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AMAZONIAN DISEASES IN ISOLATE POPULATIONS (M NACHER, SECTION EDITOR)

Leprosy Transmission in Amazonian Countries: Current Status and Future Trends

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Abstract

Purpose of Review Leprosy is one of the first pathologies described in the history of mankind. However, the ecology, transmission, and pathogenicity of the incriminated bacilli remain poorly understood. Despite effective treatment freely distributed worldwide since 1995, around 200,000 new cases continue to be detected yearly, mostly in the tropics. This review aims to discuss the unique characteristics of leprosy in Amazonian countries, which exhibit a very heterogeneous prevalence among human and animal reservoirs.

Recent Findings Groundbreaking discoveries made in the last 15 years have challenged the dogmas about leprosy reservoirs, transmission, and treatment. The discovery of a new leprosy causative agent in 2008 and the scientific proof of zoonosis transmission of leprosy by nine-banded armadillos in the southern USA in 2011 challenged the prospects of leprosy eradication. In the Amazonian biome, nine-banded and other armadillo species are present but the lack of large-scale studies does not yet allow accurate assessment of the zoonotic risk. Brazil is the second country in the world reporting the highest number of new leprosy cases annually. The disease is also present, albeit with different rates, in all neighboring countries. Throughout the Amazonian biome, leprosy is mainly found in hyperendemic foci, conducive to the emergence and transmission of drug-resistant strains.

Summary The deepening of current knowledge on leprosy reservoirs, transmission, and therapeutic issues, with the One Health approach and the help of molecular biology, will allow a better understanding and management of the public health issues and challenges related to leprosy in Amazonia.

Keywords Leprosy · South America · Amazonia · Guianas · Armadillos · *Mycobacterium leprae*

Introduction

Leprosy, also called Hansen's disease, is a chronic mycobacterial infection of the peripheral nerves and skin that generally manifests as loss of sensation and skin patches. If left untreated, the resulting nerve damage may ultimately lead to disability and disfigurement, which are the main factors responsible for social stigma against leprosy patients. The disease presents over a broad clinical and histopathological spectrum [1] and is caused by the closely related pathogens *Mycobacterium leprae* and *M. lepromatosis* [2]. These obligate intracellular bacteria are clinically indistinguishable and uncultivable in vitro, but molecular diagnostic tests were recently developed using species-specific primers for polymerase chain reaction-based detection [3, 4]. Both pathogens are cultivated in vivo in footpads of conventional and immunodeficient mouse strains (MFP) [3], and *M. leprae* in nine-banded armadillos [5]. However, only a few laboratories worldwide have this expertise to cultivate leprosy bacilli and their slow growth in vivo (12 days doubling time) impairs routinely usage of these models.

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Despite a steady but slow decrease in the new case detection rate (NCDR) of leprosy over the last three decades, associated with widespread implementation of multi-drug therapy (MDT), more than 200,000 new cases of leprosy were reported worldwide in 2018, mostly in the intertropical band [6]. Leprosy remains a significant health problem in several countries, including India and Brazil, which individually report the highest number of new leprosy cases each year with 120,334 and 28,660 respectively in 2018 [6].

Numerous unanswered questions remain regarding its transmission and ecology, as well as its zoonotic and sapronotic reservoirs. The bacilli are mainly transmitted from human to human probably through nasal droplets [7]. Persons living with an untreated multibacillary case (MB) are at higher risk for infection than those without such exposure [8]. However, the majority of new cases cannot recall interaction with a known index case, and several other potential transmission models are now being scrutinized, including zoonotic transmission.

The Amazonian biome is a vast humid tropical ecosystem extending across northwestern Brazil and parts of the following Andean countries: Venezuela, Colombia, Peru, Ecuador, and Bolivia. These countries also have regions with drier tropical ecosystems and mountainous areas. The Amazonian biome also covers the Guiana Shield located in the northern coast of South America: Guyana, Suriname, and French Guiana. In 2018, new leprosy cases were reported in all these countries [6]. Interestingly, tropical South America is also one of the principal habitats of armadillo species, including nine-banded armadillos, which has been confirmed to be a major non-human reservoir of *M. leprae* [9].

In this review, we aim to discuss the unique characteristics of leprosy epidemiology in the Amazonian countries that combine high and low prevalence areas, as well as the potential role of the sole animal reservoir known to date in this region. This is the first review focusing on global leprosy epidemiology in the Amazonian biome and its surroundings.

