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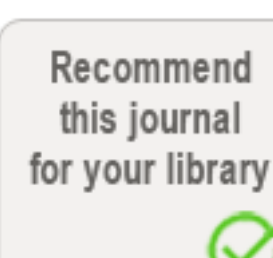
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ORIGINAL ARTICLE

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Contact with armadillos increases the risk of leprosy in Brazil: A case control study

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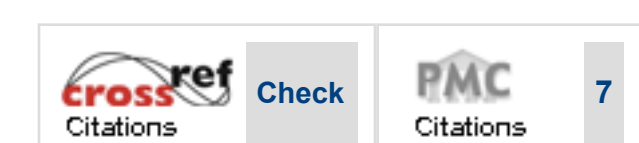
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Abstract

Background: *Mycobacterium leprae* infection has recently been detected in wild armadillos in Brazil. Leprosy is still endemic in Brazil and although its transmission is mostly by person-to-person contact, many cases report no history of previously known leprosy contact. It has been suggested that other sources of *M. leprae* may contribute to the transmission of leprosy in some areas. **Aim:** Our objective was to investigate whether contact with armadillos is associated with leprosy. **Methods:** A case-control study was carried out in Brazil. Data was collected from 506 leprosy patients and 594 controls on exposure to armadillos and age, sex, place of birth and living conditions. Univariate analysis and unconditional logistic regression were conducted to investigate whether leprosy was associated with exposure to armadillos. **Results:** Direct armadillo exposure was reported by 68% of leprosy cases and by 48% of controls ($P < 0.001$) roughly doubling the risk of leprosy, with odds ratio (OR) 2.0, 95% confidence interval (CI) [1.36-2.99]. **Conclusion:** The results of this study suggest that direct exposure to armadillos is a risk factor for leprosy in Brazil.

Keywords: Armadillo, Brazil, Case-control study, Leprosy, Transmission

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Background

Leprosy is a neglected disease that is still endemic in Brazil; in 2006, 38333 new leprosy cases were diagnosed and the prevalence was 2.1/10 000.^[1] It is well established that leprosy is transmitted by person-to-person contact,^[2] however, controversy remains as to whether other sources of *Mycobacterium leprae* such as accidental inoculation,^[3] tattooing,^[4] dog bites,^[5] and, of more relevance to this paper, direct contact with infected armadillos^[6,7,8,9,10] can contribute to its transmission.

M. leprae infection was first identified as a naturally occurring one in nine-banded armadillos from Louisiana 30 years ago. Since then, further evidence about contact with armadillos and the transmission of leprosy came from three sources: basic research on leprosy infection in armadillos, case reports of subjects with leprosy where the only possible or most likely source of infection is contact with armadillos and case-control studies investigating the association between exposure to armadillos and leprosy in humans.

Historical studies showing infection in armadillos were conducted in Mexico,^[11] in Argentina^[12] and in the USA.^[13] Serological studies found IgM antibodies to the phenolic glycolipid-I antigens of *M. leprae* in 16% of the armadillos examined in Louisiana, USA,^[14] and in 10.6% of 47 armadillos examined in the State of Espírito Santo (SES), Brazil.^[15] In USA, Job *et al.*,^[16] found *M. leprae* DNA in 53% of the inguinal lymph nodes of wild armadillos from Louisiana. In Brazil, Deps *et al.*,^[17] found *M. leprae* DNA in 12% of the blood samples and 53% of all tissue of 53 wild nine-banded armadillos in SES.^[15] Recently, four (6%) of 65 armadillos from SES were positive when tested with *M. leprae* specific primers 18 kD MLI and 65 kD ML2.^[18]

Epidemiologic case studies were carried out in USA and Brazil. Lumpkin *et al.*,^[6] attributed the leprosy diagnosed in five handlers of armadillos in USA to contact with the animals as no other contact was identified. In a survey of armadillo meat consumption among leprosy patients in the state of Syo Paulo, Brazil, Rodrigues *et al.*,^[8] reported that 101 of the 205 leprosy patients interviewed had previously consumed armadillo meat. Although the strength of their evidence is weak because these case studies had no control group, the absence of exposure to another leprosy patient is informative. Case-control studies compare the frequency of exposure to armadillos among leprosy cases with a group of controls without leprosy. In an intermediate design with two groups of cases but no healthy controls, Bruce *et al.*,^[9] in Texas (EUA) compared the frequency of direct exposure to armadillos in 32 Asiatic leprosy patients (who could easily have been infected by another patient) and 64 nonAsiatic leprosy patients. In the nonAsiatic group, 52% reported direct exposure and 19% indirect contact with armadillos, while none of the patients from the Asiatic group reported direct or indirect exposure to armadillos ($P < 0.001$).

