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Seasonal and spatial trends in the detectability of leprosy in wild armadillos

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SUMMARY

A survey for leprosy among 565 armadillos from Louisiana and Texas found IgM antibodies to the phenolic glycolipid-I antigen of *Mycobacterium leprae* in 16% of the animals. There were no geographic trends in the distribution of prevalence rates between the sites and the disease probably has a much greater range. Repeat observations in one location showed significant seasonal variations in the observable antibody prevalence rate, but the yearly average remained similar. Infected armadillos tended to be heavier, and the females usually had plasma progesterone concentrations indicative of sexual maturity. Using these characteristics to stratify the populations into adult and sub-adult cohorts, variations in the observable leprosy prevalence rate were seen to be proportional to changes in the age structure of the populations. Leprosy appears to be maintained in steady state within some regions, and nearly a third of the adult armadillos in Louisiana and Texas harbour *M. leprae*.

INTRODUCTION

Wild nine-banded armadillos, *Dasypus novemcinctus*, harbour *Mycobacterium leprae* [1]. The sylvan infection was first reported in 1975 [2] and today armadillos are recognized as a large natural reservoir of *M. leprae* [3]. The origins, range and risks of armadillo leprosy remain unclear. Besides man, armadillos are the only other natural hosts of leprosy with high rates of disease. Their wild infection may be exploitable as a model, and exposure to armadillos has been related as an important risk factor in some cases of leprosy in man [4–7].

Leprosy is either indigenous to armadillos or the animals have acquired their infection from man [8]. Armadillos are not native to the US but began expanding their range north from Mexico in about 1880. A separate group was accidentally introduced into the state of Florida in 1925. Today armadillos are found throughout the western hemisphere from Argentina to Colorado, and eastward in the US to the Carolinas [9]. But leprosy is found only among armadillos from the

southern migration and a number of disparate trends have been reported in the apparent geographic distribution of infection within that range.

Earlier investigators described enzootic leprosy prevalence using histopathological examinations to detect acid fast bacilli (AFB) within dermal nerves of armadillo ear tissues. They described its geographic distribution with point prevalence estimates, but often sampled animals in relatively small numbers from a variety of different environments. Highest prevalence rates were reported from Texas and Louisiana, the two US states historically indigenous for human leprosy. By 1986, Walsh and co-workers had examined some 1200 Louisiana armadillos and histopathological prevalence rates averaged 4% [5]. But with small individual sample numbers prevalence rates ranged by locale from 0–29·6% [10]. Surveys in Texas found a similar average prevalence rate, and, in both states, prevalence rates tended to be higher along the coastal margin than inland [1, 10]. No evidence for armadillo leprosy could be found in Florida, and prevalence rates east of the Mississippi river seemed to be lower [5]. Some felt that the disease was restricted to armadillos in Louisiana and Texas [11]. Leprosy was unknown in the new world prior to the immigration of European settlers, and Walsh and colleagues proposed that armadillos might have acquired their infection from untreated patients in the US sometimes after the animals expanded their range [10]. But Smith and co-workers found a tendency for prevalence rates to increase from north to south along the Texas coast, and suggested that armadillos may have carried leprosy with them from Mexico [1]. To date though, only 1/96 armadillos in Mexico has been reported to have leprosy [12]. The animals in Colombia, Venezuela and Paraguay are reportedly free of infection [13], but leprosy is known to occur among armadillos in Argentina [14]. Either the geographic distribution of armadillo leprosy is discontinuous, or other factors have distorted earlier prevalence estimates and supposed trends.

Histopathological examination of ear biopsies is a relatively insensitive means of detecting armadillo leprosy. The incubation period is rather long, and systemic dissemination of bacilli to ear tissues occurs only in the latest stages of the armadillo's disease [15, 16]. We have developed an enzyme linked immunosorbent assay (ELISA) [3] that detects armadillo IgM antibodies to the chemically defined and apparently species specific phenolic-glycolipid-1 antigen of *M. leprae* [17]. Results with laboratory infected animals show the assay has higher sensitivity for detecting infection than histopathological methods, yet it retains good specificity and predictive value [3, 16, 18]. To better assess the geographic distribution of armadillo leprosy, and the reliability of point prevalence estimates, we surveyed armadillos within similar low lying alluvial and coastal marsh habitats of Louisiana and Texas. We sampled one of these sites repeatedly, and examined the age structure of the population in different seasons and locations using animal weights and plasma progesterone concentrations.

