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Christon J. Hurst *Editor*

# The Connections Between Ecology and Infectious Disease

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Volume 5

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Editor

# The Connections Between Ecology and Infectious Disease

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*I am very fortunate by having been allowed two set of parents in this lifetime. My parents by birth were Hubert and Joy Hurst. They met when my father, Hubert, was a college student and would eat his noontime meal at the luncheon counter of a pharmacy in Cincinnati, Ohio near where he was studying. My mother, Joy, worked there at the pharmacy and perhaps the best part of my father's day was a smile that she served to him along with that daily lunch as the two of them fell into romance. He graduated, they married, and then they had three children which were in order of birth my brother Preston, myself, and our sister Embeth. Hubert and Joy loved their children and they generally tolerated our childhood misdeeds. That tolerance required patience because some of my misdeeds must have seemed nearly intolerable! But, most importantly for me, my parents encouraged my efforts to understand science and lovingly they showed pride of my achievements.*

Joy and Hubert Hurst on vacation in 1975

*When I still was a young child, my family moved into a large housing complex in Cincinnati, Ohio where I soon met Rebecca Wadlin and also met Becky's older sister Caroline, plus their parents Herbert and Renko. It often must have seemed as though Renko and Herbert had five children instead of just their two, Carrie and Becky, because my brother, sister, and I spent a great many hours in their apartment and Herb invited us along on numerous local adventures. Herb was from New York state and fortunately met Renko in Tokyo, Japan while he was on assignment there with the United States Air Force after the Second World War had ended. When they met, Renko was studying English*

*and her instructor had invited Herb to speak with their class. Herb and Renko corresponded when he was assigned to Okinawa. Eventually, they reunited in Tokyo, where they married and had two children. Herbert then brought their family to Cincinnati so that he could study here for a Master's Degree which he succcessfully earned. It was a sad day for me when their family moved onward to California. Decades later, Herb and Renko's family informally adopted me and that has brought me great pleasure. I happily have considered their family as also being my mother, father, and sisters.*

Renko and Herbert Wadlin with Christon Hurst in 2003

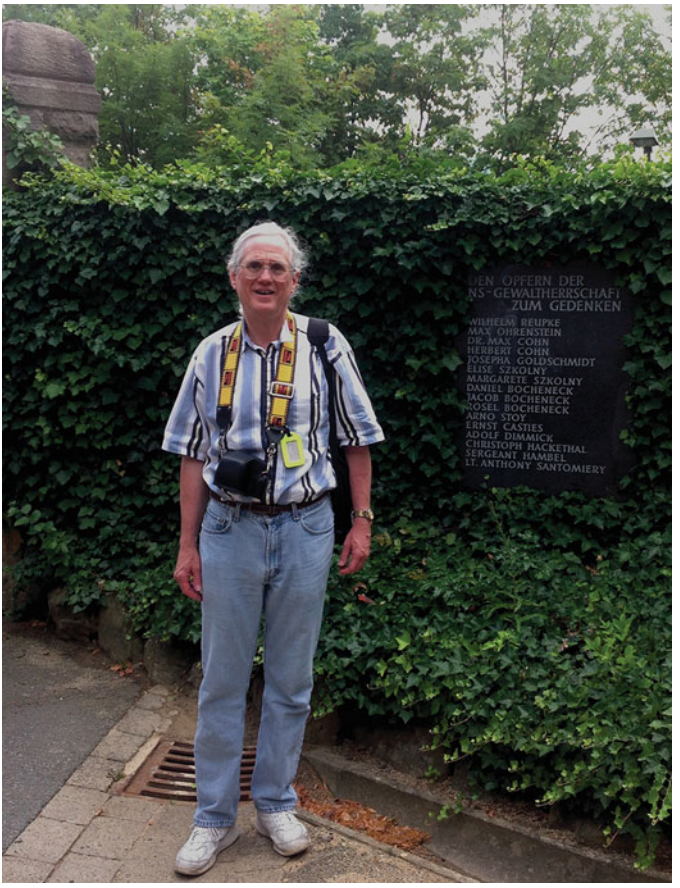
*My personal beliefs being what they are, I also wish to thank Johann Meyer and*

*Eleonora Diebold Meyer who were the parents of Guilelmina Eleonora "Mina" Meyer. John and Ellen, as they came to be known, were immigrants to the United States from the Kingdom of Württemberg. Mina was born in Sedamsville, which was then just slightly west of Cincinnati's city boundary in Hamilton County, Ohio. She was baptized on November 7th, 1868 in Saint Michael the Archangel church, located in the neighboring Lower Price Hill section of Cincinnati. Unfortunately, Mina passed away in Lower Price Hill on May 28th, 1882 and she was laid to rest at a pretty location on top of the hill, at Saint Joseph Cemetery in the Price Hill neighborhood of Cincinnati. I do as well thank Dr. Max Cohn and Anna Weiland Cohn who were the parents of Herbert Max Cohn. Herbert Cohn was born in Berlin, Germany on August 23rd, 1900. Eventually Herbert lived as a family with his parents, along with his wife Ursula Sara Gerling Cohn and their own son Charlie Max Cohn, in Bad Harzburg, Germany where his family operated their home as a pension called Haus Frohsinn and Villa Frohsinn.*


Postcard image of Haus Frohsinn in Bad Harzburg

*Sadly, Herbert Cohn was arrested from their Bad Harzburg home in November, 1938 as part of the Reichspogromnacht raids and taken to Buchenwald Concentration Camp, Germany where he became ill. Herbert Cohn died in Buchenwald Camp on November 30th, 1938 and the historical records suggest that he was shot, listing even the minute of his death. Herbert's father Max was attacked by a mob in Bad Harzburg and he thus died on December 26th, 1938. Despite the tragedy of those two months, things ended better for the rest of Herbert's family. Herbert's wife Ursula and their son Charles fortunately were sponsored into England as refugees during April, 1939 where they survived the Second World War and remained living near London. Ursula eventually had a second chance at romance and in September, 1945 remarried*

*which gave Herbert's son a new father. Herbert's son remains alive and in good health. Anna eventually returned to living in the familys Bad Harzburg home in 1940 and there she survived the war. Their home in Bad Harzburg still stands and has been well kept. Bad Harzburg thoughtfully memorialized both Herbert and his father with their names on a plaque in the local cemetery. I can attest that Bad Harzburg again is a cheerful place and that the people now residing there are very kind.*




Christon Hurst at memorial in Bad Harzburg Cemetary July 25th 2013

# Series Preface

The light of natural philosophy illuminates many subject areas including an understanding that microorganisms represent the foundation stone of our biosphere by having been the origin of life on Earth. Microbes therefore comprise the basis of our biological legacy. Comprehending the role of microbes in this world which together all species must share, studying not only the survival of microorganisms but as well their involvement in environmental processes, and defining their role in the ecology of other species, does represent for many of us the Mount Everest of science. Research in this area of biology dates to the original discovery of microorganisms by Antonie van Leeuwenhoek, when in 1675 and 1676 he used a microscope of his own creation to view what he termed "animalcula," or the "little animals" which lived and replicated in environmental samples of rainwater, well water, seawater, and water from snow melt. Van Leeuwenhoek maintained those environmental samples in his house and observed that the types and relative concentrations of organisms present in his samples changed and fluctuated with respect to time. During the intervening centuries we have expanded our collective knowledge of these subjects which we now term to be environmental microbiology, but easily still recognize that many of the individual topics we have come to better understand and characterize initially were described by van Leeuwenhoek. van Leeuwenhoek was a draper by profession and fortunately for us his academic interests as a hobbyist went far beyond his professional challenges.

It is the goal of this series to present a broadly encompassing perspective regarding the principles of environmental microbiology and general microbial ecology. I am not sure whether Antonie van Leeuwenhoek could have foreseen where his discoveries have led, to the diversity of environmental microbiology subjects that we now study and the wealth of knowledge that we have accumulated. However, just as I always have enjoyed reading his account of environmental microorganisms, I feel that he would enjoy our efforts through this series to summarize what we have learned. I wonder, too, what the microbiologists of still future centuries would think of our efforts in comparison with those now unimaginable discoveries which they will have achieved. While we study the many wonders of microbiology, we also

further our recognition that the microbes are our biological critics, and in the end they undoubtedly will have the final word regarding life on this planet.

Christon J. Hurst in Heidelberg

Indebted with gratitude, I wish to thank the numerous scientists whose collaborative efforts will be creating this series and those giants in microbiology upon whose shoulders we have stood, for we could not accomplish this goal without the advantage that those giants have afforded us. The confidence and very positive encouragement of the editorial staff at Springer DE has been appreciated tremendously and it is through their help that my colleagues and I are able to present this book series to you, our audience.

Cincinnati, OH Christon J. Hurst

# Volume Preface

Infectious disease is part of an interconnected ecology that involves a pathogenic microorganism and the host species in which that microbe causes illness. The ecology of that microorganism may include a more extended set of connections which could involve a natural environmental presence, its possible carriage by vehicles such as air, water, and food, and interactions with other host species including vectors for which the microbe either may or may not be pathogenic. This book explains these connections. The contents of this volume are divided into three sections, the first of which presents an introduction to the field of science currently titled disease ecology and explains both the role of biological community interactions plus the impact of biodiversity. The second section considers diseases directly affecting humans with a focus on waterborne and foodborne illnesses and importantly examines the critical aspect of microbial biofilms. The third section presents ecology of infectious diseases from the perspective of their impact upon mammalian livestock and wildlife plus includes understanding of the fact that those same diseases often affect humans.

Decades ago I presented two specialty courses on disease ecology at Universidad del Valle in Cali, Colombia for their graduate students in the School of Public Health and professional members of the community. The first of those courses was titled "La Ecología de la Transmisión de Infecciones y la Aplicación de Modelos en Salud Pública" [The Ecology of Infection Transmission and the Application of Models in Public Health] during August and September of 1998, which was sponsored by the Fulbright program. The second course was titled "La Ecología de Enfermedades Infecciosas Transmitidas por el Agua" [The Ecology of Infectious Diseases Transmitted by Water] during March of 2000, for which I served as an International Professor for Latin America and was sponsored by the American Society for Microbiology. Including those courses among my previous experience, I presumed that I would be qualified to write the introductory chapter for this book. However, while reading the chapters written by this books other contributing authors, the realization came to me that those others have a level of expertise which now outweighs mine and so the banner representing his subject area clearly has been

passed onward. Many of these authors and also our readers for this book represent the next generation of scientists to be exploring this field of endeavor and I feel reassurance from knowing how well they and you will proudly carry the banner forward.

I am tremendously grateful to Hanna Hensler-Fritton, Andrea Schlitzberger, and Isabel Ullmann at Springer DE, for their help and constant encouragement which has enabled myself and the other authors to achieve publication of this collaborative project.

Cincinnati, OH Christon J. Hurst

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# Part I
# Introduction to Disease Ecology

# Chapter 1
# Interkingdom Community Interactions in Disease Ecology


**M. Eric Benbow, Jennifer L. Pechal, Jeffery K. Tomberlin, and Heather R. Jordan**

**Abstract** A key tenet of community ecology is the interactions of individual organisms contribute to the ecological structure and function of ecosystems. Within these networks of interacting organisms are those taxa important for human and animal health: disease systems defined by combinations of host, pathogen, reservoir, and vector or a subset of these components. While the simplest disease system is that of the host and pathogen, more complex systems include the direct interactions of a pathogen with other hosts and the microbial communities of those hosts, reservoirs, and sometimes vectors. Each of these disease system components is made up of species that directly and indirectly interact with other species in ways that affect their individual fitness, population biology, and role in communities of the ecosystem. This chapter recognizes the direct interactions of those species that make up the primary components of disease systems; however, the focus and examples provided relate to the more indirect interkingdom (or domain) interactions that impact disease system components. The examples provided include how microbial communities mediate invertebrate and vertebrate fitness and behavior, often in systems where the

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hosts play important roles in pathogen transmission and disease emergence. The potential mechanisms of these interkingdom interactions are also developed in detail, as the mechanisms of such interactions are likely the target of future studies that could directly inform disease management strategies. Based on these examples and mechanisms, the existing literature suggests there are likely undiscovered and complex interactions of species within communities that affect disease systems.

## 1.1 Introduction

A key tenet of community ecology is the interactions of individual organisms contribute to the biological structure and function of ecosystems. Within ecosystems, infectious disease systems (i.e., the network of species involved in the existence and emergence of disease caused by pathogenic microorganisms) are defined by at least two species interacting in a way that leads to a pathogenic outcome affecting at least one of those species. This minimum system is composed of the pathogenic microorganism species (e.g., bacteria, virus, fungi, or eukaryotic parasite; hereafter referred to as pathogen) and a host species, and inherently implies a minimal interaction between the two with the host negatively affected by the pathogen (Fig. 1.1). This minimum infectious disease system is the simplest scenario in disease ecology defined as an interkingdom interaction or, as we define here, the ecological relationship where two or more species from different domains or kingdoms of life interact in a way that has a demonstrated or hypothesized biological, ecological, or evolutionary importance to one or all of the species in the disease system.

Disease ecology involves at least two different species that define a disease system within a larger community of organisms in nature. However, many disease systems of humans, other animals, and plants involve multiple intermediate species with the pathogen transmitted to one or more host or reservoir species (Fig. 1.1). These multi-species pathogen transmission pathways are also interkingdom interactions that lead to the movement of the pathogen in the environment, and ultimately to the host, resulting in pathogenesis and disease symptoms. Therefore, in its simplest form, understanding the ecology of a disease rests on defining the components, constraints, and dynamics of interacting organisms from different domains and kingdoms. The discipline of disease ecology also includes asking questions about coevolutionary relationships and the broader impact of species interactions on the community that are not a direct component of the disease system but might indeed be influential (Burdon and Thrall 2008; Ricklefs 2010). The more traditional interkingdom interactions defining many infectious disease systems will be covered in this chapter; however, this will be done with a focus on how the interactions mediate transmission and pathogenesis. This chapter will also cover how interkingdom interactions may influence infectious disease systems without the interacting organisms being any of the disease system component species (i.e., pathogen, host, reservoir, vector; Fig. 1.1). Further, because an entire book could

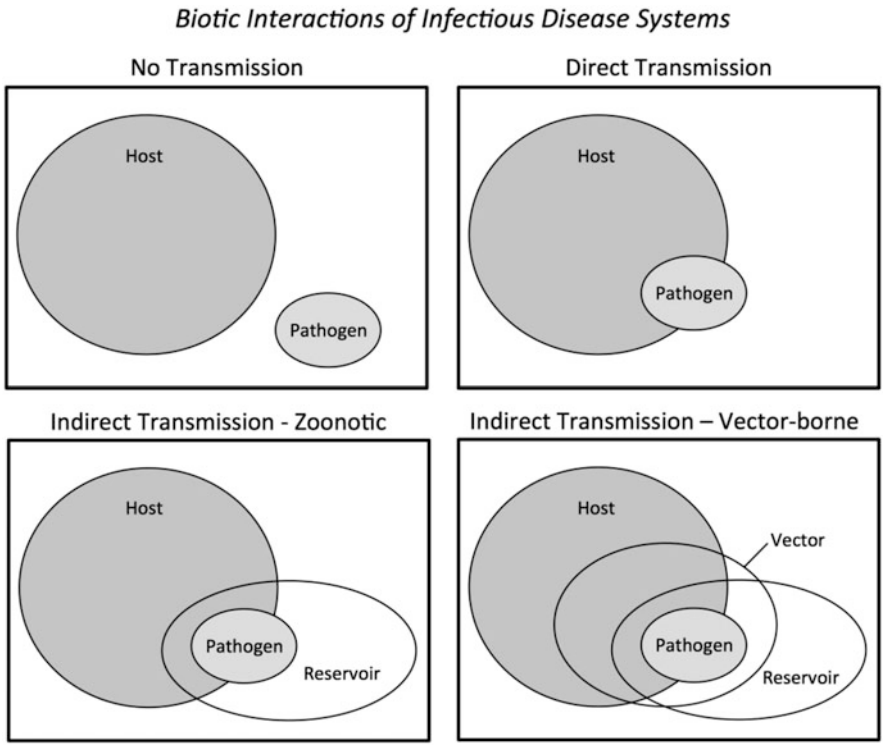


**Fig. 1.1** Conceptual depiction of the interkingdom interactions involved in an infectious disease system. When there is no transmission (top left), there are no interactions among organisms involved in the disease system. When there is direct transmission (top right), the only interaction is with the host and pathogen—the minimal disease system. Indirect zoonotic transmission involves the interaction of the host, a reservoir (often animal), and pathogen (bottom left), whereas indirect, vector-borne transmission includes these interactions but also a fourth, vector species (bottom right)

be written on all interkingdom interactions important to disease ecology, this chapter will highlight recent advancements in understanding how microbial taxa influence species of other domains and kingdoms. By introducing aspects and examples of interkingdom interactions that have been investigated in the primary ecological literature, the chapter will synthesize how this understanding can influence the scientific approach to studying disease ecology.

Lastly, disease ecology is a discipline involving the study of how hosts, reservoirs, vectors, and pathogens interact within and among populations and communities in a way that ultimately defines ecosystem structure. Since populations are inherent components of communities, we focus here on the interactions among different species within larger communities of organisms where disease systems are nested. While it is acknowledged that specific examples of interkingdom interactions occur within a broader and more complex ecological network, we focus on examples and provide discussion of interactions that often involve two or three

species with biological or ecological function, which may have relevance to the broader understanding of infectious disease ecology and evolution.

## 1.2 Background of Microbial Interkingdom Interactions

Microbes, particularly bacteria, historically were viewed as recyclers of nutrients; however, as pointed out by Janzen (1977), this role is truly limiting as they actually fill many niches in an ecosystem ranging from competitor to predator. For decades microbes have been explored for their interactions with higher life specifically from an interkingdom perspective. The idiom, "Beauty is in the eye of the beholder," rings true in the interkingdom interactions' world but with a slight twist. The idiom would be more applicable with "Relationship is in the eye of the beholder." Point being, defining the role of bacteria in a given environment truly depends on the scale one is using as well as the players identified in the process. This nuance can be a tedious road to explore and requires strict guidelines and definitions for the system being explored. One misstep and the message could be lost or confused by the researcher.

Janzen (1977) masterfully articulated in his view a world where microbes are more than simple by-standards in a realm of multicellular "giants" serving as the cogs responsible for ecosystem operations. As a great example demonstrating how bacteria are in fact competitors with animals for similar resources, consider the case of spoiling milk. Milk that spoils smells "bad" to us and thus we do not drink it. Janzen's presumed interpretation would be bacteria modified the resource in a manner resulting in reduced competition from vertebrates that would otherwise drink this resource that is common to the bacteria. While this example is anecdotal, others have tested his hypothesis and empirically demonstrated this competition does occur in nature. Burkepile et al. (2006) demonstrated reducing microbes associated with carrion in shoreline estuaries allows for crustacean scavengers to feed for longer periods of time on the decaying fish resource; however, like with the red queen (Van Valen 1973), such an arms race opens opportunities for other organisms to take advantage of these resources. Thus, as the resource decomposes and the interest of consumers of fresh carrion decreases, other animals are attracted to and consume the degraded resource.

This interaction is prominent in the vertebrate carrion literature, specifically when one considers vultures and their affinity for decomposing vertebrate remains (Kruuk 1967). These species have developed an ability to tolerate bacteria-laden resources. Even brown tree snakes are known to use volatile organic compounds (VOCs) resulting from microbial activity in mouse carcasses as a means to locate these resources (Shivik and Clark 1997).

While insects, such as blow fly (Diptera: Calliphoridae) larvae, competing for these resources can release antimicrobial products that kill competing or pathogenic bacteria (Greenberg 1968) on the resource, the bacteria may also affect the behavior (Ma et al. 2012) and ecology (e.g., oviposition) (Tomberlin et al. 2012) of flies on a resource. For a comprehensive review of these interkingdom interactions involving

bacteria and insects, see Tomberlin et al. (2017a). Briefly, bacterial communities are known to go through community succession on decomposing vertebrate remains (Pechal et al. 2013). During this process, blow fly attraction to carrion can be related to the sex and physiological state-specific aspects of the blow flies (gravid vs non-gravid) (Mohr and Tomberlin 2014, 2015). Subsequent research has determined that the potential mechanisms regulating these blow fly behavioral responses (e.g., attraction and colonization) are in part regulated by VOCs associated with microbial degradation of essential amino acids associated with the resource (Liu et al. 2016). Further, Flores et al. (2017) demonstrated that bacteria associated with blow fly larvae impact the development of larvae of a competing species. These data by Flores et al. (2017), as well as several other studies of similar interkingdom interactions (Tomberlin et al. 2012; Pechal et al. 2013; Mohr and Tomberlin 2014, 2015; Liu et al. 2016), provide new data suggesting that microbes could be serving a mechanism regulating arthropod succession patterns during colonization of vertebrate carrion. These interactions could result in pathogenic bacteria associated with a decomposing resource to either proliferate or be suppressed. Thus, complex interkingdom interaction may mediate pathogen emergence and dispersal in natural environments.

Additional studies have demonstrated that interkingdom interactions may manifest within trophic networks as well. Previous research has shown that insects, such as blow fly (Greenberg 1971a; Greenberg and Klowden 1972) and house fly [*Musca domestica* L. (Diptera: Muscidae)] (Zurek et al. 2001) larvae, consume microbes associated with decomposing organic matter. Some microbes are thought to be digested and assimilated, while others are thought to pass through the alimentary canal (Mumcuoglu et al. 2001), thus being potentially dispersed to resources in other habitats, as reviewed in Nayduch (2017). Microbial pathogen dispersal in the environment via flies is a key aspect of the transmission of some foodborne illnesses (Greenberg 1971b). For instance, Weatherbee et al. (2017) described interkingdom interactions of microbial communities with blow fly larvae on swine carcasses where taxa from the carcass became integrated into the internal microbiome of feeding and developing larvae, with distinct shifts in microbial phyla composition that showed convergence of the carcass and larval microbiomes later in decomposition.

Further Pechal and Benbow (2016) demonstrated that the internal microbial communities of aquatic insects in streams with decomposing salmon were structurally different from the same insect taxa from streams without carcasses or historic salmon spawning. Similar to Weatherbee et al. (2017), they also reported the internal microbial communities of larvae collected from masses of the salmon carcasses were similar to the carcass microbial communities. These field studies confirmed previous findings from culture-based laboratory research of bacteria that showed differential acquisition of microbial taxa from decomposing organic matter by blow flies and other filth flies (see review of Nayduch 2017). Lastly, Pechal and Benbow (2016) showed in the same field studies that the internal microbial communities of adult blow flies captured above salmon carcasses varied in time, and adults captured nearer the time of larval collections had microbiomes more similar to the larvae than adults captured 10 days later. These results suggest the microbial ecology of

carcass microbial communities in an ecosystem plays important roles in determining the internal microbial communities of both larvae that colonize and consume the resource but likely also influence adults that emerge from those larvae and then disperse into the landscape.

While descriptive, the previous examples provide evidence that the dispersal of microbes into surrounding communities and ecosystems may involve aspects of trophic ecology that provided the foundation for more deeply rooted evolutionary relationships, an area of inquiry that requires additional investigation. The current mechanisms of these potentially evolved interactions are only recently being uncovered, with recent findings showing a future for exciting avenues of research. This new area of research is especially true for those interkingdom interactions among prokaryotic microbes and eukaryotes.

## 1.3 Mechanisms of Interkingdom Interactions in Disease Ecology

Eukaryotes have variable relationships with prokaryotes, and these interactions can range from beneficial to detrimental. These associations are clearly facilitated by complex bidirectional communication taking place between them and that information exchange, physical interactions, and chemical signaling likely resulted from coevolutionary processes. Furthermore, though bacterial growth and virulence are influenced by local environmental parameters such as temperature, pH, and nutrient availability, the influence of host chemical stimuli on bacterial behavior has only recently become apparent (Rumbaugh 2007; Hughes and Sperandio 2008). Microbial receptors are able to recognize that the particular microbe is within the locality of a suitable host and, for commensals, that it is the appropriate time to initiate expression of genes involved in host colonization. Pathogens can then hijack these signals, leading to activation of their virulence genes (Rumbaugh 2007; Hughes and Sperandio 2008). Moreover, hosts and microbes have developed multiple mechanisms to protect themselves from each other and have, in some cases, also evolved mechanisms that allow a mutualistic coexistence. In this section, we discuss several mechanisms that are used for communication interaction and exchange between bacteria and their hosts. We focus particularly on methods of information transfer, as well as mechanisms for detection of and response to host signals. Deciphering such communication is needed for understanding the evolutionary biology of signal development and information exchange. Also, in terms of practical applications, greater understanding of the mechanisms mediating these processes could lead to strategies which disrupt the more damaging aspects of the information exchange and exploitation of the more beneficial and efficient segments within each process (Fig. 1.2).

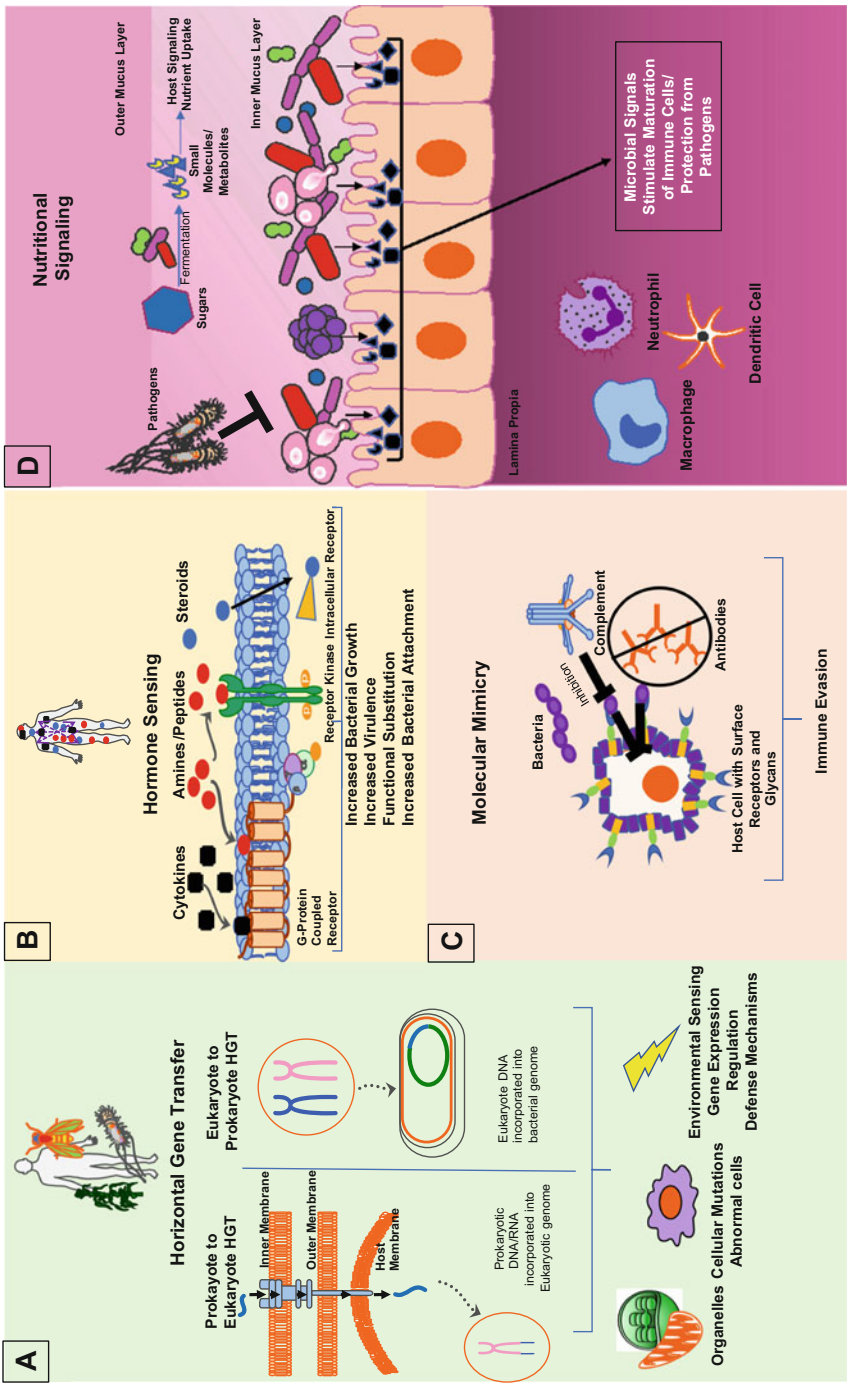


**Fig. 1.2** Conceptual mechanisms and consequences of interkingdom communication between hosts and microbes through (**a**) horizontal gene transfer, (**b**) hormone signaling, (**c**) molecular mimicry, and (**d**) nutritional signaling

### *1.3.1 Horizontal Gene Transfer*

Horizontal gene transfer (HGT) is the transfer of DNA between diverse organisms leading to acquisition of novel traits unique from those that are inherited. The advent of large-scale genome sequencing has greatly improved our understanding of the importance of HGT. Most cases of genetic transfers have been described in bacteria, where transfer between bacteria has been implicated in the acquisition and evolution of many traits including antibiotic resistance, pathogenesis, and mechanisms for bioremediation (Hooper et al. 1999; de la Cruz and Davies 2000; Werren et al. 2010; Ruzzini and Clardy 2016). The molecular interactions of gene transfer and sharing are an intimate example of how previously unknown interkingdom interactions have the potential to greatly expand the science of disease.

#### 1.3.1.1 Bacteria-to-Eukaryote HGT

Bacteria-to-eukaryote HGT may possibly transfer novel functions to plants and animals, allowing adaptation to novel niches, ultimately affecting eukaryotic evolution. This type of HGT is likely frequent because of the close and constant proximity of cells from both organisms. In addition, many bacteria have mechanisms such as types IV and VI secretion systems for interacting with eukaryotes and transferring DNA to the environment and to other organisms (Lacroix and Citovsky 2016; Joshi et al. 2017). Horizontal gene transfer may be even more common for endosymbionts that live in germ cells where transferred information from the microbe to host is likely to be passed on to future host generations. Mitochondria and chloroplasts are examples of such an event. These organelles arose from an *Alphaproteobacteria* endosymbiont and a cyanobacteria endosymbiont, respectively, and both are found in reproductive cells and are transmitted to offspring through germ cells. Such organelle transfers have played important roles in the evolution of eukaryotes (Gray 1999; Gray et al. 1999; Lang et al. 1999; Archibald 2015). One striking example is the insertion of a continuous stretch of 75% of the mitochondrial genome into the *Arabidopsis thaliana* chromosome 2. The chromosomal sequence is 99% identical to the mitochondrial genome, suggesting the transfer event was very recent (Lin et al. 1999). Horizontal gene transfers from the human microbiome also have the potential to induce human cellular mutations and, thus, may have important implications toward human health. For instance, Cui et al. (2015) identified bacterial DNA integration into gastric cancer genomes. This team looked for evidence of genetic material from *Helicobacter pylori* and the Epstein-Barr virus, both of which have been associated with gastric cancer. The researchers identified *H. pylori* integrations in 36 genes in the gastric samples, with more integrations present in the tumors than controls. Chronic *H. pylori* infection leads to DNA damage, and the authors suggested that *H. pylori* DNA may be inserted during impaired DNA repair mechanisms (Cui et al. 2015).

Additional bacteria-to-eukaryote HGT events also include transfers to invertebrates and are particularly prevalent from endosymbiotic bacteria to their host

(Lacroix and Citovsky 2016). The first *Wolbachia*-host transfer described was a variant of the bean beetle *Callosobruchus chinensis* that contained *Wolbachia* genes in its chromosome, and a later study found that approximately 30% of a *Wolbachia* genome was found on the X chromosome (Kondo et al. 2002; Nikoh et al. 2008). *Wolbachia*-host transfer has also been described in the filarial nematode *Onchocerca* spp. (Fenn and Blaxter 2006; Fenn et al. 2006). The HGT to *Drosophila ananassae* Doleschall 1858 (Diptera: Drosophilidae) from its *Wolbachia* endosymbiont is the largest as the entire *Wolbachia* genome has been integrated into the *D. ananassae* chromosome (Dunning Hotopp et al. 2007). *Wolbachia* nuclear inserts were detected in four lines of geographically diverse *D. ananassae* indicating that the insert may be widely distributed (Dunning Hotopp et al. 2007). The authors also found that some of these inserted *Wolbachia* genes were transcribed within eukaryotic cells lacking endosymbionts, suggesting lateral gene transfer occurred into eukaryotic hosts from their prokaryote symbionts, potentially providing a mechanism for acquisition of new genes and functions (Dunning Hotopp et al. 2007).

Another example involves transfers among parasitoid wasps (Hymenoptera), poxviruses, and the endosymbionts, *Wolbachia* and *Orientia tsutsugamushi* (Werren et al. 2010). The authors examined protein domain arrangements in the wasp *Nasonia vitripennis* (Walter 1836) (Hymenoptera: Pteromalidae) and found HGT involving poxviruses, *Wolbachia*, and *Nasonia*. They found 13 poxvirus protein repeats of ankyrin C-terminal domains. Phylogenetic analysis of the repeat sequences suggested that the *Nasonia* lineage acquired one or more of the proteins from *Wolbachia*, with subsequent amplification and divergence. The authors speculate that such lateral gene transfers between bacteria and eukaryotes could be an important source of evolutionary innovation (Werren et al. 2010).

#### 1.3.1.2 HGT of Small RNAs

Small RNAs (sRNAs), including small interfering RNAs (siRNAs) and microRNAs (miRNAs), are conventionally regarded as critical molecular regulators of various intracellular processes. However, recent accumulating evidence indicates that sRNAs can be transferred within cells and tissues and even across species (Skippington and Ragan 2012; Cui et al. 2015; Frohlich and Papenfort 2016). In plants, nematodes, and microbes, these mobile sRNAs can mediate interkingdom communication, environmental sensing, gene expression regulation, host-parasite defense, and many other biological functions. Zhang et al. (2012) used sensitive high-throughput sequencing methods to detect high levels of plant miRNAs in serum and tissues of humans, mice, and calves with plant-based diets and found "cross-kingdom" functional RNA transfer. However, subsequent studies further explored the emerging field of sRNA-mediated cross talk between species; some groups reported negative results and questioned its general applicability (Witwer and Hirschi 2014). Thus, careful considerations are required in future studies.

#### 1.3.1.3 Eukaryote-to-Bacteria HGT

Eukaryote-to-bacteria HGT should be expected to be widespread, as bacteria readily participate as both donors and recipients in HGT and lack the barriers to HGT possessed by eukaryotes, such as presence of a nucleus and segregated germ cells (Koraimann and Wagner 2014). Moreover, acquisition of eukaryotic genes by bacteria is of particular interest because of the possible role of such transferred genes potentially influencing bacterial pathogenicity. However, only a few eukaryote-to-bacteria transfers have been described, though spanning transfers to several bacterial phyla. For instance, *Legionella pneumophila* has been found to encode more than 100 eukaryotic-like proteins with phylogenetic analysis revealing that 29 are of eukaryotic ancestry (Lurie-Weinberger et al. 2010). Furthermore, there seems to be a modest but consistent excess of acquired eukaryotic genes in at least some parasites, such as *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*, *Xylella fastidiosa*, and *Chlamydia pneumoniae* (Koonin et al. 2001). Cyanobacterium *Synechocystis* spp. encodes a variety of proteins associated with different forms of signaling that have been considered eukaryotic (Kaneko and Tabata 1997; Ponting et al. 1999). The ancient transfer of two tubulin genes and a kinesin light chain gene to *Prosthecobacter dejongeii* has also been described (Jenkins et al. 2002). Some *Wolbachia* strains and numerous mosquito and lepidopteran species share phylogenetically related genes (Ahmed et al. 2016). Klasson et al. (2009) examined the sequenced genome of *Aedes aegypti* L. (Diptera: Culicidae) for any genes that could have originated in *Wolbachia* and found that one of the two *A. aegypti* genes identified showed female salivary gland-specific (SGS) expression. These gene and homologs in *Anopheles* are candidate *Plasmodium* sporozoite receptors, and the authors and others suggest the SGS-type mosquito genes might have arisen from an ancient transfer between *Wolbachia* and mosquitoes with HGT quite possibly happening in both directions, though this has been shown with only weak support (Korochkina et al. 2006; Klasson et al. 2009).

### *1.3.2 Hormonal Signaling*

There are three broad categories of mammalian hormones: peptides (or proteins), steroids, and amines. The structure of a hormone dictates the location of its receptor. For instance, amine and peptide hormones cannot cross the cell membrane and are instead recognized and bound to cell-surface receptors such as receptor kinases and G-protein-coupled receptors, whereas steroid hormones can cross plasma membranes and are primarily recognized and bound to intracellular receptors. Peptide hormones include the epidermal growth factor (EGF), insulin, and glucagons. Steroid hormones are derived from cholesterol, and amines are synthesized from tyrosine. Amine hormones include the catecholamines epinephrine, norepinephrine (NE), and dopamine (Freestone 2013; Kendall and Sperandio 2016). All of these

hormones are engaged in interkingdom interactions with microorganisms, where bacteria sense several of these hormones (Sperandio et al. 2003; Freestone 2013; Kendall and Sperandio 2016).

#### 1.3.2.1 Amine Hormones

Epinephrine and NE were the first hormones recognized as influencing bacterial growth and virulence gene expression. Notably, these studies were conducted with bacteria that colonize the human GI tract such as enterohemorrhagic *Escherichia coli* (EHEC) (Kendall and Sperandio 2016). For instance, epinephrine and NE have been reported to enhance the growth of EHEC, possibly by aiding in iron acquisition. More recently, NE was shown to induce enterobactin expression and iron uptake in *E. coli*. Furthermore, epinephrine and NE influence virulence gene expression in EHEC, independent of their influence on EHEC growth. Epinephrine and NE do not cross the cellular membrane; therefore, EHEC relies on the two-component systems QseBC and QseEF, to relay adrenergic signals to the intracellular environment, leading to coordinated expression of genes encoding flagella and motility, attaching-and-effacing lesion formation on enterocytes, stress responses, potassium uptake, and osmolarity (Lyte 1992; Freestone et al. 2002, 2003; Chen et al. 2003; Sperandio et al. 2003; Lyte et al. 2011; Freestone 2013; Kendall and Sperandio 2016).

#### 1.3.2.2 Peptide Hormones

Peptides constitute an extremely diverse class of hormones processed from a precursor and exported out of the cell (Freestone 2013). Gastrin, a peptide hormone secreted by stomach cells, stimulates the release of gastric acid and has been shown to increase the growth rate of *H. pylori*. This gastrin-induced phenotype was mediated by a peptide hormone, cholecystokinin, by the four C-terminal amino acids which are shared by gastrin, and a synthetic analogue, pentagastrin, showing the interaction between gastrin and *H. pylori* was specific and dependent on a defined gastrin sequence (Chowers et al. 1999, 2002). It is also noteworthy that this response is restricted to human gastrin, and this may explain the specificity of *H. pylori* to humans. Conversely, another gastric peptide hormone, somatostatin, which inhibits the secretion of gastric acid, suppresses *H. pylori* growth. Somatostatin has also been shown to directly bind to *H. pylori*, suppressing its proliferation (Yamashita et al. 1998). Furthermore, somatostatin antibody immunoglobulin G fraction blocked the antiproliferative effect of somatostatin, suggesting that *H. pylori* harbors an as-yet-unidentified receptor for this peptide (Yamashita et al. 1998; Kendall and Sperandio 2016). Moreover, *H. pylori* infection also changes the levels of these two hormones, increasing and decreasing gastrin and somatostatin levels, respectively (Joseph and Kirschner 2004; Maciorkowska et al. 2006).

Another example of interkingdom interaction involving peptide hormones is the sensing of EGFs, whose processing from an inactive membrane-associated form into

a secreted and active form requires proteolytic activation by the conserved eukaryotic membrane serine protease rhomboid (Werren et al. 2010). Although well studied in *D. melanogaster*, RHO proteins are ubiquitous, with homologies and functional conservation found in bacteria, archaea, yeast, and plants (Gallio et al. 2002). Though they show low-level sequence similarity, they possess similarity at the structural level and with conserved amino acid residues (Gallio et al. 2002). The first prokaryotic member of the RHO family to be genetically characterized was the product of the gene *aarA* from the Gram-negative pathogen *Providencia stuartii* that represses the expression of an acetyltransferase that modifies peptidoglycan (Payie et al. 1995). Gallio et al. (2002) constructed a mutant of *D. melanogaster* defective for the production of RHO and an *aarA* mutant of *P. stuartii*. They showed that *Drosophila* rhomboid-1 and AarA are able to functionally substitute for each other, indicating evolutionary convergence of the RHO proteins, providing a link between eukaryotic signaling and host-microorganism communication. However, despite these similarities, it is currently unknown whether *P. stuartii* responds to the EGF eukaryotic signal or whether the *P. stuartii* Aar-dependent signal influences eukaryotic cells (Gallio et al. 2002).

#### 1.3.2.3 Interplay Between Stress Response and Interkingdom Interactions

Adaptations to environmental, psychosocial, or physical insults in mammals are referred to as stress responses. It is well established that in an organism undergoing stress, several hormones are released to restore homeostasis. The short-term activation of the stress response involves the synchronized interaction of the neuroendocrine system to ensure that energy substrates are available to meet increasing energy demands of the body. Epinephrine and NE are classic stress hormones that have a pivotal role in the stress response (Karavolos and Khan 2014; Clark and Mach 2016; Herman et al. 2016; Myers et al. 2017). Although they increase mammal's ability to respond to the environment, there is some evidence that they can be sensed by bacteria which allows bacteria, especially pathogens, to gauge the metabolic state of the host and exploit a weakened immune system (Karavolos and Khan 2014).

In addition to epinephrine and NE, hosts may release another class of peptide hormones during stress, known as opioids, such as dynorphin (Karavolos and Khan 2014). A recent study suggested the opportunistic pathogen *P. aeruginosa* uses host dynorphin to enhance the expression of its virulence traits; it was also shown that this mammalian stress hormone cross-signals with *P. aeruginosa* quorum sensing signaling systems (a bacterial response system correlated to population density, whose influence on bacterial-eukaryotic interactions is described in more detail in later sections) (Zaborina et al. 2007; Lesouhaitier et al. 2009). Taken together, these studies have shown that there is an intricate connection between host stress signaling, bacterial response sensing, and pathogenesis, suggesting that response to stress, some of the most basic physiological functions in prokaryotic and eukaryotic cells, is central to interkingdom communication (Lesouhaitier et al. 2009).

Finally, the hypothalamic-pituitary-adrenal axis also induces glucocorticoid stress hormone release by the adrenal gland (Liu et al. 1997; Karavolos and Khan 2014). It is therefore interesting that exposure to adrenocorticotropic hormone (ACTH) increased attachment of EHEC to colonic mucosa by acting through melanocortin receptors located on enteric nerves to enhance mucosal adherence of EHEC (Schreiber and Brown 2005). Physically stressing mice by surgery or catabolic stress significantly increased the numbers of *E. coli* adherence to the cecal mucosa of the stressed mice compared to control animals. Another study showed that elevated NE and dopamine release, such as through stress exposure, modulate EHEC adherence to cecal mucosa and may influence EHEC infection susceptibility (Hendrickson et al. 1999; Chen et al. 2003). Verbrugghe et al. (2011, 2016) have shown, through murine and porcine models, that social stress results in elevated serum cortisol levels and that cortisol increased intracellular proliferation of *Salmonella* inside host macrophages. Collectively, these studies show psychological and physical stress of a host is being sensed by its microbiota, and, in the case of pathogens, host stress is leading to increased virulence.

### *1.3.3 Molecular Mimicry*

Microbial pathogens have evolved exquisite mechanisms to interfere and intercept host biological processes, sometimes through molecular mimicry of host proteins. For instance, some bacterial pathogens have capsular polysaccharides surrounding their outer membrane that are composed of linear polysaccharides known as glycosaminoglycans (GAGs) that are identical to the backbones of GAGs found in animals (Cress et al. 2014). Furthermore, a number of pathogens also have outer glycans, such as sialic acid on their cell surface. These self-associated molecular patterns (Sampson and Mazmanian 2015) allow pathogens to evade complement-mediated responses (Langford-Smith et al. 2015). Varki (2006) hypothesized that these glycans did not arise from HGT from vertebrates but, rather, from the recruitment of pre-existing homologous genes or possibly convergent evolution and that these and other forces such as faster evolution through HGT, high mutation rates, fast growth, and short life spans are likely to have further driven the diversification of species-specific glycosylation patterns during evolution (Varki 2006). Additionally, glycosyltransferase genes responsible for biosynthesizing the glycans in pathogen and host are typically not homologous (Gagneux and Varki 1999). Varki (2006) also argued that sexual reproduction-enabled mutations in host glycosyltransferases and subsequent change in glycan profile allow eukaryotic organisms to adapt to pathogenic pressure.

This ability of pathogens to exploit the similarities of their gene products with their host homologs to elude the defense response may have also contributed to drive the rapid diversification of host-defense genes in mammals. For instance, Murphy (1993) conducted a search of protein orthologs and found receptors and ligands involved in antimicrobial host-defense functions have undergone structural diversification that has resulted in species restricted function. Additionally, the authors

suggested that pathogenic microbes may have played a role through exerting selective pressure on the host (Murphy 1993).

Another example of molecular mimicry involves ubiquitination, a highly conserved eukaryotic posttranslational modification essential in determining protein fate that is often hijacked by pathogenic bacteria. The conserved SKP1/CUL1/F-box (SCF) E3 ubiquitin ligase complex plays a key role in ubiquitination of proteins in eukaryotic cells. The F-box protein component of the SCF complex provides specificity to ubiquitination by binding to specific cellular proteins, targeting them to be ubiquitinated by the SCF complex. The *L. pneumophila* protein AnkB is essential for establishing intracellular growth within macrophages and protozoa by promoting the decoration of the *Legionella* containing vacuole with polyubiquitinated proteins. The AnkB effector achieves this by mimicking the action of host cell F-box proteins, a highly conserved component of the SCF ubiquitin ligase complex found in both unicellular organisms and mammalian cells (Price et al. 2010; Price and Kwaik 2010). Importantly, *L. pneumophila*, as well as other pathogens such as *Agrobacterium tumefaciens* and *Ralstonia solanacearum*, utilizes either type III or IV secretion systems to inject into the host cell eukaryotic-like F-box effectors (AnkB in the case of *L. pneumophila*) that interact with the host SKP1 component of the SCF complex to trigger ubiquitination of specific host cell targets, a mechanism essential to promote pathogen proliferation. Price et al. (2010) identified at least 74 genes encoding putative F-box proteins belonging to 22 other bacterial species, including human pathogens, plant pathogens, and amoebal endosymbionts, suggesting that subversion of the host ubiquitination machinery by bacterial F-box proteins may be a widespread strategy among pathogenic bacteria. They also found that bacterial F-box proteins harbor ankyrin repeats that are present in F-box proteins of primitive, but not higher, eukaryotes, suggesting that acquisition of many bacterial F-box proteins may have been from primitive eukaryotic hosts rather than the mammalian host (Price et al. 2010).

### *1.3.4 Sensing the Host Immune System*

The host immune system can use bacterial signals to gauge, and respond to, bacterial infections. In kind, bacteria have also evolved the ability to sense and manipulate host immune systems. Cytokines are the chief signaling molecules employed by the immune system, which makes them a prime target to be recognized as cues by pathogens. Several cytokines, such as interleukin-1β (IL-1β), IL-2, and granulocyte macrophage colony-stimulating factor, increase the growth of virulent, but not avirulent, strains of *E. coli*, suggesting that bacteria sense these cytokines and change their behavior using a mechanism that is not due to nutritional factors (Porat et al. 1991).

High-affinity binding of IL-1β to the F1 antigen of the *Yersinia pestis* (causative agent of plague) capsule through the capsule antigen F1 assembly protein (Caf1A) has also been reported (Zav'yalov et al. 1995). The Caf1A protein is present on outer

**Table 1.1** Immune signals recognized by some pathogens and consequences of recognition

| Pathogen | Signal | Bacterial receptor | Consequence for pathogen | References |
|---|---|---|---|---|
| *Escherichia coli* | IL-1β | Unknown | Increase in growth | Porat et al. (1991) |
| *Escherichia coli* | IL-2 | Unknown | Increase in growth | Denis et al. (1991), Porat et al. (1991) |
| *Escherichia coli* | Granulocyte macrophage | Unknown | Increase in growth | Denis et al. (1991), Porat et al. (1991) |
| *Escherichia coli* | TNF-α | Unknown | Increase in growth | Luo et al. (1993) |
| *Salmonella enterica* | TNF-α | Unknown | Increased virulence | Arnold et al. (1993b), Luo et al. (1993) |
| *Salmonella enterica* and *Salmonella typhimurium* | Cationic antimicrobial peptides | PhoPQ | Modification of LPS; increased virulence | Bader et al. (2005), Dalebroux and Miller (2014) |
| *Pseudomonas aeruginosa* | IFN-γ | OprF | Quorum sensing; increased expression of PA-I lectin | Wu et al. (2005) |
| *Shigella flexneri* | TNF-α | Unknown | Increased virulence | Luo et al. (1993) |
| *Yersinia pestis* | IL-1β | Caf1A | Capsular binding | Zav'yalov et al. (1995) |
| *Candida albicans* | IL-2 | Unknown | Increased growth | Treseler et al. (1992) |

membranes and shows significant homology to the human IL-1β receptor, suggesting that the gene encoding Caf1A may have been acquired through HGT from mammals (Zav'yalov et al. 1995). Other pathogenic bacteria such as pathogenic *E. coli*, *Shigella*, and *Salmonella enterica* bind to and show increased virulence in response to tumor necrosis factor alpha (TNF-α) (Arnold et al. 1993a, b; Luo et al. 1993). More recently, TNF-α-dependent increased growth of *E. coli* was shown using both in vitro and in vivo models, but no underlying mechanism was proposed (Lee et al. 2003).

Another cytokine reportedly sensed by bacteria is gamma interferon (IFN-γ). The *P. aeruginosa* OprF surface protein binds host IFN-γ and initiates a signaling cascade in the bacterial cell that reprograms gene expression, resulting in the expression of a quorum sensing-dependent virulence determinant, PA-I lectin (Wu et al. 2005). This IFN-γ enhancement of *P. aeruginosa* pathogenesis is thought to be especially intriguing in light of the observation that IFN-γ treatment exacerbates *P. aeruginosa* infection in several mouse models and from clinical studies (Luo et al. 1993; Kendall and Sperandio 2016) (Table 1.1).

#### 1.3.4.1 Bacterial Sensing of Mammalian Antimicrobial Peptides

One of the front lines of defense of the mammalian innate immune system is the production of cationic antimicrobial peptides (CAMPs) that interact with and disrupt

bacterial membranes. Several Gram-negative bacterial pathogens modify the lipid A of their LPS to prevent CAMPs from binding to their outer membrane. For instance, salmonellae sense host cues to regulate properties important for bacterial survival and replication within host tissues. The PhoPQ two-component regulatory system senses phagosome acidification and CAMPs and then regulates the protein and lipid contents of their bacterial envelope that comprises an inner and outer membrane. In *S. typhimurium*, the PhoQ sensor kinase directly binds, and is activated by, host CAMPS. In turn, PhoQ then promotes the expression of virulence genes through a phosphorelay cascade (Bader et al. 2005). The PhoPQ regulation of genes encoding lipid A modification enzymes is vital for *S. enterica* and *S. typhimurium* virulence (Dalebroux and Miller 2014). The CAMPs alter conformation of the PhoQ, activating its kinase activity and resulting in a phosphotransfer to PhoP with subsequent activation of downstream genes necessary for invasion of epithelial cells and intramacrophage replication (Bader et al. 2005).

### *1.3.5 Nutritional Signaling*

The mammalian GI tract provides a complex, competitive, and spatially diverse environment for resident host microbiota (Hooper et al. 1998, 2001, 2002; Groussin et al. 2017). The GI tract is heavily colonized by a complex and highly adapted microbiota. Estimates of the number of bacterial species present in the human gut vary widely among studies, but it is generally accepted that individuals harbor more than 1000 microbial, species-level phylotypes (Bull and Plummer 2014). The GI microbiota has an important symbiotic relationship with its host, providing and gaining nutrients in the form of carbon and nitrogen sources. Additionally, the microbiota provides critical signals that promote maturation of immune cells and tissues, leading to protection from infections by pathogens. Gut microbiota have been shown to become perturbed during several dysbiotic disease states (Chow et al. 2010). Particularly, imbalance of the normal gut microbiota has been linked with inflammatory bowel disease, irritable bowel syndrome, and wider systemic manifestations of disease such as obesity, type 2 diabetes, and allergic dermatitis (Bull and Plummer 2014). Furthermore, once the microbiota is under immune attack, a more virulent or pathogenic profile may provide certain microbial species with a greater chance of success (Bull and Plummer 2014). Successful intestinal colonization by pathogenic bacteria is thought to depend on the microbe's ability for scavenging nutrients, sensing chemical signals present in the intestine, competing with the resident bacteria for space and nutrients, and precisely regulating the expression of virulence genes. The authors remind us that the most fundamental way of bacterial communication is the importation of substrates for metabolism and exporting its end products and that "If your neighbor eats starch and produces 5 mM succinate, you will probably take notice" (Fischbach and Sonnenburg 2011).

One example of nutritional sensing involves sugars. The mammalian intestinal epithelium is protected from direct contact with bacteria by the mucus layer whose

major component is a major intestinal secretory mucin, Muc2 (Bergstrom et al. 2010). Sugars bound to mucin are made available to the microbiota through the polysaccharide-degrading activity of glycolytic commensal anaerobes. Subsequently, the mucus layer is an important source of carbohydrates for bacterial communities colonizing mucosal surfaces (Kim and Ho 2010; Kendall and Sperandio 2016).

Fucose is also a major component of mucin glycoproteins abundant in the intestine (Robbe et al. 2004). Hooper et al. (1999) used a gnotobiotic mouse model to show *Bacteroides thetaiotaomicron*, a component of the intestinal microbiota of mice and humans, uses a repressor, FucR, as a molecular sensor of fucose availability. Importantly, FucR coordinates expression of an operon encoding enzymes in the fucose metabolic pathway with expression of another locus that regulates production of fucosylated glycans in intestinal enterocytes. Genetic and biochemical studies suggest that FucR uses fucose as an inducer at one locus and as a corepressor at the other locus. Coordinating this commensal's immediate nutritional requirements with production of a host-derived energy source underscores the bacterium's need to enter and persist within a competitive ecosystem (Hooper et al. 1999). Furthermore, fucose utilization has been found to be important for EHEC intestinal colonization (Pacheco et al. 2012).

The EHEC also regulates virulence gene expression through recognition of host glycolytic and gluconeogenic environments. The lumen is more glycolytic because of predominant glycophagic microbiota degrading complex polysaccharides into monosaccharides readily utilized by nonglycophagic bacterial species such as *E. coli* and *Citrobacter rodentium* (Yanez et al. 2003; Bertin et al. 2014). Furthermore, it has been shown that genes classified in the gluconeogenesis gene ontology category were significantly up-regulated by EHEC incubated in bovine small intestine contents, relative to EHEC grown in minimal medium (Bertin et al. 2014).

### *1.3.6 Interkingdom Signaling with Nonmammalian Hosts*

Just as signaling and recognition systems for host hormones, metabolites, and host defenses used by mammalian pathogens have been discovered, research also indicates that these mechanisms for interkingdom interactions are conserved in bacteria that infect nonmammalian hosts. Some examples have been discussed in earlier sections. However, some additional examples are highlighted here. For instance, *Edwardsiella tarda*, a pathogen leading to septicemia in fish, uses the QseBC two-component system to regulate gene expression controlling flagellar motility, fimbrial hemagglutination, and intracellular virulence (Wang et al. 2011). Moreover, *E. tarda* responds to epinephrine to promote motility, and this effect was lost by a *qseB* or *qseC* deletion strain, suggesting that this mechanism of gene regulation is conserved in diverse pathogens (Wang et al. 2011; Kendall and Sperandio 2016).

Additionally, the *eut* locus encoding ethanolamine metabolism was described as important to infection mechanisms of the insect pathogen *Photorhabdus luminescens*

and the plant pathogen *Erwinia chrysanthemi* (Munch et al. 2008). Further studies show that a disruption of *eutR* in *E. chrysanthemi* results in impaired systemic infection of African violet plants. However, it is currently unknown whether the effect was due to defects in ethanolamine metabolism or signaling through EutR-dependent regulation of virulence traits (Munch et al. 2008). Lastly, *Vibrio coralliilyticus*, a coral pathogen of *Pocillopora damicornis*, senses dimethylsulfoniopropionate (DMSP) to target heat-stressed corals, rich in DMSP. Sensing results in chemotaxis, and this signal was used solely for finding the host location, rather than for pathogen metabolism (Garren et al. 2014).

In arthropods, the presence of endosymbiotic prokaryotes plays an important role in metabolism. In some cases, genome integration has occurred from endosymbiotic-host relationships, proving that intracellular symbiosis is not simply a nutritional supplement (Fenn and Blaxter 2006; Dunning Hotopp et al. 2007; Ahmed et al. 2016). Intracellular symbiotic bacteria are also described in nematodes (Fenn et al. 2006). In particular, the presence of intracellular *Wolbachia* in filariae positively influences reproductive biology and host survival, as proved by antibiotic treatment against this bacterium (Fenn et al. 2006).

Another classic example includes the interkingdom interaction of rhizobia and host legume plants. Rhizobia are nitrogen-fixing bacteria that colonize plant roots through an evolutionarily developed association to form root nodules under nitrogen-limiting conditions. In the nodule, these bacteria fix nitrogen that can then be used by the host, and in return, the plant provides carbon useful for bacterial growth. For this mutually beneficial relationship to be established, signals are exchanged between the bacteria and the host. Specifically, legumes produce flavonoids sensed by the bacterial NodD protein. The perception of specific Nod factors by rhizobia triggers a host signaling cascade that leads, in most legumes, to formation of specialized intracellular structures called infection threads that act as a conduit for rhizobial access to the inner root tissues where they are endocytosed into nodule cells to begin nitrogen fixation (Liu and Murray 2016). Furthermore, the bacteria also produce chemicals influencing host response to infection. For example, the bacterial Nod factor may play a role in suppressing the plant immune response, thereby inducing nodule development (Toth and Stacey 2015). Studies on how rhizobia influence plant innate immunity to establish symbiotic associations highlight that interkingdom interactions are not limited to host and pathogen. Indeed, it is likely that additional studies examining interkingdom interactions between host and associated microbiota will demonstrate conserved mechanisms shared by pathogens as well as commensal bacteria. Such conserved mechanisms highlight the need for new and innovative studies in complex disease systems. Understanding such novel mechanisms may yield important advances in disease management.

### *1.3.7 Quorum Sensing Systems and Disease Ecology*

One final system for prokaryotic-eukaryotic interactions involves bacterial responses to eukaryote chemical stimuli that are dependent upon threshold bacterial concentrations, a phenomenon known as quorum sensing, which was previously mentioned. In this case, both pathogenic and nonpathogenic bacteria, and some yeast, may recognize chemical stimuli individually but will not adjust gene expression until threshold microbial densities are reached that then allow for coordinated phenotypes (Sprague and Winans 2006; Plancak et al. 2015; Hawver et al. 2016). Quorum sensing has been identified in eukaryote and prokaryote systems and has been studied extensively. However, much needed research still remains. The next section will focus on this intriguing bacterial communication pathway as a key example of complex mechanisms of microbes influencing eukaryote behavior, lending insight into the potential of similar interactions that may be important to infectious disease systems. Indeed, the precedent (see below) that bacteria influence the behavior of higher level organisms (e.g., hosts or reservoirs) suggests that these interkingdom interactions may be more important to pathogen environmental transmission than previously considered.

#### 1.3.7.1 Quorum Sensing Regulates Behavior of Eukaryotes

Limited information is available on the interkingdom interactions and microbial quorum sensing roles in inducing behavioral responses of hosts or other animals competing for similar resources as the microbes. Several reviews have been published on this topic; however, the first known review was not published until 2008 (Lowery et al. 2008). More recently, the molecular mechanisms of indole as a quorum sensing molecule and regulator of physiological responses across kingdoms or domains were published (Lee et al. 2015), while a subsequent review examined its role as a modulator of behavior and distinguished between its role as a signal and cue (Tomberlin et al. 2017b).

Specific studies exploring the role of bacteria regulating arthropod vector behavior have been published, with much research focused on the role bacteria play in regulating adult mosquito responses to oviposition (Ponnusamy et al. 2008) sites or hosts (Verhulst et al. 2009, 2010a, b, 2011). More recently, efforts have begun exploring the role of quorum sensing as a mechanism regulating mosquito behavior. Cui et al. (2015) determined that isolates of *Staphylococcus epidermidis* that were able to quorum sense attracted 74% of adult *A. aegypti* versus the mutants unable to quorum sense. This interaction has significant ramifications with regard to pathogen transmission from vector (i.e., mosquito) to hosts as the vectors utilize microbial quorum sensing as a means to locate the said hosts.

This relationship between bacterial quorum sensing and arthropod vector behavior has been demonstrated for other species as well, including filth flies that are known to transmit human pathogens (Greenberg 1971b). Research with a blow fly

model [i.e., *Lucilia sericata* (Diptera: Calliphoridae)] determined the level of attraction to food, and oviposition substrate inoculated with *Proteus mirabilis*, which is a commensal with this fly species, was partially regulated by the ability of this bacteria to swarm (quorum sensing response) (Ma et al. 2012). The response of the insect was partially regulated by its age, sex, and nutrition history (Tomberlin et al. 2012). Interestingly, the volatiles that regulated these responses, such as indole, are derivatives of essential amino acids (Liu et al. 2016). The reason this information is relevant is that it ties together microbial ecology (e.g., resource utilization), population dynamics (e.g., microbial population structure as related to resource utilization), and either potential competition or mutualistic interactions between the bacteria and the insect. If the insect were to colonize the resource at a suboptimal time point (e.g., when the bacteria are in exponential growth phase and utilizing resources quickly), survivorship of the insect offspring could be threatened. High mortality in vector offspring affects population density and potential vectorial capacity, having direct impact in models of foodborne disease transmission from filth flies.

This aspect of interkingdom interactions was described well with the house fly, *Musca domestica* L. (Diptera: Muscidae) model. Bacterial populations associated with house fly eggs shift as the eggs mature (Lam et al. 2007). Researchers determined that a regulatory threshold existed within the bacterial population that affected house fly attraction and oviposition. Attractant levels below the threshold resulted in attraction and oviposition, while levels above the threshold resulted in reduced attraction (Lam et al. 2007). They determined this was critical since colonization above the threshold might correspond to hatching of eggs associated with the microbes and the resulting larvae would cannibalize the eggs deposited by the adults after the microbial threshold was reached. Furthermore, they determined these bacteria associated with the eggs also served as nutrients for the resulting larvae (Lam et al. 2009a). They also reported that bacteria possessed antifungal properties, thereby protecting the eggs during maturation (Lam et al. 2009b). The above examples of quorum sensing interactions with invertebrates continue to identify new microbe-animal relationships that have potential to reveal new ways to manage pathogen transmission, insect vector population biology and other ecological (e.g., microbe competition) forces of disease systems. There are similar studies of interkingdom interactions that include vertebrates that may be especially important in more comprehensively understanding zoonotic diseases.

## 1.4 Microbiomes and Vertebrates and Disease Ecology

Understanding the complex dynamics and function of the microbiome in vertebrate disease systems has undergone a surge of research interest and new findings with the advent of next-generation sequencing (Cui et al. 2015). Estimates are that up to 90% of the vertebrate host genetic information is microbial (Sender et al. 2016a, b) and that this genetic material likely plays a significant role in the human condition (McFall-Ngai et al. 2013). Thus, characterization of these microorganisms, primarily

bacterial communities, in host populations has led to a better understanding of how microbes can influence behavior (Ezenwa et al. 2012; Wong et al. 2015), physiology (Nicholson et al. 2012), immunity (Eleftherianos et al. 2013), reproduction (Reid et al. 2015), nutrition (Nicholson et al. 2012), and development (Hooper 2004).

The relationships between host phylogeny and microbiomes have led to investigations into the potential mechanisms describing their coevolutionary history (Moeller and Ochman 2014; Colston and Jackson 2016). Indeed, many researchers no longer see a clear delineation between a host and its microbial constituents. Rather the host and all of its symbiotic microbes are deemed a "holobiont" (Bordenstein and Theis 2015) and should be holistically explored as a single biological entity. The complexities and integration of organisms within a holobiont have been documented for multi-host pathogen introgressions and hybridizations. For example, in West Africa there is evidence of an introgression that has been detected between a human (*Schistosoma haematobium*) and two ruminant (*S. bovis* and *S. curassoni*) schistosomes (Webster et al. 2013). Zoonotic pathogens are the cause of one billion cases of human illnesses annually (Han et al. 2015), yet the true host range of vertebrates capable of transmitting disease-causing parasites and pathogens remains underexplored.

As shown in Fig. 1.1 and mentioned in the introduction to this chapter, there are several pathways for infectious disease occurrence, but at a minimum, there must be a host species and a pathogen. An example of this minimal disease system involving a single vertebrate host species would be a human and a virus such as influenza virus. More often there is a combination of vertebrate and invertebrate host species directly and indirectly interacting with pathogens in a disease system. Pathogens can achieve host switching via rapid mutations as is seen in viruses, such as influenza A and avian flu. Thus, the breadth of potential host species for pathogen dispersal increases. Estimates of zoonotic host diversity have been attempted using predictive models based on vertebrate traits (e.g., activity cycle, dispersal age, habitat breadth, sexual maturity age, body mass) (Han et al. 2015). Several studies have determined rodents to be the most abundant zoonotic reservoir as compared to either other mammals or birds (Han et al. 2015; Johnson et al. 2015; Ostfeld and Keesing 2017). Of the extant rodent species, approximately 11% were predicted to be a zoonotic reservoir (Han et al. 2015). Many of these host species involved in disease systems are important players in an array of ecosystem services (Keesing et al. 2010; Ostfeld et al. 2010; Johnson et al. 2015; Ostfeld and Keesing 2017). However, it remains unknown as to the relationship between true pathogen prevalence and associations with vertebrate community diversity. Biodiversity within an ecosystem may play an important role in disease dynamics (Keesing et al. 2010). Increased host biodiversity may lead to a decreased pathogen transmission by reducing the probability of transmission among conspecifics (Keesing et al. 2010; Ostfeld et al. 2010; Johnson et al. 2015; Ostfeld and Keesing 2017).

This premise of how biodiversity affects transmission has occurred from suggestions in multiple disease systems. For instance, there is a documented negative correlation of *Hantavirus* in rodents when small mammal diversity increases within a habitat (Clay et al. 2009), and increased heterospecific snail populations lead to

greater probability of schistosomiasis being transmitted to a dead-end host (Laracuente et al. 1979). Continued research is needed to identify how microbiome community composition and assembly potentially impacts disease emergence and dynamics in vertebrate hosts. Furthermore, determining the response of pathogens and their hosts to dynamic selective pressures, such as shifts in host range due to climate and anthropogenic changes or disease control strategies, will be imperative for mitigating infectious disease outbreaks with a changing climate. It is of great importance to develop a better understanding for infectious disease systems with regard to the basic ecological interactions and potential robust mechanisms between microbiomes and their hosts. Below are several examples of these ecological interactions that either directly or indirectly influence disease systems.

### *1.4.1 Influence of Ontogeny and Life History on Microbiomes*

The development of an animal is linked to a multifactor suit of characters selected to optimize its fitness. Primarily the goal of the organism, whether prokaryotic or eukaryotic, is to pass along its genes through successful reproduction. In vertebrate hosts, there are a variety of reproductive strategies including oviparous, oviparity, and viviparity, and one vertebrate is known to have a mixed-mating strategy of hermaphrodites that reproduce by self-fertilization or crossbreeding—the mangrove killifish (Molloy and Gage 2006). Despite how a vertebrate is produced, a number of physiological transformations occur during development that can alter the microbiome. In the earliest stages of development, organisms can be protected against potential deleterious microbes by physical and chemical barriers (McFall-Ngai et al. 2013). These barriers include mucous and antimicrobial peptides that regulate the developing microbiome. Also, host traits can increase predictive capability of acting as a zoonotic reservoir related to an increased reproductive output, such as early age at maturity, large litters, short life span, and host geographic range, as exampled by rodents (Han et al. 2015; Ostfeld and Keesing 2017).

As a vertebrate host grows to maturity and beyond, diet and reproductive status can further regulate their microbial communities. Some research has focused on the horizontal acquisition of microbiota through feeding behaviors. Green iguana (*Iguana iguana*) hatchlings initially increase their hindgut microbiota from consuming soil within the nest chamber; shortly after hatching (*ca*. 1 week), they begin ingesting plants and soil from outside of the nest chamber; and finally, the animals associate with other conspecifics and eat the feces of older individuals, gaining access to more complex microbial communities (Troyer 1984). Other research in humans has demonstrated that the "healthy" gut microbiota is typically a stable community of *Bacteroidetes* and *Firmicutes* (Lloyd-Price et al. 2016), but there is a shift in communities as an individual ages (Backhed et al. 2004; Koenig et al. 2011; Lozupone et al. 2012; Oh et al. 2012). However, there have been significant shifts in the core gut microbiome in as little as 5 days due to changes in dietary intake (David et al. 2014). It has also been well documented that obesity in humans is positively

correlated to an increase in the *Firmicutes* to *Bacteroidetes* ratio (Turnbaugh et al. 2006, 2009; Boulangé et al. 2016). Much research is needed to further investigate the impact of other vertebrate hosts and their life history strategies on their microbiome; however, the growing body of literature suggests that the microbiome and its interaction at both molecular and cellular level have a significant role in health and disease. Some of these interactions play out through host manipulation of the microbiome, with potential influence on host and reservoir population dynamics.

### *1.4.2 Host Manipulation by the Microbiome*

Recent research on the interactions between the microbiome and host has elucidated a large number of mechanisms by which the microbiota affects animal behavior (Ezenwa et al. 2012). For example, pathogens can shift animal behavior to optimize transmission and therefore affect the entire disease system network. In this section, we will present key examples of how pathogens and other microbial groups affect vertebrate biology. These interactions likely have broad population biology and natural history effects that are important to disease ecology. A classic example of host behavior modification by a pathogen has been thoroughly described in a feline and rodent model, whereby *Toxoplasma gondii* mediates the behavior of its intermediate host (rodents) by reducing predator risk assessment (Webster 2001; Webster et al. 2017). This pathogen affects the cognitive perception of the mouse thus leading to an increased feeding rate by felines. This modification of risk assessment, however, differs between wild and domesticated feline species. Rats demonstrated an attraction to wild cats over domesticated cats when infected with *T. gondii* (Kaushik et al. 2014). There is also documented increased aggression in vertebrate populations caused by rabies, Borna disease, and Hantaan or Seoul viruses, which increased the rate of blood/saliva transmission and thus pathogen proliferation (Klein 2003).

The residing microbiota of a vertebrate host population is also known in some instances to impact sociality. In hyenas, there is a strong correlation between fermentative bacteria detected in the scent glands of different hyena species (spotted versus striped), sex, and reproductive state (Theis et al. 2013). *Firmicutes* was the predominate phylum in each species representing over 95.59–99.96% relative abundance, but constituents of *Bacteroidetes* (0.04–1.16%) and *Actinobacteria* (3.73%) were the next predominate phyla in striped and spotted hyenas, respectively (Theis et al. 2013). Furthermore there were significant differences in microbiome structure comparisons between males and females, with additional differences between lactating versus pregnant females. These differences in bacterial communities have direct implications in the chemical communication among individuals within a group and thus the overall social structure. If the same is true in other social animals, these interkingdom interactions likely play important roles in pathogen transmission in many disease systems.

For instance, in humans the gut microbiome has been linked to various mood disorders, obesity, autism, and other physical diseases, such as allergies and asthma (Cho and Blaser 2012; Han et al. 2012; Liu et al. 2012a; Hsiao et al. 2013; Christian et al. 2015). Furthermore, many studies are demonstrating associations between brain development and behavior with the microbiome (Sampson and Mazmanian 2015). For instance, Butterworth (2015) found that changes in the gut microbiome trigger changes in the central nervous system and the brain and that these changes are related to anxiety, depression, and other forms of mental illnesses in human. Christian et al. (2015) reported that the diversity of toddler's microbiome was related to temperament traits such as extroversion. In a related way, another study found that the microbiome may act to stimulate the production of serotonin and important neurotransmitter that affects mood and is related to several mood disorders (Hsiao et al. 2013). It is evident that both pathogens and commensal microbes are key in modulating host behavior, which is of importance for further exploring transmission dynamics of infectious diseases. The microbiome also affects other aspects of the human condition, with potentially important effects on disease presentation and pathogenesis.

#### 1.4.2.1 Human Hosts

The human body is a dynamic vessel for microorganisms that together are a population constantly in flux. The composition and role of microbial consortia that endogenously and exogenously comprise the human microbiome have been extensively studied for human health (The Human Microbiome Jumpstart Reference Strains Consortium 2010). The human microbiome can be extremely variable within an individual as distinct taxa of bacteria reside in different parts of the body (Grice et al. 2009). Put simply, research suggests that the human body is an ecosystem, which consists of multiple distinct natural environments (e.g., the gut, skin, eyes, mouth), with each natural environment containing a unique microbiome community. A healthy microbial community can be characterized in terms of diversity, stability, and resilience (Lozupone et al. 2012). However, a healthy microbial community that becomes disturbed will often result in a state of dysbiosis or an abnormal microbial community (structure and function) (Levy et al. 2017). Diversity is typically reduced in disease-associated dysbiosis (Le Chatelier et al. 2013; Mosca et al. 2016). The stability of the microbiome within a given body region also can have tremendous influence on the potential risk of infections by certain pathogens.

As an example, instability of the vaginal microbiome caused by bacterial vaginosis increases the risk of acquiring human immunodeficiency virus (Atashili et al. 2008). Perturbation of the "healthy" microbiota has also been documented by the overgrowth of pathogenic bacteria within *Enterobacteriaceae* in the gut during enteric infections (Stecher et al. 2013). Alternatively, modification of the disturbed microbiota can be achieved by replacing pathogenic "blooming" bacteria with commensal microorganisms. In fecal microbiota transplantation, the unhealthy gut community of an individual with inflammation caused by *Clostridium difficile* can

be improved with the introduction of *Clostridium scindens* (Buffie et al. 2015). Further, there is evidence of recurrent infection caused by antibiotic-resistant *C. difficile* being better controlled when the entire gut community was replaced from a healthy donor (van Nood et al. 2013). Understanding the mechanisms of microbiota competition may allow the development of more targeted interventions against infectious diseases in the future.

#### 1.4.2.2 Nonhuman Vertebrate Hosts

While the human ecosystem has been the most extensively studied, there is increasing empirical evidence of how nonhuman vertebrates respond to microbial communities and their function in response to various disease-causing microorganisms. While this section is not exhaustive, it provides an array of examples that illustrate the dynamic interactions between host and its microbiome and how pathogens exploit mechanisms to increase transmission. The most prevalent vector-borne disease in the Northern Hemisphere is Lyme disease; thus, its disease dynamics and mechanisms have been widely studied. Ticks (*Ixodes* spp.) are primary vectors for transmission to humans, and a tick must feed upon an infected host to acquire the causative microbe *Borrelia burgdorferi*. Classic behavioral studies for this disease have demonstrated that ticks attempting to feed on various host species have a differential response to becoming infected by the bacterium. Specifically, ticks attempting to feed on opossums are less likely to become infected than ticks that feed on mice, because of the grooming habits of the opossums (Keesing et al. 2010). However, there is also the possibility that the opossum microbiome has an impact on the colonization of *Borrelia burgdorferi*, because natural products that inhibit biofilm growth (cyclic lipodepsipeptides) derived from the internal gut microbiota have been detected in the postmortem microbiome of opossums (Motley et al. 2017).

There are also reptile hosts for Lyme disease. There is a significantly reduced diversity in the microbiome of ticks (*Ixodes pacificus*) that feed on western fence lizard hosts compared to blood meals acquired from mammalian hosts (Swei and Kwan 2017). These differences in the gut microbiota between reptilian and mammalian hosts could explain the refractory nature of these lizards against Lyme disease, but additional studies are needed to elucidate cause versus correlation.

Another example of behavior (and potential of microbiome manipulation) affecting disease dynamics is in facial tumors of Tasmanian devils. The pathogen causing these tumors is transmitted through aggressive behaviors, such as biting, that frequently occur during mating and feeding (Deakin and Belov 2012). If these social activities are mediated by the Tasmanian devil microbial communities like that of hyenas, then one would expect that transmission of this disease may be indirectly affected by the host microbiome through behavior modifications.

