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Zoonotic risk of Hansen's disease from community contact with wild armadillos: a systematic review and meta-analysis

Short title: Zoonotic risk of Hansen's disease

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Abstract

Understanding and quantifying the risk of Hansen's disease (HD) through zoonotic transmission of *M. leprae* infection from wild armadillos is important because hunting, handling and consumption of these animals is widespread in communities where HD is endemic, posing a potential threat to the health of individuals and to HD elimination. We conducted a systematic review (PROSPERO CRD42019159891) of publications in MEDLINE, EMBASE, Global Health, Scopus, LILACS, Biblioteca Digital Brasileira de Teses e Dissertações, Catálogo de Teses e Dissertações de CAPES, and Biblioteca Virtual em Saúde up to 09/05/2020 using Mesh and text terms in English, Portuguese, Spanish, and French. Random effects meta-analyses were performed including of subgroups by endemicity and type of exposure. Seven of the 9 included studies were case-control, 4 from Brazil and 3 from the USA, comprising 1,124 cases and 2,023 controls in total. The other two studies, one from Brazil and one from Colombia, were cross-sectional. The overall summary estimate (odds ratio, OR) for the relative odds of HD comparing people who had direct contact with armadillos and/or had eaten armadillo meat with those who had not was OR=2.60 (95% CI 1.78-3.80, p<0.001) with a predictive interval of OR=1.10-6.17. Summary odds ratios for specific exposures were: indirect contact, OR=1.39 (95% CI 1.02, 1.89) (p=0.04); eating, OR=2.29 (95% CI 1.13, 4.66) (p=0.02); hunting, OR=2.54 (95% CI 1.21, 5.33) (p=0.01). Most of the included studies had moderate risk of bias. Crude estimates were reduced by up to 24% when adjusted for confounders (where reported). Direct contact with wild armadillos was strongly associated with an increased risk of HD, whilst evidence for an increased risk of HD from indirect contact was weaker. The fraction of HD in endemic countries attributable to zoonotic transmission from armadillos remains unknown, but the precautionary principle needs to be adopted to protect public health.

Impacts

- Hansen's disease (leprosy) is considered a zoonosis in the USA but no recommendations have been made in countries of the Americas which have higher endemic burdens of HD and extensive armadillo populations.
- The combined results from nine studies from the USA, Brazil and Colombia show that direct contact with wild armadillos is strongly associated with an increased risk of HD.
- Living or working in areas inhabited by armadillos carries an increased risk of HD although evidence for this association is weaker.
- The fraction of HD in endemic countries attributable to zoonotic transmission from armadillos is unknown, but the precautionary principle needs to be adopted to protect public health.

Introduction

Whilst Hansen's disease (HD) is classified as zoonotic in the USA, with recommendations regarding contact with armadillos (CDC), no recommendations have been made in other countries of the Americas which have a higher endemic burden of HD and extensive armadillo populations known to carry *Mycobacterium leprae* infection (Deps *et al.*, 2020). In some of these countries, hunting, handling and eating armadillos is a common if unlawful practice (Kerr *et al.*, 2015). Here, the fraction of HD in the population attributable to contact with or consumption of armadillos will depend on the magnitude of the risk, the type and frequency of contact and consumption and how common these practices are in communities, together with the role of other (human-to-human) transmission routes for *M. leprae* and immunological susceptibility of individuals.

In countries with a low incidence of HD and in countries which are seeking to eliminate HD, zoonotic and other environmental reservoirs of infection are potentially important (Ploemacher *et al.*, 2020). Even in endemic countries, people newly-diagnosed with HD often report no known contact with a household case, a principal risk factor for HD (Deps *et al.*, 2006). Wildlife can carry multiple infectious agents (Kluyber *et al.*, 2020), therefore measures to reduce capture and consumption based on quantifying the risk for one pathogen may have wider public health benefits in preventing other zoonoses.

The risk to human health of contact with armadillos has not been systematically reviewed. The aim of our review was to identify and characterize studies which have investigated risk of HD in relation to contact with wild armadillos, whether indirectly (by living or working in and around armadillo habitats) or directly (through hunting, handling and consumption), and to quantify the relative risk of HD according to the type of contact.

Methods

Review protocol

The protocol for this systematic review was defined in advance and registered with PROSPERO (CRD42019159891). The review question was “What is the risk of Hansen's Disease (leprosy) in human populations as a result of contact with armadillos?”

Searches

We searched the following databases and libraries between October 26th-27th 2019: MEDLINE (Epub Ahead of Print, In-Process & Other Non-Indexed Citations and Daily 1946 to October 25, 2019), EMBASE (1974 to 2019 October 25), Global Health, Scopus, LILACS (Latin American and Caribbean Center on Health Sciences Information), Biblioteca Digital Brasileira de Teses e Dissertações (BDTD), Catálogo de Teses e Dissertações de CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), Biblioteca Virtual em Saúde (BVS). Grey literature sources were Global Health, Scopus, LILACS and the Brazilian academic databases.

Full search terms are provided in the supplementary appendix. In brief, we used Mesh and text search terms for: ("Hansen’s disease" OR "Leprosy") AND “Armadillos” in MEDLINE and EMBASE supplemented by Portuguese, Spanish and French equivalents (leprosy = lepra OR Hanseníase OR lepre; armadillo = tatu OR tatou). We imposed no date, language or publication type restrictions. Citations identified by the search were imported into EndNote (EndNote X9; Clarivate Analytics, Boston, MA 02210, USA) for de-duplication. Bibliographies of all included studies were searched manually.

Screening, inclusion/exclusion, quality assessment and data extraction

Screening and quality assessment were conducted independently and in parallel by three reviewers: title and abstract SC and PD; full text SC and JM; quality assessment SC, JM and PD. Disagreements were resolved by discussion between all three reviewers. References were included if they investigated the association between contact with wild armadillos in a natural (community/population) setting either directly through hunting and handling of armadillos and preparation and consumption of armadillo meat or indirectly through working in an armadillo habitat. Studies reporting individual cases or case series, or cases of infection due to exposure to armadillos captured or bred during research studies or in experimental or laboratory settings were excluded. See **File S1** for inclusion and exclusion questions applied during screening by title and abstract and screening of full texts. The methodological quality of each included study was rated using the 12-item NIH Quality Assessment Tool for Case-Control Studies or the 14-item NIH Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies (National Heart Lung and Blood Institute) (**File S1**). Each study was rated as being of 'good', 'fair' or 'poor' quality based on the average score of the two reviewers (SC and JM). Data extraction was done by one reviewer (SC) and checked for accuracy by two others (JM, PD) (**File S1**). Data extracted for the primary outcome were frequencies of exposed and non-exposed cases and controls (or non-cases in cross-sectional studies). Other extracted variables included: study location, period and design; case definition; control selection; and type of exposure (handling, hunting, consumption).