METHODS

Armadillos

We sampled armadillos at three sites in Louisiana and one in Texas. Each was selected to afford collections over a wide geographic area but from within similar

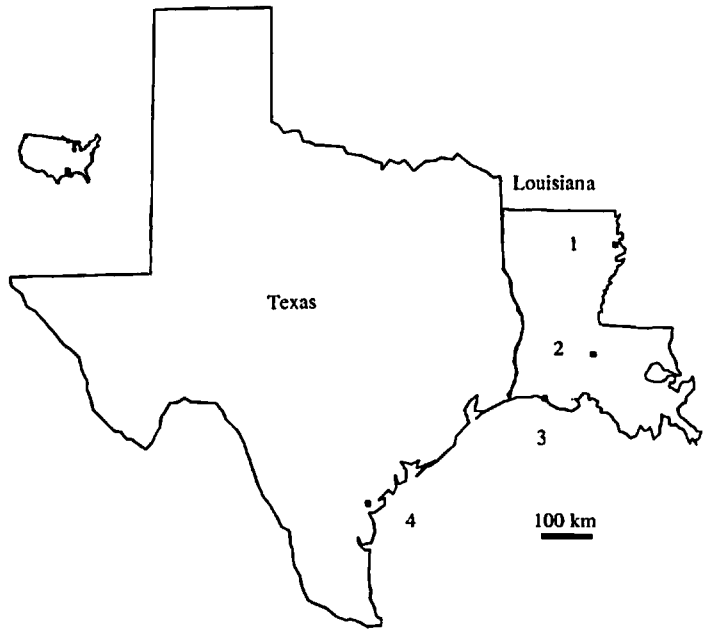

Fig. 1. Map showing locations in Texas and Louisiana where armadillos were sampled. Numbers and solid squares identify sites and correspond to location numbers listed in the Table. Inset highlights general location of Louisiana in USA.

low lying, alluvial and coastal marsh habitats. The number of animals taken and other pertinent data are shown in the Table. The general locations sampled are shown on the map (Fig. 1). The specific sites were: (*a*) The Tensas River National Wildlife Refuge (NWR) along the Mississippi river alluvial plain in northeast Louisiana; (*b*) a remote location in the Louisiana Atchafalaya basin near the Sherbourne Wildlife Management Area; (*c*) The Laccasine National Wildlife Refuge on the coastal margin of Louisiana; (*d*) The Welder Wildlife Refuge near Corpus Christi, Texas. The Welder Refuge is located approximately 835 km to the southwest of the Louisiana Tensas NWR site. We usually collected animals in each location over a period of 3–5 weeks and sought to sample 25% of the resident population. Unfortunately fewer animals were sampled in Texas than elsewhere and much of the demographic data was missing from those which we did obtain. Except for the Atchafalay site, each Louisiana location was sampled once. This same location in the Atchafalaya was used for studies in 1961 [3], 1984 [18], and 1987 [19], and it was resurveyed repeatedly over the period of this project.

Typically, armadillos were taken along 18–26 km transects of local roads. They were spotlighted at night and taken live with the aid of long handled dip nets. Upon capture each was physically restrained for sampling and the location recorded by oedometer reading or map location. Samples included the tip of one ear, which was preserved in buffered formalin, and plasma, which was harvested from blood samples obtained by sub-clavian puncture. We also recorded the sex, weight, and lengths of the inner ear and carapace before each animal was tattooed and released. Numbers and location data from animals re-captured on successive

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transects were used to estimate population parameters. The total population of armadillos in each site was computed by the Shumaker–Eschymeyer procedure [20]. Density was estimated as the ratio of the total population to the area sampled. The area sampled was derived as the length of the transect multiplied by twice the average linear movement of recaptured animals. Crowding rates were computed by Lloyd's index based on the same average linear movement interval [21]. Sufficient recapture data were available only for Louisiana sites and population parameters were not calculated for Texas.