Finally, three case-control studies were conducted comparing leprosy cases and controls without leprosy. Filice *et al.*,^[19] undertook a matched case-control study with 19 leprosy patients and 19 controls. The study did not find any significant association between armadillo contact and leprosy, but the power of the study was restricted due to the small number of patients and controls participating in the study. In a case-control study with 89 leprosy patients and 80 controls from Mexico receiving treatment in USA, Thomas *et al.*,^[7] found an increased risk of leprosy with direct armadillo exposure. The adjusted odds ratio (OR) was 6.5 in men and 4.1 in women, but again the study was small and even the approximately five-fold increase in risk did not reach statistical significance. The authors thus concluded that armadillo exposure was a potential source of infection among people born in Mexico. A case-control study in Vitória, SES, Brazil, included 136 leprosy cases (from a colony hospital and an outpatient clinic) and 173 controls with other diseases from the same outpatient clinic. The frequency of reported consumption of armadillo meat was 90.4% in cases and 15% in controls ($P < 0.001$).^[10]

This paper reports the results of a case-control study examining the hypothesis that exposure to armadillos (*Dasyus novemcinctus*) is associated with leprosy.

Methods

The study was conducted in the Metropolitan Region of Vitória (MRV) in the SES, southern region of Brazil, between June 2003 and August 2004. SES has an area of 460 778 km², a population of approximately three million people and a high prevalence of leprosy, with a case detection rate of 4 per 10 000 in 2000. Transmission is ongoing: the proportions of paucibacillary cases and of the new cases younger than 15 years of age are both still high; both are accepted indicators of recent transmission. The SES is unlikely to reach its goal of elimination of leprosy any time soon.^[1]

Cases in this study were recruited from among patients being treated for leprosy in four health units participating in the national Leprosy Control Program (LCP). All cases identified were invited to participate in the study until the required sample size was reached. We accepted the leprosy diagnosis made by the physicians of the LCP (according to WHO recommendations).^[20] Unmatched controls were recruited from among patients with other chronic diseases (mostly hypertension, diabetes and tuberculosis), who did not report leprosy and who were attending the four clinics. Recruitment to the study and interviews were carried out by a member of a six-person team (four medical students, one physician and one nurse) who visited the four health units. Cases and controls were only invited to participate in the study if they reported to the health units at the same time as the team. All suitable potential controls attending the unit were invited to participate.

Cases and controls were interviewed at the health units using a standard questionnaire on demographic characteristics (age, sex, place of birth and current residence), and were asked whether they had ever had contact with armadillos. In the case of contact with armadillos, further questions were asked about the type of exposure. Exposure to armadillos was classified as direct and indirect exposure following the classification by Thomas *et al.*,^[7] in which direct exposure includes any physical contact (hunting, eating or touching), and indirect exposure consists in residing in an area known to be an armadillo habitat. Indirect exposure was of interest because *M. leprae* can be isolated from soil,^[21] vegetation^[22] and water.^[23] making the mechanism of transmission from armadillos to humans indirect via water or soil. The categories of indirect exposure and no exposure were collapsed after an initial analysis (Table 1) clearly indicated no increase in risk with indirect exposure.

Place of birth and current residence were classified as follows: i) the metropolitan region of Vitória (MRV) includes four cities: Vitória, Vila Velha, Cariacica and Serra; ii) outside of MRV but in the SES (which will most likely be in a rural area); and iii) in other states of Brazil. Regrettably, information was not sought on the rural or urban place of residence in either the current or the past decade. Cases were grouped into four age ranges: < 15 years old, 15-40 years, 41-60 years, and > 60 years. Only cases were asked questions about contact with other leprosy patients before the initiation of the study. Information on the operational classification of leprosy (multibacillary or paucibacillary) was collected from the LCP records.

Univariate analysis and unconditional logistic regression analysis were conducted to explore the association between reported exposure to armadillos and leprosy for all cases of leprosy and separately, according to the form of contact using STATA 8. The variables included in the unconditional logistic regression model were age, sex, the health unit, place of birth and residence. These variables were chosen because they were considered *a priori* to be potential confounding variables based on the current knowledge of the disease. Ethical approval was granted by the Ethical Committee in Research of the Biomedical Centre from the Espírito Santo Federal University, Vitória, Brazil. Informed verbal consent was received after patients were given a general explanation about leprosy and the research topic. The information given to patients stated that the objective of the study was to find out more about the causes of their disease but did not mention armadillos. Written consent could not be obtained because it would be difficult to get patients' testimony about this topic as hunting and consumption of armadillos are illegal in Brazil. Another reason was the closeness and friendly environment established by the interviewer could contribute to the patients' trust in the interviewer so they could be more forthcoming about this illegal activity of hunting and consumption of armadillo meat.

Results

A total of 1100 people were interviewed (506 leprosy cases and 594 controls): 53% were men and 47% were women; the proportion of cases and controls was different in the four health units but controlling for this did not modify the OR. Patients under 40 years of age represented 42% of the cases and 57% of the controls. Exposure to armadillos increased with age: 67% of controls aged \geq 40 years reported direct exposure, but only 33% of controls < 40 years did so.

