Prevalence and risk-mapping of bovine brucellosis in Maranhão State, Brazil

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ABSTRACT

Between 2007 and 2009, a cross-sectional survey was carried out in Maranhão State, Brazil to estimate the seroprevalence of and risk factors for bovine brucellosis. In total, 749 herds and 6779 cows greater than two years of age were blood sampled. At the time of sampling a questionnaire to collect details on possible risk factors for bovine brucellosis was administered to the participating herd manager. A logistic regression model was developed to quantify the association between herd demographic and management characteristics and the herd-level brucellosis status. Spatial analyses were carried out to identify areas of the state where the presence of brucellosis was unaccounted-for by the explanatory variables in the logistic regression model.

The estimated herd-level prevalence of brucellosis in Maranhão was 11.4% (95% CI 9.2–14) and the individual animal-level prevalence was 2.5% (95% CI 1.7–3.6). Herds with more than 54 cows older than two years of age, herds that used rented pasture to feed cattle, and the presence of wetlands on the home farm increased the risk of a herd being brucellosis positive. Infected farms were identified throughout the state, particularly in the central region and on the northwestern border. Spatial analyses of the Pearson residuals from the logistic regression model identified an area in the center of the state where brucellosis risk was not well explained by the predictors included in the final logistic regression model. Targeted investigations should be carried out in this area to determine more precisely the reasons for the unexplained disease excess. This process might uncover previously unrecognized risk factors for brucellosis in Maranhão.

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exposure to infected animals and aborted fetuses (Corbel, 2006). Infected people may require prolonged periods of antibiotic treatment and may experience extended periods of convalescence (Corbel, 2006). Outbreaks of brucellosis in cattle cause abortion during the last trimester of pregnancy (Nicoletti, 1980; Corbel, 2006). In infected cows, abortions prolong intercalving intervals, reducing lifetime production of both calves and milk. The economic impact of the disease at the national level is substantial, including loss of agricultural markets and costs associated with organized efforts to eliminate the disease (Radostits et al., 2002).

Although some countries have achieved success in controlling or eradicating bovine brucellosis, mainly through test-and-slaughter programs (Godfroid and Käbohrer, 2002; Ragan, 2002; OIE, 2011), the disease still occurs, at varying levels of prevalence, particularly in countries with lower levels of economic development (Kadohira et al., 1997; Omer et al., 2000; Moreno, 2002; Poester et al., 2002; Hegazy et al., 2011).

In 2001, the Brazilian Ministry of Agriculture, Livestock, and Food Supply (MAPA) launched a national program to control and eradicate bovine brucellosis and tuberculosis (PNCEBT) in the Brazilian cattle population. The program has provided resources to undertake a series of studies to determine the prevalence, herd-level risk factors, and distribution patterns for both diseases. Once the control program was established a second investigative task was to monitor its progress, allowing adjustments to be made to avoid unnecessary waste of time and resources (Poester et al., 2009). At the time of writing, 15 out of the 26 Brazilian states and the Federal District had carried out cross-sectional studies to determine the prevalence of bovine brucellosis. The disease herd-level prevalence varied among states and within zones of the same state, ranging from 0.3% (95% Confidence Interval [CI] 0.1–0.7) in the south to 41% (95% CI 38–44) in central Brazil (Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 2009).

This current paper provides a description of the cross-sectional study of bovine brucellosis in the state of Maranhão, in the northeast of Brazil. The study aimed to estimate the prevalence, risk factors, and the spatial distribution of the disease in the state. A secondary objective of the study was to identify areas of the state where the number of brucellosis-positive herds was in excess of that predicted by the herd-level logistic regression model. We propose that targeted investigations in these areas are likely to be informative in terms of identifying previously unrecognized risk factors for brucellosis.

2. Materials and methods

2.1. Study design

A cross-sectional study was carried out between September 2007 and March 2009 in Maranhão State, located in the northeastern region of Brazil (Fig. 1). To account for the possibility of differences in the geographical prevalence of bovine brucellosis, the state was stratified into four cattle production regions, with artificial boundaries defined according to average herd size, commercial animal trade among farms, and prevailing breeding and management systems.

To estimate the serological prevalence of brucellosis at the herd and individual animal level, a two-stage sampling design was applied. Calculations were carried out in each region to determine the number of herds to be sampled to detect a herd-level prevalence of 25% with 5% precision at a 95% confidence level. For the second stage, 10 or 15 cows older than 2 years of age (in herds with <100 cows and ≥100 cows, respectively) were randomly selected within each sampled herd, using a systematic procedure. The minimum within-herd assumed prevalence was 20%.

