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Review

Prevalence of *Mycobacterium leprae* in the environment: A review

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The purpose of this review was to study the presence of *Mycobacterium leprae* in the environment and its relation with meteorological variables such as temperature and humidity. There are reports, which provide evidence that meteorological factors such as temperature and soil humidity can influence the dynamics of *M. leprae*. However, leprosy cases are registered both in the rainy and dry seasons, indicating that *M. leprae* remains viable in different environmental conditions. Therefore, it is difficult to establish the meteorological pattern(s) required to maintain the bacilli in the environment. The extensive area of endemic countries, endemicity in the bordering countries, diversity of biomes, and lack of urban infrastructure together with weather features are possible factors that influence transmission of the disease.

Key words: Leprosy, environmental health, molecular biology.

INTRODUCTION

Leprosy is a chronic infectious disease caused by the bacillus *Mycobacterium* (*M.*) *leprae*. The disease, which is prevalent in most tropical and subtropical regions of the world (World Health Organization (WHO), 2014), can manifest itself in different clinical forms depending on the type of host immune response.

In 2011, the WHO published the Enhanced Global Strategy for minimizing the leprosy burden, in order to reduce the disease incidence and its physical, social, and economic consequences. Brazil and India are responsible for 90% of the leprosy cases in the world. In 2012, 232,857 new cases of leprosy were registered worldwide. Regions with the highest number of detected cases are Southeast Asia (71%), the Americas (15.5%), India (134,752 cases), and Brazil (33,303 cases) according to the WHO (2013).

The transmission mechanism for leprosy remains unclear, despite it being studied for centuries. For a long time, it was believed that the only source of transmission of *M. leprae*, the main etiologic agent, was multibacillary patients not receiving treatment. There are, however, a considerable number of epidemiological and microbiological observations indicating that environmental sources (Loughry et al., 2009) can also play an important role in transmission of the disease by indirect contact (Kadza, 2000).

Molecular biological studies have revealed the presence of bacilli in the environment. These findings

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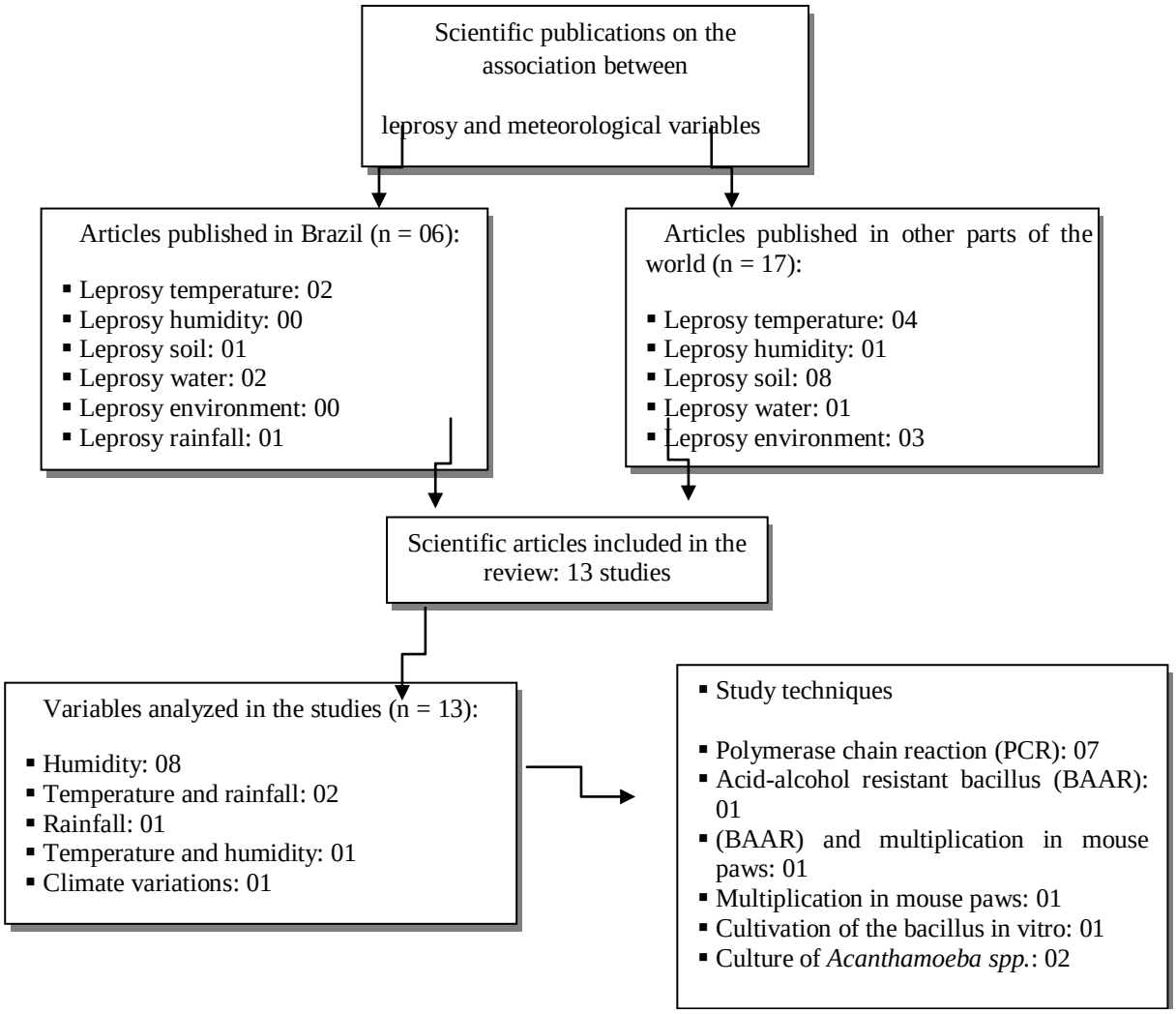

