Does leprosy in Mexico occur as a zoonosis between wild armadillos (*Dasypus novemcinctus*)?*

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QUESADA-PASCUAL, F., J. HERNÁNDEZ-SÁNCHEZ, L. SANTOS-ARGUMEDO, I. ESTRADA-GARCÍA, y S. ESTRADA-PARRA, 1998. Does leprosy in Mexico occur as a zoonosis between wild armadillos (*Dasypus novemcinctus*). *An. Esc. nac. Cienc. biol.*, Méx., **44**:37–43.

SUMMARY: Various studies have been published in which it has been demonstrated that in certain parts of southern USA a considerable percentage of wild armadillos (*Dasypus novemcinctus*) have a naturally occurring infection and that this infection is indistinguishable from leprosy. In this work the search for acid fast bacilli (*M. leprae*) in 134 wild armadillos captured in 4 Mexican states (Michoacan, Nuevo Leon, Guerrero and the State of Mexico) has been reported. In none of the examined animals was possible to find evidence of a natural infection caused by *M. leprae*. This report discusses the relevance, as a source of infection for humans, this kind of zoonosis in armadillos could represent. It is also proposed that this type of investigation should be extended to states which are highly endemic for leprosy and to those states which form the frontier with the USA, before a definite conclusion can be reached.

INTRODUCTION

Leprosy is a contagious disease causing a chronic infection in humans. The disease has been recognized for more than 2,000 years and the causative agent was identified in 1873 as the obligate intracellular parasite *Mycobacterium leprae*. Leprosy most commonly causes damage to the peripheral nerves, the skin and the mucosal tract of the upper respiratory system, however almost all organs and tissues may be affected, resulting in severe deformities, the exception being the central nervous system (Fine, 1982).

* Grant.support was supplied in part by the Dirección de Estudios de Posgrado e Investigación del IPN. The authors hold fellowships from the DEDICT-COFAA, EDD and/or SNI.

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Patients with leprosy do not fall into any uniform category, with respect to clinical manifestations, histopathology or proliferation of the mycobacteria. Such diversity is due to distinctive immune responses directed against *M. leprae* by each individual. This is reflected in the classification system of leprosy which distinguishes between two clinical poles of the disease and three shifting forms situated between the two poles (Ridley and Jopling, 1966).

Leprosy is found throughout the world, although the greatest number of patients suffering from the disease are found in the under developed parts such as south-east Asia, Africa and America. In North America, Mexico is the country with the highest incidence of leprosy (WHO, 1997).

Sixteen years ago the number of leprosy patients world wide was estimated as 11 million (Fine, 1982). Today the World Health Organization (WHO) has adjusted the number to 1'150,000 patients. This reduction, by almost 90%, has been attributed to the world wide implementation, since 1991, of multi-drug therapy. However, many epidemiologists believe that this data should be treated with caution, in particular since the WHO itself has reported that during the first 5 months of 1997, the number of new cases detected at a world level was 566,604 (WHO, 1997). Furthermore, all patients under treatment have been removed from the WHO register, even though these same patients may still be suffering from leprosy.

In spite of the many years leprosy has been known and studied, it still remains one of the least understood of the main infectious diseases of man. Such that the disease presents a complex array of characteristics and behavior, for example the manner in which it is transmitted has still to be well defined, the mechanisms of pathogenesis are not clearly understood and it is still not possible to cultivate the causative microorganism *in vitro*.

A significant advance in the study of leprosy occurred in 1971 when Kirchheimer and Storrs managed to produce an experimental disseminated infection by inoculating nine banded armadillos (*Dasypus novemcinctus)* with *M. leprae* (Kirchheimer and Storrs, 1971). Four years following this discovery another article was published in which the presence of a natural infection, indistinguishable from leprosy, was found occurring in 7 wild armadillos captured in Nueva Iberia Louisiana, USA (Walsh *et al.*, 1975). These wild armadillos were captured at a distance of between 27 and 63 km from the laboratory in which the first experiments on the inoculation of experimental armadillos with *M. leprae* were carried out. This finding has changed the traditional point of view that leprosy is a disease confined solely to humans. However there is still a prevailing and valid opinion that this "natural infection" originated from experimentally infected armadillos, which escaped from their laboratory, and/or from the inadequate disposal of cadavers and other contaminated material. The controversy was further fueled by the finding that mycobacterial infections have not been detected in any of 233 armadillos captured in the states of Louisiana, Texas and Florida (Skinsnes, 1976).