The serial testing procedure, accomplished by the Rose Bengal test (81.2% sensitivity and 86.3% specificity) and the 2-mercaptoethanol test (88.4% sensitivity and
91.5% specificity) (Gall and Nielsen, 2004), calculated for this study had a minimum of 80% sensitivity and 85% specificity. Herds that had at least one seropositive animal were classified as positive. Sample size calculations were carried out using Herdacc version 3 (Jordan, 1995).

2.2. Data collection

Details of the 100,466 cattle herds and the 2,380,555 cows older than 2 years of age registered in 2005 in Maranhão were provided by the Animal Health State Agency. The required number of herds in each of the four cattle production regions were selected using a random number generator in a spreadsheet. If the herd manager of a selected herd refused to participate in the study, another farm with similar breeding and management system was sampled and invited to take part. This process continued until a suitable replacement herd was found.

Between September 2007 and March 2009 selected herds were visited on a single occasion by the Animal Health State Agency staff. At each visit cows older than 2 years of age were yared and individual cows were selected for sampling using a systematic sampling. If 150 cows were the required sampling interval was 150/15 = 10. A random number n between 1 and 10 was selected and, as cows exited the race individuals n, n+10, n+20, n+30 and so on were selected for sampling. Sera from sampled animals were tested using a serial testing procedure with the Rose Bengal test used for screening and the 2-mercaptoetanol test used for confirmation, according to PNCEBT procedures (MAPA, 2006).

At the time of each farm visit, a closed questionnaire was administered to the herd manager by the Animal Health State Agency staff to collect information on the herd’s production system and details of possible risk factors for bovine brucellosis. The questionnaire was developed by the Veterinary Epidemiology groups from the Universities of Brasilia and São Paulo, alongside experts from the state who provided knowledge on local animal production and husbandry.

Prior to data collection all field staff were trained, all questions were thoroughly discussed and a field manual on standardization of data collection procedures was produced. The questionnaire had been extensively applied in 15 other states where the same survey had been conducted. At the time the questionnaire was administered the latitude and longitude of the main farm building of each sampled herd was recorded with a global positioning device. The procedures employed in this study were approved by the Animal Ethics Committee of the University of São Paulo, Brazil.

2.3. Statistical analysis

Data were analyzed in order to estimate herd and animal-level prevalence, and to develop a logistic regression model to identify risk factors for bovine brucellosis in Maranhão State. Since total cattle population was different among production regions, a herd sampling weight (HSW) (Dohoo et al., 2003) was calculated for sampled herds in each of the four production regions:

\[
HSW = \frac{\text{total herds in the production region}}{\text{total sampled herds in the production region}}
\]

The result of Eq. (1) reflects the number of herds that each sampled herd represents in the total cattle population registered in Maranhão. The apparent herd-level prevalence was calculated by dividing the number of test positive herds in a region, after accounting for the HSW, by the total number of herds registered in the same production region.

Apparent animal-level prevalence was estimated using a similar approach. The animal sampling weight (ASW) was calculated by:

\[
ASW = \frac{\text{cows ≥ 2 years in the farm}}{\text{sampled cows ≥ 2 years in the farm}} \times \frac{\text{cows ≥ 2 years in the region}}{\text{cows ≥ 2 years in sampled farms in the region}}
\]

The result of Eq. (2) reflects the number of cows that each sampled cow represents in the total cow (older than 2 years of age) population registered in Maranhão. The apparent individual animal-level prevalence was calculated by dividing the number of test positive cows older than 2 years of age in a region, after accounting for the ASW, by the total number of cows older than 2 years of age registered in the same production region.

The association between herd demographic and management characteristics and the herd-level brucellosis status (being a herd classified as ‘positive’ or ‘negative’) was quantified using a binary logistic regression model. For this analysis, the variable herd size was categorized into quartiles and analyzed as a categorical variable. After selecting the variables associated with the outcome herd-level brucellosis status at an alpha level <0.20, a forward stepwise variable selection, based on an alpha level <0.05, was performed. The Hosmer and Lemeshow goodness-of-fit test was used to assess the model fit. The model ability to discriminate between brucellosis positive and negative herds was assessed by calculating the area under the receiver operating characteristic (ROC) curve using ROCR package (Sing et al., 2005) in R version 2.12.2 (R Development Core Team, 2010). Statistical analyses were conducted using SPSS version 9.0 (SPSS, 1999).