Analysis

Random effects meta-analysis of odds ratios was performed in Stata (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX, USA). Between-study variance was estimated as τ^2. The proportion of variation in summary estimates attributable to between-study heterogeneity was quantified using the I^2 statistic. Evidence of heterogeneity was tested by Likelihood Ratio (LR) test comparing random and fixed effects models. Prediction intervals were estimated to show the relative risk that would be expected in similar future studies (IntHout *et al.*, 2016). We used Egger's test to detect small-study bias. A 0.5 fixed-continuity correction was added where a study contains a zero cell. Subgroup analyses by exposure type and endemicity were specified *a priori* subject to data availability.

Results

Database searches identified 1,832 references (**Figure 1**), with one additional study identified through bibliographic screening (Kerr-Pontes *et al.*, 2006). After de-duplication and screening by title and abstract, 12 references were retained for full text review, of which 9 were included for data extraction. Key features of the 9 included studies are summarized in **Table 1**.

Characteristics of included studies

Five studies were based in Brazil, two in the south eastern state of Espírito Santo (Deps *et al.*, 2003, Deps *et al.*, 2008), one in the southern state of Paraná (Schmitt *et al.*, 2010), one in the north east region state of Ceará (Kerr-Pontes *et al.*, 2006) and one in the northern state of Pará (da Silva *et al.*, 2018). All but the Pará study were case-control studies recruiting people currently affected by HD from outpatient clinics, although the Deps *et al.* 2003 study also recruited people previously affected by HD who had been segregated and

treated in a so-called ‘colony’ hospital. Controls were selected from among patients attending the same clinics for other reasons and were unmatched in all except the Ceará (frequency matched on age and sex) and Paraná study (matched on age and sex).

The Pará study was based on a single site survey visit to two villages, with 7 cases (3 previously-diagnosed, 4 diagnosed by the study team) among a sample of 146 people (da Silva *et al.*, 2018). This study also determined anti-PGL-1 antibody seropositivity by ELISA, using an optical density (OD) threshold of the average plus three times the standard deviation of healthy subjects from a hyperendemic area. It was the only one of the nine included studies to measure frequency of exposure (not at all, up to once per month, more than once per month).

The three USA studies each had a case-control design, with cases identified at an outpatient clinic of the Texas Center for Infectious Diseases in San Antonio, (Clark *et al.*, 2008), reported within the past year to the state health department or Public Health Service Hospital at Carville, Louisiana (Filice *et al.*, 1977), and attending a Los Angeles county – University of Southern California Medical Center outpatient clinic (Thomas *et al.*, 1987). Controls in the San Antonio study were selected from inpatients receiving treatment for TB (unmatched), in the Louisiana study from the neighbourhood of the person affected by HD (matched on age and sex), and in the California study from two LA County Comprehensive Health Care Clinics (frequency matched by age, sex, and area of residence).

The Colombian study differed from all the other included studies in that it recruited a group of children and adolescents (age ≤18 years) who were household contacts of people with HD whose diagnoses had been within the past 5 years (Serrano-Coll *et al.*, 2019). The

outcomes for analysis were anti-NDO-LID protein A, IgG, and IgM levels with ELISA OD cut-off values corresponding to the average OD plus two standard deviations in sera obtained from healthy individuals resident in an area not endemic for HD. Background endemicity ranged from approximately 1 incident case per 10,000 population in Colombia and Paraná (Brazil) to 3/10,000 in Ceará, 4/10,000 in Espírito Santo and >4/10,000 in Pará.

Quality assessment of included studies

All the included studies were published in peer-reviewed journals; none of the databases searched returned grey literature that could be included. Quality assessment rated risk of bias as 'moderate' in 6/9 studies; risk of bias in the other three (all case-control) was moderate-to-high (Thomas *et al.*, 1987, Filice *et al.*, 1977) or high (Deps, 2003) mainly because of weaknesses in selection of controls and lack of adjustment for confounders (**File S1**), as described below.

HD subtype classifications were reported by only three studies (Clark *et al.*, 2008, Kerr-Pontes *et al.*, 2006, Thomas *et al.*, 1987), none of which then used these in subgroup analyses (**Table 1**). The studies took different approaches to collecting and analysing data on cases and controls having been in household contact (HHC) with other HD patients: Kerr-Pontes *et al.* had selection criteria for cases and controls which excluded HHC from both groups (potentially introducing selection bias); Clark *et al.* and da Silva *et al.* collected HHC data and found no association with case status; in both Deps *et al.* studies HHC data were recorded only for cases; Schmitt *et al.* adjusted for HHC in their multivariable analysis (in which cases had 8-fold odds of HHC exposure); and Thomas *et al.* excluded HHC in a sensitivity analysis and reported no difference in their findings. In the Serrano-Coll *et al.*

study all participants were HHC by inclusion criteria. Exclusion of HD from among controls was mentioned explicitly (by self-report) by one study (Deps *et al.*, 2008).

Only five of the nine studies reported adjusted estimates (Clark *et al.*, 2008, Deps *et al.*, 2008, Schmitt *et al.*, 2010, Thomas *et al.*, 1987, Serrano-Coll *et al.*, 2019), of which two did not reported adjusted estimates for all types of exposure (**Table 2**). Adjustment for confounders reduced the crude effects of three studies in the meta-analysis by 11-24% (Clark *et al.*, 2008, Deps *et al.*, 2008, Schmitt *et al.*, 2010), whilst one study showed negative confounding that increased effect sizes by 11-19% (Thomas *et al.*, 1987). Kerr-Pontes *et al.* reported a 27% reduction in the OR for eating armadillo meat (from OR=1.14 to OR=0.83) when clustering (by municipality) was taken into account.

Case-control studies are susceptible to recall bias. This was mentioned by 5 of the 7 included case-control studies. Two groups of authors suggested that the armadillo-HD hypothesis was not commonly known among study participants in Brazil or the USA (Deps *et al.*, 2008, Filice *et al.*, 1977), whilst 4 studies designed questionnaires which asked about a range of exposures, including contacts with other wild animals, to obscure the study hypothesis (Clark *et al.*, 2008, Filice *et al.*, 1977, Kerr-Pontes *et al.*, 2006, Thomas *et al.*, 1987). Clark *et al.* reported that more cases than controls had heard of an association between animals and HD (P=0.001) but found no difference between the two groups regarding having heard specifically of an association between armadillos HD (P=0.71).