Figure 1. Flowchart of the literature review process: publications from 1980–2014 on the environmental prevalence of *M. leprae* and its association with meteorological variables.

strengthen the hypothesis of transmission of the disease independent of contact with patients, and/or maintenance of viable bacilli in the environment for long periods. As a corollary, meteorological conditions in the environment that favor the maintenance and viability of the bacilli must also be important to the disease transmission. To evaluate this hypothesis, we analyzed existing scientific literature on the presence of *M. leprae* in the environment, and its relation with meteorological variables.

LEPROSY RESEARCH: FUTURE TARGETS AND PRIORITIES

Of the 13 original articles on the association between *M. leprae* and the environment, eight involved relative humidity (%), and one each involved: temperature (°C) and rainfall (mm), only rainfall, temperature and humidity, and culture of *Acanthamoeba castellanii* and climate variations (Figure 1).

In the 1980s, studies aimed to find possible relationships between the environment and *M. leprae*. One of the techniques used in this period was Ziehl-Neelsen (ZN) staining, which is specific for acid-alcohol-resistant bacilli (BAAR) and non-cultivable acid-fast bacilli (NCAFB) (Salem and Fonseca, 1982; Kadza, 1981).

ZN staining is a bacilloscopic procedure that effectively stains acid-alcohol resistant mycobacteria; the staining intensity varies with the species of mycobacterium the microorganisms obtained from the soil or water samples (Wahyuni et al., 2010).

From 1980 to 1990, viability of the bacilli was tested under different environmental conditions. The specificity of the bacilli was determined using a multiplication method of *M. leprae* in mouse paws. Shepard in 1960

revealed their viability, the monitoring tests chemotherapeutic and levels of drug resistance using inoculation of *M. leprae* in the footpads of normal and immune-compromised mice (Azulay et al., 2008).

In 2000, research focusing on cultivation of the bacillus *in vitro* was unsuccessful, although some studies have shown evidence of metabolic activity *in vitro* (Levy and Ji, 2006).

Genome analyses of the mycobacterium have shown that cultivation on artificial media is not possible. This is because even less than half of the genome contains functional genes; the majority consists of inactivated or pseudo genes. Moreover, the genome has undergone progressive reduction, accompanied by genetic degradation and a decrease in size. These evolutionary changes originated with the elimination of important metabolic pathways and related ancillary functions of *M. leprae*, particularly those involved in catabolism (Levy and Ji, 2006).

The absence of experimental models that mimic the disease in humans, and the inability to grow *M. leprae in vitro* represent historically important limitations in the development of appropriate tools for the control of leprosy. However, owing to advances in molecular biology techniques, many studies on the *M. leprae* genome have been conducted (Silvestre, 2011).

From 2000 onwards, amplification of specific DNA sequences of the bacillus became possible by polymerase chain reaction (PCR). This technique was advantageous in that it required small numbers of the bacilli and was highly sensitive (Donoghue et al., 2001).

Recent publications on the genome sequences of *M. leprae, M. tuberculosis*, *M. bovis* and *M. smegmatis*, along with the almost-complete sequences of several other mycobacterial species (*M. avium*, *M. marinum*, *M. paratuberculosis,* and *M. ulcerans)* have enabled the identification of unique and specific proteins in *M. leprae* (Cole et al., 2001; Geluk et al., 2005).

The main method carried out in the study comprised PCR of samples from soil and water, by having high sensitivity of the bacillus, since the sequences of ribosomal RNA (rRNA) (Silvestre, 2011; Donoghue et al., 2001).