2.4. Spatial analysis

Each herd was uniquely identified by a code made up of a seven-digit city identifier, according to the Brazilian Institute of Geography and Statistics (IBGE), and a two-digit herd identifier. Because of technical errors during GPS data collection, for those herds with no geographical location details available (n = 48) easting and northing coordinates were randomly generated within the city boundaries in which each herd was located.

To describe the spatial distribution of bovine brucellosis in Maranhão two surfaces were constructed using a Gaussian-kernel smoothing function: the first represented
the number of brucellosis-positive herds per square kilometer (km²); the second represented the total number of cattle herds sampled per km². The ratio of the density surface of brucellosis positive herds to the density surface of the sampled herds at risk provided a relief map showing the distribution of brucellosis positive herds corrected for the spatial distribution of sampled herds (Bithell, 1990; Bowman and Azzalini, 1997). The bandwidth for the kernel smoothing function, calculated using the normal optimal method (Bowman and Azzalini, 1997), was fixed at 20 kilometer (km). A correction term for edge effects (coastline and state land boundaries) was applied (Diggle, 1985; Jarner et al., 2002; Marshall and Hazelton, 2010; Davies et al., 2011).

To test the uniformity of the spatial distribution of brucellosis positive herds relative to the spatial distribution of those herds that were sampled we used the technique of Hazelton and Davies (2009). This method involved placement of a regular grid of 200 cells × 200 cells over the study area. Test statistics were calculated for each cell of the regular grid corresponding to the null hypothesis of uniform risk. These test statistics, interpretable in the usual fashion with respect to a standard normal distribution, yield the asymptotically derived P-value surface given the alternative hypothesis of non-uniform risk. This analysis allowed us to superimpose contour lines on the relief map, delineating areas of significantly raised brucellosis prevalence.

To quantify the residual brucellosis risk at small scales of distance (0–10 km) relative to the study area, Pearson residuals from the logistic regression model were plotted as a binned omnidirectional semivariogram using the easting and northing coordinates of each herd as a location marker. A total of 999 Monte Carlo simulations of the data were conducted whereby the residuals were randomly allocated to each herd location and a semivariogram calculated each time. From the 999 semivariograms, minimum and maximum values for each 0.10 km increments in distance were selected and plotted as lower and upper simulation envelopes. To describe the spatial distribution in residual brucellosis risk at scales greater than 10 km, Pearson residuals from the logistic regression model were plotted using the kernel smoothed method described previously.

Spatial data analysis were carried out using the contributed R packages geoRglm (Christensen and Ribeiro, 2002), spatstat (Baddeley and Turner, 2005), and sparr (Davies et al., 2011) in R version 2.12.2 (R Development Core Team, 2010).

### 3. Results

In total, 749 herds and 6779 cows greater than two years of age were blood sampled. The herd refusal rate was not recorded, but according to the State Agency it was minimal. The estimated herd and animal-level prevalence of bovine brucellosis in Maranhão State was 11.4% (95% CI 9.2–14) and 2.5% (95% CI 1.7–3.6), respectively (Table 1).

The logistic regression analysis showed that herds with more than 54 cows older than 2 years of age (OR 4.1, 95% CI 2.5–6.7), use of rented pasture to feed cattle (OR 1.8, 95% CI 1.1–3.1), and the presence of wetlands on the home farm (OR 1.6, 95% CI 1.0–2.7) increased the odds of a herd being infected by brucellosis. Beef herds had 0.4 (95% CI 0.2–0.7) times the odds of being brucellosis positive compared with dairy and mixed herds (Tables 2 and 3). The Hosmer and Lemeshow goodness-of-fit test was not significant (P=0.73), indicating that the lack-of-fit was not sufficient to reject the model. The area under the ROC curve was 0.73, indicating that the model had moderate to good ability to discriminate between brucellosis positive and brucellosis negative herds.

The point map shows that the distribution of brucellosis positive cattle herds is widespread in the four production regions of Maranhão (Fig. 1). The kernel smoothed map of herd-level brucellosis prevalence shows that, after accounting for the spatial distribution of sampled herds, the prevalence of the disease varied throughout the state (Fig. 2). There was one single, small area of significant disease excess (P<0.05) identified close to the northwest boundary, where three out of four herds were brucellosis positive.