Epidemiology of Human Leprosy in the Amazonian Countries

A Contrasted Continental Pattern

In the Americas, 93% of new leprosy cases are detected in Brazil, followed by Paraguay, Colombia, Argentina, and Venezuela [6]. Except for Brazil, all countries reported in this review achieved the goal of leprosy elimination as defined by the World Health Organization (WHO) of < 1/10,000 inhabitants (Table 1). Nevertheless, except for Bolivia and Ecuador, new cases continue to be detected among children [6], suggesting that leprosy transmission remains active in the overall region [11]. Andean countries have an approximatively 50-fold lower NCDR compared to Brazil and an approximatively 5-fold lower rate compared to the Guiana Shield countries. The distribution of the NCDR is also contrasted within each country (Fig. 1a).

Leprosy caused by *M. lepromatosis* is rarely reported worldwide but molecular identification of the etiological agent is usually not routinely performed. So far, the species has mostly been identified in Mexico, the Caribbean, and the United States of America (USA), and has been associated with few cases in Brazil [3, 4]. Given this geographical coverage and the similar clinical outcome in both species, it is likely that *M. lepromatosis* might also be present in other Amazonian countries, but epidemiological investigations are required to improve our knowledge of the distribution of this particular leprosy agent.

Regional Epidemiological Patterns

Brazil

According to the Brazilian Ministry of Health, the spatial distribution of leprosy is highly heterogeneous, with an annual NCDR ranging from 0.1 to 13.8/10,000 inhabitants in the states of Rio Grande do Sul (South) and Mato Grosso (partly in the Amazonian region) in 2018, respectively. The average NCDR in the Amazonian states (4.7/10,000 inhabitants) is 4.2-fold higher compared to the non-Amazonian states in Brazil (1.1/10,000 inhabitants). Additionally, there is evidence of high rates of hidden prevalence of leprosy and subclinical infection among schoolchildren in the Amazonian region, which represent active foci of infection [25, 26]. During the last 2 years, improved efforts of active search have increased the NCDR from 1.22 in 2016 to 1.37/10,000 inhabitants in 2018 [27].

Andean Countries With Amazonian Regions

Leprosy was eliminated (according to the WHO definition) in these countries before 2011 [28]. In 2018, the NCDR was ranging from 0.01 in Peru to 0.08/10,000 inhabitants in Venezuela (Table 1). However, the persistence of transmission pockets has prevented eradication of the disease at sub-national level in Colombia and Venezuela. A high burden of the disease has persisted in 13 out of 32 regions in Colombia in 2016 [29] and 5 out of 23 states in Venezuela [10, 30]. In contrast, no noticeable similar transmission pockets have been reported in Ecuador, Bolivia, and Peru sub-national levels. The proportion of new cases diagnosed with grade 2 disability in these countries, however, is alarming with 5%, 19%, and 29%, respectively (Table 1). These observations may be influenced by weak health care networks

Table 1 Leprosy situation in 2018 in South America by country

	New cases in 2018 (Nb)	NCDR 2018 (per 10,000 inhabitants)	MB in new cases (%)	Children < 14 years in new cases (%)	Grade 2 disability in new cases (%)	Relapse cases (Nb)
Amazonian countries	29,498	0.84	77	6	7	1930
Brazil	28,660	1.37	77	6	7	1840
Andean countries	750	0.05	78	6	10	86
Colombia	383[a]	0.08	69	10[a]	10	47
Venezuela	245	0.08[b]	86[b]	3	6	22[b]
Bolivia	52	0.05	83	0	19	1
Ecuador	42	0.02	98	0	5	10
Peru	28	0.01	100	4	29	6
Guiana Shield	88	0.54	74	13	19	4
Guyana	50	0.64	84	14	26	0
Suriname	24	0.42	63	17	8	4
French Guiana[c]	14	0.50	57	0	14	0
Non-Amazonian countries	627	0.09	87	13	3	9%
Paraguay	345	0.50	88	3	12	52
Uruguay	6	0.02	100	0	17	0
Chile	7	0.004	57	0	0	0
Argentina	269	0.06	87	2	14	2

Reference: [6]; unless otherwise specified: [a] N. Cardona-Castro, unpublished data; [b] [10]; [c] P. Couppié, unpublished data

