

A Random Survey of Leprosy in Wild Nine-banded Armadillos in Louisiana^{1,2}

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Experimental infection of nine-banded armadillos with *Mycobacterium leprae* is now well known, and a large majority of them when infected will develop lepromatous disease (^{6, 7}). During recent years, a naturally occurring disease indistinguishable from experimental lepromatous leprosy has been reported in wild armadillos (^{9, 10, 13}). We now know that there is a large reservoir of *M. leprae* in the wild, and it can no longer be assumed that man is the only source of leprosy infection.

There is evidence that armadillo leprosy had existed in nature for at least a decade before the experimental disease was reported (¹²). How and from where the animals contracted the disease is not understood. Although there is no direct relationship between the prevalence of human disease and the infection in the armadillo, there are recent reports implicating armadillos in the transmission of leprosy to humans (⁸). Therefore, it is important to study the prevalence of the disease among wild armadillos, especially in the states of Texas and Louisiana, U.S.A., where human and armadillo leprosy co-exist.

In this study, we surveyed the prevalence of leprosy among wild armadillos by histopathological examination of both ears of armadillos found dead on the roads of Louisiana.

MATERIALS AND METHODS

Ear biopsy is a routine procedure in the screening of armadillos experimentally infected with *M. leprae* (⁴). In our experience, animals found to have generalized disease on autopsy almost always have scattered macrophages with intracellular acid-fast bacilli (AFB) in ear biopsies (³).

Armadillos are commonly killed by automobiles on the roads of Louisiana. Both ears in their entirety were collected from armadillo carcasses by the staff of the Laboratory Research Branch of the Gillis W. Long Hansen's Disease Center, Carville, Louisiana, U.S.A., who were well acquainted with the disease and also with the anatomy of the armadillo. The specimens were collected from 30 June 1984 to 29 June 1985. The animals were usually killed by automobiles during the night. The ear specimens were collected before 8:00 a.m. the next morning, were transferred immediately into a jar with buffered 10% Formalin, and then transported to the laboratory. The parish (county) and the exact site from where the specimens were collected were recorded (The Figure). The specimens were allowed to fix for at least 48 hr, and then two small representative pieces from each ear were processed for paraffin sections; 5 μ m sections were cut and stained with a modified Fite's stain (²). Since the counterstain used in this procedure is Harris' hematoxylin, the histopathological changes were easily studied. However, whenever necessary, additional sections were stained with hematoxylin and eosin (H&E) and examined. Specimens which were positive for AFB were further tested for the ability of the bacilli to retain acid-fastness after extraction with pyridine. The acid-fastness of *M. leprae* is extractable with pyridine, while that of other AFB is not (¹).

