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EDITORIAL

'Environmental' sources of *Mycobacterium leprae*: Issues and evidence

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Though *Mycobacterium leprae* is considered to be primarily a parasite of humans, there is a long history of studies, evidence and arguments which have indicated possible non-human sources of the agent. Thus different authors have suggested that *M. leprae* may be harboured in soil,[1–3] in water,[4] on plants,[5–7] or in various animal species including amoeba,[8] insects,[9,10] fish,[11–13] primates,[14] and armadillos.[15,16] The question of possible extra-human sources of *M. leprae* is an important one for leprosy epidemiology and control. If non-human sources exist, their recognition may help to explain patterns of infection and disease in human populations. Even more importantly, they would have implications for the control of the disease, and in particular for the possibility of its 'elimination' or even ultimate eradication. We consider here the nature and implications of the evidence for such extra-human sources.

There have been two different sorts of observations motivating the search for extra-human sources of *M. leprae*. One is the repeated observation of clinical leprosy in individuals with no apparent history of exposure to other known cases.[17–20] The second is the observation that clinical leprosy clusters in particular areas, such as near water sources, which has led some authors to suggest that *M. leprae* may have an extra-human source in such environments.[21,22] Neither of these lines of argument provides a strong case for extra-human sources of *M. leprae*. The long incubation period of the disease, the inability to recall contacts and encounters years after the event, the fact that stigma leads to hiding of cases in many societies, and the well-recognised fact that multibacillary cases can go undetected for long periods mean that there are substantial opportunities for unrecognised, unremembered or unacknowledged source contacts. The apparent clustering of leprosy in particular environments may simply reflect that certain environments are associated with certain social groups, health conditions or behaviours which predispose to *M. leprae* transmission or the manifestation of leprosy disease. Or they may reflect environmental

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conditions where *M. leprae* is able to persist for extended periods outside the human body, on surfaces or even in the air.

An important distinction must be made in differentiating non-human reservoirs of *M. leprae* from environmental transience. Leprosy cases can shed large numbers of bacilli into their environment through bodily secretions, or while sneezing, coughing or talking.[18,23–25] The fact that some leprosy bacilli may remain viable in certain cell-free environments for periods of hours, days or even weeks,[26–28] does not mean that they *persist* as an infectious reservoir. The distinction here is whether the bacilli can *replicate*. Given what is now known of the abbreviated genome of *M. leprae*, it is most unlikely that leprosy bacilli can replicate in any extra-cellular environment.[29] Though the literature contains several claims of culture of *M. leprae* in cell-free media,[30,31] no such claim ever has been substantiated, and there is now a strong *a priori* argument against such a possibility. Though one must be open minded about the possibility that *M. leprae* in water could reflect their association with protozoa, or with aquatic invertebrate hosts, as has been suggested for *M. ulcerans*,[32] the notion of free-living *M. leprae* persisting in the environment is implausible biologically.

It is possible that *M. leprae* could persist within other vertebrates. However, investigators seeking to propagate *M. leprae* in the laboratory have examined a long list of experimental hosts and found only a very limited host range.[33–36] Some species of primates appear to be marginally susceptible to experimental infection, and a few of them have developed spontaneous leprosy while in captivity.[37,38] However, infection among free-ranging primates has not been reported. *M. leprae's* predilection for cool body temperatures was recognised soon after Hansen described the bacillus, and as early as 1911 Couret suggested that fish might be suitable hosts for propagating leprosy bacilli.[39] However, even with the increased use of fish as experimental laboratory animals seen in recent years, there have been no credible reports of successful infection and replication of *M. leprae* in fish. Aside from the primate infections mentioned above, the only two non-human environments in which *M. leprae* are known reliably to replicate are the footpad of the mouse (*Mus musculus*), and the nine-banded armadillo (*Dasypus novemcinctus*).

The ability of the mouse footpad to support *M. leprae*, discovered by Shepard in 1960,[40] was a crucially important discovery in the history of leprosy research, but there has never been any evidence that this serves as a source of bacilli outside the artificial confines of the laboratory. Indeed, the conventional mouse is relatively resistant to *M. leprae* and will kill and eliminate the bacilli after infection in the foot pad reaches a certain threshold.[41] The armadillo story is different.