New typing methods to conclusively identify *M. leprae* have evolved with the technique of multiple-locus value analysis (MLVA). This technique ensures greater genetic differentiation in a wide range of samples with allelic diversity within a community, and thus, is useful in the detection of leprosy transmission (Young et al., 2004; Groathouse et al., 2004; Zhang et al., 2005).

Table 1 shows studies on *M. leprae* in the environment and its relation with meteorological variables published between 1980 and 2014, presented in chronological order and by the variables analyzed. In terms of temporal evolution, the highest number of studies has been published since 2000, the majority being conducted in India.

Further, analyses of soil samples have shown that *M. leprae* also has non-human reservoirs such as armadillos and protozoans. Moreover, environments favorable to pathogen survival, such as water, soil, sphagnum, as well as other factors are propitious to its transmission (Desikan and Sreevatsa, 1995; Truman, 2005; Turankar et al., 2012). The presence of *M. leprae* in water sources reflects its association with protozoans or invertebrate hosts, as well as some free-living mycobacteria (Whan et al., 2006).

Studies on free-living amoebae have revealed an association with water consumed by the population, and in some cases, with treated water (Falkinham et al., 2001). Wheat et al. (2014) showed that *M. leprae* can remain viable long-term in environmental ubiquitous free-living amoebae and retain the virulence in mouse model.

M. leprae can survive outside its main host in free-living protozoans as *Acanthamoeba castellanii* for 4 days without apparent difficult. These results show that free-living terrestrial or water-borne protozoans can act as “wild macrophages,” facilitating survival of the bacilli in the environment when expelled from the human host (Lahiri and Krahenbuhl, 2008). A recent experimental study verified that *M. leprae* remains viable for up to eight months within amoebic cysts (Wheat et al., 2014).

Multibacillary patients spread the leprosy bacilli through their nasal secretions, which in tropical regions remain viable for up to 9 days, and up to 46 days in moist soil at room temperature (Desikan, 1997). In the province of Maluku, Indonesia, where leprosy is endemic, 27% of the villagers were found to carry the bacillus within their nasal cavities (Izumi et al., 1998).

A study carried out in West Bengal, India, in 2009 analyzed 207 soil samples in areas with active cases of leprosy. *M. leprae* was viable in 28 of these samples. Single nucleotide polymorphism (SNP) testing of the bacilli found in both the environment and in patients revealed that they were of the same genotype. The study demonstrated the potential role of viable bacilli in the environment as a source of disease transmission (Turankar et al., 2012). However, it had limitations with regard to identifying the metabolic activity of the bacilli, as well as mechanisms of extended survival and transmission of *M. leprae* in different environments. Furthermore, it was observed that the proportion of samples with evidence of *M. leprae* was higher in humid areas (Izumi et al., 1998; Desikan, 1997). These findings indicate that humidity and rain helps the bacilli to survive for longer periods in the environment.

In a study conducted in Ghatampur, India, in 2008, 80 soil samples were collected, of which 40 were from residential areas housing leprosy patients, while the other 40 were from places with no patients identified (control). Of the 28 soil samples positive for viable *M. leprae*, 22 were from the residential areas, while 6 were from the control areas. Thus, the bacilli released by patients during coughing and sneezing can survive for varying periods depending on the environmental conditions. This

Table 1. Studies on the presence of *M. leprae* in the environment and its relationship with meteorological variables, published between 1980 to 2014.

Reference year of publication	Place and time of study	Variable and technique	Main findings
Humidity			
Wahyuni et al., 2010 Indonesian Journal of Tropical and Infectious Disease	Java, Indonesia 2008	Humidity PCR	Positive results in 22/90 water samples collected, 11 water samples, collected from wells that were never used by leprosy cases, were also positive.
Adriaty et al., 2010 Indonesian Journal of Tropical and Infectious Disease	Island Poteran, Sumenep, Madura and East Java, Indonesia 2009	Humidity PCR	201 samples of *M. leprae*, 91 collected from wells; 26.4% samples PCR-positive. The water used for clinical leprosy groups showed positive PCR in samples, and groups without the disease who used this water were more susceptible to leprosy.
Turankar et al., 2012 Infection, Genetics and Evolution	West Bengal, India 2009	Humidity PCR	Samples, both from the environment (soil) and the multibacilary patients exhibited the same genotype when tested by single nucleotide polymorphism (SNP) typing.
Temperature and Humidity			
Desikan e Sreevatsa, 1995 Leprosy Review	Agra, India 1993	Temperature Humidity Multiplication of *M. leprae* in mouse paws	Between the months of March and April, with temperatures between 24-33°C and atmospheric humidity of 44-28%, the bacilli survived for 14 days. During the monsoon season in August and September, with atmospheric humidity between 72-80% and temperatures of 29-33°C the bacilli survived for 28 days. In September and October, with temperatures of 25-32°C and humidity between 66-44%, the bacilli remained viable in the moist soil for 46 days.
Humidity			
Wahyuni et al., 2010 Indonesian Journal of Tropical and Infectious Disease	Java, Indonesia 2008	Humidity PCR	Positive results in 22/90 water samples collected, 11 water samples, collected from wells that were never used by leprosy cases, were also positive.
Adriaty et al., 2010 Indonesian Journal of Tropical and Infectious Disease	Island Poteran, Sumenep, Madura and East Java, Indonesia 2009	Humidity PCR	201 samples of *M. leprae*, 91 collected from wells; 26.4% samples PCR-positive. The water used for clinical leprosy groups showed positive PCR in samples, and groups without the disease who used this water were more susceptible to leprosy.
Turankar et al., 2012 Infection, Genetics and Evolution	West Bengal, India 2009	Humidity PCR	Samples, both from the environment (soil) and the multibacilary patients exhibited the same genotype when tested by single nucleotide polymorphism (SNP) typing.

