

Antibodies to the phenolic glycolipid-1 antigen for epidemiologic investigations of enzootic leprosy in armadillos (*Dasypus novemcinctus*)

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Summary Other than man, nine-banded armadillos (*Dasypus novemcinctus*) are the only known natural hosts of leprosy with high rates of disease. The origin, range and risk of their infection is not yet clear and a better description of the rate of leprosy over the armadillo's range is needed. Both histopathological examination of armadillo ear tissues and serologic screening for IgM antibodies to the phenolic glycolipid-1 (PGL-1) antigen of *Mycobacterium leprae* are good relative indices of enzootic prevalence. A survey of 216 armadillos from Louisiana and Florida detected infection only among Louisiana animals. Average antibody prevalence (12.5%) was five times higher than the fully disseminated disease rate described histopathologically (2.7%). The differences in antibody and histopathological prevalence are due to the sensitivity of the methods for detecting early infection. Histopathological examinations describe an advanced disease. The higher antibody prevalence of wild armadillos is not likely to be the result of false positive serologies from self-healing infections or other casual encounters with *M. leprae* as might be mimicked by lepromin injection. The environmental reservoir of *M. leprae* represented by infected armadillos is greater than could be previously estimated.

Introduction

A leprosy-like disease was first reported among wild armadillos (*Dasypus novemcinctus*) in 1975.¹ The aetiological agent was confirmed to be *Mycobacterium leprae* in 1983,² and today leprosy is recognized as a naturally acquired enzootic infection of nine-banded armadillos.³ The origins, range and risks of armadillo leprosy are not yet known. But exposure to armadillos could be an important risk factor in some human infections.^{4,5}

Armadillos may represent a large reservoir of potentially infectious contacts. Though they occupy a much greater range, enzootic leprosy is reported only among armadillos in Louisiana, Mississippi, Texas, Argentina and Mexico.^{2,6–11} Besides man, they are the only highly endemic natural hosts of leprosy. Their natural infection may be exploitable for modelling purposes and a better description of the rate of infection over their range is needed.

Earlier studies described enzootic prevalence by histopathologically detecting acid-fast bacilli (AFB) in dermal nerves of armadillo ear tissues. Ear biopsies successfully identify fully disseminated armadillo leprosy 92–100% of the time.^{2,7,12} But, this manifestation occurs only in the late stages of armadillo leprosy.^{12,13} We recently developed an enzyme-linked immunosorbent assay (ELISA) that detects armadillo IgM antibodies³ to the chemically defined and apparently species specific phenolic glycolipid-1 (PGL-1) antigen of *M. leprae*.¹⁴ In laboratory infected animals, the ELISA becomes positive *in a third of the time required for AFB to become detectable* in ear tissues and has good predictive value for developing fully disseminated leprosy.¹³ To determine the enzootic prevalence rates describable by these two methods in field applications, we surveyed armadillos in Louisiana and Florida, and examined the likelihood of false positive serology in armadillos exposed to killed *M. leprae* in lepromin.

Materials and methods

ARMADILLOS, SERA, AND TISSUE SAMPLES

A total of 186 wild armadillos were captured at night along open levees in 2 parishes in south central Louisiana: 131 Iberville, 55 Point Coupee. An additional 58 armadillos were similarly obtained from various field locations in Louisiana and returned to the laboratory for further study. Serum samples from 30 Florida armadillos were collected by Dr E E Storrs (Florida Institute of Technology, Melbourne, USA) and provided through the National Institutes of Allergy and Infectious Disease (Dr Darrell Gwinn, NIAID leprosy project officer). Serologic samples from Louisiana armadillos were taken by subclavian puncture or in capillary tubes following close excision of a toenail. A 1 × 2 cm snip of ear was also taken from Louisiana animals and preserved in buffered formalin for later histopathological examination. The 30 Florida armadillos had been previously necropsied with no evidence of leprosy infection found.