The occurrence of pathogens and their interactions with the microbiome for nonhuman hosts are not isolated to land-dwelling animals. There is emerging research in hosts that live in aquatic habitats. Bacterial pathogens of teleosts span a wide range of phyla and genera. These pathogens, including *Vibrio*, *Renibacterium*,

and *Mycobacterium*, are often found in the normal microbiota of healthy fish but, under certain conditions, such as when experiencing stress, can open the door for opportunistic pathogens to manifest as disease in the host (Llewellyn et al. 2014). The normal microbiota of certain fish has also been demonstrated to act as a natural barrier against fungal pathogens. For example, skin commensals of rainbow trout (*Oncorhynchus mykiss*) have been shown to inhibit *Saprolegnia australis* and *Mucor hiemalis* (Lowrey et al. 2015), which can have important economic impact for aquaculture because of disease-related mortality (Sandoval-Sierra et al. 2014). There are strategies currently being implemented in aquaculture to reduce the prevalence of many types of fish disease, especially in those species that are of global economic and cultural importance, such as *Oncorhynchus*. One strategy is to alter the gut microbiota to increase resistance against pathogens (Tellez et al. 2006), much as is performed with the use of probiotics in mammalian hosts. There is a paucity of data regarding infectious diseases in marine wildlife, even less for how the microbiome is interacting with diseased hosts. Interestingly, recent evidence has demonstrated that Gram-negative anaerobic bacteria of human origin, such as human adapted *Campylobacter*, have been detected in dolphin microbiomes (Godoy-Vitorino et al. 2017). Overall, while there is an ever-expanding amount of research about the microbiome of hosts, vectors, and pathogens, additional studies into the constituents and interactions among micro- and macroorganisms would allow to help better understand why some populations are more competent vectors and how pathogens are transmitted within an ecosystem and ultimately elucidate the mechanisms resulting in disease and effective control strategies to counter the dynamic nature of pathogens. There are many different techniques for studying these aspects of disease ecology, and one of the most recent and potentially transforming methods has been through advances in high-throughput genomic sequencing. This method is allowing scientists to identify thousands of microbial taxa, opening the doors to asking new questions about the role of interkingdom interactions in disease systems.

### *1.4.3 Current Sequencing Tools*

Characterizing a microbiome is becoming more widely available as a tool given the rapid advances in NGS. Depending on the question being investigated, one may use a bevy of approaches to better understand microbiomes and interkingdom interactions in disease ecology; see Table 1.2 for examples. All of the available platforms, however, have the same role of sequencing millions of nucleotide fragments in parallel. Several sequencing platforms can be used for NGS application (e.g., Illumina®, PacBio®, Ion Torrent™), but these platforms are constantly changing in depth of coverage, sequence read length, sensitivity, and cost. One may refer to the Advances in Genome Biology and Technology (AGBT) conference for the most up-to-date information about sequencing technologies.

Currently, Illumina® MiSeq® and HiSeq® are two of the most commonly employed platforms for assessing the structure and function of the microbiome. These are

**Table 1.2** Examples of high-throughput genomic sequencing methods and associated potential applications

| Sequencing method[a] | Application(s) |
|---|---|
| Targeted (amplicon based) | Genomics |
| De novo | Genomics |
| Nanopore DNA | Genomics |
| Whole genome | Genomics |
| Exome | Genomics |
| Single molecule real-time (SMRT) | Genomics; transcriptomics |
| Total RNA and mRNA | Transcriptomics |
| Targeted RNA | Transcriptomics |
| Small RNA and noncoding RNA | Transcriptomics |
| Methylation | Epigenomics |
| Chromatin immunoprecipitation (ChIP) | Epigenomics |
| Ribosome profiling | Epigenomics |

[a]From ten Bosch and Grody (2008), de Magalhães et al. (2010), Liu et al. (2012b), Quail et al. (2012)

versatile platforms that can be used in a wide array of applications based on the gene of interest. To assess the variability in the taxonomic microbiota composition, one may use the 16S rRNA gene, as it is the most frequently used gene to study phylogenic relationships among microbial consortia as it allows for surveys of bacteria and archaea. Because of this frequency, 16S rRNA gene databases are among the most developed. Although other, single-copy genes (e.g., ITS, 18S rRNA) have been used for phylogenetic resolution, the use of these genes for the analysis of environmental amplicons is limited by the fact that the degeneracy of protein sequences makes universal primer design difficult, and the databases are not as extensive when compared to 16S rRNA. Furthermore, after the sequencing has been performed, one must take special consideration to the bioinformatics used to analyze these millions of sequences. Due to the tremendous amount of literature available on the variation in analytical pipelines, filtering considerations, clustering approaches, and statistical and mathematical modeling available for analyzing the sequencing data, these topics will not be discussed. It is recommended that the reader explore the information pertinent to their gene(s) of interest and NGS approach. When assessing a disease system, the goal of research (e.g., Do the microbial communities differ? What is the gene expression in the diseased versus healthy state? Can variant sequences be identified?) is key in determining which sequencing platform will be most useful in providing the desired information about their study system.

## 1.5 Synthesis for Disease Ecology

The interactions of organisms of at least two taxa, the minimum disease system proposed in this chapter (Fig. 1.1), are inherently a part of disease ecology. However, the interactions of taxa from multiple domains and kingdoms of life go beyond the pathogen, host, reservoirs, and vectors, likely playing functionally important indirect roles that affect disease system dynamics such as pathogen transmission, vector attack rates, and host and reservoir behavior, among others. While much more detailed and mechanistic studies are needed, the literature to date suggests a broad arena for research into how interkingdom interactions outside of the traditional host-pathogen-reservoir-vector organisms may significantly impact the scientific understanding of infectious disease dynamics. Many of the mechanisms of such interactions are understood, but it is clear that many more pathways likely remain undiscovered in both invertebrate and vertebrate hosts, reservoirs, and vectors. Microbial direct and indirect interactions are currently being studied in many animal systems, as these domains of life clearly affect biological outcomes related to host fitness, population dynamics, and their role in communities and ecosystems. While only a few examples of these important interkingdom interactions in disease ecology are discussed in this chapter, the literature suggests that there are likely many more as part of natural ecosystem networks, within which disease systems reside. The future of scientific inquiry into novel interkingdom interactions and their importance in infectious disease ecology is exciting and promising.

### Compliance with Ethical Standards

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**Conflict of Interest** Mark Benbow declares that he has no conflict of interest. Jennifer Pechal declares that she has no conflict of interest. Jeffery K. Tomberlin declares that he has no conflict of interest. Heather Jordan declares that she has no conflict of interest.

**Ethical Approval** This article does not contain any studies with human participants or animals performed by any of the authors.

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# Chapter 2
# Biodiversity and Disease Transmission


**Serge Morand**

**Abstract** Biodiversity changes associated with the anthropogenic alteration of natural environments have been hypothesized to enhance disease transmission and to facilitate the emergence of infectious diseases. This chapter reviews the various links that may occur between biodiversity and disease transmission on scales ranging from global to local and the likely ecological mechanisms. The consequences of land usage and land cover changes on disease transmission are formulated through the overall effects on biodiversity observed from long-term observatories. Habitat fragmentation should lead to reduced diversity of pathogen species and changes in pathogen prevalence as proposed by the "perturbation hypothesis." However, habitat fragmentation that leads to increased edge, and increasing contacts between different communities of reservoirs and vectors, should increase disease transmission and pathogen prevalence according to the "pathogen pool diversity" hypothesis. Network analyses represent new tools to investigate disease transmission in a changing biodiversity context, i.e., changes in multiple hosts—multiple parasite interactions. Finally, this review advocates for manipulative experiments, theoretical studies, and long-term data collection in ecological observatories that will help in building scenarios of future health.

## 2.1 Introduction

Abiotic factors (i.e., climate and climate variability) and biotic factors (i.e., host population density, host community structure) have been demonstrated as crucial determinants of disease transmission (Anderson and May 1991; Hudson et al. 2002; Morand et al. 2013). Increasing attention has been given on the role of biodiversity as regulating disease transmission, and studies have questioned how biodiversity changes associated with the anthropogenic alteration of natural environments may

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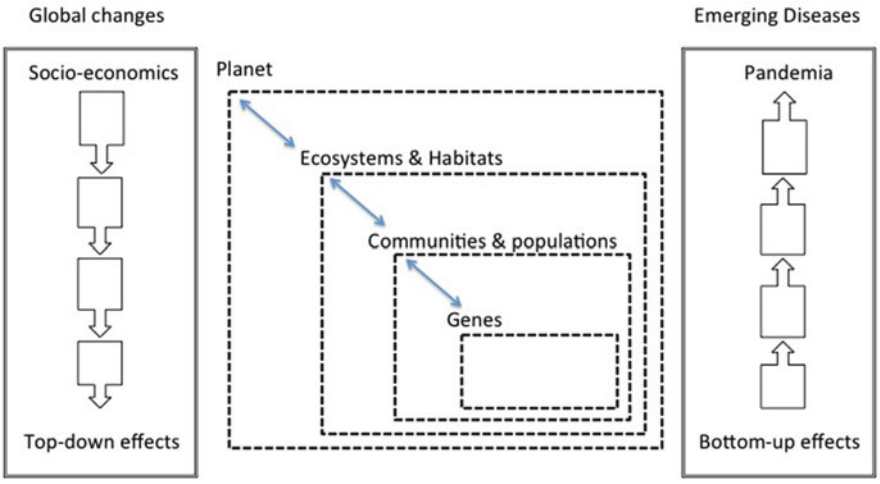


**Fig. 2.1** Top-down effects of global changes on biodiversity with bottom-up effects on infectious diseases

enhance disease transmission and potentially facilitate the emergence of new infectious diseases (Keesing et al. 2010). Human modification of habitats leads to changes in the composition of species communities often associated with a major reduction in biodiversity (Dirzo et al. 2014). Several wildlife-borne or vector-borne diseases exhibit an increasing transmission success in these human-altered environments (LoGiudice et al. 2003). This pattern was popularized as the "dilution effect hypotheses" (Schmidt and Ostfeld 2001), although hot debates challenge the potential role of a rich biodiversity as a buffer for disease transmission (Randolph and Dobson 2012; Lafferty and Wood 2013; Keesing et al. 2015).

A first aim of this chapter is to review the various links that may occur between biodiversity and disease transmission on scales ranging from global to local by presenting patterns and likely mechanisms (Fig. 2.1).

A second aim is to present network analyses as a tool for investigating disease transmission in a "biodiversity" context, i.e., multiple hosts—multiple parasites. Finally, this review advocates for long-term data collection in ecological observatories coupled with theoretical epidemiology that will help in building scenarios of future health.

## 2.2 The Links Between Biological Diversity, Cultural Diversity, and Disease Diversity

Human pathogens are not randomly distributed geographically (Dunn et al. 2010), as their number and diversity increase from higher to lower latitudes (Guernier et al. 2004; Dunn et al. 2010; Bonds et al. 2012; Cashdan 2014; Murray et al. 2015). This

pattern follows a similar trend observed for general biodiversity with increasing richness in bird and mammalian species from temperate to tropical zones (Schipper et al. 2008). Among countries, a positive relationship is observed between bird and mammal species richness and the number of human infectious diseases. A country with high biodiversity, in terms of mammals and birds, has a high diversity of human pathogens (Dunn et al. 2010; Morand et al. 2014c).

Cultural diversity appears to mirror biological diversity as emphasized by Maffi (2005). Fincher and Thornhill (2008) showed that countries with high cultural diversity, using the number of spoken languages as a proxy, have greater diversity of infectious diseases. Hence, biodiversity, cultural diversity, and human pathogens' diversity are entangled in their actual geographical distributions due to evolutionary and ecological processes (Morand 2015a) (Fig. 2.2). These large-scale patterns deserve in-depth investigations at regional and local scales. Hence, rather than to investigate at only country level, it would be worth investigating the potential associations among biodiversity, cultural diversity, and health at ecoregion and ecozone levels (Olson and Dinerstein 1998; Bailey 2014), which will allow our scientific progress to overcome nation-state entities.

However, these patterns are affected by the ongoing dramatic global changes affecting biodiversity, the epidemiological environment and the transmission of many if not all infectious diseases (Daily and Ehrlich 1996).

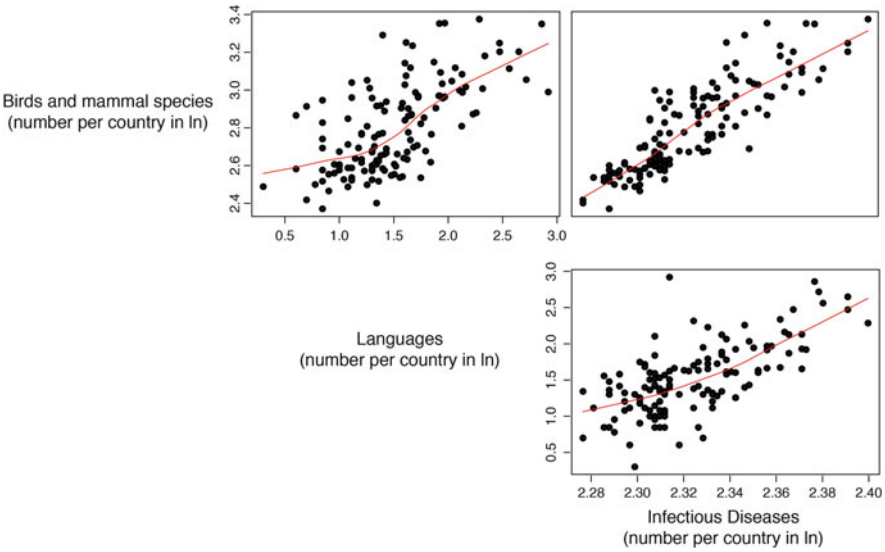


**Fig. 2.2** Cross-correlation among number of bird and mammal species, number of languages, and number of infectious diseases per country (redrawn from Morand et al. 2014c; the data are from Bird Life International (http://birdlife.org/datazone/home), the Ethnologue (https://www.ethnologue.com/), IUCN (http://www.iucnredlist.org/), and GIDEON (Global Infectious Diseases and Epidemiology Network, www.gideononline.com)

## 2.3 Global Changes

Ongoing global changes (climate change, biodiversity loss, land use change, biological invasion) are modifying the epidemiological environment as already mentioned two decades ago by Daily and Ehrlich (1996), with a sharp decrease in the burden of parasitic infection in the developed countries and a still high level in developing countries. Large declines of human parasite burdens and infectious diseases have been observed in developed countries over the last century (Armstrong et al. 1999). However, the last decades have been characterized by a dramatic increase in the number of emerging infectious diseases (Wilcox and Gubler 2005; Jones et al. 2008; Smith et al. 2014). The explanatory factors are generally associated with climate change and climate variability, increased global trade, land use change, overuse of living resources, and biodiversity loss (Chivian and Bernstein 2004; Wilcox and Colwell 2005), which are occurring on an unprecedented scale. Biodiversity loss through altered landscapes due to urbanization and agricultural intensification (Gibbs et al. 2010) appears to be linked to higher disease risks with emergence of novel pathogens resulting from increased contacts between wildlife, domesticated animals, and humans (Lloyd-Smith et al. 2009; Jones et al. 2013; Lindahl and Grace 2015; Hassell et al. 2017). Biodiversity loss also applies to the parasites themselves. Even if more than half of all biodiversity are parasites, when including viruses and pathogenic bacteria, the rate of parasite extinction in the wild is far from being estimated accurately (Dunn et al. 2009, 2010).

What has been observed over the last decades is the increasing number of global outbreaks of infectious diseases with a slight but constant increase of new pathogens worldwide (Fig. 2.3a). A second trend observed is this homogenization of global parasite distribution, which began around 1960 (Smith et al. 2007). Using network analysis, a striking decrease of the modularity of the country-pathogen network is also observed (Poisot et al. 2014), suggesting that outbreaks of infectious diseases are increasingly shared among an increasing number of countries (Fig. 2.3b). Today, an outbreak of a given infectious disease has a greater chance to spread among a larger number of countries due to globalization as countries are highly connected through trades and travels.

These above patterns strongly suggest that global changes are affecting the global epidemiological environment mostly by favoring the spread of infectious diseases among countries and by increasing the risks of pandemics (Tatem and Rogers 2006) (Fig. 2.1).

There is a good correlation between biodiversity and human pathogen diversity. It remains, however, difficult to explain how biodiversity loss can increase newly emergent infectious diseases unless we disentangle the presence of a disease in a country (i.e., its endemicity) and the patterns of epidemics or outbreaks in a country. By analyzing separately the number of diseases and the number of diseases presenting an outbreak, Morand et al. (2014a) were able to show in the Asia-Pacific region that if the number of infectious diseases was related to biodiversity

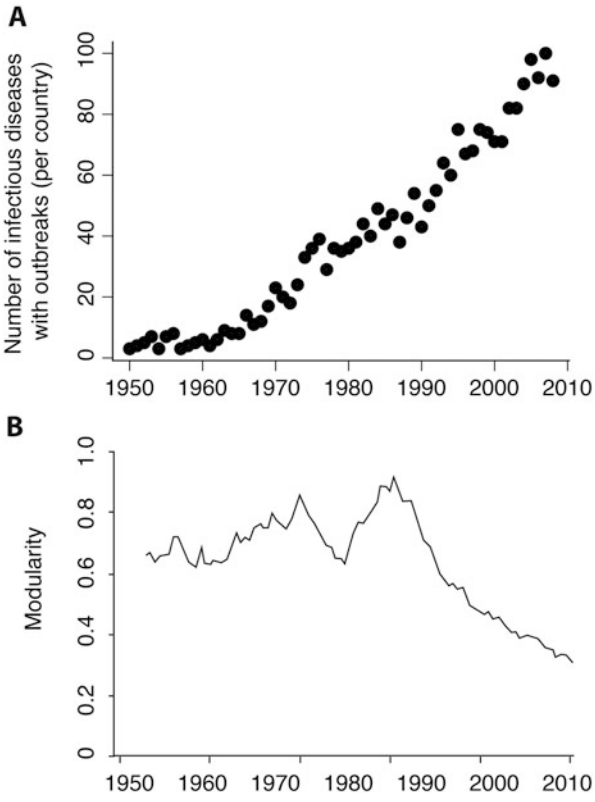


**Fig. 2.3** (**a**) Number of infectious diseases presenting outbreaks globally over the last 60 years (redrawn from Morand et al. 2014c, data from GIDEON (Global Infectious Diseases and Epidemiology Network, www.gideononline.com)). (**b**) Change in values of modularity of country-infectious diseases over time. Modularity is a measure of division of a network into modules (or groups or communities) with a high value of modularity indicating the presence of a high number of modules. The decrease of modularity values since the beginning of the 1960s depicts a global homogenization of infectious diseases (redrawn from Poisot et al. 2014)

among countries, the number of diseases presenting an outbreak was related to biodiversity loss. There was a positive association between the number of zoonotic diseases presenting an outbreak and the number of mammal and bird species at threat (taking into account potential confounding effects such as gross national product, population size, and disease surveillance and surveys). Interestingly, the number of languages at threat was also found positively associated with the number of bird and mammal species at threat (Morand and Lajaunie 2017). Hence, correlative and comparative approaches suggest (1) that biodiversity is a source of pathogen diversity but (2) that biodiversity at threat is a source of epidemics including new emerging zoonotic diseases. This may also apply to disease prevalence. For example, Derne et al. (2011) showed a negative correlation between the number of mammal species and the annual incidence of human leptospirosis among nations and territory islands worldwide. This represents a pattern that should be investigated for more zoonotic diseases. The link between transmission ecology of diseases and biodiversity should be investigated at local level, i.e., remnant habitats, than regional or global levels, i.e., among nations (Johnson et al. 2015).

## 2.4 Habitat Changes

At the larger landscape level, habitat loss and fragmentation generally lead to a significant loss of species because remnant habitats are too small and isolated for species to either persist or to recolonize (Morris 2010). The effect of fragmentation on host-parasite interactions has been examined in relation to conservation medicine and also to emerging diseases by investigating prevalence or species richness of pathogens as well as parasites (Allan et al. 2003; Lo Giudice et al. 2003; Wells et al. 2007; Gillespie and Chapman 2008; Mbora and Mc Peek 2009; Hussain et al. 2013; Fenoglio et al. 2012).

Considering parasite species richness or number of diseases, no clear results have emerged from the literature, although a comparative or meta-analysis study is needed. A recent study conducted in Southeast Asia (Bordes et al. 2015), using an extensive data set on 16 rodent species and 29 helminth species from several localities of Southeast Asia, showed that both the estimated degree of fragmentation and the rate of deforestation through the last 20–30 years have no influence on both parasite and host species richness.

On the other hand, parasite prevalence appears to be higher in fragmented places. A trend confirmed by Brearley et al. (2013), who analyzed 19 studies for changes in disease prevalence to assess the directional influence of human-modified landscapes. Nearly half of the studies examined by Brearley et al. (2013), (53%), indicated an increase in disease prevalence due to human-induced landscape change, while 21% identified a decrease in disease prevalence and 26% found varied disease prevalence (i.e., some disease increased, whereas others not). Studies in urban landscapes tend to show a consistent increase in disease prevalence (see Hassell et al. 2017). Gottdenker et al. (2014) analyzing a larger number of studies showed a similar trend with 56.9% of the studies documented increased pathogen transmission in response to anthropogenic change, and 10.4% of studies observed a decreasing pathogen transmission, although 30.4% had variable and complex pathogen responses (2.4% showed no detectable changes). The pathogen responses to anthropogenic changes should be investigated in relation to the types of land use/land cover changes, which have been rarely done.

Murray and Daszak (2013) proposed two non-exclusive hypotheses for infectious disease emergence due to land use change, which are based on different mechanisms. The first one, the "perturbation hypothesis," assumes that land use change perturbs disease dynamics in multi-host disease systems by disrupting the cross-species transmission rate. The second one, the "pathogen pool hypothesis," assumes that land use change allows exposure of novel hosts to a rich pool of pathogen diversity, which ultimately influences cross-species transmission rate.

The framework developed by Haddad et al. (2015) allows linkage between the consequences of land usage and land cover changes, and specifically habitat fragmentation, on disease transmission through the overall effects on biodiversity (such as reservoirs and vectors) (Fig. 2.4).

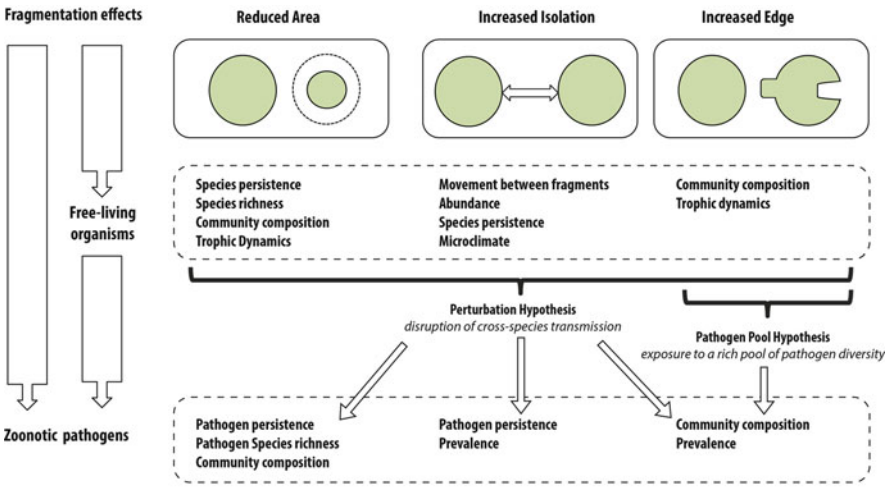


**Fig. 2.4** The framework of Haddad et al. (2015), linking the different aspects of habitat fragmentation on biodiversity, is related to infectious disease emergence through the two hypotheses developed by Murray and Daszak (2013): the "perturbation" hypothesis and the "pathogen pool diversity" hypothesis. The broad arrows on the left of the figure show the consequences of habitat fragmentation on zoonotic pathogens, directly on free-living infectious stages or through effects on free-living organisms (i.e., vectors and reservoirs). Across the top is an indication of how areal reduction, increased isolation, and increased edge can affect the diversity and dynamics of free-living species according to long-term observations (Haddad et al. 2015). Reduced area affects species persistence, species richness, community composition, and trophic dynamics, or increased isolation affects movement between fragments, species abundance, and species persistence as well as microclimate conditions. Increased edge affects mostly community composition and trophic dynamics. Across the bottom, the consequences of habitat changes are related to the "perturbation" hypothesis and the "pathogen pool diversity" hypothesis. The "perturbation" hypothesis could apply to all kinds of areal changes (reduced area, increased isolation, increased edge), whereas the "pathogen pool diversity" hypothesis should apply only to habitat fragmentation leading to increased edge. Reduced area and increased isolation should affect pathogen persistence and prevalence. Increased edge might affect the community composition of pathogens, with an increasing pathogen species richness, and pathogen prevalence

In their review on experimental nature, Haddad et al. (2015) showed that fragmentation consistently reduced species richness and community composition. They also showed that reduced territorial fragment size and increased proportion of edge habitat led to important changes in tree communities' composition, with subsequent impacts on the community composition of animals. Then, whatever the aspects of fragmentation, whether they might be either reduced fragment area, increased isolation, or increased edge, those aspects will have strong impacts on species richness, community composition, species abundance, and movement (Fig. 2.4). The consequences of habitat fragmentation, through its different aspects, can be linked to disease transmission using Murray and Daszak's hypotheses (Fig. 2.4). The

perturbation hypothesis may apply to each aspect of fragmentation and should lead to reduced diversity of pathogen species and changes in pathogen prevalence. The pathogen pool diversity may apply only to fragmentation that leads to increased edge, which should allow increasing contacts between different communities of reservoirs and vectors and ultimately to the increase of disease transmission and pathogen prevalence. Land use/land cover changes and the resulting structure of habitat fragmentation affect the structure of host-pathogen communities, modifying disease transmission. However, the prediction of outcomes, increased or decreased pathogen prevalence, is difficult to assess as several mechanisms at the level of communities and/or populations may play different roles.

## 2.5 Community Changes

### *2.5.1 Dilution Effect*

Elton (1958) pioneered the field of disease transmission and biodiversity with his "disease diversity hypothesis." As quoted by Johnson et al. (2015) Elton emphasized that "outbreaks most often happen on cultivated or planted land. . .that is, in habitats and communities very much simplified by man." According to Elton, biological diversity homogenization should favor outbreaks of diseases. The "dilution effect hypothesis" has been proposed for zoonotic or vector-borne diseases (Schmidt and Ostfeld 2001). This hypothesis suggests that host species richness and diversity act as a buffer to pathogen spread, very similar to the "biotic resistance hypothesis" formulated in biological invasions. Host communities composed of a large ratio of noncompetent host species to competent hosts should contribute to reduced parasite transmission through wasted transmissions, in comparison to host communities with high proportion of competent hosts. The transmission of a pathogen to a noncompetent host creates a dilution with the loss of infective stages, which lowers the prevalence of infection in the competent hosts. If these competent hosts act as reservoirs for human infectious diseases, the dilution effect decreases their transmission risks to humans. This phenomenon has been reported in several vector-borne diseases such as Lyme disease (LoGiudice et al. 2003) or West Nile disease (Swaddle and Calos 2008) but also for rodent-borne diseases such as hantavirus (Carver et al. 2011).

A first meta-analysis has tested the importance of dilution hypothesis in disease transmission (Salkeld et al. 2013). The meta-analysis performed on 13 studies, following their criteria, showed a weak relationship between biodiversity and disease transmission suggesting that the dilution hypothesis for disease transmission was merely idiosyncratic. The review of Johnson et al. (2015) cited 90 studies published between 2012 and mid-2014, and most of them supported the existence of a dilution effect for various diseases affecting humans, wildlife, livestock, or plants. Civitello et al. (2015) conducted another meta-analysis using 61 parasite species, which provided evidence that host diversity inhibits parasite abundance. This abundance inhibition appeared independent of host density, study design, and

type and specialization of parasites. Civitello et al. (2015) argued that a dilution effect was robust, but its magnitude was more related to frequency of focal host species than to their population density.

As a pattern of dilution can be observed in many kinds of transmission, including both direct and indirect such as those using vectors, it is likely that different epidemiological mechanisms can be operating. First, host population densities are a key parameter in parasite and disease transmission with higher host densities linked to both higher parasite and disease prevalence (Anderson and May 1979; Begon et al. 2002) and also to higher parasite species richness (Kamiya et al. 2014; Morand 2015b). Several empirical studies have emphasized the importance of this host parameter in parasite and pathogen transmission. For example, in a critical review, Jonsson et al. (2010) showed that the density of rodent populations appears to be the key factor that correlates with human infections by hantavirus. Werden et al. (2014) demonstrated the importance of rodent host densities, as compared to their diversity, on the prevalence of *Ixodes scapularis* tick nymphs infected with *Borrelia burgdorferi*. By controlling for various confounding factors, Werden et al. (2014) showed that when the relative abundance of mice was high in a site, higher small mammal species richness had little effect on nymph infection. This study suggests that high host diversity does not always reduce the prevalence of parasite infection, but host density of competent host does matter.

### 2.5.2 Invasion

Species invasion offers the opportunity to analyze the epidemiological consequences of changes in host-pathogen communities. The consequences of disease introductions in invaded environments may have dramatic effects on local species and communities, which may affect ecosystem functioning as observed for both myxomatosis and rinderpest (Dobson 1995). Diseases that are introduced either directly or through invasive species are of major concern in conservation biology with ongoing global trade and escalating new threats. As an example, 10 of 64 invasive bird species cause health impacts in Europe (Shirley and Kark 2009).

Invasion can decrease transmission of a native parasite. For instance, Telfer et al. (2005) observed a decline in the prevalence of two intracellular bacteria *Bartonella birtlesii* and *Bartonella taylorii* in native wood mouse populations concurrent with increase in the density of an invading bank vole. This prevalence decline was explained by the reduced infection having been vectored by fleas on wood mice.

### 2.5.3 Trophic Web

Predation on host reservoir species belonging to lower trophic levels may affect disease transmission. Orrock et al. (2011) in a comparative study showed that the prevalence of Sin Nombre virus (SNV) in deer mice was higher on islands harboring

fewer rodent predators. The empirical and theoretical study of Levi et al. (2012) suggested that the increase in Lyme disease in recent years in the Northeastern and Midwestern United States correlates with the decline of a key small mammal predator, the red fox. The wide decline of the red fox was explained by the competitive expansion of coyote populations, which was also found to predict Lyme disease distribution in New York State.

### *2.5.4 Multi-infection*

Parasite interspecific interactions also play a critical role in the dynamics of parasite transmission, whereas most studies have investigated only a single pathogen or parasite (Bordes and Morand 2011). Several studies conducted in rodents (Telfer et al. 2010; Bordes et al. 2015) showed that the very diversity of multi-pathogen infections affect the dynamics of each pathogen leading potentially to the generation of super-shedders (Lass et al. 2013). However, such multi-pathogen studies are still rare, although they would provide a better understanding of the epidemiological dynamics altered by biodiversity loss and habitat alterations.

### *2.5.5 Synanthropic Species*

Several studies on rodent communities in tropical disturbed landscapes have shown a shift from species-rich communities comprised mainly of native species to reduced communities dominated by generalist species, which are often invasive and may be synanthropic (Charles and Ang 2010; McFarlane et al. 2012). Increase in the density of one or more synanthropic species does appear to be a common pattern (McFarlane et al. 2012; Morand et al. 2015). As a result, the proportion of recognized zoonotic diseases is often higher in populations of those generalist rodent species inhabiting areas that are considered to have been either disturbed or urbanized (Suzán et al. 2009; Palma et al. 2012). Blasdell et al. (2011) showed that hantavirus-infected rodents in Southeast Asia primarily were rodent species associated either with settlements or agricultural environments, i.e., synanthropic rodents, and suggested a likely relationship between human-dominated habitats and the increase in rodent-borne disease occurrence. Another study showed that hantavirus prevalence for the same generalist species varied between habitats differing in their level of disturbance (Goodin et al. 2006). Moreover, by carrying and disseminating parasites across multiple habitats, synanthropic species could enhance both host-switching and spillover to other reservoirs and to humans (Bordes et al. 2017).

## 2.6 Investigating Mechanisms Using Network Analyses

Finally, all these studies, and in particular comparative and meta-analyses, plead to "move beyond debates over the generality of the dilution effect and toward a mechanistic, predictive framework for biodiversity–disease interactions" as emphasized by Civitello et al. (2015).

In contrast to ecological networks (i.e., trophic web, plant-pollinator network), host-parasite networks have been recently investigated (Hagen et al. 2012) for several host-parasite systems such as fish-parasite, mammal-flea, primate-pathogen, domestic animal-zoonotic disease, and rodent-helminth networks (Poulin 2010; Vazquez et al. 2005; Morand et al. 2014b; Pilosof et al. 2015) (Fig. 2.5).

Using a large food web data set, Chen et al. (2008) demonstrated that the number of parasite species harbored by a given host species is related to the position of this given host species in the host-parasite network. A host species with high parasite diversity, often characterized by a wide diet range, occupies a network position close to many prey species, which helps at accumulating parasites from species at lower trophic levels.

Network-based approaches have been widely used to study transmission heterogeneity (Bansal et al. 2007; Bogich et al. 2013). In particular, network topology has been investigated to determine pathogen transmission across human and wildlife populations (Gómez et al. 2013). Network topology analysis helps to visualize the overall interactions between hosts and pathogens and to estimate potential host sources of parasites/pathogens. Network centrality is a measure of the connection among each host species in the network. A central host, characterized by high value of centrality in a host-pathogen network, is the one that is infected by many pathogens that infect many other hosts in the network. The centrality of a given host species is a good estimate of its potential to be a source of pathogens to other species (Gómez et al. 2013).

Morand et al. (2014b) investigated the structure of the interactions between human and their domestic animals and the shared diseases. They used centrality, a measure of the connection among each host species (humans and domestic animals) in the network, through the sharing of parasites/pathogens, and they showed that the most central hosts in the network (i.e., high value of centrality) are the ones that are infected by many pathogens that infect many other hosts in the network. Moreover, they showed that these central domesticated hosts in the network, which favor pathogen transmission not only to humans but also to all other domesticated animals, were associated a long time ago with humans.

The application of ecological network theory to the study of host-parasite interactions has identified several patterns in disease transmission such as the existence of a negative correlation between network connectance (or link numbers) and parasite species richness and the likely nested and modular host-parasite networks.

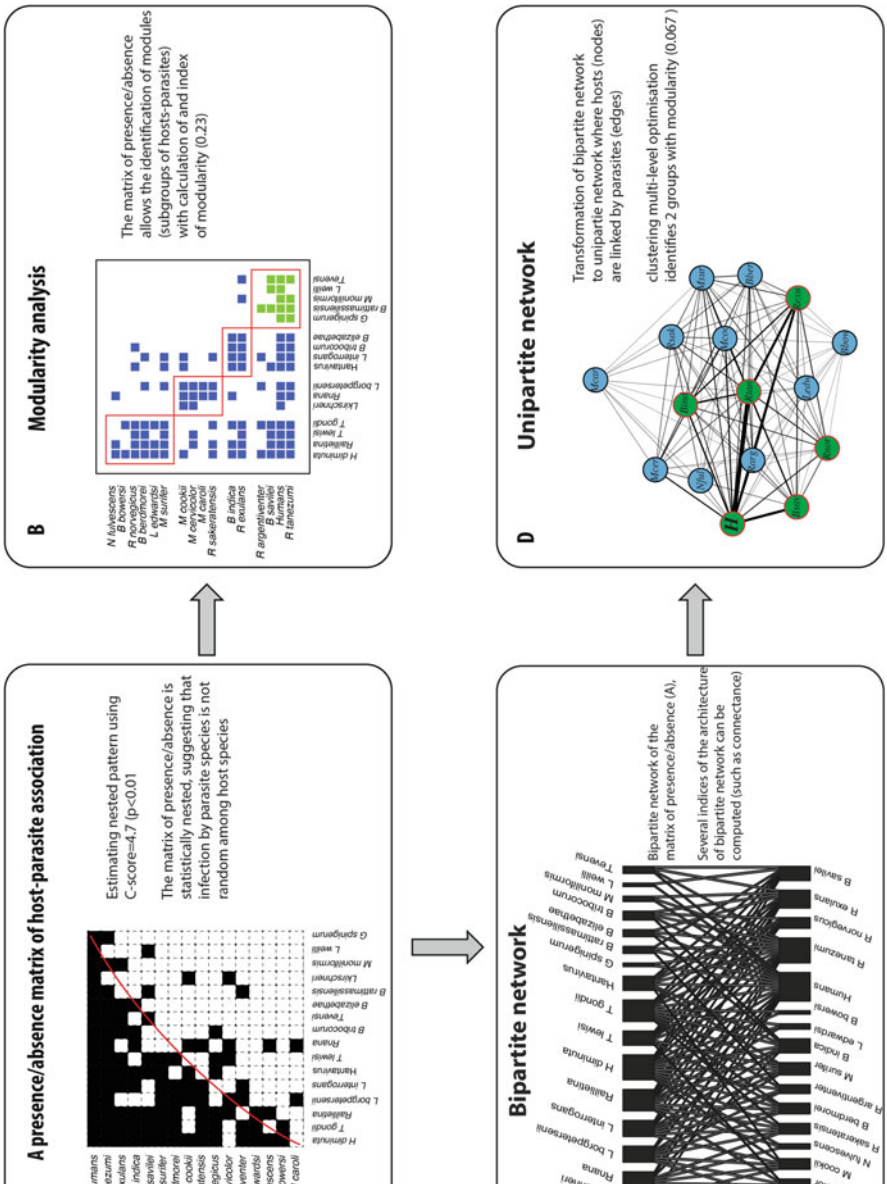


**Fig. 2.5** Example of the use of network analysis. (**a**) Matrix of presence/absence of parasite species among host species and nestedness with the filled spaces representing presence. A nested pattern is depicted as a nesting of a given subset into a larger one. Several measurements of nested pattern are available such as

Bordes et al. (2015), studying an extensive data set on 16 rodent species and 29 helminth species from several localities of Southeast Asia and using network analysis, observed that rapid habitat fragmentation impacted host-parasite interactions as the rodent-helminth network becomes less connected and thus more modular, suggesting that parasite sharing among host species may become more difficult to maintain with the increase of habitat disturbance. That study also suggested it takes time to observe changes in both reservoir and pathogen species richness in responses to habitat changes.

Few studies have tried to link how habitat fragmentation may alter host-pathogen network properties. However, an improved understanding of the properties of network structures could provide insights into the response of interacting host-pathogen communities to anthropogenic disturbances. Using field data acquired from rodent species in various habitats in Southeast Asia, Bordes et al. (2017) investigated networks based on shared parasites, shared pathogens, and shared habitats among rodent species and humans. They investigated the architectures of bipartite and unipartite networks using modularity, subgroup partitioning, and node centrality in order to assess the relative epidemiological importance of particular rodent species (Fig. 2.5). They found that three synanthropic species were found to be members of subgroups including humans in both unipartite and bipartite networks on zoonotic agents and shared habitats. Moreover, these three synanthropic rodent species showed high values of centrality in shared zoonotic agents and high centrality values of shared habitats for two of these rodent species. These three synanthropic rodent species showed high degree of connectivity with humans, and they represent a high risk for direct zoonotic spillover. Moreover, when a rodent species is central in habitat-sharing, it may also play a key role as a bridge host for pathogen spread among habitats. Finally, these rodent species identified using the network approach are some of the potential reservoir hosts for new emerging arenaviruses in similar disturbed environment of Southeast Asia (Blasdell et al. 2016).

**Fig. 2.5** (continued) C-score, or checkerboard score, which gives the randomness' statistics of the distribution of parasite presence among host species in the matrix of presence/absence (a low C-score indicates a high randomness). (**b**) Matrix of presence/absence of parasite species among host species and modularity. Modularity anlysis can help to depict modules, i.e., occurrences where there are more connected hosts and parasites in a subgroup relative to other subgroups. These modules can also be depicted in a bipartite network. (**c**) Bipartite network showing topology of (**a**) the matrix of presence/absence of parasite species among host species. Calculation of several indices, such as connectance and modularity, allow the investigation of the bipartite network architecture. (**d**) Transformation of topology from a bipartite network to a unipartite network, where the links (shared parasites) among nodes (host species) of unipartite networks depict shared parasites among hosts. Modularity analysis is used to identify subgroups of hosts that shared more parasites among them than among other subgroups. Other indices can be used such as centrality

## 2.7 Conclusion: Disease Ecology in the Anthropocene Defaunation

Anthropogenic factors have been identified as drivers of emerging diseases and have been found to favor the persistence and transmission of certain parasites (Rohr et al. 2011; Morand et al. 2014a, b, c).

The importance of human-dominated habitats in disease spread is of major concern as the risk of disease spread should be higher in human-dominated habitats. The ongoing "Anthropocene defaunation" is leading to empty tropical forests at least of large-bodied species (Dirzo et al. 2014). The sharp decline in abundance observed in many populations should have implications for zoonotic diseases by both decreasing and increasing risks. Pathogen diversity will decrease with their host diversity, but the prevalence of remaining pathogens specially those using synanthropic species as reservoirs or bridge species will increase. Indeed, rodents and small mammals in general will be the dominant mammal clades found not only in tropical forests but also in almost all human-modified environments. This may result in an increase in pathogen spillover into humans as already observed in Southeast Asian environments with critical ongoing defaunation.

The next step in research should better integrate field manipulative experiments (Suzán et al. 2009) and theoretical studies (Mihaljevic et al. 2014; White et al. 2015). Indeed, Gottdenker et al. (2014) emphasized that few studies were experimental in nature (relative to reviews). A second step is to build scenarios of future health in the face of biodiversity loss, and toward this goal we need long-term data collection such as the NEON (National Ecological Observatory Network) initiative (Springer et al. 2016), which will allow us to better explore disease transmission in relation to global and biodiversity changes.

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### Compliance with Ethical Standards

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**Conflict of Interest** Serge Morand declares that he has no conflict of interest.

**Ethical Approval** This article does not contain any studies with human participants or animals performed by the author.

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# Part II
# The Ecology of Infectious Diseases Affecting Humans

# Chapter 3
# Understanding and Estimating the Risk of Waterborne Infectious Disease Associated with Drinking Water


**Christon J. Hurst**

**Abstract** This chapter presents an understanding of how drinking water treatment reduces the risk of communitywide infectious disease. The presentation begins with data that demonstrate how a reduction of the typhoid risk in American cities paralleled the progressive implementation of filtration technology at the start of the twentieth century. Typhoid, which is caused by the bacteria *Salmonella enterica* serovar Typhi, is one of numerous infectious diseases associated with the presence of microbial contaminants in drinking water. Drinking water may serve as a primary infection route for these pathogens and the pathogens often can be transmitted within human populations by other, secondary routes. A compartment disease transmission model is presented to illustrate how the use of treatment technologies both to prevent primary acquisition of infections from drinking water and to prevent secondary spread of those infections within the community can effectively reduce the extent of a waterborne infectious disease outbreak. Additionally, a risk estimation technique is presented which has been demonstrated to accurately calculate the probability of community gastrointestinal illness in human populations based upon the numbers of pathogenic bacteria, protozoa and virus in drinking water.

## 3.1 Introduction

*Permalum bibit cave* (Beware of bad water!). A reduction of the typhoid risk in United States cities at the start of the twentieth century paralleled the progressive implementation of community drinking water filtration technology. From 1900 through 1913, as the percentage of city population served by water filtration utilities increased from 8.7% to 48%, the annual typhoid death rates in those same cities decreased from 36 to 16 per 100,000 population (Johnson 1916). Typhoid death rates

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in the United States continued that downward trend, falling to 5.3 per 100,000 population in 1921 (Editorial 1923). By 1960, the typhoid death rate in the United States had been reduced to less than 1 per 100,000 (Centers for Disease Control and Prevention 2018a). Eventually, by 2006 the incidence of typhoid in the United States had fallen to only a few hundred cases per year, and those often were associated with travelers to South Asian (Lynch et al. 2009). Drinking water is only one of many sources from which typhoid, caused by the bacteria *Salmonella enterica* serovar Typhi, and numerous other infectious diseases typically associated with the presence of microbial contaminants in drinking water can be aquired. Of the four diseases which may cause the greatest risk of suffering and death in underdeveloped parts of the world: cholera, hepatitis, malaria and typhoid, malaria is the only one that is not transmitted by ingestion of water. Infection with the water-transmissible *Orthohepevirus orthohepevirus A*, formerly called Hepatitis E virus, is particularly onerous because it causes a high incidence of death in pregnant women (Khuroo 1991; Ramalingaswami and Purcell 1988).

Not all community-distribution water supplies are treated. Often, this represents either a matter of cost or else an avoidance of disinfected water because of taste preference. At other times, the lack of community drinking water treatment may result from a mistaken assumption that water from streams, wells, or springs is by nature intrinsically pure and so water from these sources can be consumed without treatment. Even when treated drinking water is available through community distribution systems, people still may prefer to drink water obtained from untreated sources such as springs or wells. Community drinking water treatment and plumbing distribution networks are not foolproof systems and the supplied drinking water often does contain pathogenic microorganisms for which the associated health risks will be examined in this chapter. Unfortunately, the popular alternative of consuming bottled water instead of community-supplied water based upon the idea of safety may not always be a good choice from a health standpoint since commercially bottled water can contain pathogens. The current and very dangerous natural health food fad of intentionally consuming "Raw water" is a mistake which results from the philosophy that any presented untreated natural water somehow is more pure and healthful than is treated water, and that treatment removes the natural healthfulness of the water just as treatment may remove vitamins from processed food. The presumption that all things natural are more healthy than are anythings processed often is not valid. Water from all sources can be contaminated with pathogenic microorganisms and the consumption of contaminated water will produce waterborne disease.

Most of the microorganisms present in aquatic environments seem to have no effect on human health. However, some clearly do represent a public health risk and for this reason are considered to be disease causing and are referred to as pathogens. The true definition of the term "pathogen" is "something that causes pathological changes" in the appearance of cells and tissues. Often the "pathogen" is the entire organism viewed as an infectious agent. In other cases, however, the pathological change is due to a toxic substance produced by a noninfectious organism, and some toxins, such as those produced by algae and cyanobacteria, can bioaccumulate

within the food chain to extremely high concentrations. This chapter addresses organisms which are infectious for humans, and while some of these represent aquatic contaminants that come from either human or animal reservoirs, others represent organisms which are naturally present in the environment.

Drinking water treatment can be performed with varying degrees of effectiveness on a household basis. Household treatments include heating water to a temperature that is below the boiling point but high enough to destroy the microbial contaminants, heating water still higher to achieve the boiling point, and perhaps even using distillation. Unintentional water treatment is a side benefit from heating and boiling water for the purpose of preparing foods and beverages. Household cartridge filtration systems can effectively remove microbial pathogens from water. Laboratory evaluation has indicated that the practice of using clay as a coagulant for treating water on a household basis may have some success (Lund and Nissen 1986). Chemical coagulants such as alum also can have some degree of effectiveness in the treatment of drinking water (Ahmad et al. 1984). Powdered plant materials also have been evaluated as coagulants that could be used on a household basis both alone and in conjunction with other chemicals. Chemical disinfectants which can be used effectively to treat water on a small scale include chlorine and iodine. Chemical coagulants can be used in conjunction with chlorine compounds, and the later type of combined mixture is commercially available as tablets.

Two additional concepts need to be mentioned before delving further into this chapter. The first concept is the idea of primary versus secondary transmission. Primary transmission represents the initial introduction of an infectious agent into a group of susceptible individuals. Secondary transmission represents the subsequent transfer of the infectious agent from the initially infected individuals to other members of the group. Primary and secondary transmission of a disease can occur by different routes. An example used in this chapter is the disease typhoid, which often has water as the vehicle of primary transmission into a population, and for which food contaminated by handlers may then serve as a vehicle of secondary transmission within the population. The second concept is prevention of disease, which is the hallmark of public health. Prevention of infectious disease can be divided into three separate phases, termed primary, secondary, and tertiary. Primary prevention represents preclusion of disease either by reducing the exposure of new susceptible "host" individuals to the disease agent or by altering susceptibility of the host. Secondary prevention consists of early detection of the disease which hopefully leads to successful early treatment. Tertiary treatment is aimed at minimizing and hopefully eliminating long-term impairments and disabilities, and sometimes extending an individuals survival. This chapter will consider primary and secondary prevention.

## 3.2 Disease Transmission Routes

For the purpose of this chapter, the term ‘disease transmission’ refers to infectious disease. There are many mechanisms, both environmental and non-environmental, by which pathogenic microorganism are transmitted and these are termed disease transmission routes. This chapter focuses on water as a source of microbial pathogens that cause infectious disease, but many infectious diseases are aquired by direct physical contact with either a human or animal harboring an infectious agent, and other transmission routes exist as well.

Disease transmission routes can be divided into two broad categories, vertical and horizontal. Vertical disease transmission, which is passage of an infection from a mother to her embryos and offspring either prior to or during birth, is considered to represent a form of transmission by direct contact. Horizontal transmission of disease can also occur via direct contact mechanisms and encompasses those organisms transferred in saliva, transplanted body tissues, blood and blood products. The touching of contaminated skin and wounds can also result in transmission by direct contact. As such, the category of transmission by direct contact includes infections acquired from sexual partners among which are those caused by pathogens carried in semen. Included in the latter category are bacterial diseases such as syphilis, viral diseases such as hepatitis B and herpes simplex, and the chlamydial disease caused by *Chlamydia trachomatis*. For the purpose of this chapter, diseases transmitted by bites from humans, vertebrate animals, and arthropods, are also considered to represent transmission by direct physical contact.

Examples of illnesses transmitted from vertebrate animals to humans by direct physical contact with contamination on the animals body tissues including skin are the bacterial disease salmonellosis and the viral disease caused by simian immunodeficiency virus. Rabies is an example of a disease whose transmission results from animals biting humans. Diseases that can be transmitted from animals to humans are called zoonoses and animals are the natural host for those causative microorganisms. Humans usually serve only as incidental hosts in zoonoses and the humans are non-essential for the normal cycle of transmission.

The large number of diseases transmitted via arthropod vectors often involve chains of transmission that include physical contact with either humans or other vertebrates as the reservoir, invertebrates serving as the vector, and humans being the susceptible host. A variety of insects are known to serve as arthropod vectors and some of the causative microorganisms are capable of replicating in their vectors. Chiggers, which are perhaps the best known of the mite family Trombiculidae, are capable of transmitting the rickettsial disease scrub typhus. Fleas are perhaps most notorious because they transmit the bacterial disease plague. Biting flies can transmit bacterial diseases such as tularemia. Mosquitos are responsible for the transmission of many viral diseases such as dengue and encephalitis. Ticks are associated with the transmission of bacterial illnesses such as Lyme disease and tularemia, as well as the viral disease caused by tick-borne encephalitis virus. Flies which do not bite also may be associated with the transmission of gastrointestinal diseases as will be

discussed later in this chapter. In these latter instances, the flies serve only as passive carriers or vehicles when they transfer microorganisms from feces to food.

As alluded to above, many viral diseases which some people consider to be directly transmitted are in fact normally transmitted through the environment. These include rotaviral gastroenteritis, smallpox, and those so-called "childhood diseases" known as chickenpox, measles, and mumps.

### *3.2.1 Disease Transmission by Environmental Water Routes*

Water serves as the vehicle for a large proportion of environmentally transmitted disease. Bacterial diseases spread by this route include the notorious typhoid (Johnson 1916; Kehr and Butterfield 1943) and cholera. In particular, outbreaks of cholera often take the form of massive, wide-ranging epidemics, termed pandemics, which have caused fear in human populations since at least 1817. A large cholera pandemic which occurred in the Americas resulted when the causative bacteria perhaps arrived there from another part of the world in contaminated ship ballast water (McCarthy and Khambaty 1994). That pandemic resulted in approximately 350,000 cases of illness and nearly 4,000 deaths (Swerdlow and Ries 1992, 1993). An even larger cholera outbreak occurred in Haiti with the pathogen having been introduced there by international aid workers from Nepal (Transnational Development Clinic 2013). These two bacterial illnesses, cholera and typhoid, were the driving force behind the development of formal drinking water treatment utilities during the middle and end of the nineteenth century. Yet, even as we now are well into the twenty-first century, we have not escaped the historical fact that disease microorganisms consumed in conventionally treated drinking water may account for 35% of the gastrointestinal illnesses among consumers even in developed countries (Payment et al. 1991).

Public health emphasis is often placed on diseases associated with the ingestion of water, since this route of transmission has resulted in massive outbreaks. However, it is important to remember that ingesting water is not the only way in which we acquire diseases that are transmitted via contaminated water. We also acquire diseases from occupational and recreational activities performed in water, as well as from the consumption of water-contaminated shellfish and food crops, and from inhalation of water aerosols. Figure 3.1 represents water-related environmental routes by which infectious agents are transmitted to susceptible individuals. The animal reservoirs would include cows as a source of cryptosporidiosis, and rodents as a source of both giardiasis and campylosis. Table 3.1 Lists some examples of infectious diseases associated with waterborne pathogens. Drinking water often may serve as a primary infection route for these pathogens and the pathogens often can be transmitted within human populations by other, secondary routes. This chapter addresses waterborne infections affecting humans. But, it is important to remember that waterborne infections also affect other animal species including those upon which we rely as livestock, and the pathogens causing those infections would flow through the same pathways as shown in Fig. 3.1.

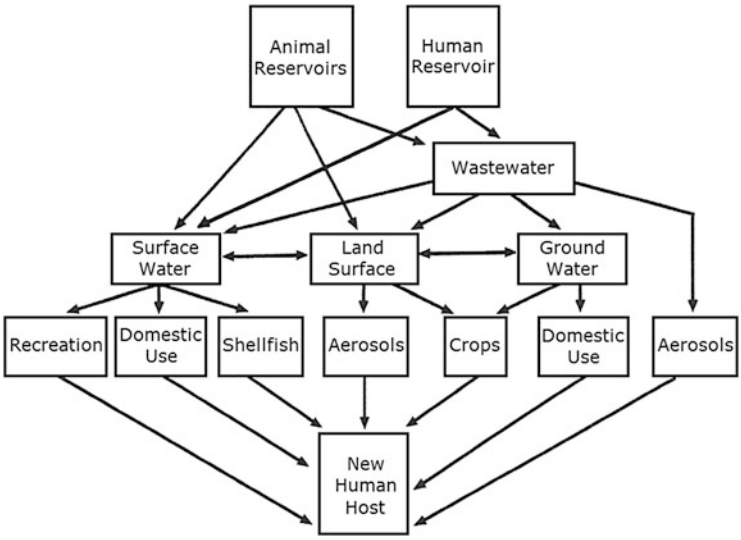


**Fig. 3.1** Water-related environmental routes by which infectious agents are transmitted to susceptible individuals. The animal reservoirs symbolically would include cows as a source of cryptosporidiosis, and rodents as a source of both giardiasis and campylosis

#### 3.2.1.1 Animal Reservoirs of Waterborne Microbial Contaminants

Continuing our examination of Fig. 3.1, we notice that animals can be an important source of microbial contaminants in surface water via two different pathways. Generally we consider vertebrate animals in this regard. The first of these pathways is fecal material and urine that animals deposit directly into the water. The second pathway represents organisms in fecal material and urine which are deposited on the land and then subsequently washed into surface waters by overland flow. Surface flow also carries its load of contaminants into groundwater via percolation through the soil and subsurface matrixes, and also water flows from the land surface into subterranean environments through geologic structures such as caves and sinkholes.

Those waterborne bacterial diseases of humans which may originate from animal reservoirs include many that are caused by microbes whose reservoirs are mammals, including campylosis contributed by not only beavers but also migratory birds, muskrats, and other rodents, and leptospirosis contributed by livestock. In humans, examples of water-associated protozoa are those microorganisms responsible for causing giardiasis and cryptosporidiosis. These protozoans are capable of cross infecting a variety of animals (Plutzer et al. 2018) including beavers and livestock, with a result that those animals may serve as reservoirs and transmit the diseases to humans via surface waters. Reoviruses and rotaviruses, which cause waterborne gastroenteritis, can cross infect humans and a variety of animals including cattle. Thus, not only wildlife but also infected livestock living in watershed areas could be reservoirs of waterborne viral gastroenteritis. Additional microbial contaminants can

**Table 3.1** Examples of infectious diseases associated with waterborne pathogens

| Exposure route | Pathogen group | Type of disease | Causative microorganism(s)[a] |
|---|---|---|---|
| Ingestion (includes contaminated drinking water and other beverages plus ice and water-associated contamination of foods) | | | |
| | Bacterial | | |
| | | Enteric fever | *Salmonella* (especially *Salmonella enterica* subsp. enterica serovar Typhi, which causes typhoid fever) |
| | | Enteritis | *Campylobacter*, *Shigella* (causes bacterial dysentery), *Vibrio* (especially *V. cholerae*, which causes cholera) |
| | | Febrile syndrome | *Francisella tularensis* |
| | | Septicemia | *Vibrio vulnificus* |
| | Protozoan | | |
| | | Enteritis | *Cryptosporidium parvum*, *Entamoeba histolytica* (causes amebic dysentery), *Giardia intestinalis*(includes the formerly named *Giardia lamblia*) |
| | Viral | | |
| | | Encephalitits | *Enterovirus* |
| | | Gastroenteritis | *Alphacoronavirus*, *Mamastrovirus*, *Norovirus*, *Rotavirus*, *Vesivirus* |
| | | Hepatitis | *Vesivirus*, *Hepatovirus* |
| | | Meningitis | *Enterovirus* |
| Body Surface Contact (usually associated with either recreational or aquatic occupational activities) | | | |
| | Bacterial | | |
| | | Enteritis | *Vibrio cholerae* |
| | | Nephritis | *Leptospira interrogans* |
| | | Wound infections | *Vibrio parahemolyticus*, *Vibrio vulnificus* |
| | Metazoan | | |
| | | Worm infestation | *Schistosoma* |
| | Protozoan | | |
| | | Encephalitis | *Naegleria* |
| | | Enteritis | *Entamoeba histolytica* |

(continued)

**Table 3.1** (continued)

| Exposure route | Pathogen group | Type of disease | Causative microorganism(s)[a] |
|---|---|---|---|
| | Viral | | |
| | | Encephalitis | *Enterovirus* |
| | | Gastroenteritis | *Alphacoronavirus*, *Mamastrovirus*, *Norovirus*, *Rotavirus*, *Vesivirus* |
| | | Meningitis | *Enterovirus* |
| | | Pharyngocon-junctival fever | *Mastadenovirus* |
| Inhalation | | | |
| | Bacterial | | |
| | | Pneumonic fever | *Legionella pneumophila* |

[a]If an organism is indicated by both its genus and species names, then the disease association is with that particular species. If only a genus name is given, then the disease association is with more than one species belonging to that genus

come from other warm blooded animals such as birds, as well as from cold blooded animals like reptiles and fish.

#### 3.2.1.2 Human Reservoirs of Waterborne Microbial Contaminants

We humans carry microorganisms both on and within our bodies, and we leave a trail of them behind us. Those microorganisms that cause intestinal illnesses are of greatest concern with regard to infections caused by ingestion of water. Humans can become infected by these same organisms from sources other than water, such as food. Because these organisms are naturally present in feces they tend to be transmitted by the fecal oral route, meaning that infection is acquired either through oral contact with or ingestion of fecally contaminated materials. The subsequent excretion of these organisms in human fecal wastes represents a reservoir in the cycle of waterborne disease transmission, as also represented in Fig. 3.1. The existance of these organisms of human origin as contaminants in wastewater is a factor that holds true for all categories of microorganisms, including bacteria (Amos et al. 2014) and virus (Melnick 1947; Melnick et al. 1954; Paul et al. 1940). Discharge of wastewater into the environment then results in microbial contamination of environmental waters that may subsequently be used as sources of potable water. Drinking water from these sources thereby completes the cycle of infection, from humans to water, and back to humans. While it is the microorganisms that are shed in human fecal material which make wastewater a major source of microbial contaminants, additional animals such as rats which dwell in sewerage collection systems will directly contribute contaminants to the wastewater. Humans also contribute microbial contaminants directly to water during the course of recreational activities.

Inhalation of water aerosols represents another route of exposure to microbial contaminants contained in wastewater. Liquid (droplet) aerosols notably are generated during wastewater aeration and also during the spray application of wastewater including sludge suspensions onto land. It is possible that aerosols generated during wastewater treatment might serve as a source of disease in wastewater workers.

#### 3.2.1.3 The Fate of Microbial Contaminants in Water

The ability of microbial contaminants to survive after they enter water is a major issue with regard to waterborne infectious disease. Microbial contaminants that are released into water will die-off if they are unable to replicate in that environment. The results of mathematical analyses have revealed that water temperature is a major factor affecting the survival of microorganisms in water, with survival increasing at lower temperatures (Hurst 1991; Hurst et al. 1992; Kehr and Butterfield 1943). Other important factors, at least from the standpoint of virus, are: the amount of nutrients available in the water which could support growth of bacterial organisms, generally exhibiting a detrimental effect upon viral survival; the level of turbidity in the water, with higher turbidity generally being beneficial to viral survival; and water hardness, likewise generally having a beneficial effect upon viral survival (Hurst 1991).

#### 3.2.1.4 Aquatic Microbes as a Reservoir of Waterborne Microbial Contaminants

There are microbes which naturally reside in water and are pathogenic for mammals. Perhaps most notably among these are *Legionella pneumophila*, which exists as an infection within free living amoeba and typically is acquired by inhalation, and *Vibrio cholerae* which resides on the chitinous shells of microcrustaceans and macrocrustaceans and typically is acquired by ingestion. The natural aquatic existance of *Vibrio cholerae* may be one of the reasons why the disease cholera can exist as an endemic focus even in areas which do not experience outbreaks of the disease (Eichold et al. 1993; Weissman et al. 1974).

But, in addition to *Legionella* and *Vibrio*, there are a large number of other bacterial genera which contain member species that naturally are found in water. Some of these species are either pathogenic or opportunistically pathogenic for mammals including humans, and the list of these genera includes: *Achromobacter*, *Acinetobacter*, *Actinobaculum*, *Aeromonas*, *Afipia*, *Alcaligenes*, *Burkholderia*, *Chromobacterium*, *Chryseobacterium*, *Citrobacter*, *Clostridioides*, *Clostridium*, *Corynebacterium*, *Edwardsiella*, *Enterobacter*, *Enterococcus*, *Eubacterium*, *Ewingella*, *Hafnia*, *Helicobacter*, *Histophilus*, *Klebsiella*, *Kytococcus*, *Leclercia*, *Lelliottia*, *Leptospira*, *Mannheimia*, *Morganella*, *Mycobacterium*, *Mycoplasma*, *Neorickettsia*, *Plesiomonas*, *Proteus*, *Providencia*, *Pseudomonas*, *Rahnella*, *Rhodococcus*, *Serratia*, *Shewanella*, *Shigella*, *Sphingobacterium*, *Sphingomonas*, *Stenotrophomonas*, *Streptococcus*, *Tsukamurella*, and *Yersinia*. Information on the

ecology of these bacteria can be found in the chapter "Opportunistic Bacteria Associated with Mammalian Livestock Disease" (Hurst 2018).

Aside from the bacteria, there also are a large number other microbial genera which contain species that naturally are found in water and similarly are either pathogenic or opportunistically pathogenic for mammals including humans. These include the fungi genera *Hortaea*, and *Verruconis*, the oomycete genus *Pythium*, and the Ichthyosporea genus *Rhinosporidium*. Information on the ecology of these genera can be found in Hurst (2016b). Additionally, there also are many groups of aquatic protozoa that infect mammals including humans.

#### 3.2.1.5 The Interconnected Environmental Flow of Water and Its Microbial Contaminants

Microbes may not stay where we place them! Microbial contaminants are not necessarily stationary within the environment and the interconnected flow of water along with its microbial contaminants, as diagrammed in Fig. 3.1, causes microbial contaminants to end up in places that we may not have intended. Wastewater and the pathogens which it contains often are disposed of by direct discharge into surface water, and once there, these contaminants in surface water are joined by microbial contaminants that initially may have been placed onto the land surface. Land disposed microbial contaminants include those contained in wastewater used for irrigation. And, we also must remember that microbial contaminants present in fecal solids can end up on the land surface either by having been directly excreted onto the land by animals including humans or by fecal solids having been applied onto the land surface intentionally as fertilizer. The collected solids from wastewater often just simply are discarded onto land with a hope that the solids then will be forgotten and either naturally or magically disappear, at which point a new doctoral student may get assigned the smelly task of studying the viruses contained in those solids (Hurst et al. 1978)! But, nothing applied to the land surface is assured of remaining there. If by no other means, then rainwater, melting snow and ice, can wash the land surface applied material into surface waters. Importantly, contaminated surface applied water can move its contaminants into the subsurface where they join with existing groundwater. In addition to the natural process of surface water percolating into and recharging groundwater aquifers, wastewater also can carry its contaminants into groundwater more directly via either engineered infiltration or direct injection with both of those processes often done for the purpose of recharging aquifers. Groundwater with its contaminants flows toward and often discharges into surface water. Groundwater also can return its contaminants to the land surface through seeps and springs. Surface water can return its contaminants to the land surface very dramatically and, at the particular moment while I am writing this chapter, the Ohio River and its tributary the Little Miami River here in southwestern Ohio, are flooding and doing exactly that! Humans may subsequently encounter these repositioned pathogens either by contact with, or consumption of, contaminated surface waters and ground waters. When surface waters return to the land

surface during floods there additionally is attendant concern about mosquito transmitted illnesses (Anders et al. 1994; Atchison et al. 1993; Cotton 1993), because flooding increases water surface area, which in turn supports the life cycle of mosquitos.

Another major issue with surface water is that the natural transport of microbial contaminants during the course of surface water flow may convey microorganisms over long distances, taking the microbes far from visible sources of contamination (Dahling and Safferman 1979). Interestingly, long distance aquatic transport of microbes also may be aided by human activities, with one possible example having been the transport of causative microbial agents of waterborne disease between different parts of the world as contaminants of ship ballast water (McCarthy and Khambaty 1994).

#### 3.2.1.5.1 Diseases Acquired from Microbially Contaminated Surface Water

The domestic use of contaminated water clearly results in disease transmission (Craun 2012; Hurst 2003) and it is possible to find microbial contaminants associated with water taps even when the water lines feeding the taps apparently are free of the suspect organism. This latter type of contamination has been described by Grundmann et al. (1993). The most commonly suspected mode of transmission associated with domestic water use is through ingestion of the contaminated water. Bacterial diseases acquired in this way include campylosis, cholera (NSF International 2011), typhoid (Johnson 1916), and tularemia (Stewart 1991). Viral diseases acquired from drinking water include diarrhea, hepatitis and poliomyelitis (Mosley 1966). Recently, the most notable protozoan diseases acquired by the ingestion of water seem to be cryptosporidiosis and giardiasis (Baldursson and Karanis 2011; Efstratiou et al. 2017; Mahmoudi et al. 2017; Rosado-García et al. 2017). Another interesting route by which diseases can be acquired from tap water is through the use of contaminated water when washing wounds (Lowry et al. 1991). Prevention of these diseases is the reason that we practice drinking water treatment at community levels.

Participation in recreational surface water activities is frequently associated with the possibility for acquisition of infectious disease caused by bacteria (Leonard et al. 2015, 2018a, b, c). Protozoan contaminants contributed in this way include the causative agents of cryptosporidiosis and giardiasis. Such disease can be contracted even from water which has no known sewage input (Sorvillo et al. 1992), suggesting that humans themselves contaminate water through the course of recreational activities. Viral contaminants contributed to water during recreational activities include causative agents of gastroenteritis and pharyngoconjunctival fever. These health problems can result from microbial pollution of both swimming pools and beaches.

Ingestion of shellfish harvested from waters that have been contaminated by discharged wastewater represents another means through which people acquire microbial illnesses from polluted water. Crustaceans can accumulate pathogenic microorganisms from the environment and then potentially pass these organisms onward to humans who consume the crustaceans. Molluscs are divided into two

categories. The first is bivalves, such as oysters and hardshell clams. The second group is gastropods, such as conch. Human pathogenic viruses, such as the species *hepatovirus A* (formerly called hepatitis A virus) of the genus *Hepatovirus*, can be detected in bivalve molluscs, and the ingestion of contaminated bivalves has caused outbreaks not only of viral disease but also of bacterial diseases such as vibriosis (Centers for Disease Control and Prevention 2018c). It is presumed that bivalve molluscs accumulate these microbial contaminants during the natural process called filter feeding. Gastropod molluscs do not filter feed, and thus would not be expected to accumulate pathogens by this same mechanism.

#### 3.2.1.5.2 Diseases Acquired from Microbially Contaminated Land Surfaces

Wastewater is sometimes used for the irrigation of vegetable crops. As mentioned previously, this practice presents a health hazard, because it may contaminate those crops with potentially pathogenic microorganisms that have been shed in feces. Human consumption of crops that were irrigated with wastewater has been implicated in the spread of cholera and it may prove possible to ameliorate this problem by disinfecting sewage effluents that are intended for agricultural usage. Particulate aerosols may be generated from wastewater sludges that have been applied onto land surfaces either for the purpose of composting that sludge or for final sludge disposal. These aerosols present a pathogenic hazard because they can contain both bacterial and fungal contaminants and may be transported by the wind.

#### 3.2.1.5.3 Diseases Acquired from Microbially Contaminated Ground Water

It is often thought that ground water is intrinsically pristine and pure, but groundwater can be contaminated with microorganisms that are pathogenic for humans. Domestic use of contaminated ground water has resulted in at least one outbreak of the bacterial illness cholera (Blake et al. 1977). Ingestion of contaminated ground water also has resulted in outbreaks of the protozoan illnesses cryptosporidiosis and giardiasis and the viral illnesses gastroenteritis and hepatitis. A heavy reliance is often placed on the use of groundwater for crop irrigation and this reliance might result in the microbial contamination of crops.

The above diseases, along with a number of others transmitted through environmental routes associated with contaminated water, are summarized in Table 3.1 along with the names of the causative microorganisms.

### 3.2.2 *Microbial Contaminants Get Transferred Around the House, Health Care Settings, and Even in Space Travel Environments*

Microbial contaminants can be transferred from one type of contaminated material to another creating a cycle of contamination. An interesting example of this is provided by an outbreak of giardiasis for which it appears that raw food initially became contaminated by washing it in tap water, after which the contaminated food was chopped on a cutting board. Other foods appear to then have become contaminated when they were later prepared on that same cutting board, which had never adequately been washed (Grabowski et al. 1989). Using microbially safe water to wash the initial raw food would have prevented the contamination process. Adequate washing of the cutting board to physically remove contaminating microorganisms using microbially safe water, or even disinfection of the cutting board, would have broken the cycle of contamination. Households are in fact broadly contaminated with fecal microbes (NSF International 2011). An edited volume in this series (Hurst 2017) recently addressed modeling the transmission and prevention of infectious disease in a variety of environmental settings, including those which are terrestrial and others which are orbital, along with an understanding of the microbial reservoirs.

## 3.3 The Concept of Modeling Transmission of Disease Through Host Populations: Epidemic Versus Endemic

Figure 3.2 presents a very basic example of the models developed for analyzing transmission of infectious disease through populations of animals and humans. Such models are commonly referred to as "Disease transmission models", and represent a type of compartmental model. In Fig. 3.2, the individual compartments are shown as rectangular boxes and they representing segments of the host population being studied. The different compartments are connected by solid arrows that represent the rates at which individual members of the population move from one compartment to another. These rates of movement are sometimes described as the "force" of flow through the model. The model presented in Fig. 3.2 has four compartments: susceptible, meaning those individuals who are susceptible to infection by the pathogenic microorganism whose affect is being studied; infectious, meaning those individuals who have become infected and are in a state where they can transmit the infectious agent to other individuals; immune, meaning those individuals who have successfully completed convalescence from the infection and who are, for at least a time, resistant to reinfection; and removed, representing individuals who at least temporarily are excluded from the population under study. In this example, it is presumed that the removed individuals are those who have died from the infection. Figure 3.2 also shows the point in the model at which transferral of an infectious agent actually occurs, represented as an arrow with a dashed line.

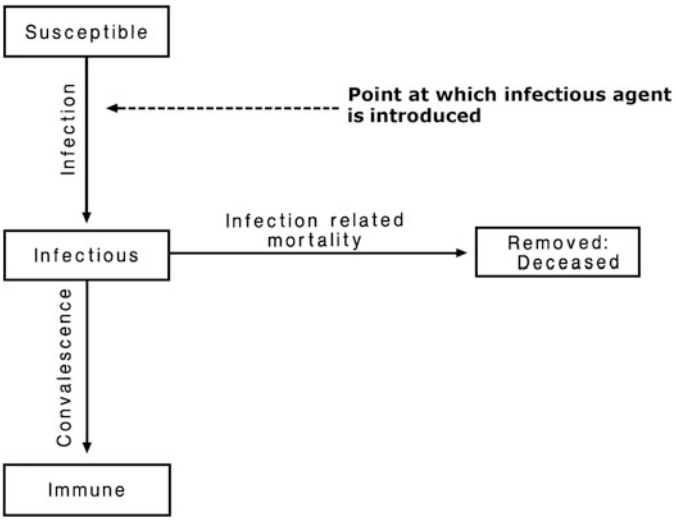


**Fig. 3.2** Basic compartment model appropriate for describing the epidemic propagation of disease through a population and the response of individuals to that disease. Models of this type are commonly termed "Disease Transmission Models". This model also shows the point at which an infectious agent (causative microorganism) is transmitted to susceptible individuals

The model presented in Fig. 3.2 was developed for epidemic disease transmission, and accordingly contains relatively few compartments. Figure 3.3 presents a model developed for endemic disease transmission and takes us a large step forward to a model that is more complex and more complete. In Fig. 3.3, live individuals can move into and out from three removed categories; susceptible, infectious, and immune. The epidemic disease model presented in Fig. 3.2 allows for disease-related mortality. The endemic disease model shown in Fig. 3.3 likewise includes disease-related mortality, and additionally allows for both natural mortality and vaccine related mortality. The model shown in Fig. 3.3 also enables the addition of susceptible individuals to the population through new births, here termed "host progeny", and returns immune individuals to the susceptible compartment through the eventual waning of immunity. The models used in this chapter pertain to the entire human population of a community.

A more detailed description of using compartment models to describe disease transmission including the transmission of disease in livestock animals has been presented by Hurst and Murphy (1996). Disease transmission models found in the literature can vary with respect to both the compartments they contain and the indicated movement of individuals. Such variation reflects the intended application of a given model, i.e., whether it is for endemic versus epidemic disease, and whether it represents a disease exposure which is pertinent to only a subset of the population, such as infants, or to the entire population (Hurst and Murphy 1996). There are considered to be four major stages of a disease. The first of those is the incubation period which occurs prior to the demonstration of symptoms. The second is a

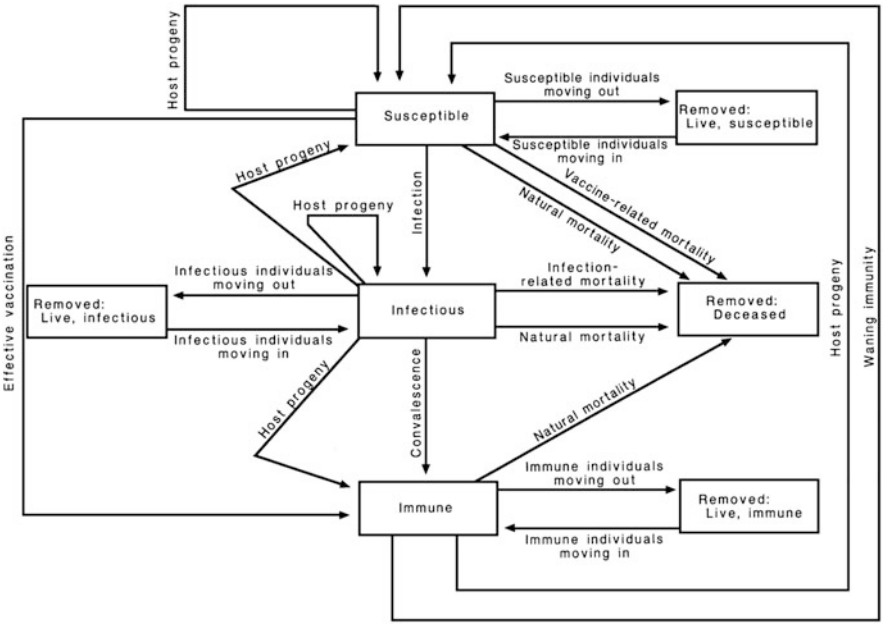


**Fig. 3.3** Compartment model appropriate for describing endemic disease propagation in a dynamic population

prodromal period when the symptoms are not severe and may not be specific, during which time the hosts usual functions still can be performed although perhaps with some amount of either distres or discomfort. The prodromal phase is followed by an illness period during which customary symptomatology may be displayed. The fourth stage is a hoped period of convalescense. These four stages can be represented by different compartments.

Figure 3.1 also could be used as a compartment model but its variables would be different and their parameter values would be harder to estimate.