M. leprae in armadillos:

Although the original demonstration that *M. leprae* can grow in the nine-banded armadillo was carried out in a laboratory,[42] there have now been repeated demonstrations that *M. leprae* is harboured in wild armadillos over a wide area of North America.[43] It is important to appreciate the nature and strength of this evidence. It includes: typical pathology,[44] skin test reactivity,[45] genomic sequence analysis,[46] *M. leprae*-specific serology[16] and PCR,[47] isolation and passage of bacilli in other animals, and epidemiological associations of armadillo contact with human cases.[19,48–50] Given the magnitude and variety of this evidence, there is no doubt that *M. leprae* persists among wild armadillos in the southern USA, and it may extend to contiguous armadillo populations in parts of Mexico. However, there is only a single report of *M. leprae* among armadillos in Mexico,[51] and further evidence is needed to clarify the southern range limits of sylvan leprosy. Whether *M. leprae* exists among armadillos elsewhere in the Americas remains an unresolved but important question.

Though negative reports from surveys are seldom published, none of the South American groups recruiting armadillos for laboratory studies in the 1970s reported evidence of naturally occurring *M. leprae* infection among the animals they collected in Brazil,[52] French Guiana[53] or Argentina,[54] and surveys on different species of armadillos in Colombia[55] and Paraguay[56] also found no (0/536) evidence for the infection. South American armadillos were thus considered to be free of *M. leprae* infection, or at least less involved than their North American counterparts. The only exception to this view were reports of a naturally occurring systemic mycobacteriosis, believed to be caused by *M. leprae*, affecting a total of nine of the 132 armadillos examined at a laboratory in northern Argentina over a 22 year period from 1979 to 2001.[57–59] Over the past few years, however, two studies have been published reporting PCR evidence for *M. leprae* infection in five out of 14 armadillos in Espirito Santos, Brazil,[60] and in nine out of 22 armadillos collected in the Andes region of Colombia.[61] Neither of these studies included other diagnostic tests to corroborate the PCR observations. Subsequent serological screening was performed in Brazil, but those later results were not correlated with the PCR data,[62,63] and separate histopathological studies were found to be negative.[64] None of these observations of possible *M. leprae* infections among South American armadillos have been confirmed by other laboratories, and a recent survey in Sao Paulo, Brazil found no evidence for *M. leprae* infection among the 44 armadillos and several other wildlife species they examined.[65] Thus it remains unclear whether these previous findings indicate a new paradigm or are somehow erroneous.

METHODOLOGICAL ISSUES

The convincing demonstration of *M. leprae*, or *M. leprae* infection, is rarely easy. This is even true in the context of diagnosing an appreciable proportion of human cases in leprosy-endemic regions. The presence of classical clinical lesions plays a major role in diagnosing human leprosy, sometimes supported by bacteriological (slit skin smear) or histopathological evidence. PCR is sometimes (rarely) used as a diagnostic aid when atypical clinical or histopathologic features obscure a clear diagnosis, but PCR itself is not thought to be highly informative when acid-fast bacilli are not detectable by light microscopy in biopsies.[66] Though several authors have reported PCR evidence for *M. leprae* in nasal smears of clinically healthy individuals in leprosy endemic communities, the validity of the evidence for *M. leprae*, and risks of false positivity are a major concern with these studies.[67,68]

The convincing demonstration of *M. leprae* in non-human material raises additional difficulties. Like humans, armadillos exhibit the full immunological and histopathological spectrum of leprosy, and many of the same laboratory methods used to help diagnose leprosy in humans can be used with armadillos. However, because the disease manifests systemically in armadillos, with few skin lesions, evidence for the infection is found earlier and more frequently with examination of reticuloendothelial (RES) tissues such as lymph nodes.[47,69] If total necropsy is not possible, PGL1 specific serology, which becomes positive after bacillary load in the RES attains a certain threshold, is the second most sensitive tool.[70,71] Though dissemination of *M. leprae* to skin or ear tissues occurs relatively late in armadillo infections and is detectable far less frequently (5–10 fold) than the other methods,[72,73] histopathological demonstration of *M. leprae* in dermal nerves remains pathognominic for leprosy in both humans and armadillos.[74] Given the well known problems of contamination and false positive reactions with PCR or serology, investigators should include a combination

of the available diagnostic techniques to confirm a diagnosis of *M. leprae* infection and corroborate their findings.[75]