Serology and histopathology

Ear tissues were prepared according to methods previously described and examined for granulomatous inflammation and acid fast bacteria within macrophages and dermal nerves [15, 22]. Plasma samples were tested in an ELISA for IgM antibodies to the phenolic glycolipid-1 (PGL-1) antigen of *M. leprae* using the method described previously [3]. The PGL-1 antigen was prepared by Dr Patrick Brennan (Colorado State University, Fort Collins) and provided through contract with the National Institutes of Allergy and Infectious Disease (Dr Darrel Gwinn, Leprosy project officer). The resulting ELISA absorbances were judged for positive and negative reaction using the earlier definitions [3]. Specificity of the reactions was confirmed by absorbing presumed positive plasmas with whole *M. leprae* and other mycobacterial species. The ELISA absorbency of a true positive was significantly reduced by absorption with *M. leprae* but not altered by absorption with the other mycobacterial species [3].

Progesterone

Concentrations of plasma progesterone were measured in a competitive inhibition radioimmunoassay (RIA). The assay used rabbit polyclonal antiserum against progesterone 11-α-BSA (Sigma, St Louis, MO.). The antiserum was diluted and used according to the manufacturer's recommendations in 0·05 M Tris-HCl buffer pH 8·0 containing 0·1 M-NaCl, 0·1% gelatin and 0·1% sodium azide. It had cross reactivity of $< 0{\cdot}3\%$ with corticosterone and $< 0{\cdot}1\%$ with 17-β-oestradiol. The labelled ligand was (1,2,6,21)-[^{3}H](*N*)-progesterone (181.6 Ci/mmol, New England Nuclear, Boston, MA). The assay was sensitive to 2 pg/tube. Plasma samples were tested in triplicate and aliquoted to glass tubes in volumes of 10, 25 and 50 μm. Steroids were extracted by vortexing each aliquot with 1 ml of ether. After freezing in a bath of dry ice and methanol, the ether was decanted to new tubes and dried under nitrogen. Tracer and antibody were added to these tubes in optimal volumes recommended by the manufacturers. Free steroid was separated from bound with dextran-coated charcoal. The bound fraction was counted on a Beckman 5801 liquid scintillation counter. The resulting counts were compared with results of known seeded standards incorporated in each run and corrected for per millilitre concentrations. Final results were the mean concentration of the triplicate tests [23].

Statistical analysis

Data were analysed on an IBM computer using standard programs in the SAS Package (Statistical Analysis Systems, Cary, NC).

RESULTS

Prevalence, distribution and general statistics

We found armadillo leprosy in each of the locations studied (Table 1). The Louisiana PGL-1 IgM antibody prevalence rate averaged 15.8% (84/530), and the prevalence rate described histopathologically averaged 3·4% (17/493). The prevalence rate found in Texas was similar. All of the animals with histopathologically detectable *M. leprae* in their ears also had PGL-1 IgM antibodies. The ELISA absorbances of histopathologically positive animals tended to be above 1·1 OD, but the frequency of histopathologically detectable infections was too low for any more extensive analysis. We found no significant trends in the geographic distribution of armadillo leprosy. Antibody prevalence rates in the northeast corner of Louisiana were similar to those in the centre and southwest parts of the state, and nearly identical to those in south Texas (Table 1).

The population characteristics varied by site (Table 1). Males were captured more frequently than females, but there was no bias in prevalence rates between the sexes. We found serologic evidence for leprosy in 44/287 (15·3%) of the known males and 39/222 (17·5%) of the known females. Animal density was the most disparate parameter. In Louisiana density was highest among armadillos along the coastal margin and tended to decrease northward and inland: (*a*) Tensas NWR = 40 armadillos/sq. mile, (*b*) Atchafalaya = 134/sq. mile, and (*c*) Lacassine NWR = 227/sq. mile. However, the number of armadillo interactions estimated by Lloyd's index of crowding [21] appeared to be similar in each of these sites, averaging near two armadillos per home range interval (range 1·83–2·17). There was no correlation between the prevalence rate of armadillo leprosy and density or crowding.

