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Surveillance for Naturally Acquired Leprosy in a Nine-banded Armadillo Population

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ABSTRACT: Samples from 77 nine-banded armadillos (*Dasypus novemcinctus*) inhabiting a 16.7 km portion of the East Atchafalaya River Levee, Point Coupee Parish, Louisiana, were serologically tested and/or histopathologically examined for evidence of naturally acquired leprosy. Five of 67 (7.5%) armadillos tested seropositive with ELISA test for IGM class antibodies to the phenolic-glycolipid-1 antigen of *Mycobacterium leprae*. One of 74 (1.3%) was histopathologically positive as determined by presence of acid-fast bacteria in nerves.

Key words: Nine-banded armadillo, *Dasypus novemcinctus*, *Mycobacterium leprae*, leprosy, survey.

Susceptibility of the nine-banded armadillo (*Dasypus novemcinctus*) to experimental infection with *Mycobacterium leprae* (Kirchheimer and Storrs, 1971; Kirchheimer et al., 1972) led to establishment of this species as an animal model for leprosy (Storrs, 1971). In 1975, a leprosy-like disease was reported from free-living armadillos in Louisiana (Walsh et al., 1975). Additional surveillance, based on histological and microbiological identification of *M. leprae*, indicated infections in wild armadillo populations of Louisiana (Walsh et al., 1977; Smith et al., 1978) and Texas (Smith et al., 1983).

Development of an enzyme-linked immunosorbent assay (ELISA) test for detection of IGM class antibodies to phenolic-glycolipid-1 antigen of *M. leprae* enabled detection of antibodies from 17 of 182 armadillo sera collected in Louisiana from 1960 to 1964 (Truman et al., 1986). An antibody prevalence of 12.5% was reported for armadillos collected from two areas in Louisiana in 1984 and it was suggested that the ELISA test would provide a sensitive means for surveying armadillo populations for the purpose of gaining an understanding of natural transmission of *M. leprae* (Truman, 1985).

Past surveys of armadillos for *M. leprae* have not reported distribution of infection within a given population. Likewise, except for a single survey (Truman, 1985), population estimates for these surveyed populations are lacking. Since this information may provide insight into possible transmission routes, a serological and histological survey for *M. leprae* directed at a single armadillo population was initiated.

The study area included the 16.7 km of the East Atchafalaya River Levee in Point Coupee Parish located between Krotz Springs and Red Cross, Louisiana. The levee is bordered on both sides by bottomland hardwood forest with some adjacent agricultural fields and water. Non-forested areas comprise approximately 35% of total border. The levee is grassy and some cattle are present. Since effective capture distance was less than 50 m from the top of the levee, the actual collecting area was less than 167 ha.

Armadillos were captured with dip nets on nine nights from 19 March to 15 April 1986. Upon capture, animals were physically restrained and bled via subclavicular puncture. An eartip was collected in 10% buffered formalin and animals were eartagged and released. Capture or recapture locations were recorded to the nearest 0.16 km by odometer reading starting from the southern end of the levee. Number of an-

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imals in the collecting area was estimated from mark-recapture data using Schumacher-Eshmeyer procedure (Schumacher and Eshmeyer, 1943; Davis and Winstead, 1980).

Histological and serological testing was performed at the National Hansen's Disease Center, Carville, Louisiana. Ear sections were examined for presence of acid-fast bacteria in nerves (Job et al., 1978). The ELISA test procedure is described (Truman et al., 1986).

Seventy-seven armadillos were captured, from which 67 sera and 74 ear sections were collected. Ten of 77 animals were recaptured with one animal recaptured twice. The number of armadillos utilizing the levee was estimated at 254 ± 60 (1.5 animals/ha). Recapture locations were available for 10 of 11 recaptures and in all but one case, where a movement of 0.8 km was observed, distance between initial and recapture locations was ≤0.16 km. Numbers of captures decreased in a northerly direction as did immediate availability of adjacent forested habitat (Fig. 1). A sex ratio of 2.1:1 for males and females was observed. This disproportionate ratio may be associated with parturition which peaks in Texas armadillo populations in March and April (Enders, 1966).