Blinding of assessors to case-control status could not be determined for any of the included case-control studies. Two groups of authors explicitly acknowledged that their studies could not assess time and duration of exposure and the onset of an infection because of the long

and variable incubation period for HD (Kerr-Pontes *et al.*, 2006, Schmitt *et al.*, 2010). The one study deemed at high risk of bias yielded very high odds ratios: OR=44.8 (95% CI 20.5, 100.6) comparing former HD patients with controls and OR=158.3 (95% CI 23.3, 6513) comparing current HD cases with the same controls. In the former HD patient analysis, control selection was non-concurrent; in the current HD patient analysis, only 1/29 cases reported not eating armadillo meat compared with 147/173 controls. Given the high risk of bias and outlier odds ratios, neither result was carried forward to our meta-analysis.

Findings of included studies

The main findings of the 9 included studies are summarized in **Table 2**; data extracted from each study are presented in **File S1.** Six of the eight studies with HD as outcome, four from Brazil and two from the USA, reported at least one positive association between contact with armadillos and HD (Clark *et al.*, 2008, da Silva *et al.*, 2018, Deps, 2003, Deps *et al.*, 2008, Kerr-Pontes *et al.*, 2006, Thomas *et al.*, 1987) whilst two, one each from Brazil and the USA, found no association (Filice *et al.*, 1977, Schmitt *et al.*, 2010).

The overall summary estimate (odds ratio, OR) for the relative odds of Hansen's disease comparing people who consumed armadillo meat and/or had direct contact with armadillos with those who did not was OR=2.60 (95% CI 1.78 to 3.80) ($p<0.001$) (**Figure 2**) with a predictive interval indicating an effect between 10% higher and 6.2-fold odds of HD. Between-study variance was $\tau^2=0.06$ and the proportion of variation in the summary estimate attributable to between-study heterogeneity was low ($I^2=27\%$) and unsupported by statistical evidence ($p=0.24$), i.e. variation was consistent with random error. Egger's test indicated no small-study bias ($p=0.54$) (**Figure S1**). The results included in this meta-analysis combined all forms of contact (hunting, handling, food preparation, and eating) except

indirect contact. Filice *et al.* noted that physical contact (when hunting) was "slight and infrequent" and none of the cases who hunted had brought armadillos home or eaten them. We added the one case in this study who had eaten armadillo to the exposed case group (of hunters); no controls had eaten armadillo meat. Where type of contact was described, all studies except Kerr-Pontes *et al.* reported that people who hunted and handled armadillo also ate armadillo: Clark *et al.* 67% of cases and 44% of controls; da Silva 100% of cases; Deps et al. (2008) 39% of cases and controls.

Summary odds ratios for direct contact by endemicity of study settings were: endemic, OR=2.23 (95% CI 1.73, 2.88) (p<0.001); non-endemic, OR=4.22 (95% CI 2.34, 7.59) (p<0.001); test-for-heterogeneity between subgroups p=0.05 (**Figure 3**). Between-study heterogeneity was negligible (I^2=0%) for both subgroups.

Summary odds ratios for specific types of exposure were: indirect contact, OR=1.39 (95% CI 1.02, 1.89) (p=0.04); eating, OR=2.29 (95% CI 1.13, 4.66) (p=0.02); hunting, OR=2.54 (95% CI 1.21, 5.33) (p=0.01) (**Figure 4**). Between-study heterogeneity was low-to-moderate (I^2=17-37%) and unsupported by statistical evidence (p≥0.2) for each subgroup except eating armadillo meat, which had strong evidence (p<0.001) of high heterogeneity (I^2=87, τ^2=0.51). Three studies provided data on eating 'only' (Filice *et al.*, Kerr-Pontes *et al.*, and Deps *et al.* 2008) with a summary estimate of OR=2.25 (95% CI 0.68, 7.43) (p=0.19).

In their cross-sectional study, da Silva *et al.* found a strong association between HD and hunting armadillo but no association with eating armadillo or other direct contact; anti-PGL-1 seropositivity was not associated with any type of contact, but there was a significantly higher median anti-PGL-1 titre in those who consumed armadillo meat more than once per

month compared with not at all (p=0.01). Similarly, among children and adolescents who had been household contacts of HD cases, Serrano-Coll *et al.* found strong evidence that anti-NDO-LID antibody levels were higher in those who had consumed armadillo meat compared to those who had not (p=0.01 for Protein A, p=0.001 for IgM and p=0.01 for IgG).

Two studies which asked parallel questions about exposure to other wild animals reported some positive associations similar to those found in the same studies for HD, specifically hunting and cleaning rabbits and birds (Clark *et al.*, 2008) and hunting and fishing in general (Kerr-Pontes *et al.*, 2006). Clark *et al.* indicated that these associations might reflect the fact that most of those who hunted and prepared armadillo also hunted and prepared other animals or that hunting in general could be a marker of exposure risk, as also suggested by the results of Kerr-Pontes *et al*. Conversely, Schmitt *et al.* found no association either for armadillo meat intake (OR=1.20, 95% CI 0.77-1.90) or other wild animal meat intake (OR=1.23, 95% CI 0.79-1.91).

Exposure to armadillo as an independent risk factor adjusted for other known risk factors was investigated by Clark *et al.* (residence in Mexico as an adult, OR=24.9 (95% CI 2.52-245); eating armadillo, OR=3.65 (95% CI 1.07-12.4); family contact, no association), Deps *et al.* 2008 (same HD risk among contacts and non-contacts, ORs not shown), and Schmitt *et al.* (7.5-fold odds of HD among HD contacts, no association with armadillo contact).

Discussion

This is the first systematic review to quantify the relative risks of Hansen's disease through contact with and consumption of wild armadillos. Our review shows overall 2.6-fold odds of HD comparing people who had direct contact or have eaten armadillo meat with those who

reported no contact or consumption, with a larger effect in non-endemic compared with endemic areas. In subgroup analyses there was an increasing trend in effect sizes from indirect contact (1.4-fold odds), to eating armadillo meat (2.3-fold odds), hunting armadillo (2.5-fold odds), and direct contact (2.9-fold odds).

