*), cercopithecine primates endemic to the Ethiopian Highlands, serve as intermediate hosts for the metacestode stage of *T. serialis* (Schneider-Crease et al. 2013; Nguyen et al. 2015). The observation of coenurosis in wild geladas expands the previously known taxonomic breadth of this neglected tropical disease to include NHP and expands the previously known array of parasites infecting NHP to include tapeworm larvae.

Table 14.1 Known primate hosts of larval tapeworm infections

Infection	Parasite species	Host		Infection site	Location	Citation
Alveolar echinococcus	*E. multilocularis*	Hominidae				
			Gorilla gorilla gorilla	Liver, kidney, lymph nodes, cerebrum, lung	Switzerland, Japan	Kondo et al. (1996), Rehmann et al. (2003)
			Pongo pygmaeus		Japan	Taniyama (1996)
			Hylobates spp.	Liver	Germany	Deplazes and Eckert (2001)
		Cercopithecidae				
			Miopithecus talapoin	Liver, lung, mesenterium	Germany	Deplazes and Eckert (2001)
			Macaca fascicularis	Liver, vertebrae, gallbladder, pancreas, lung, abdominal serosa	France, Germany, Switzerland	Rietschel and Kimmig (1994), Brunet et al. (2015), Tappe et al. (2007), Bacciarini et al. (2004), Deplazes and Eckert (2001)
			Macaca mulatta	Liver, pancreas, heart	Germany	Brack et al. (1997), Tappe et al. (2007)
			Macaca fuscata	Liver, lymph nodes, lungs, pancreas, kidney	Japan	Sato et al. (2005)
			Macaca silenus	Liver, lymph nodes, mediastinum, mesenterium, heart	Germany	Tappe et al. (2007)
			Macaca nigra	Liver, lung, lymph nodes	Germany	Deplazes and Eckert (2001)
			Macaca sylvanus	Liver, omentum, mesentery	UK	Boufana et al. (2012)
		Atelidae	*Ateles geoffroyi*	Liver, abdominal cavity, retroperitoneum, lungs	Iran	Borji et al. (2012)
		Lemuridae				
			Lemur catta	Liver, perirenal tissue, lung, lymph nodes	Germany, Japan	Kondo et al. (1996), Deplazes and Eckert (2001)

(continued)

Table 14.1 (continued)

Infection	Parasite species	Host		Infection site	Location	Citation
Cystic echinococcus	*E. granulosus*	Cercopithecidae				
			Cercopithecus ascanius	Liver	UK	Boufana et al. (2012)
			Macaca nemestrina	Liver	Germany	Plesker et al. (2001)
	E. equinus	Lemuridae				
			Varecia rubra	Liver, abdominal cavity, mesentery, omentum	UK	Boufana et al. (2012), Denk et al. (2016)
			Lemur catta	Stomach, small intestine, omentum, testes, abdominal cavity	Israel, UK	Shahar et al. (1995), Denk et al. (2016)
	E. ortleppi	Cercopithecidae	*Pygathrix nemaeus*	Lungs	Vietnam	Plesker et al. (2009)
Polycystic echinococcus	*E. vogeli*	Hominidae				
			Gorilla gorilla gorilla	Abdominal cavity, liver, intestines	US	Howard and Gendron (1980), O'Grady (1982)
Coenurosis	*Taenia* spp.	Cercopithecidae				
			Cercopithecus nictitans	Brain	Imported to UK from West Africa	Sandground (1937)
			Cercopithecus mitis	Brain, heart, subcutaneous tissue	Rwanda	Fain (1956)
			Theropithecus gelada	Viscera, subcutaneous tissue, brain, musculature	Imported to UK, US from Ethiopia	Scott (1926), Elek & Finkelstein, etc.

Cysticercosis	*Taenia spp.*	Cercopithecidae				
			Macaca fascicularis	Greater omentum	China	Tsubota et al. (2009)
			Macaca mulatta	Brain, musculature, diaphragm, pericardial, viscera, omentum	India	Walker (1936), Vickers and Penner (1968), Hobbes et al. (2003)
			Macaca cyclopis		Taiwan	Kuntz and Myers (1967)
			Macaca tonkeana	Mesentery	France	Brunet et al. (2014)
			Erythrocebus patas	Liver, mesentery, abdominal organs	Sudan	Sulaiman et al. (1986)
			Cercopithecus aethiops	Pleural cavity, pericardium, liver, mesentery, gallbladder	Sudan, Kenya	Sulaiman et al. (1986), Kuntz and Myers (1967)
		Lemuridae	*Lemur catta*	Peritoneal cavity, subcutaneous tissue	Italy, Spain	Luzón et al. (2010), De Liberato et al. (2014)

14.5 Case Study: *Taenia serialis* in Geladas

Across the academic and medical literature, *T. serialis* has traditionally been regarded as a parasite of rodent and lagomorphs in its intermediate form. In the most common iteration of the life cycle, rodents and lagomorphs ingest *T. serialis* eggs shed in carnivore definitive host feces (candidate definitive hosts include canids such as jackals, domestic dogs, and Ethiopian wolves) during foraging, and the life cycle is completed when a definitive host preys on and consumes the rodent or lagomorph (Bowman 2009). However, geladas have joined rodents and lagomorphs in hosting the *T. serialis* metacestode stage. Their inclusion into the range of intermediate host species for *T. serialis* is likely facilitated by their high degree of terrestriality and herbivory (Dunbar and Dunbar 1977), which situates them to regularly ingest *T. serialis* eggs in high numbers that are shed by sympatric carnivorous definitive hosts.

In geladas, *T. serialis* infection results in tumorous cysts full of asexually budding coenuri that grow in somatic, muscular, and visceral tissue and are frequently protuberant and visible to observers (Fig. 14.1). Cysts can also develop deep in the abdominal cavity or inside viscera and thus are observable only upon necropsy (e.g., Scott 1926). These cysts have been described since the early twentieth century in wild-caught captive geladas housed in European and North American zoos (Scott 1926; Schwartz 1926, 1927; Urbain and Bullier 1935; Elek and Finkelstein 1939; Rodhain and Wanson 1954; Bertolino 1957; Clark 1969). External cysts have been

Fig. 14.1 Protuberant *T. serialis* coenuri on the right pectoral-axillary and olecranal regions of a wild female gelada in the Simien Mountains National Park, Ethiopia Photo by author

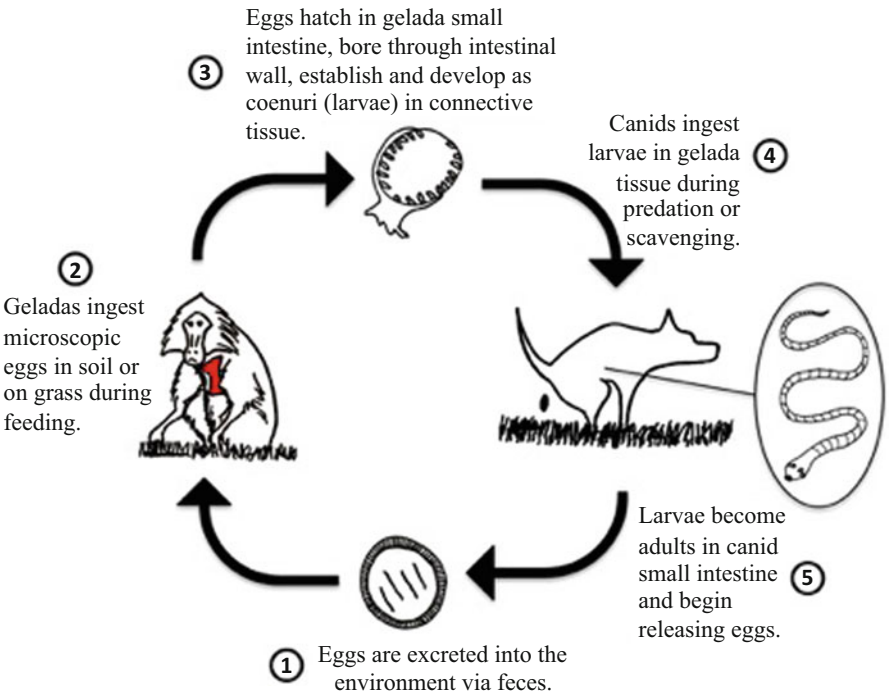

Fig. 14.2 *Taenia serialis* life cycle in geladas. (Drawing by RHG)

observed in wild geladas in the Ethiopian Highlands since researchers began systematically studying geladas (Ohsawa 1979; Dunbar 1980, Schneider-Crease et al. 2013; Nguyen et al. 2015), and recent work has confirmed the etiological agent behind the cysts as *T. serialis* with molecular tools (Schneider-Crease et al. 2013; Nguyen et al. 2015) and probed the pathological profile of *T. serialis* infection and its consequences for reproductive success and survival (Nguyen et al. 2015; Schneider-Crease et al. 2017a, 2017b). The full development of coenuri in geladas indicates that this species is not merely an accidental host and suggests instead that geladas play a role in the *T. serialis* life cycle (Fig. 14.2).

14.5.1 Pathogenesis and Fitness Effects

The physical growth of *T. serialis* larvae inside the muscular or somatic tissue (Fig. 14.3) can directly kill its gelada host, allowing the infected cadaver to be scavenged upon by the carnivorous definitive hosts, or can simply enhance its vulnerability to predation by impeding limb or organ function. Indeed, previous studies of *T. serialis* cysts in wild-caught captive geladas revealed pathologies that

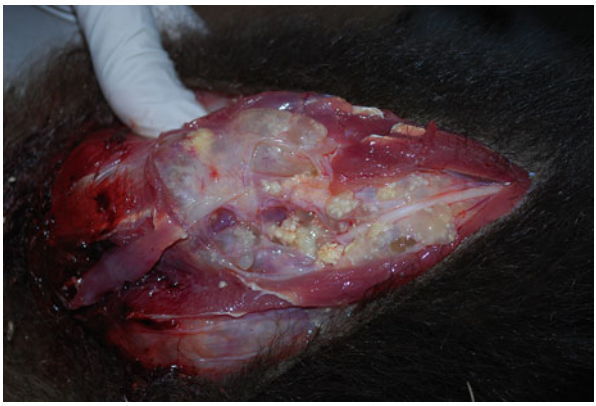

Fig. 14.3 An opened cyst revealing the fluid-filled exogenous capsules containing larvae of *T. serialis*. (Photo by JCJ)

included cachexia, spastic limb paralysis, and dysbasia in geladas (Scott 1926; Urbain and Bullier 1935; Elek and Finkelstein 1939).