## 3.4 Prevention Is the Best Solution for Infectious Disease

To quote Benjamin Franklin (1735), although out of proper context, "In the first Place, as an Ounce of Prevention is worth a Pound of Cure ...". Franklin actually was addressing fire safety, but we often use that quote when referring to health. Microbial illnesses are an important cause of morbidity and mortality in human populations. There are several means by which we try either to prevent disease transmission or to lessen the severity with which disease impacts upon the affected individuals. A compartment disease transmission model as shown in Fig. 3.4 can be used to illustrate the implementation of treatment technologies both to prevent initial

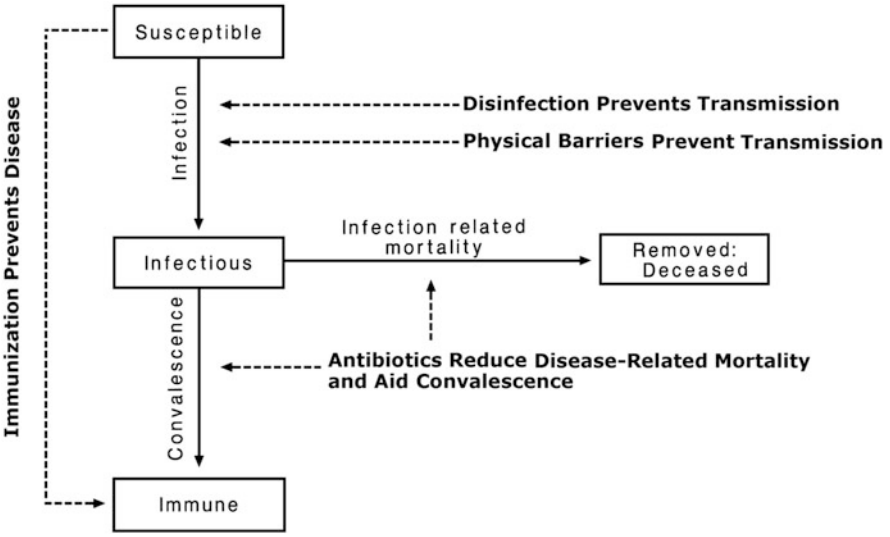


**Fig. 3.4** Basic compartment model from Fig. 3.2, modified to demonstrate the points at which different approaches may be used singly and in combination either to prevent disease transmission or to lessen the consequences of disease

acquisition of infections from drinking water and then to prevent secondary spread of the disease within the community.

Table 3.2 lists some of the categories of barriers that can be used to prevent transmission of microbial pathogens (Hurst 2016a). Figure 3.4 illustrates the points at which immunization, disinfection, physical barriers, and antibiotics are employed to reduce the transmission of pathogenic microorganisms.

### *3.4.1 Using Immunization to Prevent Infectious Disease*

Immunization is a very potent technique by which we try to both prevent the transmission of disease and to reduce deleterious post-transmission outcome for the diseased recipient. Immunization typically is used either prior to infection or, if there is a long incubation period such as is the case for rabies, then immunization can effectively be used during the incubation period. Immunization of a susceptible individual will not actually prevent transmission of the pathogen to that individual, but immunization is used in the hope of subsequently increasing the rapidity of the individuals immune response, thereby reducing replication of the pathogen within the newly infected individual. The goal is that quickly and effectively reducing reproduction of the causative microbe in this individual will prevent the acquired infection from progression to the point that the individual demonstrates overt disease

**Table 3.2** Categories of barriers used to prevent transmission of microbial pathogens

| Physical barriers | | |
|---|---|---|
| | Thermal | |
| | Acoustic (usually ultrasonic) | |
| | Pressure | |
| | | Barometric |
| | | Hydrostatic |
| | | Osmotic |
| | Radiation | |
| | | Electronic |
| | | Neutronic |
| | | Photonic |
| | | Protonic |
| | Impaction (includes gravitational) | |
| | Adhesion (adsorption) | |
| | Electrostatic | |
| | van der Waals | |
| | Filtration (size exclusion) | |
| | Geographic features | |
| | Atmospheric factors (includes such meterological aspects as humidity, precipitation, and prevailing winds) | |
| Chemical barriers | | |
| | Ionic (includes pH and salinity) | |
| | Surfactant | |
| | Oxidant | |
| | Alkylant | |
| | Desiccant | |
| | Denaturant | |
| Biological barriers | | |
| | Immunological (includes specific as well as nonspecific) | |
| | | Naturally induced (intrinsic response to microbial exposure) |
| | | Naturally transferred (lacteal, transovarian, transplacental, etc.) |
| | | Artificially induced (includes cytokine injection and vaccination) |
| | | Artificially transferred (includes injection with antiserum and tissue transfers such as transfusion and grafting) |
| | Biomolecular resistance (not immune-related) | |

(continued)

**Table 3.2** (continued)

| | | |
|---|---|---|
| Physical barriers | | |
| | | Lack of receptor molecules |
| | | Molecular attack mechanisms (includes nucleotide-based restrictions) |
| | | Antibiotic compounds (metabolic inhibitors, either intrinsic or artificially supplied) |
| | Competitive (other species in ecological competition with either the microbe, its vectors, or its hosts) | |

and thereby reduce the likelihood that the immunized individual will transmit that infection to other susceptible individuals.

Ability to mount a protective immune response naturally varies in relationship to normal factors such as increasing age or pregnancy (Gove et al. 1987). Other, unnatural conditions which can reduce the bodys ability to generate an adequate immune response thus resulting in a more severe disease outcome are underlying malnutrition (Crawford and Vermund 1988) and genetic immunosuppressive disorders (Saulsbury et al. 1980), as well as immunosuppressive viral diseases including acquired immune deficiency syndrome (Clifford et al. 1990) and measles (Crawford and Vermund 1988).

#### 3.4.1.1 Passive Versus Active Immunization

There are two basic approaches employed for immunizing individuals. The first of these is passive immunization, which typically results from the administration of immune globulin preparations by injection. Preformed antibodies present in the injected material provide immediate but short lived protection. Passive immunization sometimes is performed before an individual is exposed to the disease-causing microorganism, in which case it represents primary prevention. Passive immunization also may be performed following exposure, in which case it represents secondary prevention. Pre-exposure treatment is most likely to be done in those instances where the anticipated disease has a short incubation period. Post-exposure treatment is used in those instances where the disease either has a long incubation period, or the exposed individual is considered to be at risk of a severe disease outcome.

The second approach is active immunization, often termed “vaccination”. This involves administering antigenic materials which cause the recipient to develop an immune response. Active immunization normally is used before exposure to the causative microorganism as a means of primary prevention. The protection resulting from active immunization usually lasts for a much longer time period than does the protection afforded by passive immunization. The United States Government has

recently published an informative advisory document on immunization practices for humans (Centers for Disease Control and Prevention 2018b).

There are, however, drawbacks to reliance upon vaccination for disease control. The process of developing, commercially producing, distributing and successfully administering vaccines is both complex and costly. The complexity of this process limits the number of diseases against which we can attempt to protect people. The cost factor sadly also limits the extent to which vaccination programs are utilized. Problems can result from vaccination, with instances of vaccine-related illness and even deaths known to occur among vaccine recipients (Centers for Disease Control 1991). Also, live attenuated organisms that may be administered for vaccination purposes can occasionally revert to virulent forms (Abraham et al. 1993) and cause illness instead of preventing illness. Immunity acquired through vaccination is not necessarily permanent, and can wane with time such that immunized individuals again become susceptible to illness caused by the targeted pathogen. There often are religious objections against vaccination (Ahmed et al. 2018) and sometimes the people who administer vaccines are attacked and killed because of those religious objections (McGirk 2015).

#### 3.4.1.2 Separating the Concepts of Immunity Against Infection, Which Is Short Term, and Immunity Against Illness Which May Be Longer Term

In order to understand the risk values developed from the modeling efforts presented in this chapter, it is important to realize that infection with a given species of microorganism does not offer assurance against subsequent reinfection by that same species at some later time. This impermanence of protection allows both previously ill and also vaccinated individuals to return to the pool of susceptibles. Immunity against either infection or reinfection may last only a few months or less. Reinfection can occur even if the individual has developed a measurable immune response to that organism as determined by antibody titers (Cash et al. 1974). Studies have shown that it is possible for individuals to be reinfected with the same subgroup (Simhon et al. 1981) and even the same strain of enteric virus (Koprowski 1956), with the same strain of enteric protozoa (Nash et al. 1987), or the same phage type of enteric bacteria (Hornick et al. 1970b). It is possible for reinfection to occur as soon as 12 weeks after the initial infection (Nash et al. 1987). No information was found which indicated an upper limit to the number of times an individual could be reinfected by the same species, serotype, or strain of microorganism.

The basis for the phenomenon of naturally acquired immunity assumes that after a sufficient number of antigenic exposures to, or infections by, the same serotype of microorganism, an individual can develop a level of immune response which will be sufficiently strong and rapid so as to protect not against the possibility of subsequent infections and reinfections by that microbe, which can happen repeatedly, but rather whether the residual capacity for response can protect against the possibility that subsequent infection and reinfection events by that microbe would cause illness.

Thusly, when people mention the idea of protective long term immunity they actually should understand themselves not to be considering an "all or none" level of protection provided by their residual capability to produce an immune response. Not even naturally acquired immunity can be presumed protective against infection. Immunity is protective only against the likelihood and severity of resulting illness. While the duration of post-disease protection against future illness caused by either the same or a similar pathogen may last for perhaps 20 years, that duration period is variable and could be considerably shorter.

Natural acquired immunity has not been taken into account by the modeling exercises presented in this chapter for three reasons. First, different strains of even the same species of microorganism will vary in their immunogenicity, which is defined as the ability to illicit an immune response. Second, the duration in terms of years for the protective effect attributed to naturally acquired immunity remains uncertain and probably is variable. Third, it has been proven that reinfection of an individual by even the same strain of microorganism can result in sequential episodes of illness (Hornick et al. 1970b; Simhon et al. 1981).

This understanding about immunity and immunization is the reason why the wording in Fig. 3.4 includes "Immunization prevents disease" and not "Immunization prevents infection".

## *3.4.2 Infection Control Practices*

There are other approaches, aside from vaccination, that can be used to prevent infection. Among these are two general categories of infection control practices. The first represents establishment of physical barriers for preventing the transfer of infectious agents. The second consists of disinfection. Disinfection can include some physical barrier techniques such as irradiation, in addition to chemical barriers, and both categories of infection control practices represent primary prevention.

### 3.4.2.1 Use of Physical Barriers to Prevent Disease Transmission

The United States Department of Health and Human Services divides physical barriers for laboratory situations into two groups, primary barriers and secondary barriers. According to this classification scheme, primary barriers protect individuals and their immediate environment. As such, primary barriers would consist of protective clothing like gloves and masks, as well as personal safety equipment like filtration equipped respirators. Secondary barriers include physical containment practices and design factors intended to limit dispersion of the pathogens, among which are the use of separate rooms for handling clean versus contaminated materials, washing and other decontamination techniques that are used for work surfaces and implements, and disinfection practices including the sterilization of contaminated materials.

These barriers are applicable in different ways, some against the microbes themselves, some against vehicles, and some are used against vectors and infected hosts. Many of these same barrier concepts are applicable at different size levels. The concept of filtration, for example, applies to the use of size excluding filter materials that are designed with pores sufficiently small so as to physically retain the individual microbes themselves. Size exclusion filtration also is applied as a barrier in the form of such things as sand filters used for water treatment, and while such filters may not have a pore diameter sufficiently small as to remove individual microbes, these filters nevertheless can effectively retain particle-associated microbes and small vectors. Latex gloves have pores that similarly may not retain the microbes themselves but those pores are small enough that they do act as size exclusion barriers by retaining droplets of liquid containing the microbes, and condoms similarly prevent the transmission of sexually transmitted diseases. Protective clothing, window screens and bed nets are effective as size exclusion barriers against many insect vectors and other small arthropods, while simultaneously reducing our exposure to any disease-causing pathogens which those animals may be carrying. Fences, doors, gates, and walls are used as size exclusion barriers against numerous other vectors and also against infected hosts. The concept of physical barriers also includes quarantine practices, whereby infected individuals are isolated from susceptible individuals. Quarantine isolation has been used for humans, most notably for diseases such as tuberculosis, leprosy, influenza, and ebola. Quarantine can be effective, but may be expensive and is considered stigmatizing. These and other physical barrier concepts can be applied for public health purposes to prevent disease transmission in our daily living situations.

Table 3.3 shows the usefullness of filtration barriers for treating drinking water by illustrating the relationship between increase in percentage of United States city population supplied with community filtered drinking water and decrease in overall city population typhoid death rates. The cities used for compiling that table of data were selected for having reliable records as to cause of death (Johnson 1916). A presumption made when presenting this information is that some of the cases of typhoid illness may have been contracted directly from pathogens contained in the drinking water, and that secondary transmission of the illness subsequently may have resulted when infections initially acquired from water then were spread from the ill individuals to other, susceptible people. Additional sources of the pathogen would have including contaminated food.

Preventing disease transmission by using physical barrier techniques also can be done at the household level for treating drinking water. As an example, ceramic water filters can be used on the household level, acting by size exclusion to protect against bacteria and protozoa in contaminated water plus those filters can prevent the passage of virus that are associated with larger suspended particles. Home sandfilters were used to treat drinking water before ceramic filtration became popular, and sand filters remain in use at household levels. The efficiency of commercially designed drinking water filtration devices often is aided by particle agglomeration and sedimentation using either natural or synthetic coagulants.

**Table 3.3** Relation between increase in percentage of United States city population supplied with filtered drinking water and decrease in overall city population typhoid death rates

| Year | Percentage of city population served by water filtration utilities | Typhoid fever[a] death rates for same cities |
|---|---|---|
| 1900 | 8.7 | 36 |
| 1901 | 10.8 | 34 |
| 1902 | 11.9 | 37 |
| 1903 | 13.3 | 38 |
| 1904 | 16.0 | 35 |
| 1905 | 17.4 | 30 |
| 1906 | 20.5 | 33 |
| 1907 | 23.2 | 32 |
| 1908 | 23.3 | 25 |
| 1909 | 30.1 | 21 |
| 1910 | 34.6 | 24 |
| 1911 | 37.2 | 20 |
| 1912 | 42.4 | 16 |
| 1913 | 48.0 | 16 |

Data is presented for those United States cities having accurate registration as to cause of human death
Source: Johnson (1916)
[a]Death rates given per 100,000 population per year

#### 3.4.2.2 Use of Disinfection to Prevent Disease Transmission

Disinfection, the destruction of pathogenic microorganisms, is something which, when given sufficient time, can occur under natural environmental conditions that do not allow growth of the pathogenic microorganisms (Hurst 1991; Hurst et al. 1992; Kehr and Butterfield 1943) and this can be termed natural disinfection. Sunlight can be an effective disinfectant as are manufactured sources that generate either microwave, ultraviolet, or gamma radiations. Disinfection usually represents primary prevention. Under special medical situations, where an individual already is infected, disinfection could be considered a means of secondary prevention by either reducing the extent of infection or precluding dispersal of the pathogens.

Using disinfectants to destroy pathogens in drinking water is a practice that can be performed either at the municipal level with formal treatment facilities, or at individual and household levels. Disinfection is also performed on wastewaters to protect the quality of receiving waters.

Disinfection of drinking water on an individual or household basis can be accomplished by either boiling of the water, or distilling the water, or adding oxidizing chemicals such as chlorine and iodine.

Disinfection practices performed on an individual basis also include the washing of hands, dishes and eating implements. Many washing agents and detergents can be effective in aiding either the physical removal or destruction of microorganisms. Washing with an artificial detergent, like ordinary soap, can provide some chemical

disinfection activity (Baker et al. 1941). Although we often use soap as an aid when washing, using just water alone can have some beneficial effect (Mbithi et al. 1993). Interestingly, Schurmann and Eggers (1985) have shown that at least in the case of decontaminating skin, the use of moistened sand may be even more effective than is washing with ordinary soap. The simple practice of using chlorine bleach when washing clothing also has a disinfection effect.

### *3.4.3 Use of Antibiotics to Preclude or Lessen the Severity of Disease*

The successful development of antibiotic drugs represents one of the miracles of modern science and medicine (Taylor 1942). These drugs normally are used for secondary prevention, to reduce the severity of illness in diseased patients. However, in some instances antibiotic drugs are used prophylactically as primary prevention to preclude the initiation of an infection. Despite the miracle that these antibiotic compounds represent, microorganisms are capable of evolving drug resistance (Coronado et al. 1993). Thus, time has proven Taylor (1942) to have been overly optimistic when he stated that with discovery of the organic arsenic compound Salvarsan, and the sulphonamides, humankind had successfully conquered bacteria.

### *3.4.4 Demonstrating the Effectiveness of Techniques for Reducing the Transmission of Infectious Agents*

I will present two approaches, compartment disease transmission modeling and risk estimation modeling.

#### 3.4.4.1 Compartment Disease Transmission Modeling for Waterborne Infectious Disease

Drinking water treatment will be used as an example of our effectiveness at reducing the incidence of infectious disease in human populations. The bacterial disease typhoid is well known to be transmitted by the ingestion of contaminated water, which often represents its primary route of transmission into a population of susceptible individuals. This organism may be harbored by human carriers of the disease, and those are individuals who experience prolonged infection without necessarily displaying the symptoms normally associated with this illness. Human carriers may represent a secondary route of transmission for typhoid, the means by which the infectious agent is transferred within a human population following primary waterborne transmission. These carriers can pass the pathogen on to other members of a

population through vehicles such as contaminated food. Typhoid caused extremely high incidences of waterborne disease and death worldwide in the nineteenth century and early part of the twentieth century. While this disease still occurs both endemically and epidemically throughout the world, in developed countries its occurrence has almost completely been staunched by the introduction of adequately operated drinking water treatment facilities. Table 3.3 presented the change in urban typhoid death rates in the United States during the beginning of the twentieth century, serving as an example of the effectiveness of community drinking water treatment for reducing waterborne disease. In this case, the treatment used was water filtration, which is a form of physical barrier. In some of the cities, the water may have been chlorinated following filtration which is a combined-treatment practice whose usage began during the latter part of the time period represented in Table 3.3 (Holmquist 1924). Interestingly, the initial use for chlorination of drinking water was to control the development of water odor within distribution systems and only later was it discovered that chlorination also reduces the level of pathogenic microbes in the water. The data listed in Table 3.3 are from a publication by Johnson (1916) for cities with accurate reporting as to cause of human death. These data show that in the year 1900, for the represented cities, the percentage of urban population served by water filtration utilities was 8.7% and the concurrent annual typhoid death rate was 36 per 100,000 population. During the 13 years which followed, the percentage of population served by water filtration in those cities gradually increased to 48% and simultaneously the annual typhoid death rate gradually decreased to 16 per 100,000 population. These data dramatically suggest how drinking water treatment can be effective for reducing waterborne disease even when secondary transmission of the disease can occur within the community.

The first approach that I will present for demonstrating the potential effectiveness of drinking water treatment is the disease transmission modeling represented in Tables 3.4 and 3.5 (Hurst and Murphy 1996). The basic disease transmission model presented in Fig. 3.1 was used for this purpose, with exclusion of mortality, to estimate the social impact of a waterborne outbreak in a community of 10,000 people. It was assumed for the purpose of this modeling that: (1) all of the individuals in the community were susceptible to infection at the time of the community's initial exposure to the causative microorganism; (2) an individual became infectious one day after becoming infected; (3) the probability of an infectious individual recovering from the infection and subsequently becoming immune was 20% on any given day during their period of infectiousness; and (4) on each day that an individual was infectious, they had a 2% chance of transmitting the infection to another individual. As the percentage of susceptible individuals in the population decreased during the epidemic, transmission of the disease also became reduced. Running of the model was discontinued when the movement of individuals from the infectious category to the immune category had become less than 0.5 per day, at which point movement would have rounded down to zero individuals per day. For the models presented in Tables 3.4 and 3.5 it was assumed that exposure to the primary route of transmission, presumably microbial contaminants in the drinking water, occurred for a single day and that the initial infection rate was 10%.

**Table 3.4** Model of community wide waterborne disease epidemic caused by single day ingestion exposure to contaminated water with subsequent person to person secondary transmission

| | Number of individuals[a] | | |
|---|---|---|---|
| Day number | Susceptible | Infectious | Immune (recovered) |
| 1 | 10,000 | 0 | 0 |
| 2 | 9,000 | 1,000 | 0 |
| 3 | 8,982 | 818 | 200 |
| 4 | 8,967 | 669 | 364 |
| 5 | 8,955 | 547 | 498 |
| 6 | 8,945 | 448 | 607 |
| 7 | 8,937 | 366 | 697 |
| 8 | 8,930 | 300 | 770 |
| 9 | 8,925 | 245 | 830 |
| 10 | 8,921 | 200 | 879 |
| 11 | 8,917 | 164 | 919 |
| 12 | 8,914 | 134 | 952 |
| 13 | 8,912 | 109 | 979 |
| 14 | 8,910 | 89 | 1,001 |
| 15 | 8,908 | 73 | 1,019 |
| 16 | 8,907 | 59 | 1,034 |
| 17 | 8,906 | 48 | 1,046 |
| 18 | 8,905 | 39 | 1,056 |
| 19 | 8,904 | 32 | 1,064 |
| 20 | 8,903 | 27 | 1,070 |
| 21 | 8,903 | 22 | 1,075 |
| 22 | 8,903 | 18 | 1,079 |
| 23 | 8,903 | 14 | 1,083 |
| 24 | 8,903 | 11 | 1,086 |
| 25 | 8,903 | 9 | 1,088 |
| 26 | 8,903 | 7 | 1,090 |
| 27 | 8,903 | 6 | 1,091 |
| 28 | 8,903 | 5 | 1,092 |
| 29 | 8,903 | 4 | 1,093 |
| 30 | 8,903 | 3 | 1,094 |
| 31 | 8,903 | 2 | 1,095 |
| | Total cases of infection = 1,097 | | |

[a]Assumptions: (1) No initial immunity within community. (2) Infected persons become infectious on next day. (3) Each day 20% of previous days infectious individuals have recovered, calculated as follows: (number recovered on day $n$) = (number recovered on day $n-1$) + (0.2 × number infectious on day $n-1$). (4) Each day 2% of the infectious individuals transmit the infection to a single contact, assuming that contact is susceptible, calculated as follows: (number infectious on day $n$) = (0.8 × number infectious on day $n-1$) + [(0.02 × number infectious on day $n-1$) × (portion of initial population still susceptible on day $n-1$ expressed as a decimal value)]. This equation is valid only if there are individuals in the infectious category for day $n-1$. (5) On any given day, everyone who is neither infectious nor immune is considered susceptible, calculated as follows: (number susceptible on day $n$) = [10,000 – (number infectious on day $n$ + number immune on day $n$)]. (6) Reinfection does not occur during the course of the epidemic

**Table 3.5** Model of the same waterborne disease outbreak as in Table 3.4 with assumption that both initial transmission and secondary transmission were reduced by 80% achieved through use of physical barrier and disinfection practices

| | Number of individuals[a] | | |
|---|---|---|---|
| Day number | Susceptible | Infectious | Immune (recovered) |
| 1 | 10,000 | 0 | 0 |
| 2 | 9,800 | 200 | 0 |
| 3 | 9,799 | 161 | 40 |
| 4 | 9,798 | 130 | 72 |
| 5 | 9,797 | 105 | 98 |
| 6 | 9,797 | 84 | 119 |
| 7 | 9,797 | 67 | 136 |
| 8 | 9,797 | 54 | 149 |
| 9 | 9,797 | 43 | 160 |
| 10 | 9,797 | 34 | 169 |
| 11 | 9,797 | 27 | 176 |
| 12 | 9,797 | 22 | 181 |
| 13 | 9,797 | 18 | 185 |
| 14 | 9,797 | 14 | 189 |
| 15 | 9,797 | 11 | 192 |
| 16 | 9,797 | 9 | 194 |
| 17 | 9,797 | 7 | 196 |
| 18 | 9,797 | 6 | 197 |
| 19 | 9,797 | 5 | 198 |
| 20 | 9,797 | 4 | 199 |
| 21 | 9,797 | 3 | 200 |
| 22 | 9,797 | 2 | 201 |
| | Total cases of infection = 203 | | |

[a]Assumptions:
1–3, 5, and 6 same as stated for Table 3.3
4. Each day 0.4% of the infectious individuals transmit the infection to a single contact, assuming that contact is susceptible calculated as follows: (number infectious on day $n$) = (0.8 × number infectious on day $n-1$) + [(0.02 × 0.2 × number infectious on day $n-1$) × (portion of initial population still susceptible on day $n-1$ expressed as a decimal value)]. This equation is valid only if there are individuals in the infectious category for day $n-1$

The model shown in Table 3.4 assumes that physical barrier techniques and disinfection practices were not employed to reduce either primary (waterborne) or secondary transmission of the disease. In that case, 1,000 individuals initially would become infected. The epidemic ends with a total of 1,097 individuals having been infected, 2 of which remain infected at day 31. Ninety seven of these infections resulted from secondary transmission of the infectious agent within the community. Table 3.5 shows what would happen to this same population assuming that drinking water treatment was 80% effective at reducing the incidence of initial infection within the population, and that the use of physical barriers and disinfection practices

was similarly 80% effective at reducing secondary transmission. Using these assumptions for the efficiency of physical barriers and disinfection, Table 3.5 indicates that a total of only 203 individuals would become infected during this outbreak, with 2 of those individuals remaining infected at day 22, and only 3 of the individuals having been infected as a result of secondary transmission.

### 3.4.4.2 Risk Estimation Modeling for Waterborne Infectious Disease

Several items of information are helpful in order for us to estimate the risk of infectious disease caused by ingesting microbially contaminated water. One of these is the levels of pathogenic microorganisms present in water that is likely to be consumed by individuals. A second item is a means of calculating the probability of acquiring infection from drinking that water, along with the accompanying probabilities that infection will lead to illness, and illness ultimately progress to death. Having this information available for calculating the risk of aquiring infections from ingesting water, we can then determine the extent to which these risks could be reduced by using treatment processes to remove microorganisms before the water is consumed.

Several previous efforts have been made at estimating the disease risk to humans from consuming drinking water that contained microbial contaminants. These efforts have a long history, dating back at least to 1943 (Kehr and Butterfield 1943). The risk estimation technique being represented here has a demonstrated ability to accurately calculate the probability of community gastrointestinal illness based upon the known levels of pathogenic virus and protozoa in a communitys drinking water. The levels of pathogenic bacteria in that water had not been determined by the researchers, and so those levels of bacteria are being inferred based upon a presumption that the infectious risk not accounted for by either virus or protozoa contained in the water would have been attributable to pathogenic bacteria in the water.

#### 3.4.4.2.1 Defining Probability Values for the Health Risks Associated with Infectious Disease

The first task is to define the probabilities of infection, illness, and death associated with each microbial group, genus, species or serovar. A problem to avoid is the potentially misleading idea of curve fitting, which can alter value estimates for the minimum infectious dose of a microorganism as exampled by the information listed in Table 3.6.

Instead, I will present the suggestion (Hurst et al. 1996) that the risks be estimated as defined in Eqs. (3.1)–(3.3) and use information which came from published literature representing numbers for microorganisms and individuals. These equations determine the probability of infection per ingested pathogenic microorganism, the probability that the acquired infection will progress to illness, and that the illness will progress to death.

**Table 3.6** How the use of curve fitting can alter value estimates for the minimum infectious dose of a microorganism

| Microorganism examined for infectivity | Experimentally determined minimum infectious dose[a] (reference) | Minimum infectious dose as estimated by curve fittings[b] | Underestimation of risk which would result from using curve-fitting values[c] |
|---|---|---|---|
| *Enterovirus enterovirus B* (was Human echovirus 12) | 17 (Schiff et al. 1984) | 500.0 | 29.4 |
| *Enterovirus enterovirus C* (was Human poliovirus 1) | 20 (Minor et al. 1981) | 110.2 | 5.5 |
| *Enterovirus enterovirus C* (was Human poliovirus 3) | 0.5[d] (Katz and Plotkin 1967) | 1.93 | 3.9 |
| *Rotavirus* | 0.9 (Ward et al. 1986) | 1.62 | 1.8 |
| *Giardia* | 10.0 (Rendtorff 1954) | 49.27 | 4.9 |

[a]The number of viral organisms was determined by infectivity in cultured cells. The number of *Giardia* was determined by direct microscopic enumeration

[b]Data from Regli et al. (1991). This column contains estimated minimum infectious dose values derived by performing curve-fitting operations on the actual experimental data. The curve-fitting values listed in Regli et al. (1991) were presented as the estimated concentration of microorganisms per liter of drinking water which would give an annual risk of one infection per 10,000 individuals and assumed that each individual ingested 2 liters of water per day. Therefore, backtracking to obtain the estimated minimum infectious dose values which had been derived by curve fitting was done by multiplying the published values by $7.3 \times 10^6$, which was calculated as follows: (2 liters of water/day) × (365.25 days/year) × (10,000 individuals)

[c]If the calculation of risk were done using values derived by curve fitting in place of the actual experimentally determined values, an underestimation of risk would result. The underestimation of risk (as a factor of error) which would be associated with using those curve-fitting values was calculated as follows: (the estimated minimum infectious dose as determined by Regli et al. 1991 through curve fitting) ÷ (the experimentally determined minimum infectious dose for that organism)

[d]Actual dose was 1 $TCID_{50}$, divided by 2 to yield an estimated $TCID_{100}$ as reported in this table, using the technique described by Hurst et al. (1988)

$$\text{Probability of Infection} = \frac{1}{\text{Minimum number of microorganisms required to produce infection}} \tag{3.1}$$

$$\text{Probability of Illness} = \frac{\text{Number of individuals who showed symptoms of illness}}{\text{Total number of individuals infected}} \tag{3.2}$$

$$\text{Probability of Death} = \frac{\text{Number of individuals who died from that illness}}{\text{Total number of individuals who showed symptoms of that illness}} \quad (3.3)$$

The probability of infection estimates made by Hurst et al. (1996) were for humans experimentally ingesting typically water-associated pathogens and they are presented in Table 3.7. The probability of illness estimates made by Hurst et al. (1996) for humans experimentally ingesting typically water-associated pathogens are presented in Table 3.8. The probability of death estimates made by Hurst et al. (1996) for humans in outbreak situations caused by typically water-associated pathogens are presented in Table 3.9. The use of median values for bacteria, protozoa and virus has been chosen because the conditions, either experimental or natural, differed between those studies for which the data has been summarized. The fact that differences existed between the studies precludes averaging those data to generate mean values.

From examining the calculated median values listed in Table 3.9, it can be estimated that the overall probability of infection leading to death for illnesses caused by either enteric bacteria or virus is approximately 0.01 to 0.02, or from 1 to 2%. There are notable exceptions! As one example, human illness caused by *Orthohepevirus orthohepevirus A*, previously designated Hepatitis E virus, normally has a 0.028 probability of death (which also can be stated as either 2.8%, or 1 in 36 meaning 1 death per 36 individuals, or 1/36). However, the probability of death for women who are in either their second or third trimester of pregnancy at the time when they become ill from this virus typically ranges from approximately 0.17 (17%, or 1 in 6, Gove et al. 1987) to 0.22 (22%, Khuroo 1991). The death rate for hepatitis E illness in pregnant women can be as high as 39% (Khuroo 1991) and as many as 46% of the individuals who die during a hepatitis E outbreak situation may be pregnant women (Gove et al. 1987). As another example, Hoxie et al. (1997) observed that the overall risk of death from waterborne *Cryptosporidium* associated with a large general population of humans who experienced an outbreak of infectious illness caused by that genus in Milwaukee, Wisconsin, may have been 0.0001 (0.01%, or 1 in 10,000). Unfortunately, numbers from a publication by Crawford and Vermund (1988) indicate that the probability of cryptosporidial illness leading to death can be 0.14 (14%, or 1 in 7, or 2/14) in the case of underlying malnutrition. The risk of death from *Cryptosporidium* can be 0.20 in the case of underlying measles (20%, or 1 in 5) (Crawford and Vermund 1988) representing a 2,000-fold increase over the rate of death from *Cryptosporidium* infections in the general population. By itself, illness caused by the measles virus *Morbillivirus measles morbillivirus* is almost never fatal. However, this member of the *Morbillivirus* genus seems possibly to be the most powerfully immunosuppressive virus known to infect humans (Hurst and Adcock 2000). The single value found that represented a general population and seemed sufficiently reliable for being used in estimating the probability that illness from an enteric protozoan infection would lead to death was from a waterborne outbreak of cryptosporidiosis for which the estimated probability of death is 0.0002, or 0.02% (Marchione 1994; Mac Kenzie et al. 1994). I have used

**Table 3.7** Minimum infectious dose of enteric microorganisms for humans as determined by ingestion

| Microorganism examined | Lowest number of microorganisms required to cause infection | Probability of infection per individual microorganism ingested | Reference |
|---|---|---|---|
| Bacteria[a] | | | |
| *Salmonella enterica* subsp. enterica serovar Typhi (was *Salmonella typhi*, Quailes strain) | 100,000 | $1.0 \times 10^{-5}$ | Hornick et al. (1970a) |
| *Vibrio cholerae* (type 01) | 1,000 | $1.0 \times 10^{-3}$ | Levine et al. (1984) |
| *Vibrio cholerae* (type non-01) strain A | 1,000,000 | $1.0 \times 10^{-6}$ | Morris et al. (1990) |
| *Vibrio cholerae* (type non-01) strain B | 10,000,000 | $1.0 \times 10^{-7}$ | Morris et al. (1990) |
| *Vibrio cholerae* (type non-01) strain C | 100,000 | $1.0 \times 10^{-5}$ | Morris et al. (1990) |
| | | Median for bacteria = 0.00001 [$1.0 \times 10^{-5}$] | |
| Protozoans[b] | | | |
| *Cryptosporidium parvum* | 30[c] | 0.033 | DuPont et al. (1995) |
| *Giardia intestinalis* (was Giardia lamblia) | 10 | 0.1 | Rendtorff (1954) |
| | | Median for protozoans = 0.067 [$6.7 \times 10^{-2}$) | |
| Virus[d] | | | |
| *Enterovirus enterovirus B* (was Human echovirus 12) | 17 | 0.059 | Schiff et al. (1984) |
| *Enterovirus enterovirus C* (was Human poliovirus 1, SM strain) | 2 | 0.5 | Koprowski (1955) |
| *Enterovirus enterovirus C* (was Human poliovirus 1, SM strain) | 2 | 0.5 | Katz and Plotkin (1967) |
| *Enterovirus enterovirus C* (was Human poliovirus 1, (strain not identified) | 20 | 0.05 | Minor et al. (1981) |

(continued)

**Table 3.7** (continued)

| Microorganism examined | Lowest number of microorganisms required to cause infection | Probability of infection per individual microorganism ingested | Reference |
|---|---|---|---|
| *Enterovirus enterovirus C* (was Human poliovirus 3, Fox strain) | 0.5[e] | 2.0 | Katz and Plotkin (1967) |
| *Rotavirus* | 0.9 | 1.1 | Ward et al. (1986) |
| | | Median for virus = 0.5 [$5.0 \times 10^{-1}$] | |

Footnotes for Table 3.7:
Infection was determined serologically
[a]Number of organisms was determined by culturing
[b]Number of organisms was determined by microscopy
[c]Lower dosages were not tested
[d]Number of organisms was determined by infectivity in cultured cells
[e]Actual dose was 1 $TCID_{50}$, divided by 2 to yield estimated $ID_{100}$ of 0.5 as reported in this table, using the technique described by Hurst et al. (1988)

this value to represent probability of death from protozoan illness for the purpose of these modeling exercises. However, I understand that use of this value for protozoan illness may represent a severe understatement when it comes to estimating the risk for specific subpopulations of individuals.

There have been reports of concurrent enteric infections in individuals caused by multiple genera of either protozoa (Cristino et al. 1988) or bacteria (Mata et al. 1983), and of simultaneous infections with protozoa and virus (Grohmann et al. 1993; Crawford and Vermund 1988; Taylor et al. 1985), as well as with bacteria and virus (Mata et al. 1983). It is even possible to become concurrently infected with two different enteric virus of the same genus, as has been noted for infections either by rotaviruses (Rodriguez et al. 1983) or enteroviruses (Koprowski 1955; Tambini et al. 1993). In fact, the capability of simultaneously being infected with multiple species of the genus *Enterovirus* is what allows effective administration of oral poliovirus vaccine in a trivalent form, containing all three human poliovirus in an infectious form (Abraham et al. 1993).

I considered it important to use only data developed in humans for this modeling exercise. The reason for this decision is that the minimum number of microorganisms required to cause an infection can differ between humans and other animals by as much as several hundred fold (Koprowski 1955).

**Table 3.8** Likelihood of infection resulting in illness for enteric microbial diseases

| Causative microorganism | Ratio of illnesses to total number of infections | Probability of illness | Reference |
|---|---|---|---|
| Bacteria | | | |
| *Salmonella enterica* subsp. enterica serovar Typhi (was *Salmonella typhi*) | 34/69 | 0.4928 | Hornick et al. (1970a) |
| *Vibrio cholerae* (type 01) | 15/55 | 0.2727 | Levine et al. (1984) |
| *Vibrio cholerae* (type non-01) | 8/15 | 0.5333 | Morris et al. (1990) |
| | | Median for bacteria = 0.4928 [$4.9 \times 10^{-1}$] | |
| Protozoans | | | |
| *Cryptosporidium* | 36/65 | 0.5538 | Bongard et al. (1994) |
| *Cyclospora* | 11/50 | 0.2200 | Ortega et al. (1993) |
| *Giardia intestinalis* (was *Giardia lamblia*) | 11/34 | 0.3235 | Lopez et al. (1980) |
| *Giardia intestinalis* (was *Giardia lamblia*) | 3/5 | 0.6000 | Nash et al. (1987) |
| *Giardia*[a] | 26/133 | 0.1955 | Islam (1990) |
| *Giardia* | 977/1,211 | 0.8068 | Birkhead and Vogt (1989) |
| | | Median for Giardia = [0.4617 = $4.6 \times 10^{-1}$] Median for protozoans = 0.4386 [$4.4 \times 10^{-1}$] | |
| Viruses | | | |
| *Enterovirus enterovirus C* (was Human coxsackievirus A21) | 24/26 | 0.9231 | Couch et al. (1965) |
| *Enterovirus enterovirus B* (was Human echovirus 12) | 3/59 | 0.0508 | Schiff et al. (1984) |
| *Rotavirus* | 4/5 | 0.8000 | Kapikian et al. (1983) |

(continued)

**Table 3.8** (continued)

| Causative microorganism | Ratio of illnesses to total number of infections | Probability of illness | Reference |
|---|---|---|---|
| *Rotavirus* | 17/30 | 0.5667 | Ward et al. (1986) |
| *Rotavirus* | 15/26 | 0.5769 | Ward et al. (1989) |
| | | Median for virus = 0.5769 [$5.8 \times 10^{-1}$] | |

Infection was defined serologically
[a]Data cited here was that listed for India

#### *3.4.4.2.1.1 Using "The Blindfolded Bowler Analogy" to Understand the Calculation for Probability of Infection*

It is important to note that infection and illness are not synonymous terms. While an individual cannot become ill without first becoming infected, one can become infected without that infection progressing to illness. Studies have been done in which microorganisms were fed to otherwise healthy individuals, and those individuals were then examined for evidence of infection. Two criteria can be used for determining the existance of an intestinal infection. The first, and most important, criterion is to look for the development of a response in the individuals antibody titer against the ingested organism (Bernstein et al. 1986; Cash et al. 1974; Clark et al. 1986). Development of either a detectable antibody titer when none previously existed, or an increase in an existing antibody titer, indicates that the individual was infected. The second criterion is to determine whether the number of indicated organisms shed in the individuals feces is greater than could be accounted for by the dose that the individual was fed. Finding appreciable numbers of the same type of organism in the feces suggests that the individual was infected, if those organisms are obligate intracellular parasites such as virus and some protozoans. This technique cannot accurately be used to assess infection by organisms which live in the lumen of the intestine, such as other protozoans and bacteria.

For the purpose of the modeling exercise presented in this chapter, it is assumed that any single microorganism is capable of causing infection, and that the differences in minimum infectious dose between the various microorganisms simply represent the random statistical probabilities that the necessary combination of events leading to an infection by those microorganisms would occur. It can be seen from the information presented in Table 3.7 that the lowest number of microorganisms required to cause infection is generally greater for bacterial organisms than for either protozoans or virus. Also, it can be seen that the minimum infectious dose varies at both the genus and species level for all three categories of microorganisms examined, bacteria, protozoa, and virus. The viral data indicate that there are additional strain dependent differences regarding the minimum infectious dose for microorganisms

**Table 3.9** Likelihood of illness resulting in death for enteric microbial diseases

| Causative microorganism | Ratio of deaths to total cases of illness | Probability of death | Reference |
|---|---|---|---|
| Bacteria | | | |
| *Campylobacter* | 2/6,441 | 0.0003 | Tauxe et al. (1988) |
| *Escherichia coli* (type 0111) | 1/9 | 0.1111 | Caprioli et al. (1994) |
| *Escherichia coli* (type 0157-H7) | 1/10 | 0.1000 | Turney et al. (1994) |
| *Escherichia coli* (type 0157-H7) | 4/243 | 0.0165 | Geldreich et al. (1992) |
| *Salmonella enterica* subsp. enterica serovar Typhi (was *Salmonella typhi*) | 20,000/ 300,000[a] | 0.0667 | Johnson (1916) |
| *Salmonella enterica* subsp. enterica serovar Typhi (was *Salmonella typhi*, Zermatt, phage type E1) | 3/437 | 0.0069 | Bernard (1965) |
| *Shigella flexneri* | 1/610 | 0.0016 | Centers for Disease Control and Prevention (1994) |
| *Vibrio cholerae* (Burundi, Zimbabwe type 01) | 292/1,177 | 0.2481 | Ndayimirije et al. (1993) |
| *Vibrio cholerae* (West Africa type 01) | 20,000/ 150,000 | 0.1333 | Swerdlow and Ries (1993) |
| *Vibrio cholerae* (Bangladesh type non-01) | 1,473/ 107,297 | 0.0137 | Cholera Working Group (1993) |
| *Vibrio cholerae* (Portugal type 01) | 48/2,467 | 0.0195 | Blake et al. (1977) |
| *Vibrio cholerae* (Americas type 01) | 6,323/ 731,312 | 0.0086 | Centers for Disease Control and Prevention (1993) |
| | | Median for bacteria = 0.0180 [$1.8 \times 10^{-2}$] | |
| Protozoans | | | |
| *Cryptosporidium parvum* | 100/403,000 | 0.0002 | Marchione (1994), Mac Kenzie et al. (1994) |
| | | Median statistic not meaningful [$2.0 \times 10^{-4}$] | |
| Viruses | | | |
| *Enterovirus enterovirus A* (was Human coxsackievirus A2) | 2/403 | 0.0050 | Assaad and Borecka (1977) |
| *Enterovirus enterovirus A* (was Human coxsackievirus A3) | 2/27 | 0.0741 | Assaad and Borecka (1977) |
| *Enterovirus enterovirus A* (was Human coxsackievirus A4) | 3/580 | 0.0052 | Assaad and Borecka (1977) |

(continued)

**Table 3.9** (continued)

| Causative microorganism | Ratio of deaths to total cases of illness | Probability of death | Reference |
|---|---|---|---|
| *Enterovirus enterovirus A* (was Human coxsackievirus A6) | 2/204 | 0.0098 | Assaad and Borecka (1977) |
| *Enterovirus enterovirus A* (was Human coxsackievirus A10) | 6/351 | 0.0171 | Assaad and Borecka (1977) |
| *Enterovirus enterovirus A* (was Human coxsackievirus A16) | 2/1,654 | 0.0012 | Assaad and Borecka (1977) |
| *Enterovirus enterovirus B* (was Human coxsackievirus A9) | 8/3,039 | 0.0026 | Assaad and Borecka (1977) |
| *Enterovirus enterovirus B* (was Human echovirus 6) | 14/4,774 | 0.0029 | Assaad and Borecka (1977) |
| *Enterovirus enterovirus B* (was Human echovirus 9) | 14/5,237 | 0.0027 | Assaad and Borecka (1977) |
| *Enterovirus enterovirus C* (was Human coxsackievirus A19) | 3/18 | 0.1667 | Assaad and Borecka (1977) |
| *Enterovirus enterovirus C* (was Human poliovirus 1) | 72/8,074 | 0.0089 | Assaad and Borecka (1977) |
| *Enterovirus enterovirus C* (was Human poliovirus 1) | 95/1,031 | 0.0921 | Kim-Farley et al. (1984) |
| *Enterovirus enterovirus C* (was Human poliovirus 2) | 43/2,360 | 0.0182 | Assaad and Borecka (1977) |
| *Enterovirus enterovirus C* (was Human poliovirus 3) | 27/2,427 | 0.0111 | Assaad and Borecka (1977) |
| *Enterovirus enterovirus C* (was Human poliovirus 3) | 1/54 | 0.0185 | van Wijngaarden et al. (1992) |
| *Enterovirus enterovirus C* (were Human polioviruses 1, 2, & 3, data had been combined) | 11/204 | 0.0539 | Langmuir et al. (1956) |
| *Hepatovirus hepatovirus A* (was Hepatitis A virus) | –[b] | 0.0060[a] | Centers for Disease Control (1990) |
| *Orthohepevirus orthohepevirus A* (was Hepatitis E virus, Kashmir) | –[b] | 0.0280[c] | Ramalingaswami and Purcell (1988) |
| *Orthohepevirus orthohepevirus A* (was Hepatitis E virus, Somalia) | 87/2,000 | 0.0435 | Gove et al. (1987) |
| *Orthohepevirus orthohepevirus A* (was Hepatitis E virus, Delhi) | 90/97,600 | 0.0009 | Viswanathan (1957) |
| *Rotavirus* | 2/222 | 0.0090 | Fang et al. (1989) |
| *Rotavirus* | 7/3,439 | 0.0020 | Foster et al. (1980) |
| | | Median for virus = 0.0094 [$9.4 \times 10^{-3}$] | |

[a]Relationship between number of deaths from illness to total cases of illnesses was estimated by the cited author
[b]Values were not given in the reference
[c]Value reported for adult males

belonging to the same species. It was gratifying to notice that when values were available from two different research studies for the same strain within a single species of microorganism, the SM strain of Human poliovirus 1, both studies reported the same minimum infections dose level. The median value for probability of infection by viruses would not be changed by deleting either one of these two values for the SM strain.

When looking at the lowest number of viruses required to cause infection, it can be seen that in two instances, Human poliovirus 3 results published by Katz and Plotkin (1967), and *Rotavirus* results published by Ward et al. (1986), the infectious dose is less than one infectious unit as measured by cultured cells. This may reflect the fact that these virus evolved to replicate within cells of the intestinal wall as part of a living organ, preceded by exposure to the chemical environment within the intestines. As such, these virus are not as well suited to replicating under the conditions extant in the laboratory, where cultured cells were used to grow and quantitate the virus.

For many of the microorganisms listed in Table 3.7, the minimum infectious dose is much greater than one. This is particularly true in the case of bacteria, where the minimum infectious doses reported in Table 3.7 range from 1,000 to 10,000,000 organisms. Higher minimum infectious dose values can be found in the literature for many of the bacteria and protozoa listed in Table 3.7. However, in those other studies, it generally is the case that lower doses of microorganisms were not examined. If a unit of 100 microorganisms is sufficient to cause an infection, then so would 1,000 or even 1,000,000. For this reason, I have tried to cite studies which determined not only a minimum infectious dose, but which also tested lower dosage levels of the same organism and found the lower dosages insufficient to cause an infection.

Hurst et al. (1996) created an analogy which they named "The Blindfolded Bowler Analogy" to explain the basis for Eq. (3.1). For this analogy, it is assumed that a bowler is placed into a bowling alley and blindfolded. He is then pointed in the direction of a bowling lane and successively given objects that he is asked to roll down the lane. If the bowler is given 10 balls all of the same size, and not told whether any of them resulted in a strike until he has rolled all ten, he may find that on the whole, 10 balls is not sufficient to result in a strike. Thus, for that set of conditions, the minimum "unit" number of balls required to generate a strike is greater than 10. If allowed to use a larger number of balls, perhaps either 20, 1,000 or even 100,000 balls, the bowler will eventually find the minimum "unit" number of balls required to generate a strike. If, for example, under a defined set of conditions, there was a reliable chance that a unit of 100 balls could consistently yield a strike, and an assumption can be made that each individual ball was physically capable of producing the strike, then the probability of any individual ball producing a strike would be 0.01, the numerical inverse of the unit number of balls. It may be expected that using larger diameter balls would decrease the minimum unit number of balls required to cause a strike, and that using smaller diameter balls might increase the minimum unit number required to cause a strike. If the bowler instead were given cylinders to roll down the lane, and the length of those cylinders was greater than the diameter of the bowling balls, it might be the case that a unit of only 20 cylinders would be required to

produce a strike. In that case, the probability of a strike would be 0.05 per object rolled down the lane. Rolling cylinders whose length was equal to the width of the bowling lane might reduce the minimum unit number to 1, giving a probability of 1.0 per cylinder.

This analogy is not used to suggest that size, or overall shape, determines the minimum infectious dose for microorganisms. Instead, factors which likely influence the probability of infection per ingested organism include the number of susceptible cells in the intestinal wall, the relative number and accessibility of receptor sites on those cells, plus random genetic and phenotypic variation both on the part of the microorganism and the host. We can, of course, remove the bowlers blindfold, which enables him to understand the situation. We cannot, however, remove our own blindfold with respect to the variation in minimum unit number of virus, protozoans, or bacteria required to cause infection if ingested by a human. For the purposes of this risk modeling effort, I have again applied the Blindfolded Bowler Analogy and assumed that the probability of infection per individual microorganism ingested is the numerical inverse of the minimum infectious dose.

If we were to place a barrier across the lane used by our blindfolded bowler, we would severely decrease the probability of a rolled object producing a strike. The acidity of stomach secretions likewise represents a barrier to microorganisms which infect cells lining the intestines. Some organisms, such as enteroviruses, have evolved a natural resistance to low pH exposures, which enables them to get past the barrier posed by the stomach. Protozoan cysts and oocysts also seem to have evolved some resistance to degradation by acids. Ingesting food along with pathogenic microorganisms seems to help the microorganisms survive their trip through the stomach (Blake et al. 1980). This finding is important because humans often ingest food along with water and other beverages. Individuals who consume antacids eliminate this barrier from their stomach, a particular problem if they consume microbially contaminated water along with those antacids. Individuals who have the disease hypochlorhydria, and therefore produce an abnormally low amount of stomach acid, are correspondingly at greater risk of enteric infectious diseases such as cholera (Nalin et al. 1978).

The organism *Vibrio cholerae* has an interesting means of natural protection from stomach acids. In addition to causing massive outbreaks of disease associated with the consumption of contaminated water, this microorganism is known to cause illness associated with the consumption of macrocrustaceans, such as crabs. The natural habitat of this bacterial organism is the chitinous shells of crustaceans (Grimes 1991). Copepods and other taxa of microcrustaceans can reside as natural microfauna within community drinking water distribution systems (van Lieverloo et al. 2012). The association of *Vibrio* organisms with the chitinous shells of micro- and macro-crustaceans may facilitate survival of the microbes during passage through the stomach, and could be an important factor in waterborne disease if the *Vibrio cholerae* in source waters were attached to microcrustacean copepods and these microcrustaceans inadvertently ingested along with the water (Nalin 1976). This latter knowledge suggests that microcrustaceans may represent a potentially supportive environment for *Vibrio* contaminants that gain entrance to water distribution systems.

#### *3.4.4.2.1.2 Understanding the Calculations for Probabilities of Illness and of Death*

There are five main variables that need to be included when estimating the risk of acquiring infectious disease by ingestion of water. These are the concentration of pathogenic organisms in water which are infectious by the route of ingestion, the amount of water ingested by an individual per unit time, and the probabilities of infection, illness, and death associated with ingesting those pathogenic organisms.

The approach used in this chapter presumes that the risks of illness and death from ingesting pathogenic microbes in water are standard inverse calculations based upon knowing the numbers of infected individuals who become ill and the numbers of ill individuals who die. Illnesses that do not lead to death cover a broad range in terms of the severity of symptoms. The risk modeling exercise presented in this chapter does not address severity of illness, except in distinguishing the likelihood of infection leading to illness (Table 3.8) from the likelihood that illness subsequently results in death (Table 3.9).

There is a great deal of confidence in the numerical values used for estimating the probability of infection per ingested organism (Table 3.7) and the probability of an infection event resulting in illness (Table 3.8), because the data used for those calculations had been generated in carefully controlled prospective studies. There is somewhat less confidence in the numerical values available for calculating the probability of illness progressing to death (Table 3.9), because the latter values represent statistics that generally were compiled retrospectively from epidemiological surveys. There must also be some uncertainty as to whether the cited epidemiological studies considered the total number of illness cases attributed to a particular pathogen, or only those cases for which severe symptomatology was demonstrated. Given a fixed number of deaths for a particular disease, varying the criteria used in establishing the number of cases of illness will, in turn, change the calculated probability of illness progressing to death. The 1955–1956 waterborne disease outbreak in Delhi, India, caused by Hepatitis E virus represents an example of this variation in reporting the number of cases of illness. Viswanathan (1957) reported that 90 deaths were attributable to hepatitis contracted during this outbreak. I have chosen to cite his estimate of 97,600 cases of illness for this outbreak, since he was an epidemiologist who studied that epidemic at the time when it occurred (Viswanathan 1957). Some more recent publications (Khuroo 1991; Ramalingaswami and Purcell 1988) cited 29,300 cases of illness for this same outbreak, a number which represents only those individuals whose illness was sufficiently severe that it resulted in jaundice. If I instead had used the lower number of cases of illness in this risk modeling exercise, then the probability of illness progressing to death for that outbreak would have risen from 0.0009 to 0.0031. The choice of using overall median values to represent the probability of illness progressing to death for the categories of bacterial, protozoan, and viral diseases, rather than using corresponding mean values, helps to lessen the impact upon this modeling exercise of any cited study that may represent a particularly high or low probability value.

#### *3.4.4.2.1.3 Six Important Considerations*

Risk of infection, illness and death can be influenced by other factors in addition to these six whose implications must be understood when making risk assessment estimations for infectious diseases. The first three of these are issues that specifically apply to estimations for drinking water. The last three apply not only to drinking water but also to any infectious disease risks regardless of the source of the pathogenic organisms. I have not tried to account for these considerations when making the modeling efforts presented in this chapter.

1. Estimations of the levels of microorganisms in samples of water inherently are underestimations. This is due to the deficiencies of detection methodology.
2. To some extent, estimations of disease risk based on the levels of microbial contaminants in the municipal water distribution mains will be overestimations. This is because some of the water which people ingest is treated by heating techniques prior to its consumption, including cooking and the preparation of beverages that are consumed hot. This heating generally will reduce the level of viable microbial contaminants in the water. However, heating water and then maintaining it warm for a long time, as occurs with water-heating tanks in homes and commercial buildings including nursing facilities, can change the risk in that some pathogenic organisms such as bacteria of the genus *Legionella* can grow in water that is kept warm. Disease caused by *Legionella* usually results from inhalation of aerosolized water, rather than from ingestion.
3. Directly estimating the level of disease risk associated with pathogenic bacteria present in water is best done by enumerating those bacteria which actually are pathogenic. Most "indicator bacteria" are not considered pathogenic, and information regarding the level of non-pathogenic indicator organisms in water cannot directly be used for making risk assessment estimates.
4. If death does not occur as the outcome of an initial infection by a particular strain of a specific microorganism, then each subsequent reinfection of the same person by the same strain may carry a decreased risk that the infection would progress to illness as mentioned above.
5. Immunosuppression can increase the probability that an infection will have a severe outcome. There are some natural conditions such as pregnancy, and abnormal conditions such as malnutrition, that can result in suppression of immune system function. The result of this immunosuppression is that when some individuals become infected they may have a greater probability of death from that infection (see Sect. 3.4.4.2.1) than will other members of the general population.
6. Concurrent infections, which are incidences when a person is infected simultaneously by more than one pathogenic organism, can drastically increase the probability of death associated with any one of the involved pathogens (see Sect. 3.4.4.2.1) and many people are likely to be simultaneously infected by more than a single pathogen.

#### 3.4.4.2.2 Validation of This Risk Estimation Technique

Restating from Sect. 3.4.4.2.1.2, five main variables must be considered when estimating the risk of acquiring infectious disease by ingestion of water. These are the concentration of pathogenic organisms in water which are infectious by the route of ingestion, the amount of water ingested by an individual per unit time, and the probabilities of infection, illness, and death associated with ingesting those pathogenic organisms.

If we know the concentrations of pathogenic bacteria, protozoans and virus in drinking water, then it is possible to estimate the risks of infection, illness, and death associated with drinking the water that contains those pathogenic microbes. Two validation exercises are being presented for this risk estimation technique. Equations (3.4)–(3.7) present the format which is being used for this chapter to estimate the individual human health risks of infection, illness, and death associated with ingested microorganisms. These are based upon using Eq. (3.1) for the probability of infection per ingested organism, Eq. (3.2) for the probability of illness per incidence of infection, and Eq. (3.3) for the probability of death per incidence of illness.

$$\begin{array}{l}\text{Number of}\\ \text{organisms}\\ \text{ingested}\\ \text{annually}\end{array} = \begin{array}{l}\text{Number of}\\ \text{organisms}\\ \text{per liter}\\ \text{of water}\end{array} \times \begin{array}{l}\text{Number of}\\ \text{liters}\\ \text{ingested}\\ \text{per day}\end{array} \times \begin{array}{l}\text{365.25 days}\\ \text{of water}\\ \text{ingestion}\\ \text{per year}\end{array} \tag{3.4}$$

$$\begin{array}{l}\text{Annual}\\ \text{risk of}\\ \text{infection}\end{array} = \begin{array}{l}\text{Number of}\\ \text{organisms}\\ \text{ingested}\\ \text{annually}\end{array} \times \begin{array}{l}\text{Probability}\\ \text{of infection}\\ \text{per ingested}\\ \text{organism}\end{array} \tag{3.5}$$

$$\begin{array}{l}\text{Annual}\\ \text{risk of}\\ \text{illness}\end{array} = \begin{array}{l}\text{Number of}\\ \text{organisms}\\ \text{ingested}\\ \text{annually}\end{array} \times \begin{array}{l}\text{Probability}\\ \text{of infection}\\ \text{per ingested}\\ \text{organism}\end{array} \times \begin{array}{l}\text{Probability}\\ \text{of illness}\\ \text{per incidence}\\ \text{of infection}\end{array} \tag{3.6}$$

$$\begin{array}{l}\text{Annual}\\ \text{risk of}\\ \text{death}\end{array} = \begin{array}{l}\text{Number of}\\ \text{organisms}\\ \text{ingested}\\ \text{annually}\end{array} \times \begin{array}{l}\text{Probability}\\ \text{of infection}\\ \text{per ingested}\\ \text{organism}\end{array} \times \begin{array}{l}\text{Probability}\\ \text{of illness}\\ \text{per incidence}\\ \text{of infection}\end{array} \times \begin{array}{l}\text{Probability}\\ \text{of death}\\ \text{per incidence}\\ \text{of illness}\end{array} \tag{3.7}$$

This validation effort requires either knowing or estimating the levels of pathogenic bacteria, protozoa, and virus in community supplied drinking water. These risks will be calculated separately for bacterial, protozoan, and viral contaminants. The next item of information needed for this exercise is the amount of water ingested daily per individual. The value used in Eq. (3.4) as an estimate for the volume of

water ingested on a daily basis per individual consumer is 2 liters per day. This volume is the estimate recommended by the United States Environmental Protection Agency for use in calculating the risks from ingestion of water (United States Environmental Protection Agency 1997). More recent risk factor publications by the EPA are available but their contents are highly nuanced and do not give such a definitive number. Daily water consumption may vary greatly due to factors such as environmental temperature, differences in individual body mass, and the amount of physical exercise performed. Other estimates for water ingestion are available, including one by Payment et al. (1991) that consumers drink the equivalent of 46.5–47.3 glasses of water per week. Making an assumption that the average glass of water represents 250 ml, the value published by Payment et al. (1991) would represent approximately 1.7 liters of water ingested per day. It is possible, and perhaps likely, that not all of this ingested water will be consumed plain. Instead, some water will be used in soups, sauces, and as the basis for other beverages (Birkhead et al. 1993).

For these modeling exercises, the probability of each individual occurrence of infection, termed here to be an "infection event", is assumed to be independent of any other infection event caused by either the same or another microorganism. The probability of an infection event leading to illness is conditionally dependent upon an infection having occurred, there can be no microbially induced illness without infection although there can be infections that do not result in illness. The probability of illness progressing to death is in turn dependent upon an infection event having led to illness. It is possible that the probability of individual infection events leading to illness, and the accompanying severity of illness including progression to death, may decrease with subsequent reinfections by the same species, serotype, or strain of microorganism (Nash et al. 1987). This was discussed above in Sect. 3.4.1.2, which attempted to separate the concepts of immunity against infection and immunity against illness.

#### *3.4.4.2.2.1 First Risk Validation Exercise*

An attempt to validate the risk modeling approach described in this chapter can be done using data which Payment and colleagues have published for a section of Quebec province, Canada. Results of an epidemiological study (Payment et al. 1991) indicated that the annual risk of consumers acquiring gastroenteritis from ingesting microorganisms contained in the community-distributed tap water was 0.26. In an earlier study, it was determined that the average level of enteric viruses in the raw water entering that area's drinking water treatment plants was 3.3 cell culture infectious units per liter (Payment et al. 1985). In that same 1985 publication, it was indicated that the average cumulative viral reduction achieved by those community drinking water treatment plants was 99.97%. Table 3.10 shows that by substituting into Eqs. (3.4)–(3.6) this reported value for the level of viruses in the raw water, and taking into account the reduction in viral level achieved by community drinking water treatment, the result is a predicted individual annual risk of viral illness equal to 0.2086. The calculation used for this prediction is as follows: [(3.3 viral organisms per

**Table 3.10** For validation exercise 1: calculating the estimation of risk due to infectious, pathogenic virus in the municipality's water

| | |
|---|---|
| Concentration of organisms (virus that are infectious for mammalian cells, enumerated as infectious units) per liter of raw water | 3.3 |
| Liters of water ingested per person per day | × 2.0 |
| Days of water ingestion per year | × 365.25 |
| Probability of infection (per ingested virus capable of infecting mammalian cells) | × 0.5 |
| Probability of illness (per incidence of viral infection) | × 0.5769 |
| Estimate of residual disease risk following municipal treatment of the water (this value is included because the data provided for viral levels were determined for the water prior to processing at the treatment plant), calculated as 1.0000 – 0.9997 | × 0.0003 |
| Estimated annual risk of virus-induced illness per individual consumer | = 0.208606 |

liter of raw water) × (2.0 liters of water ingested per day) × (365.25 days of water ingestion per year) × ($5.0 \times 10^{-1}$ probability of infection per ingested virus) × ($5.8 \times 10^{-1}$ probability of illness per viral infection) × (1.0000–0.9997 as the estimate of residual viral disease risk following community drinking water treatment)].

An estimate for the amount of disease risk represented by pathogenic protozoan cysts and oocysts in the community-treated drinking water for that same geographical area can be made using results published later by Payment and Franco (1993). In that 1993 publication, the authors presented information regarding the levels of protozoans belonging to two genera, *Giardia* and *Cryptosporidium*, in the finished (treated) water produced by three water treatment plants. The levels of these protozoa in finished water from two of the treatment plants was below the researchers' limit of detection. However, the level of protozoans in finished water from the third treatment plant was 0.02 *Cryptosporidium* oocysts and 0.02 *Giardia* cysts per 100 l. In Tables 3.11 and 3.12 we see that substituting these levels of protozoa into Eqs. (3.4) through (3.6) yields an estimated annual protozoan disease risk from *Cryptosporidium* of 0.00267, plus an estimated annual risk of protozoan disease risk from *Giardia* of 0.006745.

Looking at Table 3.13 we can see that by adding together the estimated 0.2086 annual risk of viral illness per individual consumer, and the estimated annual risks from protozoan illness per consumer, gives an annual risk estimate of 0.2180 for illnesses attributed to these two categories of microorganisms. This indicates that by using the risk modeling approach presented in this chapter, we are able to account for

**Table 3.11** For validation exercise 1: calculating the estimation of risk due to *Cryptosporidium* in the municipality's water[a]

| | |
|---|---|
| Concentration of organisms (*Cryptosporidium* oocysts, enumerated by visual inspection following immunofluorescent staining) per liter of water following municipal treatment of the water | 0.0002 |
| Liters of water ingested per person per day | × 2.0 |
| Days of water ingestion per year | × 365.25 |
| Probability of infection (per ingested *Cryptosporidium* oocyst) | × 0.033 |
| Probability of illness (per incidence of cryptosporidial infection) | × 0.5538 |
| Estimated annual risk of *Cryptosporidium*-induced illness per individual consumer | = 0.00267 |

[a]This calculation does not include a value for estimation of residual disease risk following municipal treatment of the water, because the data provided for *Cryptosporidium* oocyst levels were determined for the water following processing at the treatment plant

**Table 3.12** For validation exercise 1: calculating the estimation of risk due to *Giardia* in the municipality's water[a]

| | |
|---|---|
| Concentration of organisms (*Giardia* cysts, enumerated by visual inspection following immunofluorescent staining) per liter of water following municipal treatment of the water | 0.0002 |
| Liters of water ingested per person per day | × 2.0 |
| Days of water ingestion per year | × 365.25 |
| Probability of infection (per ingested *Giardia* cyst) | × 0.1 |
| Probability of illness (per incidence of giardial infection) | × 0.4617 |
| Estimated annual risk of *Giardia*-induced illness per individual consumer | = 0.006745 |

[a]This calculation does not include a value for estimation of residual disease risk following municipal treatment of the water, because the data provided for *Giardia* cyst levels were determined for the water following processing at the treatment plant

**Table 3.13** For validation exercise 1: overall summary of risk estimations for the known levels of infectious, pathogenic virus and protozoans with the remaining risk assumed to be due to infectious, pathogenic bacteria

| | | |
|---|---|---|
| Total epidemiologically determined risk of illness per person per year associated with microbial contaminants in the municipally supplied tap water | | 0.26 (100.0%) |
| Estimated proportion of that risk attributable to virus in the municipally supplied tap water | 0.20861 (80.2%) | |
| Estimated proportion of that risk attributable to *Cryptosporidium* in the municipally supplied tap water | 0.00267 (1.0%) | |
| Estimated proportion of that risk attributable to *Giardia* in the municipally supplied tap water | 0.00674 (2.6)% | |
| Total proportion of the epidemiologically determined risk which could be accounted for by the levels of virus, *Cryptosporidium*, and *Giardia* present in the municipally supplied tap water | 0.21802 (83.9%) | 0.21802 (83.9%) |
| Difference between the actual epidemiologically determined risk of illness and the level of risk predicted by this estimation technique using data for levels of virus and protozoa in the water | | 0.04198 (16.1%) |

84% of the actual epidemiologically-determined risk. If we are able to make the assumption that the remaining 0.042 level of annual illness risk is due to bacterial contaminants in the distribution system water, then we can perform a backwards calculation to estimate the associated level of pathogenic bacteria in the distribution system water as shown in Table 3.14. According to this modeling approach, the residual individual annual illness risk of 0.042 would represent a level of approximately 11.67 pathogenic bacteria per liter in the community distributed tap water. As

**Table 3.14** For validation exercise 1: backwards calculation to estimate the level of infectious, pathogenic bacteria in the municipality's water, assuming that bacteria accounted for the risk not attributable to virus and protozoa

| | |
|---|---|
| Estimated annual risk of bacterially induced illness per individual consumer | 0.0420 |
| Probability of illness (per incidence of bacterial infection) | ÷ 0.4928 |
| Probability of infection (per ingested pathogenic bacterium capable of exhibiting growth on culture medium) | ÷ 0.00001 |
| Days of water ingestion per year | ÷ 365.25 |
| Liters of water ingested per person per day | ÷ 2.0 |
| Estimated concentration of organisms (pathogenic bacteria capable of exhibiting growth on culture medium) per liter of water following municipal treatment of the water | = 11.66698 |

[a]This back calculation does not include an estimation for the reduction of risk affected by municipal treatment of the water, because no data were provided for the extent to which pathogenic bacteria were removed by the process used at the water treatment plants

suggested earlier in this chapter, some of these bacteria could represent organisms that have persisted through the processing regimen at the community drinking water treatment plants. A second source of bacteria in water distribution systems is accidental contamination, which can result from breaks in water distribution lines and also from inadvertant cross connections with pipes that carry sewage. A third source of bacterial contaminants is the biofilm that grows on the inside walls of water distribution pipes and water storage tanks.

#### *3.4.4.2.2.2 Second Risk Validation Exercise*

Several years later, after efforts to improve the efficiency of that community's drinking water treatment facilities, Payment and coworkers performed a second epidemiological study (Payment et al. 1997) during which they found that individuals ingesting conventionally treated, community-distributed tap water had an overall 0.66 annual risk for incidence of gastrointestinal illness. This contrasted with an annual illness risk of 0.58 among a control group of consumers who drank either bottled water, or drank tap water that originated from the same community distribution system but had gone through an in-home filtration system prior to ingestion. The difference in annual risk thereby attributed to microorganisms in the tap water during the second epidemiological study was 0.08. The validation exercise presented in this chapter for the second epidemiological study of Payment et al. (validation 2) used virus and protozoa data directly published as a part of that second study

**Table 3.15** For validation exercise 2: calculating the estimation of risk due to infectious, pathogenic virus in the municipality's water

| | |
|---|---|
| Concentration of organisms (virus that are infectious for mammalian cells, enumerated as infectious units) per liter of raw water | 4.13 |
| Liters of water ingested per person per day | × 2.0 |
| Days of water ingestion per year | × 365.25 |
| Probability of infection (per ingested virus capable of infecting mammalian cells) | × 0.5 |
| Probability of illness (per incidence of viral infection) | × 0.5769 |
| Estimate of residual virus-associated disease risk following municipal treatment of the water (this value is included because the data provided for viral levels were determined for the water prior to processing at the treatment plant), calculated as 1.000000 − 0.999921 | × 0.000079 |
| Estimated annual risk of virus-induced illness per individual consumer | = 0.068749 |

(Payment et al. 1997). The calculation presented in Table 3.15 suggests that of the 0.08 annual risk of illness, the amount of that which could have been due to virus in the drinking water, per individual consumer, was 0.068749 and this suggests that virus in the drinking water could have accounted for approximately 85.9% of the observed cases of illness. The calculations presented in Tables 3.16 and 3.17 suggest that protozoa of the genera *Cryptosporidium* and *Giardia* respectively could have accounted for approximately 1.5% and 4.2% of the observed illnesses. The data which were published by Payment et al. (1997) concerning bacterial concentrations in the tap water for this study represent indicator bacterial groups rather than known waterborne bacterial pathogens. As such, the only way to estimate the proportion of observed infectious disease which would have been attributable to bacterial pathogens in the water during the time of the epidemiological studies is by subtraction.

Knowing the total risk due to pathogens in the drinking water and then subtracting the estimates for risks attributable to virus and protozoa results in the estimated proportion of illnesses due to bacterial pathogens in the tap water shown in Table 3.18. The backwards calculation presented in Table 3.19 suggests that the remaining 0.0067 estimated annual risk of illness per individual consumer potentially attributed to bacterially induced illness could have been accounted for by an estimated average concentration of 1.86116 bacterial organisms (pathogenic bacteria capable of exhibiting growth on culture medium) per liter of water following municipal treatment of the water. This validation process reveals that for the two

**Table 3.16** For validation exercise 2: calculating the estimation of risk due to *Cryptosporidium* in the municipality's water

| | |
|---|---|
| Concentration of *Cryptosporidium* oocysts (measured by visual inspection following immunofluorescent staining) per liter of raw water | 0.14 |
| Liters of water ingested per person per day | × 2.0 |
| Days of water ingestion per year | × 365.25 |
| Probability of infection (per ingested *Cryptosporidium* oocyst) | × 0.033 |
| Probability of illness (per incidence of cryptosporidial infection) | × 0.5538 |
| Estimate of residual *Cryptosporidium*-associated disease risk following municipal treatment of the water (this value is included because the data provided for *Cryptosporidium* oocyst levels were determined for the water prior to processing at the treatment plant), calculated as 1.000000 − 0.999369 | × 0.000631 |
| Estimated annual risk of *Cryptosporidium*-induced illness per individual consumer | = 0.001179 |

**Table 3.17** For validation exercise 2: calculating the estimation of risk due to *Giardia* in the municipality's water

| | |
|---|---|
| Concentration of organisms (*Giardia* cysts, enumerated by visual inspection following immunofluorescent staining) per liter of raw water | 2.00 |
| Liters of water ingested per person per day | × 2.0 |
| Days of water ingestion per year | × 365.25 |
| Probability of infection (per ingested *Giardia* cyst) | × 0.1 |
| Probability of illness (per incidence of infection by *Giardia*) | × 0.4617 |
| Estimate of residual *Giardia*-associated disease risk following municipal treatment of the water (this value is included because the data provided for *Giardia* cyst levels were determined for the water prior to processing at the treatment plant), calculated as 1.000000 − 0.999950 | × 0.000050 |
| Estimated annual risk of *Giardia*-induced illness per individual consumer | = 0.003373 |

**Table 3.18** For validation exercise 2: overall summary of risk estimations for the known levels of infectious, pathogenic virus and protozoans with the remaining risk assumed due to infectious, pathogenic bacteria

| | | |
|---|---|---|
| Total epidemiologically determined risk of illness per person per year associated with microbial contaminants in the municipally supplied tap water | | 0.08 (100.0%) |
| Estimated proportion of that risk attributable to virus in the municipally supplied tap water | 0.06875 (85.9%) | |
| Estimated proportion of that risk attributable to *Cryptosporidium* in the municipally supplied tap water | 0.00118 (1.5%) | |
| Estimated proportion of that risk attributable to *Giardia* in the municipally supplied tap water | 0.00337 (4.2%) | |
| Total proportion of the epidemiologically determined risk which could be accounted for by the levels of virus, *Cryptosporidium*, and *Giardia* present in the municipally supplied tap water | 0.07330 (91.6%) | 0.07330 (91.6%) |
| Difference between the actual epidemiologically determined risk of illness and the level of risk predicted by this estimation technique using data for levels of virus and protozoa in the water | | 0.00670 (8.4%) |

epidemiological studies published by Payment et al. (1991, 1997), bacterial pathogens could have accounted for approximately 16.1% of the observed cases of illness attributable to microorganisms in the community-distributed tap water for the first epidemiological study (Payment et al. 1991) and correspondingly could have accounted for 8.4% of the observed cases of illness for the second study (Payment et al. 1997).

**Table 3.19** For validation exercise 2: backward calculation to estimate the level of infectious, pathogenic bacteria in the municipality's water, assuming that bacteria accounted for the risk not attributable to viruses and protozoa[a]

| | |
|---|---|
| Estimated annual risk of bacterially induced illness per individual consumer | 0.0067 |
| Probability of illness (per incidence of bacterial infection) | ÷ 0.4928 |
| Probability of infection (per ingested pathogenic bacterium capable of exhibiting growth on culture medium) | ÷ 0.00001 |
| Days of water ingestion per year | ÷ 365.25 |
| Liters of water ingested per person per day | ÷ 2.0 |
| Estimated concentration of organisms (pathogenic bacteria capable of exhibiting growth on culture medium) per liter of water following municipal treatment of the water | = 1.86116 |

[a]This back calculation does not include an estimation for the reduction of risk affected by municipal treatment of the water, because no data were provided for the extent to which pathogenic bacteria were removed by the process used at the water treatment plants

## 3.5 Summary

Most disease transmission routes require that the causative microorganisms be exposed to the environment. The main purpose of this chapter was to demonstrate how mathematical models are used for describing the propagation of infectious diseases through populations, and to explain the various routes by which water associated infectious agents are transferred both to and between individuals. The major factor in determining whether an organism can be transmitted via a particular environmental route such as water, aerosols, or contact with the surface of objects, is whether or not that organism has evolved the ability to survive environmental exposure under the conditions it would encounter along that route. As an example, microorganisms which lack stability in environmental waters are unlikely to be transmitted by water. Some diseases can be transmitted by more than a single route. Tularemia, for example, can be acquired by ingesting contaminated food or water, inhalation of microbial aerosols, contact of bare skin with contaminated materials, and even acquired through insect bites.

Pathogenic microorganisms frequently can be found in source waters, and the ingestion of microbially contaminated water clearly has the potential for causing infectious disease. Microorganisms can escape even well operated, community based drinking water treatment processes. The results from this risk analysis technique indicate that direct water recycling may not be a good thing although indirect recycling occurs nearly everywhere.

This chapter described the means by which humans attempt to prevent the transmission of waterborne infectious agents of disease, and demonstrated two approaches which can be used to model the effectiveness of these prevention techniques. The first approach used historical data for typhoid to show how intercepting a primary route of disease transmission can dramatically reduce the incidence of that disease within a community even when there exist other, secondary routes for transmission of the disease. The use of a disease transmission model illustrated how this type of disease reduction can be accomplished. The second approach used a risk estimation technique.

Protozoan cysts and oocysts represent the microbial group most easily removed by filtration because of their relatively large size, although they are difficult to chemically disinfect. Virus are in particular difficult to remove by filtration because of their typically small size, although fortunately the virus seem inactivated with relative ease by using chlorine for chemical disinfection (Hurst 2001). The difficulty of virus removal by filtration, plus the fact that virus have a high probability of infection per ingested particle, likely are the reasons why virus seem to represent such a large amount of the risk from waterborne infections unless something such as a cholera outbreak is overwhelming the community.