Since the publication of this information several reports have appeared with contradictory results. Various investigations have not been successful in demonstrating the existence of "natural leprosy" occurring in wild armadillos (Kirchheimer, 1977a, 1977b, 1978; Sánchez and Kirchheimer, 1978). In contrast other authors have confirmed and extended the initial findings (Kirchheimer and Sánchez, 1978; Fox *et al.*, 1977; Smith *et al.*, 1978; Anderson, 1978; CDC Veterinary Publ. Hlth. Notes October 1978; Smith *et al.*, 1983; Job *et al.*, 1985). Only one wild armadillo has been found outside the USA,

which had "wild leprosy". It was captured in Mexico, in the municipality of Santa Ana Jilotzingo in the State of Mexico (Amezcua *et al.*, 1984).

Other animal species, found with natural leprosy infections, have been captured in Africa and include, a chimpanzee (*Pan trogloytes*) from Sierra Leone, (Donham and Leininger, 1977) and a mangabey monkey (*Cercocebus torguatus atys*) from Nigeria (Meyers *et al.*, 1980). In view of the discovery of naturally occurring leprosy in three different animal species the following question arises: Can leprosy occur as a zoonosis and if so how is this relevant to the epidemiology of the disease?

In 1980 we started to set up an armadillo (*Dasypus novemcinctus*) colony, in order to use them as an experimental model for leprosy infection (Quesada *et al.*, 1987). The establishment of the colony has permitted us to carry out a number of studies (Santos-Argumedo *et al.*, 1995; Guerra-Infante *et al.*, 1996) which include an investigation into the occurrence of natural *M. leprae* infections in wild armadillos captured in different Mexican states. The results of this investigation represent the basis for this communication.

MATERIALS AND METHODS

In this work a total of 134 armadillos (*Dasypus novemcinctus*) were studied, of which 82 were females (61%) and 52 males (39%). The animals were captured in their natural habitat in different Mexican states during the period extending from 1981 to 1996. In the state of Michoacan (108 animals), Nuevo Leon (19 animals), Guerrero (6 animals) and the State of Mexico (one animal). The animals were transferred alive to Mexico City were they were adapted to their new environment. The characteristics of the cages, in which they were maintained, as well as a description of their diet in captivity, have been published previously (Quesada *et al.*, 1987).

The animals were carefully examined for lesions which were then washed with soap and water before being treated with a 0.1% solution of benzalkonium chloride and coated with 2% iodine. They were also inspected to detect thickening of the skin or peripheral nerves as well as for the presence of nodules. From the 134 animals samples of nasal secretion were taken using a sterile cotton buds and the sample was smeared onto a slide, at the same time an ear imprint was taken under aseptic conditions. The slide containing both samples was stained by the Ziehl-Neelsen technique (Allen and Baker, 1968).

Duplicate samples were taken from 84 animals and one of these was stained using the Auramine-Phenol technique (Allen and Baker, 1968).

During the period of adaptation (4 to 6 weeks), 104 animals (78%) died of unknown causes. An autopsy was carried out on these animals, during which the animals were revised at the general macroscopic level and a systematic revision of imprints was carried out. Impression smears were taken from skin from the abdomen, cervical ganglia, tongue, liver and spleen and from any observed lesion. These organs and tissues were selected based on the observation that these are the sites in which acid fast bacilli are most often found in animals with naturally occurring leprosy (Binford *et al.*, 1977). The slides containing impressions were stained using the Ziehl-Neelsen technique and were examined using a light microscope. The slide preparations stained with Auramine-Phenol were examined by microscope under epiflurescence. In both cases oil immersion was used.

All preparations were carefully revised by at least two observers.

RESULTS

None of the animals showed thickening of the peripheral nerves or of the skin. Hepatomegaly and splenomegaly were also absent, however in some of the animals the presence of nodules was observed and specimens from these were used for impression smears. All the secretions and organ or tissues imprints were showed to be free of both acid fast bacilli when stained by Ziehl-Neelsen and fluorescent bacilli when prepared by the Auramine-Phenol technique.

In some of the animals the presence of other microorganisms was observed, including microfilaria and trypanosomes.

DISCUSSION

It is still not know with certainly the manner that leprosy is transmitted. The traditionally accepted view is that leprosy is acquired only when there is direct contact of a susceptible person with a lepromatous patient. However between 50% and 70% of the patients from well studied populations report that they have had no previous contact with other leprosy cases (Joseph *et al.*, 1985).

This discrepancy has made the occurrence of non human sources of infection a very real possibility. It has been proposed that certain mosses, water and soil may act as a habitat for *M. leprae* however due to the impossibility of culturing the bacilli *in vitro* the search has been carried out using indirect methods of identification such as monoclonal antibodies, and the results have indicated their presence although this has not been conclusively demonstrated (Blake *et al.*, 1987).

The involvement of insects as vectors has also been proposed. It has been possible to demonstrate very small numbers of *M. leprae* bacilli in mosquitoes (*Culex fatigans*) and bedbugs (*Cimex hemipterus*) homogenates, which have been allowed to feed on leprosy patients (Narayanan *et al.*, 1972).