ELISA

Using the method previously described, serum samples were tested in an ELISA for IgM antibodies to the phenolic glycolipid-1 (PGL-1) antigen of *M. leprae*.³ The PGL-1 antigen was prepared by Dr P Brennan (Colorado State University, Ft Collins, USA) and provided through contract with NIAID. Resulting ELISA absorbencies were judged for positive and negative reaction using definitions previously determined. Specificity was confirmed by absorbing presumed positive sera with whole *M. leprae* and other mycobacterial species. Absorbencies of true positive sera were significantly reduced following absorption with *M. leprae* but not altered by absorption with other mycobacterial species.³

HISTOPATHOLOGY

Ear tissues and lepromin biopsies were prepared according to methods previously described and examined for granulomatous inflammation and acid fast bacteria within macrophages and dermal nerves.^{12,15}

LEPROMIN

Lepromin, prepared from armadillo tissues, was given intradermally to the abdominal skin as 0.1 ml containing 1.6×10^7 *M. leprae*. After 21 days the test site was examined and biopsied.¹⁵ The 58 laboratory housed armadillos received varied lepromin regimen. Group 1 was 7 armadillos which eventually developed fully disseminated leprosy as a result of experimental inoculation with from 1×10^6 to 1×10^7 *M. leprae*. Lepromin was given mid-way in the experimental infection. Animals were serologically sampled prior to injection and twice again in 3-week intervals. Group 2 was 43 un-inoculated recently captured armadillos. Each received a single lepromin and was serologically sampled before injection and twice again in 3-week intervals. Group 3 consisted of 2 additional uninfected armadillos which received 8 repeated lepromin injections once every 3 months for 24 months. They were serologically sampled every 3 months at the end of each 21-day lepromin interval. Group 4 had 2 armadillos which were experimentally inoculated with *M. leprae* but resisted disseminated infection for at least 50 months prior to lepromin injection. They received 8 lepromin injections once every 3 months for 24 months and were serologically sampled at the end of each lepromin interval. Similarly, Group 5 was 4 armadillos which also had resisted an experimental infection for at least 50 months. They were given a single lepromin injection and serologically sampled before and twice again in 3-week intervals.

STATISTICAL ANALYSIS

Statistical comparisons were made using the SAS 6.03 program package (Statistical Analysis Systems, Cary, North Carolina USA).

Results

ENZOOTIC PREVALENCE

A total of 23 (12.5%) Louisiana armadillos had detectable antibodies to the PGL-1 antigen. None (0/30) of the Florida armadillos had detectable IgM antibodies to PGL-1 (Table 1). Absorption with *M. leprae* significantly reduced the resulting ELISA absorbencies of positive sera (paired-*t*; $p < 0.05$); but absorption with other mycobacterial species failed to have significant effect (data not shown). Antibody prevalence rates between Louisiana and Florida differed significantly (Fisher's exact test; $p < 0.05$); but rates between groups of Louisiana armadillos were not significantly different. Prevalence determined by histopathological examinations of armadillo ear tissues was significantly lower than antibody prevalence. Armadillos in both Louisiana parishes had detectable AFB in ear tissues (2/55 Point Coupee; 3/131 Iberville) resulting in an average histopathologic prevalence of 2.7%. All armadillos with detectable AFB in ear biopsies had detectable IgM antibodies to PGL-1. IgM antibodies to PGL-1 were also detected in the absence of AFB in ear tissues. The 30 Florida armadillos had been previously necropsied and found to be free of infection. Differences in histopathologic prevalence rates failed to show statistical significance.

Table 1. Leprosy prevalence in wild armadillos

Location	Detection method			
	Serologic		Histopathological	
	Positives per total sampled	% positive	Positives per total sampled	% positive
Louisiana	23/186	12.5 ^a	5/186	2.7
Iberville parish	14/131	10.6	3/131	2.3
Point Coupee parish	9/55	16.3 ^b	2/55	3.6
Florida	0/30	0	0/30	0

^a = Significantly higher than Florida (Fisher's exact $p=0.026$).
^b = Not significantly different from rate in Iberville parish (Fisher's exact $p=0.183$).