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Gunther Franz Craun

### Compliance with Ethical Standards

**Conflict of Interest** Christon J. Hurst declares that he has no conflict of interest.

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# Chapter 4
# The Ecology of Bacterial Agents of Foodborne Illness


**Alexander Gill and John W. Austin**

**Abstract** Eating and drinking are essential to human survival but come with an inherent level of risk. To intake nutrition, humans must introduce a foreign substance into their bodies, and this substance may be contaminated by either infectious microorganisms or microbial toxins, threats which are undetectable to human senses. Bacterial agents of foodborne illness can be divided into two groups, foodborne pathogens which infect humans and producers of foodborne toxins. For the microbiologist concerned with the bacterial agents of foodborne illness, an understanding of the ecology of these organisms is fundamental to addressing the practical issue of reducing human exposure to these organisms or their toxins. It is important to note that bacterial agents of foodborne illness are not obligate human pathogens. Humans are not a definitive, or even necessarily a desirable, host for these microorganisms, and the microorganisms may thrive in a variety of environments without ever coming into contact with human populations. Thus, the potential for these organisms to cause human illness is dependent on ecological conditions that bring the human and pathogen into contact.

## 4.1 Introduction

Though the nature of foodborne diseases and probability of risk vary regionally, depending upon the local hygiene infrastructure and food preparation customs, there is no region in which foodborne illness is not a significant contributor to morbidity and mortality. It has been estimated that, in the United States, 48 million people suffer from foodborne illness each year, with the economic cost exceeding $15.5 billion dollars annually (Hoffmann et al. 2015). In countries with weak public health and hygiene infrastructure, the impacts can be more severe. While the full burden of

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foodborne illness in low- and middle-income countries is not known (Grace 2015), the African, Southeast Asian, and Eastern Mediterranean regions have been reported to have the highest foodborne illness burden (Havelaar et al. 2015). In addition to the human cost of illness, food producers and distributors can suffer significant financial losses in responding to contamination events. The potential scale of such losses is illustrated by the proposal by European Union officials in 2011 to provide 150 million € in compensation to vegetable producers whose products were destroyed in the response to an outbreak of pathogenic *Escherichia coli*. In this chapter we discuss how an understanding of the ecology of the bacteria which cause foodborne illness can be used to identify potential food safety risks and strategies to prevent human exposure.

The term "oecologie" (ecology) was introduced to the scientific community by the German zoologist Ernst Haeckel in 1866 (Haeckel 1866). Based on the root of the Greek word *oikos* meaning house, he wrote: "By ecology we mean the body of knowledge concerning the economy of nature—the investigation of the total relations of the animal both to its inorganic and organic environment." Thus, ecology can be understood as the study of the interrelationships that occur between organisms and between organisms and their physicochemical environment.

To simplify our discussion of this topic, we have divided the ecology of foodborne bacterial pathogens into two domains. These domains are the ecology of the bacterial agent in the food production system and the ecology of the bacterial agent in the food product. The ecology of bacteria in the food production system includes identification of environments and hosts (plant and animal) which are potential sources of the bacterial agents, vectors for transmission onto food ingredients, and environmental factors which determine the potential for survival and replication of the organisms within the food processing environment. The ecology of bacterial agents in the food product is dependent upon those factors which determine the microbes' survival and replication in foods and includes factors intrinsic to food (pH, water activity, oxidation-reduction potential, nutrients, and antimicrobials), extrinsic to food (temperature, relative humidity, and gaseous environment), and interrelationships among microorganisms. The chapter concludes with several examples of foodborne pathogenic bacteria and their ecology in foods and food production systems.

## 4.2 The Ecology of Pathogens in the Food Production System

Bacteria may be introduced to foods at multiple points in the chain of production, processing, distribution, and consumer preparation. Unless controlled, once introduced bacteria may replicate on foods, resulting in an amplification of health risk and the potential for further distribution of the pathogen to other foods or food contact surfaces. Distribution and preparation are the stages in which the prevention of

**Table 4.1** Minimum and optimum growth conditions of selected foodborne pathogens

| Organism | Temperature (°C) (minimum/optimum) | pH (minimum/optimum) | Minimum water activity |
|---|---|---|---|
| *Bacillus cereus* | 4/30–40 | 4.8–5.0/6.0–7.0 | 0.93 |
| *Campylobacter jejuni* | 30/42–43 | 4.8/6.5–7.5 | 0.987 |
| *Clostridium botulinum* Group I | 10/35–40 | 4.6/7 | 0.94 |
| *Clostridium botulinum* Group II | 3.0/28–30 | 5/7 | 0.97 |
| *Clostridium perfringens* | 4/43–47 | 5/7.2 | 0.93–0.97 |
| *Escherichia coli* | 7–8/35–40 | 4.4/6–7 | 0.95 |
| *Listeria monocytogenes* | 0/37 | 4.6–5.0/7.0 | 0.90–0.93 |
| *Salmonella* | 2–7/35–43 | 3.8/7.0–7.5 | 0.93–0.94 |
| *Staphylococcus aureus* | 6.7–7/37 | 4.0/6.0–7.0 | 0.83–0.85 |
| *Yersinia enterocolitica* | −1.3/25–37 | 4.2/7.2 | 0.95 |

Minimum/optimum data from ICMSF (1996) and Stern et al. (1980)

contamination is in principle most easily achieved and safety of the product is largely dependent on appropriate packaging and handling. In contrast production and processing are the stages where initial contamination is most likely and in which process hygiene is most difficult to control.

## *4.2.1 Agricultural Environment*

The agricultural environment is a common origin for foodborne pathogens. Infectious foodborne pathogens are adapted to colonize warm-blooded hosts, with optimum growth rates typically either at or near body temperature (Table 4.1). Host animals provide an opportunity for bacteria to replicate to high concentrations in a nutrient-rich environment. The pathogen may be dispersed between livestock, wild animals, and humans by direct contact or indirectly via contaminated water or feed. Consequently, though foodborne pathogens may be distributed throughout the environment, replication within animal hosts plays an important role in their survival and distribution.

### 4.2.1.1 Poultry and Red Meats

The muscle tissue of healthy animals is effectively sterile; thus, the presence of foodborne pathogens on meat is dependent upon transfer to exposed tissue surfaces during carcass dressing and processing. Pathogens transferred to meat during these processes typically originate from populations colonizing the outer surface (skin, hide, and feathers), the gastrointestinal tract, or the esophagus (Gill and Gill 2012).

The probability of such transfer is dependent upon the hygiene of carcass dressing and breaking. The degree of hygiene achievable with good manufacturing practices varies between animals and with the diligence of individual operators. Microflora transfer is easier to control with large ruminants such as cattle than with pigs or poultry. The expectations of process hygiene are reflected by pathogen testing standards for different meat products (Gill and Gill 2014). Exceptions to this rule are the obligate pathogens *Brucella* and *Mycobacterium* which infect the internal tissues, but these bacteria cannot replicate in the tissue of dead animals. Some specialized products are prepared from tissues which, unlike muscle tissue, have a significant native microflora (e.g., pig's head, chitterlings). The initial microflora of these products cannot be controlled by harvesting practice.

#### 4.2.1.2 Seafood

As with red meats and poultry, the flesh of healthy finfish, molluscs, and crustaceans is essentially sterile, but bacterial pathogens may be present on the skin, gills, gastrointestinal tract, or other surfaces exposed to the environment. Processing may remove contaminated tissues, but typically no effort is made to prevent the transfer of microflora to other tissues during processing operations.

Of the foodborne pathogens associated with seafood, *Vibrio* spp. and *Clostridium botulinum* type E are native to aquatic environments. The presence of foodborne pathogens which cannot replicate but can survive for extended periods in natural waters (*Listeria monocytogenes*, *Salmonella*, and *Yersinia*) is indicative of contamination of water systems with agricultural runoff or sewage (Novoslavskij et al. 2016).

#### 4.2.1.3 Other Foods of Animal Origin

Potentially any pathogens which colonize dairy livestock, or are present in the production environment, may contaminate milk. Milk is invariably contaminated with microflora present on the epidermis of the udder. This may include skin flora such as Staphylococci and enteric organisms such as *E. coli*, *Salmonella*, and *Campylobacter* (Oliver et al. 2005). Microflora may also be introduced from milking equipment or from infected udder tissues. These sources of contamination can be minimized, but not eliminated, by good hygienic practice and maintenance of animal health.

The shell of eggs may become contaminated after laying, with a wide variety of organisms from the production environment. The eggshell presents an exposed environment, unsuitable for the survival of most pathogens, and the shell can be decontaminated by chemical wash treatments. The primary pathogen associated with eggs is *Salmonella*, which can colonize the reproductive tract and be vertically transmitted during egg formation (Keller et al. 1995). *Salmonella* can also be

internalized to the egg through pores in the shell, and this route has been proposed for other pathogens, including *Campylobacter* (Cox et al. 2012).

#### 4.2.1.4 Fruits and Vegetables

The control of contamination during growth and harvest is a particular concern with fruits and vegetables which are consumed raw, without the removal of an external skin or rind. Options for postharvest decontamination are highly limited; at a commercial scale, the only widespread method is washing with chlorine (Erickson 2012). Washing with sanitizers is not particularly effective and may only serve to redistribute pathogens, spreading contamination from one or two plants to a whole production lot (Zhang et al. 2009; Danyluk and Schaffner 2011). Consequently, products such as lettuce, spinach, kale, herbs, tomatoes, cantaloupes, and watermelon are increasingly recognized as causes of bacterial foodborne illness (Erickson 2012). In the United States in 2012, plant products were identified as a vehicle for 33% of foodborne outbreaks, accounting for 42% of total cases of reported foodborne illness (Bennett et al. 2014).

Contamination of fruits and vegetables with bacterial pathogens may occur before or following harvest. Prior to harvest bacteria can be introduced from the soil; manure; irrigation water; inputs such as fungicides, insecticides, and fertilizers; fecal shedding by wild and domestic animals (including birds, reptiles, and insects); and human sewage (Olaimat and Holley 2012). During harvesting bacterial pathogens may be introduced from equipment and wash water and by workers with inadequate hygiene (Olaimat and Holley 2012).

The bacterial pathogens primarily associated with outbreaks involving fruits and vegetables are *Salmonella enterica*, *E. coli*, *L. monocytogenes*, *Shigella*, and *Listeria monocytogenes*, along with *Clostridium* and *Bacillus* spp. which are commonly part of the microflora of soil and plants. It should be noted that of these listed microorganisms, *Shigella* are host specialized for humans and are not well adapted for prolonged survival in external environments; therefore, the presence of *Shigella* is indicative of direct or indirect contamination with human sewage. Pathogenic *E. coli* and *Salmonella enterica* have a wide host range and can survive for prolonged periods outside of an animal host but are not thought to constitute part of the normal microflora of plants. The risk posed by these pathogens can be reduced by efforts to control exposure of crops to domestic and wild animals and by strict monitoring and control over the hygiene of irrigation and wash waters.

The risk of illness is not equal for all commercially exploited species of plants. Sprouts are generally recognized as posing a particular risk as the seeds are germinated and grown under conditions of temperature and humidity which will support the replication of enteric pathogens such as *Salmonella enterica* and *E. coli*. The largest reported foodborne outbreak of verotoxigenic *E. coli* occurred in Europe in 2011. In Germany alone there were over 3800 cases of serious illness and 53 deaths (Radosavljevic et al. 2015). The outbreak was eventually traced to fenugreek sprouts (Radosavljevic et al. 2015).

While small populations of endophytic microorganisms have been recovered from some plant species (Samish and Etinger-Tulczynska 1963), it was thought that the internal tissues of plants are essentially sterile, and spoilage and pathogenic bacteria were localized to the plant surface. More recently, microorganisms responsible for foodborne illness have been demonstrated to penetrate the internal tissues of a wide range of plants, including leafy greens, tomatoes, mung beans, pecans, almonds, and some fruits (Deering et al. 2012). Internalization may occur via leaf stomata or lateral root junctions, but only when high concentrations of enteric pathogens are present. Internalized bacterial cells are protected prior to harvest from UV light and desiccation and are also protected from postharvest decontamination efforts (Olaimat and Holley 2012). The significance of enteric pathogens internalized to plant tissues as a cause of foodborne outbreaks remains contentious.

The risk associated with fruits and vegetables may be on the increase due to changes in the way these products are processed and consumed. In some markets an increasing proportion of fruit and vegetable products are sold as prepared convenience foods, such as premade salads. These products are minimally processed with vegetables and fruits washed and precut. Tissue damage from cutting or peeling releases nutrients that can support bacterial replication (Harris et al. 2003). Packaging can maintain product humidity to reduce drying stress on the product, but this will simultaneously protect contaminating pathogens from dissection stress and possibly maintain water activity above the minimum threshold for growth (Harris et al. 2003). Under such conditions there is a significant risk of amplification of pathogen numbers on contaminated product during storage and distribution, particularly if storage temperature is not adequately controlled.

### *4.2.2 Processing Environment*

The degree of processing that foods undergo prior to distribution to consumers is highly variable. The spectrum ranges from fresh fruits and vegetables that may simply have inedible material trimmed away and be washed to remove soil and dust to products composed of multiple ingredients which undergo complex multistep processing. An example of the latter is salami and other traditional fermented meats, which are composed of fat and muscle tissue from multiple animal species, starter culture, multiple flavoring spices, carbohydrate, and salts. Processing of these fermented meat products often consists of chopping raw meat, mixing ingredients, stuffing the resulting batter into casings (animal intestine or synthetic), fermentation, drying, and smoking. The final microflora of the product is dependent upon these processes. In the course of processing, there are multiple opportunities for microorganisms to be transferred between the food, ingredients, processing equipment, and environmental surfaces.

Though processing environments are complex and vary considerably, there are some principles to consider which determine the risk potential for pathogens, specifically whether the level of risk is constant, reduced, or amplified. A potential

for risk amplification exists because the food itself provides nutrients to support bacterial replication. It is not uncommon that replication of bacteria can occur during processing. The source of such bacteria may be the food itself, or transferred from equipment or environmental surfaces, including the clothing and protective equipment of workers (Yang et al. 2015; Youssef et al. 2013).

Whether a pathogen can replicate during food processing depends upon the environmental conditions and the adaptability of the specific organism. Pathogens may colonize the processing environment and replicate in transient communities or form persistent communities which can serve as an ongoing source of contamination. Pathogens commonly associated with this behavior include *E. coli* (Yang et al. 2015), *Salmonella enterica* (Podolak et al. 2010), and *L. monocytogenes* (Malley et al. 2015).

Bacteria transferred onto equipment surfaces in the processing environment will initially attach as single cells. Such single cells are relatively vulnerable to desiccation and removal by sanitation processes, but if growth of the bacteria occurs, a bacterial biofilm can form. Biofilms are complex communities of bacterial cells embedded in an extracellular matrix which form at the interface between two phases (solid-liquid, solid-air, liquid-air) (Stoodley et al. 2002; Costerton et al. 1995). Attachment of *Salmonella* to food processing surfaces and to other *Salmonella* may occur via amyloid fibers produced on the cell surface of *Salmonella* and other enteric bacteria that facilitate adherence to biotic and abiotic surfaces, leading to biofilm development (Austin et al. 1998b). Foodborne pathogens may form biofilms alone or be members of mixed species biofilms, in either case the cells which compose the biofilm typically display far greater resistance to stresses such as antimicrobials, desiccation, oxygen levels, and physical removal (Giaouris et al. 2015).

Control of pathogens in the food processing environment can be achieved through controlling the hygiene of the food production system. The specific details of a successful hygiene control system must match the specific physical site, production inputs, and the needs of the processor. It should be developed and applied in a systematic manner, such as Hazard Analysis and Critical Control Point (Hulebak and Schlosser 2002). However, four basic principles can be identified: prevention or minimization of bacterial transfer, prevention of bacterial replication, decontamination of the food, and sanitation of the production environment, including equipment and surfaces.

Bacteria can be transferred by any physical contact between solid surfaces, fluids, and aerosols generated by processing operations. The transfer of bacteria may introduce pathogens to foods or surfaces in the processing environment from which pathogens can circulate back to the food. Transfer can be minimized by the use of physical barriers (separation of operations, closed pipes), minimizing processing operations, and minimizing the distance food must travel through the processing environment.

There are limited means to control the growth potential of bacteria on the food itself during processing. The availability of free water can be minimized, by keeping processing as dry as possible. Free water may be present as a component of the raw

food, as an exudate, added as an ingredient or used to rinse the food and production surfaces. Temperature may be reduced to control growth potential but must also be high enough to provide a tolerable working environment.

Food products can be decontaminated to reduce pathogen numbers present. Decontamination is not possible with all foods as there are limitations on the effective treatments available due to the necessity to preserve sensory qualities or to conform to with regulatory requirements. To be successful, decontamination must be combined with prevention of bacterial transfer to prevent recontamination. An example of this approach is milk pasteurization. Milk cannot be harvested aseptically and will inevitably be contaminated with microflora. The raw milk is pumped through a pasteurizer, which applies a thermal treatment which is typically sufficient to kill pathogens associated with milk. The pasteurized milk is then transferred through a closed system to directly fill containers for commercial distribution.

The purpose of the fourth principle, sanitation of processing surfaces, is commonly thought of as killing microorganisms, but the primary role of sanitation is to deny habitat to bacteria in the processing environment. Ideally, equipment and surfaces should be designed for "cleanability," which is a combination ensuring exposure to sanitation treatments and physical removal of soil and attached cells. The removal of organic material reduces the potential for bacterial attachment and biofilm formation. Cell attachment is promoted by a coating of organic material, and nutrients are required for microbial cell replication and biofilm formation. Similarly, water is essential for the survival and replication of bacteria, and so drying of surfaces can effectively control pathogen colonization. Depending upon the type of equipment, it either may be cleaned in place (CIP) or dismantled for cleaning. Available CIP regimes vary in effectiveness in eliminating adherent bacteria (Austin and Bergeron 1995; Bremer et al. 2006), depending upon the cleaning agent used, contact time, cleaning temperature, and design of the processing equipment. Though less commonly used, equipment surfaces can also be decontaminated by physical processes such as heat (dry or steam) or UV light.

## 4.3 Survival and Replication of Bacteria in Foods

The study of microbial ecology of foods began in the 1930s. Haines (Haines 1933; Haines and Elliot 1944) and later Ingram (1949) and Westerdijk (1949) were the pioneers in microbial ecology of foods. Many of the original studies of the primary factors affecting food spoilage, such as temperature, pH, water activity, redox potential, and gaseous environment, were carried out in the two decades following the Second World War (Barnes 1994). Prior to this pioneering work on microbial ecology of foods, it was thought that most species of microorganisms could be isolated from nearly any food. This belief was based upon the isolation of microorganisms, in the absence of information on their relative abundance (Mossel et al. 1995). Westerdijk (1949), while examining spoilage fungi on decomposing organic

substrates, was the first to apply the idea of "association," or a typical microflora associated with specific substrates.

### *4.3.1 Properties Intrinsic to Foods*

Characteristics of foods play an important role in controlling bacterial growth. These characteristics are intrinsic to the food and include pH of the food, availability of water, oxidation-reduction potential of the food, the presence of naturally occurring antimicrobials, and interactions between microorganisms within the food. Foodborne microorganisms require specific conditions for growth (Table 4.1). Control of the growth of these microorganisms is often achieved by a combination of factors rather than a single factor. The use of combinations of inhibitory factors, such as refrigeration temperature, reduced water activity, lowered pH, and low redox potential to control growth of foodborne microorganisms, is referred to as "hurdle technology" (Leistner and Gould 1994).

#### 4.3.1.1 pH

Acidification of food by the addition of organic acids is the primary means of preventing the growth of human pathogens in a wide range of fermented and acidified ready-to-eat foods. Most microorganisms common to foods grow optimally at neutral pH (pH values from 6.6 to 7.5). The minimum pH limits for the growth of selected food pathogenic bacteria are listed in Table 4.1.

Acid tolerance of microorganisms is dependent upon several factors including the nutrient content and physical nature of the food itself, temperature, nature of the acidulent, the presence of preservatives, water activity, and redox potential. Some foodborne bacteria exhibit an acid tolerance response that allows them to survive typically lethal levels of acidity (Rowbury and Goodson 1998; Alvarez-Ordóñez et al. 2015). The minimum pH allowing growth of bacteria is usually dependent upon the type of acid used to adjust the pH. Hydrochloric acid and citric acid often allow growth at lower pH than acetic acid or lactic acid (Smelt et al. 1982; Álvarez-Ordóñez et al. 2010).

#### 4.3.1.2 Water Activity

Water activity ($a_w$) may be thought of as the free water within a food which is available to microorganisms. Within foods, water is present in both bound and free forms. The bound water is involved in the hydration of molecules and dissolution of ions in foods and is not available for microbial growth. The free water is not involved in interactions with the food and its constituents and is available for microbial growth. Water activity is defined as the ratio of the water vapor pressure

of the food ($p$) relative to the vapor pressure of pure water ($p0$) at the same temperature, i.e., $a_w = p/p0$. Values for water activity range from 0 to 1 (Hurst et al. 1979).

The water activity of foods can be reduced by reducing the moisture content (drying) or by the addition of solutes. In traditional food preservation, the solutes most commonly used are sodium chloride or sugar, the latter often in the form of either sucrose or honey. The solute used to control $a_w$ may influence the lower $a_w$ limits for growth. In the case of *C. botulinum*, NaCl, KCl, glucose, and sucrose show similar effects, while glycerol allows growth at lower $a_w$ (Sperber 1983). The minimum $a_w$ required for growth may be raised significantly by other factors, such as increased acidity or the use of preservatives (Baird-Parker and Freame 1967).

Decreasing water activity is an effective means of preventing the growth of spoilage organisms and bacterial pathogens on many processed or prepared foods. The water activity of most fresh foods including meat, fish, fruits, vegetables, milk, and eggs is 0.98–0.99. Breads, fruit juices, salted fish, sausages, and cheeses have water activities in the range of 0.93–0.98. Fermented or dry-cured meats have water activity in the range of 0.85–0.93. Fruit preserves typically have water activities of 0.75–0.80, and honey, dried egg, and chocolate have water activities below 0.60. The limiting water activity for most spoilage bacteria is 0.91, and most foodborne pathogens require a minimum water activity for growth of 0.93 or higher (Table 4.1). Exceptions are *Staphylococcus aureus*, which may grow at water activities as low as 0.83, and *Listeria monocytogenes* which may grow at water activities as low as 0.90. However, low water activity alone cannot ensure safety as some foodborne pathogens such as *Cronobacter*, *Salmonella enterica*, and *Listeria monocytogenes* are desiccation resistant and can survive for prolonged periods in foods with water activities well below the minimum required for growth.

#### 4.3.1.3 Oxidation-Reduction (Redox) Potential

Oxidation-reduction potential is the ease with which a substrate loses or gains electrons. When a substrate loses electrons, it becomes oxidized, while if a substrate gains electrons, it is reduced. Oxidation-reduction reactions are coupled reactions in which one substrate is oxidized and a second substrate is simultaneously reduced. The electron donor, since it reduces an oxidized substrate, is a reducing agent. The electron acceptor, since it oxidizes a reduced substrate, is an oxidizing agent. The redox potential, termed the "Eh," is measured in millivolts (mV). Oxidized substrates have larger mV values (+mV), while reduced substrates have lower values (−mV).

The redox potential of a food is dependent upon the chemical composition of the food and the storage atmosphere. Fresh foods undergoing respiration will be in the reduced state. The presence of reducing substances such as ascorbic acid, reducing sugars, and the sulfhydryl group of amino acids also contributes to a reduced state. When respiration stops, and oxygen diffuses in, fresh foods reach an oxidized state. Foods stored in air will have a larger Eh (+mV) than foods stored either under

vacuum or in a modified atmosphere containing elevated levels of either $CO_2$ or $N_2$ or reduced $O_2$.

While it is commonly assumed that *C. botulinum* cannot grow in foods exposed to $O_2$, the Eh of most foods is usually low enough to allow its growth (Kim and Foegeding 1993). *Clostridium botulinum* grows optimally at an Eh of −350 mV, but its growth initiation may occur in the range of +30 to +250 mV (Kim and Foegeding 1993).

#### 4.3.1.4 Natural Antimicrobials in Foods

Foods are highly complex in their chemical composition and may contain compounds with antimicrobial activity at sufficient concentration to impact the growth and survival of bacteria. The list of natural antimicrobials in foods is extensive, and a variety of naturally occurring antimicrobial systems include those derived from both animal and plant tissues.

Agents considered to be natural antimicrobials are diverse and include enzymes such as lysozyme and lactoperoxidase; iron-binding proteins such as lactoferrin, ovotransferrin, and lactoferricin; low molecular weight hydrophobic molecules and phenolics from plants such as eugenol, carvacrol and thymol, and phytoalexins; and peptides produced by bacteria such as nisin (Beuchat and Golden 1989; Juneja et al. 2012; Holley and Patel 2005). Some of these agents are established in commercial use, such as lysozyme and nisin which are both used to control growth of Gram-positive bacteria. Others are part of the normal composition of foods and may impact the growth and survival of microflora, including pathogens. Examples of this class are lactoferrin in milk (Masson and Heremans 1971; Rainard 1986), ovotransferrin in eggs (Wu and Acero-Lopez 2012), and the plant oil phenolics (Holley and Patel 2005).

#### 4.3.1.5 Interactions Between Microorganisms

Foods typically have a complex microflora which can significantly impact the survival and replication of foodborne pathogens by either inhibiting or enhancing growth.

Fermentation is one of the oldest forms of food preservation. Fermented foods such as bread, cheese, and wine have been produced for thousands of years and are strongly linked to culture and tradition. Lactic acid inhibits bacterial growth by lowering the pH of food. Lactic acid bacteria (LAB), those which produce lactic acid, include the genera *Lactobacillus*, *Lactococcus*, *Pediococcus*, and *Leuconostoc*. The various species of LAB produce lactic acid by utilization of hexoses via the Embden-Meyerhof pathway. The fermentative processes involved in the manufacture of sauerkraut, olives, yoghurt, and sausage all produce lactic acid as the primary acid (Blom and Mortvedt 1991). In addition to lactic acid, antimicrobial metabolic

products produced during fermentations include propionic and acetic acids, diacetyl, hydrogen peroxide, fatty acids, aldehydes, and other materials such as bacteriocins.

Destruction of competing flora may lead to unrestricted growth of a pathogen. "Pasteurization" of fish by irradiation enhances the growth of *C. botulinum* (Cann et al. 1966), probably by reduction of the normal spoilage flora which would compete with *C. botulinum*. Microorganisms with high metabolic activity may consume nutrients making them unavailable to less active organisms. Streptococci may inhibit *Staphylococcus* by consuming the B vitamins nicotinamide, niacin, and biotin (Iandolo et al. 1965; Haines and Harmon 1973).

In some cases, microorganisms may facilitate the survival or growth of foodborne pathogenic bacteria. For example, some species of free-living protozoans—such as *Acanthamoeba castellanii*, *A. polyphaga*, and *Tetrahymena pyriformis*—can act as hosts of pathogenic bacteria (Vaerewijck et al. 2014). The simultaneous finding of free-living protozoans and several enteric pathogenic bacteria on dishcloths has been used to suggest the possibility that the contaminating bacteria may have survived within the protozoans (Chavatte et al. 2014).

Metabolic activity by other flora can result in changes to the physicochemical properties of food that in turn will promote growth of the pathogen. The pH of acidic foods can be increased by the metabolic activity of some microflora, raising the pH above the level which restricts pathogen growth. For example, the growth of molds has been reported to increase the pH of tomato products sufficiently to permit the growth of *C. botulinum* (Huhtanen et al. 1976). A similar metabiotic relationship has been demonstrated where growth of proteolytic molds in tomatoes (Wade and Beuchat 2003; Wade et al. 2003) or cantaloupe (Richards and Beuchat 2005) increases the pH and promotes the growth of *Salmonella enterica*.

## *4.3.2 Properties Extrinsic to Foods*

### 4.3.2.1 Temperature

All microorganisms have temperature-defined growth limits. Cell replication ceases below a minimum temperature, maximum growth rate is observed at an optimum temperature, and there is a maximum growth temperature above which replication ceases and thermal inactivation of the microbial cells occurs. Foodborne bacterial pathogens are almost exclusively mesophilic, with their optimum growth temperatures being between 30 and 40 °C and their minimum growth temperatures typically around 5 °C (Table 4.1).

Maintaining either food or the processing environment below the minimum growth temperature by refrigeration or freezing is an effective method of preventing pathogen replication and toxin production. To be an effective control on pathogens, temperature must be maintained below the growth minimum throughout food processing and storage. Given the complexity of food production and distribution, this temperature requirement can be challenging, and significant growth or toxin

production can occur within even short periods of intermittent temperature increase. Similarly, in processing environments where the ambient temperature is maintained below the growth minimum, microbial replication can occur on equipment or surfaces that are heated by either friction or heat conduction.

Thermal treatments such as cooking and pasteurization are among the most common means used to decontaminate foods. The spores of *Bacillus* and *Clostridium* are considerably more heat resistant than either their own vegetative cells or the vegetative cells of food associated flora. Consequently, there is a potential for these spores to survive heat treatment and then outgrow in foods once the food has cooled (Gilbert et al. 1974).

#### 4.3.2.2 Gaseous Environment

Modified atmosphere packaging (MAP) is increasingly used to extend the shelf life and improve the quality of foods. MAP systems include vacuum packaging and packaging under defined gas mixtures utilizing oxygen, carbon dioxide, nitrogen, and argon (Gill and Gill 2010). It is known that MAP achieves shelf life extension primarily by reducing the availability of oxygen to aerobic microorganisms, thereby inhibiting the growth of spoilage bacteria. Carbon dioxide is inhibitory to the growth of bacteria by dissolving in water to form carbonic acid, lowering the pH (Gill and Gill 2005). Nitrogen and argon are inert gasses, which are used to flush out oxygen and provide a headspace (Gill and Gill 2005; Spencer 2005).

The bacterial pathogens of concern with MAP foods, particularly ready-to-eat foods, are *C. botulinum* and *L. monocytogenes.* Replication of *Enterobacteriaceae*, including pathogens such as *E. coli*, *Salmonella enterica*, *Shigella*, and *Yersinia*, is inhibited at pH 5.8 or below under anaerobic conditions (Gill and Gill 2005). A pH below 5.8 is typical of fresh red and fermented meats.

MAP ready-to-eat foods present a risk for botulism, as the low-oxygen environment of MAP allows the growth of *C. botulinum* and inhibits the growth of spoilage organisms. Thus, the consumer cannot rely on spoilage to indicate the food is not safe for consumption. Ready-to-eat foods, by definition, do not receive a heating process prior to consumption. Any neurotoxin that may be formed during storage of the food is not inactivated by cooking. The high incidence of type E spores in fish (Dodds and Austin 1997) has made the safety of MAP smoked fish a particular concern. *Clostridium botulinum* can grow and produce neurotoxin in MAP fish, and, depending on conditions, neurotoxin may be present before the fish is considered spoiled (Dufresne et al. 2000).

**Table 4.2** Incidence rate of illness from selected foodborne pathogens, per 100,000

| Pathogen | USA (2014) | Canada (2013) | European Union (2013) | Australia (2011) |
|---|---|---|---|---|
| *Brucella* | NA | 0.05 | 0.11 | NA |
| *Campylobacter* | 13.45 | 29.14 | 64.8 | 115.8 |
| *Listeria* | 0.24 | 0.36 | 0.44 | 0.3 |
| *Salmonella* | 15.45 | 17.57 | 20.4 | 54.3 |
| *Shigella* | 5.81 | 1.94 | NA | 2.2 |
| Verotoxigenic *Escherichia coli* | 2.35 | 1.8 | 1.59 | 0.4 |
| *Vibrio* | 0.45 | NA | NA | NA |
| *Yersinia* | 0.28 | NA | 1.94 | NA |

NA: Not available

USA 2014—CDC Food, Foodborne Diseases Active Surveillance Network (FoodNet), United States http://www.cdc.gov/foodnet/trends/tables-2014.html

Canada 2013—Public Health Agency of Canada—Notifiable Diseases On-Line—http://www.cdc.gov/foodnet/trends/tables-2014.html

European Union 2013. European Food Safety Authority. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013. EFSA Journal 2015;13(1):3991. doi: https://doi.org/10.2903/j.efsa.2015.3991

Australia 2011. The OzFoodNet Working Group. 2015. Monitoring the incidence and causes of diseases potentially transmitted by food in Australia: Annual report of the OzFoodNet network, 2011. Commun Dis Intell 2015;39(2):E236–E264

## 4.4 Case Studies

Both the reported incidence rate (Table 4.2) of specific foodborne diseases and their association with foods (Table 4.3) vary between pathogen species. Below is presented a series of case studies in which the relationship between selected foodborne pathogens and the foods they are associated with is discussed. These case studies are intended to illustrate how the hazards associated with particular combinations of pathogen and food can be understood, including why some combinations can be effectively controlled, whereas others remain a challenge.

In some of the case studies below, a value for infectious dose is presented as an indicator of the relative risk of infectivity by the pathogens. Infectious dose is not the minimum number of pathogen cells necessary to cause illness but is the number of pathogen cells required to result in a high probability (50–100%) of infecting the host (Hurst et al. 1996). Infection in volunteer exposure studies may be determined by an increase in antibody titer or shedding of the pathogen. Estimates may also be made from outbreak investigations, but these results are not consistent with volunteer studies and often indicate lower infective doses (Hurst et al. 1996; Todd et al. 2008).

The reciprocal of the infectious dose can be viewed as the probability that a single cell can establish an infection (Hurst et al. 1996). For example, if a potential host is exposed to a single cell of a pathogen with an infectious dose of 1000 cells, the probability of infection is 0.001. Variables known to affect the probability of

**Table 4.3** Identified vehicles of foodborne disease outbreaks reported to the US-CDC (1998–2013) (http://wwwn.cdc.gov/foodborneoutbreaks/)

| | | | Meat (%) | Fresh fruit and vegetables (%) | Dairy (%) | Seafood (%) | Eggs (%) | Cold prepared [a] (%) | Notable associations (%) |
|---|---|---|---|---|---|---|---|---|---|
| Infectious pathogens | | | | | | | | | |
| *Campylobacter* | Outbreaks | 245 | 30.6 | 9.0 | 51.0 | 2.9 | 0 | 4.1 | Poultry (21.2) |
| | Cases | 6101 | 15.3 | 11.6 | 59.3 | 8.2 | 0 | 4.2 | Poultry (7.9) |
| Pathogenic *E. coli* | Outbreaks | 326 | 48.2 | 27.9 | 8.3 | 1.2 | 0 | 3.7 | Beef (39.0) |
| | Cases | 9127 | 29.6 | 39.8 | 6.9 | 0.8 | 0 | 12.0 | Beef (20.4) |
| *Listeria* | Outbreaks | 35 | 20.8 | 10.4 | 33.3 | 4.2 | 0 | 2.1 | |
| | Cases | 648 | 31.1 | 26.2 | 21.3 | 1.0 | 0 | 8.0 | |
| *Salmonella* | Outbreaks | 1265 | 41.3 | 17.4 | 7.0 | 5.1 | 10.0 | 2.1 | Poultry (23.0) |
| | Cases | 44,852 | 33.1 | 29.3 | 5.6 | 2.9 | 11.1 | 1.5 | Poultry (18.6) |
| *Shigella* | Outbreaks | 155 | 28.2 | 28.2 | 2.8 | 8.5 | 0 | 23.9 | |
| | Cases | 7278 | 15.4 | 46.6 | 2.7 | 1.8 | 0 | 31.9 | |
| *Vibrio* | Outbreaks | 126 | 0.8 | 0 | 0 | 98.4 | 0 | 0 | Raw seafood (52.4) |
| | Cases | 1617 | 2.9 | 0 | 0 | 96.8 | 0 | 0 | Raw seafood (48.6) |
| *Yersinia enterocolitica* | Outbreaks | 11 | 90.9 | 0 | 9.1 | 0 | 0 | 0 | Pork (81.8) |
| | Cases | 103 | 84.5 | 0 | 15.5 | 0 | 0 | 0 | Pork (75.7) |
| Foodborne intoxication | | | | | | | | | |
| *Bacillus cereus* | Outbreaks | 440 | 35.9 | 6.6 | 2.5 | 3.9 | 0 | 6.4 | Rice (27.7) |
| | Cases | 5706 | 44.6 | 4.1 | 3.7 | 4.8 | 0 | 4.5 | Rice (23.8) |
| *Clostridium botulinum* [b] | Outbreaks | 47 | 8.5 | 2.1 | 0 | 38.3 | 4.3 | 0 | Preserved vegetables, fish, traditional northern foods, prison-prepared alcohol (pruno) |
| | Cases | 166 | 7.2 | 1.2 | 0 | 30.7 | 3.0 | 0 | |
| *Clostridium perfringens* | Outbreaks | 729 | 67.8 | 3.7 | 1.5 | 2.2 | 0 | 1.5 | Sauces, beef, poultry |
| | Cases | 26,122 | 72.1 | 1.7 | 2.3 | 2.2 | 0 | 1.8 | |
| *Staphylococcus aureus* | Outbreaks | 499 | 59.1 | 7.0 | 2.0 | 6.8 | 1.6 | 1.8 | Dairy, pork, poultry, egg products |
| | Cases | 8179 | 69.4 | 11.2 | 2.9 | 2.8 | 1.9 | 4.0 | |

Percentages do not total to 100%, since outbreak reports may list multiple food vehicles or involve foods not included in this table

[a] Cold prepared foods include pasta salad, potato salad, dips, and salsa

[b] A single case of botulism is considered to be an outbreak

infection include the specific strain of the pathogen; the food matrix, which may protect against digestive processes; and the health status of the exposed individual (Hurst et al. 1996; Todd et al. 2008). Consequently, infectious dose estimates should be considered conservatively high and that food contaminated below the infectious dose represents a reduced but potentially still significant risk to the consumer.

### *4.4.1 Brucella*

The bacteria of the genus *Brucella* are zoonotic pathogens which are known to infect a number of mammalian hosts including humans, cattle, goats, sheep, dogs, rodents, and marine mammals. Ten species of *Brucella* have been identified, and three of these species which infect domestic livestock are of particular concern as a cause of human illness, *Brucella melitensis* which primarily infects sheep and goats, *Brucella abortus* which infects cattle, and *Brucella suis* which infects swine (Godfroid 2014).

In humans *Brucella* infections cause brucellosis, a disease which is rarely fatal but can cause miscarriage and prolonged debilitation, with a wide range of symptoms depending on the tissues infected (Franco et al. 2007). Worldwide, brucellosis is one of the most common bacterial zoonoses, with an estimated 500,000 new cases each year and prevalence rates greater than 10 per 100,000 in some countries (Pappas et al. 2006). However, in some jurisdictions, brucellosis has effectively been eliminated as a disease by routine pasteurization of milk and the establishment of *Brucella*-free herds.

*Brucella* are Gram-negative bacteria forming small cocci or short rods. The members of this group are considered aerobic, although optimal growth for some strains is under microaerobic conditions. The optimal pH for growth is 6.6–7.4, and replication can occur between 20 and 40 °C, with 37 °C as the optimum (Corbel and Banai 2005). *Brucella* cells can survive for a period in water and soil but do not replicate in these environments. They are unusual among foodborne pathogens, as they are an obligate parasite that naturally replicates following invasive infection of phagocytic host cells (Atluri et al. 2011).

The primary mode of *Brucella* transmission to humans is as a foodborne disease, usually through consumption of unpasteurized dairy products (Godfroid 2014). Infection through consumption of either raw or undercooked offal of infected animals may also occur but is much less common (Chan et al. 1989; Yoo et al. 2015). Humans can become infected with *Brucella* organisms through either ingestion, inhalation, or contact with the conjunctiva of the eye and skin abrasions (Franco et al. 2007). Infection may result from close contact with infected animals or, very rarely, human-to-human contact (Franco et al. 2007; Palanduz et al. 2000).

Differences in the potential for milk and meat to serve as vehicles for the transmission of *Brucella* can be explained by the mechanism of infection. *Brucella* infection in female mammals leads to infection of the mammary glands and shedding in the milk and other mammary secretions (Meador et al. 1989; Xavier et al. 2009).

Transmission of *Brucella* in mother's milk appears to be an important mechanism for the intergenerational transmission of this parasite. Standard dairy pasteurization conditions kill *Brucella*, and the adoption of routine pasteurization effectively eliminates milk products as a vehicle for this pathogen. In raw milk, no reduction in *Brucella* was observed during 4 days of storage at 4 °C (Falenski et al. 2011). In fermented dairy products, *Brucella* numbers have been observed to decline during manufacture and storage at 4 and 24 °C, from a combination of low pH, low water activity, and interactions with starter cultures (Falenski et al. 2011; Santiago-Rodríguez et al. 2015; El-daher et al. 1990; Méndez-González et al. 2011). Individual studies have reported *Brucella* surviving at levels exceeding 100 CFU/g at up to 18–50 days of storage, making it impossible to establish manufacturing conditions or a storage period that would ensure the safety of *Brucella*-contaminated dairy products.

*Brucella* can be isolated in tissues throughout animal carcasses but is primarily concentrated in the placenta, mammary glands, lymph nodes, spleen, or localized lesions (Hutchings et al. 1951; Xavier et al. 2009; Carvalho-Neta et al. 2010). This means the probability of exposure through normal muscle tissue is low, and, unlike enteric bacteria, contamination from the gastrointestinal tract or skin during slaughter is highly unlikely. Additionally, the requirement for temperatures to be above 20 °C for *Brucella* replication means that no amplification of risk within food items can occur unless significant temperature abuse occurs. However, viable cells of *Brucella* have been reported to persist in refrigerated, frozen, or salted meat for weeks or months (Hutchings et al. 1951; Ivanova 1960). Thus meat contaminated with *Brucella* remains a hazard if consumed raw or undercooked (Kara et al. 2012).

The *Brucella* species that are a primary risk to humans have been effectively eliminated in some jurisdictions through control programs that utilize vaccination, testing, and culling to reduce the reservoir hosts of these pathogens (Moreno 2014). This strategy has been successful due to the restricted host range and limited capacity for environmental persistence of *Brucella*. Success is also dependent upon the potential for wild herds to serve as a reservoir and the willingness of authorities to commit the necessary resources for ongoing control programs (Moreno 2014).

### *4.4.2* Campylobacter

*Campylobacter* is a commonly reported cause of bacterial foodborne infection in Canada (29.1 per 100,000), the EU (64.8 per 100,000), Australia (115.8 per 100,000), and the United States (13.5 per 100,000) (Table 4.2). Compared to other major bacterial pathogens, the great majority of *Campylobacter* illnesses are sporadic cases, with disproportionally few cases associated with an outbreak (Table 4.3). Currently, 17 clinically relevant species of *Campylobacter* have been reported to be associated with human illness (Kaakoush et al. 2015); this section will discuss only two of these species, *C. coli* and *C. jejuni*, as these are primarily reported as a cause of foodborne illness.

*Campylobacter* are Gram-negative microaerophilic bacteria, with a spiral or curved rod morphology. They require 3–15% oxygen and 3–5% $CO_2$ for growth and temperatures above 29 °C with an optimum of 42 °C (Vandamme et al. 2005). Rather than generating energy by the fermentation of carbohydrates, *Campylobacter* utilize either tricarboxylic acid cycle (TCA) intermediates or amino acids as substrates for respiratory energy generation, with *C. coli* and *C. jejuni* using oxygen as a terminal electron acceptor though atmospheric oxygen concentrations are typically lethal (Vandamme et al. 2005). *Campylobacter* can be isolated from a wide range of mammals and birds, including agricultural and companion species (Horrocks et al. 2009). The physiological characteristics of *Campylobacter* indicate specialization for existence as an intracellular parasite in animal hosts, as this environment is associated with the availability of TCA cycle intermediates, low oxygen levels, and the required growth temperatures.

The infectious dose of *C. coli* is unknown, while that of *C. jejuni* has been determined as approximately 500 cells by volunteer studies (Todd et al. 2008). Both *C. jejuni* and *C. coli* infections have an onset of 24–72 h, resulting in acute gastroenteritis, with symptoms including watery or bloody diarrhea, fever, and cramps (Kaakoush et al. 2015). Gastroenteritis caused by either *C. jejuni* or *C. coli* is generally self-limiting and typically resolves within 6 days. Infection with *C. jejuni* can trigger a range of autoimmune disorders, including Guillain-Barré syndrome and reactive arthritis, as some strains produce cell surface antigens (lipo-oligosaccharides) that mimic the gangliosides on human nervous tissue (Godschalk et al. 2004).

Since replication of *Campylobacter* is inhibited below 30 °C and the physico-chemical conditions found in many foods are either inhibitory or lethal for this bacterial genus, the food safety risk associated with *Campylobacter* is dependent upon the ability of the bacteria to persist. Many *Campylobacter* strains are sensitive to oxygen and will die if exposed to atmospheric levels, but a significant minority (35.7%) of *C. jejuni* strains can survive 24 h of aerobic incubation in broth media (Oh et al. 2015). *Campylobacter* are also highly sensitive to desiccation; cells suspended in blood die as blood drops dry on an exposed surface but persist in liquid blood (Humphrey et al. 1995). The persistence of *Campylobacter* on foods and in the environment can be enhanced by their entry into a viable but non-culturable (VBNC) physiological state (Jackson et al. 2009). The VNBC state can be triggered by exposure to refrigeration temperatures (Chaisowwong et al. 2012) and pH stress (Chaveerach et al. 2003). Alternately, there is evidence that the survival of *Campylobacter* is enhanced by participation in biofilm communities and parasitism of amoebae (Indikova et al. 2015).

Both *C. jejuni* and *C. coli* are carried and shed by a wide range of domestic animals, including cattle, pigs, and sheep, but the meat primarily associated with *Campylobacter* outbreaks is poultry (Table 4.3). The prevalence of *Campylobacter* in poultry, especially broiler chickens, can be very high, and they colonize the crop and cecum at high concentrations (Musgrove et al. 2001). Cattle and other meat animals also carry and shed *Campylobacter* (Horrocks et al. 2009), so rather than the risk associated with poultry being due primarily to the presence or absence of the

pathogen, a possible explanation can be found in the differences in the processing of poultry versus larger meat animals. The infectious dose of *Campylobacter* is relatively high compared to verotoxigenic *Escherichia coli* (VTEC) and *Salmonella enterica*, and typical poultry slaughter and processing operations will result in higher loads of fecal organisms including *Campylobacter* compared to red meats (Luber and Bartelt 2007). The smaller relative size of poultry makes it more difficult to remove the gastrointestinal tract hygienically. Similarly, the transfer of fecal flora from the hides of larger animals to the carcass is easier to prevent during dehiding as the skin may be removed as a single piece, whereas poultry must be plucked and then the skin removed or retained. Additionally *Campylobacter* are highly vulnerable to desiccation, and the carcass surfaces of mammals are typically dried during cooling, while poultry is kept wet. Consequently, *Campylobacter* are more likely to be present and will be present in higher numbers and have a far better probability of survival on poultry than on red meat.

A number of strategies for the control of *Campylobacter* during poultry production have been tried. Unlike *Salmonella* which can colonize eggs to spread vertically between generations, *Campylobacter* is dependent upon horizontal transmission by host animals (Callicott et al. 2006). This creates a potential for the use of rigorous biocontrol to prevent flock colonization (Guerin et al. 2007). Changes in the processing of poultry may also result in significant reductions in *Campylobacter*, for example, freezing chicken meat for 24 h will reduce *Campylobacter* numbers by 1 $\log_{10}$ unit (Sampers et al. 2010). Such control methods can be effective, as demonstrated by the experience of New Zealand, which in 2006 introduced a multifaceted program to reduce the risk of campylobacteriosis from chicken. The success of this program was indicated by a 54% reduction in the nationwide incidence of campylobacteriosis within 2 years of implementation (Sears et al. 2011). The New Zealand program included biosecurity to reduce flock contamination, changes to processing practices and regulatory targets for poultry carcasses, freezing of chicken meat prior to retail sale, the promotion of leak proof packaging at retail, and consumer education in safe handling practices (Sears et al. 2011).

### *4.4.3 Enterobacteriaceae*

The *Enterobacteriaceae* are a large family of rod-shaped Gram-negative bacteria. They are facultative anaerobes, with both fermentative and respiratory metabolism, and demonstration of motility by peritrichous flagella is common. Individual species or strains of this bacterial family exhibit varying degrees of host or environmental specialization, but they are generally adaptable organisms, and representatives of this genus can be isolated from water, soil, and a wide variety of plants and animals. They may be pathogens of specific plants or animals, but many other members of the genus are apparent symbionts (Brenner and Farmer 2005). Five members of the *Enterobacteriaceae* are of significance as causes of human foodborne illness,

*Cronobacter* sp., *Escherichia coli*, *Shigella* sp., *Salmonella* sp., and *Yersinia enterocolitica.*

#### 4.4.3.1 *Cronobacter*

The genus *Cronobacter* is a member of the *Enterobacteriaceae* and was originally classified as *Enterobacter sakazakii*. Subsequent molecular typing led to the reclassification of these organisms as seven species within a new genus, *Cronobacter* (Iversen et al. 2008). Currently, ten species and three subspecies of *Cronobacter* are recognized (LSPN 2017). Members of the genus *Cronobacter* are not obligate human pathogens or symbionts, and, like the closely related *Enterobacter*, they may be isolated from a wide range of environmental sources (Grimont and Grimont 2005) and foods including herbs, spices, salads, grains, meat, dairy, and seafood (Jaradat et al. 2014). Thus, exposure to this organism is probably not uncommon, yet illness is associated with infants and the adults over 70 (Patrick et al. 2014). In adults, *Cronobacter* has been isolated from a wide variety of infections (Farmer 2015), most often in patients who are elderly, immunocompromised, or suffering some other underlying health condition. These adult infections appear to be the consequence of environmental exposure.

The status of *Cronobacter* as a foodborne pathogen is related to outbreaks of neonatal meningitis, bacteremia, and necrotizing enterocolitis associated with consumption of powdered infant formula contaminated with *Cronobacter sakazakii* (Farmer 2015). Though outbreaks are a rare occurrence, the results of illness can be devastating, with *C. sakazakii* neonatal meningitis having a fatality rate of 41.9%, with a high rate of neurological complications in survivors (Friedemann 2009).

The association of this opportunistic pathogen with disease in neonates can be considered to be a consequence in changes in human behavior which have created exposure routes and vulnerable populations. These changes include the adoption of powdered infant formula as a convenient source of nutrition for infants and the increased survival rate of preterm neonates, which is linked to advances in medical care (Goldenberg et al. 2008). Preterm neonates are more vulnerable to opportunistic pathogens and face barriers to breastfeeding due to their immaturity and delays in milk expression by their mothers and thus are more likely to receive artificial nutrition, including powdered infant formula (Ziegler 2011).

Why *Cronobacter sakazakii* is associated with powdered infant formula and neonatal disease is not fully clear. The thermotolerance of *C. sakazakii* is similar to other *Enterobacteriaceae*, and cells should be killed by pasteurization (Iversen et al. 2004). The presence of *C. sakazakii* in powdered infant milk formula is likely due to contamination after pasteurization during the manufacturing process (Shaker et al. 2008). The explanation seems to lie in the unusually high resistance to desiccation of *Cronobacter* species (Osaili and Forsythe 2009); however, the specific processing failures that cause contamination remain obscure (Norberg et al. 2012).

#### 4.4.3.2 Pathogenic *Escherichia coli*

*Escherichia coli* colonizes the gut of most mammalian and avian species, including humans, typically as a commensal (Scheutz and Strockbine 2005). The maximum growth rate for *E. coli* occurs under conditions found in the gastrointestinal tract of its hosts, 37 °C, pH 6–7, an environment rich in complex nutrients. However, *E. coli* is well adapted for growth and survival under a wider range of conditions, from 7 to 46 °C, pH 4.4 to 9, and water activity above 0.95. If conditions are not permissive of growth, then *E. coli* can persist for prolonged periods, with survival for months or years in water, soil, sediment, or plants (Sjogren 1994; Van Elsas et al. 2011; Pachepsky and Shelton 2011).

While the majority of *E. coli* strains are harmless to humans, some strains possess virulence factors which make them enteric pathogens. Currently, seven enteric pathotypes of *E. coli* are distinguished on the basis of pathology and the possession of specific virulence factors. These *E. coli* pathotypes have been designated as enterotoxigenic (ETEC), enteropathogenic (EPEC), diffusely adherent (DAEC), enteroaggregative (EAEC), enteroinvasive (EIEC), adherent invasive (AIEC), and verotoxigenic (VTEC) *E. coli* (Croxen et al. 2013).

The relative prevalence of the different *E. coli* pathotypes in foods is difficult to assess due to the absence of comprehensive surveys. As a cause of foodborne illness, VTEC (also known as Shiga toxin-producing *E. coli* or enterohemorrhagic *E. coli*) is the pathotype most commonly reported as a cause of outbreaks in industrialized nations. In the United States, from 1998 to 2013, there were 485 outbreaks of *E. coli* (11,600 cases) (CDC 2015). The VTEC were involved in 91.6% of reported *E. coli* outbreaks and 76.6% of reported cases of *E. coli* illnesses (CDC 2015).

The predominance of VTEC as a cause of reported outbreaks results from the low infectious dose of some strains of these pathogens, <100 cells (Todd et al. 2008), and the risk of severe patient outcomes (Karch et al. 2005; Bennish 1991). The infectious dose of EIEC can be as low as 100 cells, but the infectious dose established for other pathotypes is much greater, EPEC $10^6$, ETEC $10^7$, and EAEC $10^8$ (Todd et al. 2008). Although pathotypes other than VTEC and EIEC are associated with uncomplicated diarrhea, their impact on human health should not be underestimated. These microorganisms are a significant cause of illness and mortality, especially among infants and young children in areas where public health and sanitation infrastructure is poor, and the simple but effective treatments for diarrheal illness such as oral rehydration solutions made with potable quality water are not available (Das et al. 2014).

The foods associated with outbreaks of *E. coli* are very diverse including fresh meats, processed foods, fresh fruits and vegetables, fruit juice, sprouts, seafood, and dairy. Outbreaks of foodborne *E. coli* in the United States have predominately involved meat (48.2%) and fresh fruits and vegetables (27.9%) (CDC 2015). As a common commensal, *E. coli* is routinely transferred onto meat surfaces during slaughter and carcass breaking operations for both mammalian and avian species. Fresh fruits and vegetables are a major source of outbreaks of pathogenic *E. coli*, and

US data indicates that these food products result in a larger number of illnesses per outbreak (CDC 2015). This is attributable to the raw consumption of these food products, whereas fresh meat is typically cooked prior to consumption. Probable routes for the contamination of fresh fruits and vegetables by *E. coli* are irrigation water, processing water, harvesting techniques and equipment, and contamination from processing workers in addition to defecation onto crops by either wild or domestic animals.

Ready-to-eat meat and fermented dairy products do not typically provide environments suitable for the replication of *E. coli*. Replication of *E. coli* may be inhibited by a combination of physicochemical parameters such as pH, water activity, and organic acids and by interactions with starter cultures (Lücke 2000). Although *E. coli* often declines in these products during typical production and storage conditions, infectious doses of this pathogen can persist for months of storage (McQuestin et al. 2009; Peng et al. 2011).

#### 4.4.3.3 *Shigella*

*Shigella* spp. were historically distinguished from *E. coli* on the basis of pathology and biochemical reactions, but molecular taxonomy confirms that they have a very close relationship with *E. coli*, particularly enteroinvasive *E. coli* (EIEC) which have a very similar pathology (Strockbine and Maurelli 2005). Whether *Shigella* represents a pathotype of *E. coli* (Van Den Beld and Reubsaet 2012) or a separate species within the genus *Escherichia* (Zuo et al. 2013) remains disputed. However, to understand the role of *Shigella* in foodborne illness, it is useful to view *Shigella* as a pathotype of *E. coli* composed of strains that have become specialized for humans as hosts (Van Den Beld and Reubsaet 2012).

As with VTEC, *Shigella* has a low infectious dose, <100 cells (Todd et al. 2008), a risk of severe patient outcomes (Karch et al. 2005; Bennish 1991) and the associated outbreaks can involve a similar diversity of foods. However, due to the host specialization of *Shigella*, there are important differences in the transmission of these pathogens and the foods associated with outbreaks. In the United States, from 1998 to 2013, there were 155 foodborne outbreaks of *Shigella* (7278 cases) (Table 4.3) (CDC 2015). Unlike *E. coli* where a clear majority of outbreaks and cases involved two product type meats and fresh fruits and vegetables, *Shigella* outbreaks were divided closely between meats (28.2%), fresh fruits and vegetables (28.2%), and unheated prepared foods, such as potato or pasta salad, dips, and salsa (23.9%) (Table 4.3). The greatest proportion of cases of illness were associated with fresh fruits and vegetables (46.6%) followed by unheated prepared foods (31.9%) and meats (15.4%) (Table 4.3). Since *Shigella* is adapted for a human host, contamination of food occurs either as a consequence of poor hygiene by infected food production and service workers or the use of water for irrigation or food preparation from a source contaminated with human feces. In areas where water treatment and distribution infrastructure are relatively good, infected workers are most likely the source. This explains the association of outbreaks with prepared foods which are

consumed uncooked. The lower association of *Shigella* with meats and dairy compared to pathogenic *E. coli* (Table 4.3) probably reflects that *Shigella* is not carried by domestic animals, and the incident represents the probability of transmission to foods post cooking.

#### 4.4.3.4 *Salmonella enterica*: Typhoidal and Non-typhoidal

The genus *Salmonella* contains two species, *Salmonella enterica* and *S. bongori*, of which *S. enterica* is a human pathogen. *Salmonella enterica* is subdivided into six subspecies, which are further subdivided by serology into more than 2000 serotypes or serovars (Popoff and Le Minor 2005). Individual serotypes of *S. enterica* vary in host specificity, with some serotypes more adapted to humans and more commonly associated with human illness. Serotypes that cause human illness are distinguished by clinical pathology into two groups, typhoidal and non-typhoidal *Salmonella* (Gal-Mor et al. 2014). Infection with non-typhoidal *Salmonella* typically results in self-limiting acute gastroenteritis and watery diarrhea, with onset of symptoms within 6–12 h of ingestion and a 10-day course of illness (Gal-Mor et al. 2014). Infection with typhoidal *Salmonella* has an average incubation period of up 14 days and 21 days of illness, with symptoms of sustained fever, chills, abdominal pain, swelling of the liver and spleen, rash, nausea, diarrhea or constipation, and coughing (Gal-Mor et al. 2014). The difference in the course of illness is related to the tissues infected; both typhoidal and non-typhoidal *Salmonella* initially invade the intestinal epithelium of the small intestine (Liu et al. 1988), but non-typhoidal *Salmonella* provoke a strong inflammatory response which aids in clearing of the microorganism, while the inflammatory response to typhoidal strains is relatively weak (Gal-Mor et al. 2014). Consequently, infection of immunocompetent hosts by non-typhoidal *Salmonella* is limited to the intestinal epithelium, whereas typhoidal strains are able to cross the intestine and spread to the mesenteric lymph nodes, liver, spleen, bone marrow, and gall bladder (Gordon 2008).

Typhoidal S*almonella* serotypes such as Typhi, Sendai, and Paratyphi are specialized to human hosts (Gal-Mor et al. 2014). Like other specialized human pathogens, their ability to spread between hosts and cause disease is associated with the failure or absence of systematic hygiene systems or personal hygiene; thus, the burden of disease associated with these pathogens is borne largely by nonindustrialized countries. Typhoidal *Salmonella* have the ability to asymptomatically infect hosts, who become "carriers" and shed the bacterium in their feces. If employed in food production or preparation, such individuals can be a reoccurring source of foodborne outbreaks (Marineli et al. 2013).

Non-typhoidal serotypes typically have broader host specificity than do typhoidal strains. The health risk associated with individual non-typhoidal serotypes may vary greatly; this is reflected in the widely ranging estimates of the infectious dose, in individual outbreaks from 10 to $10^{11}$ cells (Todd et al. 2008).

A significant proportion of *Salmonella* outbreaks in the United States (41.3%) are associated with meats, particularly poultry (23.0%) (Table 4.3). *Salmonella* can

colonize practically all domestic animals and as with other enteric organisms can be transferred to meat surfaces during carcass breaking. Like *Campylobacter*, the relatively high proportion of *Salmonella* outbreaks associated with poultry probably reflects the hygiene challenges inherent in poultry slaughter and processing.

The second most common food associated with *Salmonella* outbreaks are fresh fruits and vegetables (Table 4.3). If inoculated onto plant surfaces, through contaminated water or defecation by humans or animals, the ability of *Salmonella* to survive at low water activities and its physiological adaptability can allow it to survive in the environment and colonize plant tissues. Successful colonization of plant tissues by *Salmonella* may also involve fimbriae and cellulose production for attachment and interactions with plant-associated microflora (Brandl et al. 2013). The cutting or breaking of plant surfaces intentionally or unintentionally during processing and transport may increase the availability of nutrients and moisture which in turn facilitate bacterial replication, and under such conditions temperature remains the only limit on *Salmonella* replication.

Of the foodborne bacterial pathogens, *Salmonella* has the greatest association with outbreaks involving eggs (Table 4.3). *Salmonella* are capable of colonizing the reproductive tract of birds and can internalize into the egg during the process of egg formation (Keller et al. 1995; Gantois et al. 2008). Alternatively, the shell of the egg can also become contaminated by *Salmonella* from the gastrointestinal tract of birds either during passage of the egg through the oviduct or after laying, and these subsequently contaminating bacterial cells can then penetrate the shell (De Reu et al. 2006). Once internalized into the egg, *Salmonella* may replicate in the albumen or the yolk (Humphrey and Whitehead 1993). *Salmonella* on the surface of eggs can be removed by appropriate washing methods, but internalized *Salmonella* are protected from decontamination treatments.

Low-moisture foods are generally considered safe, as the low water activity inhibits replication, but occasionally outbreaks involving *Salmonella* in low-moisture foods are reported. Products typically involved in these outbreaks include spices, chocolate (Werber et al. 2005), tree nuts, peanut butter (Viazis et al. 2015), tea, and powdered infant formula (Finn et al. 2013). Though *Salmonella* require a water activity above 0.93 for replication, similar to other *Enterobacteriaceae*, *Salmonella* can persist for prolonged periods in foods with water activities below 0.85 (Finn et al. 2013). The risk possessed by *Salmonella* in foods with low water activity may be enhanced by physiological adaptation, including cell filament formation, resulting in increased resistance to other physicochemical stresses, such as heat, pH, and sanitizers (Finn et al. 2013; Mattick et al. 2000). Alternatively, outbreaks can occur with high-moisture foods which are prepared with contaminated low-moisture ingredients and thus subsequently allow replication of *Salmonella*. High-fat, low-moisture foods may also be protective of cells during digestion following consumption, increasing the probability of infection (Aviles et al. 2013).

#### 4.4.3.5 *Yersinia enterocolitica*

*Yersinia enterocolitica* causes acute, self-limiting gastrointestinal illness, which typically resolves in 1–3 days. Importantly, *Y. enterocolitica* is notable for its cold tolerance; it can grow at 0 to 40 °C (Bottone 1997). Also notable is the fact that *Y. enterocolitica* can be isolated from a wide range of environmental sources and animal hosts, but foodborne infection is primarily associated with pork (Drummond et al. 2012). Of the 11 outbreaks with identified causes reported to the CDC from 1998 to 2013, nine (81.8%) involved pork, one outbreak involved an unspecified "deli meat," and one involved a dairy product (Table 4.3).

The intestinal contents and tonsils of pigs are important sources of *Y. enterocolitica* contamination of pork during slaughter and carcass breaking. The primacy of pork association with *Y. enterocolitica* infection may reflect that those biotypes of these bacterial species which are potential human pathogens are more common in pigs than in other hosts (Laukkanen-Ninios et al. 2014). Illness attributable to *Y. enterocolitica* associated with other foods does occur, though is far less common than pork. The capacity of *Y. enterocolitica* to survive and grow in a wide range of conditions means that, like other *Enterobacteriaceae*, contamination of plants in the field, shedding by food preparers, or cross contamination in the kitchen remain alternate routes of transmission.

### *4.4.4* Listeria monocytogenes

*Listeria monocytogenes* was first identified by E. G. D. Murray in 1924, as a Gram-positive bacillus responsible for mononucleosis in laboratory animals (Murray et al. 1926). *Listeria monocytogenes* is a nonspore-forming, facultative anaerobe, and its replication can occur from 0 to 45 °C. The species *L. monocytogenes* is ubiquitously distributed in the environment, and there is little evidence of host specificity. It has been isolated from soil, water and vegetation, silage, and both human and animal hosts (Fenlon 1985, 1989; Dowe et al. 1997). It appears that *L. monocytogenes* is a highly adaptable organism capable of life as an intracellular parasite in a wide range of eukaryotic hosts.

Of the major foodborne bacterial pathogens, *L. monocytogenes* has one of the lowest incidence rates (Table 4.2) in developed nations, but it has the highest case fatality rates, 21% in the United States in 2009–2011 (Silk et al. 2013). Patient outcomes from *L. monocytogenes* infection are highly dependent upon the underlying health status of the patient. In otherwise healthy people, listeriosis is characterized by self-limiting fever, aches, fatigue, nausea, and diarrhea (Ooi and Lorber 2005). However, in susceptible individuals (older adults, immunocompromised persons, newborns, and pregnant women), listeriosis can take the form of invasive infection, resulting in septicemia, meningitis, meningoencephalitis, or abortion (Doganay 2003).

The majority of human listeriosis cases are foodborne, and the most commonly implicated foods are ready-to-eat foods (Lianou and Sofos 2007). Due to its ubiquity in food production environments, it is not surprising that *L. monocytogenes* has been isolated from a variety of foods including milk and dairy products, meat products, egg products, seafood, vegetables, and other ready-to-eat foods (Ferreira et al. 2014; Fenlon et al. 1996; Dowe et al. 1997; Macgowan et al. 1994). The infective dose of *L. monocytogenes* is >1000 cells (Todd et al. 2008), which is relatively high compared to pathogens such as VTEC and *Salmonella.*

Outbreaks of *L. monocytogenes* are regularly traced back to the processing plant. Due to its ubiquity, *L. monocytogenes* is easily introduced into processing plants, and once introduced if sanitation and other control measures are inadequate, equipment and other environmental surfaces can support an effectively permanent population of *L. monocytogenes* (McCollum et al. 2013; Currie et al. 2015). Biofilm formation is an important factor in the persistence of such populations, and once established they may serve as a reoccurring source of outbreaks for months or years (Thimothe et al. 2004; Ferreira et al. 2014; Carpentier and Cerf 2011; Ortiz et al. 2010). This is illustrated by a human listeriosis outbreak that occurred in 2000 in the United States and was caused by a *L. monocytogenes* strain that appears to have persisted in the same food processing plant over at least 12 years, causing a sporadic case of listeriosis in 1988 and a subsequent outbreak in 2000 (Orsi 2008).

From these observations, it is clear that the prevention of *L. monocytogenes* outbreaks is the most easily controlled at the processing plant and is dependent upon establishing and maintaining an effective and rigorous sanitation plan. Due to the risk of colonization of the processing environment by *L. monocytogenes*, it is desirable to assess the effectiveness of the sanitation plan with microbiological testing which allows differentiation between transient and persistent populations of *L. monocytogenes.* This can be achieved by molecular subtyping of isolates. If the presence of a persistent population is indicated, then the sanitation plan must be thoroughly revised to address the deficiency that allows the persistent population to exist.

### *4.4.5* Mycobacterium

The members of the genus *Mycobacterium* are slightly curved or straight rods which possess a cell wall structure that is not typical of either Gram-positive or Gram-negative bacteria. Instead the cell wall contains large quantities of unique lipids. They are aerobic or microaerobic, requiring complex nutrition, and are very slow growing even under optimal conditions (Magee and Ward 2012).

Infection of humans with specific species of *Mycobacterium* can cause the diseases tuberculosis and leprosy. Tuberculosis may result from infection by either inhalation or ingestion and is caused by a number of closely related *Mycobacterium* species (*M. tuberculosis*, *M. bovis*, *M. caprae*, and *M. avium*) that may infect domestic animals or humans (Magee and Ward 2012). As a foodborne disease,

milk once was a significant vector, and foodborne tuberculosis is primarily associated with the consumption of milk contaminated with *M. bovis.* Foodborne transmission of tuberculosis is virtually unknown where the control measures of milk pasteurization and establishment of tuberculosis-free herds have been employed (Thoen et al. 2006).

In contrast to milk, the consumption of beef from infected cattle is not associated with the transmission of *M. bovis*. Three factors which limit the potential for transmission of *M. bovis* by meat have been identified (de la Rua-Domenech 2006). Firstly, *M. bovis* grows slowly even under optimal conditions and will not replicate on postmortem tissue. Secondly, the bacterium is localized in lesions which only form in the skeletal muscle of animals with advanced infection. Thirdly, *M. bovis* is not notably resistant to thermal treatment and is destroyed by normal cooking conditions.

It has been proposed that Crohn's disease, a form of chronic inflammatory bowel disease, is caused by *M. avium paratuberculosis* and that foodborne transmission plays an important role in exposure. However, a causative role for *M. avium paratuberculosis* in Crohn's disease has not been proven (Liverani et al. 2014), and though the organism has been recovered from beef and dairy products, evidence for significant foodborne exposure is scant (Gill et al. 2011).

### *4.4.6* Vibrio

Among members of the family *Vibrionaceae*, the genus *Vibrio* is composed of facultative anaerobic, Gram-negative, straight, or slightly curved rods. This genus contains a diverse range of species which are found worldwide as an essential component of aquatic ecosystems. All species are halophiles, with optimum growth occurring at sodium concentrations from 5 to 700 mM. All species are able to replicate at 20 °C, but the temperature range for individual species may range from as low as 4 °C to as high as 37 °C. The distribution and range of individual species of *Vibrio* appear to reflect specialized adaptions for specific ecological niches and localities. There is evidence that a diverse range of abiotic and biotic factors influence the proliferation of specific *Vibrio* species, and it is unclear to what extent *Vibrio* live as planktonic organisms or are dependent on symbiotic or parasitic relationships with plants, algae, animals, and protozoa (Takemura et al. 2014). Conflicting results of individual studies may reflect ecological specialization of strains within species. What is clear is that water temperature and salinity play a major role in determining the geographical range of species (Takemura et al. 2014).

Among the 70 species of *Vibrio* that have been identified, 11 species have been reported to cause human illness. Of these pathogens, three species are the most relevant for consideration as a cause of foodborne illness, *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*. These three species are genetically and phenotypically diverse with virulent and avirulent strains reported (Sanjuan et al. 2009; Ronholm et al. 2015).

Those *V. cholerae* strains capable of producing cholera toxin are the most notorious of the *Vibrio*, as they can cause large-scale outbreaks under conditions of poor water sanitation. Poor sanitation is a necessity for cholera outbreaks as the infectious dose is high ($10^6$–$10^9$ cells). Infection is most often asymptomatic, but of the patients who develop diarrhea, approximately 20% suffer acute diarrheal illness which can kill with hours of onset (WHO 2014), if treatment and supportive medical care are unavailable.

It is known that *V. cholerae* prefers water temperatures from 10 to 30 °C and 1–10 ppt saline, with the environmental presence of these organisms generally decreasing as salinity increases (Takemura et al. 2014). Crustaceans serve as a reservoir for *V. cholerae* in marine environments; the association of *V. cholerae* with the chitin exoskeleton of crustaceans has provided the microorganism with a number of advantages, including food availability, adaptation to environmental nutrient gradients, tolerance to stress, and protection from predators (Pruzzo et al. 2008). Chitinase produced by *V. cholerae* allows the microorganism to derive carbon, nitrogen, and energy from crustacean exoskeleton (Meibom et al. 2004). However, outbreaks are associated with fecal contamination of fresh water. Consequently, though cholera is endemic in some regions, the disease is practically unknown where water and sewage sanitation are well established. Foodborne transmission may occur when food or beverages are prepared with contaminated water or by infected individuals.

In America, *V. parahaemolyticus* and *V. vulnificus* are the *Vibrio* species primarily reported as a cause of foodborne disease and are almost exclusively associated with seafood, particularly uncooked products such as raw crustaceans and bivalve shellfish (Table 4.3). The two species *V. parahaemolyticus* and *V. vulnificus* differ significantly in the risks they pose. *Vibrio parahaemolyticus* has a high infectious dose of $10^5$–$10^7$ cells (Todd et al. 2008), with an incubation period of 4–90 h, with most patients experiencing disease symptoms within 24 h. The associated illness usually involves 3 days of self-limiting diarrhea, accompanied by symptoms of headache, vomiting, nausea, abdominal cramps, and low fever, though life-threating septicemia can occur in patients with underlying health conditions (Su and Liu 2007).

*Vibrio vulnificus* is a much more dangerous pathogen; it has an infectious dose estimated at less than 10 cells (Todd et al. 2008); the incubation period is short, being 26 h on average; and this organism causes septicemia with symptoms of fever, chills, nausea, and abdominal pain (Oliver 2013). In the United States, *V. vulnificus* is responsible for 95% of seafood-related deaths and has a 50% case fatality rate (Oliver 2013). The population vulnerable to *V. vulnificus* is very well defined; cases almost always (94%) involve an underlying health condition (liver disease, diabetes, immunocompromising illness), and it is most common in males over 40. In experiments with rats, the vulnerability of males has been demonstrated to be due to a protective effect by estrogen against the activity of *V. vulnificus* endotoxin (lipopolysaccharide) (Oliver 2013).

*Vibrio parahaemolyticus* is associated with water temperatures greater than 20 °C, preferring 25–35 °C, and within that temperature range, it tolerates salinity

levels from 3 to 35 ppt (Takemura et al. 2014). Cell numbers in the water column increase as temperatures increase above 15 °C, with cell density in water declining below that 15 °C, though *V. parahaemolyticus* can be isolated from marine sediments year-round irrespective of temperature (Kaneko and Colwell 1973). *Vibrio vulnificus* is associated with a wide temperature range, 10–32 °C, at 5–10 ppt salinity, but prefers water above 20 °C when salinity exceeds 10 ppt (Takemura et al. 2014). The preference of both *Vibrio* species for warmer water temperatures is reflected in the greater risk associated with the ingestion of raw oysters in summer (Cook et al. 2002). Raw oysters and other filter feeding molluscs represent an increased risk as filter feeding can very effectively concentrate *Vibrio* from the environment into the animal's tissues and result in the tissue containing concentrations several orders of magnitude higher than the surrounding waters (Takemura et al. 2014).

While there is seasonality in pathogen prevalence and numbers, the risks associated with *Vibrio* on raw seafood cannot be managed by limiting harvesting seasons. Many jurisdictions conduct bacteriological testing of harvested molluscs and either limit or expand fisheries on that basis, though this approach cannot ensure safety. Once fish or shellfish have been harvested, growth of *V. parahaemolyticus* is inhibited below 13 °C and *V. vulnificus* below 11 °C (Kim et al. 2012). Neither of these bacterial species display significant heat resistance, and so the associated illnesses are commonly linked to raw or undercooked foods.

### *4.4.7 Sporeformers*

Spore-forming bacteria can persist as spores in the environment for years or decades. This persistence makes them common contaminants of foods, especially those foods in close contact with soils. Three species of sporeformers, *C. botulinum*, *C. perfringens*, and *B. cereus*, produce toxins that can cause illness in humans and animals, and many species of sporeformers cause spoilage of foods (Setlow and Johnson 2007). Spore-forming bacteria present a special problem to food processors, as endospores can resist extreme conditions of heat, pressure, desiccation, radiation, and chemical sterilants. Endospores are metabolically inactive yet are capable of sensing their environment and will germinate into vegetative cells given the presence of appropriate conditions of moisture, temperature, and nutrients.

#### 4.4.7.1 *Bacillus cereus*

*Bacillus cereus* sensu lato is a group of species of Gram-positive, facultative anaerobic, spore-forming rods. The taxonomy of *B. cereus* sensu lato is complex and changing. To date, seven members have been described: *Bacillus cereus* sensu stricto, *Bacillus mycoides*, *Bacillus pseudomycoides*, *Bacillus thuringiensis*, *Bacillus weihenstephanensis*, *Bacillus anthracis*, and *Bacillus cytotoxicus* (Guinebretiere

et al. 2013). Unlike the closely related species *B. anthracis* and *B. thuringiensis*, *B. cereus* does not enter a pathogenic life cycle in the natural environment. *Bacillus cereus* has a saprophytic lifestyle, where spores germinate when exposed to moisture and nutrients, after which the vegetative cells multiply and then sporulate when nutrients are exhausted (Stenfors Arnesen et al. 2008). It has been suggested that *B. cereus* may be the most common aerobic sporeformer and is widely distributed in soils, sediments, dust, the rhizosphere of some plants (Halverson et al. 1993; Young et al. 1995), rain and groundwater (Brillard et al. 2015), and the intestinal tract of invertebrates (Jensen et al. 2003; Margulis et al. 1998).

Several members of *B. cereus* sensu lato are commonly isolated from foods and are associated with two food poisoning syndromes in humans—emetic (vomiting) syndrome and diarrheal syndrome (Stenfors Arnesen et al. 2008). Both syndromes are typically mild, with patients often recovering within 24 h. However, lethal cases of emetic intoxications, associated with the consumption of rice or pasta dishes, have been reported after the ingestion of food contaminated with high amounts of emetic toxin (Dierick et al. 2005; Naranjo et al. 2011; Mahler et al. 1997; Shiota et al. 2010).