study further showed that viable and dead organisms can be distinguished using DNA amplification (Mallika et al., 2008).

In another research conducted in Ghatampur and Jalma, known endemic areas of leprosy in India, 18 soil samples, two from each village from different locations near the residences of patients, were examined. The results revealed the presence of *M. leprae* DNA in 33.3%

Table 1. Contd.

Temperature and Humidity			
Desikan e Sreevatsa, 1995 Leprosy Review	Agra, India 1993	Temperature Humidity Multiplication of *M. leprae* in mouse paws	Between the months of March and April, with temperatures between 24-33°C and atmospheric humidity of 44-28%, the bacilli survived for 14 days. During the monsoon season in August and September, with atmospheric humidity between 72-80% and temperatures of 29-33°C the bacilli survived for 28 days. In September and October, with temperatures of 25-32°C and humidity between 66-44%, the bacilli remained viable in the moist soil for 46 days.
Temperature and Rainfall			
Chilima et al., 2006 Applied and environmental microbiology	Karonga, Malawi, Africa 1998 and 1999	Temperature Rainfall PCR	The rates of recovery were consistently higher for dry season samples than for wet season samples of soil. All isolates cultured from soil appeared to be strains of *M. fortuitum* and not M. leprae with a complex pattern for the environmental mycobacterial flora.
Acanthamoeba castellanii			
Lahiri and Krahenbuhl, 2008 Leprosy Review	Laboratory Research Branch, USA 2007	Climate variations	The *Acanthamoeba castellanii* phagocyte showed no apparent adverse effects. The mycobacterium survived for 4 days, thus pointing to the potential role of the amoebae in the protection of *M. leprae* under adverse environmental conditions such as desiccation, and changes in temperature and pH.
Wheat et al, 2014 Plos Negleted Tropical Diseases	Colorado State University and others, USA – 2013/2014.	Climate variations And Virulence	*M. leprae* can remain viable long-term in environmentally ubiquitous free-living amoebae and retain virulence as assessed in the mouse model.

of the soil samples (Mallika et al., 2006).

Between 1998 and 1999, research was conducted in the northern and southern parts of the district of Karonga, Malawi, Africa. Soil samples from 11 villages housing 19 families with a history of leprosy were examined at the end of the dry and rainy seasons. One hundred and thirteen and 35 samples were collected at the end of the dry (1998) and rainy (1999) seasons, respectively, from 10 families. The results from a subset of 32 samples from the same locale, harvested during the dry and rainy seasons, showed the same trends with higher rates of recovery during the dry season (66%) compared with the rainy season (34%). The authors explain that the northern part of the District of Karonga has higher rainfall than the south. This result might be closely linked to climatic changes in the environment, as the bacilli can be removed from the soil and reducing the density of these bacterial population owing to the presence of the excess rainwater. The challenge in the study was the variety of mycobacteria in the soil, which might indirectly influence human health (Chilima et al., 2006). The incidence of leprosy was three times higher in the northern part of the district, which is warmer and more humid than the southern (Fine et al., 1994).

Epidemiological, microbiological, and clinical studies indicate that 50-70% of the sporadic leprosy cases in well-studied populations is reported in people who have had no known contact with other leprosy patients (Chakrabarty and Dastidar, 2002).

The environment can be an alternative transmission pathway for the spread of the disease. *M. leprae* thrives in soil rich in fossil fuels. In 2001, soil samples containing fossil fuels were collected from different parts of the USA, Russia, and Romania. There was a high degree of correlation between the presence of fossil fuels in the soil and leprosy in the countries surveyed. According to the authors, the disease probably occurred due to soil contamination (Chakrabarty and Dastidar, 2002).