Neural involvement with *M. leprae*, the gold standard for diagnosing leprosy, has been shown only among humans, armadillos and primates. It is not seen in rodents, guinea pigs or other laboratory animals. Individuals examining hosts with atypical pathology must adjust their techniques accordingly and incorporate different combinations of methods. Investigators seeking to elaborate new and novel findings bear a substantial burden of proof to demonstrate the validity of new paradigms. In the case of *M. leprae*, this requires high standards of scientific rigour:

- Using the full array of diagnostic tests available: PCR, serology, histopathology, isolation of bacilli in animals; and critically correlating those results in a biologically plausible manner.
- With reference to PCR, one must appreciate that our knowledge of microbial and other genomes in nature is still quite limited, and there are millions of organisms that are yet to be described. PCR studies thus should be conducted with primers that amplify multiple segments of the chromosome and the resulting amplicons should be sequenced to confirm the desired product.
- Appropriate positive and negative controls should be included for the obvious pitfalls of laboratory or skin surface contamination. Ideally these should be blind coded so that their true status is unknown to the laboratory staff involved.
- Case control studies investigating contact with armadillos should control for obvious confounders including urban rural and socio-economic factors, in addition to age, sex, BCG status etc.
- Observations should be confirmed, preferably independently. This should be doable relatively easily, by a variety of methods (including simple histopathology), given the extraordinarily high prevalences reported recently from Brazilian and Colombian armadillos.[60,61]

Discussion

A critical survey of the literature on 'environmental' sources of *M. leprae*, leads us to conclude that the only convincing evidence for a non-human reservoir is that pertaining to nine-banded armadillos in the southern USA. The reports to date of evidence for *M. leprae* in armadillo populations elsewhere are intriguing, and potentially important, but require rigorous confirmation.

The issue of the range of *M. leprae* infection in armadillos is important for our understanding of leprosy. It is generally agreed that leprosy did not exist in the Americas during pre-Colombian times, and thus *M. leprae* must have been introduced into armadillos from infected humans some time in the last 500 years. Armadillos expanded their range into the USA only in the 1880s, and as a result of several separate introductions of the animals from Texas and Louisiana in the west (where *M. leprae* infection in now widespread) into Florida and other eastern states (where armadillos are considered to be free of *M. leprae*), two separate populations were formed.[76] These western and eastern populations have merged in recent decades, but there is much evidence that *M. leprae* infection is not uniformly distributed over the animal's range in the USA. Rather, it is found most commonly among

animals in low lying and coastal marsh areas of the western range.[43] A recent report of *M. leprae* infection among armadillos in Alabama suggests that the infection can spread in the region.[77] However, the apparent continued absence of *M. leprae* among armadillos in central Texas and Oklahoma, parts of Mississippi, as well as Georgia and Florida[43] provides evidence that spread of the infection is neither rapid nor certain, and it would be erroneous to presume that armadillos in all locations are reservoir hosts of *M. leprae*.

If *M. leprae* does persist in armadillos elsewhere in the Americas it will be important to map the distribution of the infections and to carry out appropriate molecular epidemiological studies to see if the bacilli reflect more than one introduction from human sources. This would inform us of the efficiency (determinants and rapidity) of spread within the armadillo populations. This will in turn have important public health implications in terms of guiding leprosy diagnostic suspicion, and leprosy control, throughout the Americas. For all such reasons rigorous studies of *M. leprae* in armadillos are of high priority.

In conclusion we note that most of the recent claims of extra-human sources of *M. leprae* have been based upon PCR evidence. It is important for researchers, journal reviewers, journal editors, and those reading the literature, to be aware of the problems with such evidence. The issues at stake are important – we must all insist upon high standards of evidence.