Supporting the hypothesis that *T. serialis*, as a parasite reliant on predator-mediated transmission, should be under selection to increase the likelihood of its host to fall victim to predation, the pathologies seen in infected wild geladas are accompanied by strikingly high mortality. Geladas with observable cysts incurred higher mortality than those without cysts at each of two long-term gelada research sites in Ethiopia—the Simien Mountains National Park (SMNP) and the Guassa Preserve (GP) (Nguyen et al. 2015; Schneider-Crease et al. 2017b). Female geladas with cysts incurred additional costs in the form of enhanced infant mortality. Offspring of mothers with cysts suffered higher mortality than those of mothers without cysts. In both populations, infant mortality was secondary effect of the impact of cysts on survival; dependent offspring of mothers with cysts inevitably died with their mothers. However, only GP offspring experienced higher mortality even when mothers survived. Overall, *T. serialis* coenurosis appears to enhance the likelihood of geladas (both hosts and host offspring) to fall victim to predation or scavenging by carnivorous definitive hosts.

14.5.2 Host Manipulation

Contrary to the nearly ubiquitous pattern of increased male host susceptibility to parasite infection and disease (Poulin 1997; Zuk 2009; Guerra-Silveira and Abad-Franch 2013), females are the preferred environment for certain larval *Taenia* spp. (Morales-Montor and Larralde 2005). The larvae of these species preferentially thrive in estrogen-rich environments and can alter the endocrinological profile of male hosts to create a more parasite-friendly environment in a process known as "parasite-induced deandrogenization" (Esch 1967; Lin et al. 1990; Sciutto et al. 1991; Terrazas et al. 1994; Larralde et al. 1995; Morales-Montor et al. 2002a, b,

2004; Gourbal and Gabrion 2004; Morales-Montor and Larralde 2005; Arteaga-Silva et al. 2009).

Larvae are armed with sex steroid receptors that bind to estrogens and androgens (Escobedo et al. 2004; Escobedo et al. 2010). In *Taenia crassiceps*, a sister taxon to *T. serialis*, ovarian hormones (17*b*-estradiol, E_2, and progesterone, P_4), stimulate larval proliferation upon binding to sex steroid receptors on the metacestode (Escobedo et al. 2004; Escobedo et al. 2010), whereas androgens (testosterone (T), and dihydrotestosterone (DHT)) inhibit it upon binding (Vargas-Villavicencio et al. 2005; Ibarra-Coronado et al. 2011). Indeed, larvae treated in vitro with E_2 and P_4 exhibit enhanced growth as compared to those treated with T and DHT (Escobedo et al. 2004, 2010; Ambrosio et al. 2015). This differential growth is due to the activity of AP-1 complex genes (*c-Fos* and *c-jun*), which underlie processes of cell proliferation and thus are important players in larval asexual reproduction (Morales-Montor et al. 1998; Escobedo et al. 2004). Estrogens increase *c-Fos* and *c-jun* activity, leading to increased larval cell proliferation and increased larval growth, whereas androgens decrease larval cell activity, leading to cell apoptosis and inhibited larval growth (Escobedo et al. 2004).

To thrive in male hosts, these species upregulate estrogen secretion and downregulate androgen secretion by increasing the synthesis of aromatase (enzyme aromatase cytochrome P-450), an enzyme that catalyzes the conversion of T to E_2 (aromatization) (Simpson et al. 1994; Terrazas et al. 1994; Morales-Montor et al. 1999a, b, 2001; Morales-Montor and Larralde 2005). Infection stimulates the production of substances critical to the induction and activation of P-450 aromatase in hosts: follicle-stimulating hormone (FSH) and the cytokine IL-6 (Spangelo et al. 1995; Morales-Montor and Larralde 2005). Male mice and swine infected with larval *T. crassiceps* and *T. solium,* respectively, exhibited increased FSH and IL-6 production, higher aromatase activity, higher estradiol concentrations, lower testosterone concentrations, and increased larval growth (while treatment with aromatase inhibitors blocked this process) (Larralde et al. 1995; Morales et al. 1996; Morales-Montor et al. 1999a, b, 2001; Gourbal et al. 2002; Morales-Montor et al. 2002a, b; Vargas-Villavicencio et al. 2005; Peña et al. 2007). Thus, parasite-driven deandrogenization is likely an adaptive manipulation by the parasite that permits taeniid larvae to optimize proliferation in male hosts.

The lack of sex differences identified either in the occurrence of visible *T. serialis* cysts or antigen presence in urine in both gelada populations under long-term study is therefore somewhat surprising (Nguyen et al. 2015; Schneider-Crease et al. 2017b). Furthermore, visible cysts were not associated with lower fecal testosterone metabolite concentrations in males in the SMNP population (Schneider-Crease 2017), suggesting that deandrogenization does not occur in this system. Further work should investigate whether *T. serialis* larvae exhibit the same estrogen affinity and androgen aversion as do its sister taxa, and, if so, whether there are adaptations in geladas that have permitted them to compensate for or mediate deandrogenization. Because the reproductive success of male geladas is closely tied to testosterone-mediated signals (Pappano and Beehner 2014), infection with a parasite that lowers testosterone production via aromatization to estradiol could act as a strong selective pressure in geladas.

14.6 Conclusion

Taeniid parasites exhibit substantial breadth in the definitive and intermediate hosts they infect. Beyond reforming the traditional view of *Taenia* and *Echinococcus* intermediate hosts, the ability of these species to infect NHP in their intermediate forms points to their substantial zoonotic potential. While decades of research have provided a thorough understanding of echinococcosis across the globe (synthesized most recently in Thompson 2017), much remains to be learned about *Taenia* species in wild monkeys. Importantly, little is known about the determinants of susceptibility. Geladas are currently the only NHP known to be integrated into the life cycle of a taeniid, and only certain species exposed to echinococcosis in captive NHP colonies become infected. For example, while four NHP species were housed in the same open-air enclosure at the German Primate Center, only three of these species were seropositive for echinococcosis (Tappe et al. 2007). Additionally, laboratory research has shown remarkable species variation in the success of experimental infections (e.g., Rogan et al. 1993). Moving forward, research should investigate whether taeniid infection in NHP is driven by elements related to exposure (e.g., terrestriality and herbivory) or whether there are biological factors that increase susceptibility to infection. For NHP in captivity that have access to outdoor areas, extra care should be given to evaluating the risks posed by the parasites carried by local primary host species.

Understanding the environmental and biological components of larval taeniid infections in NHP is essential to the development of a One Health approach to tackling the emergence of infections in novel hosts, including humans (Ing et al. 1998; Tappe et al. 2016). The viability of NHP as hosts for taeniid larval stages has pressing implications for NHP conservation, since climate change and burgeoning human population growth may increase the overlap between traditional taeniid hosts and non-hosts. If taeniids possess the capacity to rapidly expand their host repertoire, a wide array of wildlife species including numerous NHP may be at risk for infection. Given the substantial impact on survival and reproduction exacted by larval taeniid infections on intermediate hosts, the emergence of infections in new hosts may threaten the persistence of endangered species. Furthermore, as human populations expand into areas with dense wildlife populations, humans may find themselves at increasing risk for infection with larval taeniids that were previously considered to be non-zoonotic or exceedingly unlikely to emerge in humans. Preventing the transmission of taeniid tapeworms between wildlife, domestic animals, and humans will require unprecedented targeted treatment of both definitive and intermediate hosts and careful evaluation of habitat suitability for human developments.

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Chapter 15
Trypanosomiasis and Filariasis

Jan Votypka, Jana Brzonova, and Klara J. Petrzelkova

Abstract Trypanosomes and filarial nematodes are important pathogens in humans and domestic animals. However, the majority of the infections reported from nonhuman primates (NHPs) are nonpathogenic. Moreover, those hemoparasites are relatively host-specific, which means that transmission from NHPs to humans is highly unlikely with the exception of nonpathogenic *Trypanosoma rangeli* and *Trypanosoma cruzi* and the *T. brucei* complex, which cause Chagas disease and sleeping sickness in humans, respectively. NHPs may also act as reservoir hosts for some nonpathogenic human filarial parasites, e.g., *Mansonella streptocerca*. Though many studies on those hemoparasites were conducted in the last century, recent studies remain rather neglected due to the logistical, ethical, and administrative challenges associated with the collection of blood or tissue samples in wild NHPs. In this chapter, we present an overview of trypanosomes and filarial nematodes infecting NHPs with information about their distribution, biology, pathogenesis, and their zoonotic potential.

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15.1 Introduction

Hemoparasites are often an underappreciated participant in the epidemiology of some of the most important zoonosis (Burgos-Rodriguez 2011). The best-known hemoparasites are the vector-borne *Plasmodium* spp. These intracellular parasites infect humans as well as nonhuman primates (NHPs) and all five *Plasmodium* species that are causative agents of human malaria are derived from primates' ancestors (Singh et al. 2004; Cox-Singh et al. 2008; Prugnolle et al. 2011). However, also other blood parasites found in NHPs, e.g., trypanosomes and filarial nematodes, are important pathogens in humans and domestic animals. Trypanosomes from the *Trypanosoma brucei* complex are responsible for sleeping sickness in humans and nagana or surra in livestock, *Trypanosoma cruzi* causes human Chagas disease in Latin America, and filarial parasites are responsible for human and animal filariasis. The majority of the hemoparasites reported from NHPs, however, are considered to be nonpathogenic to their hosts (i.e., infections are primarily asymptomatic) and relatively host-specific. It means that transmission from NHPs to humans is highly unlikely; the only exceptions are two trypanosome species complexes, *Trypanosoma cruzi* and *T. brucei*, important pathogens that cause Chagas disease and sleeping sickness in humans, respectively. Additionally, NHPs may also act as reservoir hosts for some nonpathogenic human filarial parasites, e.g., *Mansonella streptocerca* (Van den Berghe et al. 1964).

Not only is there low pathogenicity of hemoflagellates (trypanosomes) and filarial parasites in NHP hosts (Webber 1955a, b; Baker 1972; Hoare 1972; Toft 1986; Stevens et al. 1998; Klei and Tajan 2002; Malta et al. 2010; Telleria and Tibayrenc 2017) and sporadic zoonotic transmission, but the majority of studies describing the occurrence of blood parasite in NHPs were published during the twentieth century when the species determinations of these more-or-less random findings were based only on unreliable morphological characters. Given the absence of molecular biological data in most of the publications, it is impossible to validate the reliability, prevalence, and host specificity of the described parasites. Nowadays, hemoflagellates and filarial parasites remain rather neglected in NHPs due to the logistical, ethical, and administrative challenges associated with the collection of blood or tissue samples. While some blood parasites, e.g., *Plasmodium* spp., can be reliably

detected in feces (Mapua and Votýpka 2018), detection of other blood parasites, including trypanosomes and filariae, remains challenging.