Table 2. Effect of lepromin on ELISA-IgM for PGL-1 in armadillos

Group ID and size	Status	Lepromins given	Sampling		Mean ELISA	
			Number	Interval	Initial	Change
1 n = 7	Experimentally infected and succumbed	2	3	3 weeks	0.925 ± 0.428	0.056 ± 0.093
2 n = 43	Not infected naive	1	3	3 weeks	0.300 ± 0.158	0.046 ± 0.166
3 n = 2	Not infected naive	8	9	3 months	0.134 ± 0.100	0.006 ± 0.155
4 n = 2	Experimentally infected but resistant	8	9	3 months	0.326 ± 0.154	0.022 ± 0.112
5 n = 4	Experimentally infected but resistant	1	3	3 weeks	0.390 ± 0.064	0.040 ± 0.053

ELISA absorbencies <0.580 indicate no detectable antibody; 0.580 to 0.710 are equivocal; and >0.710 indicate positive detectable antibody.

LEPROMIN AND ANTIBODY RESPONSE

Exposure to *M. leprae* through lepromin had no significant influence on humoral IgM to PGL-1 in any of the groups tested (Table 2).

Discussion

M. leprae infection in wild armadillos prompts consideration of zoonotic transmission. Some strong associations relating exposure to armadillos and human leprosy have

already been drawn.⁴⁻⁶ The relative risks for enzootic leprosy would seem dependent on a variety of host factors and the likelihood of susceptible individuals having some significant interface with infected armadillos. Estimates of simple prevalence are useful for the latter.

Earlier reports on enzootic leprosy estimated prevalence by detecting acid-fast bacteria within dermal nerves of armadillo ear tissues. In Louisiana, some 90% of the armadillos are susceptible to experimental *M. leprae* infection and eventually succumb to fully disseminated leprosy with doses as low as 10^3 organisms.¹² However, reported histopathological prevalence rates vary widely by locale from 0 to 30%. Statewide averages for Louisiana and Texas range only between 2 and 4.9%.^{2,6-9} The 2.7% histologic prevalence rate seen here among Louisiana armadillos is in keeping with those previous estimates.

Louisiana PGL-1 antibody prevalence was nearly 5 times higher than the rates described histopathologically. Antibody surveys in human populations find relatively high prevalence but appear to have low predictive values.¹⁶⁻¹⁸ Better correlation is seen with armadillos. PGL-1 IgM has good predictive value for developing fully disseminated leprosy in experimental infections. The antibodies evolve slowly and first appear only about 6 months after inoculation. There is probably marked dissemination of *M. leprae* before this time.¹³ PGL-1 IgM also is not affected by casual encounters with *M. leprae* mimicked here by lepromin injections. Though in man lepromin may elicit humoral responses to other *M. leprae* antigens;¹⁹ human volunteers receiving heat killed *M. leprae* vaccine also do not develop detectable PGL-1 IgM.²⁰ Armadillos are highly susceptible to leprosy and it seems unlikely that any significant proportion of their infections self-heal.¹² Histopathologic examinations describe an advanced disease in armadillos and the differences in armadillo antibody and histologic prevalence seem related to the stage of infection detected. The high antibody prevalence of wild armadillos indicates they are a much greater reservoir of *M. leprae* than was previously believed. How they came to be infected with *M. leprae*, and the distribution or impact of their infections is not yet known.

Leprosy is either indigenous to armadillos or they have acquired the disease; perhaps by contact with infected humans.^{6,7} Armadillos are not native to the USA; but began expanding their range north from Mexico in about 1880. Another group was accidentally introduced in the state of Florida around 1925. Today armadillos range from Argentina to as far north in the USA as Oklahoma and the Carolinas.²¹ The distribution of enzootic leprosy over their range is unclear. Most early investigators thought the disease was concentrated in Texas and Louisiana. Enzootic leprosy is found only in animals from the southern migration. Neither these or other studies have found leprosy in Florida armadillos;⁶ but Amezcua¹¹ and Martinez¹⁰ also report enzootic infection in armadillos outside the USA. To date no prevailing trends have been identified. Some of the seeming disparities may be related to the sensitivity of histopathological screening methods used in earlier studies. The low expected rate of fully disseminated disease in armadillos requires large sample sizes for reliable interpretation. Histopathological methods seem better suited for confirming the etiology of suspected cases, or for surveys that necropsy large numbers of armadillos. Serologic surveys probably have better promise for describing the actual geographic distribution of enzootic leprosy. The antibodies are detected more frequently and can describe an earlier stage of disease. Additional surveys over the range of armadillos will bring important insight into the origins and risks of armadillo leprosy.

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