The binned omnidirectional semivariogram computed using the Pearson residuals from the logistic regression model provided weak evidence of spatial autocorrelation in residual brucellosis risk at distances of 0–10 km among cattle herds, since almost all points were set within the simulation envelopes and the residual semivariogram was essentially flat (data not shown).

The kernel-smoothed plot of the herd-level residuals shows the distribution of herds throughout Maranhão State with positive and negative-sign residuals. Areas with a predominance of positive-sign residuals are interpreted as those where the observed number of brucellosis positive herds was in excess of that predicted by the logistic regression model. Areas with negative-sign residuals are interpreted as those where the number of positive herds was less than that predicted by the model. Based on the map we identified one area in the center of the state where

### Table 1

Herd and animal apparent prevalence (Ap. Prev.) level of bovine brucellosis in the four production regions of Maranhão State, Brazil.

<table>
<thead>
<tr>
<th>Region</th>
<th>Cattle herds</th>
<th>Ap. Prev. (%)</th>
<th>95% CI</th>
<th>Animals</th>
<th>Ap. Prev. (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sampled</td>
<td>Positives</td>
<td></td>
<td>Sampled</td>
<td>Positives</td>
<td></td>
</tr>
<tr>
<td>(1) North</td>
<td>149</td>
<td>6</td>
<td>4.0</td>
<td>[1.8–8.7]</td>
<td>1047</td>
<td>6</td>
</tr>
<tr>
<td>(2) Northwest</td>
<td>292</td>
<td>53</td>
<td>18.1</td>
<td>[14.1–23.0]</td>
<td>3282</td>
<td>85</td>
</tr>
<tr>
<td>(3) Northeast</td>
<td>150</td>
<td>12</td>
<td>8.0</td>
<td>[4.6–13.6]</td>
<td>1098</td>
<td>14</td>
</tr>
<tr>
<td>(4) South</td>
<td>158</td>
<td>5</td>
<td>3.1</td>
<td>[1.3–7.4]</td>
<td>1352</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>749</td>
<td>76</td>
<td>11.4</td>
<td>[9.2–14.0]</td>
<td>6779</td>
<td>112</td>
</tr>
</tbody>
</table>
the presence of disease was not entirely explained by the model (Fig. 3).

4. Discussion

This study aimed to describe the epidemiology of bovine brucellosis in the state of Maranhão, Brazil and to identify the characteristics of cattle herds that rendered them more likely to be brucellosis positive. Acknowledge of these characteristics means that control measures can be more effectively targeted toward ‘at risk’ herds.

Despite the time lag between the collection of data (2007–2009) and the analyses (2010–2011), we considered that the results provide an accurate description of the disease situation in Maranhão for the period 2007–2009. Moreover, it should be noted that the PNCEBT is in its first stage of implementation in the state and, therefore, it is very unlikely that herd-level prevalence and risk factors may have changed significantly over the specified period, given the endemic and chronic characteristics of brucellosis. Extrapolation of the findings presented here to the current brucellosis situation in Maranhão should be made with caution.

The herd and individual animal-level prevalence estimates of brucellosis for Maranhão were similar to the median prevalence estimate for the 15 other states of Brazil that have carried out related studies. Similar disease frequencies were documented in three of the four cattle production regions of Maranhão (Table 1), but in the northwest region the prevalence was significantly greater than the other three (P<0.05). This result may be partly explained by the fact that farms in the northwest are larger and stocking densities are, on average, higher compared to other regions of the state (MEC, 2001). According to responses to the PNCEBT questionnaire, vaccination rates in these herds were generally lower compared with other areas of the state, cattle are commonly moved between farms and use of veterinary services is not frequent. All of these factors are likely to contribute to the occurrence and spread of bovine brucellosis (Nicoletti, 1980; Crawford et al., 1990; Corbel, 2006; Stringer et al., 2008). Moreover herds having more than 54 cows older than 2 years of age