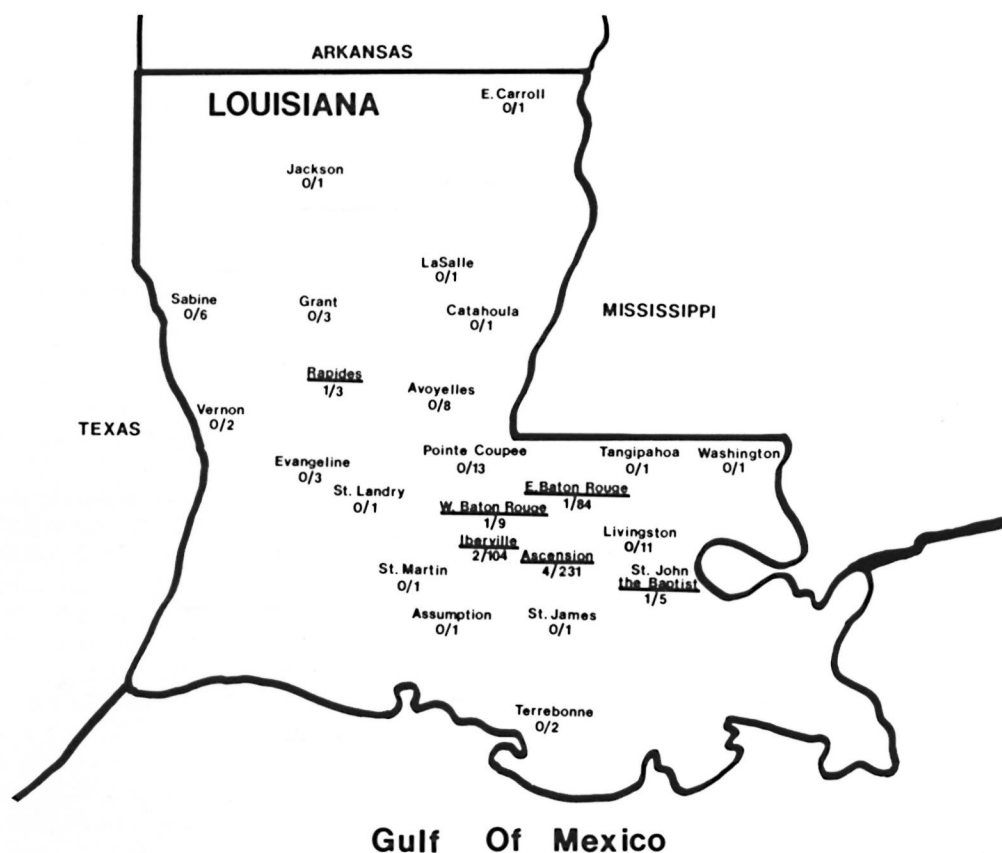
RESULTS

The numbers of specimens obtained and studied from each parish are given in Table

¹ Wayne M. Meyers, M.D., Ph.D., kindly served as Editor in regard to the submission, review, revision, and acceptance of this manuscript.

² Received for publication on 8 January 1986; accepted for publication in revised form on 6 March 1986.

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THE FIGURE. State of Louisiana, showing the distribution of 10 leprosy-infected armadillos among 494 animals collected from 24 parishes.

1. Of the 567 specimens collected, 73 were found to be autolysed and were discarded; 494 specimens were processed for histopathological examination and studied.

Table 2 shows the distribution of animals which had granulomas composed of macrophages containing AFB in the ear specimens. The granulomas varied in appearance from one animal to another. Some had a few, small scattered foci of macrophages in the subepithelial tissue, which on acid-fast stain showed many intracellular bacilli. In some there were large areas of granulomatous inflammation composed of collections of bacilli-filled macrophages infiltrating subepithelial connective tissue and occasionally striated muscles. In all of the specimens positive for AFB, organisms were present within cutaneous nerves, and they lost their acid-fastness on treatment with pyridine. Ten (2%) of the 494 animals ex-

amined showed lepromatous disease of the ear. Of the 64 parishes in Louisiana, animals from 24 were examined, and diseased animals were detected in six of these parishes (The Figure).

Table 3 shows the number of specimens collected during various seasons of the year and the number of animals with the disease. In the spring and summer there were large numbers of animals available for collection of specimens. In the autumn there were fewer animals, and in winter there were very few animals. The differences in the prevalence of the disease among the animals during the various seasons of the year was not statistically significant (χ^2 test).

DISCUSSION

Histopathological examinations of both ears of the armadillos were used in this study to diagnose disseminated leprosy. In an ear-

TABLE 1. *Distribution of armadillos.*

Parish	Total no. ear specimens collected	Decomposed specimens	No. studied
Ascension	256	25	231
Iberville	109	5	104
East Baton Rouge	94	10	84
Livingston	28	17	11
Pointe Coupee	15	2	13
West Baton Rouge	10	1	9
Avoyelles	8	0	8
Sabine	8	2	6
St. John the Baptist	6	1	5
Evangeline	4	1	3
Grant	3	0	3
Rapides	3	0	3
Terrebonne	3	1	2
Tangipahoa	3	2	1
Vernon	2	0	2
Assumption	2	1	1
St. James	2	1	1
St. Landry	2	1	1
St. Martin	2	1	1
Catahoula	1	0	1
East Carroll	1	0	1
Jackson	1	0	1
LaSalle	1	0	1
Washington	1	0	1
Iberia	1	1	0
St. Charles	1	1	0
Total	567	73	494

lier study, Meyers, *et al.* (9) reported that 46 of the 50 wild armadillos found with disseminated leprosy were positive for AFB in ear smears or ear biopsy studies. Conversely, autopsies on 353 animals with negative ear smears did not show any with disseminated disease (9). In our experience, 19 of 20 armadillos autopsied with disseminated disease had lepromatous granulomas of the ear (3). Therefore, histopathological examination of the ears for acid-fast organisms is a good method for detecting armadillos with disseminated disease. In all the 10 specimens positive for AFB, the organisms were found inside cutaneous nerves and their acid-fastness disappeared on pyridine treatment, confirming that they were *M. leprae*.