Nb number, *NCDR* new case detection rate, *MB* multi-bacillary

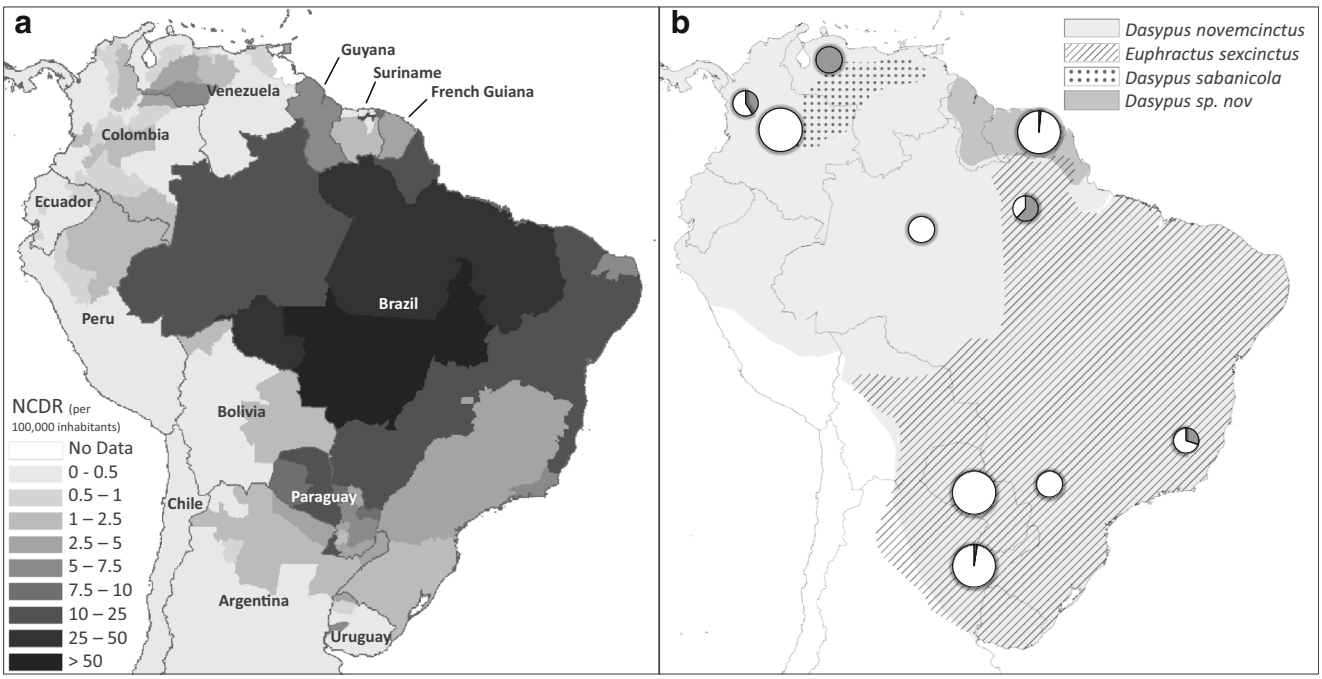

Fig. 1 Leprosy new case detection rates (NCDR) in human population in 2018 and armadillo *Mycobacterium leprae* infection point-estimate prevalence in Amazonian countries. **a** Map of leprosy NCDR per 100,000 inhabitants in 2018, per state. NCDR is reported by state/region for each country, except Guyana and French Guiana. NCDR are for 2018, except 2017 for Ecuador. **b** Map of the armadillo's species range (only species known to be naturally infected by *M. leprae*) and research studies on leprosy detection in wild armadillos. Larger circles indicate studies with at least 50 specimens analyzed. The proportion of positive specimens appear in gray. Details of represented investigations are in Table 2

and a lack of awareness about leprosy among dermatologists and the general population, contributing to underdetection of the real NCDR and delayed diagnosis in these countries.

The Guiana Shield

Guyana, Suriname, and French Guiana have a NCDR of 0.42, 0.50, and 0.64/10,000 inhabitants, respectively (Table 1). In French Guiana, only 22% of new cases from the period 2007–2014 were considered autochthonous. Some 56% of the cases originated from Brazil, mainly among illegal gold miners [31], who are subject to harsh living conditions, poor health, and poor access to health care [32]. Similar conclusions were recently drawn in Guyana, which has seen a re-emergence of leprosy following human migration from endemic regions [33]. In 2018, patients diagnosed with a grade 2 disability represented 8%, 14%, and 26% of new leprosy cases, respectively, in Suriname, French Guiana, and Guyana (Table 1), an unfortunate indicator that there is insufficient awareness about leprosy in the community.