15.2 Trypanosomiasis

15.2.1 Trypanosomes in NHPs

The genus *Trypanosoma* Gruby, 1843 (Euglenozoa: Kinetoplastea: Trypanosomatidae) is a member of the class Kinetoplastea Cavalier-Smith 1981, previously known as the order Kinetoplastida Honigberg 1963 (for more information see Gibson 2017). The group is named after the kinetoplast, a unique cell organelle consisting of the tightly packaged mitochondrial DNA, which forms a stainable structure within the single mitochondrion. Trypanosomes infect all classes of vertebrate hosts, but most attention is directed to the species that cause serious forms of human and animal diseases and heavy economic losses. The trypanosomes range from nonpathogenic species to those that are highly pathogenic for their hosts including humans. Two examples for the latter are the causative agents of Chagas disease (*T. cruzi* complex) in Latin America or sleeping sickness (*T. brucei* complex) in sub-Saharan Africa. Moreover, the family Trypanosomatidae includes, along with the trypanosomes, the genus *Leishmania*, the causative agent of human cutaneous, mucocutaneous, and visceral leishmaniasis (e.g., *Leishmania tropica*, *L. braziliensis,* and *L. donovani*).

The trypanosomes are best known as free-swimming flagellates in vertebrate blood. However, species-dependent, they may become sequestered in the capillaries of certain organs (e.g., rodent species *T. lewisi*, which was repeatedly found in Latin American monkeys), attached to peripheral capillary endothelium (e.g., *T. congolense*), or leave the vascular system and invade the lymphatics and connective tissue fluid (e.g., *T. brucei* and *T. evansi*). The most dangerous representative of trypanosomes, *T. cruzi*, invades and multiplies as amastigotes inside many different host cell types including muscle cells, macrophages, and fibroblasts.

Trypanosomes and leishmanias are transmitted to a vertebrate host by an invertebrate vector, mostly an insect, but with significant differences in their survival strategies and the life cycles. African trypanosomes (*T. brucei* complex and related Salivarian trypanosomes) undergo a complex development in tsetse flies, which results in the production of infective trypanosomes in the salivary glands and are transmitted by a bite. On the other hand, *T. cruzi* is transmitted orally or intradermally through feces (via contamination) of infected reduviid bugs (trypanosomes with this type of transmission are called Stercoraria). The genus *Leishmania* is transmitted to mammals by a bite of phlebotomine sand flies and evades elimination from the bloodstream by propagation in a vertebrate host's phagocytic cells. Trypanosome infections are usually diagnosed by finding of free-swimming stages in the peripheral blood; leishmanias are detectable by the skin biopsies or using various serological methods (for details, see Garcia 2016).

15.2.1.1 Zoonotic Potential of NHP Trypanosomes

The vast majority of trypanosomes reported in NHPs are considered to be nonpathogenic. In addition, it is generally assumed that a large proportion of trypanosome species are highly host-specific and therefore have only a very low potential to be transmitted to humans from NHP hosts. Based on previous studies, it could be suggested that at least a part of NHP trypanosome infections represents accidental infections (e.g., from rodent hosts) and therefore in these cases, NHPs cannot act in the role of reservoir animals for humans. The only exceptions are the following three trypanosome species/complexes: (i) in humans nonpathogenic *Trypanosoma rangeli*, (ii) in NHPs very rarely occurring subspecies of the *Trypanosoma brucei* complex, and (iii) the most important *Trypanosoma cruzi* (complex), which is a serious human pathogen and the causative agent of Chagas disease.

Trypanosoma cruzi is a complex of several closely related trypanosome species (see Sect. 15.2.1.2) distributed throughout South and Central America with extension into the southern and southwestern regions of the United States (Desforges and Kirchhoff 1993). It is worth mentioning that this parasite was first found in the black-penciled marmoset (*Callithrix penicillata*). In 1908, Carlos Justiniano Ribeiro Chagas was sent as a public health official to the interior of the Brazilian state of Minas Gerais to control malaria among railroad construction workers. At that time, he was already familiar with trypanosomes and when he discovered a new trypanosome species in the blood of a monkey, he named this parasite *Trypanosoma minasense*. At the same time, residents pointed out some blood-sucking bugs feeding on various mammals including humans, the possible vector, determined by Chagas as *Panstrongylus megistus* (initially called *Conorhinus megistus*). When he examined intestinal contents of these hematophagous bugs in his makeshift laboratory, he encountered flagellated organisms that he inferred to be the intermediate forms of the trypanosomes diagnosed in marmosets. To confirm this hypothesis, Chagas sent some infected bugs to Oswaldo Cruz, his mentor and employer in Rio de Janeiro, who succeeded in passing the infection from the insects to common marmosets (*Callithrix jacchus*) that were kept in captivity. Cruz's experiments resulted in the visualization of flagellates in the peripheral blood of the infected monkeys; however, the observed trypanosomes were morphologically distinct from *T. minasense*. Shortly thereafter, Chagas established that this new trypanosome, which was named *Trypanosoma cruzi* in honor of his mentor Oswaldo Cruz, could be passed experimentally to many other hosts, including dogs, cats, and rabbits, and also that it could be grown on blood agar (Kirchhoff 2001; Jansen et al. 2017).

Subsequently, different species of NWM mostly from the families Cebidae and Callitrichidae (namely, squirrel monkeys, owl monkeys, marmosets, tamarins, spider monkeys, woolly monkeys, cebus monkeys, and uakaris) are commonly found to be naturally infected by *T. cruzi* (Jansen et al. 2015). *Trypanosoma cruzi* can only be found in the Western Hemisphere, where it primarily infects wild and domestic mammals. Due to the fact that *T. cruzi* is an important human pathogen, the

occurrence of this parasite in NHPs has been described and investigated in greater detail than the occurrence of other trypanosomes in NHPs.

A high proportion of *T. cruzi*-infected NWM can be explained primarily by their behavior. Night refuges of NHPs in hollow trees are often shared with triatomine bugs, the situation which brings the vector and the mammalians host in close proximity (a passive process in which parasite and host meet through chance) and allows contaminative transmission of the parasite (Carcavallo et al. 1998). In addition to this, some NHP species frequently consume invertebrates including triatomine bugs infected by trypanosome, which facilitates transmission through the oral route. A congenital infection in the colony-born squirrel monkey (*Saimiri sciureus*) has also been reported (Eberhard and D'Allessandro 1982). The prevalence of infection varies significantly depending on locality and NHP host species, but it was demonstrated that in some areas, nearly half of the wild population of, e.g., golden lion tamarin (*Leontophitecus rosalina*) is infected by *T. cruzi* (Lisboa et al. 2000). It has to be taken into consideration that conservation programs often include exchange, translocation, and reintroduction of NWM, which can lead to an introduction of infected animals in *T. cruzi*-free areas and trigger the establishment of a new transmission cycle (Jansen et al. 2017). Similar to other reservoir mammals, it is considered that infection of *T. cruzi* in wild NHPs is less harmful to their hosts and does not result in the serious sequelae that are seen in humans. However, during experimental infections of NWM (*Callithrix* spp., *Cebus* spp., and *Saimiri* spp.) and OWM (*Macaca mulatta*) NHPs by *T. cruzi*, some animals showed symptoms resembling Chagas disease, such as a low frequency of cardiac abnormalities and the very rare occurrence of megasyndromes (principally megacolon and megaesophagus) and systemic changes (Monteiro et al. 2006).

15.2.1.2 Diversity and Occurrence of Trypanosomes in NHPs

The vast majority of simian trypanosome species were described primarily according to the trypomastigote morphology found in the blood and based on the "one host–one parasite" hypothesis (Maslov et al. 2013). However, it was experimentally demonstrated that trypomastigotes of *Trypanosoma minasense* display high polymorphism depending on the host infected (Ziccardi and de Oliveira 1999). Therefore, it is likely that some of the trypanosome names are only junior synonyms of the previously described species. It could be nicely demonstrated in the case of *Trypanosoma rangeli* Tejera, the species with at least two junior synonyms – *T. diasi* Deane and Martins or *T. saimirii* Rodhain (Rosenblum and Cooper 1968; Ziccardi et al. 2005) or in the case of several trypanosome names – *T. advieri*, *T. brimoti*, *T. devei*, *T. escomeli*, *T. florestali*, *T. manguin-hense*, and *T. mycetace* – which all are most likely only junior synonyms of *Trypanosoma minasense* Chagas (Rosenblum and Cooper 1968).

The validity and the taxonomic status of the below-mentioned NHP trypanosome species, previously listed in Baker (1972), Toft (1986), and Strait et al. (2012), has never been confirmed by any type of molecular study or by an experimental infection

demonstrating the stability of the morphological features or verifying the supposed host specificity. *Trypanosoma conorhini* is defined as a parasite of *Rattus rattus* transmitted by *Triatoma rubrofasciata*; however, the natural infection of rats is very low and experimental infection identified Asian monkeys of the genus *Macaca* as a possible reservoir host of this species (Deane et al. 1968; Cross et al. 1983; Denning and Karcher 1986). Two trypanosome species, *T. irangiense* and *T. perodictici*, were described from potto (*Perodicticus potto*) and Thomas's bushbaby (*Galagoides thomasi*) in the Democratic Republic of Congo (Reichenow 1917). Two more trypanosome species were described from NHPs in Colombia: *T. lambrechti* from white-fronted capuchin (*Cebus albifrons*) and *T. sanmartini* from squirrel monkeys (*Saimiri* spp.) (Deane 1969; Deane et al. 1970). Finally, the trypanosome species *T. primatum* was described more than 100 years ago (Reichenow 1917) from chimpanzees (*Pan troglodytes*) and gorillas (*Gorilla gorilla*).

Unlike the list of six trypanosome species noted above and described many years ago, the reliability and validity of the species discussed below are significantly higher, owing to the sequencing data provided by different authors. NHP trypanosomes, with available sequencing data, could be found in six main trypanosome groups/clades recognized by molecular phylogenetic analyses (see Hamilton and Stevens 2017).

One of the most common simian trypanosome species, *Trypanosoma minasense* Chagas, is a member of the *T. irwnini* clade. The species was molecularly detected for example in a wild population of saddleback tamarin (*Leontocebus weddelli*) in southeastern Peru (Erkenswick et al. 2017), in wild howler monkeys (*Alouatta caraya*) in northeastern Argentina (Martínez et al. 2016), or in a South American red-handed tamarin (*Saguinus midas*) (Sato et al. 2008). However, many older studies demonstrated the infection by *T. minasense* in a number of NWM species (e.g., marmosets, capuchins, squirrel monkeys, spider monkeys, howler monkeys, and wooly monkeys) only based on the trypomastigote morphology (see Toft 1986; Sato et al. 2008). The trypanosome species was originally described from black-penciled marmosets (*Callithrix penicillata*) in Brazil (Chagas 1909). *T. minasense* was detected at a prevalence of approximately 20% in 11 NHP species in Peru and Colombia (Table 15.1) (Dunn et al. 1963); moreover, the same trypanosome species was later recorded in three wild Panamanian species, *Saguinus geoffroyi*, *Cebus capucinus*, and *Ateles fusciceps* (Sousa et al. 1974). The occurrence of *T. minasense* in Colombia has been confirmed also in a later study where five NHP species (Table 15.1) have been found infected, while in Brazil this trypanosome was detected only in two species of squirrel monkeys (*Saimiri sciureus* and *S. ustus*) (Ziccardi and de Oliveira 1997).