References

[1] Lavania M, Katoch K, Katoch VM *et al.* Detection of viable *Mycobacterium leprae* in soil samples: insights into possible sources of transmission of leprosy. *Infect Genet Evol*, 2008; **8**: 627–631.

[2] Kazda J, Ganapati R, Revankar C *et al.* Isolation of environment-derived *Mycobacterium leprae* from soil in Bombay. *Lepr Rev*, 1986; **57**(Suppl 3): 201–208.

[3] Blake LA, West BC, Lary CH, Todd JR. Environmental non-human sources of leprosy. *Rev Infect Dis*, 1987; **9**: 562–577.

[4] Matsuoka M, Izumi S, Budiawan T *et al. Mycobacterium leprae* DNA in daily using water as a possible source of leprosy infection. *Indian J Lepr*, 1999; **71**: 61–67.

[5] Mostafa HM, Kazda J, Irgens LM, Luesse HG. Acid-fast bacilli from former leprosy regions in coastal Norway showing PCR positivity for *Mycobacterium leprae*. *Int J Lepr Other Mycobact Dis*, 1995; **63**: 97–99.

[6] Kazda J, Irgens LM, Muller K. Isolation of non-cultivable acid-fast bacilli in sphagnum and moss vegetation by foot pad technique in mice. *Int J Lepr Other Mycobact Dis*, 1980; **48**: 1–6.

[7] Kazda J. Occurrence of non-cultivable acid-fast bacilli in the environment and their relationship to *M. leprae*. *Lepr Rev*, 1981; **52**(Suppl 1): 85–91.

[8] Lahiri R, Krahenbuhl JL. The role of free-living pathogenic amoeba in the transmission of leprosy: a proof of principle. *Lepr Rev*, 2008; **79**: 401–409.

[9] Saha K, Jain M, Mukherjee MK *et al.* Viability of *Mycobacterium leprae* within the gut of Aedes aegypti after they feed on multibacillary lepromatous patients: a study by fluorescent and electron microscopes. *Lepr Rev*, 1985; **56**: 279–290.

[10] Sreevatsa. Leprosy and arthropods. *Indian J Lepr*, 1993; **65**: 189–200.

[11] Hutchinson J. On Leprosy and Fish-Eating. A Statement of Facts and Explanations. Archibald Constable and Co, 1906.

[12] Chaussinand Rea. Inoculation of Hansen and Stefansky bacilli in the rainbow perch *Eupomotis gibbosus.* Preliminary note. (Abstract). *Int J Lepr Other Mycobact Dis*, 1952; **20**: 420–421.

[13] Couret M. The behavior of bacillus leprae in cold-blooded animals. *J Exp Med*, 1911; **13**: 576–589.

[14] Gormus BJ, Xu K, Baskin GB *et al.* Experimental leprosy in rhesus monkeys: transmission, susceptibility, clinical and immunological findings. *Lepr Rev*, 1998; **69**: 235–245.

[15] Walsh GP, Meyers WM, Binford CH. Naturally acquired leprosy in the nine-banded armadillo: a decade of experience 1975–1985. *J Leukoc Biol*, 1986; **40**: 645–656.

[16] Truman RW, Shannon EJ, Hagstad HV *et al.* Evaluation of the origin of *Mycobacterium leprae* infections in the wild armadillo, *Dasypus novemcinctus*. *Am J Trop Med Hyg*, 1986; **35**: 588–593.

[17] Taylor CE, Elliston EP, Gideon H. Asymptomatic infections in leprosy. *Int J Lepr Other Mycobact Dis*, 1965; **33**: 716–731.