The Emergence of Regional Antimicrobial Resistance

In the face of the development of resistance following dapsone monotherapy, the WHO has recommended the use of MDT regimen since 1981 [34]. MDT is efficient and includes a combination of dapsone, rifampicin, and clofazimine. However, dapsone and rifampicin drug-resistant strains are still circulating in the population following the monotherapy era [35••]. Resistance to ofloxacin, a second-line drug that is used in the case of rifampicin resistance or intolerance, is also subject to emergence, perhaps because of its routine use in many other common diseases [36]. Minocycline and clarithromycin are the only other drugs used in case of resistance or intolerance to rifampicin [36]. MFP assay is the gold standard method for antimicrobial susceptibility testing in leprosy. However, this method is time-consuming (requiring 6 to 9 months to complete) and requires highly trained technicians. Therefore, the method is not suitable for routine use in a surveillance program, and molecular screening techniques are now more the norm. Molecular assays for drug susceptibility screening have been developed for rifampicin, dapsone, and ofloxacin [37]. The mechanism of action of clofazimine remains to be elucidated and, so far, *M. leprae* resistance to minocycline and clarithromycin has not yet been detected in leprosy patients.

Over the last decade, a surveillance program implemented by WHO investigated the rate of *M. leprae* drug resistance to dapsone, rifampicin, and ofloxacin worldwide among relapse and new leprosy cases, with special emphasize on rifampicin [35••]. Rifampicin is the cornerstone drug of leprosy treatment due to its rapid bactericidal activity, so MDT efficiency is severely compromised in case of resistance. The 2009–2015 report showed that Brazil, Colombia, and India were among countries reporting more than five rifampicin-resistant cases [35••]. In Colombia, rifampicin- and dapsone-resistant cases are reported in relapse patients (9/37) previously treated with dapsone monotherapy [38], while resistances were found in both primary (5/32) and relapse (27/321) cases in Brazil [39]. Recent literature also reported dapsone and ofloxacin resistance as well as circulation of multidrug-resistant strains in Brazil [40••]. In a hyper-endemic village that was once a leprosarium in Pará (Brazilian amazon), 43% of patients had a *M. leprae* strain resistant to one or more anti-leprosy drugs, in both new patients and relapse cases. Furthermore, the authors observed both familial and community-level clustering of resistant strains in this village, indicating that this particular setting with high leprosy prevalence in a genetically susceptible population was driving hyper-endemicity with the emergence and transmission of drug-resistant *M. leprae* strains [41].

Except for Brazil and Colombia, none of the Amazonian countries were part of the drug resistance surveillance program [35••]. In Venezuela and Bolivia, during a 3-year survey published in 2011, with 197 patients and 10 patients respectively, only one case of dapsone resistance-associated mutation was observed [42]. No information is available for Ecuador, Peru, nor Suriname. Still, all three countries showed a high number of relapse cases compared to their low NCDR, with 10, 6, and 4 respectively in 2018 (Table 1). These data suggest the possible presence of drug-resistant strains as in Colombia (12% relapse cases) or reflect poor treatment compliance in the patient.

Reinfection might also be a major driver of the persistence of (hyper)endemicity in the Amazonian region [43]. *M. leprae* strains from the same patients or closely related individuals may differ only with few single nucleotide polymorphisms (SNPs) [40••, 44] and these differences can be best investigated through whole-genome sequencing (WGS) approach [45] which are currently not performed routinely.

Environmental Sources of Leprosy Bacilli

Armadillo as a *M. leprae* Reservoir

One of the major features of leprosy in the Americas is the presence of nine-banded armadillos (*Dasypus novemcinctus*), the most important non-human *M. leprae* reservoir known to date. Naturally infected nine-banded armadillos were first discovered in 1975 in the USA [46]. The presence of *M. leprae* among armadillos was later confirmed in Mexico [47], non-Amazonian parts of Brazil [12, 15, 17–19, 48], Argentina [49], and Colombia [20], and recently was confirmed in the Brazilian Amazonia [14•].

The first evidence of zoonotic leprosy transmission from nine-banded armadillos to humans was provided by Truman

et al. in 2011, who showed that 64% of patients from the same areas who had a possible endemic exposure to armadillo-borne *M. leprae* were carrying the same *M. leprae* strain as 88% of the naturally infected nine-banded armadillos in Southern USA [50••]. Later, a second zoonotic strain was discovered in Florida, where 42% of patients harbored one of the two known zoonotic strains [51]. The zoonotic transmission risk is likely to increase in the USA, as armadillo range is expanding, leprosy is spreading in armadillo populations, and armadillo-human interactions are intensifying as a result of increasing urbanization [52]. High density and humidity are the only factors linked to *M. leprae* prevalence in nine-banded armadillos so far [9].

There are 9 described armadillo species in Amazonia and the Guianas [53], and a tenth in the Guiana Shield, *Dasypus* sp. nov., pending description [54]. Apart from *D. novemcinctus*, at least three other species may be naturally infected with *M. leprae* (Fig. 1b): *Euphractus sexcinctus* [12, 15], *Dasypus* sp. nov. [22], and *D. sabanicola* (A. Paniz-Mondolfi, P. Singh; unpublished data). In South America, point-prevalence rates of *M. leprae* infection in wild armadillos range from 0 to 100% (Table 2). *D. septemcinctus* is experimentally susceptible to *M. leprae* infection and would need further assessment in wild animals [55].