Stevens et al. (1998) found a simian trypanosome from Southeast Asia, *Trypanosoma cyclops*, to be related to the *T. theileri* clade. This clade contains trypanosomes from marsupial as well as placental mammals, mainly deer and cattle. The simian trypanosome species *T. cyclops* was originally isolated from the genus *Macaca* (*M. nemestrina* and *M. ira*) in Malaysia and the insect vector of this species is still unknown; however, transmission by reduviid bugs was suggested (Weinman 1972). In 1984, Weinman et al. described another simian trypanosome,

Table 15.1 List of selected trypanosomes, *Leishmania* spp., and filariae found in NHPs; occurrence in humans and the zoonotic potential is mentioned

Species	OW Monkeys/Lemurs	NW Monkeys	Great Apes	Humans	Other Hosts	References
Trypanosomatids						
Trypanosoma cruzi clade						
Trypanosoma cruzi complex		*Alouatta caraya, Ateles fusciceps, A. geoffroyi grisescens, Cacajao* sp., *Callithrix jacchus, C. penicillata, Cebus albifrons, C. apella, C. capucinus, Lagothrix* sp., *Leontophitecus chrysomela, L. rosalia, Saguinus boliviensis, S. geoffroyi, S. leucopus, Saimiri sciureus, S. ustus, Tamarinus nigricollis*		YES	YES	Chagas (1909), Dunn et al. (1963), Marinkelle (1966), Sousa et al. (1974), Eberhard and D'Allessandro (1982), Ziccardi and Lourenço-de-Oliveira (1997), Fernandes et al. (1999), Lisboa et al. (2000), Ndao et al. (2000), Kirchhoff (2001), Jansen et al. (2015), Martínez et al. (2016), Jansen et al. (2017)
Trypanosoma rangeli		*Aotus* sp., *Cebuella pygmaea, Cebus capucinus, Saimiri boliviensis, S. sciureus, S. ustus, Saguinus bicolor, S. geoffroyi, S. labiatus labiatus, S. midas*		YES	YES	Sousa et al. (1974), Ziccardi and de Oliveira (1997), Marinkelle (1966), Maia da Silva et al. (2004), da Silva et al. (2008), Sato et al. (2008)
Trypanosoma conorhini	*Macaca fascicularis, M. cyclopis, M. mulatta*				YES	Deane et al. (1968), Hoare (1972), Cross et al. (1983), Denning and Karcher (1986)
"Madagascar subgroup"	*Indri indri, Propithecus diadema*					Larsen et al. (2016)

(continued)

Table 15.1 (continued)

Species	OW Monkeys/Lemurs	NW Monkeys	Great Apes	Humans	Other Hosts	References
"Unnamed trypanosome"	*Cercopithecus nictitans*					Hamilton et al. (2009)
Trypanosoma brucei clade						
Trypanosoma brucei complex	*Cercocebus albigena, C. cephus, C. mona, Cercopithecus neglectus, C. nictitans, C. torquatus, Colobus guereza, Miopithecus talapoin, Perodicticus potto*		*Gorilla gorilla, Pan troglodytes*	YES	YES	Herder et al. (2002), Njiokou et al. (2006), Jirků et al. (2015)
Trypanosoma congolense	*Cercopithecus cephus*				YES	Herder et al. (2002)
Trypanosoma vivax	*Cercopithecus cephus, C. mona, C. nictitans, Miopithecus talapoin, Perodicticus potto*				YES	Herder et al. (2002)
Other trypanosomes						
Trypanosoma cyclops	*Macaca nemestrina, M. ira*					Weinman (1972)
Trypanosoma lucknowi	*Macaca mulatta*					Weinman et al. (1984)
Trypanosoma lewisi		*Aotus* spp., *Callithrix jacchus*			YES	da Silva et al. (2010)
Trypanosoma minasense		*Alouatta caraya, Aotes trivirgatus, Ateles fusciceps, A. geoffroyi grisescens, A. paniscus, Callithrix*				Chagas (1909), Dunn et al. (1963), Rosenblum and Cooper (1968), Sousa et al. (1974), Toft (1986), Ziccardi

		penicillata, Cebuella pygmaea, Cebus albifrons, C. albifrons hypoleucus, C. apella, C. capucinus, C. griseus, Lagothrix infumata, Leontocebus weddelli, Saguinus geoffroyi, S. ustus, S. midas, S. oedipus, Saimiri boliviensis, S. sciureus, Tamarinus nigricollis				and de Oliveira (1997), Ziccardi and de Oliveira (1999), Sato et al. (2008), Martínez et al. (2016), Erkenswick et al. (2017)
Trypanosoma irangiense[a]	*Galagoides thomasi*					Reichenow (1917)
Trypanosoma lambrechti[a]		*Cebus albifrons*				Deane et al. (1970)
Trypanosoma primatum[a]			*Gorilla gorilla, Pan troglodytes*			Reichenow (1917)
Trypanosoma perodictici[a]	*Perodicticus potto*					Reichenow (1917)
Trypanosoma sanmartini[a]		*Saimiri* spp.				Deane (1969)
Leismania spp.						
Leishmania (*Leishmania*) *major*	*Cercopithecus aethiops*		*Gorilla gorilla* (?)	YES	YES	Dedet (1993), Ashford (1996), Hamad et al. (2015) (vs. Bastien et al. (2015), Votýpka et al. (2018))

(continued)

Table 15.1 (continued)

Species	OW Monkeys/Lemurs	NW Monkeys	Great Apes	Humans	Other Hosts	References
Leishmania (*Leishmania*) *infantum*		*Alouatta guariba, Aotus nigriceps, Callicebus nigrifrons, Cebus xanthosternos, Leontopithecus chrysomelas, Pithecia irrorata, Saguinus imperator*		YES	YES	Malta et al. (2010)
Leishmania (*Leishmania*) *amazonensis*		*Ateles paniscus, Saguinus geoffroyi*		YES	YES	Herrer et al. (1973)
Leishmania (*Viannia*) *braziliensis*		*Aotus azarae, A. trivirgatus*		YES	YES	Herrer and Christensen (1976), Lima et al. (2012)
Leishmania (*Viannia*) *shawi*		*Cebus apella, Chiropotes satanas*		YES	YES	Lainson et al. (1988), Lainson et al. (1989), Acardi et al. (2013)
Filariae						
Dipetalonema graciliformis		*Ateles chamek, Saguinus midas, S. mystax, S. niger*				Correa et al. (2016)
Dipetalonema caudispina		*Alouatta guariba, Ateles paniscus, Brachyteles arachnoides, Callicebus personatus, Cebus apella, C. capucinus, Cebus* sp., *Leontopithecus chrysopygus, L. rosalia, Saguinus bicolor, Saimiri sciureus*				Correa et al. (2016)

Dipetalonema robini		*Sapajus nigritus*				Vanderhoeven et al. (2017)
Dipetalonema gracilis		*Ateles paniscus, Brachyteles arachnoides, Callithrix jacchus, Cebus apella, C. capucinus, C. cay, C. libidinosus, Cebus* sp., *Lagothrix lagotricha, Leontopithecus chrysopygus, L. rosalia, Saginus bicolor, Saimiri sciureus*				Correa et al. (2016)
Dipetalonema yatesi		*Ateles chamek*				Notarnicola et al. (2007)
Dipetalonema petteri	*Avahi occidentalis, Eulemur macaco macaco, E. fulvus, E. albifrons, E. rufus, E. mongoz, Lepilemur ruficaudatus, Microcebus murinus, Propithecus coquereli*					Irwin and Raharison (2009)[b]
Dipetalonema barbascalensis		*Aotus trivirgatus*				Esslinger and Gardiner (1974)
Dirofilaria corynodes	*Cercopithecus* spp., *Cercocebus* spp., *Erythrocebus* spp.					Orihel and Seibold (1972)
Dirofilaria immitis			*Pongo* sp.	YES	YES	Orihel (1970)
Dirofilaria magnilarvatum	*Macaca fascicularis*					Wong and Brummer (1978)

(continued)

Table 15.1 (continued)

Species	OW Monkeys/Lemurs	NW Monkeys	Great Apes	Humans	Other Hosts	References
Edesonfilaria malayensis	*Macaca irus, M. fascicularis*					Nonoyama et al. (1984), Notarnicola et al. (2007)
Mansonella tamarinae		*Saguinus nigricollis*				Bain et al. (2015)[b]
Mansonella mariae		*Saimiri sciureus*				Bain et al. (2015)[b]
Mansonella perstans			*Gorilla gorilla, Pan troglodytes*			Bain et al. (2015)[b]
Mansonella gorillae			(unspecified)			Bain et al. (2015)[b]
Mansonella leopoldi			*Gorilla gorilla*			Bain et al. (2015)[b]
Mansonella lopeensis			*Gorilla gorilla gorilla*			Bain et al. (2015)[b]
Mansonella streptocerca			*Gorilla gorilla, Pan paniscus, P. troglodytes schweinfurthii*	YES		Bain et al. (2015)[b]
Mansonella rodhaini			*Pan troglodytes schweinfurthii*, *P. t. troglodytes, P. paniscus*			Bain et al. (2015)[b]
Mansonella vanhoofi			*Gorilla gorilla, Pan paniscus*			Bain et al. (2015)[b]
Mansonella marmosetae		*Alouetta* spp., *Ateles paniscus, Saguinus geoffroyi, S. oedipus, Saimiri*				Bain et al. (2015)[b]

		boliviensis, S. sciureus, S. oerstedii oerstedii				
Mansonella atelensis atelensis		*Ateles fusciceps rufiventris, A. geoffroyi*				Bain et al. (2015)[b]
Mansonella atelensis amazonae		*Cebus olivaceus*				Bain et al. (2015)[b]
Mansonella barbascalensis		*Aotus trivirgatus*				Bain et al. (2015)[b]
Mansonella colombiensis		*Cebus apella, Saimiri sciureus*				Bain et al. (2015)[b]
Mansonella mystaxi		*Saguinus mystax mystax*				Bain et al. (2015)[b]
Mansonella obtusa		*Cebus albifrons, C. capucinus, Saimiri oerstedii oerstedii*				Bain et al. (2015)[b]
Mansonella panamensis		*Aotus lemurinus zonalis, A. trivirgatus, Cebus apella, C. capucinus, Saimiri oerstedii oerstedii*				Bain et al. (2015)[b]
Mansonella parvum		*Cebus capucinus, Saimiri oerstedii oerstedii*				Bain et al. (2015)[b]
Mansonella peruviana		*Saimiri sciureus*				Bain et al. (2015)[b]
Mansonella saimiri		*Saimiri sciureus*				Bain et al. (2015)[b]
Mansonella tamarinae		*Saguinus nigricollis*				Bain et al. (2015)[b]

(continued)

Table 15.1 (continued)