As might be expected, specific types of exposure in the three higher-risk subgroups overlapped, with people who hunted and handled armadillo also tending to eat armadillo meat (although not in all cases). Similarly, some people who reported eating armadillo will also handle the meat. Whilst there was no association with HD of 'only eating' armadillo, this estimate was based on just three studies, one of which had a single exposed case. It was therefore not possible for us to establish whether this exposure alone might confer lower or no risk, given that cooking armadillo meat will sterilize any *M. leprae*.

If we adopt a cautious interpretation that unmeasured and residual confounding, biases and clustering would reduce our overall summary estimate by at least 40%, then the effect of direct contact tends towards 2-fold odds. That a true effect remains after adjustment is plausible, given that several of the studies showed independent effects of armadillo contact or consumption on HD risk regardless of exposure to or stratification by known risk factors, including in the Serrano-Coll *et al.* study where all participants had been exposed to household HD cases. A causal argument is strengthened by the dose-response effect apparent in our subgroup meta-analyses and by anti-PGL-1 antibody titres increasing with frequency of armadillo meat consumption as reported by Kerr-Pontes *et al.* We also have circumstantial evidence of people newly-diagnosed with HD often reporting no known contact with a household case, including 55% (280/506) of the cases in the Deps *et al.* 2008 study (Deps *et al.*, 2006), which might suggest an exogeneous pool of *M. leprae* infection.

Perhaps the strongest supporting evidence for the likelihood of HD risk from exposure to wild armadillos at community level is provided by well-documented case reports and case series in low-incidence countries which, although inadmissible for our review, substantiate zoonotic transmission at individual level (Domozych *et al.*, 2016, Lumpkin *et al.*, 1983). Also consistent with our review findings are sporadic cases implicating occupational exposure to armadillo habitats as a risk factor for HD (Mohan & Fairley, 2020), probably through *M. leprae* persistence in the environment (Ploemacher *et al.*, 2020, Tió-Coma *et al.*, 2019). We found that living or working in armadillo-inhabited areas increased HD risk by 39%, albeit with relatively weak evidence of an association. The average prevalence (by meta-analysis) of *M. leprae* infection in wild armadillos in Brazil was equivalent to 1 in 10 armadillos being infected, albeit with wide variation (Deps *et al.*, 2020). Recent studies have reported similar overall prevalences in multiple states in the USA (Ploemacher *et al.*, 2020).

Further studies like those reviewed here are unlikely to further greatly our understanding of HD risk in relation to armadillo capture and consumption in communities. We would argue that it has been established beyond reasonable doubt that armadillos carry *M. leprae* infection in endemic and non-endemic American countries (Deps *et al.*, 2020, Truman *et al.*, 2011), and that this poses a risk to human health. A wide variety of contact with armadillos through hunting, butchering, cooking and consumption has been described among residents of the state of Ceará, in north-eastern Brazil (Kerr *et al.*, 2015). This included hunting for pleasure as much as for economic reasons. The latter was reported to have declined when the government provided more financial support to families through poverty reduction programmes. Stricter legislation and punitive measures might be effective in curtailing armadillo-hunting where it is done mainly for sport but are unlikely to have much impact

where armadillos are captured as a source of additional income. Instead, efforts to effect behaviour change need to be focused on educational programmes coordinated by national and state HD agencies, adopting a One Health approach in partnership with national or regional animal conservation and ecology groups perhaps aimed at younger generations (Gazzinelli *et al.*, 2016). In addition, recommendations regarding zoonotic reservoirs need to be incorporated into official guidelines for the control and elimination of HD in Brazil.

Strengths and limitations

The main strength of our review is that its scope was very focused, and we are confident that all relevant studies have been identified from regions where armadillos and humans interact and HD cases occur, and is therefore unlikely to be affected by publication bias. Despite differences in settings and study designs, the degree of between-study heterogeneity was mostly in the low-to-moderate range and was not supported by statistical evidence (I^2 p-values were ≥0.2 for all subgroups except eating armadillos), i.e. the observed variability could be attributed to sampling rather than differences between studies. The main limitations have been described in our quality assessment of the included studies, principally that the meta-analyses were based on crude measures of effect, that observational studies are susceptible to multiple sources of bias, and that the nature of the exposure and the outcome preclude determination of a temporal relationship between contact with armadillos and development of HD. Overlapping exposures preclude completely reliable estimation of subgroup-specific effects. Residual and unmeasured confounding is of concern, given the strong socioeconomic determinants of HD risk (Nery *et al.*, 2019).

Conclusion

Our review has shown that direct contact with wild armadillos is associated with an increased risk of HD at community level even in endemic settings. Whilst the fraction of HD in endemic countries attributable to zoonotic transmission from armadillos is unknown, the precautionary principle should prevail to protect public health, with educational efforts directed towards changing behaviour and improving community knowledge of risks associated with capture and consumption of wild animals.

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Conflict of interest

The authors declare no competing financial interests or other source of conflict of interest.

Ethical considerations

No human or animal subjects were directly involved in this study.

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Table 1: Characteristics of included studies investigating risk of Hansen's disease (HD) due to exposure to wild armadillos