Sampling year and season

We sampled the Louisiana Atchafalaya site repeatedly from July 1984 [18] to January 1989. Leprosy prevalence rates remained stable from year to year but there were significant seasonal variations in the apparent antibody prevalence rate (Fig. 2). Prevalence rates were significantly higher in the winter and summer months than in the spring (spring *vs.* other seasons, χ^2, $P = 0{\cdot}03$). The lower spring prevalence was first observed in 1986 [19] and confirmed here in additional samplings. The total number of armadillos estimated to populate the site remained similar each season (mean = 271 ± 114) and there was no evidence for calamity. The spring samplings were characterized by highly disparate male:female ratios (Table 1). Both the sex ratio and leprosy prevalence rate of the seasonal samples changed rapidly from spring to summer intervals. Since armadillos are synchronized breeders which give birth in the spring [24], we sought to determine if the significant decline in prevalence could be related to age structure changes in the population.

Delimiters for armadillo age

We examined the possible relationship that certain morphological characteristics and sex hormones had with detecting the enzootic infection. We did not record these morphologic characteristics prior to 1987 and they were not available

Table 1. *Locations and characteristics of armadillos sampled*

Location (map site number)	Sampling season-year	Number sampled	Percent antibody positive	Percent histologic positive	Male/female ratio	Total pop.*
1. Tensas NWR	SM-88	77	23·4	6·4	0·9	158±36
2. Atchafalaya	SP-86	77	7·7†	1·3	2·1	254±60
	SP-88	74	9·5†	1·4	1·7	239±74
	SM-85	55	16·4	3·6	ND	274±106
	SM-88	73	17·8	4·1	1·2	286±128
	FW-87	70	15·7	5·7	1·3	302±195
	AW-88	37	18·9	ND	1·1	ND
3. Lacassine NWR	AW-88	78	20·6	1·5	1·2	88±30
4. Welder	SM-88-9‡	35	17·1	5·7	ND	ND

* Mean ± standard deviation estimated total population of armadillos.
† Significantly different from other sites or seasons.
‡ Animals taken in summers of 1988 and 1989.
NWR, National Wildlife Refuge; ND, not done, incomplete or meaningless; SP, spring; SM, summer; AW, autumn/winter.

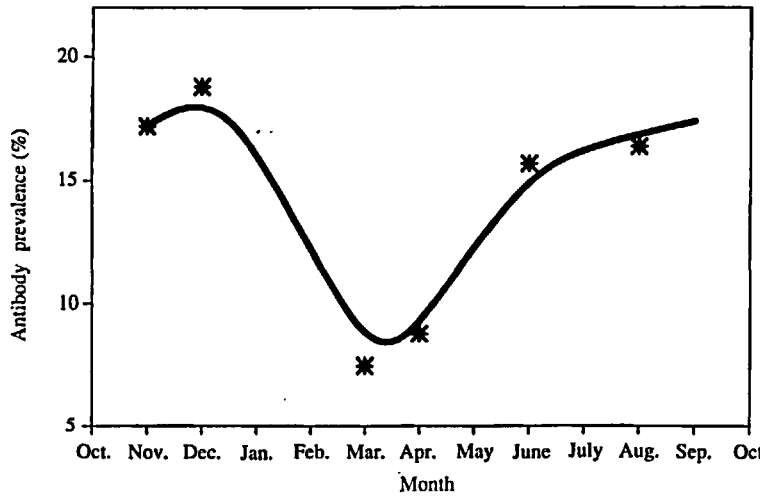

Fig. 2. Monthly variations in the observable PGL-1 IgM antibody prevalence rate among Atchafalaya armadillos. Data compiled from six surveys over 4-year period and show significant decline in observable prevalence rate for spring samplings.

for a few of the animals taken since that time. The average armadillo sampled weighed 3·7±0·5 kg, its inside ear length was 3·6±0·4 cm, and the carapace length was 34·9±3·3 cm. Each of these characteristics was highly correlated (range of r values: 0·712–0·894; $n = 347$). Since weight seemed to be the most reproducible measure, we used it for additional analyses.