To date, the precise mode of transmission of *M. leprae* from armadillos to humans remains unclear. The bacilli may pass through direct contact with armadillo skin, blood, and body fluids, perhaps through hunting, cleaning, and preparing the meat [5, 14, 56]. The bacilli may be shared, through the respiratory routes [57], or spread by contact when keeping animals in an enclosure to purge or fatten it [14, 56], or for traditional medicine or using parts of it to make objects [58]. Several studies have evaluated the risk of leprosy from eating armadillo meat but the results remain inconclusive [48, 59–62]. Furthermore, the outcome of these studies should be taken with caution because of potential biases and given that risk assessments may be impacted by the high proportion of people naturally immune to leprosy [63] and the very long incubation period [64].

Despite a ban by most South American countries on hunting armadillos, except for native populations, the consumption of armadillo meat is widespread. In Brazil, armadillo hunting is frequent for both food and leisure [56]. In French Guiana, it is legal to hunt and sell *Dasypus* armadillo meat. In Venezuela, consumption of armadillo as well as other game has been traditional in many areas and has increased in the context of the humanitarian crisis.

We lack important information to understand the discrepancies observed in animal *M. leprae* infection prevalence in similar ecosystems [5]. Furthermore, pockets of high endemicity and high animal *M. leprae* infection prevalence do not necessarily overlap [13], part of which could be attributable to the role of host genetic susceptibility to leprosy which may vary in different ethnic groups/populations [65–68]. The assessment of animal reservoir extension and drivers of animal *M. leprae* infection prevalence and susceptibility [9], as well as the animal-to-animal and animal-to-human transmission pathways [69] including the role of the environment in its transmission, would require further investigation.

Questioned Role of Other Species

The recent discovery of red squirrels (*Sciurus vulgaris*) infected with *M. leprae* and *M. lepromatosis* in the British Isles [70••] pushes even further our understanding of leprosy ecology and suggests the existence of other reservoirs of the bacilli. In the Amazonian and Guiana Shield regions, where the biodiversity is among the richest in the world [71], molecular surveys in lowland tapirs (*Tapirus terrestris*), owl (*Aotus trivirgatus*), and capuchin (*Sapajus apella*) monkeys and margay cats (*Leopardus wiedii*) in Mato Grosso state in Brazil have detected *M. leprae* DNA from nasal swabs [72]. The nose may serve as a filter of the environment and there is no other evidence that these animals may serve as reservoir of the bacilli, but detecting *M. leprae* DNA on nasal mucosa reinforces the hypotheses of an environmental presence of the bacilli made possible by excretion from human and animal carriers.

The Role of Soil and Water

Indirect contact with leprosy patients through the bacilli they shed to the environment, such as when bathing [60], could facilitate transmission and is one of the possible explanations for the disease persistence in human despite the effectiveness of MDT [73]. *M. leprae* DNA has been found in water and soil samples taken from the immediate peridomicile area of leprosy patients in India [74–76], as well as in soil samples of leprosy patients' house in Bangladesh [77•]. In the Northeastern Brazilian state of Ceará, *M. leprae* DNA was found in 54.4% of natural water sources (lakes, dams, streams, and wells) used by locals [78]. As many as 76.7% of these samples were harboring viable *M. leprae* [79].

Another hypothesis is indirect transmission of leprosy bacilli through soil contaminated by infected animals, to which people can be exposed when cultivating or gardening [51], or hunting [56]. Armadillos dig burrows for shelter and feeding. *M. leprae* DNA has been detected in armadillo burrow soil in Suriname [77•]. Shedding leprosy bacilli from infected armadillos might occur during digging and sheltering, leading to accumulation of bacilli in burrows where they remain protected from sunlight and less subject to desiccation [80].

It is unclear how an obligate intracellular parasite like *M. leprae*, which cannot be cultivated on artificial media in

Table 2 Research studies of *M. leprae* infection in wild armadillos in tropical and subtropical South America