Study	Country (state)	Period	Design	Types of contact	Cases†	Controls	Remarks
(Clark *et al.*, 2008)	USA (Texas)	01/2001 to 06/2005	Case-control	Hunting, cleaning, eating, direct (= working with armadillos or keeping as pets), indirect (= working in armadillo habitat),	Public health facility HD outpatient, n=28, mean age 52 (range 21-76) years, 75% (21/28) male; 7 TT, 1 BT, 3 BB, 4 BL, 8 LL, 5 I/unclassified	Public health facility TB inpatient, n=59, mean age 43 (range 22-70) years, 88% (52/59) male	Contact with other animals (rabbits, deer, birds, squirrels) was investigated for comparison
(da Silva *et al.*, 2018)	Brazil (Pará)	Not reported	Cross-sectional	Hunting, cleaning, eating (with frequency)	Sample: N=146 people (all ages) from two villages, 43% (63/146) male, 3 HD case previously diagnosed and treated plus 4 HD cases diagnosed by study team		The study also tested for anti-PGL-1 antibodies by ELISA, finding 63% (92/146) positive; HD >4/10,000 people/year
(Deps *et al.*, 2003)	Brazil (Espírito Santo)	10/2000 to 02/2001	Case-control	Eating armadillo meat	Former HD patients from Pedro Fontes hospital, n=107; current HD outpatients from dermatology service, n=29	Dermatology service patients, n=173	Age and sex in each group not reported; HD 4/10,000 people/year
(Deps *et al.*, 2008)	Brazil (Espírito Santo)	06/2003 to 08/2004	Case-control	direct = any physical contact (hunting, eating or touching); indirect = residing in an area known to be an armadillo habitat	Current HD outpatients at 4 health units, n=506	Patients attending the same health units for other reasons, n=594	Age and sex in each group not reported; 53% male overall; 42% of cases and 57% of controls age <40y; HD 4/10,000 people/year
(Filice *et al.*, 1977)	USA (Louisiana)	1975	Case-control	Touched, hunted, eaten (all but 2 cases and 3 controls had seen wild armadillos). Two cases had overseas military experience in HD-endemic countries "long before their onsets"	Louisiana residents who had leprosy between 1966-1975, n=19 (from initial 39 cases), mean age 54 (range 19-81) years, sex not reported	Neighbourhood controls matched on age (within 5y if <30y or within 10y if ≥30y) and sex	20 excluded cases comprised: 13 HHC, 3 who moved to Louisiana <1y before HD onset, 1 refused, 2 deceased, 1 with dementia
(Kerr-Pontes *et al.*, 2006)	Brazil (Ceará)	03/2002 to 08/2002	Case-control	Hunting or eating 'peba' (= armadillo species *Euphractus sexcinctus*) in past 10 years; indirect (our definition) = working in forest in past 10 years	Current HD outpatients at 4 primary care centres diagnosed in past 2 years, n=226, median age 38-51 (range 20-87) years, 47% (107/226) male; 88 TT, 58 B, 43 LL, 33 I	Patients attending the same primary care centre for non-dermatological reasons, n=857, median age 30-48 (range 20-87) years, 40% (345/857) male	Multiple socioeconomic, envrionmental and behavioural variables collected, including hunting, keeping animals, working in forest or fields; HD 3/10,000 people/year

Study	Country (state)	Period	Design	Types of contact	Cases†	Controls	Remarks
(Schmitt *et al.*, 2010)	Brazil (Paraná)	2005 to 2009	Case-control	Eating armadillo at any time prior to HD diagnosis. Patients who could not recall exactly whether they had or had not consumed any armadillo meat were excluded.	HD outpatients attending Pró-Hanseníase Foundation clinic, n=121, mean age 48 years, 58% (70/121) male	Non-HD dermatological patients attending same clinic, n=242, mean age 49 years, 58% (140/242) male	Controls had: psoriasis (13%), acne (7%), basal cell carcinoma (7%), actinic keratosis (7%), onychomycosis (6%), melasma (5%), vitiligo (5%); HD 1/10,000 people; HD 1/10,000 people
(Serrano-Coll *et al.*, 2019)	Colombia (3 regions)	2015 to 2016	Cross-sectional	Eating armadillo meat	Sample: N=82 children and adolescents ≤18 years old who had HHC with a HD patient whose diagnosis was within the past 5 years. Case definition was anti-NDO-LID protein A, IgG, and IgM ELISA optical density (OD) > 0.127, 0.183, 0.226		Anti-NDO-LID OD also analysed in linear regression; HD 1/10,000 people/year
(Thomas *et al.*, 1987)	USA (California)	Not reported	Case-control	direct = physical contact, whether occupational, recreational, or dietary; indirect = residence in an area known to be a habitat to any of a list of game animals, including armadillos	Mexican-born HD outpatients at LA County-USCMC clinic, n=88, mean age (men) 40 (range 23-65) years (women) 42 (range 26-84) years, 64% (56/88) male; 3 BT, 2 BB, 8 BL, 76 LL	Mexican-born outpatients at LA County health clinics, n=79, mean age (men) 42 (range 21-65) years (women) 49 (range 20-84) years, 51% (40/79) male	

† Hansen's disease classifications: TT = 'tuberculoid'; BT = 'borderline tuberculoid'; BB = 'borderline borderline'; BL = 'borderline lepromatous/virchowian'; LL = 'lepromatous/virchowian'

Table 2: Findings from included studies investigating risk of Hansen's disease (HD) due to exposure to wild armadillos

Study	Cases	Type of exposure	Unadjusted odds ratio (95% CI)[a]	Adjusted odds ratio (95% CI)	Other findings	Remarks
(Clark *et al.*, 2008)	HD outpatients	Hunting	4.17 (1.49, 11.7)	n/r		
		Cleaning	4.91 (1.56, 15.4)	n/r		
		Direct	3.54 (1.21, 10.4)	n/r		
		Indirect	1.68 (0.62, 4.60)[a]	n/r		
		Eating	4.82 (1.72, 13.5)	3.65 (1.07, 12.4)		
		Any	7.03 (1.80, 39.5)[a]	n/r		
(da Silva *et al.*, 2018)	HD current and new	Hunting	6.73 (1.41, 32.1)	n/r	PGL-1 OR = 1.22 (0.50, 2.94)	There was a significantly higher median anti-PGL-1 titre in those who consumed armadillo meat most frequently (p=0.01), OR = 1.77 (95%CI 0.64, 4.89)[c]
		Eating	3.81 (0.45, 32.6)	n/r	PGL-1 OR = 1.09 (0.54, 2.17)	
		Direct	3.27 (0.38, 27.9)	n/r	PGL-1 OR = 0.94 (0.46, 1.91)	
(Deps *et al.*, 2003)	Former HD patients	Eating	44.8 (20.5, 100.6)[a]	n/r		
	Current HD patients	Eating	158 (23.3, 6513)[a]	n/r		
(Deps *et al.*, 2008)	Current HD patients	Direct	2.34 (1.78, 3.06)	2.01 (1.36, 2.99)		'Eating only' exposure odds ratio was calculated from data provided by the author (Deps)
		Indirect	0.99 (0.64, 1.53)	n/r		
		Any	2.04 (1.56, 2.68)[a]	n/r		
		Eating	4.19 (2.90, 6.06)	n/r		
(Filice *et al.*, 1977)	HD patient ('66-'75)	Indirect	1.59 (0.16, 21.2)[a]	n/r		
		Direct	0.74 (0.12, 4.32)[a]	n/r		
		Hunting	1.42 (0.20, 11.3)[a]	n/r		
		Eating	no exposed controls	-		
(Kerr-Pontes *et al.*, 2006)	HD outpatient <2y	Hunting	1.42 (1.12, 1.79)	n/r		For 'Indirect' exposure we used data for 'working in forest in past 10 years'
		Eating	0.83 (0.65, 1.05)	n/r		