Weights ranged from 0·4–6·4 kg. Obviously some very young animals were sampled. More than 60% of the infected armadillos (both sexes) weighed greater than the mean weight of all of their particular sample (season or location). None of the infected animals weighed less than 2·7 kg (Fig. 3). Though leprosy tended to be more frequent among heavier armadillos, the infected animals showed a broad range of weights.

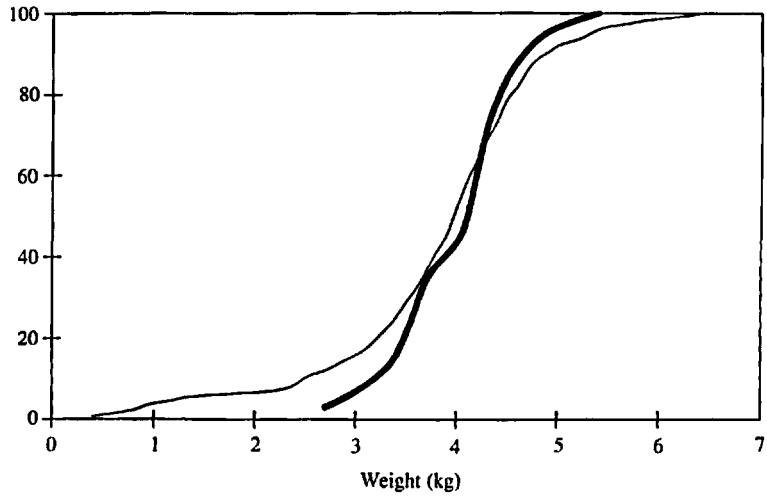

Fig. 3. Comparison of the weights found for infected armadillos (—) and for all wild armadillos (—) sampled by cumulative percent in the population.

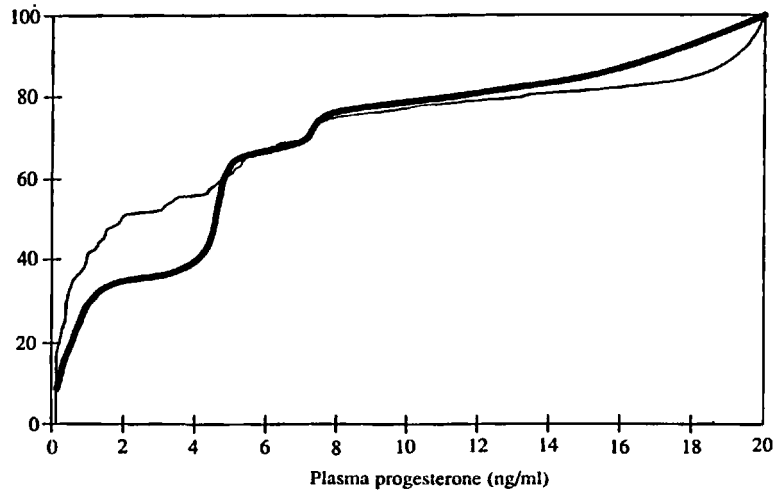

Fig. 4. Comparison of the plasma progesterone concentrations found for leprosy infected female armadillos (—) and for all wild female armadillos (—) sampled by cumulative percent in the population.

Concentrations of many sex hormones increase as animals reach maturity. Among four female armadillos born in our laboratory, plasma progesterone concentrations remained consistently less than 1·5 ng/ml until the animals reached about 20 months of age and the level increased rapidly (data not shown). Among the wild female armadillos we sampled, plasma progesterone concentrations ranged from 0 to 20 ng/ml. The average concentration tended to be higher in samples taken during winter months, the season associated with gestation for armadillos [24]. The distribution of progesterone concentrations among wild females was bimodal around 4 ng/ml (data not shown) and we used this level to mark mature sexual function. We found a broad range of plasma progesterone concentrations among the leprosy infected wild females, but 77%