Both cases and controls reported frequent exposure to armadillos. Direct armadillo exposure was higher among leprosy patients than among the controls and this was found to be highly significant ($P < 0.001$, (Table 1)). There was no increase in risk of leprosy with indirect exposure compared to no exposure to armadillos (OR = 0.99, 95% CI = 0.64-1.53).

(Table 2) shows OR of leprosy for direct exposure to armadillos, using a collapsed category of no exposure and indirect exposure as a baseline. Adjustments were made for age, sex, place of birth and residence. There was a statistically significant two-fold increase in the risk of leprosy associated with direct exposure to armadillos after controlling for confounding variables. Among the 631 cases and controls with direct exposure to armadillos, 44% had only eaten its meat, 18% had handled it and 39% had both eaten and handled it. No significant difference was found between the type of exposure in exposed cases and controls. When the place of birth was analyzed, a higher proportion of cases and controls that had had direct armadillo exposure, were born in small cities from the SES, outside the MRV. The majority of those who were not exposed were born in the MRV. Multibacillary cases were more likely to have had direct armadillo exposure than paucibacillary cases, but this was of borderline significance ($P = 0.05$). Those with no previous contact with another leprosy case were at similar risk as those with known leprosy contact (KLC).

Discussion

Our results show that direct exposure to armadillos is associated with a two-fold increase in the incidence of leprosy; and this increase in risk is significant after controlling for confounding. In subgroup analysis, the risk was significant for those with known contact with another leprosy case and increased but not significantly so, in those without a KLC. The frequency of armadillo consumption was found to be high.

The only limitation of the design was that current residence was not controlled for, in terms of whether they lived in the large conurbation MRV. The pattern of contact with armadillos is complex, and by no means restricted to those living in rural areas. Although it is true that the majority of the people with some armadillo exposure were born in the countryside of the SES, armadillos are also found in the suburban areas of MRV, where they are captured and slaughtered for consumption. Armadillo meat can be found on the black market in MRV, and its meat is frequently sold and consumed, despite the government's prohibitions. So the impact of any rural/urban divide on exposure to armadillos is likely to be more complex than envisioned.

Hunting and eating of armadillos might also be related to poverty which may be the reason why this activity is so common in the area: the risk of leprosy increases with poverty in Brazil.^[24] We obtained no information on the socio-economic status of cases and controls in the study and therefore, did not control for social characteristics. Information on exposure to armadillos was collected after the patients were diagnosed with leprosy, and there may have been a degree of recall bias, although the hypothesis of armadillos causing leprosy is not widely known in Brazil. Two studies in Brazil stated patients' beliefs on what had caused their leprosy. In Vitória, none of > 100 patients mentioned contact with armadillos^[10] and in Rio de Janeiro, patients mentioned other animals-rats and dogs-but not armadillos.^[25] The study was not matched for age or sex, but this was controlled for in the analysis. Controls were not asked about contact with cases of leprosy. Due to this limitation, previous contact rather than a confounding variable, was used for subgroup analysis.

We expected exposure to armadillos to play a larger role among patients without a known contact with another case of leprosy (or KLC), but in fact there was an increase in both groups. A possible explanation for this is that if armadillo exposure is a risk, it would remain a risk for cases with another known cause for leprosy. Finally, some patients did not report a history of either KLC or contact with armadillos. Although contact with cases not known to have leprosy remains a possibility, this confirms the importance of further investigations of the nature of the exposure, the pattern of responses, and the possibility of other reservoirs of infection.^[26]

It is biologically plausible that armadillos play a role in the transmission of leprosy: *M. leprae* was isolated in armadillos^[15] including *D. novemcinctus* from the SES in Brazil using molecular biology techniques.^[19] Past research is consistent with armadillos playing a role in transmission, and the lack of definitive evidence in the past may have resulted from the small size of previous studies. Exposure to armadillos should be confirmed by further studies as a risk factor for leprosy in humans, the relative contribution varying according to the frequency of exposure to armadillos in each place. The lack on emphasis on armadillo exposure in control programs might have resulted from the fact that armadillos are not present in most endemic countries outside the Americas.

In conclusion, *M. leprae* has been identified in armadillos in the SES, and more leprosy cases than controls have reported increased frequency of contact with armadillos. This is strong evidence suggesting that armadillos may play a role in leprosy infection in Brazil. We recommend that further studies be undertaken in areas where armadillos and leprosy are frequent; further epidemiological and laboratory studies to clarify how *M. leprae* from armadillos can infect humans, and molecular biology studies comparing the *M. leprae* strains isolated in humans and armadillos. In terms of public health, we recommend an educational program to inform the local population about the increased risk for leprosy associated with the hunting and eating of armadillos.

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