Study	Cases	Type of exposure	Unadjusted odds ratio (95% CI)[a]	Adjusted odds ratio (95% CI)	Other findings	Remarks
		Indirect	1.43 (0.90, 2.29)	n/r		
(Schmitt *et al.*, 2010)	HD outpatient	Eating	1.20 (0.77, 1.90)	1.07 (0.56, 2.04)		
(Serrano-Coll *et al.*, 2019)	NDO-LID protein A	Eating	12.6 (3.3, 47)	8.1 (0.92, 70)		
	NDO-LID IgM	Eating	17.6 (4.2, 74)	9.4 (0.97, 90)		
	NDO-LID IgG	Eating	5.5 (0.9, 31)	7.4 (0.32, 170)		
(Thomas *et al.*, 1987)	HD outpatient (male)	Direct	6.5 (2.3, 18.1)	6.5 (1.5, 28.5)		
		Indirect	2.4 (0.8, 7.4)	2.7 (0.7, 10.5)		
		Any	4.5 (1.8, 10.9)	4.0 (1.3, 13.0)		
	HD outpatient (male)	Direct	3.5 (1.0, 9.1)	4.1 (0.8, 21.7)		
		Indirect	3.0 (1.0, 12.3)	3.5 (0.9, 14.0)		
		Any	3.2 (1.2, 8.4)	3.7 (1.0, 13.4)		
	HD outpatient (both)	Direct	5.59 (2.40, 13.2)[a]	n/r		
		Indirect	2.69 (1.14, 6.36)[a]	n/r		
		Any	3.97 (1.96, 8.09)[a]	n/r		

[a] Unadjusted odds ratios marked with this superscript were not reported in the original publication but were calculated for this review from the reported frequencies

[b] Anti-PGL-1 antibody titre OD>0.295

[c] Armadillo meat eaten frequently (more than once per month) compared to less than once per month

Figure legends

Figure 1. PRISMA Flow Diagram

Figure 2. Relative risk of Hansen's disease (odds ratios) comparing groups exposed to wild armadillos with unexposed groups

Figure 3. Relative risk of Hansen's disease (odds ratios) comparing groups exposed to wild armadillos with unexposed groups by endemicity of study setting

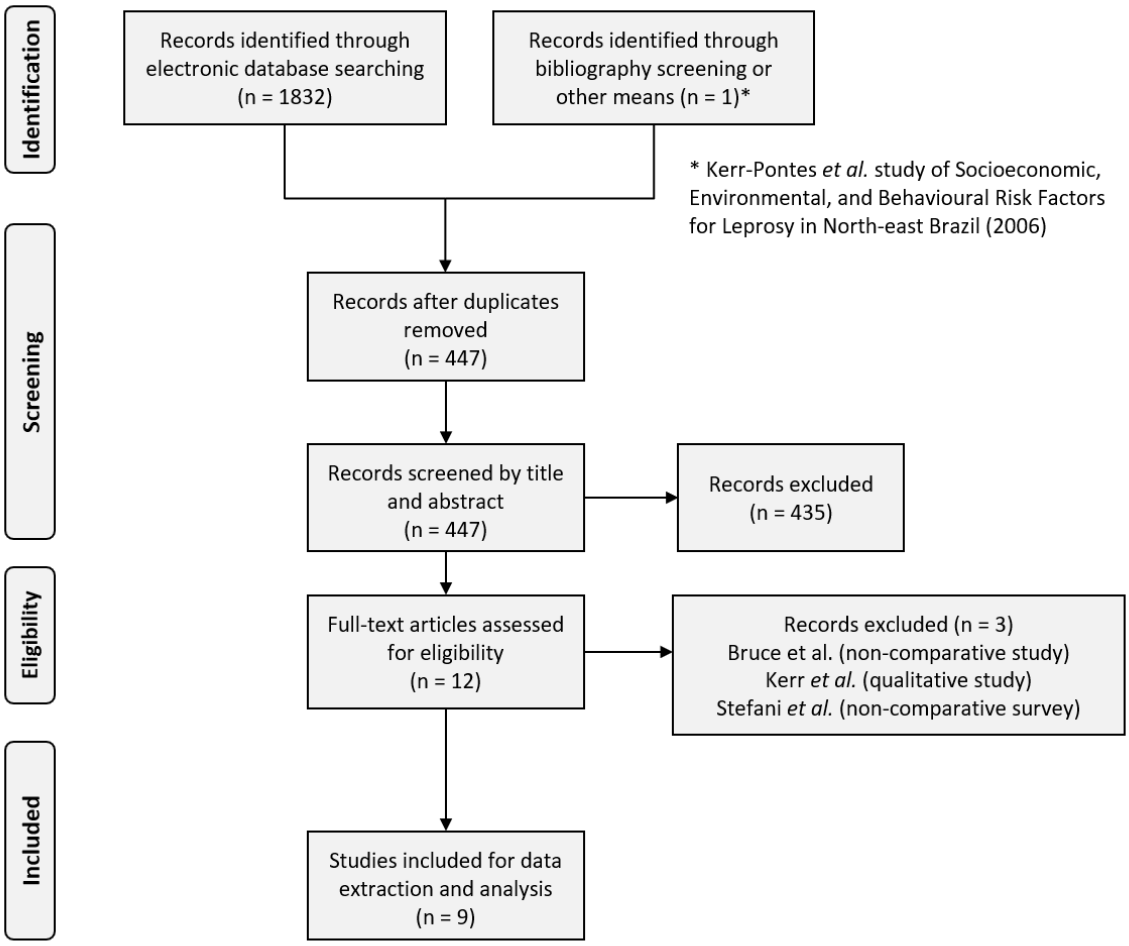
Figure 4. Relative risk of Hansen's disease (odds ratios) comparing groups exposed to wild armadillos with unexposed groups by type of exposure