In 1981, Kadza conducted a study across nine countries, where 729 samples were collected as follows: 273 from Norway (32.9% positive), 71 from Ivory Coast (23.9% positive), 36 from Portugal (55.6% positive), 20 from India (30.0% positive), 30 from Peru (40.0% positive), and 67 from Louisiana, USA (25.4% positive), 40 from Sweden, 77 from Scotland, and 115 from Germany, all of which were negative for the presence of the bacillus. *M. leprae* from positive samples was inoculated in the footpads of mice and armadillos. Through technique of isolation NCAFB it was possible to show characteristic growth in the footpads of mice and armadillos. The results suggested since more than 30 years that leprosy is transmitted not only by direct contact, but also indirectly by environmental means. However, the researchers could not culture the bacilli using the Lowenstein-Jensen and Middlebrook methods (Kadza, 1981).

A study conducted at the Institute for Leprosy in Agra,

India, found important differences in viability of the bacilli in adverse conditions during dry and rainy seasons. The first experiment was carried out in dry soil in the months of March and April, at temperatures of 24–33°C and atmospheric humidity of 28%. Under these conditions, the bacilli could not survive for more than 14 days. Upon repeating the experiment during the rainy season (August and September) with an atmospheric humidity ranging between 72–80% and temperatures of 29–33°C, the bacilli survived for at least 28 days. In the months of September and October, at temperatures of 25–32°C and humidity between 66–44%, the bacilli remained viable in moist soil for 46 days. Throughout the year, *M. leprae* remained viable for up to five months in soil that was dry, but under the shade. When exposed to direct sunlight for 3 h/day, the bacilli survived for 7 days. Furthermore, the bacilli remained viable for 2 months when stored between 4 and −20°C but when frozen at −70°C, they remained viable for only half the time. When exposed to antiseptics such as Savlon® and alcohol, the bacilli were rapidly killed, while in saline solution at room temperature, they survived for 60 days. These results indicate different survival rates of the bacilli outside the human body under different environmental conditions in India, where the disease is endemic. The transmission by indirect contact and the occurrence of new cases in the absence of known sources is consistent with viable bacilli outside the body. However, the study presented limitations in the management of refrigeration equipment to preserve the bacilli (Desikan and Sreevatsa, 1995).

WATER

Other studies indicate that *M. leprae* can also survive in water. In a study conducted in Poteran Island, Sumenep, Madura, and East Java, Indonesia, 201 samples were collected and divided into three groups: 91 water samples collected from wells, 42 nasal swabs from household contacts, and 68 histological sections from leprosy patients. Upon analyses of the samples, 26.4% isolates from the water sources, 61.9% from the nasal swabs, and 35.3% from the skin biopsies tested positive. PCR results show that water used by leprosy clinics tested positive, and groups without leprosy that used this water were more susceptible to the disease. Therefore, water is considered a possible reservoir and source of infection for leprosy, because detection of *M. leprae* DNA was significantly higher in individuals using the water than in individuals who did not (Adriaty et al., 2010).

Thus, cases of leprosy in individuals with no history of exposure to other known cases might be explained by exposure to viable *M. leprae* in water (Turankar et al., 2012).

Meanwhile, the research in East Java, Indonesia showed that 22 of the 90 samples of water examined were *M. leprae*-positive. Forty-eight samples were collected from wells used by leprosy patients; 11 of these tested positive for *M. leprae*. Interestingly, water samples collected from wells that were never used by leprosy patients also tested positive; *M. leprae* was found in free-living aquatic amoeba-like protozoa. Therefore, existence of the bacilli in water resources used by inhabitants of endemic areas does not seem to be influenced by the presence of leprosy patients living in the same area (Wahyuni et al., 2010).

Finally, the findings of a study conducted in 2002 in an endemic area of Ceará in northeastern Brazil, in the municipalities of Juazeiro, Morada Nova, Sobral, and the state capital Fortaleza, also suggested that infections arise from contact with contaminated bodies of water. The prevalence of infection among individuals using the water for bathing was higher than that among individuals who did not. Therefore, water might be an important carrier of the disease in this region. Streams and rivers have running water only in the rainy season. Thus, when precipitation stops, stagnant pools of water remain and these might serve as potential reservoirs for the bacilli. One limitation of the survey was the small number of counties investigated (Kerr-Pontes et al., 2006).

Molecular-based studies have revealed the importance of meteorological and climatic factors in the life cycle of *M. leprae*. The bacillus is known to remain viable as a probable source of infection leading to disease, especially under conditions of high humidity and temperature that characterize the tropical regions of the world. However, the bacilli can also survive in environments with broad variations in temperature and humidity. Therefore, basic infrastructures including sewers, water supply, and hygiene are the most important factors in protecting against the disease (Silva et al, 2010).