Other experiments have been carried out using flies from different genera (*Musca, Caliphora* and *Stomoxys*) which were fed on nasal secretions or on ulcerating skin lesions of leprosy patients. *M. leprae* bacilli were discovered on the legs, mouthpieces, abdominal wall and in the stomachs of these insects. This suggests that flies may play a part in the mechanical transport of *M. leprae* (Huang, 1980).

In order to consider the possibility that animals act as reservoirs, such animals should mainly be found in endemic areas. Regarding the chimpanzee (Donham and Leininger, 1977) and the mangabey monkey (Meyers *et al.*, 1980) caught in Africa with natural leprosy, no connection was established between these infected animals and any leprosy patients. Therefore these findings do not have any epidemiological meaning.

A possible reservoir is the armadillo since a substantial number of them have been found naturally infected in the southern USA (Walsh *et al.*, 1986) but only one in Mexico (Amezcua *et al.*, 1984). Nevertheless, the armadillo habitat is found exclusively in temperate regions of the American continent and it is therefore extremely unlikely that the armadillo could play a relevant role, as a reservoir host, in zones such as India, Indonesia and Myanmar (Burma) where leprosy occurs most frequently. In South America where leprosy, is a highly endemic, no naturally occurring infections have been found in armadillos (Convit and Pinardi, 1974).

In order to search for the presence of *M. leprae* in Mexican wild armadillos, in this study we used tissues imprints and smears stained selectively by the Ziehl-Neelsen or the fluorochrome procedure using Auramine. In leprosy it is well established that the determination of bacteriological index by microscopy in patients with the indeterminate form or polar tuberculoid disease is often unproductive, since this is a relatively insensitive technique (Rao *et al.*, 1991). In our hands this low sensitivity technique could have accounted for the lost of detection of some naturally infected animals, however this was the previously employed method that allowed the finding of natural leprosy in armadillos (Walsh *et al.*, 1975).

Now a days more sensitive methods for the detection of a few bacteria have been developed; possibly the most widely used is the detection of specific DNA or RNA sequences from the microorganism in question, through the amplification of these by the polymerase chain reaction (PCR). This method has been applied in the last years for the diagnosis of leprosy (Hartskeerl *et al.*, 1990; Williams *et al.*, 1990; Santos *et al.*, 1997). The PCR is highly sensitive and has allowed the detection as few as 10^2 bacilli of *M. leprae* (Williams *et al.*, 1990). Unfortunately this technique is not free of pitfalls such as: false positives by cross-contamination, false negatives, non-specific amplification, etc. (Wright and Wynford-Thomas, 1990).

In 1991 Job *et al.*, compared the PCR technique for the detection of *M. leprae* in wild armadillos against others methods, the highest sensitivity judged by positivity was for the PCR (53.3%), but this gave false positives and false negatives reactions. However the presence of acid fast bacilli detected in one or more tissues by light microscopy examination was positive in 46.6% of the cases.

Although there have been anecdotal reports of the association between leprosy in humans and contact with armadillos (Lumpkin *et al.*, 1983; Thomas *et al.*, 1987) there have also been other reports in which there has been no such association (Filice *et al.*, 1977).

It is therefore not easy, with the available data, to confirm the role of the armadillo as a reservoir for leprosy. The fact that in certain areas of the USA leprosy has been found as a zoonosis merits further discussion and further investigation in order to determine how this zoonosis was initiated.

In Mexico the search for armadillos with naturally occurring infection with *M. leprae* needs to be extended, such that a larger number of states in Mexico are included. In particular those states which are highly endemic for leprosy, such as Jalisco, Sinaloa, Colima, Nayarit and Guanajuato. Also included would be those Mexican states which form the frontier with the USA, since it is known that 150 years ago armadillos migrated from Mexico to the Rio Grande valley in the USA (Storrs, 1978).

RESUMEN

Se publicaron varios estudios en los que se demostró que en algunos lugares del sur de los Estados Unidos de Norteamérica, un porcentaje importante de armadillos silvestres (*Dasypus novemcinctus)* presentan una infección natural indistinguible de la lepra. En este trabajo se informa de la búsqueda de bacilos ácido alcohol resistentes (*M. leprae*), en 134 armadillos silvestres capturados en cuatro estados (Michoacán, Nuevo León, Guerrero y Estado de México) de la República mexicana. En ninguno de los animales examinados fue posible encontrar alguna evidencia de la infección natural causada por

M. leprae. Se discute la relevancia que podría tener, en caso de existir esta zoonosis entre los armadillos silvestres, como fuente de contagio para los humanos y se propone extender este tipo de pesquisa en los estados con mayor edemia de lepra y en los estados fronterizos con los Estados Unidos, antes de llegar a conclusiones definitivas.

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Recibido: febrero de 1998. Aceptado: junio de 1998.