Species	OW Monkeys/Lemurs	NW Monkeys	Great Apes	Humans	Other Hosts	References
Sandnema digitatum	*Hoolock hoolock, H. leuconedys, Macaca arctoides*					Bain et al. (2015)[b]
Mansonella ozzardi	*Erythrocebus patas* (experimental infection)	(unspecified)	(unspecified)	YES		Bain et al. (2015)[b]
Macacanema formosana	*Macaca cyclopsis*			YES		Schad and Anderson (1963), Lau et al. (2002)
Meningonema peruzzii	*Chlorocebus pygerythrus, Miopithecus ogouensis*			YES		Orihel and Esslinger (1973), Boussinesq et al. (1995)
Brugia malayi	*Macaca* spp.			YES	YES	Laing et al. (1960), Orihel and Seibold (1972)
Brugia pahangi	*Macaca* spp.			YES	YES	Laing et al. (1960), Orihel and Seibold (1972)
Loa loa	*Cercocebus albigena, Chlorocebus pygerythrus, Mandrillus leucophaeus, Papio cynocephalus*		*Gorilla beringei, G. gorilla, Pan troglodytes*	YES		Rodhain and van den Berghe (1939), Van den Berghe et al. (1964), Bain et al. (1995), Sandground (1936)
Onchocerca volvulus			*Gorilla beringei*	YES		van den Berghe et al. (1964)
Paulianfilaria pauliani	*Lepilemur ruficaudatus, Propithecus verreauxi, P. coquereli*					Irwin and Raharison (2009)[b]
Courduriella courdurieri	*Indri indri*					Irwin and Raharison (2009)[b]

Protofilaria furcata	*Varecia rubra, Hapalemur griseus, Propithecus coquereli*					Irwin and Raharison (2009)[b]
Cercopithifilaria degraaffi	*Papio ursinus*				YES	Bain et al. (1982)
Cercopithifilaria eberhardi	*Papio anubis*					Lefoulon et al. (2014)
Cercopithifilaria kengensis	*Papio cynocephalus*					Lefoulon et al. (2014)
Cercopithifilaria narokensis	*Papio anubis*					Lefoulon et al. (2014)
Cercopithifilaria verveti	*Cercopithecus aethiops*					Lefoulon et al. (2014)

Note: the spectrum of hosts may not be complete
[a]taxonomic validity is unclear
[b]original citations in the review

Trypanosoma lucknowi, most likely related to *T. cyclops*. The trypanosome culture was established in one out of 126 *Macaca mulatta* originated from the vicinity of Lucknow, Uttar Pradesh, India (Weinman et al. 1984).

In Brazil, 200 free-ranging and 160 captive monkeys have been examined, and only three captive owl monkeys (*Aotus* spp.) and a common marmoset (*Callithrix jacchus*) were found to be infected with *Trypanosoma lewisi* (da Silva et al. 2010). *T. lewisi* represents a separate clade (the subgenus *Herpetosoma*) on the phylogenetic trees. The species is globally distributed, naturally infects rodents, and is transmitted by fleas. The study of da Silva et al. (2010) suggests that proximity of NHPs and infected rats may be responsible for the host switching from their natural rodent hosts to NHPs in which this trypanosome can cause sporadic and opportunistic flea-borne infection.

The very well-known *T. brucei* clade contains mostly trypanosomes of African mammals (e.g., *T. brucei* complex, *T. vivax*, *T. congolense*), with two human pathogenic subspecies of the *T. brucei* complex, *T. b. gambiense* and *T. b. rhodesiense*. In southern Cameroon, trypanosomes from the *T. brucei gambiense* group were found in wild collared mangabey (*Cercocebus torquatus*) and greater spot-nosed monkeys (*Cercopithecus nictitans*), whereas the *T. brucei* non-*gambiense* group was detected in moustached monkeys (*Cercopithecus cephus*) and greater spot-nosed monkeys (*C. nictitans*) (Herder et al. 2002). In a subsequent study, which included a greater sample size, infection with the *T. brucei* non-*gambiense* group was reconfirmed in nine NHP species (*Cercocebus torquatus, C. albigena, Cercopithecus neglectus, C. nictitans, C. mona, C. cephus, Colobus guereza, Miopithecus talapoin*, and *Perodicticus potto*), while the *T. brucei gambiense* group was detected in only two NHP species (*Cercocebus torquatus* and *Cercopithecus nictitans*) (Njiokou et al. 2006). A recent study by Jirků et al. (2015) demonstrates the occurrence of trypanosome species from the *T. brucei* complex also in African great apes.

Similar to other species of African trypanosomes (such as *T. congolense* and *T. vivax*), *T. brucei brucei*, *T. b. rhodesiense*, and *T. b. gambiense* are transmitted to various mammals by tsetse flies (*Glossina* spp.). In addition to the canonical defenses that are usually encountered in mammals, these African trypanosomes of the *T. brucei* complex need to defy a novel innate immune mechanism evolved in humans and some NHPs (e.g., *Papio*, *Cercocebus*, *Mandrillus*, *Gorilla*) – a highly efficient trypanolytic factor (TLF) that is present in serum (Pays et al. 2006). The trypanolytic activity was shown to be associated with high-density lipoprotein particles (spherical particles that comprise a hydrophobic lipid core surrounded by a hydrophilic layer). In contrast to *T. b. brucei*, two trypanosome species, which are responsible for African human trypanosomiasis (sleeping sickness), *T. b. gambiense* and *T. b. rhodesiense*, have developed mechanisms for escaping from lysis mediated by the trypanosome lytic factor (Lugli et al. 2004; Wheeler 2010; Jirků et al. 2015). Resistance to TLF is primarily composed of Apolipoprotein L1 (APOL1) and haptoglobin-related protein (HPR) (Pays et al. 2006; Raper and Friedman 2013). The sera of some African NHPs (e.g., baboons, sooty mangabeys, mandrills, and gorillas) were shown to be capable of APOL1-mediated killing of the flagellates,

while the serum of chimpanzees showed no trypanolytic activity due to secondary loss of the APOL1 gene (Lugli et al. 2004; Thomson et al. 2014; Jirků et al. 2015). Moreover, early studies showed that experimental infections with *T. b. rhodesiense* and *T. b. brucei* mostly caused the death of untreated chimpanzees, while infections with *T. b. gambiense* were mild and did not result in apparent clinical symptoms (Hoare 1972).

The other two members of the *T. brucei* clade have been found in wild NHPs in southern Cameroon (Herder et al. 2002). *Trypanosoma vivax* has been detected in five species (Table 15.1) while *T. congolense* "savannah type" has been found only in the moustached monkey (*C. cephus*). Although the epithet of this trypanosome ("savannah") seducing to the speculation of its occurrence on open savannah area in association with grazing cattle, the "savannah type" was found in various biotopes including forest habitat (Votýpka et al. 2015). *Trypanosoma vivax* and *T. congolense* are causative agents of nagana and infect cattle mainly in West Africa but *T. vivax* has been introduced in South America as well.

The last phylogenetic clade accommodating simian trypanosomes is the *Trypanosoma cruzi* clade, which consists of several groups, sometimes referred to as subgroups or subclades (Hamilton and Stevens 2017; Espinosa-Álvarez et al. 2018). The most important part of the *T. cruzi* clade is the *T. cruzi cruzi* group, also called *T. cruzi* sensu stricto or *T. cruzi* complex, which has very low host specificity, resulting in a high number of mammalian hosts including humans and some NHPs (Telleria and Tibayrenc 2017). A nomenclature for the *T. cruzi* complex has been adopted since 2009 and includes six discrete taxonomic units (sometimes considered as separate species), namely, *T. cruzi* I (TcI), *T. cruzi* II (TcII), *T. cruzi* III (TcIII), *T. cruzi* IV (TcIV), *T. cruzi* V (TcV), and *T. cruzi* VI (TcVI), based on different molecular markers and biological features (Zingales et al. 2009; Hamilton and Stevens 2017). The majority of *T. cruzi* isolates from NHPs belong to the TcII lineage (which is the causative agent of the significant part of human cases); however, other lineages (TcI, TcII, and TcIV) have also been detected in NHPs.

In a survey of more than 200 NHPs from Peru and Colombia, eight *Trypanosoma cruzi* complex or *T. cruzi*-like strains were identified in squirrel monkeys (*Saimiri boliviensis*) and marmosets (*Tamarinus nigricollis*) (Dunn et al. 1963). Later on in Panama, *T. cruzi* was found in Geoffroy's tamarin (*Saguinus geoffroyi*; 12%), white-faced capuchins (*Cebus capucinus*; 5%), squirrel monkeys (*Saimiri sciureus*; 2%), and black spider monkeys (*Ateles fusciceps*; 1%) (Sousa et al. 1974). In neighboring Colombia, *T. cruzi*-like parasites were detected in six species of NHPs (Table 15.1) (Marinkelle 1966). Approximately one-fourth of squirrel monkeys (*Saimiri sciureus*), exported from Guyana and Peru between 1985 and 1998, were tested positive for *T. cruzi* (Ndao et al. 2000). In Brazil, simian trypanosomes were studied in more localities and 10 to 15% of squirrel monkeys (*Saimiri sciureus* and *S. ustus*) (Ziccardi and de Oliveira 1997) and tamarins (*Leontopithecus chrysomelas* and *L. rosalia*) (Fernandes et al. 1999) were found to be infected by *T. cruzi*. In northeastern Argentina, almost half of the wild population of howler monkeys (*Alouatta caraya*) was PCR positive for *T. cruzi* (Martínez et al. 2016). Although the United States is not a typical endemic country for Chagas disease, infections

by *T. cruzi* in NHPs have been repeatedly reported from southern states. Free-ranging Old World Monkeys (OWM) released on St. Catherine's Island, Georgia, were tested for infection of *T. cruzi* as part of a surveillance study. The parasite was detected in 11 lion-tailed macaques (*Macaca silenus*) and one ring-tailed lemur (*Lemur catta*) (Pung et al. 1998). Twenty-one rhesus monkeys (*Macaca mulatta*) kept in the outdoor colony were found to be infected by *T. cruzi* in Texas (Kasa et al. 1977).

The *T. rangeli* group, within the *T. cruzi* clade, also includes trypanosome infecting humans (Espinosa-Álvarez et al. 2018). However, unlike the *T. cruzi* complex causing the life-threatening Chagas disease, the infection by *T. rangeli* is asymptomatic to the vertebrate hosts. Except in humans, the presence of *T. rangeli* was molecularly confirmed in two NHPs, squirrel monkey (*Saimiri boliviensis*) and red-handed tamarin (*Saquinus midas*), both imported to Japan (Sato et al. 2008) from South America. This trypanosome species has been also found in 40% of wild bare-faced tamarin (*Saguinus bicolor*), living in the Brazilian Amazon rainforest (da Silva et al. 2008). Many other studies also indicate the occurrence of this species based on the trypomastigote morphology. In Panama, *T. rangeli* was detected in Geoffroy's tamarin (*Saguinus* geoffroyi; 56%) and white-headed capuchin (*Cebus capucinus*; 13%) (Sousa et al. 1974), while in Colombia the parasite was detected in two white-headed capuchin monkeys (*C. capucinus)* by xenodiagnoses, a diagnostic method demonstrating the presence of parasites by exposing possibly infected animals to a sensitive vector (Marinkelle 1966). In the Brazilian Amazon, *T. rangeli* was found in several NHP species (*Aotus* sp., *Cebuella pygmaea*, *Saguinus labiatus labiatus*, and *Saimiri sciureus*) (Maia da Silva et al. 2004), while in the Brazilian state of Rondonia, *Trypanosoma rangeli* or *T. rangeli*-like parasites were detected in one-third of examined wild squirrel monkeys (*Saimiri sciureus* and *S. ustus*) (Ziccardi and de Oliveira 1997).