The reported prevalence of leprosy among wild animals varies considerably. In Louisiana, 47 (6.8%) infected animals were found among 689 animals examined. The prevalence varied from 4% to 30%, depending on the site (9). Smith, *et al.* (10) reported that the prevalence of leprosy among armadillos

TABLE 2. *Distribution of lepromatous animals.*

Parish	No.
Ascension	4/231
Iberville	2/104
East Baton Rouge	1/84
West Baton Rouge	1/9
Rapides	1/3
St. John the Baptist	1/5
Other 18 parishes	0/58
Total	10/494

in Texas was 4.7%. In those studies, groups of armadillos from a particular area were trapped and examined. In our study the prevalence rate is only 2.0%. This may be because the specimens were collected from animals which had come out of their burrows and were crossing a road when struck by an automobile. It is quite possible that the prevalence of the disease in the general population of armadillos is higher than 2.0% and that many of the sick animals remain in or near their burrows and were, therefore, not sampled in the present study. Of the 24 parishes from which armadillo ear specimens were collected, only six had animals with the disease. However, in all three parishes from which a reasonable number of specimens were collected and examined there were armadillos with leprosy.

Many more armadillos were apparently killed by automobiles during the spring and summer than in the autumn, and very few were found in winter (Table 3). The body temperature of the armadillo varies from 32°C to 35°C with an environmental temperature varying between 16°C and 28°C.

TABLE 3. *Seasonal variation in collection of specimens.*

Season	No. animals studied	No. animals infected
Spring		
21 March–20 June	174	0
Summer		
21 June–20 September	176	5
Autumn		
21 September–20 December	107	4
Winter		
21 December–20 March	37	1
Total	494	10

At lower temperatures the armadillos are unable to maintain a constant high body temperature (¹¹). Therefore, during the autumn and winter months they prefer to stay within their burrows and their movements are relatively limited. Although there is an apparent difference in the prevalence of the disease during the different seasons, it is not statistically significant.

An armadillo with disseminated disease may have a bacterial load of up to 10^{12} *M. leprae* in its organs (⁵). When it is killed on the road up to that many bacilli may be released into the environment. The implications of such a dissemination of *M. leprae* into the environment need further study.

SUMMARY

On the basis of the finding that lepromatous granulomas were almost always seen in the ears of armadillos with disseminated leprosy, a random survey of the prevalence of the disease among wild armadillos in the state of Louisiana, U.S.A., was conducted by examining histopathologically both ears of armadillos killed on the roads by automobiles. Ten (2%) of the 494 animals examined had disseminated leprosy. If a killed lepromatous armadillo releases up to 10^{12} *Mycobacterium leprae* into the environment, the importance of the event to the epidemiology of leprosy must be considered.

RESUMEN

En base al hallazgo de que siempre se encuentran granulomas lepromatosos en las orejas de los armadillos con lepra diseminada, se hizo un estudio (al azar) sobre la prevalencia de la enfermedad entre los armadillos "salvajes" en el estado de Louisiana, U.S.A. El estudio se basó en el examen histopatológico de las dos orejas de los armadillos muertos por automóviles sobre las carreteras del estado. Diez (2%) de los 494 animales examinados tuvieron lepra diseminada. Si un armadillo libera hasta 10^{12} *Mycobacterium leprae* en el ambiente, debe considerarse muy seriamente la importancia del evento en la epidemiología de la lepra.

RÉSUMÉ

On sait que les tatous atteints de lèpre disséminée présentent presque toujours des granulomes lépromateux au niveau des oreilles. En se servant de ce critère, on a mené une enquête randomisée de la prévalence de la maladie chez les tatous sauvages en Louisiane, aux Etats-Unis. On a procédé, pour ce faire, à l'examen histo-pathologique des deux oreilles de tatous

tués sur les routes par des automobiles, un tel échantillonnage étant considéré comme tiré au hasard. Lorsqu'un armadillo lépromateux tué libère 10^{12} bacilles de la lèpre dans l'environnement, l'importance de ce fait pour l'épidémiologie de la lèpre doit être prise en considération.

Acknowledgments. We acknowledge with gratitude the assistance we received from the staff of the Laboratory Research Branch of the Gillis W. Long Hansen's Disease Center, Carville, Louisiana, in collecting the ear and nose specimens. We are grateful to Mr. Greg McCormick for technical assistance and Mrs. Mary M. Jackson for secretarial assistance.

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