The bacterium is so commonly isolated that it is often simply declared as a "contaminant" when isolated from clinical specimens and is given little concern (Bottone 2010). As a result of the ubiquitous distribution of *B. cereus* in foods, the bacterium is ingested in small numbers and becomes part of the transitory human intestinal flora (Jensen et al. 2003; Turnbull 1985). The spores of *B. cereus* are particularly a concern in the food industry because these spores are not inactivated either by pasteurization (Carlin et al. 2000; Choma et al. 2000; Crielly et al. 1994) or gamma radiation (Mtenga et al. 2012, 2013; Thayer and Boyd 1994), and their hydrophobic nature allows them to adhere to food processing surfaces (Faille et al. 2002; Tauveron et al. 2006).

The emetic syndrome is caused by the cyclic dodecadepsipeptide cereulide, which is produced in food before ingestion (Agata et al. 1995), causing a foodborne intoxication. Emetic food poisoning by *B. cereus* is most often associated with consumption of contaminated cooked rice and pasta dishes that were not properly cooled. Some strains have the ability to grow at low temperatures and can be regarded as psychrotrophs with a minimal growth temperature of 4 °C (van Netten et al. 1990). Rice can become contaminated with microorganisms from either soil, irrigation water, animal feces, or insects (Kim et al. 2014; Haque and Russell 2005; Choi et al. 2014), with *Bacillus* spp. being one of the primary contaminants (Cottyn et al. 2001). In a recent study (Kim et al. 2014), *B. cereus* spores were isolated from 96.8%, 80.6%, and 57.8% of rough, brown, and white rice samples, respectively. Levels of *B. cereus* in unhusked rice have been estimated to be as high as 1.2 to $3.5 \times 10^3$ cfu $g^{-1}$ (Sarrías et al. 2002). Spores prepared from *B. cereus* strains isolated from rice are heat resistant, having $D_{90}$-values ranging from 3.23 to 23.26 min (Sarrías et al. 2002). Thus spores survive the cooking process, to germinate, grow, and produce cereulide in the cooked product.

*Bacillus cereus* can also cause a diarrheal syndrome due to release of enterotoxins produced during growth in the small intestine after ingestion of high numbers of viable cells or spores (Clavel et al. 2004; Granum et al. 1993). Diarrheal syndrome is

most often associated with sauces and vegetables, meat products, soups, puddings, and milk products (Kotiranta et al. 2000; Stenfors Arnesen et al. 2008).

#### 4.4.7.2 *Clostridium botulinum*

Popoff (2015) has described diseases due to toxigenic clostridia as "an accidental connection between a host and an environmental bacterium which synthesizes a specific toxic compound, rather than resulting from bacterial strategy to invade an organism and subsequently survive in this new environment sheltered from host defenses."

Since the sole virulence factor of *C. botulinum* is botulinum neurotoxin, neurotoxigenic clostridia are characterized into seven different types, A through G, based on the serological specificity of the neurotoxin produced. Human botulism, including foodborne, wound, and infant and adult colonization botulism, is associated with types A, B, E, and, very rarely, F. Neurotoxigenic clostridia are also divided into six groups based on phenotype, and this division is supported by genomic analysis. Group I includes BoNT/A-, BoNT/B-, and BoNT/F-producing *C. botulinum*. These strains are proteolytic, grow at a minimum temperature of 10 °C, and produce spores of high heat resistance. Group II includes BoNT/B-, BoNT/E-, and BoNT/F-producing *C. botulinum*. These strains are nonproteolytic, grow at refrigeration temperatures, and produce spores of low heat resistance. Group III includes BoNT/C- or BoNT/D-producing *C. botulinum*. These strains cause botulism in animals, but not in humans. Group IV includes BoNT/G-producing *Clostridium argentinense*. Group V includes neurotoxigenic BoNT/F-producing *Clostridium baratii*; and Group VI includes neurotoxigenic BoNT/E-producing *Clostridium butyricum* (Smith et al. 2015). The genes encoding botulinum toxin types B, E, and F are not limited to strains with similar genomic backgrounds but have crossed species. Type B toxin is produced by Groups I and II *C. botulinum*; type E toxin is produced by Group II *C. botulinum* and neurotoxigenic strains of *C. butyricum*, while type F toxin is the most promiscuous toxin being produced by both Group I and II *C. botulinum* and by neurotoxigenic strains of *C. baratii*.

Spores of *C. botulinum* are ubiquitously distributed in soils and in marine and freshwater sediments. They are commonly isolated from many animals, including animals processed for food. Global distribution of *C. botulinum* roughly indicates *C. botulinum* type A spores predominate in soils in the western United States, China, Brazil, and Argentina and type B spores in the eastern United States, the United Kingdom, and much of continental Europe. Of the type B spores, most American type B strains are proteolytic (Group I), while most European strains are nonproteolytic (Group II). The majority of botulism cases caused in northern latitudes are a result of *C. botulinum* type E, indicating the predominance of type E strains in northern regions and in most temperate aquatic regions and their surroundings. Types C and D are found more frequently in warmer environments. The reasons for this distribution pattern are not understood well. Type A appears to be favored by neutral to alkaline soil with low organic content; this is consistent with

its virtual absence in the highly cultivated soils of the eastern United States and Europe. Type E is psychrotolerant, which undoubtedly plays a role in its prevalence in the North and in many aquatic environments. The high rate of botulism in Inuit communities of southern Ungava Bay correlates with the high prevalence of *C. botulinum* type E found in that environment (Leclair et al. 2013).

One of the primary hurdles to inhibit the growth of *C. botulinum* is low pH. The minimum pH allowing growth of *C. botulinum* group I is 4.6; for group II, it is approximately pH 5.0. Many fruits are sufficiently acidic to inhibit *C. botulinum* by their pH alone, while acidulents frequently are used to preserve other products. The rate of acidification of foods can be important in inhibition of microorganisms, and notably *C. botulinum* can grow in some acidified foods if excessively slow pH equilibration occurs.

Importantly, *C. botulinum* is not considered to be a successful "competitor" in terms of growing in foods with mixed bacterial flora. Lactic acid bacteria, including *Lactobacillus*, *Pediococcus*, and *Streptococcus*, can inhibit growth of *C. botulinum*, largely by reducing the pH, but also by the production of bacteriocins (Rodgers et al. 2003; Okereke and Montville 1991). An example of this is the "Wisconsin process" which uses *Pediococcus acidilactici* and a fermentable carbohydrate for producing bacon with 40 or 80 ppm sodium nitrite rather than conventional bacon made with 120 ppm nitrite in the United States (Tanaka et al. 1985).

The growth of other microorganisms may also protect consumers by causing spoilage that would make a toxic product less likely to be consumed. For example, *C. botulinum* was observed to grow and produced toxin in ready-to-eat bagged salads, but only after the salads were visibly spoiled (Austin et al. 1998a). In the United States and Canada, smoked fish is only permitted for sale when packed in a film permeable to oxygen, ensuring growth of aerobic spoilage flora and hence spoilage of the product when stored for an extended time at abusive temperatures (Dufresne et al. 2000).

Traditional northern foods in Canada, Alaska, and Greenland are common causes of botulism. Meat and fat from seal, whale, and walrus are aged without control of temperature, followed by consumption without cooking. This tradition has led to high rates of botulism in Arctic, and near-Arctic, communities. While this form of food preparation has been incorrectly referred to as "fermentation," the lack of fermentable carbohydrates in muscle and fat tissue results in the pH of these traditional foods remaining near neutral pH, with the resultant growth of *C. botulinum* type E.

Pruno, an alcoholic beverage prepared by prison inmates, has caused several botulism outbreaks in US prisons over the past several years (Adams et al. 2015; Vugia et al. 2009; Walters et al. 2015; Williams et al. 2014; Yasmin et al. 2015). Pruno is prepared from food scraps, typically fruits, sugar, and water. Notably, the pruno batches associated with botulism all had potatoes added to them (Walters et al. 2015). Potatoes were reported to produce higher levels of alcohol in a shorter fermentation time. Addition of potatoes, a food that has a history of causing botulism (Angulo et al. 1998; Bhutani et al. 2005; Seals et al. 1981), may have introduced

spores to the pruno formula and would not contribute to a decrease in pH as addition of fruits would.

#### 4.4.7.3 *Clostridium perfringens*

Similar to the other spore-forming bacteria, *C. perfringens* is widely spread in the environment. The species *C. perfringens* is classified into one of the five types (A–E) based on the production of four major toxins (alpha, beta, epsilon, and iota). This species is frequently present in the normal intestinal microbiota of humans and animals. Food poisoning caused by *C. perfringens* is attributed to a subset of type A strains which produce *C. perfringens* enterotoxin (CPE); CPE is a single polypeptide with a molecular weight of 3.5 kDa and is produced during sporulation of vegetative cells after large numbers have been ingested ($>10^6$ bacteria $g^{-1}$) which requires growth in temperature-abused foods.

*Clostridium perfringens* was the second most common cause of bacterial foodborne disease outbreaks in the United States during 1998–2010 (Grass et al. 2013) and the most common bacterial source of foodborne illness in Canada during 2000–2010 (Thomas et al. 2013).

The species *C. perfringens* has an extensive history of use as an indicator for fecal contamination, based on its association with the gastrointestinal tract of humans and other animals and the presence of its spores in sewage (Mueller-Spitz et al. 2010). Specifically, *C. perfringens* has been shown to be a conservative indicator for fecal excreta from carnivorous wildlife and human-associated sewage (Vierheilig et al. 2013). Important for its use as an indicator is the fact that *C. perfringens* does not reproduce in aquatic systems (Wright 1989; Desmarais et al. 2002). The spores may be detected long after a pollution event has occurred and far from the source (Vierheilig et al. 2013).

## 4.5 Conclusions

Exposure to bacterial foodborne pathogens and their potential to cause disease are not random, though these organisms are widely dispersed in the environment and part of the common microflora of agricultural environments. Even the most ubiquitous and adaptable of foodborne bacterial pathogens are associated with specific foods. These associations can be understood as arising from the ecological relationship of the individual pathogen with the food production environment, the food product itself, and its associated microflora.

Knowledge of these relationships is invaluable in informing the identification of potential control strategies and excluding control strategies which will be ineffective. For example, *Mycobacterium* and *Brucella* have been effectively eliminated as foodborne diseases in some jurisdictions by reduction in the availability of susceptible animal hosts through such measures as immunization and testing, as well as

culling of herds to eliminate the presence of infected animals. But the success of these strategies is dependent upon the limited host range and poor survival of these pathogens outside a host. Consequently, it can be predicted that this approach is unlikely to be successful with pathogens such as *E. coli* and *Salmonella* which can survive and replicate in a far wider range of potential hosts and environmental niches. Similarly, some ready-to-eat foods, such as cheeses and fermented meats, have a surprisingly good history of safety with traditional production methods, which do not include a decontamination step, as the food itself is an unfavorable environment for pathogen survival and growth.

In general, the hazard potential for infectious foodborne pathogens depends on the probability of exposure of the consumer to an infectious dose of the organism. This in turn is dependent upon the probability of inoculation of the food with the pathogen and its potential for survival on the product throughout the shelf life of the product. Replication of the pathogen either on or within the food amplifies the risk but is not required for illness to occur. The key strategies for the control of infectious foodborne pathogens can be understood to be decontamination and the prevention of contamination, and decontamination is not always available for ready-to-eat products. In contrast, bacterial agents of intoxication present a risk not simply through their presence but through bacterial growth and toxin production. For these pathogens, food safety can be established by formulating, storing, and packaging food to prevent toxin production, even if the pathogen is present.

The value of the examples presented in this chapter is in illustrating the relationship between pathogens and food commodities and the factors which determine these relationships. The ecology of foodborne pathogens is not static but should be understood as an evolving system. New pathogens and new food pathogen associations may emerge with changes to food production and distribution. The potential causes of these changes are as diverse as the food production system. The locations in which foods are grown or harvested may alter due to changes in the environment or economic opportunity. The geographic range of pathogens may change in response to changes in host range or climatic conditions. Food processing, packaging, and distribution may be changed by industries seeking new opportunities. Fashions in food consumption and preparation regularly change among consumers. The principles presented in this chapter will hopefully be of value to microbiologists attempting to address the food safety challenges of the future.

## Compliance with Ethical Standards

**Conflict of Interest** Alexander Gill declares that he has no conflict of interest. John W. Austin declares that he has no conflict of interest.

**Ethical Approval** This article does not contain any studies with human participants or animals performed by either of the authors.

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# Chapter 5
# Impact of Mycobacterial Biofilms on Public Health


**Anil K. Ojha**

**Abstract** The genus *Mycobacterium* represents over 150 bacterial species of the actinomycetes family, inhabiting a wide range of ecological niches—from soil and aquatic environments to intracellular phagosomes in human bodies. *Mycobacterium tuberculosis (Mtb)*, the etiological agent of tuberculosis in human, is the predominant mycobacterial pathogen that resides in an estimated one-third of the world's human population, causing disease in hundreds of millions and killing over a million people every year. In addition, several environmental mycobacteria including *Mycobacterium avium* and *Mycobacterium abscessus* are opportunistic pathogens that can establish infection in immunocompromised individuals. A common characteristic of all mycobacterial infections, regardless of the species, is their extraordinary recalcitrance to antibiotic regimens, although the underlying basis of drug resistance remains unclear. Recent studies suggest a possible linkage between mycobacterial tolerance to antibiotics and their propensity to grow as organized multicellular aggregates, called biofilms. This chapter describes the linkage and its implication in controlling mycobacterial infections in humans.

## 5.1 Introduction

Homeostasis in the interfaces with commensal polymicrobial communities is a critical aspect of human life, perturbation of which is both a cause and a consequence of infection by pathogenic microbes. The defining feature of microbial pathogens is their ability to colonize the human body and inflict tissue damage, typically associated with the microbes either inducing inflammation, or producing toxins, or both. However, the line of separation between infectious and commensal microbes is blurred by those microbial species that can successfully establish infections inside

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human body but cause disease only under conditions of compromised host immunity. Mycobacteria represent a large number of species that belong to this category, of which the etiological agent of tuberculosis (TB) *Mycobacterium tuberculosis* (Mtb) is the most prominent and pervasive species. One in every three individuals on this planet is estimated to be infected with Mtb, of which only a fraction (about 5–10%) present the disease symptoms of clinical TB (WHO 2012). The remainder population, clinically termed as possessing latent TB, carry the risk of reactivation into symptomatic disease upon compromised immunity from various origins such as HIV infection (WHO 2012). The complexity of the global TB epidemic is further compounded by the inefficient treatment regimens, which involve at least 6-month-long administration of multiple antibiotics (Jindani et al. 2003). This extraordinary level of Mtb persistence against both the host immunity as well as antibiotics raises pertinent questions about the underpinning causes, which seem to be shared by several other mycobacterial species that also share many genetic and phenotypic characteristics with the TB pathogen. In addition to Mtb and related species that form Mtb complex (MTC), several nontuberculous mycobacteria (NTMs) also colonize human hosts. Of major significance among the NTMs are *Mycobacterium avium*, *Mycobacterium abscessus*, and *Mycobacterium ulcerans*, all of which establish chronic infection in diverse host niches and are extremely difficult to eradicate (Gonzalez-Santiago and Drage 2015; Stout et al. 2016).

Identification of factors underlying the persistent characteristics of mycobacteria has been a hot pursuit for a long time. Among the most established contributing factors is the thick, waxy, and uniquely architectured bacterial cell wall, constituted of a myriad of atypical lipids and lipoproteins, which forms a barrier against physicomechanical stress as well as chemical diffusion (Brennan and Nikaido 1995). In addition to physical recalcitrance, physiological adaptation through acquisition of a nonreplicating but viable state is also argued to facilitate mycobacterial survival under external threats, presumably by a "ride-through" mechanism (Parrish et al. 1998). However, the molecular basis underlying the development of persistent bacilli remains unclear. An ever-mounting body of evidence in recent years suggests that the natural propensity of mycobacteria to grow into self-organized multicellular communities, called biofilms, significantly facilitates their survival (Ojha et al. 2005, 2015; Richards and Ojha 2014), presumably through both physical occlusion as well as physiological adaptation. This chapter reviews the biofilm paradigm and its implications in the understanding of mycobacterial persistence in the context of public health.

## 5.2 Biofilm Paradigm: The Whole Is More Than Sum of All

### *5.2.1 Early Discoveries*

In discovering the etiological agents of infectious diseases including TB, Robert Koch laid down the key criteria for establishing the causal relationship between

pathogen and the disease. These criteria, called Koch's postulates, later had tremendous influence on the field of microbiology. Among the most striking contribution of the Koch's postulates is the widely held view of microbes as free-living unicellular organisms. This was founded on the widespread practice of excessive manipulation of culturing conditions in the laboratory, presumably to fulfill the second tenet of the postulate that requires clonally pure culture of a presumptive pathogen. Several microbiologists in the early twentieth century however cautioned on the view of microbial unicellularity as a laboratory artifact by providing evidence of surface-associated sessile occurrence of microbes in communities (Henrici 1933; Zobell 1943), although little attention was paid to their arguments in the following years, perhaps because of the needs for pure cultures in the rapidly emerging field of molecular microbial genetics. In the early 1980s, Costerton and colleagues reestablished the view of microbial plurality through a series of microscopic observations of infected tissues, medical implant devices, and environmental specimens (Costerton et al. 1981; Marrie et al. 1982). The overriding conclusion of Costerton's studies was that microbes invariably grow and live in complex multicellular communities, which appeared to be encased in extracellular polymeric substance. These microbial communities were called biofilms. Later investigations rendered further insights into the inner architecture of biofilms, providing evidence of water channels and movement of solutes in extracellular space (de Beer et al. 1994). An implication of this finding was an ordered organization of resident microbes in biofilms, raising basic questions about the underlying mechanisms. Clinical significance of biofilms was rendered by studies that found unusually high levels of antibiotic tolerance associated with microbes in biofilms as compared to their planktonic counterparts (Nickel et al. 1985). Interestingly, the biofilm-associated antibiotic tolerance is a metastable phenotype that disappears when cells are re-dispersed into planktonic suspensions (Nickel et al. 1985; Ojha et al. 2015), implying that tolerance phenotype develops within the environmental context of biofilms.

### 5.2.2 *Molecular Insights into Developmental Process of Biofilms*

Investigations using genetically tractable species like *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Vibrio cholera*, and *Staphylococcus aureus* have provided knowledge about the molecular process underlying the development of biofilms and differentiation of resident microbes within the architecture (Chai et al. 2008; Hall-Stoodley et al. 2004; Kolter and Greenberg 2006; Kolter and Losick 1998). Although each species appears to have evolved with specific environmental and genetic requirements for growth and development of biofilms, they also share multiple aspects of the developmental processes. Biofilms of a majority of the species proceed through distinct stages of development, initiated by attachment of single-cell planktonic bacilli on a substratum, followed by aggregated growth of

bacilli into a multicellular community which matures through encapsulation of the community by extracellular matrix (Hall-Stoodley and Stoodley 2002). The processes of attachment, aggregation, and maturation (matrix synthesis) are regulated by distinct set of genetic network (Hall-Stoodley and Stoodley 2002). Upon attachment to a substratum, bacteria change their global gene expression pattern, and those changes include downregulation of their motility-associated flagellar biosynthesis genes with concomitant upregulation of adhesins, pillin, and other substratum-anchoring proteins (Richards and Ojha 2014). This transcriptional reprogramming facilitates transition from a motile existence as planktonic cells to their surface-associated sessile growth. Substratum attachment by *Pseudomonas aeruginosa* occurs in two stages. First the cells attach "reversibly" to a surface and then transition into an irreversibly attached state through expression of a surface-attachment protein, SadB (Caiazza and O'Toole 2004; Hinsa et al. 2003). This two-stage process is argued as a niche sampling process by the organism, although evidence of such stepwise attachment in other microbes remains undiscovered. Genetic distinctions between the process of substratum attachment and intercellular aggregation can be derived from studies in *V. cholerae* and *S. aureus*. *Vibrio cholerae* expresses Bap1, a matrix-associated protein, for substratum attachment and Rmb1 for cell-to-cell aggregation (Absalon et al. 2011). Similarly, *S. aureus* uses MSCRAMMs (microbial surface components recognizing adhesive matrix molecules) for substratum attachment but polysaccharide intercellular adhesion for intercellular aggregation (Conrady et al. 2008; Cramton et al. 1999; Cucarella et al. 2001; Heilmann et al. 1996). While substratum and intercellular aggregation are key early-stage events in the developmental process, the process of maturation is never completed without the synthesis of extracellular polymeric substances (EPS) that constitute the matrix in which the cells are "glued" together. Polysaccharides, proteins, DNA, and lipids generally constitute EPS, although relative abundance of these molecules in EPS varies across the species (Branda et al. 2005; Fux et al. 2005).

### 5.2.3 *Spatial Differentiation of Microbes in Biofilms*

Development of three-dimensional architecture is associated with occlusion of nutrient and oxygen to the resident cells within biofilms (Kolter and Losick 1998; Stoodley et al. 2002). Thus, the interior cells must adapt to the self-generated restrictions on oxygen and nutrient supplies in order to grow and survive. Indeed, gene expression analyses in *B. subtilis* and *P. aeruginosa* using transcriptionally fused reporter systems have shown that cells in spatially different regions of biofilms produce different sets of gene products (Chai et al. 2008; Rani et al. 2007; Werner et al. 2004).

Strikingly only a minority of cells in *B. subtilis* biofilms are engaged in matrix synthesis. Moreover, the signaling mechanisms for matrix synthesis in *B. subtilis* are intricately linked to a sporulation pathway through an upstream regulator, SpoA (Chai et al. 2008). The linkage between the two biological processes—with

seemingly disparate energy commitment—could possibly be viewed as a part of a feedback loop mechanism that signals bacilli to switch from a high-energy commitment state (biofilm producer) to a conservation state (sporulation) upon experiencing starvation. This is suggested by the distinct subpopulations of matrix producers and spore producers within a single biofilm of *B. subtilis*. Similarly, active metabolism in biofilms of *P. aeruginosa*, measured by transcription and DNA replication, was seen only in the top 25% of the total depth of the biofilms (Rani et al. 2007; Werner et al. 2004). The sharp decrease in metabolism between interior and exterior locations within a biofilm correlates with the microavailability of oxygen, and most cells (~90%) residing under low oxygen conditions seem to remain viable (Werner et al. 2004), suggesting a programmed metabolic slowdown in the majority of the cells. Further support for metabolic differentiation was obtained by real-time microsensor measurements, which indicated an anoxic environment and associated metabolic inactivity, without loss of viability, within the interiors of the dental biofilms (von Ohle et al. 2010).

Besides physiological and metabolic heterogeneity, resident cells in biofilms also display structural and morphological heterogeneities (Wimpenny et al. 2000). In *E. coli* biofilms, the fibrous surface proteins known as curli form the outer regions of the architecture encasing ovoid bacterial cells, whereas cells at the bottom of the biofilms are elongated and entangled in mesh-like structures (Serra et al. 2013). Importantly, development of these structural patterns is orchestrated by stationary phase sigma factor (RpoS) as well as alarmone ppGGpp and the secondary messenger cyclic di-GMP (Serra et al. 2013).

### 5.2.4 Interspecies Interactions in Polymicrobial Biofilms

Although the studies described above were performed in monospecies biofilms, the findings have profound implications in the context of polymicrobial biofilms. The changes in microenvironment which occur within the biofilms of primary colonizers could create new niches for the growth of secondary colonizers (Elias and Banin 2012). The secondary colonizers could also induce wide-ranging defense mechanisms in the primary colonizer (Rendueles and Ghigo 2012), that together maintain species balance within the community. A continuum of interspecies dynamics is perhaps best exemplified by the studies of polymicrobial biofilms in oral ecosystem, in which the primary colonizers, *Streptococcus mutans* and *Actinomyces* species, are important for growth of *Lactobacillus* species at a later time (Benedict et al. 1992). Similarly, the EPS of another primary colonizer in oral biofilms, *Streptococcus gordonii*, facilitate recruitment of *Porphyromonas gingivalis* (Kuboniwa et al. 2006), which appears to support subsequent recruitment of *Treponema denticola* (Yamada et al. 2005). Lactic acid produced by *Streptococcus oralis* is utilized by *Veillonella* sp. for colonization in oral biofilms (Periasamy and Kolenbrander 2010).

Evidence of competition for niche control is also widespread in polymicrobial biofilms (Rendueles and Ghigo 2012). For example, a surfactin required for

swarming motility in *B. subtilis* inhibits biofilm formation of multiple species including *E. coli* and *Salmonella enterica* (Mireles et al. 2001), while polysaccharides produced by uropathogenic *E. coli*, called capsule 2, inhibits surface attachment and aggregation of various gram-positive and gram-negative species (Valle et al. 2006). In addition to preventing colonization, many species also employ strategies to actively disrupt an existing biofilms of other species. For example, dispersin B from *Actinobacillus actinomycetemcomitans* is a hydrolase of a major EPS component, poly-*N*-acetylglucosamine, that can hydrolyze the matrix and disperse biofilms of several bacterial species (Kaplan et al. 2004). Many bacterial species target other matrix components like DNA and proteins to break down the preexisting biofilms of other bacilli (Iwase et al. 2010; Nijland et al. 2010). A cross-kingdom competition for niche control was demonstrated by a study on mixed biofilms of *Candida albicans* and *Pseudomonas aeruginosa* that often co-colonize in lungs of cystic fibrosis patients. (Hogan and Kolter 2002). *Pseudomonas aeruginosa* biofilms appear to impair the colonization of *Candida* species through secreted factors (Holcombe et al. 2010), while farnesol from *Candida albicans* can inhibit quinolone synthesis in *P. aeruginosa* and associated gene regulation mechanisms that control many attributes of that bacterial species including biofilm formation and virulence (Cugini et al. 2007).

### 5.2.5 *Intercellular Communication in Biofilms*

From the studies described above, it is evident that microbes are sensing not only their environment but also other microbial cells around them. The most well-studied type of intercellular sensing among microbes is quorum sensing, in which individual cells sense the density of their own species (intraspecies quorum sensing) as well as other species (interspecies quorum sensing) through accumulation of secreted signals that trigger a response only above a threshold concentration (Camilli and Bassler 2006; Waters et al. 2008). While quorum sensing controls a wide range of bacterial phenotypes in both planktonic and biofilm cultures, activities in biofilms directly impact the structural integrity and subsequent existential consequences on the involved population of cells. Quorum sensing is employed by *Vibrio* sp. to exit biofilms, perhaps to recolonize a new niche (Hammer and Bassler 2003), whereas the mechanism appears to induce aggregation and matrix synthesis in other species (Hogan et al. 2004). In the context of polymicrobial communities, interspecies quorum sensing can induce either cooperative or competitive behavior in the participating cells depending on the involved species. An interesting example for coexistence of agonistic as well as antagonistic effects of quorum sensing can be found in oral microflora. Interspecies quorum sensing between *S. gordonii* and *P. gingivalis* induce biofilms of both these species (McNab et al. 2003). However, a serine protease called challisin secreted by *S. gordonii* can interfere with activity of competence-stimulating peptide (CSP) of *S. mutans*, thereby impairing the ability of *S. mutans* to from biofilms (Senadheera and Cvitkovitch 2008). Whereas these

dynamics likely play themselves out during development of dental biofilms and associated caries, additional such scenarios are likely possible.

### 5.2.6 *Persistence of Microbes in Biofilms*

As stated earlier in this chapter, one of the defining aspects of microbial existence in biofilms is their recalcitrance to external threats including but not limited to physicomechanical stress, chemical stress, antibiotics, and host immunity (Fux et al. 2005; Stoodley et al. 2002; Van Acker et al. 2014). We have at present only a limited understanding of the factors underlying such tolerance. Studies of monospecies biofilms have revealed that tolerance primarily arises from two factors: (a) diffusion barrier created by extracellular matrix and (b) physiological adaptation to unique microenvironments (Mah and O'Toole 2001). The property of matrix as a diffusion barrier however depends on both the bacterial species as well as the nature of chemical challenge (Mah and O'Toole 2001). While *P. aeruginosa* could restrict the diffusion of piperacillin, antibiotics like vancomycin and rifampicin are restricted by *S. epidermis* (Mah and O'Toole 2001). These differences could perhaps explain perceptions of greater drug tolerance by polymicrobial biofilms, relative to homospecies biofilms of the participating communities (Elias and Banin 2012). It is argued that cooperative behavior among various species is the primary basis of induced resistance in polymicrobial biofilms. This is evident from induced resistance in a slime-negative mutant of *S. epidermis* when cultured in biofilms with *C. albicans* (Adam et al. 2002). While the slime-negative mutant of *S. epidermis* was sensitive to vancomycin in a monospecies culture, it acquired resistance to that antibiotic when co-cultured with *C. albicans* (Adam et al. 2002).

Physiological tolerance to antibiotics in biofilms is linked to their slower growth rate and adaptation to self-generated stresses from limiting nutrients and oxygen, although the extent of resistance varies for different antibiotics (Fux et al. 2005; Mah and O'Toole 2001). By comparing chemostat planktonic cultures with their biofilm counterparts for many bacterial species, investigators have discovered that planktonic cells were comparable to biofilms in ciprofloxacin sensitivity under slow-growing condition but gained sensitivity when growth rate was increased (Desai et al. 1998; Duguid et al. 1992). Downregulation of metabolisms, and subsequent decrease in molecules targeted by antibiotics, is postulated to give rise to growth rate-related persistence (Lewis 2008). Toxin-antitoxin (TA) modules like *mazEF*, *relE*, and *hipA*, as well as *glpD* and *plsB*, have been implicated in maintaining persistent cells under antibiotic exposure by regulating growth and cell division (Lewis 2008). However, the role of these genes in controlling biofilm-associated persistence remains unknown. Adaptation to stresses in biofilms is supported both by upregulation of the stress-related sigma factor RpoS and by many nutrient assimilation pathways in the multicellular architectures (Mah and O'Toole 2001; Serra et al. 2013). The linkage between these pathways and stress tolerance is

supported by the fact that mutation in RpoS sensitizes *P. aeruginosa* biofilms to antibiotics (Mah and O'Toole 2001).

### 5.2.7 Dispersal of Microbes from Biofilms

Dispersal of cells from established biofilms is the final developmental stage. This process is particularly significant from a clinical perspective for novel intervention strategies to disrupt infectious biofilms and thus facilitate rapid clearance. Dispersal could either be auto-induced or forced by a competing species as described in Sect. 5.2.4. Auto-induced dispersion is considered either a bet-hedging process to diversify the colonization capacity or an exit strategy from an existing condition (McDougald et al. 2012; Petrova and Sauer 2016). Generally, dispersal is triggered by environmental cues, such as nutrient availability, oxygen tension, nitric oxide, temperature, etc., and requires auto-induced signaling pathways that involve self-produced extracellular molecules. For example, carbon limitations induce detachment of many bacterial species from surfaces (Delaquis et al. 1989; Gjermansen et al. 2005; Sauer et al. 2004). Dispersal of *Pseudomonas putida* under conditions of carbon limitation is triggered by degradation of a major matrix component, LapA, by a protease LapG. Expression of LapG is negatively regulated by a secondary messenger cyclic di-GMP, such that downshift of the messenger under a condition of nutrient limitation induces LapG activity (Gjermansen et al. 2010) favoring microbial dispersal. In *V. cholerae*, high cell densities result in the induction of a master regulator HapR which represses cyclic di-GMP and EPS synthesis, triggering dispersal of cells from biofilms (Hammer and Bassler 2003). In addition to downregulation of EPS synthesis in the above instances, other species utilize degradation mechanisms to dissolve matrix material for dispersal purposes (Karatan and Watnick 2009). Localized degradation of matrix is associated with production of hydrolases, proteases, and nucleases to degrade EPS, environmental DNA (eDNA), and proteins of the matrix that are thought to facilitate programmed dispersal of cells from biofilms (Allison et al. 1998; Baty et al. 2000; Gjermansen et al. 2010; Mann et al. 2009). Interestingly, localized and programmed cellular death in biofilms of *P. aeruginosa* by rhamnolipids has also been linked to large-scale dispersal of cells (Boles et al. 2005). Another mechanism of dispersal in *P. aeruginosa* involves localized lysis of cells, presumably to provide nutrients to neighboring interior cells as an exit cue (Purevdorj-Gage et al. 2005).

## 5.3 Biofilm Paradigm in Mycobacteria

### 5.3.1 A Historical Perspective

Long before microbial multicellularity was hypothesized and conceptualized, microscopic observations of Mtb found "cords" or parallel filamentous bundles of bacilli (Jones 1896). However, despite a linkage between cording and virulence of Mtb,

these studies went underappreciated due to the influence of Koch's postulates (Silva et al. 1985). In fact, the propensity of mycobacteria to cord was considered a major hurdle in mycobacteriology that involved clonal purity of strains, to the extent that deliberate inclusion of dispersing agents like Tween 80 in medium has been the mainstream technique of in vitro culturing of Mtb for over six decades (Dubos and Middlebrook 1948). This is despite the fact that cording has been attributed to the virulence of Mtb, and detergent has been shown to compromise cell wall integrity and bacterial resistance to antibiotics (Hunter et al. 2006).

### *5.3.2 Manifestation of Mycobacterial Multicellularity*

Detergent-free liquid cultures of Mtb and other mycobacteria invariably form microscopic aggregates of varied morphologies, from unstructured clusters to cords (Richards and Ojha 2014). If left unperturbed, these aggregates organize on the air-medium interface into macroscopic textured pellicles (Richards and Ojha 2014). Similarly, on a solid substratum, the aggregates form colonies (Richards and Ojha 2014). These morphological developments are not restricted to Mtb, but are characteristics of all mycobacterial species including the environmental species like *Mycobacterium smegmatis*. In the past decade, these morphological developments of mycobacteria have been closely examined in the context of the broader paradigm of biofilms, and it is increasingly becoming clear that the aggregated growth of mycobacteria fits into the definitions of biofilms from multiple criteria. First, the formation of pellicles has specialized genetic requirements, which are dispensable for planktonic growth (Islam et al. 2012; Richards and Ojha 2014). Second, the pellicles harbor 100–1000-fold more drug-tolerant persisters than do planktonic cultures of similar cell density, and mutations that disrupt pellicle formation also reduce the frequency of persisters (Ojha et al. 2008). Third, mycobacteria in pellicles have distinct sets of gene-expression profiles as compared to their planktonic counterparts, suggesting that physiological adaptation of cells is associated with their multicellular architecture (Ojha and Hatfull 2007). For example, *M. smegmatis* cells in pellicles induce high levels of siderophore synthesis genes, required for iron acquisition, despite having enough iron in the medium to keep the genes repressed in planktonic cells (Ojha and Hatfull 2007). A reasonable interpretation from this observation is that cells in pellicles likely experience localized depletion of iron due to limited availability from diffusion restriction, leading to induction of the iron sequestration pathway. Consistent with this idea, multiple nutrient transporters are also induced in pellicle cultures of *M. smegmatis*, as compared to their planktonic counterparts. Induction of several candidate multidrug efflux pumps in pellicles suggests the necessity of efficient efflux pathways in pellicle cells for maintaining metabolic homeostasis (Ojha and Hatfull 2007). Taken together, these bodies of evidence indicate that the aggregated growth of mycobacteria represents an organized multicellular community of cells, similar to the biofilms of other microbial species.

### 5.3.3 *Biofilms of Environmental Mycobacteria and Their Impact on Human Health*

Our environment is replete with mycobacterial species, particularly NTMs and rapid-growing mycobacteria (RGMs), which inhabit the soil and water ecosystems as well as engineered water distribution systems (van Ingen et al. 2009; Whiley et al. 2012). Perhaps the most striking evidence of mycobacterial prevalence in our immediate surroundings emerges from a study that reports large abundance of NTMs in polymicrobial communities found in showerheads in American cities (Feazel et al. 2009). Coexistence of NTMs in polymicrobial communities not only highlights the shared habitat for mycobacteria and other bacterial species but also opens up questions about their interactions with the other participating species (Whiley et al. 2012). One example of such interactions is elucidated by a role of *Acinetobacter calcoaceticus* in nucleating co-aggregation of *Mycobacterium mucogenicum* in polymicrobial biofilms formed in water distribution systems (Simoes et al. 2008). The public health impact of polymicrobial biofilms with mycobacteria is accentuated by high incidence of contamination with NTMs and RGMs of the water distribution systems in nosocomial settings (van Ingen et al. 2009; Whiley et al. 2012; Williams et al. 2005). This is further aggravated by high levels of mycobacterial tolerance to chloramine, a common disinfectant used for potable water treatment in urban and hospital settings (Steed and Falkinham 2006; van Ingen et al. 2009; Whiley et al. 2012; Williams et al. 2005). Of the NTMs and RGMs, *Mycobacterium avium* complex and *Mycobacterium abscessus* stand out for their alarmingly high rate of incidence in clinical settings.

#### 5.3.3.1 *Mycobacterium avium Complex*

*Mycobacterium avium complex* (MAC) refers to a select group of very closely related mycobacterial species—*Mycobacterium avium* ssp. *avium*, *Mycobacterium avium* ssp. *hominissuis*, *Mycobacterium avium* ssp. *paratuberculosis*, *Mycobacterium avium* ssp. *paratuberculosis*, and *Mycobacterium intracellulare* (Whiley et al. 2012). Members of the MAC group cause pulmonary and extrapulmonary infections in immunocompromised individuals. Prior to the development of AIDS therapeutics, about 40% of AIDS patients developed MAC infection and a vast majority of them ultimately succumbed to it (Karakousis et al. 2004). In addition, patients undergoing transplant surgery also carry high risk of MAC infection, thereby increasing the burden of MAC-related mortality in developed countries (Whiley et al. 2012). Additionally, it must be noted that MAC infections, particularly those caused by *M. intracellulare*, are also prevalent in individuals with potentially increased susceptibility even in the absence of overtly obvious compromised immunity (Field et al. 2004; Han et al. 2005; Sugita et al. 2000). Infections caused by MAC are also attributed to Crohn's disease (Pierce 2009), although causal relationship between MAC infection and those disease symptoms

remains controversial (Chamberlin et al. 2001). We can recognize that MAC infections share characteristics of other mycobacterial infections including the fact of their being highly recalcitrant to antibiotics, and successful treatment often requires multiple drugs administered simultaneously over a period of 12 months.

Relationship between MAC biofilms and their persistent and virtually untreatable infections can be viewed from two perspectives. First, the ability of MAC to establish biofilms in environmental polymicrobial communities and their extraordinary recalcitrance to disinfectants increases our exposure to these species, thereby increasing the incidence of nosocomial infections associated with MAC. Second, biofilms of MAC can facilitate the persistence of these microbes against the challenges of host immunity and antibiotic treatment (Falkinham 2007; Rose and Bermudez 2014), thereby making the infections often chronic and untreatable. Interestingly, mutants of *M. avium* that fail to form biofilms in vitro also have impaired ability to invade and translocate bronchial epithelial cells (Yamazaki et al. 2006a), suggesting that colonization of host tissues could also be associated with biofilm formation of MAC. Thus, pharmacological or physical disruption of MAC biofilms could potentially be an effective strategy to not only prevent but also treat infections. Structural and genetic analyses of *M. avium* biofilms have revealed interesting insights that could possibly yield suitable targets for disruption. Bermudez and colleagues recently showed the presence of eDNA in biofilms of clinical isolates of *M. avium* and also demonstrated that treatment of *M. avium hominissuis* isolate 5 (MAH 5) biofilms with DNAse reduced the biomass by half (Rose et al. 2015), thus opening the possibility of using DNAse as one of the agents to disrupt biofilms. Gene expression analysis revealed induced expression of several key genes in biofilms of *M. avium*, among the most notable of which were genes involved in the tricarboxylic acid (TCA) cycle, GDP-mannose biosynthesis, and glycopeptidolipid (GPL) biosynthesis (Yamazaki et al. 2006b), suggesting that these genes might serve as candidates for targeting biofilms.

#### 5.3.3.2 *Mycobacterium ulcerans*

*Mycobacterium ulcerans* is another NTM and it causes a highly persistent skin and soft tissue infection, called Buruli ulcer, which often remains refractory to treatment with most of the common antibiotics (Yotsu et al. 2015). *Mycobacterium ulcerans* is largely associated with freshwater ecosystems, and it is believed that contact with contaminated water leads to infection (Garchitorena et al. 2015). Although the precise mechanisms of transmission of *M. ulcerans* remain elusive, both biotic and abiotic factors have been attributed to its dynamics in aquatic ecosystem and subsequent infection (Garchitorena et al. 2015). However, the outcome of infection with *M. ulcerans* is highly dependent on the pathogen's ability to produce a toxin called mycolactone, which is considered to be a primary factor for tissue damage (George et al. 1999). Interestingly, the synthesis of mycolactone has been linked to the ability of *M. ulcerans* to form biofilms (Marsollier et al. 2005), thus raising the possibility of a direct relationship between biofilms and pathogenesis of *M. ulcerans.*

A molecular insight into this relationship however has been limited by genetic intractability of the pathogen that demands new tools of molecular genetics for further investigation.

#### 5.3.3.3 *Mycobacterium abscessus*

*Mycobacterium abscessus* is an environmental RGM found both in natural aquatic ecosystems as well as in engineered water distribution systems. *M. abscessus* can cause chronic infections in lungs and post-traumatic wounds on skin upon contact with contaminated water (Benwill and Wallace 2014). Infections with *M. abscessus* have been increasingly common in recent years (Benwill and Wallace 2014). Individuals with cystic fibrosis are particularly at high risk of developing infection with *M. abscessus*. The often necessity of a 24-month-long multidrug treatment regimen for *M. abscessus* infections poses numerous challenges, particularly in the context of immunocompromised patients. The chronic and drug-tolerant characteristics of *M. abscessus* infections share similarities with the characteristics of other microbial species that establish biofilms in host tissues. Recent advancements in genetic techniques have offered insight into the molecular relationship between biofilms and pathogenesis of *M. abscessus* (Medjahed and Reyrat 2009; Medjahed and Singh 2010). Strains which demonstrate rough colony morphology when growing on agar plates generally are devoid of glycopeptidolipids (GPL) on the cell surface, these strains can readily form cords and biofilms, and they can colonize the host more efficiently as compared to smooth colony mutants (Bernut et al. 2014; Catherinot et al. 2007; Nessar et al. 2011). However, deficiency of GPL-negative mutants in substratum attachment also suggests that initial colonization of the pathogen is distinct from aggregated growth and biofilm formation, which is perhaps more critical for its pathogenesis and persistence (Nessar et al. 2011). This idea is clearly elucidated by a recent finding that biofilm defect in *M. smegmatis* due to deficiency in aggregation can be restored by suppressor mutation in GPL biosynthesis (Yang et al. 2017). In another interesting study, the switch from rough to smooth colonies was found to be a reversible process induced by exposure to subinhibitory concentrations of aminoglycosides, suggesting an active process of adaptation by the pathogen to a drug-tolerant state under challenged condition (Tsai et al. 2015).

### 5.3.4 *In Vitro Biofilms of Mtb Complex: From an Assay for Drug Discovery to the Riddle of Persistent TB*

Even though the evidence for Mtb biofilms in the host tissues remains to be established, the fact that these biofilms can exist as structured multicellular drug-tolerant communities in vitro (Richards and Ojha 2014) offers a new growth model

to understand the mechanisms of physiological adaptation by the pathogen to environmental stresses, which in turn could be relevant for understanding its persistence inside the host. For example, mutation in *mma4*, which synthesizes oxygenated mycolic acids in Mtb, abrogates biofilm growth of Mtb (Sambandan et al. 2013). Given that oxygenated mycolic acids increase the envelope permeability (Takayama et al. 2005), it could be argued that a *mma4*-dependent envelope permeability is perhaps critical for enhanced nutrient uptake of Mtb in restrictive biofilm environments. A role of *mma4* in Mtb survival inside of its host further argues for overlap between the survival mechanisms employed by the pathogen during development of biofilms in vitro and growth inside the host. Similarly, identity of genetic loci for antibiotic tolerance in biofilms could potentially lead to novel targets for therapeutics against persistent TB.

## 5.4 Future Outlook

In summary, the large abundance of mycobacteria in our environment and their extraordinary ability for persistence against the common antibacterial agents poses a significant public health risk. Novel mitigation strategies would require better understanding of the molecular mechanisms underlying their persistence characteristics and process of biofilm development. Urgency in development of new molecular genetics tools for manipulation of mycobacterial pathogens like *M. abscessus* is necessitated by the rapidly increase in their clinical incidence. New and powerful high-throughput sequencing platforms and advanced microscopic techniques have the potential to lead to new methods for investigations into these areas of research.

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**Conflict of Interest** Anil K. Ojha declares that he has no conflict of interest.

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# Part III
# The Ecology of Infectious Diseases Affecting Livestock and Wildlife

# Chapter 6
# Opportunistic Bacteria Associated with Mammalian Livestock Disease


**Christon J. Hurst**

**Abstract** This chapter presents a current compendium of knowledge regarding the opportunistically bacteria associated with those livestock mammals used as sources of meat and milk for consumption by humans. All of these mammalian species are terrestrial and the majority are bovids. However, as important as the bovids are to us, they do indeed represent only part of a diverse collection which includes rodents as small as the Edible dormouse and cervids as large as the moose or Eurasian elk. The opportunistic microorganisms mentioned in this chapter represent a critical concern with respect to human food supplies. This chapter lists the pathogens by genus along with their higher taxonomy, provides information regarding the ecology of these genera, and summarizes their associated disease information with respect to livestock mammals. The genus listings include the individual pathogen species by name and also identify those host mammals which are known to be affected by each pathogen species.

## 6.1 Introduction

Humans are by definition omnivores, which means that we can and indeed generally do eat as food almost anything whose ingestion is not either physically or chemically lethal. Animals certainly are an important source of dietary protein for most humans and as livestock those animals are most easily and inexpensively fed if either they are herbivores or can otherwise forage for themselves. The biggest factors in our choices of food animals are the affordability and availability of the animals and, of course, our level of hunger. A major challenge for us when keeping the animals as livestock is that we maintain their health, which includes a need to understand the pathogens which affect these animals.

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This is a companion article for Hurst (2016), which presented the subject of opportunistic algae, fungi and ichthyosporea associated with mammalian livestock disease.

## 6.2 Livestock and Their Use

Livestock, in its broader definition, can include any type of animal that either is domesticated, semi-domesticated, or captured from the wild and then maintained under agricultural supervision. That supervision is termed animal husbandry and it has made a big change in the cultural evolution of our species by allowing a longer term control of meat resources and enabling dairy industry. Maintaining livestock freed us from continually hunting wild animals in order to meet our food requirements and subsequently enabled the development of larger human communities. The list of animals considered to be livestock includes not only mammals, but also amphibians, most notably frogs; those numerous avian groups which are categorized as poultry; crustaceans, most notably decapods such as crab, crayfish, lobster, prawn and shrimp; fish including eels; insects, most notably bees, crickets and silk worms; gastropod molluscs including abalone and edible terrestrial snails; bivalve molluscs including clams, mussels and oysters; and among the reptiles we keep as livestock not only snakes and turtles, but also various members of the order Crocodilia including alligators, caimans, and crocodiles.

While the definition of livestock is not limited either to mammals or the other groups of animals that are intended as food for humans, this chapter must have a more limited focus and therefore considers only those mammals maintained for the goal of consumption by humans. Some of the mammals listed in this chapter as livestock serve us for more than a single function. In addition to eating them as a source of meat, many of the larger mammals are of course considered to produce sufficient milk and be adequately passive in terms of their temperament that they are used as dairy animals. Those animals utilized in dairy industries generally are represented by the bovids, but the category of dairy animals also includes such cervids as moose and reindeer, camelids including camel and to a far lesser extent llama, and among the equines we use both the horse and donkey as sources of milk. Body fat from many of these animals is considered a food in its own right and also perceived as an important cooking ingredient. The larger mammals often have been used as a source of labor and transportation, either as beasts of burden for animal portage or harnessed for use as drayage animals. Indeed the very largest often are ridden.

There are, of course, many additional mammalian species kept as livestock and primarily of note are three species perceived as fur animals with their meat generally used to feed other animals. These three fur animals are the American mink (*Neovison vison*) and the two remaining species of chinchilla, Short-tailed chinchilla (*Chinchilla chinchilla*) and Long-tailed chinchilla (*Chinchilla lanigera*). Still other

terrestrial mammals such as rats are hunted and eaten by humans but they generally are not kept as livestock.

Component parts of livestock that neither are eaten by humans nor fed as meat to other animals are utilized for a variety purposes with a few of those examples mentioned here. Body fat and butter fat from mammals has been burned as a source of lighting. We make not only health and beauty products, but also household furnishings and decorations from animal materials. Body fat serves as an ingredient for making soap. Hydrolysed keratin is a common cosmetic ingredient. Animal body fat, butter fat, and waxes such as lanolin are used for skin emollients. The hides of larger animals often are used as a source of leather, collagen, and derivatives of collagen including gelatin. Bones and connective tissue similarly are used as a source of collagen for making gelatin. Bones also are used as an ingredient in ceramic furnishings. Mammals which have long hair that easily can be spun and woven often become a source of fiber for clothing and other domestic items made of cloth. Mammals are, of course, not the only livestock animals that we view as raw materials. As an example, the shells of crustaceans and molluscs can be used as ingredients in dietary supplements for other animals. Birds often produce desirable feathers used for decorative purposes including clothing. Chemical industries have developed commercial applications for a broad range of additional products manufactured from shells and feathers.

### *6.2.1 The Mammals Kept as Livestock for Human Consumption*

The mammalian species addressed in this chapter are those used as sources of either meat, milk, or both, for human consumption. These mammals are listed in Table 6.1 and taxonomically described in this section. All of the listed mammals are used as sources of meat. Those mammals which also are used as sources of milk for human ingestion are members of the genera *Alces*, *Bos*, *Bubalus*, *Camelus*, *Capra*, *Cervus*, *Ovis*, and *Rangifer*.

Examining the list of mammals maintained as livestock intended for human consumption may reveal some interesting surprises. What we either now raise to eat or have raised in the past varies tremendously from one part of the world to another and the dietary choices include some cultural preferences whose origins often were distinctly local in nature. Our preferential decisions include aspects that are religious, perhaps most notably being the considerations regarding consumption of cattle and pig. There also is the truth that different societies often view as pets those same animals which other people would consider to be traditional foods, and this difference frequently has become a point of contention when cultural groups meet and intermix. Local taste preferences include choices of such mammals as cat, dog, dormouse, horse and guinea pig. It is unfortunately often considered easier to negatively criticise someone else's cultural practices when we should instead present

**Table 6.1** Mammals kept as livestock for human consumption

| Infraclass | Order | Family | Subfamily | Genus | Common name |
|---|---|---|---|---|---|
| Eutheria | Artiodactyla | Bovidae | Bovinae | *Bison* | American bison |
| | | | | *Bos* | Cattle<br>Yak<br>Gayal |
| | | | | *Bubalus* | Carabao<br>Water buffalo |
| | | | Caprinae | *Capra*<br>*Ovis* | Goat<br>Sheep |
| | | Camelidae | Camelinae | *Camelus*<br>*Lama*<br>*Vicugna* | Camel<br>Llama<br>Alpaca |
| | | Cervidae | Odocoileinae | *Alces*<br>*Rangife* | Moose<br>Reindeer |
| | | | Cervinae | *Cervus* | Elk<br>Red deer |
| | | | | *Dama* | Fallow deer |
| | | Suidae | Suinae | *Sus* | Pig |
| | Carnivora | Canidae<br>Felidae | Caninae<br>Felinae | *Canis*<br>*Felis* | Dog<br>Cat |
| | Lagomorpha | Leporidae | Leporinae | *Oryctolagus* | Rabbit |
| | Perissodactyla | Equidae | Equinae | *Equus* | Donkey (Ass)<br>Horse |
| | Rodentia | Caviidae<br>Hydrochaeridae<br>Gliridae<br>Myocastoridae | Caviinae<br>Hydrochoerinae<br>Glirinae<br>Not assigned | *Cavia*<br>*Hydrochoerus*<br>*Glis*<br>*Myocastor* | Guinea pig<br>Capybara<br>Dormouse<br>Coypu (Nutria) |
| Metatheria | Diprotodontia | Macropodidae | Not assigned | *Macropus* | Kangaroo |

All of these listed mammals are raised as sources of meat for human consumption. Some members of the following genera also are used as a sources of milk for human consumption: *Alces*, *Bos*, *Bubalus*, *Camelus*, *Capra*, *Cervus*, *Equus*, *Ovis*, and *Rangifer*. The Water buffalo also is called the Asian buffalo and sometimes those two names are combined as Asian water buffalo. The African buffalo or Cape buffalo does not seem to ever have been domesticated owing to its unpredictable nature and for which reason it is considered dangerous to humans. The terms yak, goat, sheep and pig respectively refer to the Domestic yak, Domestic goat, Domestic sheep, and Domestic pig. My usage of Camel refers to both the Bactrian camel and the Dromedary camel. The species indicated in this table as dormouse commonly is known as the Edible dormouse or Fat dormouse, it is live-trapped and then raised domestically. My usage of the term kangaroo refers as a group to three species, the Red kangaroo, Eastern grey kangaroo, and Western grey kangaroo. Of those three kangaroo species it is the Red kangaroo which seems to be the most suggested for domestication as livestock. Kangaroo indeed are bred in captivity and raised on farms but to date their production for human consumption generally is considered unprofitable because of cost competition against hunted animals. I have kept use of the order name Artiodactyla although Cetartiodactyla seems now to be on the road to replacing that order name

acceptance and offer understanding of the differences between the many personal choices which are made by societal groups. We must not criticize what is eaten in other, and in particularly less wealthy cultures, simply because those who are fortunate enough to have wealthier lives can afford to have multiple options and be finicky eaters. We also should not allow our personal preferences to influence control over what human groups either can or cannot choose to eat.

### 6.2.2 Terminology for the Mammalian Livestock Groups Included in This Chapter

The following briefly summarizes some of the terminology that I have used in this chapter when referring to mammalian livestock.

The Carabao often is called a water buffalo, but currently these two names are not considered synonymous. For this chapter the common name Carabao corresponds to the species (*Bubalus carabanensis*). The common name buffalo is used in this chapter as a reference to the Water buffalo (*Bubalus bubalis*) which also is called the Asian buffalo, and sometimes those names are combined in common practice to form the name Asian water buffalo. The African buffalo or Cape buffalo (*Syncerus caffer*) seems not to have been domesticated owing to its unpredictable nature, for which reason it is considered dangerous to humans. The term bison, when used in this chapter, indicates the American bison (*Bison bison*) unless specifically identified as a referral of information pertaining to the European bison (*Bison bonasus*). The terms yak, goat, sheep and pig respectively refer to the Domestic yak (*Bos grunniens*), Domestic goat (*Capra hircus*), Domestic sheep (*Ovis aries*), and Domestic pig (*Sus scrofa*). My usage of camel refers to both the Bactrian camel (*Camelus bactrianus*) and the Dromedary camel (*Camelus dromedarius*).

Not all of the mammals listed in Table 6.1 are considered domesticated, a few such as the dormouse and kangaroo are captured wild and then raised in captivity. The species indicated in Table 6.1 as dormouse is that commonly known as the Edible dormouse or Fat dormouse (*Glis glis*), it is live-trapped and then raised domestically. My usage of the term kangaroo refers as a group to three species, the Red Kangaroo (*Macropus rufus*), Eastern grey kangaroo (*Macropus giganteus*), and Western grey kangaroo (*Macropus fuliginosus)*. Of those three kangaroo species it is the Red kangaroo which seems to be the most suggested for domestication as livestock. Kangaroo indeed are bred in captivity and raised on farms but to date their production for human consumption generally is considered unprofitable because of cost competition against hunted animals.

Bovid is a category that refers to members of the family Bovidae, and is represented in this chapter by both bovines and the caprids, with members of the latter group also identified by the term caprines.

Bovine is a term which refers to members of the subfamily Bovinae, is represented in this chapter by the following species: the American bison (*Bison bison*); Cattle (*Bos taurus*);

Gayal (*Bos frontalis*); Yak (referring in this chapter to the Domesticated yak, *Bos grunniens*); Water Buffalo (*Bubalus bubalis*); and Carabao (*Bubalus carabanensis*). Neither of these bovines biological relatives the European bison (*Bison bonasus*) nor the African buffalo or Cape buffalo (*Syncerus caffer*), seem to have been either domesticated or otherwise considered as livestock and thus those two relatives are not included in this chapter. Interestingly, the American bison currently is raised agriculturally in Europe.

Canine is a term which represents those members of the subfamily Caninae that are grouped within the family Canidae. The category of canines is represented in this chapter by only one subspecies recognized as Dog or Domestic dog (*Canis lupus familiaris*).

Caprid and caprine are terms which refer to members of the subfamily Caprinae, and that group is represented in this chapter by the following species: goat, which for the purposes of this chapter represents only the Domestic goat (*Capra hircus*), and sheep, which herein refers to only the Domestic sheep (*Ovis aries*).

Camelid is a term which refers to members of the family Camelidae, and they are represented in this chapter by the following animals: Alpaca (*Vicugna pacos*); camel, referring in this chapter to both the Bactrian camel (*Camelus bactrianus*) and the Dromedary camel (*Camelus dromedarius*); and Llama (*Lama glama*).

Caviid is a term which refers to members of the family Caviidae, a group that is represented in this chapter by the Guinea pig (*Cavia porcellus*).

Cervid is a term which refers to members of the family Cervidae, and they are represented in this chapter by the following animals: Elk (*Cervus canadensis*); Fallow deer (*Dama dama*); Moose or Eurasian Elk (*Alces alces*); Red deer (*Cervus elaphus*); and Reindeer, also called Caribou (*Rangifer tarandus*). The information provided for pathogens of moose does not distinguish between species of *Alces*, all of which are termed to be moose.

Equine is a term that refers to members of the subfamily Equinae, which includes the genus Equus. That group is represented in this chapter by two species, those being the Donkey or Ass (*Equus asinus*) and the Horse or Domestic Horse (*Equus ferus caballus*).

Feline is a term which refers to members of the subfamily Felinae within the family Felidae, as represented in this chapter by only that species know as Cat or Domestic cat (*Felis catus*).

Glirid is a term which refers to members of the family Gliridae, a group that is represented in this chapter by the name dormouse, herein referring to only the Edible dormouse or Fat dormouse (*Glis glis*).

Leporid is a term which refers to members of the family Leporidae, and that group is represented in this chapter by the rabbit, also known as the European rabbit or Common rabbit (*Oryctolagus cuniculus*).

Macropod is a term which refers to members of the family Macropodidae, and three of its member species are represented in this chapter under the general name kangaroo; those are the Eastern grey kangaroo (*Macropus giganteus*), Western grey kangaroo (*Macropus fuliginosus*), and Red Kangaroo (*Macropus rufus*).

Rodent is a term that refers to members of the order Rodentia, which includes both the caviids and glirids as well as the hydrochaerids. That order also includes the Coypu or Nutria (*Myocastor coypus*).

Ruminant is a term which in this chapter refers only to those mammals belonging to the suborder Ruminantia, as represented by members of the families Bovidae and Cervidae. Camelids also are foregut fermenters but not classed as ruminants because of differences in stomach structure.

Suid is a term which refers to members of the family Suidae, and that group is represented in this chapter by only one species, the Pig or Domestic pig (*Sus scrofa*).

## 6.3 Opportunistic Pathogenicity

Opportunistic pathogens are those which typically cause disease subsequent to an opportunity. Some opportunistic pathogens are environmental residents that may live naturally in soil and water. But, if one of these opportunistic environmental organisms arrives at a location either on or within a potential host animal or plant which suits the metabolic capabilities and requirements of that environmental organism, then an infection can ensue and the host may become diseased. Many other opportunistic pathogens reside naturally either at various times or exclusively as symbionts or commensals of host plants and animals, and yet should the host become weakened in its capacity to mount a protective defense, that otherwise harmless symbiotic or commensal organism may reveal its pathogenic potential. The ability of a host to defend against the pathogenic potential inherent in a symbiotic or commensal organism often involves a component of site specificity. For example, a microorganism which normally is commensal in either the mouth or colon of an animal may cause disease if it reaches a cutaneous wound on that same animal. The term 'primary pathogen' often is used as a defining reference to those pathogenic organisms which can cause disease in otherwise healthy hosts. Correspondingly, opportunistic pathogens are frequently, although often confusingly, termed 'secondary pathogens' perhaps suggesting that opportunistic pathogens cannot be the sole attacker of a host. Opportunistic pathogens very often can act alone as the sole cause of pathogenicity related to an infection. Perhaps a clearer usage of the term secondary pathogen would involve a more restricted reference to uniquely those organisms whose pathogenicity can be expressed only as a compounding factor to an underlying infection that has been caused by some other pathogen.

There are numerous factors which affect opportunistic pathogenicity and although they strongly overlap, it is possible to group these as: host factors, microbial factors, ecological factors, and medical factors. Some of them are included in Table 6.2. A general introduction to disease transmission was published by Hurst and Murphy (1996).

### *6.3.1 Host Factors*

There are several component host factors including immunosuppression and immune dysfunction which can increase the susceptibility of mammals to pathogenic attack.

**Table 6.2** Factors Affecting Opportunistic Pathogenicity

| |
|---|
| Host factors |
| Immunosuppression |
| Concurrent infection |
| Diabetes |
| Dietary deficiency |
| Injury |
| Medically induced effects |
| Stress |
| Underlying immune dysfunction |
| Secretory systems |
| Adaptive immunity, also termed specific defense |
| B cells |
| T cells |
| Innate immunity, also termed nonspecific defense |
| Defensins |
| Extrachromosomal histone H2B |
| Lysozymes |
| Macrophage |
| Magainins |
| Pattern recognition receptors |
| Transferrins |
| Microbial factors |
| Zoonotic potential and host range |
| Ecological factors |
| Demography of an infectious disease |
| Presence of a susceptible animal host |
| Source of pathogenic organism |
| Environmental presence |
| Presence of another infected host serving as reservoir |
| Presence of suitable vector |
| Medical factors |
| Severity and duration of disease and symptoms |
| Acute versus chronic |
| Overt versus subacute |
| Appropriate diagnosis |
| Prevention and treatment |
| Antimicrobial compounds |
| Microbial treatments |
| Probiotics |
| Prebiotics |
| Synbiotics |
| Vaccines |

#### 6.3.1.1 Immunosuppression

Immunosuppression can be present because of such conditions as concurrent infections, diabetes, dietary deficiencies, injury, medically induced suppression which generally is a side effect of treatment for some other illness, and stress whose components include not only climatic factors like seasonal weather but also the effects of transport and relocating animals including capture and release programs.

#### 6.3.1.2 Immune Dysfunction

Underlying immune dysfunction generally is due to genetic deficiencies and often the impaired immune functions can be improved by selective breeding programs. Table 6.2 mentions the category of secretory system dysfunctions, representing either the absence, overproduction, or functional failure of metabolic components including those normally associated with mucosal surfaces of the respiratory and genitourinary tracts. An example of secretory failure is the chloride channel dysfunction which causes cystic fibrosis and increases susceptibility to respiratory infections. Dysfunction also can occur in the adaptive and innate immune systems, which in turn will compromise those systems and often results in the host more easily being targeted by potentially pathogenic organisms.

##### 6.3.1.2.1 Adaptive Immunity

Adaptive immunity also is termed specific defense. In mammals, its main components are B cells and T cells. The B cells produce secretory proteins including antibodies as well as signaling proteins termed cytokines. The central role of T cells is termed cell mediated immunity, and its component categories include Helper T cells which assist other lymphocytes, Cytotoxic T cells which destroy tumor cells and virus-infected cells, Memory T cells which allow the immune system to respond more quickly to subsequent infections by similar pathogens, Suppressor T cells which help maintain immunological tolerance, and Natural killer T cells which are perceived as connecting the adaptive and innate immune systems. Some of the T cells also release cytokines.

##### 6.3.1.2.2 Innate Immunity

Innate immunity also is termed nonspecific defense. There are numerous components which comprise the innate immune system and only some examples are mentioned here. Defensins are cationic proteins that typically bind to and assist in the destruction of microbes by forming pore-like defects. Defensins are present in some types of immune cells and many are secretory in nature. Extrachromosomal

histone H2B represents a category of proteins that typically seem to remain in the cytoplasm where they aid in antiviral defense by attacking intracellular double stranded DNA. Lysozymes are proteins that damage bacterial cell walls by catalyzing hydrolysis, they are present in some types of immune cells and also are secretory. Macrophage are a category of phagocytes which act in conjunction with both adaptive and innate immune responses. The macrophage can engulf and then destroy microbes, and yet some pathogens have evolved a capability to survive inside the host animal by establishing residence within macrophage. Magainins are secretory peptides which act at least in part by permeabilizing cell membranes. Pattern recognition receptors also are proteins, of which some types are secretory, that act by recognizing molecules termed pathogen-associated molecular patterns. Transferrins are secretory plasma proteins that bind and control the level of available iron.

### *6.3.2 Microbial factors*

Among the microbial factors are zoonotic potential and host range. These are evolutionarily related to the success of past host-pathogen interactions and determined by metabolic requirements of the pathogen in conjunction with the pathogens ability to resist the hosts defenses. Zoonotic potential of environmental microbes can be influenced by ambient environmental conditions that affect genetic expression of the pathogens metabolism. An example of environmental preconditioning occurs when ambient environmental conditions such as elevated temperature activate the genetic expression of microbial metabolic traits that act as pathogenic traits, priming the capability of a microbe to initiate infection when it enters the warm body of a mammalian host.

### *6.3.3 Ecological Factors*

Ecological factors can influence the demography of an infectious disease, often acting in conjunction to determine the likelihood of pathogen acquisition. From the perspective of a pathogen, demographic success requires a source of the pathogenic organism, simultaneous presence of a susceptible animal host, and some way in which the pathogen can encounter the host. The source of many pathogenic organisms arises from their possessing a natural environmental presence, such presence may range from ubiquitous to geographically limited and also can be seasonal. Other pathogenic species are acquired from infected hosts and importantly for these pathogens the likely simultaneous presence of both infected reservoir hosts and susceptible hosts can be geographically limited and also may be seasonal. Some pathogens require transferral by a suitable vector, but the presence of their potential vector species likewise can be geographically limited and also include a component of seasonality.

### 6.3.4 Medical Factors

There are a large number of medically related factors which can influence the incidence of opportunistic pathogenicity and outcome. Prevention of disease is the best goal. Among the disease prevention measures, those with proven success are good general animal husbandry practices, use of vaccines, and administration of prophylactic antimicrobials. Vaccines often protect the majority of animals but also will kill some animals and so their usage involves considering the net balance of risk. Prophylactic use of antimicrobials clearly can benefit animals but potentially also may have a negative affect by increasing the antibiotic resistance capabilities of microbes.

In addition to understanding that environmental conditions and host-microbe interactions can affect the occurrence of opportunistic infections, we must recognize the role of microbe-microbe interactions. It is for this reason that preventative treatments based upon the concept of assisting an animal's protective commensal microbial populations have seen an increased level of interest in recent years. These treatments often are categorized as probiotics, prebiotics, and synbiotics. Probiotics are microbes administered to commensally compete against pathogens, but some of the microbes used as probiotics are themselves opportunistic pathogens and their administration can cause disease. Prebiotics are compounds administered with the intention of benefiting commensal microbes, with an example being nutritional supplements. Synbiotics is a concept that represents simultaneous administration of probiotic microbes and prebiotic compounds.

When disease does occur, obtaining an appropriate diagnosis in a timely fashion better allows for successful treatment of the affected animal. Disease may be acute, meaning that it has either a rapid onset or short duration, versus chronic, meaning that the disease has long term persistence. Overt disease is more likely to be recognized and adequately treated as compared with subacute disease. Following successful diagnosis, the issues of key importance in supporting the affected animals become reducing the duration of disease and severity of symptoms. Sometimes supportive care is sufficient, but when that accompanied by antimicrobial compounds becomes inadequate then further measures must be taken including the possibility of destroying the affected animals to protect healthy members of the host population.

## 6.4 Listing of Opportunistic Bacteria Affecting Livestock Mammals

Many of the listed bacteria naturally are environmental organisms and most typically they reside either in soil or water. Others are naturally associated with plants. Often these environmental organisms are saprophytically associated with decaying organic material and their association with animals typically arises from either a passive

presence on the skin, accidental introduction into wounds, ingestion or inhalation. Some of the listed fungi either are opportunistic dermatophytes or commensal residents of the mucosal tissues, and many of these microbes may have either no natural environmental presence or only a brief presence in the natural environment as contaminants associated with carcasses and naturally shed materials including animal excretions.

Some pathogen species are very specific with respect to their host range and in particular that tendency exists with viruses, while other pathogens are generalists capable of infecting many different host animals. The bacterial pathogens tend to be generalists in terms of their infective capability and you could presume that any association listed between a member of these pathogen groups with one member of a mammalian genus could suggest that the pathogen similarly has an ability to infect other members of that same mammalian genus. Some disease associations found to exist for the European bison but not for the American bison are listed in this chapter under the presumption that a pathogen which affects the European bison likely would also be capable of affecting the American bison. I have listed all of the potentially opportunistic associations that I could find by internet researching. The fact that a disease association between some particular pathogen species and host species cannot be found listed in this chapter should not result in the readers presumption that such an association does not exist.

Some mammalian groups seem to be represented in more detail among these listings. That fact represents differences in the amount of modern veterinary research which is conducted regarding the different pathogen and host relationships. The ability to conduct those studies depends upon availability of research funding, the relative value of the animal in question plus acceptable cost of treating opportunistic infections where the animal is raised, and indeed financial capacity for treating the animal. More research is done on larger animals and on those animals considered as pets because these categories tend to have more financial value per individual animal. Historically traditional veterinary studies would always have been a part of animal husbandry. Those historical studies identified diseases but generally not the affecting pathogens. I suggest the following references as good sources of additional information on these opportunistic pathogens: Kahn and Line (2010), Pathogen Safety Data Sheets and Risk Assessment (2015), Sumbali (2011), The Center for Food Security and Public Health (2015), and Vetbook (2015).

Note: use of brackets around an assigned species name indicates that either part or all of that assigned name has not officially been approved.

### *6.4.1* Acholeplasma

Phylum: Tenericutes; Class: Mollicutes; Order: Acholeplasmatales; Family: Acholeplasmataceae; Genus: *Acholeplasma*. *Acholeplasma* species rely upon saprophytic nutrition which is an extracellular chemoheterotrophic digestion process that digests and decays organic material. The members of this genus vary with regard to the expansiveness of their host range. Their associated symptomatology varies from

conjunctivitis or keratoconjunctivitis to systemic infections including those of the lung leading to pneumonia, and of either the fetus or placenta leading to abortion. *Acholeplasma laidlawii* is the only mollicute that is capable of existing free of any host and it can do so provided that the environment provides its necessary nutritional needs. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Acholeplasma axanthum* (wide host range in mammals), *Acholeplasma cavigenitalium* (guinea pig), *Acholeplasma equifetale* (horse). *Acholeplasma hippikon* (horse), *Acholeplasma laidlawii* (wide host range in mammals including horse and camel), and *Acholeplasma oculi* (wide mammalian host range including sheep, goat, horse, camel).

### *6.4.2* Achromobacter

Phylum: Proteobacteria; Class: Betaproteobacteria; Order: Burkholderiales; Family: Alcaligenaceae; Genus: *Achromobacter*. The species *Achromobacter xylosoxidans* is an aquatic organism that causes lung infections including pulmonary nodules (pig, rabbit, coypu).

### *6.4.3* Acinetobacter

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Pseudomonadales; Family: Moraxellaceae; Genus: *Acinetobacter*. Members of the genus *Acinetobacter* commonly are found in soil and water including fresh water and sewage. They notably can grow in drinking water distribution systems. The *Acinetobacter* are associated with necrotising fasciitis, urinary tract infections, and pneumonia. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Acinetobacter baumannii* (cattle, pig), *Acinetobacter beijerinckii* (livestock), *Acinetobacter calcoaceticus* (cattle, coypu), *Acinetobacter gyllenbergii* (potentially in livestock), *Acinetobacter haemolyticus* (cattle, sheep, pig, coypu), *Acinetobacter lwoffii* (cattle, goat, sheep), *Acinetobacter radioresistens* (livestock), *Acinetobacter rudis* (cattle), *Acinetobacter schindleri* (ruminants), and *Acinetobacter ursingii* (potentially in livestock).

### *6.4.4* Actinobacillus

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Pasteurellales; Family: Pasteurellaceae; Genus: *Actinobacillus*. The members of this genus are naturally part of the subgingival ecosystem. Members of the genus *Actinobacillus* can affect lymph nodes of the head, notably the face, mouth, esophagus and neck,

and their symptoms include the formation of granulomas; granulomas also can form in the lungs associated with pleuropneumonia, and in the udder. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Actinobacillus capsulatus* (alpaca, llama), *Actinobacillus equuli* (horse), *Actinobacillus lignieresii* (camel, cattle, horse, pig, sheep, dog), *Actinobacillus pleuropneumoniae* (cattle, sheep, pig), *Actinobacillus suis* (pig, horse, cattle, goat, alpaca, dog, cat), and *Actinobacillus ureae* (found in coypu although a disease association remains unclear).

### *6.4.5* Actinobaculum

Phylum: Actinobacteria; Class: Actinobacteria; Order: Actinomycetales; Family: Actinomycetaceae; Genus: *Actinobaculum*. The members of this genus typically are found as saprophytes in soil and water. The species *Actinobaculum suis* is importantly known as the cause of ascending urinary tract infections in swine and that disease is known as Porcine Cystitis-Pyelonephritis Complex (pig).

### *6.4.6* Actinomyces

Phylum: Actinobacteria; Class: Actinobacteria; Order: Actinomycetales; Family: Actinomycetaceae; Genus: *Actinomyces*. The members of this genus typically are found in soil and compost. The disease symptoms associated with members of the genus *Actinomyces* are varied. They include secondary invasion of decayed teeth and mandible leading to oral abscesses, osteomyelitis characterized by dislodgement of teeth, mandibular fractures (called lumpy jaw) and cervical abscesses. This bacterial genus can cause soft tissue infections including bursitis, abscesses in the gastrointestinal tract and lungs, meningitis, meningoencephalitis, and endophthalmitis. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Actinomyces bovis* (cattle, sheep, pig, moose, caribou, presumably the cause of lumpy jaw in kangaroo), *Actinomyces canis* (dog), *Actinomyces hordeovulneris* (dog), *Actinomyces hyovaginalis* (goat, sheep, pig), *Actinomyces israelii* (pig, cattle), *Actinomyces naeslundii* (pig), *Actinomyces ruminicola* (cattle), *Actinomyces suimastitidis* (pig), and *Actinomyces viscosus* (dog).

### *6.4.7* Aerococcus

Phylum: Firmicutes; Class: Bacilli; Order: Lactobacillales; Family: Aerococcaceae; Genus: *Aerococcus*. The members of this bacterial genus are saprophytes commonly

isolated from air and dust, plus they also have been isolated from vegetation and soil. *Aerococcus suis* has been noted to cause meningitis (pig). *Aerococcus urinae* is found in urine and associated with urogenital infections including bovine reproductive disease (cattle, horse). *Aerococcus urinaeequi* similarly is found in urine of cattle and horse, this bacterial species previously was named *Pediococcus urinaeequi*, and although it causes disease in humans it seems to not yet have been found causal of livestock infections. *Aerococcus vaginalis* may be associated with urogenital inflammation (cattle). *Aerococcus viridans* is broadly infective and its host range includes fish, lobsters, and sea turtles, with respect to mammalian livestock this bacterial species is noted for causing clinical and also subclinical mastitis particularly in cattle and it also causes Bovine Severe Respiratory Syndrome (cattle, pig).

### *6.4.8* Aeromonas

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Aeromonadales; Family: Aeromonadaceae; Genus: *Aeromonas*. The members of this genus typically are found in aquatic environments. Members of the genus *Aeromonas* typically are associated with diarrhea, hemorrhages, and septicemia. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Aeromonas caviae* (guinea pig), *Aeromonas hydrophila* (dog, cattle, horse, pig, sheep), and *Aeromonas veronii* (found in coypu although a disease association remains unclear).

### *6.4.9* Afipia

Phylum: Proteobacteria; Class: Alphaproteobacteria; Order: Rhizobiales; Family: Bradyrhizobiaceae; Genus: *Afipia*. The species *Afipia felis* typically is found residing in hospital water supplies. It causes wound infections and also has been associated with aseptic facial granulomas and pyoderma (cat).

### *6.4.10* Alcaligenes

Phylum: Proteobacteria; Class: Betaproteobacteria; Order: Burkholderiales; Family: Alcaligenaceae; Genus: *Alcaligenes*. Members of this genus typically are found in soil and aquatic environments. The species *Alcaligenes faecalis* correspondingly is found in moist environmental areas such as soil and water, and additionally is found in the alimentary tract of vertebrates. The species *Alcaligenes faecalis* is an opportunistic pathogen typically associated with postoperative infections in humans and

thus its presence in livestock animals might also prove to have a disease association (found in coypu although a disease association remains unclear).

### *6.4.11* Anaplasma

Phylum: Proteobacteria; Class: Alphaproteobacteria; Order: Rickettsiales; Family: Anaplasmataceae; Genus: *Anaplasma*. The members of this genus typically reside in host blood cells as obligately intracellular microorganisms. The clinical signs associated with *Anaplasma* infections are highly variable, ranging from subclinical infection in animals under a year of age to severe peracute disease in naive adults. Symptoms include infertility, anemia, jaundice, dark yellow urine, weight loss, meningitis, encephalitis, encephalomyelitis, abortion and death. Recovered animals typically become persistent carriers and reservoirs of infection for life. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Anaplasma bovis* (cattle and possibly others as well), *Anaplasma marginale* (American bison), *Anaplasma ovis* (goat, sheep), *Anaplasma phagocytophilum* (sheep, cattle, dog, horse, cat, llama), and *Anaplasma platys* (dog).