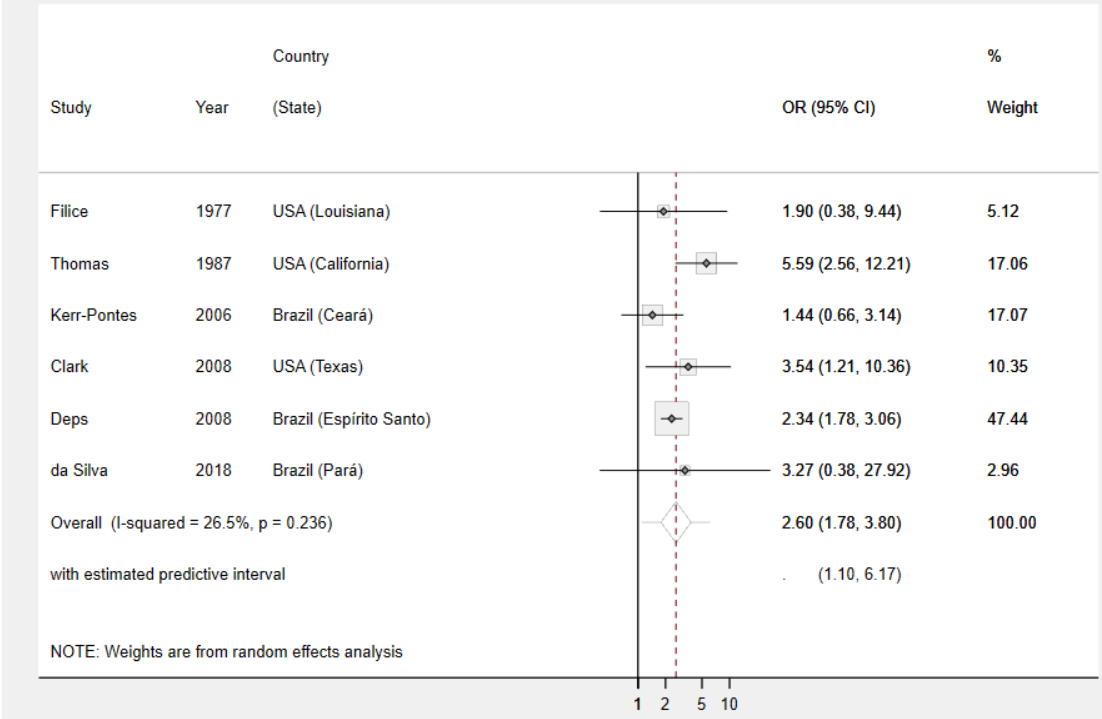
Appendices

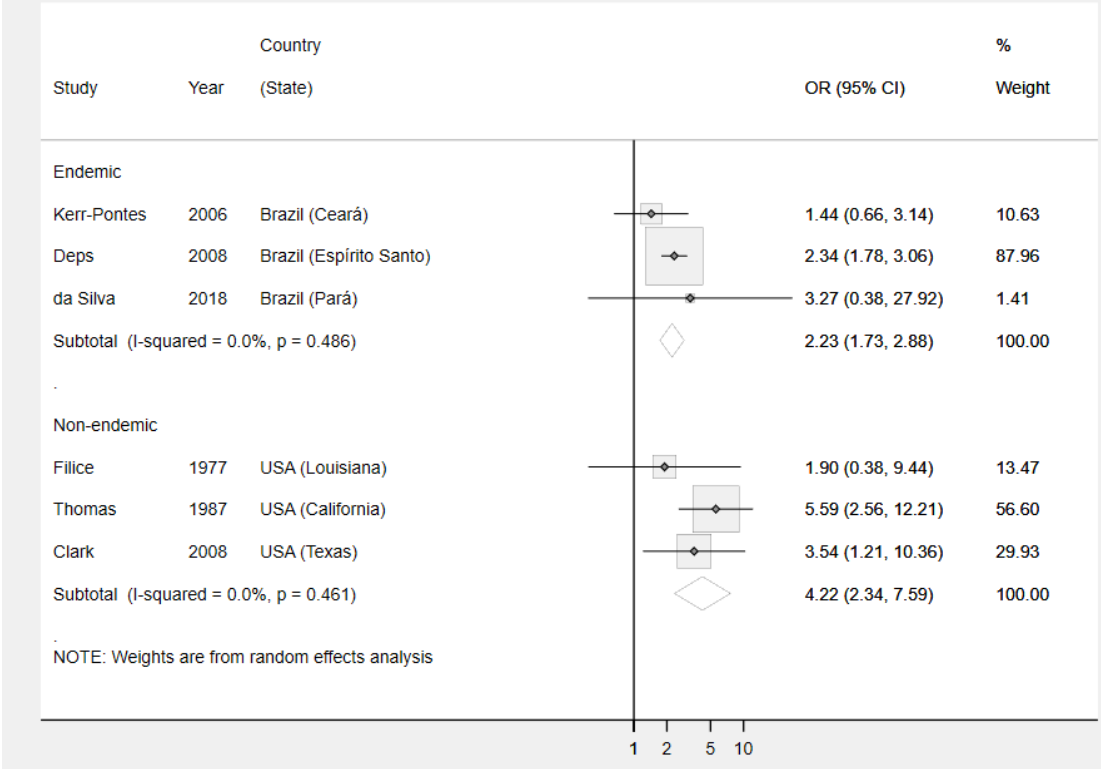
Figure S1. Funnel plot with pseudo 95% confidence limits of *M. leprae* prevalence in wild armadillos in Brazil detected using PCR methods (corresponding to Fig 4).