Another part of the *T. cruzi* clade is represented by the Madagascar subgroup (similar to the Australian group), which consists of unnamed trypanosomes found only in the blood of two endangered wild lemurs (*Indri indri* and *Propithecus diadema*) in Madagascar (Larsen et al. 2016).

The last part of the *T. cruzi* clade is the *T. conorhini* group, which accommodates trypanosomes naturally infecting rats. Yet, the trypanosome species that resemble *Trypanosoma conorhini* has been described also in Indonesian monkeys of the genus *Macaca* (Deane et al. 1968; Weinman 1977; Denning and Karcher 1986). The authors suggested that these Indonesian trypanosomes could be a primate-adapted strain of *T. conorhini* (Deane et al. 1968; Hoare 1972).

The last simian infecting member within the *T. cruzi* clade is unnamed trypanosomes from greater white-nosed monkey (*Cercopithecus nictitans*) isolated in a study that examined trypanosome diversity in a wide range of wild vertebrates in Cameroon (Hamilton et al. 2009).

15.2.1.3 *Leishmania* Parasites in NHPs

Compared to trypanosomes, studies focusing on *Leishmania* occurrence in NHPs are rare. In 1996, Ashford published a review on *Leishmania* reservoirs and NHPs are listed only as a minor or incidental host species. In the OWM, only the grivet monkey (*Cercopithecus aethiops*) has been described as a host for *Leishmania major* (Dedet 1993; Ashford 1996). However, in 2015, the highly unexpected finding of *Leishmania major* parasites in feces of wild western lowland gorillas from southern Cameroon (Hamad et al. 2015) raised concerns and prompted a controversial discussion on the validity of these findings (Bastien et al. 2015; Votýpka et al. 2018).

Interestingly, reports suggest that NWMs are much more likely to be infected by leishmania parasites than OWMs (Roque and Jansen 2014). One of the first studies addressing leishmania occurrence in the NWM resulted in the isolation of the parasite from the tufted capuchin monkey (*Cebus apella*) and the black bearded saki (*Chiropotes satanas*) in Brazil (Lainson et al. 1988). Subsequently, this leishmania species was described as *Leishmania* (*Viannia*) *shawi* (Lainson et al. 1989). Later on, Geoffroy's tamarin (*Saguinus geoffroyi*) and the owl monkey (*Aotus trivirgatus*) in Panama were found to be infected by *Leishmania* (*Leishmania*) *amazonensis* and *L.* (*Viannia*) *braziliensis*, respectively (Herrer and Christensen 1976; Herrer et al. 1973). Unknown species of *Leishmania* from the subgenus *Viannia* were found in four Argentinean owl monkeys (*Aotus azarai*) (Acardi et al. 2013). Malta et al. (2010) diagnosed *Leishmania (Leishmania) infantum* (syn. *chagasi*) in seven NHP species kept in captivity in Brazilian Belo Horizonte (Table 15.1) *and Leishmania amazonensis* was detected in spider monkey (*Ateles paniscus*) in another Brazilian zoo in São Paulo (Lima et al. 2012).

While NHPs can be considered as reservoir hosts for humans in the case of *Trypanosoma cruzi*, they do not play a relevant role for *Leishmania* infection in humans. Nevertheless, it is worth noting that NHPs are used as an animal model for both, *Trypanosoma* and *Leishmania* infection (e.g., Deane et al. 1968; Seah et al. 1974; Ouwe-Missi-Oukem-Boyer et al. 2006; Grimaldi Jr 2008; Thuita et al. 2008; Chanyalew and Hailu 2013; Roque and Jansen 2014).

15.3 Filariasis

15.3.1 Filarial Parasites in NHPs

Filariae are nematodes of the superfamily Filarioidea with a worldwide distribution of their vertebrae definitive host and are transmitted by blood-feeding arthropods, the intermediate hosts. Most of Filarioidea develop in wild host species (mammals, birds, reptiles, and amphibians) without any symptoms (Klei and Tajan 2002). Yet some, especially those of the family Onchocercidae, infect mammals including

humans and animals, causing a disease known as filariasis. Our current, rather scrappy knowledge suggests that the majority of NHP infections are asymptomatic.

The adult filariae are slender thread-like worms that live in tissues, body fluids, or body cavities of their definitive hosts. Female filariae are typically much larger than the males, their length varies from a few to 30 cm. The adult worms may survive for years, the fertilized females continuously producing motile embryos (primitive larvae) called microfilariae circulating in the blood or living in the skin and subcutis of the definitive host. Microfilariae measure 100–400 μm and represent a diagnostic stage. The indirect life cycle includes a variety of biting or blood/lymph suckling insect or other arthropods. Microfilariae must succeed in invading its vector organism fairly soon, because, unlike adult filarial worms, they only survive for a few weeks to less than a few hours, depending on the species. There is no further development outside of a suitable blood-feeding vector. They seek out host tissue suited to the nature of the vector species. For example, if the vector is a skin-piercing fly (vessel-feeder; solenophagy), such as mosquitoes (Culicidae), the microfilaria must enter the peripheral blood circulation, whereas species to be borne by skin-rasping flies (pool-feeder; thelmophagy), such as black flies (Simuliidae), and skin-cutting flies, such as horse flies (Tabanidae), tend to establish in hypodermal tissues. The larvae undergo daily migrations to body regions favored by the vector ectoparasites and corresponding with the insect activity (diurnal vs. nocturnal species). Microfilariae are ingested by a vector during feeding or actively migrate into its mouthpart, continue to develop in vector tissues, and undergo morphological changes, molt twice, and become third-stage infective larvae. Infective larvae migrate to the mouthpart and when the insect feeds on a suitable vector, the larva invades tissues of the animal and migrates to the appropriate site in the body where it develops into sexual maturity (Orihel 1970; Klei and Tajan 2002).

Most of the literature on filarial infections in wild NHPs was published in the last century (focused on filarial identification and pathogenesis). Recently, the research on filarial parasites seems to be rather neglected in part because of the logistical and ethical constraints associated with the collection of blood or tissue samples from protected NHP populations.

Filarial infection is usually diagnosed by a demonstration of microfilariae in fresh blood. Blood smears with different staining or concentration techniques (e.g., Knott technique) as well as skin biopsies (for details, see Orihel 1970; Garcia 2016) are most suited. With a few exceptions, the morphology of the larvae (microfilaria) is not sufficient for species determination and adult worms, necessary for reliable taxonomical identification, can be obtained only during necropsies (e.g., Bain et al. 1995). Studies on filariae in NHPs utilizing molecular methods are rare but have been conducted (Toure et al. 1999; Sato et al. 2008; Springer et al. 2015; Erkenswick et al. 2017; see also Lefoulon et al. 2015). Springer et al. (2015) and Erkenswick et al. (2017) encountered slight discordance in microfilariae infection status, from 10% to 16% blood smear positive samples were PCR negative, while only Springer et al. (2015) detected 11% of cases when the infection was revealed by PCR only. Thus, neither method seems to be 100% sensitive. However, these new studies

employing molecular tools try to focus on demographic, temporal, and ecological aspects of filarial infections.

Sequence data, however, are not always capable of resolving the taxonomic classification, since reference data from morphologically determined adults are mostly nonexistent. This problem can only be overcome when adult parasites that are collected during necropsies are subsequently morphologically identified, genetically characterized, and made readily available to the scientific community. Based on current data, a reliable classification is challenging and sometimes impossible; it is still not clear which species represent synonyms, some genera seems to be poly- or paraphyletic, while others have been (repeatedly) renamed in the past; e.g., *Cercopithifilaria* was established as a subgenus of *Dipetalonema* (Eberhard 1980) and, subsequently, Bain et al. (1982) elevated *Cercopithifilaria* to full genus level and assigned to it several newly described species and some species previously placed in *Dipetalonema*.

15.3.1.1 Diversity of Filarial Parasites in NHPs

According to Strait et al. (2012), at least 13 different filarial species infect NWM, but the genera *Dipetalonema* and *Mansonella* are the most frequently reported (Dunn and Lambrecht 1963; Esslinger and Gardiner 1974; Petit et al. 1985; for review on *Mansonella* see Bain et al. 2015, Table 15.1). Prevalence often exceeds 70%, and multiple infections with two to four species are common in endemic areas for NWM (Sato et al. 2008). Filariae of these two genera live in the abdominal or thoracic cavities and can cause fibrinopurulent peritonitis or pleuritic with associated fibrinous adhesions resulting in entrapment of the worms, or they live in the subcutaneous tissues causing little or no inflammation (Orihel and Seibold 1972).

Four filarial species have been described in lemurs, namely, *Dipetalonema petteri*, *Paulianfilaria pauliani*, *Courduriella courdurieri*, and *Protofilaria furcata* (Irwin and Raharison 2009). *Dirofilaria corynodes* is reported to be the most prevalent filarial parasite of African OWM (Table 15.1). These are large parasites (length up to 30 cm; see Webber 1955a) that are found in the subcutaneous tissues of the trunk and lower extremities where their presence causes very little tissue reaction (Orihel and Seibold 1972). Other *Dirofilaria* species (Table 15.1), inhabiting the peritracheal connective tissue and the diaphragm of the infected host, have been reported from Asian OWM (Orihel and Seibold 1972; Table 15.1). *Edesonfilaria malayensis* has been described in OWM (Table 15.1); the adult worms are usually found free in the peritoneal cavity but have been also reported from the subserosal connective tissue of the abdominal and thoracic cavities causing various clinical outcomes (for details see Gardiner et al. 1982; Nonoyama et al. 1984). The genus *Cercopithifilaria* with five described species occurs in Cercopithecidae hosts and lives in subcutaneous connective tissues (Lefoulon et al. 2014).

Several species of *Mansonella* have been detected in both species of gorilla and chimpanzee (reviewed in Bain et al. 2015; see also Table 15.1). The preferred sites of *Mansonella* are subcutaneous tissues and intramuscular fascia, the microfilariae of

M. streptocerca and *M. rodhaini* remain in the dermis, while the others circulate in the peripheral blood (Bain et al. 1995). Filariae are also often found in association with various organs and structures in the body of the host, e.g., *M. vanhoofi* inhabits various mesenteries and the connective tissue adjacent to several organs (Orihel 1970). *Dirofilaria immitis* was found in the heart and the abdominal cavity of the orangutan (Orihel 1970). *Loa loa* also has been reported from the chimpanzee and both species of gorilla (Rodhain and van den Berghe 1939; van den Berghe et al. 1964; Bain et al. 1995; see also below). More detailed information about filarial diversity in NHP can be found in numerous reviews: Webber (1955a, b), Orihel (1970), Toft (1986), Strait et al. (2012), and Dunn and Lambrecht (1963) (South American), Dunn and Ramachandran (1968) (Southeast Asia); for great apes see van den Berghe et al. (1964), Orihel (1970), and Bain et al. (1995).