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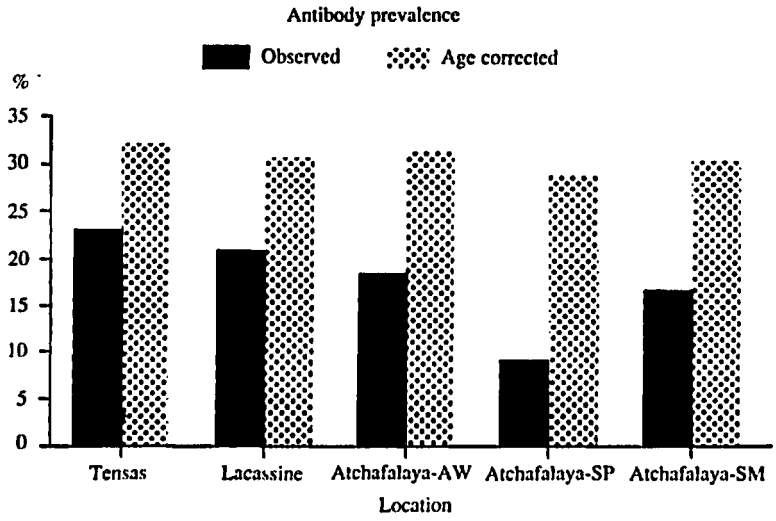

Fig. 5. Observed and age-corrected PGL-1 IgM antibody prevalence rates for armadillos from three sites in Louisiana. Observed rate is the quotient of antibody-positive animals per the number of animals sampled. The age-corrected rate assumes that all antibody-positive animals are adults. Tensas, Tensas River National Wildlife Refuge; Lacassine, Lacassine National Wildlife Refuge; Atchafalaya, Atchafalaya basin area in Louisiana. AW, autumn/winter; SP, spring; SM, summer.

(30/39) of them showed concentrations in excess of 4 ng/ml and appeared to be sexually mature (Fig. 4).

Weights and plasma progesterone concentrations were highly correlated ($r = 0{\cdot}762$) and in combination they showed a significant tendency for infected females to have higher weights, or, higher progesterone levels ($P = 0{\cdot}02$). Approximately 90% (35/39) of the infected females weighed more than one standard deviation above the mean weight of their sample period, or had plasma progesterone concentrations in excess of 4 ng/ml: 54% had both characteristics, 23% had only higher progesterone and 13% had only higher weights. Ten per cent of the infected females had neither characteristic. Using these combined characteristics to define maturity, only 30% of the Atchafalaya females sampled during the spring could be classed as adults, compared with an average of 56% during the other seasons. We used the maturity ratio found among the females to estimate the structure of the overall population. Assuming that all of the antibody positive animals are adults, then the prevalence rates observed in each season and habitat could be age corrected to between 28·5 and 32% (Fig. 5). Nearly a third of the adult armadillos harbour *M. leprae*.

DISCUSSION

Leprosy is a slow chronic disease which would be expected to manifest itself primarily among adult armadillos. Histopathologically detectable leprosy infections are not found among obviously immature armadillos [27]. The age structure of any animal population can change dramatically in different seasons and environments. Unfortunately, there are no established methods for estimating armadillo ages and it has not been possible to discern what influence population

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age structure differences may have had on some of the disparate geographic trends reported for armadillo leprosy. Weight and sexual function are somewhat relative to an animal's age. Among female armadillos, ovarian follicles mature at around 20 months of age and there is an accompanying increase in the constitutive concentration of plasma progesterone to above 4 ng/ml [25, 26]. But a number of factors besides age can effect an animal's weight and, regardless of their age, not all females will function sexually each year. We used a combination of animal weights and plasma progesterone concentrations to stratify armadillo populations into adult and sub-adult cohorts. The application here confirms that the observable prevalence rate of armadillo leprosy can be significantly influenced by differences in population age structure and it provides evidence for long-term maintenance of leprosy in armadillo populations.

Leprosy seems to present little disadvantage to armadillos. Infected animals do not develop overt clinical signs. As the disease progresses large numbers of bacilli disseminate throughout most of their body tissues [15], and infection can only be detected with histopathology or serology. The prevalence rate of histopathologically detectable leprosy seen here is similar to the rates reported in earlier studies [1, 8, 10, 28]. PGL-1 IgM antibody prevalence rates, though, are much higher than histopathological prevalence rates. The two rates differ by the stage of disease the methods can detect but both describe well established infections.