### *6.4.12* Arcanobacterium

Phylum: Actinobacteria; Class: Actinobacteria; Order: Actinomycetales; Family: Actinomycetaceae; Genus: *Arcanobacterium*. The members of this genus typically are soil dwelling and they infect through either commensal activities or via trauma. Members of the species *Arcanobacterium* are considered normal flora of the pharynx but can cause internal ear infections, upper respiratory infections including pharyngitis and sinusitis, endocarditis, lower respiratory infections including pneumonia, and necrotising fasciitis. Notably, the bacterial species previously classified as *Arcanobacterium pyogenes* now is classified as *Trueperella pyogenes*. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Arcanobacterium canis* (dog), *Arcanobacterium haemolyticum* (dog, cattle), *Arcanobacterium hippocoleae* (dog), *Arcanobacterium phocae* (dog), and *Arcanobacterium pluranimalium* (dog).

### *6.4.13* Arcobacter

Phylum: Proteobacteria; Class: Epsilonproteobacteria; Order: Campylobacterales; Family: Campylobacteraceae; Genus: *Arcobacter*. Arcobacter species are found in feces including those of animals that may not be displaying symptomatology and

Arcobacter are highly abundant in sewage, which may represent their natural ecology. *Arcobacter butzleri* has been associated with enteritis and diarrhea (pig, cattle, horse). *Arcobacter skirrowii* is associated with diarrhea (sheep) and aborted fetuses (pig, sheep, cattle). *Arcobacter thereius* has been isolated from tissue of an aborted fetus (pig).

### *6.4.14* Arthrobacter

Phylum: Actinobacteria; Class: Actinobacteria; Order: Micrococcales; Family: Micrococcaceae; Genus: *Arthrobacter*. The members of this genus commonly are found in soil. Members of the genus *Arthrobacter* typically are associated with genital infections including vaginitis. The species belonging to this microbial genus which has been indicated to have a potentially opportunistic disease association with mammalian livestock is: *Arthrobacter gandavensis* (cattle).

### *6.4.15* Atlantibacter

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Enterobacteriales; Family: Enterobacteriaceae; Genus: *Atlantibacter*. The species *Atlantibacter hermannii* has been isolated from soil and presumably resides naturally in soil, but *Atlantibacter hermannii* also is broadly infective of mammals notably including cattle. This species generally is considered nonpathogenic but has been isolated from wounds, eye infections, periodontitis, sputum and stool. *Atlantibacter hermannii* previously was named *Escherichia hermannii*.

### *6.4.16* Bacillus

Phylum: Firmicutes; Class: Bacilli; Order: Bacillales; Family: Bacillaceae; Genus: *Bacillus*. The members of this genus typically are found in soil. Many of the species belonging to the genus *Bacillus*, including some sold as probiotics, are opportunistic and there have been instances in which *Bacillus* administered as probiotics resulted in overt infectious disease. The range of opportunistic disease caused by *Bacillus* species includes actinomycotic-like lesions as well as chronic infections of the eye, kidney, skin and udder. Their ability to cross the placenta can result in abortion. This opportunistic nature contrasts with that of some other member species, notably *Bacillus anthracis*, which are considered to be a obligate pathogens. Toxins produced by many of the *Bacillus* species can complicate the subject of infection. Curiously notable among these is *Bacillus cereus*, whose growth in improperly stored steamed rice has resulted in a foodborne association for humans that is termed fried rice syndrome. Those species belonging to this microbial genus which have

been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Bacillus cereus* (broadly infective of mammals including camel and water buffalo), *Bacillus licheniformis* (bovines), *Bacillus mycoides* (cattle), *Bacillus pumilus* (broadly infective of ruminants including cattle), and *Bacillus subtilis* (cattle, sheep).

### *6.4.17* Bacteroides

Phylum: Bacteroidetes; Class: Bacteroidia; Order: Bacteroidales; Family: Bacteroidaceae; Genus: *Bacteroides*. The members of this genus typically are found naturally existing only in a symbiotic relationship with the mammal within which they inhabit the mucins covering colonic mucosa. Various members of the genus *Bacteroides* contribute to inflammatory bowel disease, meningitis, meningoencephalitis, periodontal disease, and pleuropneumonia. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Bacteroides fragilis* (sheep, cattle, horse, pig), *Bacteroides helcogenes* (pig), *Bacteroides ovatus* (cow), and *Bacteroides pyogenes* (pig).

### *6.4.18* Bartonella

Phylum: Proteobacteria; Class: Alphaproteobacteria; Order: Rhizobiales; Family: Bartonellaceae; Genus: *Bartonella*. The members of this genus typically are obligate intracellular parasites and known to be carried by mites, fleas, body lice and ticks. Members of the genus *Bartonella* have been associated with wound infections and oral inflammation. They also may complicate other diseases such as respiratory infections. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Bartonella alsatica* (rabbit), *Bartonella australis* (kangaroo), *Bartonella bovis* (cattle), *Bartonella capreoli* (deer, elk), *Bartonella chomelii* (cattle), *Bartonella clarridgeiae* (cat is the presumed host), *Bartonella henselae* (cat), *Bartonella melophagi* (sheep), *Bartonella rochalimae* (dog), *Bartonella schoenbuchensis* (cattle, deer), and *Bartonella vinsonii* (dog).

### *6.4.19* Bergeriella

Phylum: Proteobacteria; Class: Betaproteobacteria; Order: Neisseriales; Family: Neisseriaceae; Genus: *Bergeriella*. The species *Bergeriella denitrificans* has been

isolated from biofilm present on oral, throat, and nasopharynx mucosa (found in guinea pig but the disease association remains unclear.

### *6.4.20* Bergeyella

Phylum: Bacteroidetes; Class: Flavobacteriia; Order: Flavobacteriales; Family: Flavobacteriaceae; Genus: *Bergeyella*. The species *Bergeyella porcorum* has been isolated from the lungs and tonsils of pigs and may be normal flora in those locations. The species *Bergeyella zoohelcum* is considered normal oral flora and that seems to be its natural environment. The species *Bergeyella zoohelcum* has, however, been associated with bite wounds and potentially with bacteremia after ingestion of goat blood (cat, dog, goat).

### *6.4.21* Bordetella

Phylum: Proteobacteria; Class: Betaproteobacteria; Order: Burkholderiales; Family: Alcaligenaceae; Genus: *Bordetella*. The species *Bordetella bronchiseptica* can persist in the environment but presumably is not natural to the environment, instead it naturally resides in the respiratory tract. In particular, the association of this bacterial species with rabbits is nearly asymptomatic although it has been associated with both acute pneumonia and also can affect the upper respiratory tract. Infection outbreaks may have a high morbidity but mortality usually is low (broadly infective for terrestrial mammals including dog, cat, pig, rabbit, sheep, goat, kangaroo, guinea pig, donkey, horse). It is important to note that *Bordetella bronchiseptica* often acts synergistically with *Pasteurella multocida* to cause disease with paroxysmal cough and sneezing being among the most notable signs and those can be accompanied by oculonasal discharge and rhinitis with the possible consequence of sudden death.

### *6.4.22* Borrelia

Phylum: Spirochaetes; Class: Spirochaetia; Order: Spirochaetales; Family: Borreliaceae; Genus: *Borrelia*. The normal ecology of this genus is to be maintained as an infection of ticks and it might not have an ecology that is independent of host vertebrates and invertebrates. The species belonging to *Borrelia* are associated with systemic disease which can affect the joints, as well as causing renal, neurologic, and cardiac abnormalities. The most common syndrome includes recurrent lameness accompanied by anorexia, lethargy, and lymphadenopathy. Renal failure generally is fatal and this bacterial genus also can cause abortion. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic

disease association with mammalian livestock are: *Borrelia coriaceae* (cattle), and *Borrelia theileri* (cattle).

### *6.4.23* Borreliella

Phylum: Spirochaetes; Class: Spirochaetia; Order: Spirochaetales; Family: Borreliaceae; Genus: *Borreliella*. Possibly, as with *Borrelia*, the normal ecology of this genus is to be maintained as an infection of ticks and it might not have an ecology that is independent of host vertebrates and invertebrates. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Borreliella afzelii* (Edible dormouse), and *Borreliella burgdorferi* (dog, cat, deer, horse, cattle, sheep, Edible dormouse). *Borreliella afzelii* formerly was named *Borrelia afzelii* and *Borreliella burgdorferi* formerly was named *Borrelia burgdorferi*.

### *6.4.24* Brachyspira

Phylum: Spirochaetes; Class: Spirochaetia; Order: Brachyspirales; Family: Brachyspiraceae; Genus: *Brachyspira*. The normal environment for members of this genus is within the intestine and they typically also are transiently identifiable in moist feces excreted by infected animals. The *Brachyspira* cause intestinal edema with haemorrhage. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Brachyspira canis* (dog), *Brachyspira hyodysenteriae* (pig, guinea pig), and *Brachyspira pilosicoli* (pig).

### *6.4.25* Brucella

Phylum: Proteobacteria; Class: Alphaproteobacteria; Order: Rhizobiales; Family: Brucellaceae; Genus: *Brucella*. The *Brucella* can survive for periods of years in soil and be found in aerosols, but their natural ecology seems to be residing in association with a host mammal. Infections caused by the member species of *Brucella* often demonstrate no outward clinical signs. Chronically infected animals may present with evidence of only decreased milk production. Other symptoms include epididymitis, seminal vesiculitis, orchitis, testicular abscess, spondylitis and arthritis. They can cause placentitis and endometritis. The most severe consequences include unfavorable reproductive outcomes such as mid to late term abortion, stillborn offspring, and neonatal weakness that results in death. The disease name associated with this bacterial genus bears specific mention, and it is brucellosis. Those species belonging to this microbial genus which have been indicated to have a potentially

opportunistic disease association with mammalian livestock are: *Brucella abortus* (capybara, cervids including elk, yak, camel, cattle, bison, water buffalo, llama, goat, sheep), *Brucella canis* (dog), *Brucella melitensis* (camel, goat, sheep), *Brucella neotomae* (rodents), *Brucella ovis* (sheep goat), and *Brucella suis* (dog, rabbit, reindeer, pig).

### *6.4.26* Burkholderia

Phylum: Proteobacteria; Class: Betaproteobacteria; Order: Burkholderiales; Family: Burkholderiaceae; Genus: *Burkholderia*. The members of this genus typically are found in soil and water. The severity of disease outcome associated with infections caused by members of the genus *Burkholderia* varies tremendously depending upon the combination of bacterial and host species that are involved. In general, the members of this genus are associated with ulcerative nodules of the skin, internal organs including lung, and the upper respiratory tract. One member of this genus, *Burkholderia mallei*, causes an acute form of cervical lymphadenopathy in horse and donkey which is called glanders. Glanders can be extremely deleterious and the causative infection can be transmitted to cats that subsequently are fed meat from diseased animals. Those cats infected by consuming infected horse meat consequently develop localized nodules on nasal mucosa and bloody nasal discharge. *Burkholderia mallei* also causes a chronic form represented by generalized lymphadenopathy and ulcerated skin nodules. Thus, it should be understood that the severity of illness associated with *Burkholderia mallei* varies greatly and is dependent upon the site of infection. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Burkholderia cepacia* (cattle, rabbit, sheep), *Burkholderia mallei* (cat, camel, goat, kangaroo, coypu), and *Burkholderia pseudomallei* (camel, alpaca, kangaroo, cattle, pig, goat, horse, cat, dog, sheep, pig, guinea pig).

### *6.4.27* Campylobacter

Phylum: Proteobacteria; Class: Epsilonproteobacteria; Order: Campylobacterales; Family: Campylobacteraceae; Genus: *Campylobacter*. The members of this genus typically colonize the intestinal mucosa and often seem to be commensal. They correspondingly are found in manure and they do survive to be found in environmental water and soil. The disease symptomatology associated with members of the genus *Campylobacter* varies from none to severe gastroenteritis. They can also cause periodontal disease, infertility, abortion and stillbirth. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Campylobacter coli* (cattle, pig, seldom in camel), *Campylobacter fetus* (sheep, goat, cattle, camel, alpaca, llama,

buffalo), and *Campylobacter jejuni* (horse, cattle, sheep, goat, rabbit, kangaroo, camel).

### *6.4.28* Chlamydia

Phylum: Chlamydiae; Class: Chlamydiia; Order: Chlamydiales; Family: Chlamydiaceae; and Genus: *Chlamydia*. The members of this genus seem unable to survive outside a eukaryotic host cell and for many their lifecycle in fact requires growth within a eukaryotic host cell, they can be found within free living amoeba. The members of this genus are associated with conjunctivitis, keratoconjunctivitis, rhinitis, abortion, and pneumonitis. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Chlamydia abortus* (cattle, sheep, goat, fallow deer, red deer), *Chlamydia caviae* (guinea pig) *Chlamydia felis* (cat), *Chlamydia pecorum* (sheep, goat, cattle, pig), *Chlamydia pneumoniae* (camel, cattle), *Chlamydia psittaci* (sheep, cattle, goat, cat, pig, horse, dog), *Chlamydia suis* (pig), and *Chlamydia trachomatis* (cattle, pig, guinea pig). *Chlamydia abortus* previously was named *Chlamydophila abortus*, *Chlamydia caviae* previously was named *Chlamydophila caviae*, and *Chlamydia felis* previously was named *Chlamydophila felis*.

### *6.4.29* Chromobacterium

Phylum: Proteobacteria; Class: Betaproteobacteria; Order: Neisseriales; Family: Chromobacteriaceae; Genus: *Chromobacterium*. The species *Chromobacterium violaceum* is considered to reside naturally in water and soil of tropical and sub-tropical regions. Infections cause by the species *Chromobacterium violaceum* begin as either localized skin infections or localized lymphadenitis. They can progress to septicemia and abscesses of internal organs including the liver, lung, spleen, skin, lymph nodes, and brain resulting in fatal multiorgan failure (broadly infective as an opportunistic pathogen of mammals including macropods).

### *6.4.30* Chryseobacterium

Phylum: Bacteroidetes; Class: Flavobacteriia; Order: Flavobacteriales; Family: Flavobacteriaceae; Genus: *Chryseobacterium*. The members of this genus typically are found soil, plants, water, and milk. The *Chryseobacterium* often are found to be present without an apparent disease association, and yet they can cause a variety of disease consequences including mastitis and meningoencephalitis. Those species belonging to this microbial genus which have been indicated to have a potentially

opportunistic disease association with mammalian livestock are: *Chryseobacterium bovis* (cattle, also found in coypu although a disease association remains unclear for coypu), *Chryseobacterium gleum* (coypu—disease association remains unclear), *Chryseobacterium haifense* (cattle), *Chryseobacterium indologenes* (coypu—disease association remains unclear), and *Chryseobacterium oranimense* (cattle).

### *6.4.31* Citrobacter

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Enterobacteriales; Family: Enterobacteriaceae; Genus: *Citrobacter*. The members of this genus mainly are found in the open environment including soil, water, and sewage. The species *Citrobacter freundii* is opportunistic and can cause a number of different disease syndromes including urinary tract infections, joint infections, pneumonia, sepsis, meningitis, brain abscesses, and neonatal sepsis (camel, guinea pig, coypu).

### *6.4.32* Clostridioides

Phylum: Firmicutes; Class: Clostridia; Order: Clostridiales; Family: Peptostreptococcaceae; Genus: *Clostridioides*. The species *Clostridioides difficile* typically is found in soil, aquatic sediments, sewage, river water, lake water, and swimming pool water. *Clostridioides difficile* can cause symptoms ranging from diarrhea to life-threatening inflammation of the colon (pig, guinea pig, rabbit, bovines, equines). *Clostridioides difficile* previously was classified as *Clostridium difficile*.

### *6.4.33* Clostridium

Phylum: Firmicutes; Class: Clostridia; Order: Clostridiales; Family: Clostridiaceae; Genus: *Clostridium*. The members of this genus typically are found in soil, aquatic sediments, and sewage. The members of this genus are infamous for the toxins which they produce and some species such as *Clostridium tetani* and *Clostridium botulinum* are associated with muscular spasms and paralytic toxicoses. Respiratory or cardiac paralysis can occur and usually causes death, typically a mortality of 80% is expected with those infections. *Clostridium botulinum* also causes a chronic toxicosis. But, given that understanding, *Clostridium tetani* causes illness only when associated with deep narrow wounds that typically result from punctures. *Clostridium moniliforme* causes ascending urinary tract infections, meningitis and meningoencephalitis. *Clostridium perfringens* can cause gas gangrene, a myonecrosis with gas production. The bacterial species listed here typically reside

naturally in the intestine although they can cause ulcerative colitis, chronic intestinal toxicosis, myonecrosis (gangrene), haemoglobinuria, infectious necrotic hepatitis, malignant edema, and abomasitis. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Clostridium botulinum* (cattle, chicken, deer, donkey), *Clostridium cadaveris* (horse, sheep, guinea pig, rabbit), *Clostridium chauvoei* (bison, cattle, deer, sheep), *Clostridium moniliforme* (dog), *Clostridium novyi* (cattle, sheep, bison), *Clostridium perfringens* (elk, moose, alpaca, llama, cattle, guinea pig, camel, dog, goat, sheep, red deer, fallow deer, pig, yak, kangaroo), *Clostridium septicum* (cattle, sheep, goat, pig), and *Clostridium tetani* (cattle, donkey). *Clostridium moniliforme* previously was named *Eubacterium moniliforme*.

### *6.4.34* Corynebacterium

Phylum: Actinobacteria; Class: Actinobacteria; Order: Corynebacteriales; Family: Corynebacteriaceae; Genus: *Corynebacterium*. The members of this genus typically are found in soil and water, they also are commensal on the skin and mucous membranes. The *Corynebacterium* species are associated with a wide range of symptoms including ascending urinary tract infections causing cystitis and pyelonephritis. They also cause septic peritonitis, lymphadenitis, respiratory infections, mastitis, posthitis (infection of the penus forskin) and vulvitis. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Corynebacterium amycolatum* (cattle), *Corynebacterium auris* (cattle), *Corynebacterium auriscanis* (dog), *Corynebacterium bovis* (cattle, camel), *Corynebacterium camporealensis* (sheep), *Corynebacterium canis* (dog), *Corynebacterium cystitidis* (cattle, sheep, goats), *Corynebacterium glucuronolyticum* (pig), *Corynebacterium jeikeium* (cat), *Corynebacterium lactis* (cattle), *Corynebacterium mastitidis* (sheep), *Corynebacterium pilosum* (cattle), *Corynebacterium pseudotuberculosis* (goat, sheep, horse, cattle, camel, water buffalo, llama, alpaca, cervids), *Corynebacterium renale* (cattle), *Corynebacterium striatum* (cattle), *Corynebacterium suicordis* (pigs), *Corynebacterium ulcerans* (camel, cat, cattle), *Corynebacterium ulceribovis* (cattle), *Corynebacterium urealyticum* (cat, dog), and *Corynebacterium uterequi* (horse).

### *6.4.35* Coxiella

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Legionellales; Family: Coxiellaceae; Genus: *Coxiella*. The species *Coxiella burnetii* is an opportunistic commensal that survives in the environment. It is shed in urine (particularly in association with pregnancy), milk, and feces. During birth, this species can be found in high levels within the amniotic fluids and placenta. *Coxiella burnetii* can be environmentally

acquired by inhalation of dust from contaminated materials. The species *Coxiella burnetii* causes Q fever with both acute and chronic presentations. It often presents as subclinical disease with symptoms including anorexia, infertility, metritis, late term abortion, or retained placenta. Abortions often are followed by recovery of the dam without complications (cattle, sheep, goat, camel).

### *6.4.36* Cutibacterium

Phylum: Actinobacteria; Class: Actinobacteria; Order: Propionibacteriales; Family: Propionibacteriaceae; Genus: *Cutibacterium*. The species *Cutibacterium acnes* is virtually ubiquitous as a commensal in the oral cavity and on the skin of animals, including residence both in and around sweat glands and sebaceous glands, and surprisingly it can become a lethal opportunist. In particular, *Cutibacterium acnes* which causes the benign skin condition termed acne also causes chronic inflammatory disease of the eyes, and additionally is a cause of placentitis and infection of the fetus leading to abortion (cattle, dog). *Cutibacterium acnes* previously was classified as *Propionibacterium acnes*.

### *6.4.37* Dermatophilus

Phylum: Actinobacteria; Class: Actinobacteria; Order: Micrococcales; Family: Dermatophilaceae; Genus: *Dermatophilus*. The species *Dermatophilus congolensis* may be a saprophyte in soil but its presumed normal habitat and ecology is as a commensal on the skin. The species *Dermatophilus congolensis* causes exudative dermatitis (broadly infective of mammals although affecting more frequently cattle, horse, sheep, goat).

### *6.4.38* Dichelobacter

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Cardiobacteriales; Family: Cardiobacteriaceae; Genus: *Dichelobacter*. The species *Dichelobacter nodosus* survives in soil but its normal ecology may be in association with the paw or hoof where it can cause interdigital dermatitis (moose, sheep, kangaroo, cattle).

### *6.4.39* Edwardsiella

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Enterobacteriales; Family: Enterobacteriaceae; Genus: *Edwardsiella*. The *Edwardsiella* typically are found in natural water, including water in which aquarium fish are raised. The members of this species typically are associated with the gastrointestinal tract of healthy fish. They can cause necrotic skin wounds and gastroenteritis, from the gastrointestinal tract they can spread extraintestinally to become septicemia. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Edwardsiella ictaluri* (dog), and *Edwardsiella tarda* (dog, pig, cattle).

### *6.4.40* Ehrlichia

Phylum: Proteobacteria; Class: Alphaproteobacteria; Order: Rickettsiales; Family: Anaplasmataceae; Genus: *Ehrlichia*. The members of this genus typically are obligately intracellular pathogens and their ecology is similar to that of the bartonella which involves invertebrate (mites, lice, fleas, ticks) and vertebrate host cycles. Illnesses caused by members of the genus *Ehrlichia* typically are non-specific and range from either subclinical or mild to severe including abrupt death. The associated symptoms include lethargy, thrombocytopenia, anorexia, diarrhea, lymphadenopathy, weight loss, muscle or joint pain, hepatocellular enzyme abnormalities, lesions of the visceral membranes, and respiratory or neurologic signs. *Ehrlichia canis* has been identified as causing fatal central nervous system disease in a dog for which the fungal species *Cladophialophora bantiana* (that fungal species previously was named *Xylohypha bantiana*) caused a contributing secondary infection in the form of a systemic mycosis affecting the liver, spleen, kidney, and adrenal glands. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Ehrlichia canis* (dog), *Ehrlichia chaffeensis* (cat, dog), *Ehrlichia ewingii* (dog), *Ehrlichia ovina* (sheep), and *Ehrlichia ruminantium* (cattle, sheep, goat, cervids, buffalo).

### *6.4.41* Enterobacter

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Enterobacteriales; Family: Enterobacteriaceae; Genus: *Enterobacter*. The members of this genus typically are found in soil and water, and they are as well found on seeds and plants. Many *Enterobacter* species frequently are found in the gastrointestinal tract, feces, and dairy products. Members of this genus produce a variety of illnesses among which are

respiratory infections including pleuropneumonia and septic peritonitis. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Enterobacter cancerogenus* (coypu—disease association remains unclear), *Enterobacter cloacae* (guinea pig, rabbit, coypu), and *Enterobacter hormaechei* (sheep).

### *6.4.42* Enterococcus

Phylum: Firmicutes; Class: Bacilli; Order: Lactobacillales; Family: Enterococcaceae; Genus: *Enterococcus*. The members of this genus are normal intestinal flora and characteristically are found in sewage contaminated water. Members of the genus *Enterococcus* typically are associated with urinary system infections. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Enterococcus avium* (dog), *Enterococcus canintestini* (dog), *Enterococcus canis* (dog), *Enterococcus casseliflavus* (capybara), *Enterococcus durans* (camel), *Enterococcus faecalis* (capybara, moose, cattle, coypu, dog), *Enterococcus faecium* (llama, moose, coypu, dog), *Enterococcus gallinarum* (coypu—disease association remains unclear), *Enterococcus hirae* (llama), and *Enterococcus villorum* (pig).

### *6.4.43* Erysipelothrix

Phylum: Firmicutes; Class: Erysipelotrichia; Order: Erysipelotrichales; Family: Erysipelotrichaceae; Genus: *Erysipelothrix*. The members of this genus typically reside on the skin, but they also can be found in sewage and fecally contaminated soil. The disease symptoms associated with members of the genus include anorexia, depression, stilted gait, exercise intolerance, lameness, enlarged joints and death. The species *Erysipelothrix rhusiopathiae* can cause an acute rash of the upper dermis (erysipelas) often appearing patterned. *Erysipelothrix tonsillarum* often causes tonsillitis. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Erysipelothrix rhusiopathiae* (very broadly infective of vertebrate animals perhaps most notably pig, kangaroo, sheep, cattle, moose, and rabbit, but the host range also encompasses fish, birds, reptiles and invertebrates including lobster) and *Erysipelothrix tonsillarum* (pig).

### *6.4.44* Escherichia

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Enterobacteriales; Family: Enterobacteriaceae; Genus: *Escherichia*. The members of this genus

typically are intestinal commensals, but they also can be found naturally in subtropical and tropical soil and water. Infections caused by the bacterial genus *Escherichia* typically can be asymptomatic but also include enteritis, mastitis, endometritis, sepsis, septic peritonitis, ascending urinary tract infections, peritonitis, pleuropneumonia, otitis externa, meningitis and meningoencephalitis. The most commonly noted member of this genus is *Escherichia coli*, which normally is an intestinal commensal whose various genetic strains are infamous for causing potentially fatal disease when ingested by an inappropriate, non-host adapted animal species. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Escherichia albertii* (cattle, pig, rabbit), *Escherichia coli* (broadly infective of terrestrial mammals including pig, sheep, goat, cattle, capybara, water buffalo, yak, horse, dog, rabbit, cat, camel, alpaca, llama, kangaroo, elk, moose, red deer, guinea pig, reindeer, coypu), *Escherichia fergusonii* (cattle, goat, horse), and *Escherichia vulneris* (widely indicated as affecting animals but specific information was not found on associations with mammalian livestock).

### *6.4.45* Eubacterium

Phylum: Firmicutes; Class: Clostridia; Order: Clostridiales; Family: Eubacteriaceae; Genus: *Eubacterium*. The members of this genus typically are found as saprophytes in soil and water. Some also are normal inhabitants of human skin and cavities, occasionally causing infection of soft tissue. The species *Eubacterium rangiferina* is associated with reindeer although the disease association remains unclear.

### *6.4.46* Ewingella

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Enterobacteriales; Family: Yersiniaceae; Genus: *Ewingella*. The species *Ewingella americana* can be found in domestic sources of water including air conditioning units, ice baths and wound irrigation systems. In human, this bacterial species typically is associated with wound infections, pneumonia and peritonitis (coypu—disease association remains unclear).

### *6.4.47* Finegoldia

Phylum: Firmicutes; Class: Tissierellia; Order: Tissierellales; Family: Peptoniphilaceae; Genus: *Finegoldia*. It is not known if the members of this species naturally have an environmental presence. Their understood ecology is as

commensals representing part of the normal skin biota although they are as well found in the oral cavity, genitourinary tract, and intestine. The *Finegoldia* are associated with infections of the skin and soft tissues, additionally causing infections of internal organs following surgery. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Finegoldia magna* (coypu, dog), *Finegoldia sp. feline oral taxon 341* (cat), and *Finegoldia sp. feline oral taxon 342* (cat).

### *6.4.48* Francisella

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Thiotrichales; Family: Francisellaceae; Genus: *Francisella*. The species *Francisella tularensis* seems to be obligately intracellular and broadly associated with vertebrates as well as invertebrate host animals. It primarily infects macrophages in vertebrate host organisms and hemocytes of invertebrates. This bacterial species is capable of lengthy survival in the environment where it can be found in water, grassland and haystacks. The fact that *Francisella tularensis* can survive in *Acanthamoeba castellanii*, which is a free living opportunistically pathogenic soil amoeba, suggests that amoeba might represent an environmental reservoir for *Francisella tularensis* and hints at some of the complex ecology that can be involved with opportunistic infections. The species *Francisella tularensis* causes the disease tularemia which bears notation for the severity of its associated illness. This bacterial species generally is considered to affect all endothermic animals. Despite being quite lethal in some host species such as human, *Francisella tularensis* may qualify as an opportunistic pathogen in some rodents and other mammalian groups (rabbit, Edible dormouse, coypu).

### *6.4.49* Fusobacterium

Phylum: Fusobacteria; Class: Fusobacteria; Order: Fusobacteriales; Family: Fusobacteriaceae; Genus: *Fusobacterium*. The natural ecology of this genus seems to be residence in the oral mucosa where its member species typically cause subgingival dental plaque. No information could be found that would suggest *Fusobacterium* species exist openly in nature. Members of the genus *Fusobacterium* cause calf diphtheria and necrotic abscesses particularly of the tongue, throat, jaw and liver. They can cause pleuropneumonia, meningitis, and meningoencephalitis. *Fusobacterium necrophorum* can contribute to the interdigital dermatitis associated with *Dichelobacter nodosus*. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Fusobacterium canifelinum* (cat, dog), *Fusobacterium equinum* (horse), *Fusobacterium necrophorum* (cattle, kangaroo, bison, water

buffalo, alpaca, llama, cervids including elk and reindeer), *Fusobacterium nucleatum* (dog), and *Fusobacterium russii* (dog).

### *6.4.50* Gemella

Phylum: Firmicutes; Class: Bacilli; Order: Bacillales; Family: (not assigned); Genus: *Gemella*. The members of this genus primarily are found in the mucous membranes of the oral cavity and upper digestive tract, although they can grow in drinking water distribution systems. Members of the genus *Gemella* typically cause infection of previously damaged tissue and have been associated with abscesses including those of the brain. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Gemella cuniculi* (rabbit) and *Gemella morbillorum* (cattle, coypu, rabbit, pig, goat, sheep, horse).

### *6.4.51* Haemophilus

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Pasteurellales; Family: Pasteurellaceae; Genus: *Haemophilus*. The species [*Haemophilus*] *parasuis* is found in the upper respiratory tract of healthy pigs and no information was found suggesting that it naturally exists in the open environment. As an opportunistic pathogen, this bacterial species is associated with meningoencephalitis, peritonitis including inflammation of the internal viscera, and polyarthritis. It can additionally contribute to bacterial pneumonia (pig).

### *6.4.52* Hafnia

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Enterobacteriales; Family: Hafniaceae; Genus: *Hafnia*. The members of this genus typically are found in soil, water and sewage. They also are commensals of the gastrointestinal tract. Members of the genus *Hafnia* cause systemic infections including septicemia and pneumonia. They also are suspected of causing gastroenteritis. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Hafnia alvei* (rabbit, guinea pig, coypu, sheep, cattle, goat) and *Hafnia paralvei* (cattle).

### *6.4.53* Hathewaya

Phylum: Firmicutes; Class: Clostridia; Order: Clostridiales; Family: Clostridiaceae; Genus: *Hathewaya*. The species *Hathewaya histolytica* can be found in feces and soil. *Hathewaya histolytica* causes gas gangrene, a myonecrosis with gas production (guinea pig, rabbit, cattle). *Hathewaya histolytica* previously was classified as *Clostridium histolyticum*.

### *6.4.54* Helicobacter

Phylum: Proteobacteria; Class: Epsilonproteobacteria; Order: Campylobacterales; Family: Helicobacteraceae; Genus: *Helicobacter*. The members of this genus typically are found in surface water, soil, dung, and flies. When associated with a host animal, the *Helicobacter* typically live in the lining of the upper gastrointestinal tract. Members of the genus *Helicobacter* characteristically are associated with gastrointestinal symptoms, notably including ulcerative gastritis. They also can cause hepatic disease in some host species. Alpaca and llama seem particularly susceptible to stress-related stomach ulcers caused by *Helicobacter pylori*. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Candidatus Helicobacter bovis* (cattle), *Helicobacter canadensis* (rodents, pig), *Helicobacter canis* (cat, dog), *Helicobacter equorum* (horse), *Helicobacter felis* (cat), and *Helicobacter pylori* (broadly infective of mammals including alpaca, llama, camel, goat, sheep, cattle, cat, horse, donkey).

### *6.4.55* Histophilus

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Pasteurellales; Family: Pasteurellaceae; Genus: *Histophilus*. The species *Histophilus somni* is considered to be an obligate parasite, typically found commensally on mucosal surfaces of the reproductive and respiratory tracts of healthy animals. It also can be acquired environmentally from inhalation of dust and aerosolized water containing the bacteria. This bacterial species is associated with otitis media and otitis interna, pneumonia, and abortion (alpaca, cattle, llama, donkey).

### *6.4.56* Janthinobacterium

Phylum: Proteobacteria; Class: Betaproteobacteria; Order: Burkholderiales; Family: Oxalobacteraceae; Genus: *Janthinobacterium*. The species *Janthinobacterium lividum* naturally lives in soil and is capable of causing opportunistic infections including fatal septicemia in human. Its associated with rabbits is of potential interest (rabbit—disease association remains unclear).

### *6.4.57* Klebsiella

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Enterobacteriales; Family: Enterobacteriaceae; Genus: *Klebsiella*. The members of this genus typically are found in soil, water, plants, and insects.

Members of the genus *Klebsiella* also are normal fecal and oral cavity inhabitants. They can, however, cause septicemia, septic peritonitis, multiple organ inflammation, abscesses including those of the liver, endometritis, pleuropneumonia and meningitis. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Klebsiella aerogenes* (capybara, camel, coypu, goat), *Klebsiella oxytoca* (guinea pig) and *Klebsiella pneumoniae* (capybara, coypu, llama, camel, cattle, equines, goat).

### *6.4.58* Kocuria

Phylum: Actinobacteria; Class: Actinobacteria; Order: Micrococcales; Family: Micrococcaceae; Genus: *Kocuria*. The members of this genus typically are associated with soil and also are commensals of the skin. The disease symptoms associated with members of this genus represent an association with urinary tract infections and they also can cause peritonitis in the form of cholecystitis (inflammation of the gallbladder). Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Kocuria kristinae* (sheep) and *Kocuria varians* (sheep).

### *6.4.59* Kytococcus

Phylum: Actinobacteria; Class: Actinobacteria; Order: Micrococcales; Family: Dermacoccaceae; Genus: *Kytococcus*. The species *Kytococcus sedentarius* is a

marine dwelling microbe which causes internal infections following damage to mucosal tissues (sheep).

### *6.4.60* Lawsonia

Phylum: Proteobacteria; Class: Deltaproteobacteria; Order: Desulfovibrionales; Family: Desulfovibrionaceae; Genus: *Lawsonia*. The species *Lawsonia intracellularis* is obligately intracellular and although it can be found in feces from infected animals the duration of its environmental survival is uncertain. This species typically causes enteritis in the form of a proliferative enteropathy (dog, guinea pig, rabbit, pig, horse). There has to have been some fun found in all of this serious microbiology research and so I will relate to you that when I was a very young child my father managed a Lawson's Dairy store in Dayton, Ohio. My role in the family business was washing the store windows. The name of that chain of stores is unrelated to this bacterial genus and I never got diseased by their products.

### *6.4.61* Leclercia

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Enterobacteriales; Family: Enterobacteriaceae; Genus: *Leclercia*. The species *Leclercia adecarboxylata* most likely is ubiquitous in the natural environment. It has been found in food, water, and terrestrial molluscs. As an opportunistic pathogen *Leclercia adecarboxylata* causes infection of wounds and mastitis (cattle, coypu).

### *6.4.62* Legionella

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Legionellales; Family: Legionellaceae; Genus: *Legionella*. The species *Legionella pneumophila* is naturally associated with environmental freshwater. The typical source is warm water, and that association includes such domestic reservoirs as hot water storage tanks and recirculating heated water systems. It can be acquired from free living amoeba such as *Acanthamoeba* and also by inhalation of vesicles produced by *Acanthamoeba*. *Legionella pneumophila* causes pneumonia (dog, goat, horse, cattle, sheep, pig, guinea pig, rabbit).

### *6.4.63* Lelliottia

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Enterobacteriales; Family: Enterobacteriaceae; Genus: *Lelliottia*. The species *Lelliottia amnigena* is found in drinking water, surface water, and soil. This species is known to cause endophthalmitis in human and thus its association with other mammals is of potential interest (coypu—disease association remains unclear). I would suggest researching under the previous name of this microbe which was *Enterobacter amnigenus*.

### *6.4.64* Leptospira

Phylum: Spirochaetes; Class: Spirochaetia; Order: Leptospirales; Family: Leptospiraceae; Genus: *Leptospira*. The natural habitat for members of this genus is shallow stagnant water including puddles, ponds, lakes, and bogs. Members of the genus *Leptospira* typically cause either none or modest symptomatology in carrier animals, but in non-adapted animals they can cause persistent infections that vary in severity from inapparent to fatal. Kidney infections are a typical consequence of the disease, and can include renal failure that often is accompanied by concurrent hepatitis. Persistent infections associated with members of this bacterial genus typically involve the kidney and genital tract. Abortion commonly can result within the first 5 weeks after infection. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Leptospira fainei* (pig), *Leptospira inadai* (rodents), *Leptospira interrogans* (alpaca, llama, camel, dog, elk, cattle, pig, American bison), *Leptospira weilii* (kangaroo), and *Leptospira wolffii* (water buffalo, coypu, dog).

### *6.4.65* Listeria

Phylum: Firmicutes; Class: Bacilli; Order: Bacillales; Family: Listeriaceae; Genus: *Listeria*. The members of this genus typically are distributed widely throughout the environment, particularly in soil and fecal matter. Members of the genus *Listeria* characteristically are associated with septicemia and meningoencephalitis, but there also is an ophthalmic form. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Listeria ivanovii* (ruminants, mainly sheep) and *Listeria monocytogenes* (alpaca, llama, reindeer, horse, cattle, camel, sheep, goat, pig, donkey, coypu, additionally found in several species of wallaby which belong to the genus *Macropus* and therefore *Listeria monocytogenes* also likely occurs in kangaroo).

### *6.4.66* Macrococcus

Phylum: Firmicutes; Class: Bacilli; Order: Bacillales; Family: Staphylococcaceae; Genus: *Macrococcus*. The members of this genus tend to live naturally on the skin and are found in milk suggesting a potential involvement with mastitis although a definitive disease association remains unclear. Those species belonging to this microbial genus which may have a potentially opportunistic disease association with mammalian livestock are: *Macrococcus bovicus* (cattle), *Macrococcus brunensis* (llama), *Macrococcus carouselicus* (horse), *Macrococcus caseolyticus* (horse, sheep, pig, cattle, goat), *Macrococcus equipercicus* (horse), *Macrococcus hajekii* (llama), and *Macrococcus lamae* (llama).

### *6.4.67* Mannheimia

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Pasteurellales; Family: Pasteurellaceae; Genus: *Mannheimia*. The members of this genus typically are found in pasture grass, drinking water, soil, and straw bedding. They normally are commensals that variously inhabit the nasopharynx, tonsils and rumen. The *Mannheimia* often cause secondary respiratory infections, they also have been associated with otitis media and otitis interna, focal proliferative granulomatous inflammation of subcutaneous adipose tissue, mastitis which can become gangrenous, pneumonia, and meningoencephalitis. They also are a major cause of stress related respiratory illness in animals that recently have been transported, termed shipping fever or transit fever. Additional information on *Mannheimia haemolytica* can be found by researching its previous name which was *Pasteurella haemolytica*. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Mannheimia caviae* (guinea pig), *Mannheimia glucosida* (sheep), *Mannheimia granulomatis* (cattle), *Mannheimia haemolytica* (camel, alpaca, llama, cattle, sheep, goat, horse, donkey), and *Mannheimia ruminalis* (sheep).

### *6.4.68* Micrococcus

Phylum: Actinobacteria; Class: Actinobacteria; Order: Micrococcales; Family: Micrococcaceae; Genus: *Micrococcus*. The members of this genus typically are found in water, dust, and soil. Members of the genus *Micrococcus* typically are associated with mastitis. There have been general associations between members of this bacterial genus with camelids and equines, but in those studies the discovered *Micrococcus* organisms were not identified by species. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic

disease association with mammalian livestock are: *Micrococcus cohnii* (rabbit) and *Micrococcus luteus* (cattle, water buffalo).

### *6.4.69* Morganella

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Enterobacteriales; Family: Morganellaceae; Genus: *Morganella*.

The species *Morganella morganii* is found in soil, water, and fecal flora. *Morganella morganii* has a commensal relationship in the intestinal tract. As a pathogen it is associated with urinary tract, wound and respiratory infections (kangaroo).

### *6.4.70* Moraxella

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Pseudomonadales; Family: Moraxellaceae; Genus: *Moraxella*. The members of this genus generally are only isolated from animal tissues and fluids. They typically are commensals of the upper respiratory tract although they can cause infections of the lower respiratory tract. The members of this genus also are notable for their ability to colonize the ocular surface and cause both conjunctivitis and keratoconjunctivitis. They do as well cause infections of bite wounds. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Moraxella boevrei* (sheep, goat), *Moraxella bovis* (cattle), *Moraxella bovoculi* (cattle, reindeer), *Moraxella canis* (cat, dog), *Moraxella caprae* (sheep), *Moraxella caviae* (guinea pig), *Moraxella cuniculi* (pig), *Moraxella equi* (horse), *Moraxella ovis* (sheep, goat, cattle), *Moraxella lacunata* (alpaca, llama), and *Moraxella porci* (pig).

### *6.4.71* Mycobacterium

Phylum: Actinobacteria; Class: Actinobacteria; Order: Corynebacteriales; Family: Mycobacteriaceae; Genus: *Mycobacterium*. Some species of *Mycobacterium* are free living, typically in soil and water including chlorinated tap water, and also in food sources. Other *Mycobacterium*, however, such as *Mycobacterium tuberculosis* seem to have evolved from being soil microorganisms and reached dependency upon animal hosts with concomitant loss of their environmental capability. Animals typically are infected by members of this genus either in utero or during the first few months of life and these early infections may be assymptomatic. Some members of this genus cause vulvitis and vaginitis. The various member species also can cause

mastitis, lymphadenitis, chronic enteritis, systemic infections including peritonitis, and pneumonia. *Mycobacterium* species can contribute to urinary system infections associated with other microorganisms. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Mycobacterium asiaticum* (red kangaroo), *Mycobacterium avium* (thus far identified in all of the mammalian livestock animals listed in this chapter except Edible dormouse, and capybara), *Mycobacterium bovis* (camel, llama, elk, fallow deer, bison, cattle, water buffalo, cat, goat, sheep, horse, pig, rabbit), *Mycobacterium caprae* (sheep, goat, cattle, pig, red deer), *Mycobacterium chelonae* (pig, cattle), *Mycobacterium farcinogenes* (cattle), *Mycobacterium fortuitum* (pig), *Mycobacterium genavense* (rabbit, kangaroo), *Mycobacterium intracellulare* (capybara, pig, cattle, horse), *Mycobacterium kansasii* (alpaca), *Mycobacterium lepraemurium* (rodents, cat), *Mycobacterium microti* (rodents, llama), *Mycobacterium porcinum* (cattle, pig), *Mycobacterium scrofulaceum* (cattle, buffalo, pig), *Mycobacterium smegmatis* (cat, dog, sheep), *Mycobacterium tuberculosis* (camel, horse, pig, cattle, rabbit), *Mycobacterium vaccae* (cattle), and *Mycobacterium xenopi* (pig).

### *6.4.72* Mycoplasma

Phylum: Tenericutes; Class: Mollicutes; Order: Mycoplasmatales; Family: Mycoplasmataceae; Genus: *Mycoplasma*. The members of this genus are ecologically diverse. Typically the *Mycoplasma* are not free-living albeit some are saprotrophic and members of this genus can be found as aquatic residents in natural water, water tanks, water baths, and on the cooling coils of refrigeration units. Many of these species can survive and replicate within protozoa which may in turn facilitate their existence in the natural environment. Members of the genus *Mycoplasma* have been associated with general loss of body condition, decreased milk production, and decreased reproductive rates. Specific disease associations include both upper and lower respiratory tract illnesses including sinusitis and pleuropneumonia (especially tuberculosis), otitis media and otitis interna, conjunctivitis, genital infections including posthitis, vulvitis and vaginitis, and chronic polyarthritis which can be severe and result in death. Carrier animals which demonstrate few or no signs of illness typically are found within mammalian populations. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Candidatus Mycoplasma haemolamae* (alpaca, llama, donkey), *Mycoplasma arginini* (wide host range in mammals including sheep, goat, cattle, pig, cat, camel), *Mycoplasma arthritidis* (cattle, sheep, goat, rodents), *Mycoplasma bovigenitalium* (cattle), *Mycoplasma bovirhinis* (cattle), *Mycoplasma bovis* (bison, buffalo, camel, cat, cattle, deer, goat, pig, rabbit), *Mycoplasma bovoculi* (cattle), *Mycoplasma canis* (dog), *Mycoplasma capricolum* (goat, sheep, cattle, camel), *Mycoplasma caviae* (guinea pig), *Mycoplasma cavipharyngis* (guinea pig), *Mycoplasma conjunctivae* (sheep, goat), *Mycoplasma equigenitalium* (horse),

*Mycoplasma equirhinis* (horse), *Mycoplasma feliminutum* (cat), *Mycoplasma felis* (cat, horse), *Mycoplasma haemocanis* (dog), *Mycoplasma haemofelis* (cat), *Mycoplasma hyopharyngis* (pig), *Mycoplasma hyopneumoniae* (pig), *Mycoplasma hyorhinis* (pig), *Mycoplasma hyosynoviae* (pig), *Mycoplasma mucosicanis* (dog), *Mycoplasma mycoides* (bison, cattle, camel, goat, sheep, water buffalo, yak), *Mycoplasma ovipneumoniae* (sheep, goat), *Mycoplasma ovis* (sheep, goat), *Mycoplasma pulmonis* (rodents, horse), *Mycoplasma subdolum* (horse), *Mycoplasma suis* (pig), and *Mycoplasma wenyonii* (cattle).

### *6.4.73* Neisseria

Phylum: Proteobacteria; Class: Betaproteobacteria; Order: Neisseriales; Family: Neisseriaceae; Genus: *Neisseria*. The members of this genus naturally exist as colonizers of mucosal surfaces and although *Neisseria* can be found in fluids shed from the host, their environmental survival in those fluids is considered limited. Members of the genus *Neisseria* have a general association with dental plaque in many mammalian livestock animals including cattle, yak, cervids, kangaroo, sheep, cattle, llama and cat, and potentially those bacteria may contribute to oral cavity disease. More specific disease associations include otitis media, otitis interna, and meningitis. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Neisseria animalis* (guinea pig), *Neisseria canis* (dog), *Neisseria dentiae* (cattle), *Neisseria meningitidis* (guinea pig), and *Neisseria weaveri* (dog).

### *6.4.74* Neorickettsia

Phylum: Proteobacteria; Class: Alphaproteobacteria; Order: Rickettsiales; Family: Anaplasmataceae; Genus: *Neorickettsia*. The bacterial species *Neorickettsia risticii* causes a syndrome whose symptoms include gastroenteritis, lymphadenopathy, abortion, and laminitis. This bacterial species may be transmitted by trematodes associated with snails, and in some cases the infection is acquired by the horse during ingestion of water that contains aquatic insects and helminths harboring the bacterially infected trematode metacercaria (horse). *Neorickettsia risticii* previously was named *Ehrlichia risticii*.

### *6.4.75* Nocardia

Phylum: Actinobacteria; Class: Actinobacteria; Order: Corynebacteriales; Family: Nocardiaceae; Genus: *Nocardia*. The members of this genus typically are found in

soil that contains a high amount of organic material. Members of the genus *Nocardia* often are associated with abscesses. The symptoms associated with *Nocardia* infections include mastitis as well as other cutaneous and subcutaneous lesions, peritonitis, pneumonia, meningitis and meningoencephalitis. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Nocardia asteroides* (cattle, water buffalo, dog, and camelids including llama), *Nocardia brasiliensis* (kangaroo), *Nocardia farcinica* (cattle), *Nocardia nova* (cattle), and *Nocardia otitidiscaviarum* (cattle).

### *6.4.76* Paeniclostridium

Phylum: Firmicutes; Class: Clostridia; Order: Clostridiales; Family: Peptostreptococcaceae; Genus: *Paeniclostridium*. The species *Paeniclostridium sordellii* is found in soil as well as animal feces and can be isolated from the intestine. There exist both pathogenic and non-pathogenic strains of this bacterial species and both groups are considered normal vaginal flora. Its pathogenic strains are known to cause toxic shock syndrome in humans and enteritis in sheep and cattle. The species *Paeniclostridium sordellii* additionally causes pneumonia, endocarditis, arthritis, peritonitis, myonecrosis, and infects the umbilical stump of newborns (alpaca, llama, guinea pig, cattle, sheep). The species *Paeniclostridium sordellii* previously was assigned the name *Clostridium sordellii*.

### *6.4.77* Pantoea

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Enterobacteriales; Family: Erwiniaceae; Genus: *Pantoea*. The species *Pantoea agglomerans* can be isolated from plant surfaces including seeds and fruits, as well as human and animal feces. *Pantoea agglomerans* typically is a pathogen of fish in which it causes hemorrhaging ocular infections. In humans, this bacterial species is opportunistically associated with skin and eye infections. Following puncture wounds of humans caused by thorn and wood splinter injuries, this bacteria has been associated with septic arthritis, osteomyelitis and meningitis, and these illnesses particularly have been noted in immunosuppressed individuals and newborns. The discovery of this bacterial species in other mammals causes suspicion that it may similarly be an opportunistic pathogen in those other mammals (coypu, dog).

### *6.4.78* Pasteurella

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Pasteurellales; Family: Pasteurellaceae; Genus: *Pasteurella*. The members of this genus naturally reside on mucosal membranes as a part of oral, respiratory, genital, and gastrointestinal floras; those which naturally reside in the mouth are shed by nasal secretions. They can survive for at least a period of weeks in water, fecal slurries, and on moist surfaces. Members of the genus *Pasteurella* are associated with general symptoms including depression. More specific illness associations for infections caused by *Pasteurella* include those typical of the upper and lower respiratory tracts among which pneumonia and pleuropneumonia are perhaps the most notable, and they additionally cause bite wound abscesses, otitis media and otitis interna, mastitis, arthritis, gastroenteritis, urinary system infections, meningitis and meningoencephalitis. The symptoms range in severity from subclinical to peracute and fatal. The *Pasteurella* often cause secondary respiratory infections. They also are a minor but still significant cause of stress related respiratory illness in animals that recently have been transported, termed shipping fever or transit fever. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: [*Pasteurella*] *aerogenes* (this microorganism currently is considered Incertae sedis) (pig), *Pasteurella caballi* (pig, horse), *Pasteurella canis* (horse, dog, cattle, sheep), *Pasteurella dagmatis* (dog, cat), [*Pasteurella*] *mairii* (this microorganism currently is considered Incertae sedis) (pig), *Pasteurella multocida* (coypu, cervids including red deer and fallow deer, camel, alpaca, cattle, sheep, goat, pig, guinea pig, rabbit, cat, dog, kangaroo, yak), and *Pasteurella stomatis* (dog, cat).

### *6.4.79* Peptoniphilus

Phylum: Firmicutes; Class: Tissierellia; Order: Tissierellales; Family: Peptoniphilaceae; Genus: *Peptoniphilus*. The natural ecology of the species *Peptoniphilus indolicus* presumably includes soil and mud. It additionally is found in milk and other udder secretions. The typical disease associations for *Peptoniphilus indolicus* are wound infections and skin lesions including mastitis (cattle, pig). *Peptoniphilus indolicus* previously has been named *Peptococcus indolicus* and *Micrococcus indolicus*.

### *6.4.80* Peptostreptococcus

Phylum: Firmicutes; Class: Clostridia; Order: Clostridiales; Family: Peptostreptococcaceae; Genus: *Peptostreptococcus*. The members of this genus

typically are found in soil. They also are commensal organisms living predominantly on the skin as well as in the mouth, vagina, gastrointestinal and urinary tracts. Members of the genus *Peptostreptococcus* characteristically are associated with periodontal disease but they also cause pleuropneumonia, meningitis and meningoencephalitis. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Peptostreptococcus anaerobius* (dog, cat, coypu), *Peptostreptococcus canis* (dog), and *Peptostreptococcus russellii* (found in pig although the disease association remains unclear).

### *6.4.81* Plesiomonas

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Enterobacteriales; Family: Enterobacteriaceae; Genus: *Plesiomonas*. The species *Plesiomonas shigelloides* typically is found in freshwater. It also causes gastroenteritis which can be followed by septicemia if there is underlying immunodeficiency (cattle, goat, pig, cat, dog).

### *6.4.82* Porphyromonas

Phylum: Bacteroidetes; Class: Bacteroidia; Order: Bacteroidales; Family: Porphyromonadaceae; Genus: *Porphyromonas*. The members of this genus naturally reside in the mouth below the gingival surface. No information could be found that indicated their having an ecological presence in the natural environment. Members of the genus *Porphyromonas* have been associated with periodontal disease, vulvitis and vaginitis. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Porphyromonas asaccharolytica* (dog), *Porphyromonas cangingivalis* (dog), *Porphyromonas canis* (dog), *Porphyromonas canoris* (dog), and *Porphyromonas crevioricanis* (dog). The previously named species *Porphyromonas cansulci*, which affects dog, has been reclassified and currently is considered part of the species *Porphyromonas crevioricanis*.

### *6.4.83* Prevotella

Phylum: Bacteroidetes; Class: Bacteroidia; Order: Bacteroidales; Family: Prevotellaceae; Genus: *Prevotella*. Although some members of this bacterial genus are found in flooded agricultural soils (rice fields), the members of this genus typically are considered to be oral, vaginal, rumenal and colonic flora. The most typical illness

symptoms associated with members of the genus *Prevotella* are periodontal disease and foot abscesses, but the scope of illness caused by this bacterial genus also includes severe intestinal disease, bacteremia, systemic infections and osteomyelitis, infections of wounds such as those caused by bites, infections of the genital tract, and infections of both the upper and lower respiratory tracts. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Prevotella buccae* (dog), *Prevotella melaninogenica* (cat, dog, cattle), and *Prevotella oris* (dog).

### *6.4.84* Proteus

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Enterobacteriales; Family: Morganellaceae; Genus: *Proteus*. The members of this genus typically are saprophytes found in soil and water, decomposing animal matter, sewage, manure soil, human and animal feces. Members of the genus *Proteus* are associated with septic peritonitis, urinary tract infections, uterine infections, and otitis externa. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Proteus mirabilis* (camel, cattle, cervids, kangaroo, goat), *Proteus penneri* (coypu—disease association remains unclear), and *Proteus vulgaris* (camel, equines, kangaroo, water buffalo, goat).

### *6.4.85* Providencia

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Enterobacteriales; Family: Morganellaceae; Genus: *Providencia*. These listed members of the genus *Providencia* are associated with water, soil, and sewage. They also inhabit the skin and gastrointestinal tract. Members of the genus *Providencia* have been associated with bacteremia and also isolated from animals suffering from wounds including burns, urinary tract infections, pneumonia, meningitis, endocarditis, ocular infections, dermatitis, cellulitis, and cystitis associated with indwelling urinary catheters. Those species belonging to this microbial genus which have been indicated to have a potentially opportunistic disease association with mammalian livestock are: *Providencia alcalifaciens* (dog), *Providencia rettgeri* (kangaroo), and *Providencia rustigianii* (pig).

### *6.4.86* Pseudarthrobacter

Phylum: Actinobacteria; Class: Actinobacteria; Order: Micrococcales; Family: Micrococcaceae; Genus: *Pseudarthrobacter*. The members of this genus commonly are found in soil. Members of the genus *Pseudarthrobacter* also are associated with genital infections including vaginitis. The species belonging to this microbial genus which has been indicated to have a potentially opportunistic disease association with mammalian livestock is: *Pseudarthrobacter equi*, previously named *Arthrobacter equi* (horse).

### *6.4.87* Pseudomonas

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Pseudomonadales; Family: Pseudomonadaceae; Genus: *Pseudomonas*. The members of this genus typically are found in water and soil, with an increased presence in manure amended soils. Members of the genus *Pseudomonas* have been associated with otitis media and otitis interna, wound infections notably including those associated with burns (I can attest to you from having studied microbiology in a hospital as a ten year old child, that *Pseudomonas* produces a characteristic and unforgettable sickeningly sweet odor in burn wounds), mastitis, lung infections, ascending urinary tract infections, and endometritis. Those species belonging to this microbial genus which have been indicated as having a potentially opportunistic disease association with mammalian livestock are: *Pseudomonas aeruginosa* (camel, cattle, cat, dog, donkey, guinea pig, horse, kangaroo, sheep, goat, water buffalo), *Pseudomonas alcaligenes* (cattle), *Pseudomonas fluorescens* (dog, coypu), *Pseudomonas luteola* (cat, dog, guinea pig, goat), *Pseudomonas mendocina* (cattle, coypu), and *Pseudomonas stutzeri* (sheep).

### *6.4.88* Pseudopropionibacterium

Phylum: Actinobacteria; Class: Actinobacteria; Order: Propionibacteriales; Family: Propionibacteriaceae; Genus: *Pseudopropionibacterium*. This species *Pseudopropionibacterium propionicum* is virtually ubiquitous as a commensal in the oral cavity and on the skin of animals, including residence both in and around sweat glands and sebaceous glands. It can infect the lacrimal apparatus and surprisingly it can become a lethal opportunist (cattle). In human this bacterial species has caused pelvic inflammatory disease following hysteroscopic surgery. *Pseudopropionibacterium propionicum* previously has been classified as *Propionibacterium propionicum*, *Propionibacterium propionicus*, *Actinomyces propionicus*, and *Arachnia propionica*.

### *6.4.89* Psychrobacter

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Pseudomonadales; Family: Moraxellaceae; Genus: *Psychrobacter*. The species *Psychrobacter pulmonis* typically is found in soil, with an increased presence in manure amended soils. This microbial species has been associated with lung infections (sheep).

### *6.4.90* Rahnella

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Enterobacteriales; Family: Yersiniaceae; Genus: *Rahnella*. The species *Rahnella aquatilis* typically is found in freshwater, soil, and in association with some invertebrates notably snails and beetles. The fact that *Rahnella aquatilis* interestingly also can be isolated from parasitic nematodes potentially suggests that those nematodes might be involved with transmission of this bacterial species. The species *Rahnella aquatilis* causes respiratory infections (cattle, goat).

### *6.4.91* Rhodococcus

Phylum: Actinobacteria; Class: Actinobacteria; Order: Corynebacteriales; Family: Nocardiaceae; Genus: *Rhodococcus*. The species *Rhodococcus equi* thrives in water and soil, particularly being found in areas that receive animal manure. This bacterial species causes peritonitis (goat, sheep, horse, cattle, camel, llama, alpaca, pig, cervids).

### *6.4.92* Rickettsia

Phylum: Proteobacteria; Class: Alphaproteobacteria; Order: Rickettsiales; Family: Rickettsiaceae; Genus: *Rickettsia*. The *Rickettsia* are obligate intracellular pathogens. Some members of the genus *Rickettsia* can survive in amoeba, and indeed some amoeba contain endosymbiotic *Rickettsia*. However, these listed species seem to have no presence in the natural environment and instead alternate in a cycle between arthropod and mammalian hosts. The arthropod hosts for these *Rickettsia* species tend to be ticks, mites, lice, or fleas. Members of the genus *Rickettsia* have been associated with a variety of non-specific illnesses and although these often are mild or non-clinical, the infections also can become severe to the point that they result in death of the animal. The infections can be either short term or long term. Sometimes the animals demonstrate no symptoms at all, albeit they meet the defining

criterion of infection in response to the microorganism by producing serologically detectable levels of antibodies which react against the microorganism. The most common clinical signs associated with *Rickettsia* infections include lethargy, anorexia, ataxia, rash, swollen lymph nodes, and localized edema. Those species belonging to this microbial genus which have been indicated as having a potentially opportunistic disease association with mammalian livestock are: *Rickettsia aeschlimannii* (camel), *Rickettsia africae* (camel), *Rickettsia felis* (cat), *Rickettsia rickettsii* (capybara, coypu, cat, dog), *Rickettsia sibirica* (camel), and *Rickettsia typhi* (Edible dormouse—also called the Fat dormouse).

### *6.4.93* Rodentibacter

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Pasteurellales; Family: Pasteurellaceae; Genus: *Rodentibacter*. Presumably, as with the *Pasteurella*, members of this genus naturally reside on mucosal membranes as a part of oral, respiratory, genital, and gastrointestinal floras and might be shed by nasal secretions with a potential for some environmental survival. The species belonging to this microbial genus which has been indicated to have a potentially opportunistic disease association with mammalian livestock is *Rodentibacter pneumotropicus*, previously named *Pasturella pneumotropica*, (guinea pig, dog, cat, rodents including coypu, camel).

### *6.4.94 Salmonella*

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Enterobacteriales; Family: Enterobacteriaceae; Genus: *Salmonella*. The species *Salmonella enterica* typically is found in compost, manure amended soil, and crops grown in manure amended soil. The association of mammals with *Salmonella enterica* varies from subclinical intestinal carriage to septicemia and death. This bacterial species is considered to be an opportunistic pathogen and most of the infections which it causes tend to produce relatively inconsequential disease including gastroenteritis and ocular lesions. *Salmonella enterica* also can be present in the tonsils. More severe disease outcomes can occur including septic peritonitis, bacteremia, multifocal petechial hemorrhage, inflammation of tissues lining the visceral organs (serositis), polyarthritis, bronchopneumonia, meningitis, and meningoencephalitis. Importantly, the septicemic infections caused by *Salmonella* often are fatal without prior observed clinical signs (camel, alpaca, llama, pig, cattle, coypu, cervids including elk and red deer, kangaroo, guinea pig, horse, goat, sheep, cat, dog).

### *6.4.95* Serratia

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Enterobacteriales; Family: Yersiniaceae; Genus: *Serratia*. The *Serratia* are ubiquitous in the natural environment, being found in soil, water, and growing on plant material. Members of the genus *Serratia* colonize the respiratory and urinary tracts, and are associated with mastitis, respiratory illnesses, sepsis, and septic abortion. Those species belonging to this microbial genus which have been indicated as having a potentially opportunistic disease association with mammalian livestock are: *Serratia ficaria* (goat), *Serratia fonticola* (goat), *Serratia liquefaciens* (cattle, water buffalo, coypu), *Serratia marcescens* (capybara, coypu, cattle, sheep, goat), *Serratia odorifera* (goat), and *Serratia rubidaea* (cattle).

### *6.4.96* Shewanella

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Alteromonadales; Family: Shewanellaceae; Genus: *Shewanella*. The species *Shewanella putrefaciens* typically is isolated from marine environments where it may have potential to remediate petroleum spills, and it is as well found anaerobic sandstones. This species of *Shewanella* is noted for causing open lesions in fish, and it opportunistically causes both soft tissue infections and bacteremia in human (coypu—disease association remains unclear).

### *6.4.97* Shigella

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Enterobacteriales; Family: Enterobacteriaceae; Genus: *Shigella*. The *Shigella* typically are found in water, soil, and animal feces. Members of this genus can be carried asymptomatically. The disease that they typically cause is a form of enteritis which generally is self limiting but can become severe and fatal. They are the common cause of bacterial dysentery in human. Complications due to *Shigella* bacteremia are possible, mainly in immuno-compromised individuals, and those complications include arthritis, neuritis, vulvo-vaginitis, chronic colitis, conjunctivitis, and eventually death. Death can occur swiftly following the appearance of severe symptoms. Those species belonging to this microbial genus which have been indicated as having a potentially opportunistic disease association with mammalian livestock are: *Shigella dysenteriae* (camel, dog), *Shigella flexneri* (cattle), and *Shigella sonnei* (goat).

### *6.4.98* Sphingobacterium

Phylum: Bacteroidetes; Class: Sphingobacteriia; Order: Sphingobacteriales; Family: Sphingobacteriaceae; Genus: *Sphingobacterium*. The members of this genus typically are found in soil, plants, and water. The disease association with *Sphingobacterium multivorum* in livestock mammals remains unclear but this bacterial species is opportunistically pathogenic in humans where it causes respiratory tract infections and septicemia (found in cat, dog, and sheep, although the disease association with these livestock mammals remains unclear).

### *6.4.99* Sphingomonas

Phylum: Proteobacteria; Class: Alphaproteobacteria; Order: Sphingomonadales; Family: Sphingomonadaceae; Genus: *Sphingomonas*. The species *Sphingomonas paucimobilis* can be found in freshwater, seawater, and terrestrial habitats including plant root systems. This species can grow in drinking water distribution systems. *Sphingomonas paucimobilis* also can be found associated with equipment in hospitals including respiratory therapy items, humidifiers, water, air, bedside water bottles, sinks, and temperature probes. This species often is associated with bite wounds, the result of infection can include both ulcerations and abscess as well as bacteremia (cat, dog, rodents including coypu). The previous name of this species was *Pseudomonas paucimobilis*.

### *6.4.100* Staphylococcus

Phylum: Firmicutes; Class: Bacilli; Order: Bacillales; Family: Staphylococcaceae; Genus: *Staphylococcus*. The members of this genus typically are found as a minor component of the soil microbial community. In association with mammals they normally inhabitant the skin and mucous membranes including those of the upper respiratory tract. Members of the genus *Staphylococcus* commonly cause infections of the skin, eye, external ear and mammary gland, the severity of which can range from minor to severe including skin redness, pustules, and abscesses. The various *Staphylococcus* species can, however, affect every organ in the host's body. Encompassing the fullness of their disease consequences is a lengthy task and the description must include genito-urinary infections that may ascend the urinary tract, as well as infections which can begin in the lung to cause pneumonia and pleuropneumonia. *Staphylococcus* infections can progress to cause bacteremia, septic peritonitis, and endocarditis. These bacterial species also can affect the skeleton and joints causing osteomyelitis, and invade the central nervous system resulting in meningitis, encephalitis, meningoencephalitis and encephalomyelitis. These

consequences of infection can prove fatal for immunocompromised animals. The toxins produced by *Staphylococcus* may generate symptoms that typically would be associated with food poisoning. *Staphylococcus* species also can cause food poisoning from enterotoxin contamination in meat. It is important to note that in older kangaroo *Staphylococcus aureus* sometimes is considered a primary pathogen rather than being opportunistic. Those species belonging to this microbial genus which have been indicated as having a potentially opportunistic disease association with mammalian livestock are: *Staphylococcus aureus* (coypu, dog, sheep, moose, camel, guinea pig, yak, water buffalo, pig, equines, cattle, kangaroo), *Staphylococcus auricularis* (cattle, coypu, sheep), *Staphylococcus capitis* (dog, kangaroo), *Staphylococcus caprae* (goat), *Staphylococcus chromogenes* (equines), *Staphylococcus cohnii* (dog, rabbit), *Staphylococcus delphini* (dog), *Staphylococcus epidermidis* (camel, cat, coypu, dog, kangaroo, horse, cattle, cervids), *Staphylococcus equorum* (cat, horse), *Staphylococcus felis* (cat), *Staphylococcus gallinarum* (sheep), *Staphylococcus haemolyticus* (horse, cat, dog), *Staphylococcus hominis* (coypu, dog), *Staphylococcus hyicus* (camel, coypu, cervids, pig, cattle), *Staphylococcus intermedius* (camel, cat, coypu, dog, equines, cattle), *Staphylococcus lentus* (dog, sheep), *Staphylococcus lugdunensis* (dog), *Staphylococcus pseudintermedius* (dog), *Staphylococcus saccharolyticus* (coypu—disease association remains unclear), *Staphylococcus saprophyticus* (cattle, sheep), *Staphylococcus schleiferi* (dog), *Staphylococcus sciuri* (pig, cattle, coypu, sheep, goat), *Staphylococcus warneri* (coypu, dog), and *Staphylococcus xylosus* (kangaroo, cattle, goat).

### *6.4.101* Stenotrophomonas

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Xanthomonadales; Family: Xanthomonadaceae; Genus: *Stenotrophomonas*. The species *Stenotrophomonas maltophilia* ubiquitously is found in water, soil, and associated with plants. This bacterial species is notably resistant to antibiotics and produces severe infections in immunocompromised hosts. It is a growing source of latent pulmonary infections in human. *Stenotrophomonas maltophilia* typically causes infection following wounds, it also can cause pneumonia, urinary tract infections, and bacteremia (horse, dog, cat, goat, cattle, sheep, rabbit, coypu). The previous names assigned to *Stenotrophomonas maltophilia* include *Pseudomonas maltophilia* and a great deal of literature can be found by searching under that older bacterial species name.

### *6.4.102* Streptobacillus

Phylum: Fusobacteria; Class: Fusobacteria; Order: Fusobacteriales; Family: Leptotrichiaceae; Genus: *Streptobacillus*. Members of this genus are found in soil

and contaminated water. *Streptobacillus felis* causes pneumonia (cat). *Streptobacillus moniliformis*, previously named *Actinomyces muris*, is perhaps the most notable member of this bacterial genus. The natural habitat of *Streptobacillus moniliformis* seems to be the oral cavity, nasopharynx, respiratory tract and middle ear of numerous rodent species, especially rats, with natural colonization rates in apparently healthy rats sometimes far greater than 50%, and *Streptobacillus moniliformis* seems to be an opportunistic pathogen in rats that occasionally causes otitis media, conjunctivitis, and pneumonia. *Streptobacillus moniliformis* may similarly affect those rodent species that are kept as livestock, and it does indeed cause throat infections (capybara) including cervical abscess (guinea pig), pneumonia (guinea pig), plus scrotal infections (capybara). *Streptobacillus moniliformis* causes rat bite fever in humans which is a systemic illness classically characterized by fever, rigor (fever with shivering and a feeling of chills), polyarthralgia, including more rarely pneumonitis, endocarditis and meningitis. The syndrome of rat bite fever caused by *Streptobacillus moniliformis* seems perhaps rare or unknown in livestock mammals. *Streptobacillus moniliformis* has, however, been carried and transmitted by cats, dogs, and pigs, thus potentially being an opportunistic pathogen in those livestock species. Cats, dogs, and pigs presumably acquire *Streptobacillus moniliformis* from eating rodents.

### *6.4.103* Streptococcus

Phylum: Firmicutes; Class: Bacilli; Order: Lactobacillales; Family: Streptococcaceae; Genus: *Streptococcus*. The members of this genus typically are found in water, soil, and vegetation. The *Streptococcus* are considered normal animal flora. However, members of the genus *Streptococcus* do cause infections whose severity ranges from asymptomatic to severe depending upon the age and immune status of the infected animal. These bacteria can cause mastitis and cellulitis, ocular discharge, otitis externa, otitis media and otitis interna, cervical lymphadenitis (glanders), and abscesses. *Streptococcus* infections can ascend the urinary tract and also cause endometritis. The bacteria belonging to this species can invade both the upper and lower respiratory tracts, cause septic peritonitis, pleuropneumonia, meningitis and meningoencephalitis. It is important to note that some subspecies of *Streptococcus equi* are considered primary pathogens rather than being opportunistic. Those species belonging to this microbial genus which have been indicated as having a potentially opportunistic disease association with mammalian livestock are: *Streptococcus agalactiae* (camel, cattle, dog, cat), *Streptococcus anginosus* (coypu, dog), *Streptococcus caballi* (horse), *Streptococcus canis* (dog, cat, cattle, rabbit), *Streptococcus caprae* (goat), *Streptococcus dentisani* (donkey), *Streptococcus dysgalactiae* (camel, equines, kangaroo, water buffalo, cattle), *Streptococcus entericus* (cattle), *Streptococcus equi* (alpaca, llama, camel, dog, horse, donkey, cattle, sheep, goat, pig, guinea pig), *Streptococcus equinus* (cattle, alpaca, sheep), *Streptococcus gallolyticus* (horse, dog, kangaroo, rabbit, guinea pig, goat), *Streptococcus orisasini* (donkey), *Streptococcus orisuis* (pig—disease association

remains unclear), *Streptococcus ovis* (sheep), *Streptococcus pluranimalium* (cattle, sheep, goat, cat), *Streptococcus pneumoniae* (camel, guinea pig, equines, rabbit, rodents including coypu, dog), *Streptococcus porci* (pig), *Streptococcus porcinus* (pig), *Streptococcus porcorum* (pig), *Streptococcus pyogenes* (cattle, dog), *Streptococcus rupicaprae* (goat), *Streptococcus sanguinis* (llama), *Streptococcus suis* (pig, cattle, bison, sheep, goat), *Streptococcus uberis* (camel, water buffalo, cattle), *Streptococcus viridans* often generally termed 'Viridans streptococci' (alpaca, llama, cattle, sheep, goat, rabbit).

### *6.4.104* Treponema

Phylum: Spirochaetes; Class: Spirochaetia; Order: Spirochaetales; Family: Spirochaetaceae; Genus: *Treponema*. Members of the phylum Spirochaetes represent a diverse group of bacteria, many of whose members can be found in soil and marine sediments. Members of the genus *Treponema*, however, are strongly dependent upon their hosts and many seem to be obligate parasites meaning that they do not have a prolonged survival independent of a host. Various members of the genus *Treponema* normally dwell within the oral cavity and cause necrotic periodontal disease, others are associated with necrotic skin ulcers typically of the ear and foot, and some affect the genital organs. Many *Treponema* species also are found as natural residents in the rumen and colon of cattle and sheep where they contribute to the digestion process and where disease associated with their presence often remains unclear except for ulcerative colitis. All of these listed *Treponema* species are found in animal feces and presumably that presence plays a role in their transmission. There is a strong likelihood that fecal presence contributes to the risk of a common *Treponema* disease association for cattle which is called Bovine digital dermatitis. Those species belonging to this microbial genus which have been indicated as having a potentially opportunistic disease association with mammalian livestock are: *Candidatus Treponema suis* (pig), *Treponema brennaborense* (cattle), *Treponema bryantii* (cattle—disease association remains unclear), *Treponema denticola* (cattle—disease association remains unclear), *Treponema medium* (cattle—disease association remains unclear), *Treponema pedis* (cattle), *Treponema phagedenis* (cattle), *Treponema porcinum* (pig), *Treponema saccharophilum* (cattle—disease association remains unclear), and *Treponema zioleckii* (sheep—disease association remains unclear).

### *6.4.105* Trueperella

Phylum: Actinobacteria; Class: Actinobacteria; Order: Actinomycetales; Family: Actinomycetaceae; Genus: *Trueperella*. The members of this genus are found as soil dwellers and typically infect animals in association with commensal

relationships or trauma related wounds. The *Trueperella* can be found in the urogenital tract where they cause both metritis and ascending urinary tract infections. They also can cause abscesses, otitis media and otitis interna, dermatitis of the footpads, mastitis, non-specific lesions involving the visceral organs, tenosynovitis (a type of infectious arthritis associated with the connective tissue surrounding tendons), lymphadenitis, gastroenteritis, pneumonia for which a severe outcome can include embolic pneumonia, and attack the upper respiratory tract. The *Trueperella* additionally have been isolated together with various other bacterial species under different disease conditions associated with opportunistic infections. The bacterial species *Trueperella bialowiezensis* and *Trueperella bonasi* are listed here because they affect the European bison and thus likely also would be infective of the American bison. The European bison has the taxonomic name *Bison bonasus* and currently seems not to be raised as a livestock animal. Notably, the previous names assigned to *Trueperella pyogenes* were *Corynebacterium pyogenes*, *Actinomyces pyogenes*, and *Arcanobacterium pyogenes*. Those species belonging to the genus *Trueperella* which have been indicated as having a potentially opportunistic disease association with mammalian livestock are: *Trueperella abortisuis* (cattle, pig), *Trueperella bialowiezensis* (European bison), *Trueperella bonasi* (European bison), and *Trueperella pyogenes* (broadly infective of mammals including camel, alpaca, llama, cattle, water buffalo, cervids including fallow deer and elk, pig, sheep, kangaroo, goat, horse).

### *6.4.106* Tsukamurella

Phylum: Actinobacteria; Class: Actinobacteria; Order: Corynebacteriales; Family: Tsukamurellaceae; Genus: *Tsukamurella*. Members of the genus *Tsukamurella* are saprophytes found in soil and water. The species *Tsukamurella inchonensis* is opportunistically associated with wound infections, as well as bacteremia, lung and gastric infections in human. For that reason, the discovery of this bacterial species in livestock mammals potentially is of agricultural interest (cattle and sheep, although the disease association for sheep remains unclear).

### *6.4.107* Tyzzerella

Phylum: Firmicutes; Class: Clostridia; Order: Clostridiales; Family: Lachnospiraceae; Genus: *Tyzzerella*. The species [*Clostridium*] *piliforme* (use of brackets around an assigned species name indicates that either part or all of that assigned name has not officially been approved) is found in soil and feces, and animals typically acquire it from contaminated food and water. This bacterial species also resides in organs of the digestive system and is associated with gastroenteritis. From residence in the ileum and cecum, [*Clostridium*] *piliforme* can pass through the hepatic portal into the liver,

and from there progress to infect other tissues in the body. The taxonomic name [*Clostridium*] *piliforme* is not currently approved and this species has been assigned at least temporarily to the genus *Tyzzerella*. The previous name assigned to this species was *Bacillus piliformis*. This bacterial species opportunistically affects a number of mammalian livestock species and it is important to note that in rabbit this bacterial species may be a primary pathogen rather than opportunistic (cervids, cattle, kangaroo, alpaca, llama, rabbit).