[18] Fine PEM. Leprosy: the epidemiology of a slow bacterium. *Epidemiol Rev*, 1982; **4**: 161–187.
[19] Deps PD, Alves BL, Gripp CG *et al.* Contact with armadillos increases the risk of leprosy in Brazil: a case control study. *Indian J Dermatol Venereol Leprol*, 2008; **74**: 338–342.
[20] Abide JM, Webb RM, Jones HL, Young L. Three indigenous cases of leprosy in the Mississippi delta. *South Med J*, 2008; **101**: 635–638.
[21] Sterne JA, Ponnighaus JM, Fine PE, Malema SS. Geographic determinants of leprosy in Karonga District, Northern Malawi. *Int J Epidemiol*, 1995; **24**: 1211–1222.
[22] Kerr-Pontes LR, Barreto ML, Evangelista CM *et al.* Socioeconomic, environmental, and behavioural risk factors for leprosy in North-east Brazil: results of a case-control study. *Int J Epidemiol*, 2006; **35**: 994–1000.
[23] Huang CLH. The transmission of leprosy in man. *Int J Lepr Other Mycobact Dis*, 1980; **48**: 309–318.
[24] Davey TF, Rees RJW. The nasal discharge in leprosy: clinical and bacteriological aspects. *Lepr Rev*, 1974; **45 IS**: 121–134.
[25] Rees RJ, Meade TW. Comparison of the modes of spread and the incidence of tuberculosis and leprosy. *Lancet*, 1974; **1**(7846): 47–48.
[26] Desikan KV, Sreevatsa. Extended studies on the viability of *Mycobacterium leprae* outside the human body. *Lepr Rev*, 1995; **66**: 287–295.
[27] Truman RW, Krahenbuhl JL. Viable *M. leprae* as a research reagent. *Int J Lepr Other Mycobact Dis*, 2001; **69**: 1–12.
[28] Desikan KV. Viability of *Mycobacterium leprae* outside the human body. *Lepr Rev*, 1977; **48**: 231–235.
[29] Cole ST, Eiglmeier K, Parkhill J *et al.* Massive gene decay in the leprosy bacillus. *Nature*, 2001; **409**(6823): 1007–1011.
[30] Murohashi T. Cultivation of *M. leprae* in cell-free, semi-synthetic media. *Acta Leprol*, 1980; **80**: 17–34.
[31] Rees RJW. The microbiology of leprosy: leprosy. In: Hastings RC (ed). Churchill Liningstone KW – N1, New York, 1985; pp. 31–52.
[32] Mosi L, Williamson H, Wallace JR *et al.* Persistent association of *Mycobacterium ulcerans* with West African predaceous insects of the family *belostomatidae*. *Appl Environ Microbiol*, 2008; **74**: 7036–7042.
[33] Scollard DM, Adams LB, Gillis TP *et al.* The continuing challenges of leprosy. *Clin Microbiol Rev*, 2006; **19**: 338–381.
[34] Meyers WM, Gormus BJ, Walsh GP. Experimental leprosy: leprosy. In: Hastings RC (ed). Churchill Livingstone KW – N1, New York, 1994; pp. 385–408.
[35] Rees RJ. A century of progress in experimental leprosy. *Int J Lepr Other Mycobact Dis*, 1973; **41**: 320–328.