Besides leprosy patients without treatment, those in subclinical stages or those who exhibit spontaneous remissions may also be sources of bacillary spread, providing a transitional period of pathogen excretion via the nasal and/or oral routes (Cree and Smith, 1998).

Literature provides evidences that support the presence of *M. leprae* in the environment, having been found in different abiotic and biotic substrates. It was found in water (Wahyuni et al., 2010) and soil (Mallika et al., 2008) near leprosy clinics. It was also found in sphagnum (Kadza et al., 1980) and in a number of0species ranging from protozoa (Lahiri and Krahenbuhl, 2008) to more complex organisms such as mammals (Truman and Fine, 2010).

The viable bacilli found in water and soil can be an important disseminator of the disease, indicating extra-human sources of *M. leprae*. Locales with moist soil and associated ambient temperatures guarantee the viability of the pathogen (Ooi and Moschella, 2001).

The finding that *M. leprae* can survive ingestion by amoebae suggests that protozoans can significantly improve the survival of these bacilli in the soil, and therefore be instrumental in the transmission of leprosy

(Lahiri and Krahenbuhl, 2008). This shows the potential role of amoebae in the protection of *M. leprae* under adverse environmental conditions such as temperature and pH changes.

The handling and consumption of armadillo meat is also a possible source of *M. leprae* infection, chiefly in patients with no history of contact with other leprosy patients before their diagnosis (Deps et al., 2003). The mechanism of this transmission, however, has not been elucidated yet.

In 2011, a research conducted in Louisiana and Texas, in the southern region of the United States, revealed cases of leprosy in Native Americans who had never been outside the country. The exact mechanism of transmission remains unclear, but armadillos appear to be the possible reservoir, since the patients and the armadillos were shown to carry the same strain of *M. leprae* (Truman et al., 2011).

Before the *M. leprae* genome was decoded in 2001, availability of new antigens was limited mainly because the bacilli could not be grown in axenic culture. Until then, *M. leprae* had remained an enigma mainly due to its inability to be cultured *in vitro* (Cole et al., 2001). Subsequently, comparison of the genomes and proteomes of *M. tuberculosis* and *M. leprae* revealed that the latter suffers from reduced evolutionary potential. It presented a genome of only 3.3 mega bases compared with 4.4 mega bases of *M. tuberculosis*. This reduction in the *M. leprae* genome has resulted in the elimination of many important metabolic pathways, explaining its intracellular habitat and inability to be cultivated in vitro (Cole et al., 2001).

Since 2000, considerable advances have been made with sequencing of the bacillus DNA. In particular, the 16S rRNA sequence has been used in viability assays, whereas detection of the *M. leprae* mRNAs has been proposed as a promising tool for rapid detection and measurement of viability of the bacilli in the environment (Kurabachew et al., 1998). The major advantage of PCR is its high sensitivity and specificity for detecting DNA from *M. leprae*, without the bacterial culture(Goulart and Goulart, 2008). The technical advances in determining the presence of *M. leprae* in the environment has been complemented by many new findings, such as the elucidation of its 16S rRNA sequence, facilitated by methods such as PCR and Real Time (RT)-PCR (Kadza, 1981; Opromolla, 1997; Abreu et al., 2006).

There were some limitations to the studies discussed in this review, though. First, in the 1980s, detecting acid-alcohol resistant bacilli was not possible due to difficulty in cultivating the bacilli (Salem and Fonseca, 1982; Kadza, 1981). *M. leprae* is deficient in the transport of iron, which is required for cell division, thus making it unlikely that the bacilli can replicate by artificial means (Kato, 1994). The reduction in the *M. leprae* genome might also explain this difficulty (Cole et al., 2011). Secondly, the problem in experimental research in 1995 was the management of refrigeration equipment to preserve the bacilli (Desikan and Sreevatsa, 1995). Exposure to very low temperatures could cause the water to form crystals and harm the bacilli. Moreover, freeze-thaw cycles could also destroy the microorganisms. Thirdly, the small number of counties was an obstacle encountered during research in the state of Ceará (Kerr-Pontes et al., 2006) because of which, the results might not be similar in other parts of the state.

CONCLUSION

This review provides evidence that meteorological factors such as temperature and soil humidity can influence the dynamics of *M. leprae*. The occurrence of this disease is associated with variations in temperature and humidity. However, leprosy cases are registered equally in the rainy season as well as in the dry season, suggesting that *M. leprae* remain viable in various environmental conditions. Therefore, it is very difficult to establish the meteorological pattern to maintain the bacilli in the environment, but there are no doubts about the presence of the bacillus in water, soil as well protected by free-living amoebas. The key aspect in the environment-human transmission appears to be the intensity of exposure to contaminated soil and water that differs between developed and developing countries.

The extensive land area of endemic countries, endemicity in the bordering countries, diversity of biomes, the lack of urban infrastructure, together weather features are possible factors that could influence disease transmission.