Five of 67 (7.5%) armadillos tested seropositive. Four of these seropositives were located at 2.4, 2.8, 4.1, and 4.8 km corresponding to area of peak use (Fig. 1). Location of the fifth seropositive animal was lacking. One of 74 (1.3%) animals was histopathologically positive and this animal was captured at 5.6 km. All seropositive animals were histopathologically negative and a serum sample was not collected from the single histopathologically positive animal.

Truman (1985) reported antibodies to the phenolic-glycolipid-1 antigen in nine of 55 (16.3%) armadillos sampled in 1984 from the same location as the present study. Two of these 55 (3.6%) were histopathologically positive. While higher than the

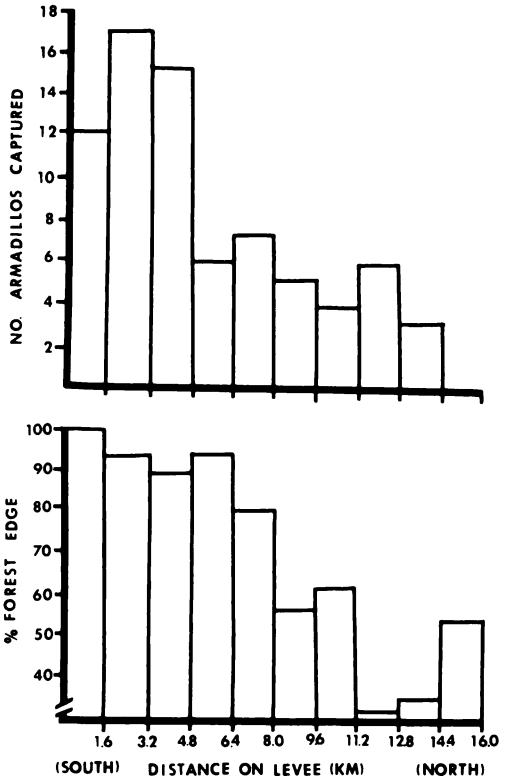


FIGURE 1. Distribution of armadillos along a 16 km stretch of the East Atchafalaya River Levee, Point Coupee Parish, Louisiana.

7.5% serologically positive and 1.3% histopathologically positive results reported here, significant differences were not detected between 1984 and 1986 serological ($\chi^2 = 2.356$, df = 1, $P = 0.125$) and histological ($\chi^2 = 0.725$, df = 1, $P = 0.394$) results with chi-square analysis.

Previous studies in Louisiana by Walsh et al. (1977) and Smith et al. (1978) report a 7% and 10% prevalence of histopathologically positive animals, respectively. Prevalence from 11 different areas in Louisiana ranged from 4 to 30% (Walsh et al., 1977) and from 0 to 15% for different counties in Texas (Smith et al., 1983). Based on histological findings, prevalence of *M. leprae* in armadillos collected in this study area is relatively low.

The reason for these differences in prevalence between areas are unclear, but may

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relate to population density and/or environmental conditions influencing transmission of *M. leprae*. Seropositive and histopathologically positive armadillos in this study were restricted to a 3.2 km section of the 16.7 km levee. Although speculative, this distribution may indicate a density or environmental relationship. Differences in prevalences between populations also could be influenced by population age structure. However, because there is a lack of defined age criteria for this species, this information is not available from this and other studies.

Serologic surveillance of armadillo populations coupled with significant population parameters such as distribution, density, and age structure, could be used to study the epidemiology of *M. leprae* infection in free-ranging armadillo populations. Armadillo populations under conditions encountered in this survey can be sampled with ease. Variations in distribution can be detected within relatively small areas allowing for recognition of possible density-dependent relationships. Likewise, movement of individual animals is minimal, as reflected in this study and by previous home range estimates (Layne and Grover, 1977; Galbreath, 1982), allowing for recognition of specific animal or soil type associations.

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