Country	State and location	Sample period	Sample size and species	*M. leprae* molecular biology detection results (method)	*M. leprae* serology detection results (method)	AFB/*M. leprae* histology detection results (method)	Reference
Bolivia	No studies known						
Brazil	Rio Grande do Norte: 20 distinct locations within 5 rural municipalities	2016	20 *Euphractus sexcinctus*	100% + (RLEP PCR on tissue)	100% + anti-PGL1 Ab and 5% + anti-LID1 Ab (ELISA); 85% + NDO-LID1; 80% + ML flow test	ND	[12]
	Amazonas: Coari municipality	2015	12 *Dasypus novemcinctus*	0% + (RLEP qPCR on tissue)	ND	0% + (Fite-Faraco staining)	[13]
	Pará: 2 rural communities in Belterra (western Pará)	NS	16 *D. novemcinctus*	62% + (RLEP PCR on tissue)	ND	Unknown proportion of PGL1 Ag-positive spleen sections (immunohistochemical staining)	[14•]
	Ceará: 12 endemic municipalities all across Ceará state	2007	27 *D. novemcinctus* and 2 *E. sexcinctus*	19% + DN and 50% + ES (RLEP nested PCR on tissue)	ND	ND	[15]
	São Paulo: 4 municipalities. Mato Grosso do Sul: Pantanal da Nhecolândia	NS	17 *D. novemcinctus*, 3 *E. sexcinctus*, 2 *Cabassous tatouay*, and 1 *C. unicinctus*	0% + (RLEP PCR on tissue)	ND	0% + (Ziehl-Neelsen staining)	[16]
	Espírito Santo: Alegre municipality	2004–2006	20 *D. novemcinctus*	ND	20% + PGL1 (ELISA)	ND	[17]
	Espírito Santo: unspecified rural area	1999–2006 (?)	37 *D. novemcinctus*	ND	29.7% + ML flow test	ND	[18]
	Espírito Santo: unspecified rural area	1999–2001 (?)	14 *D. novemcinctus*	35.7% + (RLEP PCR on blood)	ND	ND	[19]
Colombia	Antioquia: rural areas of Barbosa municipality	2007–2008	22 *D. novemcinctus*	41% + (RLEP nested PCR on tissue)	ND	ND	[20]
	–	–	205 (*D. novemcinctus* and other unspecified species)	–	–	0% (unspecified technique)	[21]
Ecuador	No studies known						
French Guiana	Petit-Saut dam lake area	1994–1995	120 *Dasypus* sp. nov. and 42 *D. kappleri*	ND	6.7% + anti-PGL1 D. sp. nov.; 0% + anti-PGL1 DK; 0% + anti-LID1 *D.* sp. nov. and DK (ELISA)	ND	[22]
	All French Guiana	2015–2019	67 *D.* sp. nov. and 8 *D. kappleri*	1.5% + *D.* sp. nov. and 0% + *D. kappleri* (RLEP qPCR on tissue)	ND	PCR-positive specimen confirmed by AFB on Fite-Faraco staining	[22]
Guyana	No studies known						
Peru	No studies known						
Suriname	Pikin Slee and Gujaba	2018–2019	3 *Dasypus novemcinctus* or sp. nov. (?)	0% (RLEP qPCR on tissue)	0% + anti-PGL1 (ELISA)	ND	W. Faber, A. Geluk; H. Menke, K. Sewpersad, and T. Pieters, unpublished data
Venezuela	All across Venezuela	1980s–1990s	Hundreds of *D. novemcinctus*	ND	ND	0% + AFB (Fite-Faraco staining)	A. Paniz-Mondolfi, personal data
	Portugesa: Ospino	2014	1 *D. sabanicola*	ND	ND		

Table 2 (continued)

Country	State and location	Sample period	Sample size and species	*M. leprae* molecular biology detection results (method)	*M. leprae* serology detection results (method)	AFB/*M. leprae* histology detection results (method)	Reference
						100% + AFB (Fite-Faraco staining)	A. Paniz-Mondolfi and P. Sing, unpublished data
Argentina	Corrientes: Mercedes	NS	83 (unspecified species)	–	2.4% + PGL1	0% + AFB (staining method not described)	[9]
	Corrientes	NS	132 *D. novemcinctus*	ND	ND	6.8% + AFB (Ziehl-Neelsen, Fite-Faraco, and King-Young staining)	[23]
Paraguay	5 different locations	1973–1977	104 (*D. novemcinctus*, *Chaetophractus villosus*, and *C. vellerosus*)	ND	ND	0% + AFB (staining method not described)	[24]
Uruguay	No studies known						