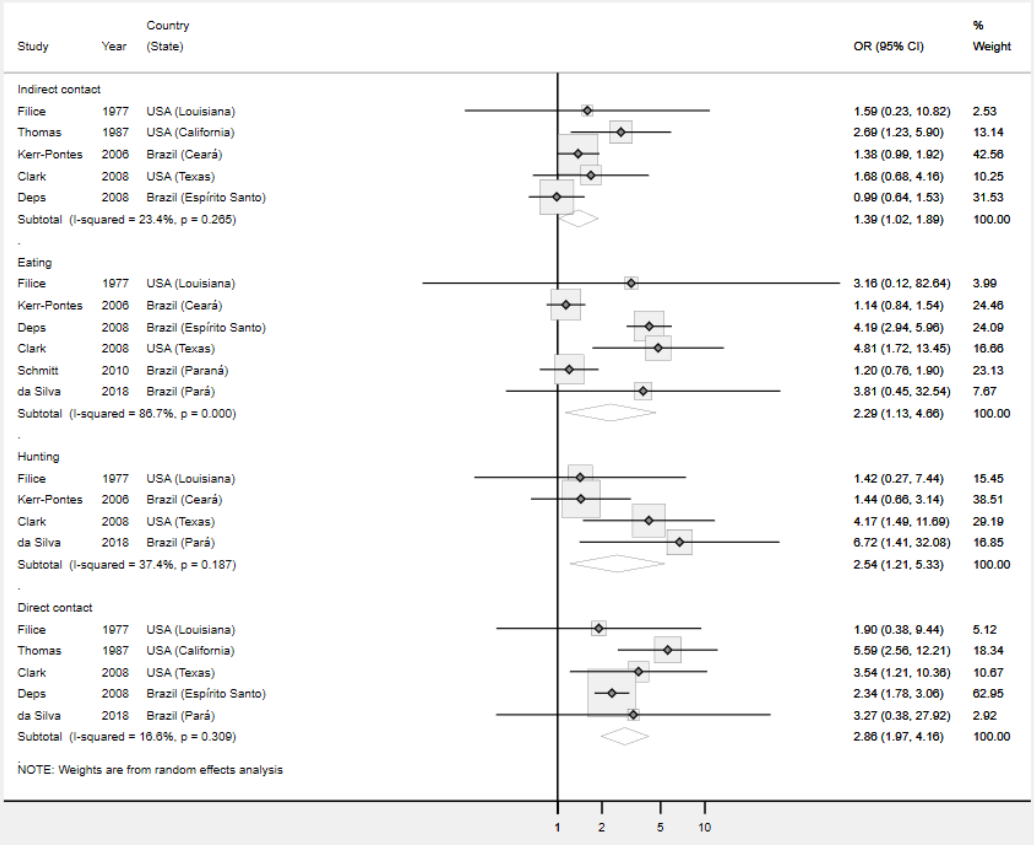
File S1. Supplementary Appendices (search terms, quality assessment (QA) tools, QA scores, extracted data)

Checklist S1: PRISMA Checklist


Identification
Screening
Eligibility
Included
Records identified through electronic database searching (n = 1832)
Records identified through bibliography screening or other means (n = 1)*
* Kerr-Pontes et al. study of Socioeconomic, Environmental, and Behavioural Risk Factors for Leprosy in North-east Brazil (2006)
Records after duplicates removed (n = 447)
Records screened by title and abstract (n = 447)
Records excluded (n = 435)
Full-text articles assessed for eligibility (n = 12)
Records excluded (n = 3)
Bruce et al. (non-comparative study)
Kerr et al. (qualitative study)
Stefani et al. (non-comparative survey)
Studies included for data extraction and analysis (n = 9)


Study
Year
Country (State)
OR (95% CI)
% Weight
Filice 1977 USA (Louisiana) 1.90 (0.38, 9.44) 5.12
Thomas 1987 USA (California) 5.59 (2.56, 12.21) 17.06
Kerr-Pontes 2006 Brazil (Ceará) 1.44 (0.66, 3.14) 17.07
Clark 2008 USA (Texas) 3.54 (1.21, 10.36) 10.35
Deps 2008 Brazil (Espírito Santo) 2.34 (1.78, 3.06) 47.44
da Silva 2018 Brazil (Pará) 3.27 (0.38, 27.92) 2.96
Overall (I-squared = 26.5%, p = 0.236) 2.60 (1.78, 3.80) 100.00
with estimated predictive interval . (1.10, 6.17)
NOTE: Weights are from random effects analysis
1 2 5 10


Study
Year
Country (State)
OR (95% CI)
% Weight
Endemic
Kerr-Pontes 2006 Brazil (Ceará) 1.44 (0.66, 3.14) 10.63
Deps 2008 Brazil (Espírito Santo) 2.34 (1.78, 3.06) 87.96
da Silva 2018 Brazil (Pará) 3.27 (0.38, 27.92) 1.41
Subtotal (I-squared = 0.0%, p = 0.486) 2.23 (1.73, 2.88) 100.00
.
Non-endemic
Filice 1977 USA (Louisiana) 1.90 (0.38, 9.44) 13.47
Thomas 1987 USA (California) 5.59 (2.56, 12.21) 56.60
Clark 2008 USA (Texas) 3.54 (1.21, 10.36) 29.93
Subtotal (I-squared = 0.0%, p = 0.461) 4.22 (2.34, 7.59) 100.00
.
NOTE: Weights are from random effects analysis
1 2 5 10


Study
Year
Country (State)
OR (95% CI)
% Weight
Indirect contact
Filice 1977 USA (Louisiana) 1.59 (0.23, 10.82) 2.53
Thomas 1987 USA (California) 2.69 (1.23, 5.90) 13.14
Kerr-Pontes 2006 Brazil (Ceará) 1.38 (0.99, 1.92) 42.56
Clark 2008 USA (Texas) 1.68 (0.68, 4.16) 10.25
Deps 2008 Brazil (Espírito Santo) 0.99 (0.64, 1.53) 31.53
Subtotal (I-squared = 23.4%, p = 0.265) 1.39 (1.02, 1.89) 100.00
Eating
Filice 1977 USA (Louisiana) 3.16 (0.12, 82.64) 3.99
Kerr-Pontes 2006 Brazil (Ceará) 1.14 (0.84, 1.54) 24.46
Deps 2008 Brazil (Espírito Santo) 4.19 (2.94, 5.96) 24.09
Clark 2008 USA (Texas) 4.81 (1.72, 13.45) 16.66
Schmitt 2010 Brazil (Paraná) 1.20 (0.76, 1.90) 23.13
da Silva 2018 Brazil (Pará) 3.81 (0.45, 32.54) 7.67
Subtotal (I-squared = 86.7%, p = 0.000) 2.29 (1.13, 4.66) 100.00
Hunting
Filice 1977 USA (Louisiana) 1.42 (0.27, 7.44) 15.45
Kerr-Pontes 2006 Brazil (Ceará) 1.44 (0.66, 3.14) 38.51
Clark 2008 USA (Texas) 4.17 (1.49, 11.69) 29.19
da Silva 2018 Brazil (Pará) 6.72 (1.41, 32.08) 16.85
Subtotal (I-squared = 37.4%, p = 0.187) 2.54 (1.21, 5.33) 100.00
Direct contact
Filice 1977 USA (Louisiana) 1.90 (0.38, 9.44) 5.12
Thomas 1987 USA (California) 5.59 (2.56, 12.21) 18.34
Clark 2008 USA (Texas) 3.54 (1.21, 10.36) 10.67
Deps 2008 Brazil (Espírito Santo) 2.34 (1.78, 3.06) 62.95
da Silva 2018 Brazil (Pará) 3.27 (0.38, 27.92) 2.92
Subtotal (I-squared = 16.6%, p = 0.309) 2.86 (1.97, 4.16) 100.00
NOTE: Weights are from random effects analysis
1
2
5
10