[36] Johnstone PA. The search for animal models of leprosy. *Int J Lepr Other Mycobact Dis*, 1987; **55**: 535–547.
[37] Meyers WM, Gormus BJ, Walsh GP. Nonhuman sources of leprosy. *Int J Lepr Other Mycobact Dis*, 1992; **60**: 477–480.
[38] Leininger JR, Donham KJ, Rubino MJ, Meyers WM. Naturally acquired leprosy in a chimpanzee necropsy findings and experimental transmission to other animals. *Lab Invest*, 1980; **42**: 132.
[39] Couret M. The behavior of bacillus leprae in cold-blooded animals. *J Exp Med*, 1911; **13**: 576–589.
[40] Shepard CC. The experimental disease that follows the injection of human leprosy bacilli into footpads of mice. *J Exp Med*, 1960; **112**: 445–454.
[41] Levy L, Ji B. The mouse foot-pad technique for cultivation of *Mycobacterium leprae*. *Lepr Rev*, 2006; **77**: 5–24.
[42] Kirchheimer WF, Storrs EE. Attempts to establish the armadillo (*Dasypus novemcinctus Linn.*) as a model for the study of leprosy. I. Report of lepromatoid leprosy in an experimentally infected armadillo. *Int J Lepr Other Mycobact Dis*, 1971; **39**: 693–702.
[43] Truman RW. Leprosy in wild armadillos. *Lepr Rev*, 2005; **76**: 198–208.
[44] Binford CH, Meyers WM, Walsh GP *et al.* Naturally Acquired Leprosy-Like Disease in the Nine-Banded Armadillo *Dasypus-Novemcinctus* Histo Pathologic and Microbiologic Studies of Tissues. *J Reticuloendothel Soc*, 1977; **22**: 377–388.
[45] Meyers WM, Kvernes S, Binford CH. Comparison of reactions to human and armadillo lepromins in leprosy. *Int J Lepr Other Mycobact Dis*, 1975; **43**: 218–225.
[46] DeWitt MYL, Klatser PR. *M. leprae* isolates from different sources have identical sequences of the spacer region between the 16S and 23S ribosomal RNA genes. *Microbiology*, 1994; **140**: 1983–1987.
[47] Job CK, Drain V, Williams DL *et al.* Comparison of PCR techniques with other methods for detection of *M. leprae* in tissues of wild armadillos. *Lepr Rev*, 1991; **62**: 362–373.
[48] Thomas DA, Mines JS, Mack TM, *et al.* Is armadillo exposure a risk factor for leprosy. 34[1], 91A EP - 1986. Ref Type: Generic.
[49] Bruce S, Schroeder TL, Ellner K *et al.* Armadillo exposure and Hansen's disease: an epidemiologic survey in southern Texas. *J Am Acad Dermatol*, 2000; **43**(2 Pt 1): 223–228.
[50] Clark BM, Murray CK, Horvath LL *et al.* Case-control study of armadillo contact and Hansen's disease. *Am J Trop Med Hyg*, 2008; **78**: 962–967.
[51] Amezcua ME, Escobar-Gutierrez A, Storrs EE *et al.* Wild Mexican armadillo with leprosy-like infection [letter]. *Int J Lepr Other Mycobact Dis*, 1984; **52**: 254–255.