### *6.4.108* Ureaplasma

Phylum: Tenericutes; Class: Mollicutes; Order: Mycoplasmatales; Family: Mycoplasmataceae; Genus: *Ureaplasma*. There is no indication that members of the genus *Ureaplasma* have a presence in the natural environment. The *Ureaplasma* are common inhabitants that colonize the oral cavity, oropharynx and genitourinary tract. Members of this genus are associated with urinary and possibly also respiratory tract infections, vulvitis, vaginitis, posthitis, salpingitis (infection and inflammation in the fallopian tubes) which can produce infertility, endometritis, abortion from early embryonic death, weak calves and decreased conception rates after pregnancy. Those species belonging to this microbial genus which have been indicated as having a potentially opportunistic disease association with mammalian livestock are: *Ureaplasma canigenitalium* (dog); *Ureaplasma cati* (cat); *Ureaplasma diversum* (cattle, pig); *Ureaplasma felinum* (cat), *Ureaplasma parvum* (sheep), and *Ureaplasma urealyticum* (rodents including guinea pig).

### *6.4.109* Veillonella

Phylum: Firmicutes; Class: Negativicutes; Order: Veillonellales; Family: Veillonellaceae; Genus: *Veillonella*. The members of this genus typically are found in saliva and also found in feces, which can play a significant role in their environmental transmission between hosts. The members of this bacterial species are considered to be normal residents of the intestine and oral mucosa of mammals, they also are present in the respiratory and genitourinary tracts. As infections, the *Veillonella* typically are associated with bite wounds. As opportunistic pathogens, notably in the case of *Veillonella parvula*, they have been implicated as a cause of endocarditis, meningitis, and osteomyelitis. Those species belonging to this microbial genus which have been indicated as having a potentially opportunistic disease association with mammalian livestock are: *Veillonella caviae* (guinea pig—disease association remains unclear) and *Veillonella parvula* (coypu, dog).

### *6.4.110* Vibrio

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Vibrionales; Family: Vibrionaceae; Genus: *Vibrio*. The members of this genus are ubiquitous in the environment. They characteristically reside in and are acquired from water where they typically exist by growing on the chitin shell of aquatic macroinvertebrates and microinvertebrates including copepods. Correspondingly, the *Vibrio* are noted for their chitinase activity. Some members of this bacterial genus can be primary pathogens but most are opportunistic and cause secondary disease. Members of the genus *Vibrio* cause both acute as well as chronic forms of disease and often the symptoms are nonspecific. The *Vibrio* species often specifically attack the skin causing a darkening or erythema including petechial hemorrhages, wound infections and ulcerations. They also can cause otitis, produce sight-threatening ocular infections, attack the host's central nervous system including that of a developing fetus, cause gastroenteritis and coelomic distension, and cause respiratory problems. *Vibrio* also produce toxins that frequently play a role in pathogenesis. Some animal species that are noted for their toxicity can naturally harbor resident *Vibrio alginolyticus* as symbionts and this bacterial species has been suggested as one possible source of the potent neurotoxin tetrodotoxin associated with many vertebrates (puffer fish of the family Tetraodontidae, porcupine fish of the family Diodontidae, and the rough-skinned newt *Taricha granulosa*) and invertebrates (moon snails of the family Naticidae and blue-ringed octopus of the genus *Hapalochlaena*). Those species belonging to this microbial genus which have been indicated as having a potentially opportunistic disease association with mammalian livestock are: *Vibrio alginolyticus* (coypu) and *Vibrio cincinnatiensis* (cattle).

### *6.4.111* Yersinia

Phylum: Proteobacteria; Class: Gammaproteobacteria; Order: Enterobacteriales; Family: Yersiniaceae; Genus: *Yersinia*. The members of this genus can be environmentally present in urine and feces. It also is possible that they may be vectored through the environment in the free living amoeba *Acanthamoeba castellanii*, which naturally is found in soil and water. Members of the genus *Yersinia* cause mastitis, lymphadenopathy, abscess, hemorrhage, bacteremia, enteritis, abdominal pain, and pneumonia. The bacterial species *Yersinia pestis* is particularly notable because it causes the disease named plague. The severity of illness caused by *Yersinia pestis* ranges from subclinical to a chronic wasting, and peracute mortality can occur without other obvious signs of illness. In some host animals *Yersinia pestis* infections produce mortality rates as high as 80%–100%. However, infections caused by *Yersinia pestis* are subclinical in resistant host species and despite being quite lethal in human, *Yersinia pestis* may qualify as either a commensal or opportunistic pathogen in the Edible dormouse. Those species belonging to this microbial genus

which have been indicated as having a potentially opportunistic disease association with mammalian livestock are: *Yersinia enterocolitica* (camel, horse, coypu, guinea pig, cervids, pig, cattle, cat, dog), *Yersinia pestis* (Edible dormouse), and *Yersinia pseudotuberculosis* (dog, cat, coypu, guinea pig, rabbit, cattle, pig, sheep, kangaroo, cervids, alpaca, llama, capybara).

### Compliance with Ethical Standards

**Conflict of Interest** Christon J. Hurst declares that he has no conflict of interest.

**Ethical Approval** This article does not contain any studies with human participants or animals performed by the author.

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# Chapter 7
# Infectious Disease in Wild Animal Populations: Examining Transmission and Control with Mathematical Models


**Sergey S. Berg, James D. Forester, and Meggan E. Craft**

**Abstract** The mathematical modeling of ecological interactions is an essential tool in predicting the behavior of complex systems across landscapes. The scientific literature is growing with examples of models used to explore predator-prey interactions, resource selection, population growth, and dynamics of disease transmission. These models provide managers with an efficient alternative means of testing new management and control strategies without resorting to empirical testing that is often costly, time-consuming, and impractical. This chapter presents a review of four types of mathematical models used to understand and predict the spread of infectious diseases in wild animals: compartmental, metapopulation, spatial, and contact network models. Descriptions of each model's uses and limitations are used to provide a look at the complexities involved in modeling the spread of diseases and the trade-offs that accompany selecting one modeling approach over another. Potential avenues for the improvement and use of these models in future studies are also discussed, as are specific examples of how each type of model has improved our understanding of infectious diseases in populations of wild animals.

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## 7.1 Introduction

Infectious diseases of both humans and animals have played a large role in shaping the world as it is today, from the role of smallpox in the fall of the Aztec (McNeil 1998) to the 2001 foot-and-mouth disease epidemic that cost the United Kingdom over $6 billion (Thompson et al. 2002), and will continue to do so in the future. Although most research into this topic has focused on human and domesticated animals as hosts, the study of wildlife diseases has been growing rapidly since the 1980s (Anderson et al. 1981; Kawata 2010; McCallum 2016). This interest has been driven by desires to minimize risks to human health (e.g., zoonotic diseases such as leptospirosis; Munoz-Zanzi et al. 2014), decrease loss of livestock [e.g., transmission of brucellosis from elk (*Cervus canadensis*) to cattle (*Bos taurus*); Xie and Horan 2009], and preserve either native or endangered species [e.g., canine distemper in the black-footed ferret (*Mustela nigripes*); Thorne and William 1988].

Mathematical models have been a useful tool in the study of wildlife infectious diseases. These models have been used for tasks as varied as predicting the spatial spread of rabies in Eastern Europe (Källen et al. 1985), comparing the benefits of vaccination versus culling in eradicating bovine tuberculosis from England (Smith and Cheeseman 2002), investigating the role of bird feeders in promoting the spread of conjunctivitis in house finches (*Carpodacus mexicanus*) in North America (Hosseini et al. 2004), and even to show that flea-borne transmission is insufficient to maintain plague epizootics in Colorado prairie dogs (*Cynomys ludovicianus*; Webb et al. 2006). Here we present a review of four types of models used in studies of wildlife infectious diseases—compartmental, metapopulation, spatial, and contact network—and discuss their uses, limitations, and contributions to the understanding of infectious diseases. It is important to note that although we present these four models as separate for the purpose of this review, there is in fact substantial overlap between these categories (e.g., a metapopulation model can also exhibit properties of a spatial model).

## 7.2 Compartmental Models

The majority of disease models are based on categorizing hosts within a population based on their disease status. The most basic form of such a model describes the proportions of the population that are susceptible to ($S$), infected with ($I$), and recovered from ($R$) a particular disease (a complete list of model variables and parameters used in this chapter is provided in Table 7.1). For a pathogen that confers lifelong immunity from future infections, this compartmentalization results in the following set of differential equations that describe how individuals transition between different disease categories:

**Table 7.1** Description of variables and parameters commonly used in mathematical models of infectious diseases

| Symbol | Definition |
|---|---|
| $S$ | Proportion of the population that is susceptible to a particular disease |
| $I$ | Proportion of the population that is infected with a particular disease |
| $R$ | Proportion of the population that is recovered from a particular disease |
| $\upsilon$ | Influx of new susceptible into the population through births |
| $\mu$ | Removal of individuals from the population through deaths |
| $\beta$ | The product of contact rate and transmission probability for a particular disease |
| $\gamma$ | Rate of recovery from a particular disease |
| $X$ | Number of individuals that are susceptible to a particular disease |
| $Y$ | Number of individuals that are infected with a particular disease |
| $Z$ | Number of individuals that are recovered from a particular disease |
| $N$ | Total number of individuals in the population |
| $R_0$ | Basic reproductive ratio |
| $\lambda$ | The force of infection for a particular disease |
| $\rho_{ij}$ | The relative strength of transmission from one subpopulation, $i$, to a different subpopulation, $j$ |
| $P$ | The proportion of subpopulations infected with a particular disease |
| $r$ | The reinfection (coupling) rate from an infected subpopulation to an uninfected subpopulation |
| $e$ | The rate of local extinction for a subpopulation |
| $D_X$ | Diffusion rate for individuals that are susceptible to a particular disease |
| $D_Y$ | Diffusion rate for individuals that are infected with a particular disease |
| $D_Z$ | Diffusion rate for individuals that are recovered from a particular disease |
| $\nabla^2$ | Local diffusion rate of individuals through space |
| $\boldsymbol{G}$ | Adjacency matrix that represents the presence, duration, or frequency of contact between individuals |

$$\frac{dS}{dt} = \upsilon - \beta SI - \mu S, \tag{7.1}$$

$$\frac{dI}{dt} = \beta SI - \gamma I - \mu I, \tag{7.2}$$

$$\frac{dR}{dt} = \gamma I - \mu R, \tag{7.3}$$

where $\upsilon$ is the influx of new susceptible individuals through births, $\mu$ represents the removal of individuals through death, $\beta$ is the product of contact rate and transmission probability, and $\gamma$ is the recovery rate (Keeling and Rohani 2008). This formulation is known as the susceptible-infected-recovered (SIR) model, which was originally proposed by Kermack and McKendrick (1927). A slightly different version of these equations can be used to model the *number* of individuals in each disease category:

$$\frac{dX}{dt} = \upsilon - \beta XY/N - \mu X, \tag{7.4}$$

$$\frac{dY}{dt} = \beta XY/N - \gamma Y - \mu Y, \tag{7.5}$$

$$\frac{dZ}{dt} = \gamma Y - \mu Z, \tag{7.6}$$

where $X$, $Y$, and $Z$ are the number of susceptible, infectious, and recovered individuals and $N$ is the total number of individuals ($N = X + Y + Z$). Such compartmentalization ignores many details of the progression of the disease, as well as heterogeneity in individual responses and contact probabilities (i.e., all individuals have the same probability of contacting every other individual in the entire population), but nonetheless provides some very important insights into disease dynamics (Keeling and Eames 2005; Keeling and Rohani 2008; Kawata 2010).

Many modifications have been made to this basic framework, usually involving further subdivision of the disease classifications to reflect either more complex pathogen biology (e.g., waning immunity, fatal infection, or the presence of a latent period), multiple pathogens [e.g., canine distemper and sarcoptic mange in fishers (*Martes pennanti*); Keller et al. 2012], multiple hosts [e.g., louping ill virus in red grouse (*Lagopus lagopus scotica*), mountain hare (*Lepus timidus*), and red deer (*Cervus elaphus*); Gilbert et al. 2001], or greater structure within the host population (e.g., age, sex, or social status). These modifications include the susceptible-infectious (SI) and susceptible-exposed-infectious-recovered (SEIR) models, among others. For structured host populations, differences in contact rates between different subgroups often become important, replacing the single parameter $\beta$ with a matrix that describes the transmission of infection between and within different groups. It is important to note that the assumption of random mixing still remains despite these modifications, although now it is limited to transmission within each subgroup (Keeling and Eames 2005; Keeling and Rohani 2008).

### 7.2.1 Uses and Limitations

Some of the first and most influential uses of compartmental models involved establishing the conditions necessary to ensure either disease persistence or eradication (Smith and Cheeseman 2002; Keeling and Rohani 2008). Two of the most important quantities in epidemiology emerged from such analyses. The first, $R_0$, defines the average number of secondary cases that would arise from an average primary case in an entirely susceptible population. The exact formulation of this variable depends on the type of model used (i.e., SIR versus SEIR); however, for the SIR model described above, it is given by $R_0 = \frac{\beta}{\gamma+\mu}$. The force of infection, $\lambda$, on the other hand, defines the per capita rate at which susceptible individuals contract the infection, and is equal to $\beta I$ and $\beta Y/N$, for the proportions and number of individuals formulations, respectively. The values that these quantities take in different host-pathogen systems can determine

the final outcome of the disease (e.g., $R_0$ must be greater than unity for a disease to spread and less than unity to ensure eventual disease eradication; Keeling and Eames 2005; Keeling and Rohani 2008; Kawata 2010).

Anderson et al. (1981), for example, used a compartmental model to demonstrate that vaccinating at random at least a proportion $p = 1 - 1/R_0$ of the population ensures the eventual eradication of rabies from foxes. Although numerous methods have been devised to improve the calculation of this value (e.g., incorporating age or transmission heterogeneity), the original formulation remains one of the most fundamental and often-used discoveries in epidemiology (Keeling et al. 2003; Garnett 2005; Yip et al. 2007). Multi-host models, on the other hand, have been used to investigate the effects of multiple hosts on maintaining infections in situations where a single host is insufficient to sustain the ongoing spread of the disease (e.g., Dobson and Meagher 1996; Gilbert et al. 2001).

The main limitation of these models is the mean-field assumption, wherein all individuals in the population contact each other with equal probability. Although certain modifications have been devised to address this concern (e.g., introducing host structure such that certain groups mix more preferentially with each other), it still remains the main drawback of compartmental models. Additionally, because such models are aspatial, they cannot be used to investigate the dynamics of host populations that exhibit significant spatial heterogeneity or to model the *spatial* spread of an invading disease (Keeling and Eames 2005; Keeling and Rohani 2008; Kawata 2010).

Another issue with compartmental models has centered on how best to model the transmission between susceptible and infectious individuals. For most of the field's history, density-dependent transmission was used because of its clear analogy to how the rate of interaction between two types of molecules is directly proportional to their densities. While this framework, which yields the familiar $\beta SI$ transmission term, has been applied with some success, certain populations may not display such a characteristic. For a sexually transmitted disease in humans, for example, the number of transmissible contacts (i.e., sexual partners) is often independent of population density (Lloyd-Smith et al. 2004; Ryder et al. 2007). In these situations, a frequency-dependent rate of transmission is required, which alters the transmission term to $\beta SI/N$ (McCallum et al. 2001; Begon et al. 2002; Keeling and Rohani 2008).

Although these examples may give the impression that transmission operates only at two extremes (i.e., either independent or as a function of host density), in most situations, it is in fact a continuum of values and may even change throughout the course of an epidemic. Antonovics et al. (1995), for example, modeled this continuum based on the Type II Holling functional response curve, wherein contacts at low densities are proportional to the host density but eventually reach a maximum at higher densities. Because the exact formulation of the transmission term is crucial to a model's predictive and descriptive capabilities, as well as the qualitative behavior of the system itself, the relationship of contact rates to density is still an area of active research. In general, however, frequency-dependent transmission is used primarily in models of vector-borne pathogens and human populations, while density-dependent transmission is generally considered to be more applicable to

plant and animal diseases (McCallum et al. 2001; Begon et al. 2002; Keeling and Rohani 2008).

Another common use of compartmental models has been to investigate the role of seasonality in producing either annual, semiannual, or even chaotic patterns of disease prevalence. Many wildlife populations, for example, experience significant changes in transmission rates throughout the year as a result of either flocking behavior (Hosseini et al. 2004), seasonal migration (Bradley and Altizer 2005), or congregation during the breeding and molting season (Swinton et al. 1998). An additional source of seasonality that is almost exclusive to wildlife populations arises from birth pulses which recruit a large number of susceptible individuals into the population at approximately the same time each year (Gremillion-Smith and Woolf 1988; Keeling and Rohani 2008). If a time-dependent component is incorporated into the transmission term, then increasing the amplitude of seasonality can increase the number of prevalence peaks throughout the year, eventually resulting in chaotic dynamics (Ireland et al. 2004).

### *7.2.2 Specific Applications*

The earliest application of compartmental models to infectious diseases in wildlife was presented in the seminal work of Anderson et al. (1981), who developed a deterministic SEI (susceptible-exposed-infectious) model for the fatal rabies virus in European red foxes (*Vulpes vulpes*; Kawata 2010). This model demonstrated that the latent period of rabies could act as a time-delayed density-dependent regulator of fox population growth and result in the 4-year oscillations in population size and disease prevalence observed in fox populations throughout Europe. The authors also concluded that culling alone was unlikely to be effective once rabies becomes established within a fox population. Vaccination, on the other hand, significantly increased the odds of disease eradication, especially when combined with culling in regions with low fox density (Anderson et al. 1981).

Gilbert et al. (2001) used a multi-host approach to investigate how the persistence of a tick-borne virus (louping ill virus) is affected by the presence of grouse, hare, and deer in upland Britain. This model demonstrated that neither deer nor grouse alone could sustain such a virus, but that the combination of the two could and that the inclusion of hare into this system greatly increased the likelihood of virus persistence (Gilbert et al. 2001). These results were later used by Laurenson et al. (2003) to investigate whether decreasing hare density could be used as a strategy to decrease virus prevalence in grouse. They discovered that not only would this strategy decrease infections in grouse, but it would also increase their fecundity as a consequence of increased life expectancy. The authors concluded that mountain

hares serve as a reservoir species that can maintain the virus as long as hare density remains above five individuals $km^{-2}$.

Packer et al. (2003) used a simple compartmental SI model to demonstrate a very interesting principle—that predator control programs aimed at increasing prey abundance could prove harmful to prey populations that are regulated by infectious diseases rather than by predation. The authors showed that because infected prey live longer in the absence of predators, they are able to infect many more susceptible animals than they would be able to otherwise. Although this effect is more pronounced when predators selectively remove infected prey (e.g., if the infection makes prey easier to capture or locate), nonselective predation is still beneficial to host populations by reducing the life span of infectious individuals. This principle was used to retroactively explain how the introduction of private gamekeepers (who removed predators) in red grouse habitats destabilized the grouse population in North Yorkshire, England (Packer et al. 2003).

### *7.2.3 Future Directions*

As illustrated above, mean-field compartmental models have been used in a wide variety of settings and have provided the foundation for much of the epidemiological literature and theory. Unfortunately, the mean-field assumption and the aspatial nature of these models limit their ability to provide accurate predictions of the likely spread of a disease through a wildlife population. This limitation has led to the development of several more sophisticated methods to describe the spread of infectious diseases, which are detailed throughout the remainder of this chapter. Despite these advances in infectious disease modeling, there are still several aspects of disease ecology that can be best investigated with compartmental models.

For example, compartmental models can be used to explore the role of seasonal variation in mortality rates in the spread of infectious diseases. Previous research has investigated the role of seasonality in births (e.g., Ireland et al. 2004), demonstrating how increases and decreases to the magnitude of this seasonality can have profound consequences to the spread of the disease. Unfortunately, similar theory does not yet exist for the effects of altering the magnitude of seasonality in mortality rates. This avenue for future research would be particularly appealing because, unlike for births, newly developed theory could be tested by manipulating the timing, extent, and demographic characteristics of mortality through changes to harvest regulations. Because harvest constitutes a substantial portion of deaths in many wildlife populations (e.g., Erb et al. 2013; Ramsey et al. 2014), understanding how changes in harvest intensity will impact the spread of disease could prove to be a powerful tool for wildlife managers.

## 7.3 Metapopulation Models

Although the spread of infectious diseases is predominantly a localized process (i.e., between individuals in the same location), the movement of individuals between aggregate groups can help to facilitate the geographical spread of the disease. Metapopulation models, originally developed for application in ecology (Levins 1969; Hanski 1999), provide a simple but powerful means of capturing this spatial structure by subdividing the entire population into distinct groups known as subpopulations. These models typically assume that transmissible contacts will occur at a higher rate between individuals of the same group than between individuals of different groups, allowing each subpopulation to exhibit largely independent dynamics (Jesse et al. 2008; Ball et al. 2015).

In such a framework, the original SIR equations can be modified as

$$\frac{dX_i}{dt} = \upsilon_i N_i - \lambda_i X_i - \mu_i X_i, \tag{7.7}$$

$$\frac{dY_i}{dt} = \lambda_i X_i - \gamma_i Y_i - \mu_i Y_i, \tag{7.8}$$

$$\frac{dZ_i}{dt} = \gamma_i Y_i - \mu_i Z_i, \tag{7.9}$$

where $i$ refers to parameters that are particular to subpopulation $i$ and may vary substantially between subpopulations due to differences in the local environment (Broadfoot et al. 2001; Langlois et al. 2001; Keeling and Rohani 2008). Although most of the useful quantities derived from mean-field compartmental models still apply, they must now incorporate, $\rho_{ij}$, the relative strength of transmission from one subpopulation, $i$, to a different subpopulation, $j$ (e.g., $\lambda_i = \beta_i \sum_j \rho_{ij} Y_j / N_i$ vs. $\lambda = \beta Y/N$; Keeling and Rohani 2008).

Although metapopulation structure is inherently based on the host population of interest, these models usually fall into one of three categories. Wind- and vector-borne transmission models, for example, assume that subpopulations are epidemiologically sessile, forcing coupling (i.e., the strength of transmission) to decrease with distance. These models have been used extensively to model the spatial dynamics of livestock diseases by treating each farm as a sessile subpopulation (e.g., Ferguson et al. 2001; Keeling 2001). Commuter models, which are most commonly used for human population, are instead based on the premise that permanent relocation between subpopulations is rare but temporary travel between them is sufficient for disease spread (Keeling and Rohani 2008). Finally, most models of animal diseases rely on either the migration or permanent movement of individuals to spread the pathogen (Keeling and Rohani 2008).

Once the appropriate relations are defined, the system of equations can be solved in either a deterministic or stochastic manner. Because this framework is able to capture the spatial clustering of the host population, it has been used extensively not only for the study of infectious diseases but also in the ecological literature to derive

such concepts as the extinction-colonization balance, rescue effect, and isolation paradigm (Hanski 1998, 1999).

### 7.3.1 Uses and Limitation

One of the most common uses of metapopulation models is to investigate the influence of substructure on disease thresholds and persistence. Group size heterogeneity, for example, can increase disease persistence by allowing smaller subpopulations, where the pathogen would normally be unable to persist, to continually become reinfected from larger ones where the disease is maintained (Broadfoot et al. 2001). The level of substructure has also been shown to be an important consideration, with higher numbers of subdivision (e.g., dividing subpopulations into smaller groups) leading to longer disease outbreaks and increased persistence (Swinton et al. 1998). Similar approaches have also been used to understand the rate and heterogeneity of the spatial spread of a pathogen (e.g., Langlois et al. 2001; López et al. 2009).

A general trend that has emerged from this modeling framework is that given certain levels of aggregation and coupling, a pathogen can persist within the host population for much longer than would be predicted by a mean-field compartmental model (Swinton et al. 1998; Fulford et al. 2002; Keeling and Rohani 2008). Although many modeling studies have concluded that persistence increases with the intergroup connectivity, Jesse et al. (2008) demonstrated that the relationship is strongly nonlinear. More specifically, a peak in epidemic duration appears at small movement rates (due to asynchrony in the disease dynamics between subpopulations), followed by a global maximum at larger rates (when the pathogen "perceives" the metapopulation as a single well-mixed population). These results are particularly applicable to outbreak management because they demonstrated that while restricting animal movement (a common response to disease outbreaks in cattle farms) will reduce the total *number* of subpopulations that experience infection, it may also prolong epidemic duration instead of decreasing it. At these lower movement rates, any infectious animal that does move between subpopulations has a large probability of encountering a fully susceptible subpopulation, thereby preventing the disease from dying out for an increased length of time (similar to the rescue effect in ecology). Another important conclusion is that although increasing mixing between subpopulations via habitat corridors is an effective means of species conservation (Hanski 1999), their potential as conduits for the spread of infectious diseases should be carefully considered before implementation (Hess 1994, 1996).

Metapopulation models have also been used to evaluate the benefits of different control strategies that take into account the local nature of spatial transmission. Beyer et al. (2012), for example, demonstrated that rabies fade-out in Tanzania became increasingly more likely once vaccination reduced the number of susceptible dogs in each village subpopulation below 150. Fulford et al. (2002) used a similar

approach to show that for subpopulations that occur in a chain configuration (e.g., along rivers or roads), limited culling applied to alternate patches was significantly more effective in reducing disease prevalence than extensive culling applied in large batches along the chain.

Apart from the fact that metapopulation models still assume homogeneous mixing (although at the intra-subpopulation level), their main limitation is that they require detailed information on the number of individuals in each subpopulation, which can be difficult to collect and computationally intensive to explicitly model (Keeling and Rohani 2008). An alternative approach, originally proposed by Levins (1969), models each subpopulation as being either pathologically empty (disease-free) or occupied (having infection). The intuitive way to conceptualize this is to assume that localized extinctions and successful recolonizations are extremely rare, so that each subpopulation spends the vast majority of its time either disease-free or close to the endemic equilibrium. For a large number of subpopulations with equal probabilities of infecting each other, the proportion of infected subpopulations, $P$, is governed by

$$\frac{dP}{dt} = r(1 - P)P - eP, \tag{7.10}$$

where $r$ measures the reinfection (coupling) rate from an infected subpopulation to an uninfected one and $e$ is the rate of local extinction (Keeling and Rohani 2008). Although this approach has been used much less extensively than the previously described standard approach, it has nonetheless been used to derive some interesting results [e.g., see Smith et al. (2002) below].

### 7.3.2 *Specific Applications*

Plowright et al. (2011) created a metapopulation model to provide a mechanistic understanding of the causal links between anthropogenic change and emergence of new zoonoses from wildlife in Australia. As urbanization destroyed most of the contiguous forest cover on the eastern coast, fruit bats (*Pteropus* spp.) began switching their diet from the more patchily distributed nectar and fruit sources to urban gardens that provide abundant food year-round. Unlike the old diet that required long-distance foraging and migration, the new one required minimal migration, which decreased the connectivity between subpopulations (identified by the location of their daytime roosts). By incorporating data on within- and between-camp transmission, the authors demonstrated that the decreased migration led to increased epidemic sizes and divergence in both amplitude and frequency from epidemics in rural camps. This was due to the fact that as fewer individuals migrated, the probability of infected hosts moving between camps decreased, which lowered the probability of camps becoming reinfected after local viral extinction and increased the time over which subpopulations could recruit susceptible individuals via births. This resulting decline in herd immunity across the metapopulation shifted disease

dynamics toward sporadic, shorter, and more intense local epidemics with larger numbers of infected individuals. However, if this connectivity were to drop below a certain threshold level, then the resulting movement would become insufficient to reinfect distant camps. Thus, the same conditions that are currently responsible for large and sporadic epidemics could eventually lead to viral extinction, rendering the entire population vulnerable to a large synchronized outbreak (Plowright et al. 2011).

Another application of this modeling framework was recently employed by Pons-Salort et al. (2014) to explore the role of four different species of bats in the persistence of rabies across three cave-dwelling colonies on the Balearic Islands in Spain. By successively removing each of the four species from the transmission cycle, they were able to demonstrate that the common bent-wing bat (*Miniopterus schreibersii*) is the only species essential for the persistence of the virus, because it serves as a regional reservoir in the system. In fact, if this species were removed, then even increased contact rates and interisland exchanges between the remaining three species would be insufficient to maintain rabies infections. Additionally, Pons-Salort et al. (2014) demonstrated that contrary to expectation, the most abundant species of bat on the islands, the greater mouse-eared bat (*Myotis myotis*), plays a relatively minor role in the persistence of the virus.

A large wave of rabies infections began on the Virginia-West Virginia border in the mid-1970s and generated interest in how landscape structure (e.g., the location of roads, bodies of water, and human population centers) affects the spread of disease. Smith et al. (2002) used a Levins-type metapopulation model to study a small portion of this wave as it traveled through raccoons (*Procyon lotor*) in 169 townships across Connecticut from 1991 to 1996, concentrating on the underlying spatial heterogeneity of the habitat. A spatial SI model (used instead of an SIR because there is no recovery from infection at the township level) was compared to the observed data of reported rabies outbreaks and resulted in a best fit model where rivers reduced transmission by 87% compared to land boundaries; local transmission accounted for most but not all of the spatial spread; and human population density played a small but positive role in transmission (Smith et al. 2002). Although this formulation ignored within-subpopulation dynamics (i.e., a newly infected township can transmit infection as strongly as when endemic equilibrium is reached), it allowed for far richer parameterization without the need to estimate the number of raccoons in each township.

### *7.3.3 Future Directions*

As illustrated above, metapopulation models have provided important insights into the complexities and spatial variation observed in disease prevalence and outbreaks. Despite the progress that has been made in the development and application of these models, however, there are a number of limitations that still require improvement.

For example, there is substantial interest in devising a practical framework for ascertaining the optimum timing and duration of vaccination pulses to promote the extinction of a pathogen at the population level. Although theoretical research has

demonstrated that pulsed vaccination has the ability to synchronize epidemic behavior and thereby limit pathogen rescue effects, understanding how this benefit interacts with the resulting buildup of susceptibles between pulses can have enormous public and animal health consequences (Keeling and Rohani 2008).

Another exciting avenue for future research is to develop a method for using data from the first phase of an epidemic to accurately parameterize all aspects of a metapopulation model (Ball et al. 2015). Unfortunately, data collected during this phase are prone to a number of biases that are not yet fully understood (e.g., underestimation of fatality rates due to the time delay from disease onset to death; Nishiura et al. 2009). Overcoming these challenges could greatly improve the capability of metapopulation models to accurately predict the likely progression of an outbreak and the efficacy of different control strategies.

Finally, several studies have noted that assuming homogeneous mixing within subpopulations tends to overestimate the spatial spread, time to peak incidence, and the peak number of cases of epidemics when compared to more refined models (Ajelli et al. 2010; Keeling et al. 2010). Identifying situations where these differences are most prominent and devising methods to adjust model predictions accordingly still remains a major challenge in metapopulation modeling theory (Ball et al. 2015).

## 7.4 Spatial Models

Spatial models subdivide the area occupied by the host species into a grid and thus provide a way to model the spatial structure of a population without dividing it into distinct social groups. The most common approach is to model interactions as occurring only between individuals occupying either the same or neighboring grid cells while allowing individuals to move between them. These spatial lattice-based models can be thought of as a special case of metapopulation models (where each cell represents its own subpopulation) and as such are governed by a very similar set of equations:

$$\frac{dX_i}{dt} = \upsilon - \beta X_i \frac{\left(1 - \sum_j \rho_{ji}\right) Y_i + \sum_j \rho_{ij} Y_j}{\left(1 - \sum_j \rho_{ji}\right) N_i + \sum_j \rho_{ij} N_j} - \mu X_i, \tag{7.11}$$

$$\frac{dY_i}{dt} = \beta X_i \frac{\left(1 - \sum_j \rho_{ji}\right) Y_i + \sum_j \rho_{ij} Y_j}{\left(1 - \sum_j \rho_{ji}\right) N_i + \sum_j \rho_{ij} N_j} - \gamma Y_i - \mu Y_i, \tag{7.12}$$

$$\frac{dZ_i}{dt} = \gamma Y_i - \mu Z_i, \tag{7.13}$$

where $\rho_{ij}$ is equal to one if $i$ and $j$ are neighbors and zero otherwise.

An alternative approach is to treat each grid cell as being large enough to only contain a single individual (i.e., a subpopulation of one). This results in a finite number of states for these subpopulations, wherein each cell is either empty or

occupied by a susceptible, infectious, or recovered individual (Keeling and Rohani 2008; Miksch et al. 2013). This formulation follows a fire ecology framework originally developed by Bak et al. (1990), where each cell was either empty or occupied by a healthy or burning tree (reflecting a recovered, susceptible, and infectious host, respectively). Burned trees that died left empty spaces that could then be colonized (reflecting recovery and births), and fire (reflecting infection) could spread between neighboring trees. Although this framework makes a number of simplifying assumptions (e.g., trees do not always die from fire), it has nonetheless been used as the foundation for some interesting spatial models of infectious diseases [e.g., see Tischendorf et al. (1998) below].

### *7.4.1 Uses and Limitations*

Like all mathematical models, lattice-based spatial models are a clear abstraction of reality because, except for agricultural settings such as orchards (e.g., Gibson 1997; Poggi et al. 2013), individuals do not exist on a regular grid. Thus, spatial models are mainly used to investigate how the effects of spatial separation and nonrandom mixing cause disease dynamics to deviate from mean-field approximations in a spatial framework. For example, these models have demonstrated that an invading infection is characterized by a clear wave-like spread because the disease must first spread to neighboring sites before reaching the rest of the population. Similar to metapopulation models, spatial models have also been used to evaluate the broad-scale consequences of different control efforts including vaccination (e.g., Tischendorf et al. 1998) and culling (e.g., White and Harris 1995; Deal et al. 2004).

Although spatial models are a clear abstraction of the spatial structure of most host populations, certain types of wildlife populations actually lend themselves very naturally to be modeled using such an approach. Populations that occur in either a one-dimensional chain (e.g., along a river) or loop (e.g., surrounding an impassable urban center) can be thought of as having only nearest-neighbor connectivity and as such can easily be modeled using a lattice-based approach.

Despite these uses, the separation of populations into a lattice framework has several drawbacks. Imposing a square grid onto a habitat, for example, forces interactions in particular directions (e.g., north, south, east, and west) to play a disproportionately larger role in disease transmission than interactions in other directions. The use of a hexagonal grid, however, helps to alleviate this problem and often better recreates observed spatial patterns (Anneville et al. 1998; Van Baalen and Rand 1998; Kao 2003; Birch et al. 2007). A more general issue is that the discretization of space forces intracell interactions to be far stronger than intercell ones, regardless of the actual distance between individuals in each cell. Two individuals that occupy adjacent corners of two separate cells, for example, would have weaker interactions than two who occupy the same cell but are at opposite ends, despite the fact that the former are closer to each other than the latter (Keeling and Rohani 2008).

Another disadvantage of this lattice-based approach is that dividing the space occupied by a population into discrete cells limits how precisely we can model the location of each individual (i.e., we only know which cell an individual is in but not where inside the cell they are). An alternative approach is to treat this space as continuous, which allows us to model the *exact* location of each individual in the population. This approach is essentially an extension of the lattice-based framework, where the size of each cell has become infinitely small, and is the foundation of reaction-diffusion models (Keeling and Rohani 2008). The standard reaction-diffusion model assumes that individuals move randomly throughout the landscape and that infections are only transmitted between individuals sharing the same location (Källen et al. 1985; Murray et al. 1986; Yachi et al. 1989; Kawata 2010), resulting in the following set of equations:

$$\frac{\partial X}{\partial t} = \upsilon - \beta XY/N - \mu X + D_X \nabla^2 \mathrm{X}, \tag{7.14}$$

$$\frac{\partial Y}{\partial t} = \beta XY/N - \gamma Y - \mu Y + D_Y \nabla^2 \mathrm{Y}, \tag{7.15}$$

$$\frac{\partial Z}{\partial t} = \gamma Y - \mu Z + D_Z \nabla^2 \mathrm{Z}, \tag{7.16}$$

where $X$, $Y$, and $Z$ are now functions of both space and time, $\nabla^2$ describes the local diffusion of individuals through space, and $D_X$, $D_Y$, and $D_Z$, are the diffusion rates for the three classes of individuals (Keeling and Rohani 2008; Kawata 2010).

An extension of this approach is to incorporate a transmission kernel that models how transmission risk decreases with distance. This kernel acts to relax the assumption that transmission is solely a localized process, allowing individuals not in the immediate vicinity of an infectious individual to still become infected, albeit at a significantly lower rate (Schofield 2002; Keeling and Rohani 2008). Because reaction-diffusion models provide a very broad-scale view of disease outbreaks, their application has mostly been limited to investigating either epidemics that span large spatial scales (Conner and Miller 2004) or the presence and role of rare but long-range disease transmission (e.g., dispersing animals) in accelerating the spatial diffusion of a newly introduced pathogen (Schofield 2002; Keeling and Rohani 2008).

### *7.4.2 Specific Applications*

In 1997, a large-scale and long-term immunization of European foxes against rabies was under consideration to be either terminated or cut back due to diminishing returns and despite lasting sporadic incidences. Tischendorf et al. (1998) used a spatial model to investigate the effectiveness of this program and assess the consequences of either reducing or eliminating it on the persistence of rabies. This approach involved simulating the mating, dispersal, and vaccination of fox social groups across a lattice grid, as well as the contact between and within groups. Unlike the traditional approach, each grid was able to hold one of six possible states—the

three "simple" states of infectious, empty, and susceptible and three new "mixed" states of susceptible plus immune, infectious plus immune, and empty plus immune (meaning that only immune foxes are present). The results of this model showed that even in a highly immunized fox population such as the one throughout Europe, rabies can still persist in the form of moving clusters of infection. The probability of disease eradication rises sharply after a mean immunization rate of 70% is reached, although a further 6 years of maintaining this level was required to guarantee rabies eradication (Tischendorf et al. 1998).

In South West England, efforts to eradicate bovine tuberculosis from cattle were complicated by the presence of a wildlife reservoir for the disease in European badgers (*Meles meles*). White and Harris (1995) developed a stochastic lattice-based model that incorporated density-dependent fecundity and cub survival and used badger social groups as the basic unit of measurement. The major result of this model showed that although bovine tuberculosis could persist for a long time in populations with a disease-free equilibrium group size of only four individuals, a group size threshold of six was required for the disease to become endemic. Additionally, increasing intergroup contact rates significantly improved the probability and rate of spread of infection and lowered the group size threshold required for endemicity. As such, perturbations of badger social groups caused by control operations could actually increase the probability of persistence (or spread) of an infection instead of preventing it (White and Harris 1995).

In 1977, a single rabies-positive raccoon was identified in Pennsylvania and determined to be a by-product of a restocking program that transported raccoons from Florida to Virginia and West Virginia in the mid-1970s. By 1996, the rabies virus spread to almost all counties in Pennsylvania, resulting in 3912 confirmed cases of infected raccoons and a further 2137 cases in other animals. Moore (1999) used the timing of first cases in each county to construct a reaction-diffusion model for the entire state and explore what factors influenced the speed and direction of the spread. The resulting contours revealed that the infection first spread northward along the corridors of the Appalachian Mountain and Great Valley sections of the state, before twisting to the west once it reached the high plateau areas in the north. These results differed from the homogeneous point diffusion process originally proposed for the rabies outbreak and were used to inform a strategic oral-bait vaccination strategy (Moore 1999).

Sayers et al. (1985) used a similar approach to investigate how geographic features influenced the diffusion of fox rabies through cities in Germany. The derived pathogen velocity vectors demonstrated that propagation was fastest along a broad range of limestone with elevations up to 600 m and diverse land cover and vegetation. These results were later used by Källen et al. (1985) to design a deterministic model for the spread of this epizootic front westward across Europe that helped identify the width of a control barrier that would prevent the disease from entering the rabies-free United Kingdom. This model predicted a front wave speed of 50 km year$^{-1}$, consistent with empirical data from the German-Polish border, and resulted in an estimated barrier width of 15 km (Källen et al. 1985). This westward spread was eventually halted through the delivery of a highly effective oral vaccine (Jackson and Wunner 2002).

### 7.4.3 Future Directions

As illustrated above, the use of spatial models has provided managers with not only the tools to evaluate different control strategies but also with explanations for observed disease distributions. However, there are still many interesting aspects of spatial disease models that warrant additional research.

For example, there is interest in exploring how spatial structure influences basic epidemiological characteristics such as $R_0$ and the epidemic threshold (Riley et al. 2015). Although calculating these values is relatively simple in models that exhibit homogeneous mixing (i.e., compartmental and metapopulation models), the clumping of individuals in spatial models substantially complicates the calculations. Mollison and Kuulasmaa (1985), for example, demonstrated that the epidemic threshold (i.e., the value of $R_0$ required for the disease to spread through the population) for a nearest-neighbor lattice-based model is between 2 and 2.4, compared to the usual value of 1. A better understanding of how clumping and other spatial characteristics of a population influence this and other aspects of disease spread can greatly improve the use of spatial models.

Another exciting avenue for future research is to investigate the effects of spatial and temporal resolution on the accuracy of spatial models. Although it may be tempting, for example, to select the finest scale possible based on the available data, it may be more helpful to model the spread of the disease at the same scale as that used for control and management (e.g., counties or townships; Riley et al. 2015). Finer temporal scales may also be tempting, but require substantially more data on all aspects of animal behavior that could contribute to disease spread. Kjaer (2010), for example, used a 2-hour time step to model the spread of chronic wasting disease in a hypothetical population of white-tailed deer (*Odocoileus virginianus*). Berg (2016), on the other hand, modeled a similar system using a 1-week time step. Although this later approach allowed for far richer parameterization of the progress of the disease in individual animals, it came at the expense of being unable to include events that occurred at time intervals shorter than 1 week (e.g., occasional daylong excursions made by deer outside of their home range). Developing generalities about how different spatial and temporal scales influence the modeled spread of a disease can greatly improve the use of spatial models in the management and control of wildlife diseases.

## 7.5 Network Models

Although many infectious disease models assume random mixing either within the host population as a whole or within each subpopulation or grid cell separately, the number of contacts each individual has in a real population is significantly smaller than the (sub)population size and is highly heterogeneous between individuals. The contact network approach, originally developed for application in statistical physics,

provides another means of studying disease dynamics in these heterogeneous populations (Keeling and Eames 2005; Keeling and Rohani 2008; Craft and Caillaud 2011). In this framework, individuals (or groups of individuals) are represented as nodes, and connections between them are referred to as edges. These edges can represent transmission events, contact through which infections could spread, and even movement of animals between groups (Pellis et al. 2015), and can be either undirected (for infections that can pass in both directions with equal probability) or directed (for infections that can pass better in one direction than the other; Keeling and Eames 2005). These connections may also be either binary (representing whether any contact has occurred or not) or weighted (representing the duration or frequency of contact) depending on the host-pathogen system of interest (Godfrey 2013).

The simplest way to represent a contact network is to construct a $N \times N$ adjacency matrix, $\boldsymbol{G}$, where $N$ is the number of individuals in the study population and $\boldsymbol{G_{ij}}$ represents either the presence, duration, or frequency of contact between individual $i$ and individual $j$. Although this matrix is usually symmetric (i.e., $\boldsymbol{G_{ij}} = \boldsymbol{G_{ji}}$), directed networks yield nonsymmetric matrices (i.e., $\boldsymbol{G_{ij}} \neq \boldsymbol{G_{ji}}$; Keeling and Eames 2005). A number of useful quantities have been derived using adjacency matrices to describe how connections within a network are structured. These include individual-level metrics such as degree (number of edges connected to a single node) and centrality (how important and influential a node is within the network; White et al. 2015), alongside population-level metrics such as clustering coefficient (the extent to which an individual's neighbors are connected to each other; Godfrey 2013) and "small-world-ness" (i.e., the interaction of local clustering and average path length; Humphries and Gurney 2008).

Once the appropriate contact network and associated adjacency matrix are constructed, transitions between disease states are evaluated in discrete time steps (e.g., once every 3 months) on an individual basis using predetermined probabilities (e.g., a susceptible individual who is in contact with an infectious one has a 0.4 probability of becoming infected, while the infectious individual has a 0.2 probability of recovering; Keeling and Rohani 2008). This framework is able to capture complex individual-level structure and heterogeneity in a relatively simple manner and as such has been used not only for animal diseases but also for a variety of human ones such as HIV (Sloot et al. 2008) and SARS (Small and Tse 2005). Network models have also been used in other disciplines, such as sociology, to measure actor prestige (Korfiatis and Sicilia 2007) and to investigate dynamics of rumor spreading (Moreno et al. 2004).

### *7.5.1 Uses and Limitation*

One of the most common uses of network models is to investigate the role of degree heterogeneity and well-connected individuals (i.e., super-spreaders) in driving disease dynamics (Böhm et al. 2009; Hamede et al. 2012; White et al. 2015). For

example, because these super-spreaders can have a disproportionately high impact on the basic reproductive ratio and the epidemic threshold, network models are often used to evaluate how either vaccinating or quarantining super-spreaders will influence disease spread (Craft and Caillaud 2011; Tompkins et al. 2011). Normally, such well-connected individuals would be considered to be at highest risk of infection (Godfrey 2013). However, while this relationship does exist for many wildlife populations [e.g., brushtail possums (*Trichosurus vulpecula*); Corner et al. 2003], it appears to be absent in others [e.g., meerkats (*Suricata suricatta*); Drewe 2009]. It is also important to remember that although super-spreaders have been identified in many wildlife populations, they appear to be absent in others [e.g., Tasmanian devils (*Sarcophilus harrisii*); Hamede et al. 2009].

A general trend that has emerged from analyzing network models is that high variation in the degree distribution tends to lower the epidemic threshold and increase the basic reproductive ratio when compared to mean-field compartmental models that use the average degree as a proxy for transmission rates (Bansal et al. 2007). Although several researchers have proposed methods to account for this heterogeneity in mean-field models (e.g., Anderson et al. 1986; Newman 2002), their explicit inclusion via network modeling still provides the best predictor of disease spread (Hamede et al. 2012).

Comparing alternative networks, on the other hand, has been used to gain insights into the importance of different types of animal behavior in disease transmission and to evaluate support for alternative working hypotheses (Godfrey 2013). Drewe (2009), for example, developed separate networks for grooming and aggressive interactions in meerkats and found that not only are the two highly directional (e.g., groomers had a higher risk of tuberculosis infection than groomees), but that grooming was the most important type of social interaction. Fenner et al. (2011) used a similar approach to demonstrate that connectivity to dispersers was more important than connectivity to resident individuals in predicting nematode loads in pygmy blue-tongue lizards (*Tiliqua adelaidensis*).

Networks have also been used to show that group size alone does not necessarily reflect transmission rates. For example, there appears to be no relationship between group size and risk of infection in gidgee skinks (*Egernia stokesii*; Godfrey et al. 2009). Larger primate groups, on the other hand, offset the increased parasite risk associated with group size by imposing within-group substructure (Griffin and Nunn 2011). Network models have been used to show that social interactions are often more important in disease spread than either spatial proximity or home-range overlap (Bull et al. 2012), address the issue of using different spatial scales (Davis et al. 2008), and investigate if density influences contact rates (Ji et al. 2005). Alongside these more specific applications, network models have also been used in much the same way as earlier model types—to evaluate a wide range of disease control measures such as oral vaccines (Delahay et al. 2009) and culling (Ramsey et al. 2002).

As has been illustrated above, one of the main advantages of network models is their versatility, allowing them to be applied to most host-pathogen systems (e.g., both directly and indirectly transmitted viruses, bacteria, and parasites; Godfrey 2013). In

fact, they can be parameterized to recreate almost any other model type (e.g., a compartmental model is simply a fully connected network, while a lattice-based model can be replaced by a lattice network with nearest-neighbor connectivity; Craft and Caillaud 2011). The main limitation of these models, however, is that accurate parameterization requires detailed data on the contact and movement patterns of every individual within the study population at a time scale appropriate for the transmission process (Godfrey 2013). Although ongoing advances in GPS and proximity sensor technology are helping to alleviate this problem (e.g., Leu et al. 2010; Hamede et al. 2012; Reynolds et al. 2015), accurate sampling of every individual is still neither financially nor logistically feasible except for the smallest of wildlife populations (Tompkins et al. 2011; Godfrey 2013). Because of this, most studies (e.g., Craft et al. 2010; Hamede et al. 2012; Reynolds et al. 2015) use a "representative" subset of the population to generate contact networks that recreate observed measures of connectivity (e.g., average number of contacts, degree distribution, or amount of clustering).

Another challenge in using contact networks is defining what constitutes a transmissible contact (e.g., what proximity or duration is required to transmit an aerosol pathogen; Craft and Caillaud 2011; Tompkins et al. 2011). Because it is often impossible to determine exactly how transmission occurs without resorting to controlled transmission experiments that are often infeasible, most studies use either spatial proximity (e.g., Fenner et al. 2011) or home-range overlap (e.g., Godfrey et al. 2010) as proxies of contact and transmissible events. Additionally, wildlife networks often, if not always, exhibit temporal variation (e.g., higher contact rates while females are in estrous; Rushmore et al. 2013) that can drastically alter disease dynamics, requiring long-term observations for accurate parameterization (Volz and Meyers 2007, 2009; Hamede et al. 2012).

### *7.5.2 Specific Applications*

Although most contact networks are extrapolated from a supposedly representative subset of the study population, certain situations (e.g., endangered or very large animals) may still allow researchers to explicitly capture the full network of contacts. Leu et al. (2010), for example, constructed a directed transmission network for a subpopulation of sleepy lizards (*Tiliqua rugosa*) to determine whether asynchronous use of overnight refuges (a form of indirect and directional transmissible contact) could account for observed ectoparasite loads in individual lizards. Simulating disease spread on this network provided a powerful predictor of ectoparasite loads and revealed that individuals who use many refuges had significantly lower loads as a result of a lower probability of using a previously occupied refuge. This study helped to not only identify increasing the number of available refuges as the optimum strategy for reducing disease in sleepy lizards but also provided one of the first frameworks for using contact networks to investigate the dynamics of indirectly transmitted diseases (Leu et al. 2010).

Network models have been used to demonstrate that heterogeneity in either the connectivity or degree of individuals can have important consequences for the epidemic threshold and rate of spread of a disease. However, it is important to realize that a population's contact structure often operates on multiple hierarchical scales (i.e., individuals form groups that exhibit their own heterogeneous contact network; Craft and Caillaud 2011). Caillaud et al. (2013) constructed an undirected, two-level (i.e., individuals interacting within groups alongside intergroup connectivity) hierarchical model for a hypothetical infectious disease to demonstrate that variation in group size significantly reduces the epidemic thresholds, as well as increases the mean and variance of small outbreak sizes. This suggests that incorporating group size heterogeneity into disease models can greatly improve their predictive capabilities, much like the incorporation of degree heterogeneity described above. The authors also introduced the concept of an epidemiological effective group size, defined as the group size in a homogeneous population that would result in the same epidemic thresholds as the heterogeneous one. By applying this concept to lions (*Panthera leo*) in the Serengeti National Park, Caillaud et al. (2013) demonstrated that the observed network, which exhibited highly variable pride sizes, is comparable to a homogeneous population with approximately 10–20% larger prides.

Recognizing the importance of temporal variation in contact structure, Hamede et al. (2012) used proximity-sensing radio collars to derive seasonal patterns of contact in a distinct population of Tasmanian devils. These patterns were then used to generate sets of contact networks with suitable characteristics (e.g., mean degree and transitivity) that regenerated associations once every 3 months. By stochastically simulating the spread of devil facial tumor disease through these dynamic contact networks, the authors demonstrated that failure to account for seasonal contact structure significantly overestimated the time to host extinction as well as the transmissibility threshold necessary for an epidemic to occur. Interestingly, the differences in time to host extinction between network and compartmental models became negligible for transmission rates close to either zero or one, suggesting that contact heterogeneity has little effect on the dynamics of host extinction from diseases with either very low or very high transmissibility. Unfortunately, because transmission rates are likely to be intermediate for most wildlife diseases, ignoring contact structure can have negative consequences for wildlife conservation (Hamede et al. 2012).

### *7.5.3 Future Directions*

As illustrated above, the use of network models to study the patterns of and driving forces behind disease spread has been growing in the wildlife literature despite many challenges (Craft 2015). As technological advances continue to lessen these challenges, network models will undoubtedly provide the means to investigate many new facets of disease ecology.

For example, Pellis et al. (2015) suggest investigating how specific network characteristics influence the spread of an infectious disease through a system. Previous research has demonstrated the effect of degree and group size heterogeneity on the epidemic threshold and basic reproductive ratio (Hamede et al. 2012; Caillaud et al. 2013). Similar research is needed to investigate other effects such as the impact of clustering and degree correlation on $R_0$ and the likely size and duration of an outbreak. The role of the distribution and correlation of weights in weighted networks deserves similar attention. A better understanding of the epidemiological consequences of these and other characteristics can greatly improve the use of network models in controlling the spread of infectious diseases (Pellis et al. 2015).

Another exciting avenue for future research is to consider how infections themselves alter the topography and characteristics of a network. Croft et al. (2011), for example, found that the presence of infection in female guppies (*Poecilia reticulata*) decreased the duration of contact between individuals and significantly lowered the clustering of the network. Although this study demonstrated a mechanism by which individuals modified their contact structure in a way that depressed the spread of a disease, other infections may instead alter behavior and the corresponding network in a way that promotes disease spread. Identifying and understanding the consequences of these alterations to the likely spread of a disease is an important step in improving the predictive capabilities of network models.

There is also a need to develop more tractable and realistic methods of incorporating the dynamic nature of real-life contact structures into network models (Pellis et al. 2015). The presence, strength, and direction of contacts between individual animals are neither stagnant nor permanent, but instead change over time due to a variety of intrinsic and extrinsic factors (e.g., resource abundance, migration, or injury). Properly modeling the formation and dissolution of these contacts remains a major challenge in accurately parameterizing dynamic network models of wild animal populations.

Finally, network models can be used to explore how infections have influenced the evolution of social organization in wild animals (Godfrey 2013). One hypothesized cost of this evolution is the increased risk of infection caused by higher contact rates and local population densities (Moller et al. 1993; Altizer et al. 2003). Using network models to investigate how these costs are offset by social behavior (e.g., the use of alternate sleeping or roosting sites) can greatly improve our understanding of social structures and the evolution of certain behaviors in a variety of animals (Godfrey 2013).

## 7.6 Conclusion

The use of epidemiological models in the study of wildlife diseases began in the early 1980s and has since then grown to be a major component of the wildlife disease literature. Although mean-field compartmental models laid much of the foundation for current epidemiological practices (e.g., the basic reproductive ratio and

vaccination threshold), their inability to capture spatial dynamics led to the use of metapopulation and spatial models (Keeling and Rohani 2008; Kawata 2010). More recently, the use of contact networks has emerged as an intuitive and versatile means of studying disease dynamics. As ongoing advances in GPS and proximity sensor technology make it more feasible to capture the level of data required to more accurately parameterize contact structure, network modeling will continue to provide useful insights into infectious diseases of wild animals (Keeling and Eames 2005; Tompkins et al. 2011; Godfrey 2013).

Although much research has evaluated the uses, limitations, and dynamics of different infectious disease models, many potential avenues for future research still exist for each one. For example, although previous research using compartmental models has assessed the role of birth and transmission seasonality in producing annual, semiannual, and even chaotic patterns of disease prevalence (Ireland et al. 2004; Hosseini et al. 2004), little research investigating the role of seasonal patterns of mortality has been undertaken. The development of generalities about what ecological variables (e.g., resource or predator distribution) govern the establishment of contact structures is also of high priority (Craft and Caillaud 2011; Tompkins et al. 2011).

Several more general concerns also exist. For example, there is a substantial need to improve the analytical and computational tools available to infectious disease modelers; emerging methods developed for the analysis of "big data" are particularly intriguing for discerning the contact structure within populations of wild animals (Pellis et al. 2015). These tools include accurate and computationally efficient methods of calculating the basic reproductive ratio, epidemic threshold, and other epidemiologically important quantities from complex model structures (Ball et al. 2015). Identifying ways to combine two or more of the modeling approaches discussed in this chapter (Ball et al. 2015; Pellis et al. 2015), or to combine these models with other tools such as pathogen phylogenies (Frost et al. 2015), to provide a more realistic representation of the complexities involved in the spread of infectious diseases also deserves additional attention.

The ultimate goal of epidemiological models such as those discussed in this chapter is to allow scientists to understand the mechanisms behind and predict the future course of the spread of infectious diseases. As the use and advancement of these models continue, they will undoubtedly continue to aid efforts to minimize risks to human health, decrease loss of livestock, and preserve native and endangered species of wildlife.

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**Ethical Approval** This article does not contain any studies with human participants or animals performed by any of the authors.

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# Chapter 8
# The Ecology of Pathogen Spillover and Disease Emergence at the Human-Wildlife-Environment Interface

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**Abstract** Novel diseases are increasingly emerging into human populations through the complex—and often, unseen—stepwise process of spillover from a combination of wildlife, livestock, vectors, and the abiotic environment. Characterizing and modeling the spillover interface are a key part of how eco-epidemiologists respond to the growing global burden of emerging infectious diseases; but the diversity of pathogen life cycles and transmission modes poses a complex challenge for ecologists and clinicians alike. We review our current understanding of the spillover process and present a framework that relates spillover rates and human-to-human transmissibility to the basic reproduction number ($R_0$). Using pathogens that exemplify important transmission pathways (anthrax, Ebola, influenza, and Zika), we illustrate key aspects of the spillover interface and discuss implications to public health and management of emerging infectious disease.

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## 8.1 Introduction

The spread of environmental or animal pathogens into human populations is a natural and perpetual process that dates back as far as human records extend. One of the earliest recorded epidemics, the plague of Athens (also called the Thucydides syndrome), took place between 430 and 425 B.C.E. and is believed to have been an outbreak of Ebola virus (Olson et al. 1996) or typhoid fever (Papagrigorakis et al. 2006). Measles emerged in human populations after evolving from rinderpest, a disease of cattle, sometime around the eleventh or twelfth century, and perhaps the most famous disease outbreak in recorded history, the Black Death, was caused by plague (*Yersinia pestis*), a vector-borne zoonosis. In this sense, the emergence of diseases is a natural process, the by-product of pathogen "chatter" between susceptible host populations of different species, with the emergence of diseases into human populations only a small subset of the broader cross talk among mammals, and animals writ large.

This process of spillover is stochastic and, from the perspective of pathogen success, fairly imperfect. The majority of mammalian viruses lack the capacity to even infect humans, and the majority of spillover events that do occur fail to establish circulating infection in the new host. But despite the long and complex history of peoples and plagues, scientific consensus indicates that the rate of infectious disease emergence has been accelerating in more recent history. Differentiating the next pandemic from background noise is a nearly insurmountable challenge and one that is ongoing as new pathogens evolve or are discovered. As participants in clinically relevant research settings, the task of ecologists studying spillover revolves around three key issues:

1. Describing the process of spillover and interactions and state changes between hosts, pathogens, and the environment that lead to disease emergence
2. Developing predictive conceptual and quantitative models that can explain spillover patterns and forecast disease emergence, epidemic behavior, and/or intervention impact
3. Applying ecological and clinical knowledge to develop interventions that mitigate, control, and potentially prevent epidemics and pandemics

Each of these independently poses a substantial challenge. The diversity of pathogens, transmission modes, and eco-epidemiological interfaces further complicates the development of a conceptual framework that will adequately address any one of these challenges, let alone all three.

In this chapter, we review existing research on the spillover interface and the relevant ecological advances that have been made especially in the last two decades. We begin by reviewing the most recent work characterizing the process of pathogen emergence. Building on this, we present a conceptual framework that engages the stages of pathogen emergence as a dynamic continuum rather than a binary, or even linear, set of sequential steps. We use this framework to evaluate a number of pathogens that illustrate key features important to the spillover process. We conclude by discussing how this approach lends itself to a more quantitative and focused

assessment of spillover itself—the key element shaping pathogen emergence potential at the human-animal-environmental interface.

## 8.2 The Usual Suspects: What Makes an Emerging Zoonosis?

**Zoonotic disease** emergence arises as a consequence of transmission of a multi-host pathogen from an animal host (direct or indirect transmission) to a human, with or without onward human-to-human transmission and establishment (Antia et al. 2003). We define a **zoonotic reservoir** as one or more epidemiologically connected animal populations and/or environments where a pathogen can be maintained and transmitted to humans (Haydon et al. 2002) and, more broadly, domestic animals as well. Regardless of transmission mode, the process by which a pathogen moves from one host population (or environmental reservoir) to another host population is referred to as **spillover** and arises from complex bidirectional interactions among people, animals, pathogen communities, and environments. This is a key step in zoonotic disease emergence but remains poorly understood and often not explicitly quantified in transmission models (Lloyd-Smith et al. 2009; Iacono et al. 2016), which tend to focus on dynamics within a single population.

Some of the most significant quantitative work at the spillover interface has focused not on predicting rates of zoonotic spillover but on identifying and predicting the common features of spillover diseases and the pathways they tend to exploit. Viruses have been best studied in this context; basic rules of thumb are well established, such that single-stranded RNA viruses are predisposed to become zoonoses, likely due to a predisposition toward evolvability and host switching. Other rules have emerged from studying the networks of viral transmission between different host groups and humans. For example, a study by Johnson et al. (2015) demonstrated a number of key rules, perhaps most importantly that viruses with greater host plasticity in animal reservoirs are more likely to demonstrate human-to-human transmissibility. Some virus families, such as arenaviruses and filoviruses, are predisposed to human-to-human transmission. Correspondingly, some groups of mammals were especially common reservoirs of zoonotic viruses, including rodents (especially for arena- and bunyaviruses), primates (retroviruses), and bats (paramyxoviruses and rhabdoviruses). Pathways of spillover were also noted to differ, with bush meat hunting being a notable source of exposure to nonhuman primate viruses, while proximity of homes to fields was a significant driver for exposure to rodent-borne viruses (Dearing and Dizney 2010). Although sometimes not considered a "pathway of spillover," vectors can also act as a critical component in spillover dynamics with, for example, Johnson et al. showing that virus spillover from bird reservoirs was disproportionately vector borne.

A recent study by Olival et al. (2017) refined and elaborated these findings, confirming that RNA viruses are more commonly zoonotic and that the phylogenetic breadth of viral hosts is the strongest predictor of zoonotic potential (analogous to

Johnson's findings about viral plasticity). Olival et al. also confirmed that bat viruses were disproportionately zoonotic, an unsurprising finding given that many well-known viruses have originated from bat reservoirs [e.g., Ebola and Marburg viruses, Hendra and Nipah viruses, and even severe acute respiratory syndrome (SARS) and, likely, Middle East respiratory syndrome (MERS)]. Olival et al. confirmed that viruses with arthropod vectors have a greater plasticity in their mammalian hosts and showed that viral replication in the cytoplasm also predicts zoonotic potential. Finally, their study shows that human population density in host ranges and the phylogenetic relatedness of wildlife reservoirs to humans were important predictors of zoonotic emergence. These aspects are likely related to the evolutionary processes that occur at the spillover interface, where viruses coevolve with new hosts and incrementally develop greater emergence potential over time (which we discuss below).

Studies like Johnson's and Olival's are a key part of how disease ecologists consolidate information about the emergence of zoonotic diseases and reveal important information about the patterns—and, ideally, processes—that transpire at the spillover interface. Given the recent preponderance of devastating viral outbreaks, such as Ebola and Zika, it is unsurprising that so much attention has been paid to the factors that drive viral emergence. Comparatively less work has been done to consolidate these types of results for other major pathogen groups, like bacteria, protozoans, and fungi. While broad studies like these elucidate general patterns and help develop predictive tools for identifying the zoonotic potential of recently discovered pathogens, these studies do little to explain outbreak process and spillover dynamics for known zoonoses.

## 8.3 Characterizing Pathogen Spillover

The diversity of existing emerging pathogens can be overwhelming, with over 300 documented emerging infectious diseases occurring between 1940 and 2004 (Jones et al. 2008), and several more, which have since emerged. Greater still, it must be expected, is the diversity of potential zoonotic threats that have not yet emerged into human populations but could do so in the coming years as viruses, hosts, and landscapes experience accelerated change. Frameworks provide an opportunity to generalize patterns across pathogens, supporting model development and interventions that can be especially valuable for diagnosing epidemic profiles and responding during the early days of a pathogen's emergence. Several frameworks have been developed that organize and describe pathogens based on stages of emergence, but the spillover interface is still poorly captured for many diseases, and most frameworks have limited direct applicability to quantitative methods. In this section, we review existing frameworks and, building on this, present our own framework that is explicitly directed at modeling the unique mathematical behavior of pathogen spillover.

### 8.3.1 Existing Frameworks

In contemporary disease ecology literature, two major frameworks have been proposed that describe the stages of pathogen emergence. The first, and most commonly referenced, was proposed by Wolfe et al. (2007) and divides pathogen emergence into five progressive stages through which a pathogen may pass sequentially:

**Wolfe's Stages of Spillover**

I. Agent only in animals
II. Primary infection in humans
III. Limited outbreak in humans
IV. Long outbreak in humans
V. Exclusive human agent

In this classification scheme, zoonotic emergence occurs between Stages II and IV. In the most constrained case (Stage II), spillover only ever leads to primary infection in humans with no onward transmission within human populations. Whereas Stage III is characterized by short chains of transmission ("stuttering chains"), Stage IV features human-to-human sustained epidemic transmission. The line between the two is obviously not only subjective but may create difficulties when characterizing expected pathogen behavior. For example, outbreaks of Ebola virus beginning in 1976 were typically small and situated in rural areas where outbreaks could have been classified as Stage III. However, since 2014, Ebola has been considered a Stage IV zoonosis, and modeling work undertaken during the 2014–2015 Ebola epidemic in West Africa showed that "the same epidemiological conditions that were present in 1976 could have generated a large outbreak purely by chance" (Camacho et al. 2014). The line, therefore, between short-chain and epidemic transmission for a pathogen is sometimes unclear.

A second framework, proposed by Lloyd-Smith et al. (2009), refines the Wolfe classification scheme by more formally considering the mathematical definitions of the three intermediate categories:

II. Spillover into humans (no human-to-human transmission; $R_0 = 0$)
III. Stuttering transmission in humans ($R_0 \leq 1$)
IV. Long outbreak in humans ($R_0 > 1$)

Here, $R_0$ is defined as the number of secondary infections caused by a typical single infective individual in a wholly susceptible population during its period of infectiousness (Diekmann et al. 1990). Incorporating an explicitly quantitative aspect to the definition of the stages improves the framework in obvious ways and facilitates the more direct integration of epidemiological modeling and zoonosis

research. However, the same data limitations pose a problem for this framework, as a fairly newly emerged zoonosis is unlikely to have enough accompanying surveillance data to characterize its basic $R_0$, let alone elucidate situational variance due to socioeconomic and ecological factors. Later, we will discuss modeling approaches that can resolve some of these problems.

What do these frameworks contribute to disease ecology? At the most basic level, frameworks for classifying pathogens help us abstract and generalize patterns across hosts, pathogens, and landscapes. Furthermore, developing a more standardized language for describing spillover and disease emergence helps bridge the gap between ecology, infectious disease research, and clinical and public health. With the increasing burden of emerging infectious diseases and the accelerating rate of emergence of novel zoonoses, frameworks for classifying zoonotic disease emergence can help develop and refine prioritization schemes.

### *8.3.2 Advancing the Spillover Framework*

The past few decades of ecological research have shown that pathogen life history is far more flexible and dynamic than any linear set of steps characterizing emergence might capture. A framework oriented on a sequential progression can be useful for conceptualizing the emergence of some pathogens like measles or human immunodeficiency virus (HIV); but the diversity of emerging zoonoses contains many more life histories. Moreover, the development of a framework with an inherent directionality risks "adaptationism," misconstruing the random evolutionary trajectories of pathogens that include humans as a (non-special) part of a broader ecosystem including wildlife, vectors, livestock, and even the soil and water. To improve our understanding of spillover into human populations, we must first better conceptualize the complicated dynamics of interspecies spillover (human or not; Fig. 8.1).

As discussed previously, spillover and emergence of zoonotic pathogens have generally been represented within a framework that focuses on the objects of systems (hosts, pathogens, entities in the environment) (Daszak et al. 2000; Childs et al. 2007) rather than the interacting and cascading systems' processes themselves. It is the active steps of emergence (exposure, contact, invasion, and onward transmission), influenced by host-pathogen evolutionary processes, that ultimately determine the outcome of interactions among entities. In short, spillover and emergence are stochastic processes with outcomes that depend on the probabilities of the occurrence of underlying events. As we will make clear, the variability associated with spillover and emergence is large because the size of groups involved (vis-à-vis number of infected individuals) is small and hence subjects to the vagaries of sampling: it is only after sufficiently many of these events have been observed that we can begin to meaningfully characterize spillover and emergence in terms of expected rates of pathogen transmission. Using our framework, three key processes are delineated that determine (1) the occurrence of pathogen spillover from reservoirs either to an intermediary or directly to a focal host; (2) the occurrence of primary transmission from an intermediary to a focal host, when intermediary hosts

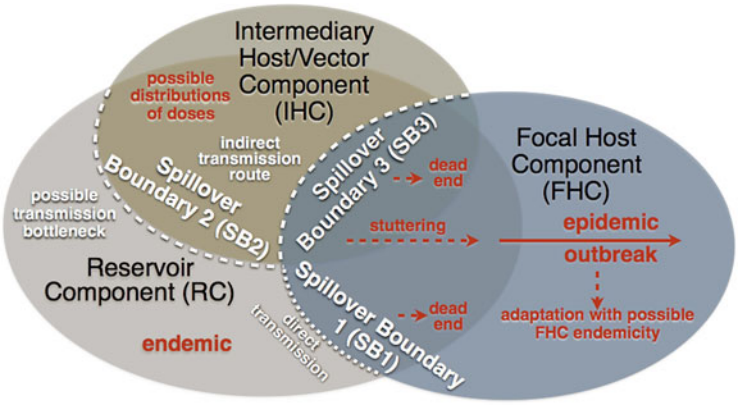


**Fig. 8.1** Conceptual model of the process of pathogen spillover and emergence. The ellipses represent three conceptual components: reservoir (RC, either environmental or wildlife populations serving as an ecological system in which the pathogen is endemically maintained), intermediary host/vector (IHC) population that serves to transfer the pathogen from the reservoir to the focal host, and focal host (FHC), generally human or species of special concern (e.g., domestic or highly threatened species). The dotted lines represent three spillover boundaries between RC-FHC (SB1 direct transmission route), RC-IHC (SB2), and IHC-FHC (SB3) (the concatenation of these latter two constitutes the indirect transmission route)

play a role (which is in most cases); and (3) the occurrence of secondary focal host-to-host transmission required for true disease emergence in the focal host. We use the term true emergence to contrast with the observation of multiple focal host cases of an infectious disease arising exclusively from spillover (Antia et al. 2003). Using emerging zoonotic disease examples, we explore this framework and provide recommendations for evaluating zoonotic outbreaks, managing interventions during outbreaks, and planning future research.

#### 8.3.2.1 Structural Elements

The model has three main compartments: a reservoir component (RC, self-sustaining or endemic source of pathogen that may be purely environmental or involve at least one host species as well), an intermediary host/vector component (IHC) where prevalence would drop to zero if the reservoir were removed (if this component is self-sustaining, then it just becomes part of the reservoir), and a focal host component (FHC) where epidemic outbreaks may occur and the potential for endemic establishment of the pathogen exists through evolutionary processes. Each of these compartments in the model is influenced by various factors and interactions (e.g., factors which influence the state of the host, pathogen, and environment and the resultant interactions; see Table 8.1 for examples), as well as the processes of source-host contact, intensity and duration of exposure, pathogen invasion potential for individual hosts (i.e., interactions between pathogens and the immune system), and onward transmission and adaptation within host populations. We find it insightful to

**Table 8.1** The disease emergence process is divided into four main steps. Examples of both factors and zoonotic pathogen systems are provided for each emergence step

| | Example | | |
|---|---|---|---|
| Process | Factor | Disease | Mechanism |
| Host exposure (spatial overlap between host and pathogen) | Host behavior | Chagas disease | Sedentary lifestyles and rearing of domestic animals in particular, such as the Brazilian guinea pigs (*Cavia aperea*), were important in creating favorable conditions for the domiciliation (successful home invasion) of the vector (Triatominae) (Alexander and McNutt 2010) |
| | Habitat alteration and fragmentation | *Borrelia burgdorferi*, Lyme disease | Deforestation and habitat fragmentation results in decreasing mammalian species diversity and increasing population densities of the reservoir with concomitant increased human exposure (Allan et al. 2003) |
| | Agricultural processes | *Cryptosporidium parvum* | Fecal contamination of water sources in agriculture production systems and food processing (Orlandi et al. 2002) |
| | Commercial resource use | Ebola | Logging of forests and exposure to virus, commercial sale of bush meat |
| | Climate | *Yersinia pestis*, plague | El Niño Southern Oscillation events increase the number of *Yersinia pestis* flea vectors and rodent host populations and are linked to prairie dog colony extinction events due to this pathogen (Stapp et al. 2004) |
| | Movement of infected hosts | *Trypanosoma brucei rhodesiense*, sleeping sickness | Movement of infected cattle is linked to an outbreak of human sleeping sickness in a previously unaffected area of Uganda (Fèvre et al. 2005) |
| | Seasonal dynamics | Lassa virus | Dry season shifts increases in rodent host densities in houses may increase the risk of contact with rodent host and excreta (Fichet-Calvet et al. 2007) |
| | Host density | Hantavirus | Increases in rodent populations and density are |

(continued)

**Table 8.1** (continued)

| Process | Example | | |
|---|---|---|---|
| | Factor | Disease | Mechanism |
| | | | associated with increased contact and pathogen spillover to humans (Wells et al. 1997) |
| | Disruption of reservoir host social structure | *Mycobacterium bovis*, bovine tuberculosis | Culling of badgers to control TB exposure induced badger long-distance movement and dispersal (Donnelly et al. 2003) |
| Contact (nature of contact with the pathogen will determine if pathogen can invade host) | Predation of infected hosts | Bluetongue virus | Bluetongue virus, a vector-borne pathogen, infects ruminants, shrews, and some rodent species. Ingestion of infected prey allows virus transmission to African predators without vector involvement (Alexander et al. 1994) |
| | Predation of infected hosts | African horse sickness virus | African horse sickness is a vector-borne disease that principle affects equids. Domestic dog and African predators are thought to be infected from ingestion of infected equid species (Alexander et al. 1995) |
| | Diet | Ebola | Hosts that eat certain fruits are brought together where transmission of the virus can occur from the bat reservoir through saliva contamination of fruit and ingestion by other species such as gorillas (Dobson 2005) |
| | Socioeconomic status of human communities | Leptospirosis | Poverty and compromised sanitation infrastructure increase contact with environmental sources of *Leptospira* (Reis et al. 2008) |
| | Pathogen release and change in immune protection in the human host | Monkeypox virus | Cross-immunity between smallpox and monkeypox virus and cessation of vaccination have allowed host immunologic release and emergence of monkeypox virus in the human host (Rimoin et al. 2010) |

(continued)

**Table 8.1** (continued)

| Process | Example | | |
|---|---|---|---|
| | Factor | Disease | Mechanism |
| Pathogen invasion and adaptation (pathogen enters and replicates in the susceptible host) | Virus evolution and strain variability | Influenza viruses | Influenza interspecies transmission is influenced by strain variability. New strains can evolve when two different viruses infect individual cells. Segments derived from each of the infecting "parents" may reassort and create a new strain with a changed host invasion potential (Webster et al. 1997) |
| | Host physiology, coinfection | *Cryptococcus neoformans* | Immunosuppression of the human host with HIV/AIDS can increase pathogen invasion risk (Chuck and Sande 1989) |
| | Coinfection can decrease macroparasite infestations | Nematode infections | The gastrointestinal nematode *Heligmosomoides polygyrus* decreases infestation of the tick *Ixodes ricinus* in free-living yellow-necked mice, *Apodemus flavicollis* (Ferrari, Cattadori et al. 2009) |
| | Tissue tropism | Hendra and Nipah virus | Viral transmission and spillover is influenced by the tissue tropism of the virus and access to the exterior of the host (Hooper et al. 2001; Childs 2004 |
| | Human behavior | Sudden acute respiratory syndrome (SARS) | Wet markets allow different hosts and viruses to have concentrated contact with each other and human, supporting viral change and host adaptation (Brown 2004) |
| | Mutation frequency, genetic diversity | Venezuelan equine encephalitis virus | RNA mutations allow production of amplification-competent (high equine viremia) viral strains, which can then invade the human host through vector-mediated transmission (Anishchenko et al. 2006) |
| | Virus evolution and strain variability | SARS | Genetic variations in critical genes, such as the *Spike* gene |

(continued)

**Table 8.1** (continued)

| Process | Example | | |
|---|---|---|---|
| | Factor | Disease | Mechanism |
| | | | (protein responsible for host cell receptor binding) influence pathogen invasion potential (Song et al. 2005) |
| | Human sexual behavior | HIV | Persons at an increased risk of transmitting or being infected by the HIV virus were more likely to practice unprotected sex (Halperin 1999) |
| Sustained onward transmission in new host(s) (secondary cases of human-human transmission) | Culture and human behavior | Ebola virus | Culturally driven practices such as ritual handwashing and sharing of communal meals at funerals of Ebola-infected individuals have been significantly associated with Ebola viral transmission (Griffin et al. 2003) |
| | Globalized travel and contact | SARS | Air travel and global traffic facilitate the spread of the pathogen (Ali and Keil 2006) |
| | Within human host adaptive mutation potential | SARS | Non-synonymous changes in the spike gene are found in viruses with sustained human-to-human transmission. These genetic changes are not found in viruses circulating in the reservoir host (palm civets) or spillover viruses that did not successfully move to the host epidemic space where sustained human-to-human transmission occurs (Pepin et al. 2010) |

consider pathogen spillover as typically involving four conceptually distinct, transmission processes: spillover from the reservoir component (RC) directly to the focal host component (FHC) (i.e., spillover boundary 1, SB1), spillover from the RC to the intermediary host/vector component (IHC) (i.e., spillover boundary 2, SB2), spillover from the IHC to the FHC (i.e., spillover boundary 3, SB3), and host-to-host transmission within the FHC itself.