15.3.1.2 Zoonotic Potential of NHP Filarial Parasites

Human-infecting filaria *Brugia malayi* and *Brugia pahangi* are found in various animals and have been reported from a wide variety of Asian NHP, particularly *Macaca* species (e.g., Laing et al. 1960; Orihel and Seibold 1972). The adult parasites are found in the lymphatic and perilymphatic tissues. Symptoms and histopathologic changes in the lymphatic system, similar to that seen in human Malayan filariasis, have not been reported in infected NHPs (Orihel and Seibold 1972). Yet, *Meningonema peruzzii*, a filarial parasite originally described from African OWM vervet monkey (*Chlorocebus pygerythrus*) and Gabon talapoin (*Miopithecus ogouensis*) almost half a century ago (Orihel and Esslinger 1973), has been relatively recently reported in human cerebrospinal fluid. A female fourth stage larva of probably *M. peruzzii* was recovered from the cerebrospinal fluid of a Cameroonian patient harboring *Loa loa*, but without any neurological signs (Boussinesq et al. 1995). In its natural simian hosts, the worms were found only in the subarachnoid space along the dorsum of the brain stem at the level of the medulla oblongata. Symptoms and lesions associated with infection by this parasite were not reported (Orihel and Esslinger 1973).

Loa loa, commonly known as the "eye worm," occurs in humans in the western part of Africa and has been reported from a variety of OWM (drills, baboons, mangabeys, and vervets, as well as African great apes) (Strait et al. 2012). This filaria was previously reported by Treadgold (1920) as a separate species, *Loa papionis*, because although it is morphologically almost identical to *L. loa*, it differs in size, microfilariae circulate in the peripheral blood with different circadian rhythm, and the parasite is transmitted by different horse fly *Chrysops* species with twilight activity (Noireau and Gouteux 1989). However, Duke (1964) reported the capacity to produce fecund hybrids with the human-infecting *L. loa* and thus these parasites appear to belong to the same species that infects humans. The overall contribution of simian hosts to human loiasis is considered minimal due to spatial and temporal separation of transmission between the human and simian strains, but the possibility of some cross-transmission between host species should not be

completely ruled out, although Duke (2004) failed to experimentally infect himself with simian *Loa* strain derived from wild drill (*Mandrillus leucophaeus*). *L. loa* infections in NHP are usually asymptomatic, but see Duke (1960).

Human filaria *Onchocerca volvulus*, causing human onchocerciasis (river blindness) in sub-Saharan Africa, was found in a subcutaneous fibrous nodule in the mountain gorilla (*Gorilla beringei*) (van den Berghe et al. 1964). *Mansonella perstans* causes serous cavity filariasis; the species infects humans in many parts of sub-Saharan Africa, parts of Central and South America, and the Caribbean and has been reported also in western lowland gorillas (*G. gorilla gorilla*) and chimpanzees (*Pan troglodytes schweinfurthii*) in Cameroon (Reichenow 1917). *M. streptocerca* causes subcutaneous filariasis in humans inhabiting rainforests in West and Central Africa but has been found also in both species of chimpanzee in the Democratic Republic of Congo (Peel and Chardome 1947) and in mountain gorilla (Van den Berghe et al. 1964).

15.3.2 Infectious Diseases and NHP Zoonoses

Zoonoses represent a public health risk recently pointed out by the spreading of previously unknown human infectious diseases emerging from animal reservoirs. Frequently, NHPs serve as a possible reservoir of zoonotic diseases; however, not all pathogens possess equal zoonotic potential. Approximately a quarter of the human emerging infectious diseases, predominantly of viral or bacterial origin, are shared with NHP hosts. Parasites, including hemoparasites transmitted by invertebrate vectors, are common in both wild and captive NHPs and occur in both clinically asymptomatic and diseased animals. Based on studies published over more than a 100 years, it seems that trypanosome and filaria infections are rather common in NHPs and new species are described regularly. However, compared to *Plasmodium* parasites with a very high zoonotic potential (e.g., *P. knowlesi*, an NHP protozoan transmitted by mosquitoes, commonly reported to cause human malaria), the role of other hemoparasites in the maintenance of zoonotic transmissions is quite limited. The only exception is *Trypanosoma cruzi*, the causative agent of human Chagas disease transmitted by the triatomine bug, which has been repeatedly reported in both captive and free-ranging NHPs.

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Chapter 16
Forming, Storming and Norming Your Way Into One Health: The Gombe Case Study

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Abstract Multidisciplinary approaches are critical to address the increasingly complex issues at the intersection of nonhuman primates and neglected infectious diseases. In this chapter, we use the Gombe Ecosystem Health Project in Tanzania to demonstrate how team science can be launched to tackle complexity in health. The diverse interactions among humans, nonhuman primates, and domestic animals within and outside the park highlight the need for collaborative research in order to thoroughly understand the role of monkeys in pathogen transmission. We offer three steps for the creation of a multidisciplinary team that can perform research in the context of ecosystem health: (1) problem formulation and conceptual mapping, (2) stakeholder consideration, and (3) team formulation and practice. This case study illustrates the expansion from a "Chimpanzee Health Project" to an "Ecosystem Health Project" that was only successful through the use of multidisciplinary team science.

Keywords Stakeholders · Baboons · Ecosystem

16.1 Introduction

This volume has established that the issue of nonhuman primates (NHP) and largely neglected infectious diseases is indeed a Grand Challenge of complexity. In Chap. 3, we discussed why multidisciplinary team science needs to be a key part of the solution, and that many models exist for designing, implementing, conducting, and evaluating research under the paradigm of team science. Given the fact that careful creation of a new team with adequate time and funding is a luxury that rarely exists, in this final chapter, we offer a way forward in three steps — (1) problem formulation and conceptual mapping, (2) stakeholder consideration, (3) team formulation and practice — using the case of the Gombe Ecosystem Health Project in Tanzania to illustrate each point.

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Tanzania's Gombe National Park is a small (56 km^2) reserve of forest (35 km^2) and lake (21 km^2), located on a narrow strip of land along the rift escarpment that rises eastwards from the shore of Lake Tanganyika (Pusey et al. 2008). Gombe National Park is directly bordered by the growing villages of Mwamgongo (to the north) and Mtanga (to the south), as well as Chankele, Bubango, and Mgaraganza to the east. Each village houses thousands of people plus their domestic animals, such as cattle, goats, chickens, and dogs, as well as pests such as rats and mice, all of which could serve as reservoirs for pathogens. Residents of Mwamgongo and Mtanga fish in Lake Tanganyika and do farming near the park borders, but no longer have direct (free and/or legal) access to natural resources and supporting ecosystem services within park boundaries. The park also has staff dedicated to community engagement, which sets the stage for discussions about conservation, health, and resource management across the interface of park and community.

Gombe is most famous for its population of eastern chimpanzees (*Pan troglodytes schweinfurthii*), but the park is also home to five monkey species, including olive baboons (*Papio anubis*), red colobus monkeys (*Piliocolobus tephrosceles*), vervet monkeys (*Chlorocebus pygerythrus*), red-tailed monkeys (*Cercopithecus ascanius*), and blue monkeys (*Cercopithecus mitis*). Gombe's *Cercopithecus* population also includes red-tailed-blue hybrids (*Cercopithecus mitis x C. ascanius* hybrids). Olive baboons range throughout the park, interacting with chimpanzees and humans, and sometimes with domestic animals along the park borders. Intensive efforts have gone into habituating chimpanzees, baboons, and several other monkey species for scientific study. Positive outcomes of NHP habituation include that the park has become a popular destination for tourism, which also brings economic support to the local community and additional protection of the wildlife within from poaching and further habitat encroachment (Pusey et al. 2007). As a result of habituation and tourism, human-NHP interaction — through means such as human presence in NHP habitat, NHP crop-raiding, and shared water sources — is common (Parsons et al. 2014, 2015), which is in addition to the NHP-NHP interactions – through shared habitat and food resources, as well as predator-prey interactions (e.g., chimpanzees and baboons prey on other NHP). Together, these interactions magnify the potential for infectious disease introduction and transmission (Gilardi et al. 2015), particularly among the baboons and chimpanzees, which are more terrestrial and thus more likely to interact with humans directly and indirectly.

The complexity of the interface between the park and the areas immediately surrounding the park can be visualized in a conceptual map (Fig. 16.1), which reinforces the need for multidisciplinary and multisectoral partnership development. To understand the role of all human and NHPs in pathogen transmission in this system, we must develop multidisciplinary research that can be employed in the context of ecosystem health. Therefore, we must consider all aspects of the ecosystem that can play a role in monkey disease patterns, including humans, chimpanzees, domestic animals, shared food and water resources, and interspecies interactions. A multidisciplinary approach is necessary to properly assess not just ecological interactions but also the potentially competing interests and perceptions of the park and how it is used by local communities, government, and many others.