[52] Andrade LMC, Silva CO. Experimental Work With the Armadillo in Brazil. *Pan Am Health Organ Sci Publ*, 1979; **366** (Armadillo as an animal model in Bio-medical research): 85–88.
[53] Barrenton G. The leprosy research program in Frencg Guiana. *Pan Am Health Organ Sci Publ*, 1978; **366**: 96–97.
[54] Balina LM, Cardama JE, Gatti JC *et al.* Research On Armadillos in Argentina. *Pan Am Health Organ Sci Publ*, 1978; **366**: 103–110.
[55] Munoz-Rivas G. Notes on Granulomatosis in Armadillos Innoculated with *M. leprae*. *Pan Am Health Organ Sci Publ*, 1978; **366**: 99–102.
[56] Innami S. *The study of the armadillo in Paraguay. The Armadillo as an Experimental Model in Biomedical Research*. Pan American Health Organization, Washington, D.C., 1978; pp. 89–95.
[57] Resolagi E, Martinez A, Resoahli J *et al.* Comminicacion de un caso de micobactericas. *Leprologia*, 1979; **21**: 18–25.
[58] Resolagi E, Martinez A, Resoahli J *et al.* Micibacteriosis natural en armadillos similar a lepra humana. *Gacete Veterinaria*, 1982; **45**: 674–676.
[59] Zumarraga MJ, Resoagli EH, Cicuta ME *et al.* PCR-restriction fragment length polymorphism analysis (PRA) of *Mycobacterium leprae* from human lepromas and from a natural case of an armadillo of Corrientes, Argentina. *Int J Lepr Other Mycobact Dis*, 2001; **69**: 21–25.
[60] Deps PD, Santos AR, Yamashita-Tomimori J. Detection of *Mycobacterium leprae* DNA by PCR in blood sample from nine-banded armadillo: preliminary results. *Int J Lepr Other Mycobact Dis*, 2002; **70**: 34–35.
[61] Cardona-Castro N, Beltran JC, Ortiz-Bernal A, Vissa V. Detection of *Mycobacterium leprae* DNA in nine-banded armadillos (*Dasypus novemcinctus*) from the Andean region of Colombia. *Lepr Rev*, 2009; **80**: 424–431.
[62] Deps P, Antunes J, Tomimori-Yamashit J. Detection of *Mycobacterium leprae* infection in wild nine-banded armadillos (*Dasypus novemcinctus*) using the rapid ML Flow test. *Revista da Sociedade Brasileira de Medicina Tropical*, 2007; **40**: 86–87.
[63] Deps PD, Antunes JM, Faria C *et al.* Research regarding anti-PGL-I antibodies by ELISA in wild armadillos from Brazil. *Rev Soc Bras Med Trop*, 2008; **41**(Suppl 2): 73–76.
[64] Deps PD, Michalany NS, Tomimori-Yamashita J. False positive reaction of the immunohistochemistry technique using anti-BCG polyclonal antibodies to identify *Mycobacterium leprae* in wild nine-banded armadillos. *Int J Lepr Other Mycobact Dis*, 2004; **72**: 327–330.
[65] Pedrini SC, Rosa PS, Medri IM *et al.* Search for *Mycobacterium leprae* in wild mammals. *Braz J Infect Dis*, 2010; **14**: 47–53.
[66] Scollard DM, Gillis TP, Williams DL. Polymerase chain reaction assay for the detection and identification of *Mycobacterium leprae* in patients in the United States. *Am J Clin Pathol*, 1998; **109**: 642–646.
[67] Warndorff DK, Glynn JR, Fine PE *et al.* Polymerase chain reaction of nasal swabs from tuberculosis patients and their contacts. *Int J Lepr Other Mycobact Dis*, 1996; **64**: 404–408.
[68] Fine PE. Commentary: is it really *M. leprae*? *Int J Lepr Other Mycobact Dis*, 2004; **72**: 317–319.
[69] Job CK, Drain V, Truman RW *et al.* Early infection with *M. leprae* and antibodies to phenolic glycolipid-I in the nine-banded armadillo. *Indian J Lepr*, 1990; **62**: 193–201.
[70] Truman RW, Morales MJ, Shannon EJ, Hastings RC. Evaluation of monitoring antibodies to PGL-1 in armadillos experimentally infected with *M. leprae*. *Int J Lepr Other Mycobact Dis*, 1986; **54**: 556–559.
[71] Truman RW, Job CK, Hastings RC. Antibodies to the phenolic glycolipid-1 antigen for epidemiologic investigations of enzootic leprosy in armadillos (*Dasypus novemcinctus*). *Lepr Rev*, 1990; **61**: 19–24.
[72] Job CK, Sanchez RM, Hastings RC. Manifestations of experimental leprosy in the armadillo *Dasypus novemcinctus*. *Am J Trop Med Hyg*, 1985; **34**: 151–161.
[73] Paige CF, Scholl DT, Truman RW. Prevalence and incidence density of *Mycobacterium leprae* and *Trypanosoma cruzi* infections within a population of wild nine-banded armadillos. *Am J Trop Med Hyg*, 2002; **67**: 528–532.
[74] Job CK, Harris EB, Allen JL, Hastings RC. A random survey of leprosy in wild nine-banded armadillos in Louisiana USA. *Int J Lepr Other Mycobact Dis*, 1986; **54**: 453–457.
[75] Truman RW, Kumaresan JA, McDonough CM *et al.* Seasonal and spatial trends in the detectability of leprosy in wild armadillos. *Epidemiol Infect*, 1991; **106**: 549–560.
[76] Talmage RV, Buchanen CD. The armadillo (*Dasypus novemcinctus*) A review of its natural history, ecology, anatomy, and reproductive physiology. XLI Number 2 ed., Rice Institute, Houston, 1954; pp. 1–135.
[77] Loughry WJ, Truman RW, McDonough CM *et al.* Is leprosy spreading among nine-banded armadillos in the southeastern United States? *J Wildl Dis*, 2009; **45**: 144–152.