In experimentally induced armadillo leprosy, PGL-1 IgM antibodies appear in a third the time required for the bacilli to disseminate and become histopathologically detectable in ear tissues [16]. The antibodies remain detectable over the course of infection and are not elicited in response to inoculations with killed *M. leprae* [18]. Armadillos must harbour at least 10^6 actively proliferating *M. leprae* in their reticuloendothelial tissues in order to effect PGL-1 IgM seroconversion [29]. Those animals with histopathologically detectable *M. leprae* in their ears usually have much higher bacterial loads [15]. Approximately 92% of the armadillos in Louisiana appear to be susceptible to *M. leprae* [22]. They will succumb to experimentally induced infections with doses as low as 1000 bacilli [15]. Those few armadillos which can resist the infection do not develop PGL-1 IgM antibodies [16]. If they would survive long enough in the wild, most of the antibody-positive armadillos would probably go on to develop histopathologically detectable *M. leprae* in their ears. The high antibody prevalence found in the wild indicates that active leprosy is sustained by a large proportion of the armadillo population.

In the Louisiana Atchafalaya site the relative rate of leprosy in the adult segment of the population remains similar throughout the year. The significant decline in the observable leprosy prevalence rate seen each spring is the result of seasonal age structure changes in the population. Armadillos are synchronized breeders and pregnant females sequester themselves from the rest of the population during the spring for parturition and nurturing of their young [24]. The aberrantly low observable spring prevalence rate returns to higher levels by mid summer when the adult females become active again. Newman [30] also noted this behaviour and its effect on sampling armadillo populations as early as 1910. Age structure differences of armadillo populations may have contributed to some

of the disparities previously noted in the apparent geographic distribution of armadillo leprosy. The histopathologic detection method used in earlier studies is potentially more biased to detect higher rates of disease in communities which host older age structures, and most other investigators collected their animals from over a number of different seasons and environments [1, 10]. We found no significant trends in the distribution of antibody prevalence rates between these similar habitats and see no indication that the disease might be restricted to only Louisiana and Texas. Leprosy appears to be common among armadillos within these bottomland habitats and may extend to other populations in similar environments elsewhere in the Americas.

Leprosy appears to be hyperendemic among Louisiana armadillos and is maintained in steady state within some regions. Average yearly prevalence rates in the Atchafalaya site remained similar over the period of this study and were not significantly different from the rate we described for armadillos taken from there in the years 1960–4 [3]. Long-term maintenance of such a high rate of infection suggests that leprosy has occurred among armadillos for several generations. It seems unlikely that sylvan leprosy might have originated in only some hypothetical US nidus within recent history. If armadillos acquired leprosy from humans at all, they really could have done so in a number of more highly endemic foci and they would probably have repeated the event at several locations over many years. Under such circumstances armadillo leprosy might be expected to have a wide geographic distribution with high prevalence rates in areas where conditions are conductive to transmission. The animals may even be continuing to exchange *M. leprae* with humans in some of those regions today.

Some strong associations relating exposure to armadillos and the incidence of leprosy in man have already been drawn [4–7]. Nearly a third of the adult armadillos in some regions harbour *M. leprae* and the size of this reservoir alone suggests that it could contribute to at least some cases of human infection. But the relative importance of non-human reservoirs in leprosy transmission is not yet clear. Many wild animal populations harbour infectious agents that are potentially harmful to man. Their impact on human health is generally dependent on the degree of susceptibility to the agent, the rate of infection in the animals, and the likelihood that susceptible people have some significant interface with the infected animals. Most earlier studies concentrated on armadillos in the south central USA, where the incidence of leprosy in man is low. But more than 95% of the US citizenry is estimated to be naturally immune to leprosy [31], and perhaps few of these people really have significant contact with armadillos. Both the susceptibility for leprosy and human use of armadillos is much higher elsewhere in the Americas. Little is yet known about leprosy in armadillos outside the US and the role that armadillos may play in perpetuating leprosy among people in high endemic areas of the Americas merits additional investigation.

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