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Survey for Leprosy in Nine-banded Armadillos (*Dasypus novemcinctus*) from the Southeastern United States

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ABSTRACT: Ears from 853 nine-banded armadillos (*Dasypus novemcinctus*) from Alabama, Arkansas, Florida, Georgia and Mississippi were examined microscopically for evidence of leprosy. All were negative for both acid-fast bacteria (*Mycobacterium leprae*) and lesions compatible with leprosy.

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

The nine-banded armadillo (*Dasypus novemcinctus*) was first reported in the United States in 1854 from southern Texas; it currently occurs throughout much of the southcentral and southeastern United States. Armadillo populations in the southeastern United States resulted from northward and eastward range expansion from Texas and from populations introduced in Florida in the early 1920's which expanded into Georgia and South Carolina (Cleveland, 1970).

Leprosy in armadillos, which are the only free-living animals in North America known to be naturally infected with *Mycobacterium leprae,* has recently been reviewed by Walsh et al. (1986). Since the first report of a leprosy-like disease in an armadillo from Louisiana (Walsh et al., 1975), serological and histopathological surveys have well established the existence of naturally occurring leprosy in armadillos in Louisiana (Walsh et al., 1977; Smith et al., 1978; Truman et al., 1986) and Texas (Smith et al., 1983). Other than a report of a single armadillo with histologic evidence of leprosy from the Natchez, Mississippi area (Walsh et al., 1986), the existence of naturally occurring leprosy in armadillos throughout the rest of its southeastern range has not been demonstrated. This report presents the findings of a histopathological survey for *M. leprae* in armadillos from Alabama, Arkansas, Florida, Georgia and Mississippi.

This study utilized ear specimens from armadillos collected in five southeastern states (Alabama, Arkansas, Georgia, Mississippi and the northern panhandle of Florida of the United States). Most samples (approximately 700) were obtained from animals killed by vehicles on roads; the rest were from animals collected for other research purposes or animals that were shot. Ears were collected primarily by state wildlife personnel who were instructed to collect ear tips from only freshly killed animals (judged to be dead for <24 hr). The collections were made from April to October 1987. Specimens were collected in 10% buffered formalin solution, embedded in paraffin, sectioned at 5 μm and stained by a modified acid-fast technique (Fite-Faraco; Armed Forces Institute of Pathology, Washington, D.C. 20366, USA). Tissue from an armadillo experimentally infected with *M. leprae* served as a positive control for the acid-fast stain. When inflammation was present, additional sections were stained with haematoxylin and eosin. Specimens that were deemed too desiccated to examine histopathologically were not included in the study.

Eight hundred sixty-six specimens were collected, of which 853 were considered adequate for histopathological examination (Table 1). These included 144 samples from 15 counties in Alabama, 93 samples from 11 counties in Florida, 60 samples from 8 counties in Arkansas, 246 samples

TABLE 1. Distribution of 853 armadillos examined histologically for evidence of *Mycobacterium leprae* infection.

State	*n*	Number of animals examined by county
Alabama	144	Baldwin (7), Barbour (1), Butler (3), Choctaw (12), Clarke (57), Coffee (6), Covington (23), Geneva (1), Greene (3), Henry (1), Mobile (2), Sumter (25), Tuscaloosa (1), Washington (1), Wilcox (1).
Arkansas	60	Arkansas (13), Hempstead (8), Howard (2), Jefferson (7), Miller (18), Monroe (8), Prairie (3), Sevier (1).
Florida	93	Bay (26), Calhoun (12), Escambia (13), Gadsden (1), Gulf (23), Jackson (3), Okaloosa (2), Santa Rosa (2), Taylor (1), Walton (1), Washington (9).
Georgia	246	Atkinson (3), Baker (2), Ben Hill (11), Berrien (7), Brantly (1), Bryan (12), Bullock (1), Camden (100), Chatham (2), Chattahoochee (2), Clinch (1), Coffee (6), Decator (8), Dougherty (1), Effingham (3), Glynn (6), Irwin (3), Jeff Davis (4), Lanier (4), Liberty (7), Long (3), Lowndes (4), McIntosh (26), Mitchell (2), Pierce (12), Talbot (1), Ware (13), Wilcox (1).
Mississippi	310	Adams (38), Amite (13), Bolivar (6), Calhoun (13), Carroll (8), Claibourne (19), Clarke (59), Coahoma (1), Copiah (18), Franklin (26), George (9), Grenada (7), Harrison (4), Holmes (7), Issaquena (1), Jackson (7), Jefferson (3), Jones (2), Lafayette (8), Lincoln (1), Marion (1), Montgomery (7), Neshoba (2), Newton (5), Panola (1), Pearl River (3), Rankin (3), Sharkey (14), Stone (10), Sunflower (2), Tallahatchie (1), Washington (1), Webster (2), Yalobusha (2), Unknown (6).

from 28 counties in Georgia, and 310 samples from 34 counties in Mississippi.