AFB, acid-fast bacilli; +, positive; *RLEP*, *M. leprae*-specific repetitive element; *PCR*, polymerase chain reaction; *PGL1*, phenolic glycolipid-1 or ND-O; *Ab*, antibodies; *LID1*, fusion of ML0405 and ML2331 proteins; *ELISA*, enzyme-linked immunosorbent assay; *NDO-LID*, ND-O antigen and LID1 fusion protein conjugate; *ML flow test*, immunochromatographic test detecting IgM Ab to PGL1; *ND*, not done; *qPCR*, quantitative real-time PCR; *NS*, not specified; *Ag*, antigen; *DN*, *Dasypus novemcinctus*; *ES*, *Euphractus sexcinctus*; *DK*, *Dasypus kappleri*

the laboratory, might survive in the natural environment. Recently, however, it was shown that free-living amoebas could provide a convenient refuge for *M. leprae* by ingesting the bacilli shed from infected human/animal hosts. Recent studies show that ingested bacilli can remain viable for extended durations and could enable ongoing transmission [76]. Ubiquitous environmental amoebas like *Acanthamoeba* and *Vermamoeba* have been found in armadillo burrows [81] that might benefit bacterial survival. *Acanthamoeba castellanii* are capable of ingesting *M. leprae* and after 72 h, the extracted bacilli's viability is intact [82]. In addition, *M. leprae* can remain virulent for at least 35 days and viable up to 8 months after phagocytosis in encysted *A. castellanii* and *A. polyphaga* [83]. Infected amoebas have not yet been observed in nature. Nevertheless, with its very high humidity throughout the year [84], Amazonia might provide a perfect habitat for a prolonged survival of the bacilli in the environment [85].

The role of blood-sucking arthropods is also under scrutiny, and to date, kissing bugs and ticks, parasites of both humans and armadillos, have been experimentally demonstrated to be capable of ingesting *M. leprae*. Bacilli can remain alive in their digestive tract for at least several days and later excreted. Experimentally, kissing bugs from the *Rhodnius* genus are capable of excreting viable and infective *M. leprae* in their feces [86]. Some kissing bugs feed on armadillos, including species from the terrestrial genera *Panstrongylus* [87], *Rhodnius* [88], and *Triatoma* [89–92], and their habitat is associated with armadillo burrows [93, 94]. Whether kissing bugs from the *Panstrongylus* and *Triatoma* genera are also able to excrete *M. leprae* is not yet known. Ticks, mainly from the genera *Amblyomma*, are also frequent parasites of armadillos in Brazil [95, 96] and in French Guiana [97]. Ticks may ingest viable *M. leprae* while taking blood meals as armadillos do periodically show bacteremia [5]. Experimentally, ticks from the *Amblyomma sculptum* species are capable of vertical transmission of *M. leprae* from the infected female to her larvae and are therefore potential competent vectors of *M. leprae* and may be implicated in transmission between armadillos and to humans [98]. However, the presence of different species of kissing bugs and ticks naturally infected with *M. leprae* still remains to be assessed.

Molecular Epidemiology

M. leprae strains possess strikingly low levels of genetic diversity [99, 100] and the genomic approach has provided insights into the intriguing biology of *M. leprae* with extensive reductive evolution [101].

Comparative genomics analysis of *M. leprae* strains has identified SNPs which enable to distinguish SNP types 1 to 4 [102]. The remarkable genetic conservation of *M. leprae* became further evident when four strains from different parts of the world (India, Brazil, Thailand, and USA) were

compared at the genome level, revealing only a few hundred variants. Upon analyzing these genomic markers in a set of over 400 strains, the phylogeographic association of *M. leprae* revealed an association of the SNP types with the routes of ancient human migration. These investigations also led to the development of a more robust genotyping schemes comprising of 16 SNP subtypes under the four major SNP types described earlier [45, 103]. Several sets of primers were developed for molecular characterization of strains in endemic countries. Such information has further helped in identifying unique genomic markers specific to a particular genotype, such as the 11 bp deletion at position 17915 in the 3I strains which can be identified even on 2% agarose gel without any sequencing [50••]. Another approach of identifying locally predominant genotypes such as SNP type 3I in samples from Colombia based on PCR-restriction fragment length polymorphism of the SNP 7614 has been described [58] and similar approach can be developed for SNP type 1D strains, as these are predominant in India [104–106].

However, the limitation of SNP typing for monitoring local transmission dynamics was evident from the fact that most of the strains from a given geographic area belonged to the same SNP type. Hence, for monitoring local transmission, a set of selected VNTRs are considered very useful [107], though some of these VNTR loci can be hyper-variable, i.e., some VNTR loci can differ between different lesions in the same patient [108]. In addition, the use of VNTRs alone has a drawback that deriving a reliable inference regarding which strains are ancestral compared to the others is very difficult. Hence, a combined genotyping scheme using a selected panel of SNPs and VNTRs has been successfully used for confirming the zoonotic link between armadillo and human leprosy in Southern USA [50••, 51].

Recent advents in the next-generation sequencing technologies and target DNA enrichment methods have enabled detailed comparative genomic investigations into a large number of *M. leprae* strains representing all known SNP types [4, 40, 44, 109]. These approaches allowed to make progress on issues related to relapse and re-infection, which was not feasible even with the combined analysis of SNP genotyping and VNTRs [45].