Direct transmission across SB1 occurs between focal susceptible individuals and either individuals from reservoir species, the environment, or fomites (e.g., infected intermediary host fecal material or carcasses). Transmission across SB2 often presents an important bottleneck to pathogens ultimately entering FHC. Transmission

across SB3 is generally the focal spillover process, particularly those vectored by mosquitoes and parasites. Transmission across these spillover boundaries may either result in a dead-end infection of a focal host, a stuttering transmission chain in the focal host ($R_0 < 1$ in FHC), or an epidemic outbreak ($R_0$ necessarily $> 1$ in FHS). One or more such outbreaks may lead to adaptation and FHC endemicity.

Situations may exist where some focal hosts have been infected across the SB1 or SB3, resulting in mixed transmission. Emergence within the FHC (i.e., a zoonotic outbreak) requires that novel host(s) then transmit the pathogen to other members of the same species, with the degree of onward transmission determining the nature of the epidemic in the FHC. In some cases, pathogens may routinely spillover without resulting in any onward transmission in the FHC. In this case, the novel host is a dead end, and the epidemic is purely driven by spillover events. In humans, we see examples of this with anthrax and rabies. Once a spillover proceeds to include direct focal host-to-host transmission, if $R_0 < 1$, the process ends in a transmission chain that has a geometric distribution with an expected length of $(1 - 1/R_0)$ (distribution of chains that arise from several such transmission events is referred to as stuttering chains, Antia et al. 2003). On the other hand, if $R_0 > 1$, then the probability of an epidemic outbreak in FHC rises steeply with the value of $R_0$ (Antia et al. 2003).

Finally, it is worth stressing that the reservoir (RC) may itself be a vector-host system with another vector acting as the intermediary (i.e., IHC) between RC and the focal host (FHC). This is the case for West Nile virus (WNV) where RC is maintained by a passerine bird and *Culex* mosquitoes host-vector system but where *Aedes* mosquitoes are an intermediary host/vector component (Kilpatrick 2011; Petersen et al. 2013). Thus, when humans get bitten by WNV-infected *Culex* individuals, transmission can be regarded as direct (i.e., occurring across SB1); but when humans get bitten by WNV-infected *Aedes* individuals, transmission can be regarded as indirect with the pathogen needing to cross both SB2 and SB3 to be transferred from RC to FHC. The reason for this discussion relates to how we ultimately use our model to assess probabilities of outbreaks of zoonotic disease in humans and other focal species of interest.

#### 8.3.2.2 IHC Computational Elements

It is important to note that all models are abstractions. Deciding on the appropriate level of detail to include in a model depends on the questions to be addressed and an understanding of the processes needed in the model to adequately address these questions. In our framework, we assume that little is known about the structure and dynamics of the reservoir. Thus, we simply represent the reservoir in terms of a spatiotemporal risk-of-infection function $R(x, t)$, where $x$ is a point in space (typically 2D) and $t$ is a point in time. More ambitiously, we endeavor to represent the intermediary host in terms of both its population density $Y(x, t)$ and the prevalence of infection $IY(x, t)$ over space and time. Calculation of $PY(x, t)$ in terms of $R(x, t)$ and $Y(x, t)$ represents the real challenge, particularly if the impacts of various critical factors, as elaborated in the sections below, are to be incorporated. The primary focus is on the IHC where most of the interesting dynamics take place

prior to an outbreak within the FHC. Once an outbreak begins in the focal host population, then the emphasis switches to the dynamics of infection within the FHC. This will only progress beyond the dead-end or stuttering phases if the pathogen is sufficiently virulent within FHC to have an associated $R_0 > 1$.

As will become clear from our discussion, many different types of zoonotic systems exist, each requiring its own approach to the development of an appropriate model. Here, we focus on general concepts, as well as some novel elements that have not been introduced elsewhere in the literature. The essence of an IHC model is the intermediary population. Without going into detail regarding age, sex, or spatial structure, the dynamics of this population should minimally be described by a model that includes a demographic recruitment process (e.g., births and immigration), a natural mortality process, a pathogen exposure process, a disease class structure (e.g., susceptible S, infected but not yet infectious E, infectious I, recovered with some level of immunity R that may wane over time), a disease-induced mortality process, and a disease progression transfer matrix. The latter may be rather simple when considering transfers from E to I to R and possibly back to S, but the real challenge lies in modeling the transfer of individuals from class S to class E—i.e., the disease transmission process.

Disease transmission can be unpacked as a concatenation of (1) a source/susceptible host contact process that possibly includes notions of the intensity and duration of exposure to a dose of pathogen and (2) the probability of succumbing to infection given the characteristics of the contact. Contact itself requires an understanding of host behavioral and movement ecology, while dose-exposure computations require explicit characterization of the pathogen encounter risk distribution function $R(x, t)$ introduced above. Note that the movement of susceptible individuals in IHC may alter their movement behavior in response to the distribution of $R(x, t)$, as is the case for anthrax. Finally the probability of succumbing to infection given a contact ($C$) of intensity $w$ and duration $\tau$ will depend on the immunological state of the susceptible in IHC. If a susceptible is immunologically naive, then we would expect the probability of succumbing to be given by a function $C(w, \tau)$ that has the following properties: $C(0, \tau) = 0$ for all $\tau \geq 0$ and $C(w, 0) = 0$ for all $w \geq 0$ imply no dose and no transfer; $C(w, \tau) \geq 0$ is an increasing function of $w$ for fixed $\tau$ and of $\tau$ for fixed $w$ but is constrained to satisfy $C(w, \tau) \leq 1$ for all $w \geq 0$ and $\tau \geq 0$; and, most importantly, $C(w, k)$ is an increasing function of $w$ when $w\tau = k$. The latter implies that for a constant dose $k$, it is more contagious to be exposed to a higher dose rate (dose per unit time) for a shorter time than a lower dose rate for a longer time. Perhaps the simplest two-parameter function that satisfies these properties is

$$C(w, \tau) = \left(\frac{c_1 w\tau}{1 + c_1 w\tau}\right)\left(\frac{c_2 w}{1 + c_2 w}\right) \tag{8.1}$$

where $c1 > 0$ and $c2 > 0$ are the parameters in question. If a susceptible is not naive, then we can reduce the value of $C(w, \tau)$ accordingly. This function for $C$ is of course a model, and therefore an oversimplification (and should not be taken to suggest that low doses imply negligible exposure), but offers a useful conceptual framework and is a starting point for more complicated epidemiological models.

#### 8.3.2.3 FHC Empirical Elements

Susceptible individuals within FHC have three possible sources of infection: from RC across spillover boundary SB1, from IHC across spillover boundary SB3, and from host-to-host transmission within FHC. With recent advances in RNA and DNA sequencing technology, we now have the ability to trace the lineage of pathogens that have high mutational rates, in particular single-stranded RNA viruses (Duffy et al. 2008) within families such as the Coronaviridae (including SARS-CoV and MERS-CoV), Flaviviridae (e.g., yellow fever, West Nile, dengue, Zika, and hepatitis C viruses), and Filoviridae (Ebola virus and Marburg virus). Monitoring molecular data over time allows an opportunity to assess the spillover rates of viruses across SB1 and SB3. Genetic sequences from pathogens originating in RC and IHC are necessary here but not always available. Contact tracing can also be used in human populations to infer whether transmission is primary (i.e., across SB1 or SB3) or secondary (i.e., human-to-human within FHC). Finally, once secondary transmission has been identified in the FHC, then contact tracing can also be used to construct next-generation distributions where estimates of $R_0$ can be developed. Only if $R_0 > 1$ can an outbreak occur, and even then if $R_0 > 1$ is close to 1, epidemic fade-out may be more likely than an epidemic outbreak (Parrish et al. 2008; Lloyd-Smith et al. 2005).

The quantity $R_0$ is often considered the most important element studied in epidemiology, providing critical insight into epidemic behavior (Heesterbeek 2002). The value of $R_0$ can be reduced by shortening the period of infectiousness, decreasing the rate of new infections, or by increasing pathogen mortality (Hudson et al. 2008). In a full FHC epidemic with eventual fade-out, host factors themselves may drive the process through changes in contact behavior, treatment, and/or other interventions that reduce $R_0$, leading to fade-out and cessation of the epidemic. For example, interventions such as quarantining infected individuals limited the SARS outbreak in China in 2003 to fewer than 10,000 cases, even though the international travel of infected individuals puts hundreds of millions of individuals at risk (Smith 2006). Alternatively, the pathogen may establish itself in the new host population, sustained by secondary transmission, leading ultimately to endemic infection (e.g., HIV/AIDS; see below) or eventual epidemic fade-out. In the latter case, the pathogen cannot persist in the population as might happen, for example, when the proportion of susceptible individuals has been greatly reduced by the action of the epidemic itself [e.g., 1918 influenza pandemic, "the Spanish flu" (Taubenberger and Morens 2006)]. Ultimately, it is the convergence and interplay of specific factors from the involved compartments and host-pathogen interactions that will determine if pathogen transmission will successfully occur and the nature of the resultant epidemic(s).

#### 8.3.2.4 Factors Influencing the Disease Emergence Process

A unique suite of factors will influence each step of the pathogen invasion process, determining the potential for disease emergence (Table 8.1). As alluded above,

pathogen exposure is the fundamental requirement for pathogen spillover in a new host and is influenced by the spatial overlap and density of infected and susceptible hosts. Here, various factors can influence the outcome of exposure. External forcings such as climate or extreme weather events are able to drive host and pathogen distributions and disease occurrence (Alexander et al. 2012b). For example, El Niño Southern Oscillation events have been linked to increases in the number of *Yersinia pestis* flea vectors and rodent host populations, leading to increased pathogen invasion and mortality in prairie dog (*Cynomys ludovicianus*) colonies and colony extinction events (Stapp et al. 2004). Likewise, human behavior can influence domestic animal pathogen exposure to wildlife. For example, cattle herding behavior of dog owners influenced contact between African wild dogs (*Lycaon pictus*) and domestic dogs, exposing the wild dogs to canine pathogens and causing catastrophic declines in wild dog populations (Alexander and McNutt 2010).

While spatiotemporal overlap between a potential host and a pathogen reservoir may occur, the nature of the contact between host and pathogen must be appropriate to support host invasion (Morris et al. 2016). For example, socioeconomic status can influence human exposure to leptospirosis where poverty and compromised sanitation infrastructure increase contact with environmental sources of *Leptospira* (Reis et al. 2008). Similarly, bluetongue virus was historically thought to infect only ruminants, shrews, and some rodent species with pathogen transmission being vector dependent. Bluetongue virus in African predators, however, appears to be related to the ingestion of virus-infected prey with prevalence levels associated with feeding behavior and organ access (Alexander et al. 1994).

Pathogen spillover and replication in the new host can be influenced by the presence of other pathogens (Rigaud et al. 2010). Cross-immunity between pathogens can influence disease outcomes, as seen with smallpox and monkeypox virus, where cessation of smallpox vaccination allowed host immunologic release and emergence of monkeypox virus in the human host (Rimoin et al. 2010). Evolutionary change can also influence pathogenicity of an infectious disease organism or modify host resistance and pathogen invasion potential (Tack et al. 2012). In this case, coevolutionary selection occurs in response to variation in a myriad of processes acting on both the host and pathogen affecting their interactions across space and time. These effects make it difficult to generalize pathogen invasion behavior and predict host-pathogen interaction outcomes.

Pathogen factors pertaining primarily to evolutionary change [neutral drift, coevolution with the host, or adaptive evolution (Antia et al. 2003)] often become critical in determining the final outcome of the invasion in situations where regular outbreaks of the pathogen lead to a strain endemic to the FHC. This is the case for all human diseases that have clear origins in wildlife populations. For example, non-synonymous changes in the Spike gene were found only in SARS viruses where sustained human-to-human transmission occurred. These genetic changes were not found in viruses circulating in the reservoir host (palm civets; *Paradoxurus hermaphroditus*) or in those spillover viruses that did not successfully move to the FHC where sustained human-to-human transmission occurred (Pepin et al. 2010). A more dramatic case is HIV-AIDS that has two different simian sources, resulting in two distinct groups of HIV pathogens: one from gorillas (*Gorilla gorilla*; HIV-1) and

the other from sooty mangabeys (*Cercocebus atys*; HIV-2) (Lemey et al. 2003; Keele et al. 2006).

#### 8.3.2.5 Zoonotic Disease Emergence and $R_0$

Many studies of multi-host pathogen systems have mathematically incorporated the process of spillover (McCormack and Allen 2007; Kilpatrick et al. 2006; Dobson 2004), but failure to monitor spillover rates long term has limited our ability to assess the magnitude of spillover as isolated phenomena from within host species transmission. For example, in diseases such as rabies (Zinsstag et al. 2009) and brucellosis (Zinsstag et al. 2005), where $R_0$ in humans is zero, we often find calculations of $R_0$ developed for reservoir populations, without any explicit quantitative consideration of the magnitude of spillover into the FHC. This is also seen in a recent paper that explicitly models dog-to-human transmission for rabies (Zinsstag et al. 2009), including the development of clinical disease in both human and dog populations. $R_0$ is calculated for dog-to-dog transmission in IHC, but no specification of spillover rates across SB3 is made. This tells us that there is sustained transmission in the dog population, prior to any introduction of interventions; but the estimated dog-to-human contact rate is never used to provide an explicit corresponding estimation of the size of the spillover event. In the context of vector-borne diseases, a similarly approached assessment can be found with *Plasmodium knowlesi* transmission from monkeys to humans through *Anopheles leucosphyrus* mosquitoes, an interesting example of spillover (Cox-Singh and Singh 2008). A recent model of this system (Yakob et al. 2010) could be usefully extended to include the elaboration of spillover values: monkeys to humans and humans back to monkeys, in both cases through mosquitoes. Incorporation of such measures would be useful in understanding and characterizing the dynamic process of disease emergence.

#### 8.3.2.6 $R_0$, $H_T$, and Spillover

When transmission has a significant density-dependent component, $R_0$ is related to the establishment threshold $H_T$ (Diekmann et al. 1990) (but see Lloyd-Smith et al. 2005), defined as the minimum density of susceptible hosts necessary for establishment of the pathogen in a new host population. This threshold may disappear when frequency-dependent transmission predominates (Getz and Pickering 1983), as is the case for sexually transmitted diseases. Establishment of the pathogen will only occur if the necessary threshold density of susceptible individuals is identified and is required to ensure that $R_0 \geq 1$; otherwise, failing to have the necessary density of susceptibles, the pathogen will fade out with $R_0 < 1$. Disease control or eradication efforts are then focused on using this threshold principle to manipulate pathogen fade-out in the new host population through the reduction of susceptibles as, for example, with H1N1 and the use of vaccination and school closures to control the outbreak. However, $R_0$ and $H_T$ do not apply to transmission across spillover boundaries SB1 and SB3 where primary transmission of zoonotic disease occurs. These concepts are,

by definition, only applicable to the transmission within the reservoir or spillover host populations themselves. Further, spillover and onward transmission differ in an important respect: spillover is a series of onetime events, while onward transmission can either lead to stuttering chains, involving small groups of individuals from the animal and human populations interacting in the FHC, or full-blown outbreaks. Consequently, relative to time, the accumulation of spillover events is a linear process coupled to the nonlinear exponential processes of transmission in the reservoir and spillover host populations (Fig. 8.2). To assess the potential for outbreaks in the FHC, we need estimates of both spillover rates across SB1 and SB3, as well as estimates of the $R_0$ that will ensue once host-to-host transmission is established in the FHC.

By way of illustration, we can examine how public health systems consider zoonotic diseases. Human health objectives are focused on minimizing and preventing human morbidity and mortality associated with zoonotic disease transmission, and control efforts are directed at reducing or even eliminating initial spillover events. This public health perspective highlights the importance of characterizing spillover both qualitatively and quantitatively. Many emerging zoonotic pathogens present as a spillover epidemic only in the human population with $R_0 = 0$ (e.g., anthrax) or a mixed epidemic type where there is spillover with highly inefficient human-to-human transmission ($R_0 < 1$ in the human host, e.g.,

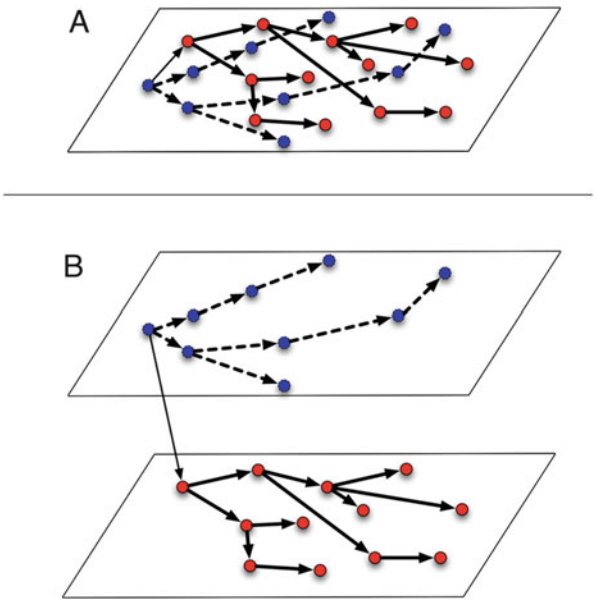


**Fig. 8.2** Mixed epidemic dynamics (**a**) shows spillover and onward transmission in a mixed epidemic (spillover epidemic and host epidemic). Blue circles are members of the reservoir population; red, the new host. Edges represent transmission. (**b**) shows exactly the same graph but explicitly separating the two populations. In (**b**), it is obvious that there are two separate processes, linked by a single event. The single spillover event does not lead to a geometrically growing number of spillovers, whereas onward transmission in the new host may. The appropriate interventions and surveillance methods for the two processes are different

monkeypox virus). The immediate focus of control for many emerging zoonoses is not the reduction of infectious animals in the reservoir populations (the reservoir species may not even be known) but minimizing human behaviors and other factors that are thought to contribute to pathogen exposure and invasion risk in humans (e.g., Ebola; see below). In these instances, $R_0$ in the human or in the reservoir population is not the central factor of interest. Rather, we need to focus on the complex processes of zoonotic pathogen spillover that must occur in the first place. Quantifying spillover rates provides information on the number of cases that can be expected in the FHC over time, a process that is not captured by the traditional $R_0$ in either the IHC or FHC.

Applying $R_0$ to our framework suggests that emergence outcomes can therefore be characterized in one of four ways (Lloyd-Smith et al. 2005):

1. The infected host is a dead end—dying and not passing the pathogen on to another human because human-to-human transmission is not possible ($R_0 = 0$ in the FHC).
2. The epidemic stutters along and inevitably fades out because $R_0 \leq 1$ in the FHC.
3. Though $R_0 > 1$ in the FHC, the infected host may or may not pass it on to another human, but the chain of transmission dies out by chance with probability $p_{\text{fadeout}} = 1 - 1/R_0$ (**epidemic fade-out**).
4. $R_0 > 1$ in the FHC, and the transmission chain takes off with probability $p_{\text{breakout}} = 1/R_0$ (**epidemic breakout**).

Fortunately, when $R_0 > 1$ in the FHC, a clear statistical demarcation exists between the epidemic fade-outs (#3) and breakouts (#4), with indicated probabilities. The distribution of sizes of total number of individuals infected is bimodal, with the low-end mode associated with fade-outs and the much larger, upper-end mode with breakouts [cf. simulated distributions of Ebola outbreaks in Getz et al. (2015)]. The emergence of a zoonotic disease thus requires at least a stuttering process in the FHC to occur sufficiently often to ensure an index case for a full epidemic in the FHC.

### *8.3.3 Applying the Framework*

Effective management of emerging disease threats requires that we differentiate among spillover and FHC processes when designing interventions. Our framework lends itself to its own classification scheme for differentiating zoonoses:

**Our Framework for Classifying Epidemics**

A. Spillover epidemic, either fading or breaking out after initial spillover
B. Mixed epidemic (outbreak in both RC and FHC)
C. Human host epidemic of zoonotic origin (initial spillover, with subsequent adaptation of pathogen to become endemic in the FHC)

(continued)

D. Animal host epidemic of human origin (as in previous case with roles of human and animal hosts reversed)
E. Human host epidemic with zoonotic genetic source contribution
F. Animal host epidemic with human genetic source contribution

To illustrate these points, we describe four systems of pathogen emergence that represent the epidemic types along the continuum (Table 8.2).

**Table 8.2** Epidemic types, examples, and their description

| # | Name | Examples | Description |
|---|---|---|---|
| 1 | Spillover epidemic | Zoonoses—rabies, anthrax (humans)<br>Anthropozoonoses | Only spillover transmission occurs, as the spillover host is a dead-end host for the pathogen, i.e., never enters the host epidemic space. Three kinds of transmission are identified:<br>(a) Zoonoses—animal-to-human pathogen transmission. Also known as obligate zoonotic when referring to pathogens of animal origin<br>(b) Anthropozoonses—human-to-animal pathogen transmission |
| 2 | Mixed epidemic | Ebola virus (human)<br>Paramyxoviruses (great apes)<br>Human tuberculosis (elephants; persistent mixed transmission can occur bidirectionally) | Spillover (zoonotic and anthropozoonotic) may be persistent (with varying quiescent periods) but is accompanied by limited secondary human-to-human or animal-to-animal transmission in the host epidemic space |
| 3 | Human host epidemic of zoonotic origin | SARS, HIV | A zoonotic pathogen makes a species jump from the zoonotic host departing from the spillover epidemic space, becoming human host adapted with sustained human-to-human transmission |
| 4 | Animal host epidemic of human origin | *Mycobacterium bovis* | A human source pathogen makes a species jump from the human host departing from the spillover epidemic space, becoming animal host adapted with sustained animal-to-animal transmission |
| 5 | Human host epidemic with zoonotic genetic source contribution | Swine-origin influenza A H1N1 | A pathogen co-circulates in human or animal reservoirs, where genetic reassortment and transmission occur |
| 6 | Animal host epidemic with human genetic source contribution | Human H1N2 and human-swine reassortant H1N2 and H1N1 influenza A viruses in pigs | |

#### 8.3.3.1 Anthrax (Spillover and Epidemic Fade-Out, Obligate Spillover Pathogen: Almost Exclusively RC)

Anthrax is a zoonosis caused by the spore-forming, gram-positive bacterium, *Bacillus anthracis*. This zoonosis affects livestock and wildlife nearly worldwide (Hugh-Jones and Blackburn 2009), with recent reemergence in humans in several agricultural areas (Kracalik et al. 2015). Under certain environmental conditions, spores can persist for long periods of time in the soil and cause subsequent outbreaks (Blackburn et al. 2007; Cherkasskiy 1999; Dragon and Rennie 1995). Across its known geographic distribution, epizootics can range from a few cases (sporadic) to massive outbreaks (Blackburn 2006).

**Spillover** As a soilborne pathogen, spillover begins at the host-environment interface, and epizootics can persist for weeks to months (Hugh-Jones and Blackburn 2009). Infection may be from either direct ingestion of spores from soil or contaminated vegetation (primarily in grazing herbivores), ingestion of leaves contaminated with blowfly emesis (primarily for browsers) (Blackburn et al. 2010; Braack and De Vos 1990), or direct inoculation from biting flies (Blackburn 2010; Krishna et al. 1958; Blackburn et al. 2014b). Though poorly studied, animal inhalation of spores is not implausible (Turnbull et al. 1998). It has been suggested that during large epizootics, high case numbers of a primary species (e.g., American bison; *Bison bison*) may cause enough environmental contamination that secondary host species (e.g., moose, *Alces alces*, or elk, *Cervus canadensis*) may succumb later in the outbreak (Fig. 8.3a) (Hugh-Jones and Blackburn 2009; Dragon et al. 1999). Specific mechanisms for transmission remain poorly understood and require further research. Human infection is considered secondary and often linked with handling contaminated carcasses or slaughtering infected animals (Woods et al. 2004) and therefore constrained to the spillover. The spatial boundaries of the spillover are limited by the distribution of environmental conditions that support the pathogen; though when livestock control is limited, contaminated meat movement can increase spillover into urban areas (Kracalik et al. 2013, 2015). Limited mechanical transmission may occur within or between herbivorous hosts if tabanid flies do in fact play a role in the transmission cycle (Blackburn et al. 2014a). However, this is likely limited by the seasonality of fly life cycles and conditions that promote pathogen survival. Onward transmission in humans is unlikely. Fade-out results as a consequence of outbreak control interventions [e.g., carcass burning or burial, livestock vaccination campaigns (Kracalik et al. 2014)] or seasonal limits on the pathogen or mechanical vectors, though these conditions are not fully understood.

**FHC** Limited onward transmission may occur within an ungulate host species if tabanid flies do in fact play a role in the transmission cycle. Onward transmission in humans is highly unlikely. Fade-out results as a consequence of either outbreak control interventions (e.g., carcass burning or burial and sustained preventative vaccination when tenable) or seasonal limits on either the pathogen or mechanical vectors.

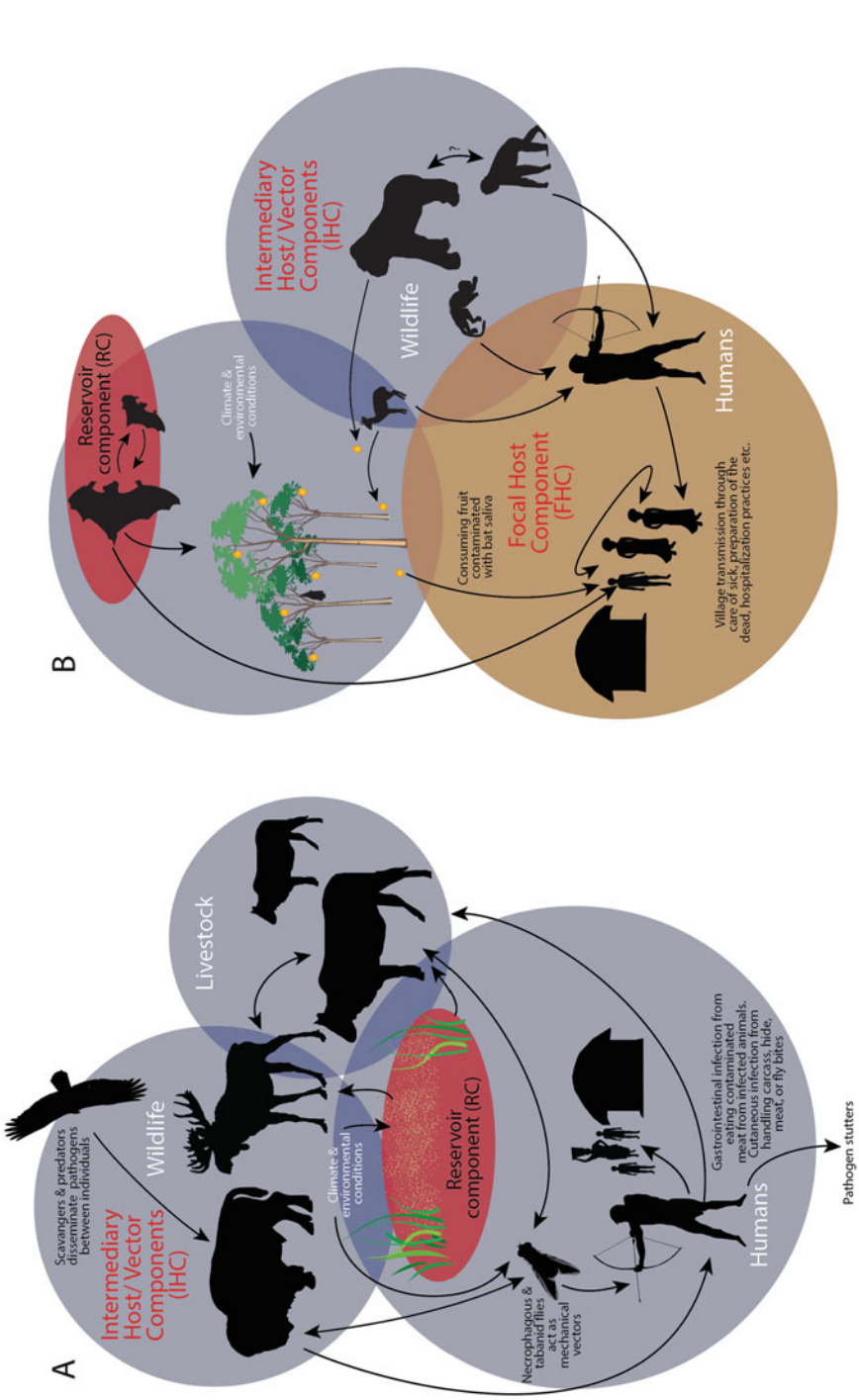


**Fig. 8.3** Anthrax disease (**a**) is caused by the spore-forming soil bacterium *Bacillus anthracis* with spillover occurring directly in the environmental reservoir. Host behavior and environmental conditions affect epizootics, with onward transmission limited to nonhuman hosts affected by biting flies. Human cases are

#### 8.3.3.2 Ebola (Mixed Epidemic Type, Mixed Pathogen Type: Spillover Pathogen from RC/IHC and within FHC Transmission)

Ebola hemorrhagic fever is an emerging zoonotic viral disease in West and Central Africa causing severe morbidity and high mortality in humans and wildlife (Alexander et al. 2015). Outbreaks are sporadic with viral quiescent periods upward of 20 years.

**Spillover** Successful spillover of the pathogen appears to be a complex process involving a number of coupled networks and seasonal drivers (Pinzon et al. 2004), linking the human host-to-virus reservoirs (Fig. 8.3b). Transmission to humans results from direct contact with infected wildlife species through handling and eating of bush meat [duiker; primates, SB3; bats, SB1 (Leroy et al. 2009)] or ingestion of fruit contaminated with Ebola-infected bat saliva [(Alexander et al. 2015), SB1]. Three bat species are considered putative virus reservoirs (Alexander et al. 2015). Spillover epidemics are necessarily limited to the spatial distribution of the reservoir host or distribution network of infected bush meat.

**FHC** Sustained onward transmission in humans results from close contact with blood, secretions, or tissues of infected individuals. The spatial extent of the host epidemic is then limited to the distribution of human-to-human contacts necessary for successful transmission of the pathogen. Fade-out generally results as a consequence of outbreak control interventions rather than the biology of the pathogen (barrier techniques, quarantine).

#### 8.3.3.3 HIV/AIDS (Human Epidemic of Zoonotic Origin, Human Host Adapted: Initially Spillover, Now Solely FHC Transmission)

HIV-1 and HIV-2 are the causative agents of AIDS in humans.

**Spillover** Historically, HIV originated from spillover of simian immunodeficiency virus (SIV) pathogens from nonhuman primate species to humans in Africa [SB3 (Heeney et al. 2006)]. On adaptation to the human host, the virus has departed from the spillover.

**FHC** Successful adaptation of these viruses to sustained human-to-human transmission is a rare event. Of 35 different primate species infected with lentiviruses, only a few viruses from two primate species (chimpanzees, *Pan troglodytes troglodytes*, and sooty mangabeys) successfully invaded and have persisted in the human host population causing global pandemics (Heeney et al. 2006). Here, human-to-human virus transmission dynamics are largely driven by sociocultural factors that influence human behavior (Halperin 1999).

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**Fig. 8.3** (continued) secondary to animal infections, and onward transmission is unlikely. Ebola (**b**) is an emerging zoonotic disease where spillover dynamics appear to include up to four coupled systems. Population dynamics and seasonal influences appear to be primary drivers of the process of pathogen invasion (Alexander et al. 2012b)

#### 8.3.3.4 Influenza (Epidemic with Reservoir Genetic Source Contribution: Spillover with Potential Bidirectional Transmission Within and Between IHC and FHC)

**Spillover** Influenza viruses circulate in a wide array of domestic animal and wildlife species with frequent spillover to humans (SB3). Spillback from humans to animals can also occur.

**FHC** Reassortment of genetic material from these animal reservoirs has been associated with changes in virulence, invasion potential, and adaptation in the human host (Smith et al. 2009; Olsen 2002). Some strains (e.g., H5N1, "bird flu") demonstrate limited human-to-human transmission potential while being highly virulent (Yang et al. 2007). Other strains (e.g., H1N1, "swine flu") are highly adapted to the human host, have relatively low virulence, and cause pandemic disease (Lagace-Wiens et al. 2010).

### *8.3.4 Evaluating the Framework*

Characterization of epidemic types within our framework allows us to more clearly identify direct implications for outbreak control. For example, zoonotic pathogen transmission at SB1 or SB3 will be spatially restricted in occurrence to where the RC or IHC species and humans intersect. In contrast, once a pathogen has evolved and adapted to the human host niche (FHC), the pathogen is freed from this spatial restriction. This moves the outbreak from being described principally by ecological variables such as host range or environment (e.g., Ebola, anthrax) to an outbreak described principally by socioculturally shaped transmission dynamics (e.g., HIV). A caveat here is the movement of infected animal products in the form of bush meat, which can expand the spatial restrictions in spillover also driven by sociocultural influences (Alexander et al. 2012a). The movement of bush meat is a growing concern in the public health and agricultural sectors.

Our framework identifies important gaps in the quantitative evaluation of the zoonotic outbreaks by identifying the need to link together the concatenated stochastic processes of spillover (SB1-3) and onward transmission in the FHC. The real problem, however, of characterizing transmission spillover boundaries is that sample sizes are rather small and observations are only partial: we may have some observations of successful spillovers (e.g., Ebola outbreaks over the past 40 years), but we have no idea how many infected animal-susceptible human contact events actually occurred. Further, during the early stages of a zoonotic outbreak, we do not have data that provides what proportion of transmission events took place across SB1 and SB3 versus that occurring within the FHC. For example, a hunter may be infected with Ebola virus from eating infected gorilla meat (Fig. 8.4). This infected individual may then go on to infect four other people in his family that care for him. Alternatively, five people in this family may handle and eat the meat of an infected gorilla directly and become infected, with no human-to-human transmission occurring. In both cases, five

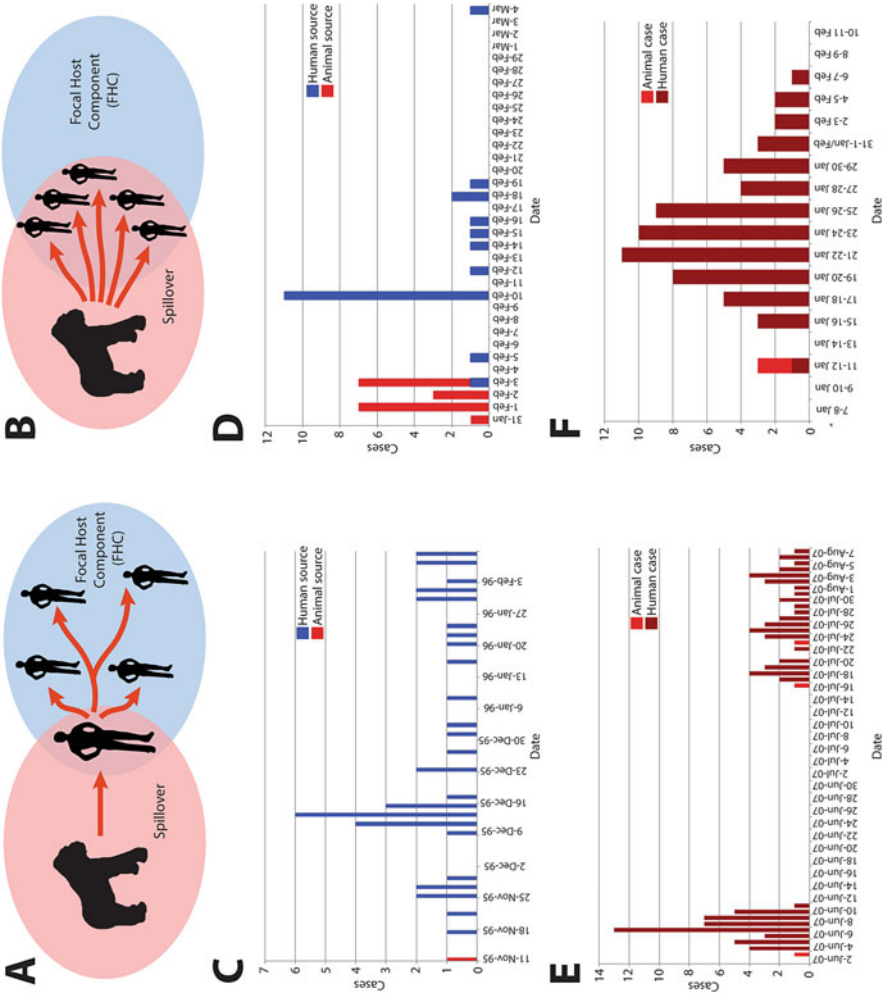


**Fig. 8.4** Scenarios of Ebola spillover. In (**a**), the spillover is limited with the majority of transmission occurring in the host-epidemic focal host component (FHC). In (**b**), there is only a spillover epidemic with no FHC ($R_0 = 0$). Insets **c** and **d** illustrate Ebola outbreaks in Gabon (Georges et al. 1999) where both spillover types

individuals are infected, but in the first case, two separate parameters must be estimated to predict the pathogen emergence process (transmission rates in both the spillover and the FHC), while in the second case, only the transmission rate in the FHC is needed, given that a spillover event has occurred. It is possible that genetic data may allow us to differentiate between these two cases, though we will need to know something about the rates of Ebola virus strain evolution within animal and humans hosts, respectively. In the second case, however, estimating the probability that isolated spillover events occur over time requires long-term samples of outbreak rates and the assumption of stationary conditions (i.e., the environmental and human population factors involved remain unchanged over time). While the temporal dynamics of these two cases would be different, in a mixed ongoing epidemic, characterization and application of how the two stochastic processes, one at the spillover boundary and one in the FHC, are intertwined are essential to gaining the necessary insight into the outbreak etiology and efficacy of different management actions.

Explicitly integrating the separate stochastic processes of spillover and onward transmission facilitates consideration of new questions that are not routinely discussed in emerging infectious disease literature. Increasing our research focus on this integration from both the mathematical and conceptual perspective will be important to our ability to model the dynamics of coupled spillover and FHC transmission processes. Emphasis is given to the possibility that some spillovers will stutter to extinction even though we may have $R_0 >> 1$ in the human population. Conversely, some spillovers with $R_0 < 1$ in the human population will, nevertheless, lead to outbreaks sustained by continuous transmission from animal to human spillover. When the public health focus is on the pathogen, not the strain (as in influenza), we can then consider the case of multiple spillovers of different strains with a distribution of $R_0$ in the human population. The public health question is then not so much will an HkN$\ell$ (k = 1, ..., 18; $\ell$ = 1, ..., 11) variant of influenza spillover and seed an epidemic as it is how often will a reassorted influenza virus spillover and seed an epidemic. The spillover component is then the critical ingredient in an analysis.

## 8.4 Ongoing Quantitative Challenges at the Spillover Interface

The spillover interface is, epidemiologically, unique and complex; and, as we have highlighted here, unique quantitative approaches are correspondingly needed to describe the rate and dynamics of spillover. The most basic concepts like $R_0$ can

**Fig. 8.4** (continued) (**a**, **b**) are illustrated. Red indicates human cases directly from animals; blue indicates human-to-human transmission. (**e**) and (**f**) illustrate livestock anthrax outbreaks with human spillover in Bengal (Ray et al. 2009) and Zimbabwe (Gombe et al. 2010). In both, human cases (dark red) were directly from animals (light red), illustrating anthrax is limited to the intermediary host/vector component (IHC)

be less meaningful and may need special formulations that more accurately summarize the different classes of epidemics we describe above. At the actual spillover boundary (Fig. 8.1), basic concepts like the force of infection break down. In normal epidemiology, for a pathogen circulating within a population, the force of infection λ is conventionally expressed as

$$\lambda = \frac{I}{N}\,\beta \tag{8.2}$$

where $\beta$ is the transmission rate. Within a single species, contact rates can be defined based on assumptions of density or frequency dependence, using the number or proportion of infected individuals, respectively, to parameterize $\lambda$. Between two species, contact rates are harder to define based on $I$ alone, and so the force of infection is a problematic concept at the spillover interface. Lloyd-Smith et al. (2015) suggested a customized definition of the **spillover force of infection**, adapted to the language we use here:

$$\lambda_s = \left(\frac{I_{RC}}{N_{RC}}\right) \times (\text{RC} : \text{FHC contact rate}) \times \beta_s \tag{8.3}$$

where

$$\beta_s = P\,(\text{infection}|\text{contact}) \tag{8.4}$$

Parameterizing the RC:FHC contact rate—especially as a single model variable—poses a serious quantitative challenge. Increasingly complex models will be needed that break down the different processes at the RC:FHC interface—as well as those that separate the role of the IHC—helping researchers more readily conceptualize and parameterize models.

Recent work on Lassa fever has especially highlighted just how far models at the spillover interface have come in the last 5 years. A model recently published by Iacono et al. (2016) establishes a "unified framework" for modeling zoonotic spillover with horizontal transmission, at the most fundamental level based on the notion that spillovers are a Poisson point process with rate $\lambda$, such that the probability of a given number $k$ of spillover events during interval $\tau$ is

$$P(k) = \frac{e^{-\lambda\tau}(\lambda\tau)^k}{k!} \tag{8.5}$$

They suggest that spillovers are a **self-exciting** process, as new human infections generate more human infections, but are also a **self-correcting** process, as acquired immunity in human populations depletes susceptible populations and decreases future transmission rates. Different framings of the relative importance of those processes lend themselves to different corresponding mathematical formulations, such as self-correcting Poisson processes or—if random variation in $\lambda$ is stochastic enough to be important to model accuracy—a Poisson-gamma mixture with

feedback. In their model, the force of infection is expressed as a combination of zoonotic and human-to-human components:

$$\widehat{\lambda}(t) = S_{\mathrm{H}}(t)\eta_{\mathrm{R}}(N_{\mathrm{R}})Pr_{\mathrm{R}}(N_{\mathrm{R}})\chi R + S_{\mathrm{H}}(t)\eta_{\mathrm{H}}(N_{\mathrm{H}})Pr_{\mathrm{H}}(N_{\mathrm{H}}, t)\chi_{\mathrm{H}} \tag{8.6}$$

where $S_{\mathrm{H}}$ is the susceptible human population size; $Pr_{\mathrm{R}}$ and $Pr_{\mathrm{H}}$ are the prevalence of infected rodents and humans, respectively; $N_{\mathrm{H}}$ and $N_{\mathrm{R}}$ are population sizes of humans and rodents, respectively; and $\chi_{\mathrm{R}}$ is a parameter that represents "the ability of the reservoir to excrete a suitable dosage of the virus and the human response to it" (with a corresponding $\chi_{\mathrm{H}}$ for human-to-human transmission). In their application to Lassa fever, the authors show that the flexibility of this model produces dramatic responses to subtle changes. For example, moving from a constant rate of spillover to a seasonally peaking process (as a mode of hypothesis testing) reduces human-to-human contributions to outbreaks from roughly 90% to 20%.

With expanding accuracy in models of the spillover process, simulation methods have now begun to be developed that appropriately predict spatial risk patterns for Lassa fever. A study by Redding et al. (2016) combined a (simpler) model of RC: FHC transmission ($\lambda$, which they term the "force of zoonotic infection") with habitat suitability modeling for reservoir hosts to develop an "environmental-mechanistic zoonotic spillover model." Models like these, which consider spatial variation in human-reservoir contact and spillover risk, represent a tremendous advancement in the quantification of the spillover interface. Most spatial patterns in zoonotic disease emergence are currently studied with more correlative methods like **ecological niche models**, which are tremendously powerful but subject to a number of user-end decisions, only relate patterns of occurrence to environmental variables (transmission risk rather than transmission rates), and suffer from problems relating to lack of consensus among conflicting models. Both modeling frameworks offer the possibility of extending models for predictions under global change; but models that explicitly and mechanistically account for human case burdens arising from spillover (rates, not risks) have a clear advantage when the data exists to parameterize them.

## 8.5 Conclusion

Articulation of zoonotic outbreaks as a set of processes that take place dynamically (Fig. 8.1) allows a deeper understanding of how to computationally categorize different classes of zoonotic diseases, with important implications to both public health policy and management of zoonotic outbreaks. Using this approach it becomes clearer why influenza is so different from many other zoonotic pathogens because the spillover-FHC concatenation can be bidirectional between humans and animals as well as animals and the environment. While $R_0$ is often ubiquitously applied to analysis of all infectious disease, this framework helps us understand why this concept is only part of the story for zoonotic diseases where key stochastic events and interdependent processes influence spillover probability at any or all

interfaces. This emphasis on the spillover component of zoonotic disease emergence points to a need for greater computational and mechanistic focus on spillover itself.

### Compliance with Ethical Standards

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**Ethics Approval** This article does not contain any studies with human participants or animals performed by any of the authors.

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# Chapter 9
# Integrating Landscape Hierarchies in the Discovery and Modeling of Ecological Drivers of Zoonotically Transmitted Disease from Wildlife

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**Abstract** Changes in landscape and land use can drive the emergence of zoonoses, and hence, there has been great interest in understanding how land cover change and the cascade of ecological effect associated with it are associated with emerging infectious diseases. In this chapter, we review how a spatially hierarchical approach can be used to guide research into the links between landscape properties and zoonotic diseases. Methodological advances have played a role in the revival of landscape epidemiology and we introduce the role of methodologies such as geospatial analysis and mathematical modeling. Importantly, we discuss cross-scale analysis and how this would provide a richer perspective of the ecology of zoonotic diseases. Finally, we will provide an overview of how hierarchical research strategies and modeling might be generally used in analyses of infectious zoonoses originating in wildlife.

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## 9.1 Introduction

Zoonotic diseases, or zoonoses, are infectious diseases transmitted to humans from animals and may be bacterial, viral, or parasitic in origin. Approximately 58% of the pathogens associated with infectious diseases in humans have originated through spillover from wildlife—e.g., ebolaviruses, hantaviruses, coronaviruses, henipaviruses (Jones et al. 2008; Lloyd-Smith et al. 2009; Smith et al. 2014; Woolhouse and Gowtage-Sequeria 2005). Since 1980, zoonotic pathogens represent the bulk of the outbreaks in human populations in both number (87% versus 13% by vector-borne pathogens) and diversity (Smith et al. 2014). The recently reported increase in zoonoses has been attributed to a variety of reasons, although a major driver is changes in land use resulting from the increased demands of human populations on the natural environment through agriculture intensification and deforestation (Jones et al. 2013; Woolhouse and Gowtage-Sequeria 2005). Land alteration for food production, broadly defined to include both agriculture and pastoral activities, has produced profound changes in the type and structure of the earth's vegetation cover. It has also altered the way humans interact with their environment, for example, in some geographical areas suppressing wildlife-human interaction (e.g., contact with large predators common among hunter-gathers) and instead favoring contact between humans and peridomestic species such as rodents, which are common reservoirs for many zoonotic pathogens. Numerous examples of emergent zoonoses have often accompanied land clearance, and hence, there has been great interest in understanding how land cover change and the cascade of ecological effects associated with it are ecologically associated with emerging infectious diseases (McFarlane et al. 2013; Woolhouse and Gowtage-Sequeria 2005).

A persistent question in modeling of pathogen emergence is what is the spatial scale at which these processes occur? How do we measure and integrate the impact of processes that occur at varying and nested spatial scales that result in the observed disease distribution (Watts et al. 2005)? For example, climate (which can vary in scale from local to continental) and landscape are each associated with disease patterns and have been cited as factors in zoonotic disease outbreaks. Land cover change and land-use intensification often occurs at finer scales than climate and can thus be thought of as nested within the climate system. At still finer scales, population dynamics and habitat interactions of the pathogen and its reservoir communities (and, indeed, with the human populations vulnerable to disease transmissions from these communities) occur within the climate and landscape scales and are influenced, but not necessarily determined, by them. This hierarchical nesting (Fig. 9.1) of these processes complicates the overall study and modeling of the spillover and transmission dynamics of wildlife pathogens to human populations. However, there are existing bodies of theory which can help shed light on these processes. Hence, we review some of these conceptual ideas, focusing especially on landscape epidemiology, a body of theory first developed in the 1930s and more recently updated to include modern tools and techniques for studying both disease and environmental process (Ostfeld et al. 2005), and hierarchy theory, a framework which incorporates the idea of multiple, nested spatial scales (Allen and Starr 1982).

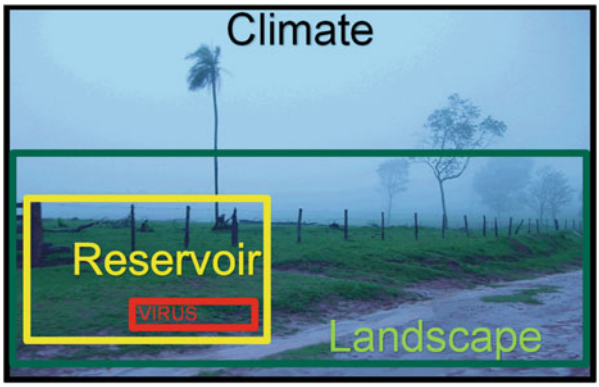


**Fig. 9.1** Illustration of hierarchical nesting of possible key components in pathogen emergence in wildlife. Each process outlined with a "box" represents a distinct spatial scale to consider in developing models

In the following, we will review how a spatially hierarchical approach can be used to guide research into the links between landscape properties and zoonotic diseases. This will show how landscape concepts have been used to analyze the occurrence and spatial patterning of zoonotic diseases and how many of these studies are being conducted at particular spatial scales. This review will be followed by an illustration of how an integrated, cross-scale analysis might be employed to gain a deeper understanding of the ecology of zoonotic diseases, using an example from our own research. As earlier noted, methodological advances have played a role in the revival of landscape epidemiology. We will therefore discuss the role of methodologies such as geospatial analysis and mathematical modeling—again using examples drawn from our current research. We will show how cross-scale analysis might be employed to gain a deeper understanding of the ecology of zoonotic diseases. Finally, we will provide an overview of how hierarchical research strategies and modeling might be generally used in analyses of infectious zoonoses originating in wildlife.

## 9.2 Landscape Epidemiology and Ecology

Landscape epidemiology is the study of spatial patterns of disease and disease risk arising from underlying environmental causes. The fundamental concepts of landscape epidemiology, first proposed by Evegeniĭ Pavlovskiĭ, stem from the idea that the spatial occurrence of disease could be understood by studying landscape and environmental factors associated with the disease (Pavlovskiĭ 1966). Pavlovksiĭ's ideas have undergone a revival, stimulated in part by widespread availability of geospatial data, analysis tools, and models. In particular, satellite remote sensing data analyzed within a geographic information systems (GIS) framework has equipped landscape epidemiologists with a powerful suite of tools for analyzing environmental patterns associated with disease occurrence. Landscape epidemiology has also benefited from the theoretical perspective of landscape ecology, another relatively new discipline that attempts to understand the relationship between spatial pattern and ecological process (Meentemeyer et al. 2012). For example, landscape

epidemiologists have made use of ecological concepts such as fragmentation to analyze disease vectors (Brownstein et al. 2005; Reisen 2010). One aspect of landscape ecology that remains relatively unexplored in infectious disease applications is the concept of spatial hierarchy.

### *9.2.1 Hierarchy Theory*

Hierarchy in ecology is a multifaceted theory incorporating elements of nonlinear dynamics and complexity; for a comprehensive treatment of hierarchy theory, see Allen and Starr (1982). The fundamental concept of hierarchy theory is that processes occurring at finer scales (i.e., "lower" in the spatial hierarchy) are constrained by processes at higher levels. Hierarchical levels can also be distinguished by the rates at which ecological processes occur—faster at finer scales, slower at coarser ones.

Hierarchy theory in ecology arose as a response to the need for a rigorous method of handling middle-number systems, that is, systems whose components are too few to treat statistically but too many to address with classical Newtonian mathematics. Hierarchy provides a framework by which these middle number systems can be decomposed into a series of manageable units, whose environmental drivers can be characterized by the scale (and thus the rate) at which they occur. Such a framework is amenable to the study of landscape epidemiology, since the linkages between environmental factors and disease are often multivariate, nonlinear, and not confined to a specific spatial scale.

Spatial scale is a crucial aspect of hierarchy theory. Any discussion of the relationship between ecological process and scale therefore must first define how the term is used and to what characteristic dimensions these terms apply. These definitions are complicated by the number of ways that the term "scale" is used. Scale is frequently referred to by descriptive adjectives such as "large" or "small," which Meentemeyer (1989) noted can have opposing meaning depending on whether one is referring to cartographic scale (where small scale refers to less spatial detail) or ecological scale, where smaller scale equates to smaller spatial area and greater detail. Ecologists generally distinguish between the grain and extent of a process, where extent refers to the area over which a process occurs, and grain denotes the smallest resolvable component of the process. Typically, grain can be described as either "fine," indicating small resolvable elements, or "coarse," indicating larger elements. Thus, we might characterize the scale process like tropical deforestation as occurring at relatively large extent, because a large area is affected, but at relatively fine grain, because the individual deforested units can be quite small. Csillag et al. (2000) argue that for ecological uses of scale, it is preferable to adopt specific terminology to distinguish between extent and grain. In hierarchy theory, spatial extent is probably the more commonly used sense of scale (Jenerette and Wu 2000). In describing spatial hierarchies, it is often useful to apply descriptive names to realms of scale. Terms such as "global," "continental," "regional," and "local" are often used, although the precise areas referred to often vary. For our review of hierarchy in infectious disease analysis, we will operationally define continental scale as areas exceeding $10^6$ km$^2$, regional scales as ranging from $10^3$ km$^2$–$10^6$ km$^2$,

local scale from $10^1$–$10^3$ km$^2$, and microscale $<10^1$ km$^2$. The lower end of the microscale represents the general size range of a small rodent's world.

Landscape analyses across spatial scale frequently use remote sensing data (Kitron et al. 2006; Wu 1999). Currently, imagery is available from an array of orbital sensors with widely varying spatial, temporal, and spectral resolutions (Table 9.1). Grain size (or resolution, as it is more commonly termed in remote sensing) is an engineered property of these sensors, dependent on the optical characteristics of the sensor and orbital characteristics of the platform. Theoretically,

**Table 9.1** Partial list of current of current and past orbital remote-sensing instruments, frequently used in landscape epidemiological studies

| Instrument | Platform | Spatial resolution (m) | Notes |
|---|---|---|---|
| Pleiades 1A | Pleiades | 2.0 m (VNIR)<br>0.5 m (PAN) | Available 2011–Present |
| Worldview | Worldview 1–4 | 1.41–1.84 (VNIR)<br>0.31m–0.46 m (PAN) | Resolution is at nadir. Finer resolution sensor on WV-3 and -4. Available 2007–present |
| GeoEye-1 | GeoEye | 1.84 m (VNIR)<br>0.46 (PAN) | Available 2008–present Resolution is at nadir value |
| QuickBird | QuickBird | 2.63 (VNIR)<br>0.73 (PAN) | Available 2001–2015 |
| IKONOS | IKONOS | 3.2 (VNIR)<br>0.82 (PAN) | Available 2007–2105 |
| HRV | SPOT 1–3 | 20 m (VNIR)<br>10 m (PAN) | Available 1986–1997 |
| HRVIR | SPOT 4–5 | 20m (VNIR, SWIR)<br>10 m (Pan) | Available 1998–2015 |
| Vegetation | SPOT 4–5 | 1000 m | Produces a vegetation index (NDVI) product |
| Azersky | SPOT 6–7 | 6 m (VNIR, SWIR)<br>1.5 m (PAN) | Available 2012–present |
| Multispectral scanner (MSS) | Landsat 1–5 | 79 m | Available 1972–1999 |
| Thematic Mapper/ Enhanced Thematic Mapper + (TM/ETM+) | Landsat 4,5,7 | 30 m (VNIR, SWIR)<br>120 m (TIR)<br>20 m (Pan) | Available 1984–2013 (ETM+ experienced partial failure in 1999) |
| OLI/TIRS | Landsat 8 | 30 m (VNIR, SWIR)<br>100 m (TIR)<br>15 m (Pan) | Available 2013–present |
| LISS | IRS 1C/1D | 23.5 m (VNIR, SWIR)<br>7.5 PAN | Available 2003–present |
| Sentinel-2 | Sentinel-2 | 10 m (VNIR)<br>20 m (SWIR) | Available 2015–present |
| Terra/Aqua | MODIS | 250 m (Red, NIR<br>500 m (VNIR, SWIR)<br>1000 m (VNIR, SWIR, TIR) | Available 1999–present |
| AVHRR | NOAA polar orbiters | 1000 m (VNIR, TIR) | Available 1979–present |

there are no limitations to the extent of any of these systems, although practical limitations (particularly cost of data acquisition) dictate a rough correspondence between extent and resolution. Thus, the extents listed in Table 9.1 are based on these practical limitations. The variety of remote sensor data available has certainly facilitated hierarchical landscape analysis, but in some sense, it has also imposed limitations. Each remote sensor represents a "window" through which ecological process at some combination of grain size and extent can be observed. These limitations also extend to the range of spectral wavelengths each sensor can detect and the number and width of bands in which these wavelengths are detected. Combining these discrete views into an integrated picture is a central challenge for hierarchical analysis of infectious disease processes.

## 9.3 Landscape and Zoonotic Disease: A Selected Review of Literature

There is a large body of work relating infectious disease to environmental factors. Most of these studies have concentrated on a single class of causative factor operating over a characteristic spatial scale. In the following, we will briefly review several examples which represent different classes of landscape factors that have been shown to impact zoonotic viral emergence. Since our intent is to show how landscape spatial hierarchies influence disease processes, we will concentrate on examples that show the influence of landscape and land cover processes at various spatial scales, using the concept and terminology of scale developed in the previous section.

For convenience, we will group the literature reviewed here into two categories. First, we will review the relationship between land cover disturbance (including anthropogenic and natural disturbance) and zoonotic disease emergence. Included in this category are causes due to landscape structure, including natural landscape barriers, land cover change and disturbance, and fragmentation. This category also includes disturbance due to agricultural practices. The second class of landscape impacts that we will review are climate-driven landscape changes, which include climate-influenced changes in vegetation phenology patterns as well as persistent or transient superficial changes such as flooding. Processes contained within these two categories are not exclusive; however we will review them based on their predominant process.

### *9.3.1 Disturbance-Driven Landscape Change*

Landscape structure refers to the pattern and arrangement of habitats on the Earth's surface. Like all landscape variables, structure is complex. It varies with scale and per organism. The same landscape might be structurally very different for birds, small mammals such as rodents, and larger animals, and since zoonotic disease

reservoirs vary in their body size and habitat, structural effects on disease also vary. Structure can arise naturally due to topographic, edaphic meaning related to the soil, or climatic conditions, as well as through human landscape alteration. Both types of structural changes can be relevant to disease processes. Landscape structure has been related to zoonotic disease at a variety of spatial scales. At regional scale, Russell et al. (2006) show how natural landscape barriers such as rivers and preferred habitat can limit rabies spread by raccoons and be used to manage wildlife through vaccination efforts. Smith et al. (2002) used landscape heterogeneity as a predictor of the spread of rabies. Langlois et al. (2001) showed that the distribution of hantavirus-bearing rodents in North America was influenced by landscape fragmentation. Their analysis was unusual in that the extent of the study was continental, but the grain size was local (≈1 km). Estrada-Peña and Oteo (1991) showed that landscape structure, particularly landscape connectivity, showed a strong influence on the abundance of Lyme disease vectors in Spain.

Land cover changes are known to affect zoonotic diseases through controls on the population dynamics of reservoir species (especially wild mammals) as well as disease vectors (Patz et al. 2008). Giraudoux et al. (2003) used regional-scale land cover changes in France and China to show how host mammal communities affect transmission dynamics of the endoparasite *Echinococcus multilocularis.* Using the ROMPA (ratio of optimal to marginal patch area) hypothesis of Lidicker and others, it has been shown that regional-scale landscape dynamics of intermediate host species can in turn affect parasite egg survival and transmission (Lidicker 1995). The Giraudoux et al. (2003) study also considered the role of landscape change on establishing minimum thresholds, which they termed "filters" or "screens" of suitability for disease transfer.

Agriculture practices led to the Nipah virus outbreak in Malaysia (Epstein et al. 2006; Pulliam et al. 2012). Using a combination of field, laboratory, and modeling approaches, these efforts have supported the hypothesis that emergences of viruses such as Nipah are due to ecological and not evolutionary drivers. These findings underscore the importance of having multidisciplinary teams work together to build predictive models for discovery of the relationships between anthropogenic environmental change and the transmission or spillover of infectious agents.

### *9.3.2 Climate-Driven Landscape Change*

Climate-driven landscape change in this context refers to the effect of atmospheric processes (most notably precipitation) on the habitat of zoonotic host organisms. Variations in rainfall magnitude and frequency have notable effects on vegetation phenology, causing variations in surface greenness that can be tracked using remote-sensing instruments (De Beurs and Henebry 2004; Reed et al. 2009). The emergence of several zoonotic diseases including hantavirus pulmonary syndrome (HPS) (Yates et al. 2002), Argentine hemorrhagic fever (Simone et al. 2010), and Bolivian hemorrhagic fever (Kilgore et al. 1995) can be clearly linked to landscape.

Several studies have linked climate-driven changes to patterns of disease occurrence at different spatial scales. Two groups have evaluated the relationship between temporal patterns of Normalized Difference Vegetation Index (NDVI) and occurrence of Ebola virus in West Africa (Pinzon et al. 2004; Tucker et al. 2002). They found that NDVI trajectories showed distinctive "trigger events" prior to occurrences of the disease in humans and apes, which they hypothesized might be used to forecast conditions conducive to outbreaks of Ebola hemorrhagic fever. The remotely sensed data for this analysis came from the NOAA-AVHRR sensor, with continental spatial extent and observation grain size (pixel resolution) of 1 $km^2$. The observed NDVI trajectories were related to precipitation patterns, reinforcing the link between climate and disease occurrence. Estrada-Peña and Oteo (1991) and Estrada-Peña et al. (2006) used coarse resolution vegetation index data to model and predict the continental-scale relationship between climate-driven landscape change and Lyme disease. Again, the resolution and extent of this study were consistent with the idea that climate constrains disease processes at higher levels in the spatial hierarchy. At finer spatial scales, Glass et al. (2000) used patterns of reflectance in Landsat Thematic Mapper (TM) data to statistically model the presence of HPS in the Southwestern United States. At this spatial scale, the spectral response of the surface incorporates climatic factors (especially antecedent precipitation) but also integrates structural and compositional factors of the vegetation canopy itself. Models based on these techniques have shown some utility for predicting hantavirus cases (Glass et al. 2002).

## 9.4 Hierarchical Analysis of an Emergent Zoonosis: An Example

One of the important advantages of a hierarchical approach is that it allows a multifactorial explanation for the occurrence of the infection and disease. The environmental, landscape, and climatic processes each contribute to processes that may alter species interactions within their habitat. These extrinsic factors can alter the reservoir population dynamics, drive extinction, and affect maintenance (persistence) of the microorganism. Essentially, these factors can create constraints within their spatial scale and across scales. In this section, we explore an example of hierarchical observation that can be used in conjunction with modeling to test hypotheses regarding the effects of environment, landscape, and climate upon zoonotic pathogen distribution. For this we draw upon our work and others in the study of *Hantavirus* with a focus on South America (Jonsson et al. 2010; Palma et al. 2012).

### 9.4.1 Continental Scale: Phylogeography

Hierarchy theory suggests that processes at coarser grain also occur over longer time frames and can often be assumed to be static with respect to finer-scale processes. The phylogeographical patterns of South American hantaviruses within the Southern Cone ("El Cono Sur," a subcontinental region roughly defined as consisting of the country Argentina, often including Chile, plus sometimes considered to include Uruguay and Paraguay) mapped with environment at the coarsest spatial scales (Chu et al. 2006). In other words, the phylogenetic clades of hantaviruses from the Southern Cone of South America appear tied to coherent spatial patterns consistent with subcontinental-scale biogeographic features such as the major biomes (Chu et al. 2006). In Fig. 9.2, the locations of strains from three major subclades of South American hantaviruses are shown in the context of the major biomes based on the World Wildlife Fund terrestrial ecoregions data (Olson et al. 2001). For example, we find members from one subclade (i.e., Laguna Negra, Rio Mamoré, and Alto Paraná viruses) carried by rodent reservoirs that span the tropical grass and shrubland and dry broadleaf forest areas along the western, central regions of South America (Chu et al. 2006; Johnson et al. 1997; Richter et al. 2010; Yahnke et al. 2001). In contrast, a second subclade of rodents that harbor Jabora, Maporal, and Necocli hantaviruses inhabit mainly the moist broadleaf forest biome stretching from Venezuela and Colombia into Paraguay (Chu et al. 2009; de Oliveira et al. 2011; Fulhorst et al. 2004; Londoño et al. 2011). Knowing the extent of prevalence of these closely related viruses over this vast region would certainly be fascinating from a phylogeographical point of view. It is interesting that these viruses are not yet associated with cases of HPS. There are numerous strains of hantaviruses that have been identified in the Atlantic Forest (extends along the eastern coast of Brazil and into eastern Paraguay) and in the temperate grass and shrubland of Argentina. The third clade of rodents, those that harbor the Juquitiba, Oran, and Lechiguanas viruses, resides in the more humid Lower Chaco (a region that encompasses the flooded savannas of Southern Paraguay and forms a transitional environment between the arid Gran Chaco) and temperate coniferous forests in Argentina, Brazil, and Uruguay (De Araujo et al. 2015; Delfraro et al. 2008). Andes, Maciel, and Pergamino viruses reside in rodents in the temperate grass and shrubland biome in Argentina (Bohlman et al. 2002; González-Ittig et al. 2014). Andes virus is also found in Chile in Mediterranean woodland and shrub biome (Torres-Perez et al. 2004). Araraquara viruses are associated with rodents that largely reside within agriculturally transformed areas of Brazil (i.e., sugarcane production fields) that were formerly areas of moist broadleaf forest biome (De Araujo et al. 2015; de Sousa et al. 2008; Suzuki et al. 2004).


**Fig. 9.2** Illustration of the major biomes of South America from the World Wildlife Fund terrestrial ecoregions data (Olson et al. 2001). Selected strains of closely related hantaviruses are presented from three distinct subclades as indicated by the color of the yellow or pink dot at their location. The viruses represented by the pink dot and pink with yellow represent two distinct lineages from one subclade

### *9.4.2 Regional-Scale Land-Cover Association with Pathogen Prevalence*

Coarse-scale analysis has shown a relationship between hantavirus genetics and broad ecological pattern. Narrowing this view to a more regional scale begins to reveal how land cover (and land cover disturbance) affects the spatial variability of hantaviruses. Like all zoonotic disease, the ecology of each species of *Hantavirus* is closely related to that of its host organism; thus, generalization of virus-landscape relationships cannot be made without considering the habitat characteristics of the reservoir host. We do not have abundant data on the microhabitat characteristics of many host species (Lozada and Guthmann 1998). However, general habitat types defined at grains sizes of ~1 km might be useful to study preferences among various

host species, and be relevant to the landscape epidemiology of hantaviruses, regardless of whether certain land cover types are more closely associated with the presence of the virus.

A regional-scale analysis of rodent reservoirs of hantaviruses in Paraguay (Goodin et al. 2006) showed that the host species do indeed show patterns of land cover preference, even when land cover is mapped into very general categories. The most common hantavirus host rodents in Paraguay, *Akodon montensis* and *Oligoryzomys* spp., both showed disproportionately high probabilities of occurring in areas subjected to large-scale agricultural disturbance (Goodin et al. 2006). Even more significant, however, was the fact that rodents found to be antibody positive for hantavirus (indicating exposure to the virus at some point) were more likely to be associated with the human-disturbed land cover types. This relationship held even when the underlying differences in habitat preference were controlled. This finding suggests that some aspect of land cover disturbance or change increases the likelihood that a member of a host species will be infected with the virus. While analysis at the regional scale shows that human land cover alteration is associated with hantavirus presence, discovering the specific nature and causes for this observation cannot be addressed at this scale. Questions of causation must be addressed at finer spatial scales, in large part because that is the scale to which most host populations and individuals are interacting with their environment (Lozada and Guthmann 1998; Owen et al. 2010).

### *9.4.3 Local Scale: Host and Habitat Associations*

Simply presumed, because rodents species have specific requirements with respect to habitat, and because each hantavirus species seemingly persists only in certain rodents species, one might assume that a predictive map can be made for of the prevalence of a particular hantavirus based on knowledge of the associated rodent species. Unfortunately, for most rodents, we know very little about their general life cycles and other important biological information that is critical for modeling (e.g., age at sexual maturity, birth rates, litter sizes). Further, we have only a minimal amount of information on their habitat preferences, although some recent studies have indicated that fine-grained evaluations are necessary to understand rodent species distributions and community composition (Goodin et al. 2009; Lozada and Guthmann 1998; Poindexter et al. 2012; Schnell et al. 2010).

### *9.4.4 Microscale: Virus and Host Interactions*

At the microscale level, pathogen survival and reproduction depend on the dynamics within the reservoir host, which in turn depend on the habitat or the reservoir. A rodent may live within an approximate 1 km range for most of its life barring fire,

flooding, and other natural disasters. In the case of a viral pathogen, the virus must overcome physical barriers of the host and be compatible with cell receptors to gain entry into a target cell (Allen et al. 2012). Viral replication depends on host and viral genetics and other host factors such as prior pathogen or other immunogenic exposure, nutritional status, coinfection, age, sex, reproductive status, and the host immune response (Allen et al. 2012). These and other factors determine outcome when a pathogen enters a host, with the possibilities including severity of any associated disease and whether the pathogen either is to be cleared by the host immune system, or the pathogen will persists in the host. For example, the survival of hantaviruses in nature depends on maintenance of persistent infections within their specific rodent reservoir. Hantaviruses infect and persist only in the rodent reservoir with which the virus has coevolved, and the infection is believed to last the life of the animal (Meyer and Schmaljohn 2000). Notably, persistent infection of rodent reservoirs by hantaviruses shows continuous virus replication, without complete clearance by the immune system, and no pathological changes (Jonsson et al. 2010; Vaheri et al. 2013). Humans are not a natural reservoir for these viruses, and as such humans typically become infected only upon contact with aerosolized excreta from the rodent reservoir. In humans, hantavirus infection can result in severe disease although outcomes vary with different hantaviral species. The molecular basis for different disease outcomes in humans has been attributed to difference in receptor preferences of nonpathogenic and pathogenic hantaviruses. Models that connect data on the outcomes associated with immune response in reservoir versus human hosts are just beginning to be developed.

## 9.5 Role of Mathematical Modeling in Spatial Ecology of Infectious Diseases

Mathematical models are valuable tools for synthesizing information and testing hypotheses to provide insight into how and why disease outbreaks or spatial patterns of infections might arise in wildlife populations. Several books and review articles summarize some of the modeling efforts on zoonotic infectious diseases (Alexander et al. 2012; Allen et al. 2012; Grenfell and Dobson 1995; Heesterbeek et al. 2015; Hudson et al. 2002; Lloyd-Smith et al. 2009). A variety of modeling formats, deterministic and stochastic, have been applied to the study of zoonotic diseases. These models include compartmental, agent-based, individual-based, metapopulation, network, and ecological niche, but these classifications overlap. Agent-based, individual-based, and metapopulation models may be classified under network models, where the nodes are infectious agents, individuals or populations, connected via an underlying network (Riley et al. 2015). Simple compartment models (SEIR—susceptible, exposed, infectious, and recovered) are connected via a dispersal network in what is often called a patch model or metapopulation model (Allen et al. 2009; Arino et al. 2005; McCormack and Allen 2007b). Ecological

niche models contain less detail about individual dynamics, and instead they are closely related to landscape, presence/absence data, and GIS-based climatic and environmental data (Alexander et al. 2012; Peterson 2014).

Mathematical tractability and the complexity of interactions among pathogens, reservoirs, and human hosts and the environment have often restricted the model formulation to the reservoir host and to a single spatial scale—landscape, population, or within-host. Coupling temporal scales (hours to years or longer), biological complexity levels (genes to cells to ecosystems), and spatial scales (local to global) have been a continuous challenge to modelers (Heesterbeek et al. 2015). Recent theoretical investigations on coupling within-host and between-host models are advancing (Feng et al. 2013; Gilchrist and Coombs 2006; Mideo et al. 2008).

Hantaviruses and rabies are two examples where spatial patterns of infection involving multiple species have been observed (Chu et al. 2009, 2006; Haydon et al. 2002; Rhodes et al. 1998; Smith et al. 2002). Spatially explicit computer simulations that incorporate landscape heterogeneity and spatial genetic structure are being applied to study control of rabies and other zoonotic diseases (Alexander et al. 2012; Parratt et al. 2016; Real and Biek 2007; Rees et al. 2013). Models with multi-host species and multi-pathogens are being investigated. For example, mathematical models for hantavirus infection in rodents have been studied in the context of multiple host species, spatial spread, and environmental variability (Abramson and Kenkre 2002; Abramson et al. 2003; Allen et al. 2006a, b, 2009; McCormack and Allen 2007a, b). These models have shown that those random or seasonal variations which impact an ecosystems carrying capacity for a particular rodent species can trigger outbreaks when rodent densities and contacts rates are high (Allen et al. 2006b). In addition, theoretical analyses have shown that when multiple species, as opposed to a single species, are involved in the transmission process, there may be a dilution or amplification effect that impacts disease persistence (Dobson 2004; McCormack and Allen 2007a). Models for spatial spread among discrete patches have shown the importance of there being at least one patch where the disease persists (Arino et al. 2005; Allen et al. 2009; McCormack and Allen 2007b).

## 9.6 Conclusions

Wu and Loucks (1995) have suggested that the most significant contribution of hierarchy theory is as a framework for explicitly incorporating heterogeneity and scale into ecological analysis. In this chapter, we have tried to show some ways in which hierarchical consideration of spatial (and to some extent, temporal) scale can be incorporated into ecological analyses of zoonotic diseases. Our review of the literature also suggests some of the opportunities in infectious disease research resulting from the hierarchical consideration of scale but also some of the challenges. Spatial patterns of zoonotic hosts, the pathogens they harbor, and the host-zoonosis relationships may appear quite different depending on the scale at which we view

them. Although patterns derived from different size scales are often referred to as "emergent" patterns, it is unclear whether the newly recognized large-scale or the small-scale patterns might not be emergent from scale levels either above or below them. Although we could view these scales to be nested subsets of one another, it may be more useful both conceptually and in practice, to view the patterns derived from different scales as complementary information requiring integration, rather than as contradictory results requiring amelioration.

One area of opportunity for the application of hierarchical concepts in zoonotic disease ecology corresponds to scale-related questions facing the global change research community in general; how does global environmental change, especially global warming, manifest itself spatially? It has long been recognized that the spatial distribution of both individuals and communities of species is linked to climate and that these are two-way linkages; however much uncertainty remains about where, when, and how species will respond to climate change (Potter et al. 2013). Fundamentally, the issue reduces to one of scale; the potential impacts of climate change are most often conceptualized at the macroscale (i.e., regional or global) but operate across a range of scales including those more proximate to the host or reservoir organisms (Ashcroft et al. 2009; Diffenbaugh et al. 2005; Suggitt et al. 2011). Adoption of the complementary, hierarchical view of scale provides a framework to address these and similar questions in disease ecology.

Model selection among the wide array of potential formats depends on the virus-host ecological system being investigated, data availability, and the questions to be addressed. Many challenges remain in model formulation, analysis, and simulation of zoonotic disease dynamics that relate to landscape and climate and the wide range of temporal and spatial scales (Allen et al. 2012; Buhnerkempe et al. 2015; Heesterbeek et al. 2015; Lloyd-Smith et al. 2009; Pellis et al. 2015). Addressing the challenges of scale can be met through mathematical and computational approaches and methods being developed in a variety of fields including computer science, ecology, geography, immunology, genetics, mathematics, statistics, and virology. This will require the continued close collaboration across numerous disciplines to converge toward models that reflect the immense biological diversity of pathogen-host ecology.

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### Compliance with Ethical Standards

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**Conflict of Interest** Douglas G. Goodin declares that he has no conflict of interest. Colleen B. Jonsson declares that she has no conflict of interest. Linda J. S. Allen declares that she has no conflict of interest. Robert D. Owen declares that he has no conflict of interest.

**Ethical Approval** This article does not contain any studies with human participants or animals performed by any of the authors.

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