Conflict of interests

The authors did not declare any conflict of interest.

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REFERENCES

Abreu MAMM, Michalany NS, Weckx LM, Pimentel DRN, Hirata CHW, Alchorne MM de A (2006). The oral mucosa in leprosy: a clinical and histopathological study. Rev. Bras. Otorrinolaringol. 72:312-316.

Adriaty D, Wahyuni R, Iswahyudi I, Agusni I, Izumi S (2010). TTC repeats variation of *Mycobacterium leprae* isolates for analysis of leprosy transmission in leprosy endemic area in East Java, Indonesia. Indones. J. Trop. Infect. Dis. North Am. 1(1):38-41.

Azulay RD, Azulay DR, Azulay-Abulafia L (2008). Dermatologia. 5th. ed., rev. e atual. Rio de Janeiro: Guanabara Koogan. 322-346.

Chakrabarty AN, Dastidar SG (2002). Is soil an alternative source of leprosy infection? Acta Leprol. 12(2):79-84.

Chilima BZ, Clark IM, Floyd S, Fine PE, Hirsch PR (2006). Distribution

of environmental mycobacteria in Karonga District, northern Malawi. Appl. Environ. Microbiol. 72(4):2343-2350.
Cole ST, Eiglmeier K, Parkhill J, James KD, Thomson NR, Wheeler, PR, Garnier T, Churcher C, Harris D et al. (2001). Massive gene decay in the leprosy bacillus. Nature 409(6823):1007-1011.
Cree IA, Smith WC (1998). Leprosy transmission and mucosal immunity: towards eradication? Lepr. Rev. 69(2):112-121.
Deps PD, Faria LV, Gonçalves VC, Silva DA, Ventura CG, Zandonade E (2003). Epidemiological features of the leprosy transmission in relation to armadillo exposure. Hansenol. Int. 28:138-144.
Desikan KV (1997). Viability of *Mycobacterium leprae* outside the human body. Lepr. Rev. 48(4):231-235.
Desikan KV, Sreevatsa (1995). Extended studies on the viability of *Mycobacterium leprae* outside the human body. Lepr. Rev. 66(4):287-295.
Donoghue HD, Holton J, Spigelman M (2001). PCR primers that can detect low levels of *Mycobacterium leprae* DNA. J. Med. Microbiol. 50(2):177-182.
Falkinham JO, Norton CD, LeChevallier MW (2001). Factors influencing numbers of *Mycobacterium avium, Mycobacterium intracellulare*, and other mycobacteria in drinking water distribution systems. Appl. Environ. Microbiol. 67(3):1225-1231.
Fine PE, Sterne JA, Ponnighaus JM, Rees RJ (1994). Delayed-type hypersensitivity, mycobacterial vaccines and protective immunity. Lancet 344(8932):1245-1249.
Geluk A, Klein MR, Franken KL, van Meijgaarden KE, Wieles B, Pereira KC, Buhrer-Sékula S, Klatser PR. et al. (2005). Postgenomic approach to identify novel *Mycobacterium leprae* antigens with potential to improve immunodiagnosis of infection. Infect. Immun. 73(9):5636-5644.
Goulart IM, Goulart LR (2008). Leprosy: diagnostic and control challenges for a worldwide disease. Arch. Dermatol. Res. 300(6):269-290.
Groathouse NA, Rivoire B, Kim H, Lee H, Cho SN, Brennan PJ, Vissa VD (2004). Multiple polymorphic loci for molecular typing of strains of *Mycobacterium leprae*. J. Clin. Microbiol. 42(4):1666-1672.
Izumi S, Budiawan T, Saeki K, Matsuoka M, Kawatsu K (1999). An epidemiological study on *Mycobacterium leprae* infection and prevalence of leprosy in endemic villages by molecular biological technique. Indian J. Lepr. 71(1):37-43.
Kadza J (1981). Occurrence of non-cultivable acid-fast bacilli in the environment and their relationship to *M. leprae*. Lepr. Rev. 52(Suppl 1):85-91.
Kadza J (2000). The ecological approach to leprosy: Non-cultivable acid-fast bacilli and environmentally derived *M. leprae*. In: The ecology of mycobacteria ed. Kadza J, Kluwer Academic Publishers: Dordrecht/Boston/London. 7:40-47.
Kadza J, Irgens LM, Muller K (1980). Isolation of non-cultivable acid-fast bacilli in sphagnum and moss vegetation by foot pad technique in mice. Int. J. Lepr. Other. Mycobact. Dis. 48(1):1-6.
Kato L (1994). Water soluble palmitic acid in media for cultivation of leprosy derived psychrophilic mycobacteria from *Mycobacterium leprae* infected tissues. Hansenol. Int. 19:17-27.