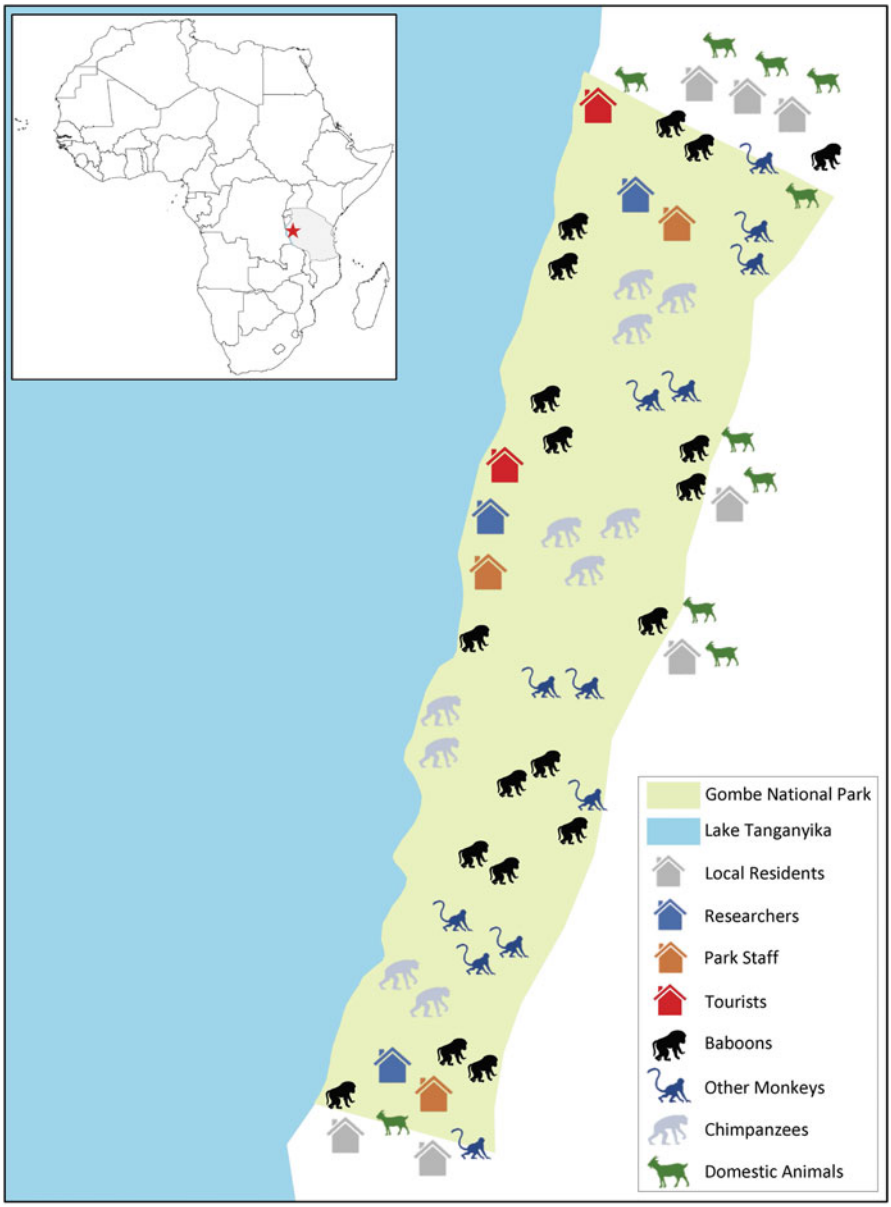

Fig. 16.1 Complexity of interaction. This conceptual map displays the complexity of the interactions among humans, nonhuman primates, and domestic animals at the interface of Gombe National Park and the immediate surrounding area. Baboons and other monkeys range throughout the park, where they interact, directly and indirectly, with humans, chimpanzees, and occasionally domestic animals. This complex interface highlights the need for collaborative multidisciplinary research in order to thoroughly understand the role monkeys play in pathogen transmission

16.2 Problem Formulation and Conceptual Mapping

Primate research in Gombe National Park began in 1960 and initially mostly concentrated on chimpanzees and baboons (Goodall 1986; Morris and Goodall 1977; Nash 1976; Ransom 1981; van Lawick-Goodall et al. 1973). Over time, however, studies of the other monkey species and their ecology have been conducted (Bakuza 2018; Clutton-Brock 1973, 1975; Detwiler 2002; Kamenya 1997). Like other research in GNP, the Gombe Ecosystem Health Project had its origins as a project focused on chimpanzees (Lonsdorf et al. 2006, 2011; Williams et al. 2008); however, a growing appreciation about the threat infectious diseases pose to all primate populations in and around the park (Tapanes et al. 2016; Wallis and Lee 1999) led to a multispecies approach. This in turn resulted in a more comprehensive approach that included collaboration with researchers studying different monkey species in this ecosystem. Further, researchers, tourists (domestic and international), park management staff, and Tanzanian field researchers reside inside the park, but the park border is not fenced; therefore, local villagers and their domestic animals have some access to the park and often use trails cutting through the park (Parsons et al. 2014). This is a system where monkey health is intricately connected to the health of great apes, humans, and domesticated animals and involves many different stakeholders with a variety of interests, thus, an ideal system for an ecosystem health approach that includes as many stakeholders as possible.

There are varying degrees of interaction among the six NHP species in Gombe National Park and existing knowledge about those interactions are also diverse. Red colobus monkeys are the preferred focal species for hunting by chimpanzees, while blue and red-tailed monkeys (and their hybrids) are also hunted by chimpanzees. The population structure and large numbers of baboons facilitate intricate (i.e., a network of) interactions with humans and other NHPs. Among the monkey species, baboons have the broadest range throughout the park and at park boundaries and interact with human settlements in and around the park, and also directly interact with chimpanzees (e.g., youngsters of both species sometimes play, while young baboons also may be hunted and consumed by chimpanzees) (Goodall 1986). Thus, baboons are a potentially powerful indicator of overall health and disease risk in the system.

16.3 Stakeholder Consideration

Stakeholder analysis is a formal process of evaluating and including all those with a stake in the health outcomes being studied and/or managed. It involves the development of an inclusive list of all interested and affected parties, their priorities or interests, and how construction of the team may provide adequate stakeholder representation. The prioritization of health for many stakeholders, whether it be that of humans or NHPs, is a common theme, but in areas with high human-NHP interaction, optimization of health outcomes for all (humans, NHPs, and domestic

Table 16.1 Consideration of stakeholders. This is a conceptual representation of the stakeholders considered in the full stakeholder analysis related to health in the Greater Gombe Ecosystem. Their perceived priorities of interest are indicated by an X. Sustainable Health is highlighted as a priority of interest to all stakeholders

	Perceived Priorities of Interest				
Stakeholder	Conservation/ Management	Sustainable Livelihood	Research	Sustainable Health	Financial Profit
Nonhuman primates (apes and monkeys)	X	–	–	X	–
Domestic animals (goats, sheep, dogs)	–	X	–	X	–
Local residents (living adjacent to the park)	X	X	X	X	X
Tanzania National Parks (park management, infrastructure, and some research)	X	X	X	X	X
Jane Goodall Institute (JGI) (Gombe Stream Research Center and TA CARE project)	X	X	X	X	–
Tanzania Wildlife Research Institute (TAWIRI)	X	X	X	X	–
Independently permitted researchers	X	X	X	X	–
Tourists and tour operators	X	?	–	X	X
Other nongovernmental organizations	X	?	?	X	–

animals) needs to be a priority. In this case study, the shift from monitoring health threats for chimpanzee conservation to a focus on several species of monkeys as well as humans and domestic animals was a necessary but complicated shift, and the development of partnerships and funding strategies is ongoing. Represented here (Table 16.1) is a simplified version of the stakeholders considered in the full analysis related to health in the Greater Gombe Ecosystem.

16.4 Team Formulation and Practice

Questions at the ecosystem scale require far-reaching collaborations among stakeholders in Tanzania, local and international researchers, and people living around the park. Understanding and communicating the intersection of the requisite scientific and stakeholder perspectives is a necessary part of the planning process; an example from this case is presented in Fig. 16.2 below. The figure presents the core scientific

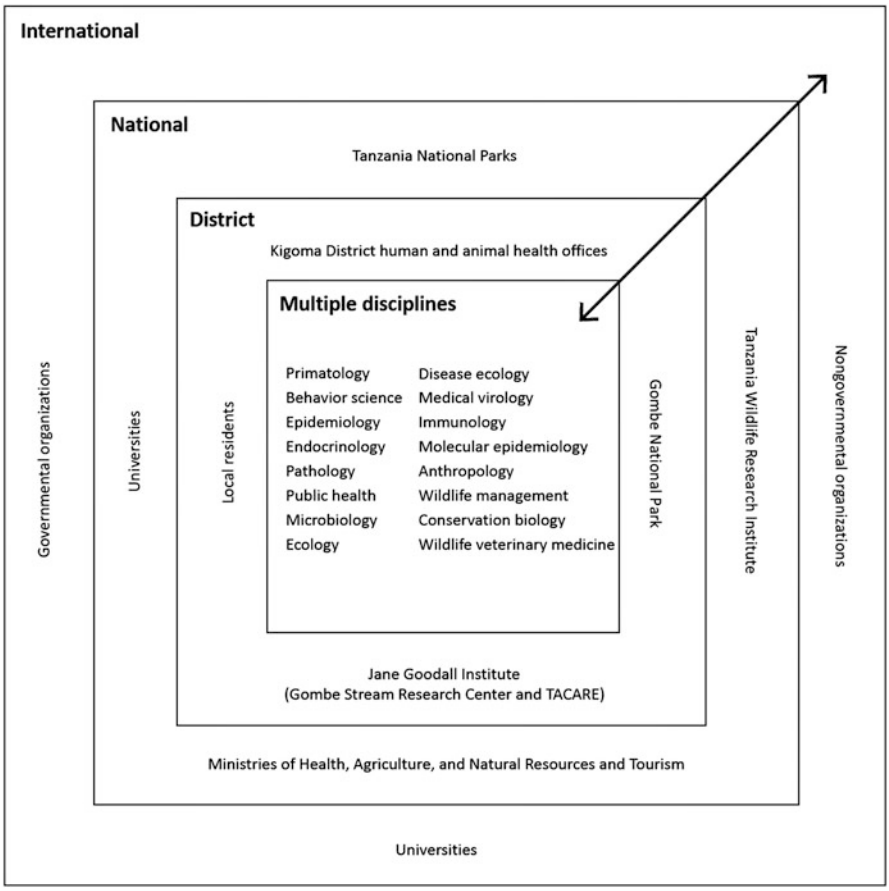

Fig. 16.2 Scale of collaboration. Disciplines working in Gombe National Park must interact with partners at multiple levels for successful multidisciplinary research to be accomplished. Core scientific disciplines are necessary as they respond to and collaborate with partners at all levels (district, national, and international)

disciplines in the center square and geographical levels of stakeholder/partnership/funding involvement in concentric squares. The disciplines are not mutually exclusive; thus, there may be some overlap in skillsets represented. One of the most rewarding aspects of this approach is that international organizations and district (or local) organizations can have a productive dialogue. Ultimately, the success of the program has largely been due to engagement and optimization between the priorities of those represented in Fig. 16.2, which is a blend of global and local research priorities.

In most cases, team formulation is an iterative, dynamic process progressing through conceptual stages of formulation (coming together, defining roles and research questions), adaptation (operationalizing plans, learning multidisciplinary

language, and starting work), and functionality (streamlining processes and starting on the road to successful outcomes) that must be carefully managed for success. This process is often represented as forming, storming, and norming to reflect three distinct phases of team development. Good leadership, inclusivity, and communication are key to managing teams through these stages, but good team formulation and stakeholder inclusion can reduce conflict and increase equity further down the road. This is simply stated, but complex and difficult to implement in the real world.

The expansion from a "Chimpanzee Health Project" to an "Ecosystem Health Project" changed the scope and practice of health research in Gombe National Park to include NHPs, humans, and livestock across the park boundary (Parsons et al. 2014). Successful collaboration with the baboon research team to explore health around this important NHP is exemplified through several published studies (Bakuza 2020; Chuma et al. 2018; Deere et al. 2019; Harper et al. 2012; Parsons et al. 2015; Terio et al. 2018; Wolf et al. 2016). This proof of concept has resulted in the end of phase I of the larger project, incorporating 15 years of health research in Gombe National Park. The team is now in the planning stages for phase II, which will be designed more holistically from the onset to account for all desired health outcomes in Gombe National Park and the surrounding ecosystem.

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