Studies have shown that dominant strains among humans in Brazil are SNP subtypes 4P and 4N, as well as SNP subtype 3I [40, 110]. In Colombia, SNP subtype 4N is mainly found in the Northeast and Caribbean coast and SNP type 3 in the Andes, according to the origin of the Colombian population [111], while in Venezuela, a majority of human strains are SNP subtype 3I with some 4P, 1D, 4O, and 4N [42, 103]. For the Guiana Shield, a SNP subtype 1A was found in a patient in Guyana [103] and a SNP type 4 in a one-century-old skeleton has been uncovered from a cemetery in a former leprosarium in Suriname [112]. This broad diversity of *M. leprae* genotypes indicates that there have been multiple introductions, e.g., SNP type 3I from Europe likely got introduced during the period of colonialism; SNP type 4 from West Africa likely reached America with the slave trade and SNP type 1 most likely arrived through Asian migrations [103]. Besides, additional admixture was observed inside the SNP type 3I suggesting several introductions from Europe [40••].

Zoonotic strains in *E. sexcinctus* in northeastern Brazil and in *D. novemcinctus* in the Southern USA are reported as SNP type 3 and 3I-2 types respectively [15, 50••, 51]. In the positive *D. sabanicola* found in Venezuela, preliminary results suggest the presence of a mixed genotype infection (3I and 1D) which needs further investigation (A. Paniz-Mondolfi, P. Singh; unpublished data). Interestingly, SNP type 3I is the most prevalent genotype in Venezuelan patients, followed by the 1D genotype, clearly correlating from an epidemiological standpoint. Moreover, genotypes 3I and 1D are also the most prevalent (73% and 13% respectively) among clinical specimens in hyperendemic areas [42]. There is currently no information on putative genetic differences of strains circulating in low and high endemic areas at genome level.

Even if it has not yet been described in naturally infected armadillos, the genetically distant SNP type 4P, which is predominant in patients in South America, is capable to experimentally infect and impair armadillo's health similar to the 3I genotype zoonotic strains [113•], suggesting that other human genotypes may have the potential of infecting armadillos. Although not directly detected in armadillos yet, *M. leprae* DNA with SNP type 1 or 2 have been found in armadillo burrow soil in Suriname [77•]. Also, the SNP types of *M. leprae* detected in soil/water samples and the leprosy patients living in those areas are often the same, whether in India [73] or in Brazil [78], which reinforces the hypothesis of an environmental reservoir of *M. leprae* likely contributing in continuous leprosy transmission.

Conclusions

This is the first time that all studies carried out in this region have been brought together and put into perspective, highlighting the unique characteristics concerning the epidemiology of leprosy in the Amazonian biome and surrounding regions. Pockets of high human leprosy endemicity within global areas of medium endemicity and the presence of armadillos, the most important non-human reservoir of *M. leprae* known to date, characterize leprosy epidemiology in this region. The discovery of the zoonotic nature of leprosy in the USA and the recent identification of another animal reservoir in the British Isles marked the beginning of a renewed interest in the field. It also introduced a new paradigm for leprosy epidemiology, prevention, and control, which shifted from a typically human-only disease, that could theoretically be eradicated, to a zoonotic disease, at least in the Americas, questioning the likelihood of leprosy eradication in a near

future. Emergence of drug resistance also challenges the leprosy elimination goal. Drug resistance in primary leprosy cases urged health authorities to implement systematic drug susceptibility testing for all leprosy cases. This is especially important in a high endemic area such as Brazil and in countries reporting high relapse case rates, to prevent emergence and transmission of drug-resistant strains. As an example, the Brazilian government have recently implemented a drug resistance surveillance network in which samples are screened centrally in a reference laboratory. Such an initiative should be acknowledged, encouraged, and might be used for future collaboration between the Amazonian countries where drug resistance surveillance is not routinely performed. Besides, the existence of such a laboratory could also allow to use specific techniques such as WGS to be implemented for large-scale studies. So far, only few genomes from South America are available and additional investigation would help to identify bacterial markers possibly linked with relapse and high endemicity. There are still many gaps to fill in order to better characterize the leprosy eco-epidemiology and to evaluate the burden of zoonosis part in leprosy transmission in the Amazonian countries. Broadly speaking, this region offers a unique and promising opportunity to increase our understanding of leprosy agent's eco-epidemiology worldwide.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent All reported studies/experiments with human or animal subjects performed by the authors complied with all applicable ethical standards.

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- •• Of major importance

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