Kerr-Pontes LR, Barreto ML, Evangelista CM, Rodrigues LC, Heukelbach J, Feldmeier H (2006). Socioeconomic, environmental, and behavioural risk factors for leprosy in North-east Brazil: results of a case–control study. Int. J. Epidemiol. 35(4):994-1000.
Kurabachew M, Wondimu A, Ryon JJ (1998). Reverse transcription-PCR detection of *Mycobacterium leprae* in clinical specimens. J. Clin. Microbiol. 36(5):1352-1356.
Lahiri R, Krahenbuhl JL (2008). The role of free-living pathogenic amoeba in the transmission of leprosy: a proof of principle. Lepr. Rev. 79(4):401-409.
Levy L, Ji B (2006). The mouse foot-pad technique for cultivation of *Mycobacterium leprae*. Lepr. Rev. 77(1):5-24.
Loughry WJ, Truman RW, McDonough CM, Tilak M, Garnier S, Delsuc F (2009). Is leprosy spreading among nine-banded armadillos in the southeastern United States? J. Wildl. Dis. 45(1):144-152.
Mallika L, Katoch K, Katoch VM, Gupta AK, Chauhan DS, Sharma R et al. (2008). Detection of viable *Mycobacterium leprae* in soil samples: insights into possible sources of transmission of leprosy. Infect. Genet. Evol. 8(5):627-631.
Mallika L, Katoch K, Sachan P, Dubey A, Kapoor S, Kashyap M, Chauhan DS, Singh HB, Sharma VD, Jadhav RS, Katoch VM (2006). Detection of Mycobacterium leprae DNA from soil samples by PCR targeting RLEP sequences. J. Commun. Dis. 38(3):269-273.
Ooi WW, Moschella SL (2001). Update on Leprosy in immigrants in the United States: status in the year 2000. Clin. Infect. Dis. 32(6):930-937.
Salem JI, Fonseca OJ (1982). Acid-alcohol resistance bacilli in the water of the Aleixo lake. Hansenol. Int. 7(1):25-35.
Silva DRX, Ignotti E, Souza-Santos R, Hacon SS (2010). Hanseníase, condições sociais e desmatamento na Amazônia brasileira. Rev. Panam. Salud Publica. 27(4):268–75.
Silvestre MPSA (2011). Associação do polimorfismo do gene humano NRAMP1 na Susceptibilidade/resistência para hanseníase em áreas Endêmicas do Estado do Pará. (PhD dissertation presented at the Federal University of Pará). Available: http://repositorio.ufpa.br/jspui/handle/2011/3961
Truman R (2005). Leprosy in wild armadillos. Lepr. Rev 76(3): 198-208.
Truman R, Fine PE (2010). Environmental' sources of *Mycobacterium leprae*: issues and evidence. Lepr. Rev. 81(2):89-95.
Truman RW, Singh P, Sharma R, Busso P, Rougemont J, Paniz-Mondolfi A, Kapopoulou A, Brisse S, Gillis TP, Cole ST (2011). Probable zoonotic leprosy in the southern United States. N. Engl. J. Med. 364(17):1626-1633.
Turankar RP, Mallika L, Singh M, Siva Sai KS, Jadhav RS (2012). Dynamics of *Mycobacterium leprae* transmission in environmental context: deciphering the role of environment as a potential reservoir. Infect. Genet. Evol. 12(1):121-126.
Wahyuni R, Adriaty D, Iswahyudi CRSP (2010). Mycobacterium leprae in daily water resources of inhabitants who live in leprosy endemic area of East Java. Indones. J. Trop. Infect. Dis. 1(2):65–68.
Whan L, Grant IR, Rowe MT (2006). Interaction between Mycobacterium avium subsp. paratuberculosis and environmental protozoa. BMC. Microbiol. 6:63.
Wheat WH, Casali AL, Thomas V, Spencer JS, Lahiri R, et al. (2014) Long-term Survival and Virulence of Mycobacterium leprae in Amoebal Cysts. PLoS Negl. Trop. Dis. 8(12):e3405.
WHO (2013). Global leprosy: update on the 2012 situation. Weekly epidemiological record. 88(35):365-380.
WHO (2014). World Health Organization. Weekly epidemiological record. Global leprosy situation. Available: http://www.who.int/wer/2013/wer8835.pdf
Young SK, Taylor GM, Jain S, Suneetha LM, Suneetha S, Lockwood DN, Young DB (2004). Microsatellite mapping of *Mycobacterium leprae* populations in infected humans. J. Clin. Microbiol. 42(11):4931–4936.
Zhang L, Budiawan T, Matsuoka M (2005). Diversity of potential short tandem repeats in *Mycobacterium leprae* and application for molecular typing. J. Clin. Microbiol. 43(10):5221-5229.