All ears were microscopically negative for acid-fast bacilli. Forty-seven of the ears had dermal inflammation which was usually suppurative or pyogranulomatous and often associated with defects in the overlying epidermis. A few ears had granulomas with no identifiable causative agent. In no instance was the inflammation considered compatible with leprosy. Twenty-seven ears (3%) contained embedded plant material which was surrounded by a thin rim of granulomatous inflammation (primarily foreign body giant cells). Job et al. (1986b) suggested that such embedded plant material may serve as a fomite for transmission of *M. leprae* among armadillos. Seven ears had unidentified mites in the stratum corneum.

Histopathological examination of ears for acid-fast organisms is considered a good method for detecting armadillos with disseminated disease. Job et al. (1985) reported that 19 of 20 armadillos with disseminated disease had lepromatous granulomas of the ear. In another report, 46 of 50 wild armadillos with disseminated leprosy were positive for acid-fast bacilli in ear specimens, whereas 353 animals with negative ear specimens showed no disseminated disease (Walsh et al., 1977).

The failure to detect *M. leprae* in this study does not insure that animals from these areas are free of infection. It is possible that leprosy is present but that the prevalence is too low to be detected by the sampling technique and screening method employed. Assuming a 100% detection probability, negative histological results from 853 specimens indicate that *M. leprae*, if present, is limited to $<1\%$ of the study population (95% confidence limit) (Steel and Torrie, 1980).

A prevalence of 2% was reported for a similar histopathologic survey of armadillos killed on the road in Louisiana (Job et al., 1986a). Higher prevalences ranging from 4 to 30%, however, were reported from histologic studies of localized armadillo populations in Louisiana (Walsh et al., 1977; Smith et al., 1978). Job et al. (1986a) suggested that surveys utilizing animals killed on roads may be biased if sick animals remain in or near burrows. Although no information is available, prevalence estimates may also be biased if the risk of being killed by vehicles is associated with specific segments of the population (i.e., sex and age classes). Therefore, more intensive sampling at particular sites throughout the southeastern United States may still reveal the presence of leprosy.

There is evidence also that serological testing is a more sensitive indicator of *M. leprae* in armadillo populations than histological examination. In a single armadillo population in Louisiana, Truman (1985) reported that 16% of animals were seropositive but only 4% of animals were histopathologically positive. In a subsequent survey at the same location, 8% and 1% of the armadillos were seropositive and histopathologically positive, respectively (Stallknecht et al., 1987).

There is increasing concern that people who handle armadillos may be at risk for contracting leprosy and that armadillos should be considered a potential source of infection for humans. Leprosy has been reported in seven native-born Texans who had handled armadillos in parts of Texas known to have armadillos with lepromatous lesions (Walsh et al., 1986). Armadillo contact also was found to be an important risk factor in the development of the disease in Mexican-born residents in Los Angeles, California (Thomas et al., 1987), and leprosy is known to occur in armadillos from Mexico (Walsh et al., 1986). More recently, six time-clustered cases of leprosy have occurred in a nonendemic area of northern Louisiana. None of these patients had contact with known human cases, but they did reside in an area with an armadillo population that has a 30% prevalence of leprosy (West et al., 1988).

In the continental United States, endemic leprosy occurs in humans living in the Gulf coastal areas of Texas, southern Louisiana and California (Enna et al., 1978) with greater than one-half of indigenous cases in persons born in Texas (Joseph et al., 1985). The known distribution of leprosy in armadillos in the United States roughly parallels the distribution of endemic human leprosy in the southern United States. Negative findings in this report coincide with the low incidence of human cases in native-born citizens from the states surveyed. With the exception of one positive animal reported from 40 armadillos in southwestern Mississippi (Walsh et al., 1986), evidence of *M. leprae* in armadillos in Alabama, northern Florida, Arkansas, Mississippi and Georgia is lacking. Leprosy was not detected in 178 armadillos from southern Mississippi (Walsh et al., 1977), and there was no evidence of leprosy in over 800 armadillos sampled from Florida (Walsh et al., 1977; Kirchheimer and Sanchez, 1978; Smith et al., 1983). Although the results of this study are not definitive, the risk of acquiring leprosy from armadillos in Alabama, Arkansas, Florida, Georgia and Mississippi probably is low based on the apparently low prevalence of infection in these areas.

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