<table>
<thead>
<tr>
<th>Variable</th>
<th>Seropositive Exposed</th>
<th>Seropositive Total</th>
<th>Seronegative Exposed</th>
<th>Seronegative Total</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herd size with more than 54 cows older than 2 years of age</td>
<td>40</td>
<td>76</td>
<td>316</td>
<td>669</td>
<td>&lt;0.00</td>
</tr>
<tr>
<td>Herd size with more than 142 cattle</td>
<td>37</td>
<td>76</td>
<td>150</td>
<td>673</td>
<td>&lt;0.00</td>
</tr>
<tr>
<td>Absence of calving paddock</td>
<td>32</td>
<td>75</td>
<td>455</td>
<td>671</td>
<td>&lt;0.00</td>
</tr>
<tr>
<td>Absence of vaccination against bovine brucellosis</td>
<td>56</td>
<td>74</td>
<td>583</td>
<td>665</td>
<td>0.00</td>
</tr>
<tr>
<td>Beef herd</td>
<td>23</td>
<td>76</td>
<td>316</td>
<td>669</td>
<td>0.00</td>
</tr>
<tr>
<td>Mixed herd</td>
<td>42</td>
<td>76</td>
<td>277</td>
<td>669</td>
<td>0.02</td>
</tr>
<tr>
<td>Use of rented pasture to feed cattle</td>
<td>25</td>
<td>76</td>
<td>144</td>
<td>672</td>
<td>0.02</td>
</tr>
<tr>
<td>Purchase of breeding animals</td>
<td>49</td>
<td>76</td>
<td>347</td>
<td>672</td>
<td>0.03</td>
</tr>
<tr>
<td>Absence of veterinary assistance</td>
<td>56</td>
<td>75</td>
<td>551</td>
<td>658</td>
<td>0.04</td>
</tr>
<tr>
<td>Place of breeding animals slaughter</td>
<td>8</td>
<td>76</td>
<td>131</td>
<td>673</td>
<td>0.05</td>
</tr>
<tr>
<td>Presence of wetlands on the home farm</td>
<td>43</td>
<td>75</td>
<td>306</td>
<td>668</td>
<td>0.05</td>
</tr>
<tr>
<td>Use of artificial insemination</td>
<td>5</td>
<td>75</td>
<td>18</td>
<td>668</td>
<td>0.07</td>
</tr>
<tr>
<td>Presence of horse</td>
<td>65</td>
<td>76</td>
<td>515</td>
<td>673</td>
<td>0.07</td>
</tr>
<tr>
<td>Presence of shared lands among farms</td>
<td>15</td>
<td>76</td>
<td>166</td>
<td>668</td>
<td>0.32</td>
</tr>
<tr>
<td>Destiny given to the abortion products</td>
<td>45</td>
<td>63</td>
<td>466</td>
<td>611</td>
<td>0.39</td>
</tr>
<tr>
<td>Dairy herd</td>
<td>11</td>
<td>76</td>
<td>76</td>
<td>669</td>
<td>0.42</td>
</tr>
<tr>
<td>Presence of wild animals (like capybara and deer)</td>
<td>9</td>
<td>76</td>
<td>63</td>
<td>673</td>
<td>0.48</td>
</tr>
<tr>
<td>Use of intensive breeding system</td>
<td>22</td>
<td>76</td>
<td>171</td>
<td>671</td>
<td>0.51</td>
</tr>
<tr>
<td>Presence of birds</td>
<td>62</td>
<td>76</td>
<td>528</td>
<td>673</td>
<td>0.52</td>
</tr>
<tr>
<td>Presence of swine</td>
<td>28</td>
<td>76</td>
<td>272</td>
<td>673</td>
<td>0.54</td>
</tr>
<tr>
<td>Presence of sheep and goat</td>
<td>19</td>
<td>76</td>
<td>150</td>
<td>673</td>
<td>0.59</td>
</tr>
<tr>
<td>Presence of dog</td>
<td>57</td>
<td>76</td>
<td>486</td>
<td>673</td>
<td>0.60</td>
</tr>
<tr>
<td>Presence of cat</td>
<td>37</td>
<td>76</td>
<td>348</td>
<td>673</td>
<td>0.61</td>
</tr>
<tr>
<td>Occurrence of abortion during the previous twelve months</td>
<td>15</td>
<td>72</td>
<td>130</td>
<td>659</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Table 3
Multivariable analysis of risk factors for herd-level bovine brucellosis in Maranhão State, Brazil.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>b</th>
<th>SE (b)</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herd size (cows older than 2 years of age)</td>
<td>≥54 cows</td>
<td>1.4</td>
<td>0.25</td>
<td>4.1</td>
<td>[2.5–6.7]</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>&lt;54 cows</td>
<td>0.8</td>
<td>0.27</td>
<td>0.4</td>
<td>[0.2–0.7]</td>
<td>0.00</td>
</tr>
<tr>
<td>Herd type</td>
<td>Beef</td>
<td>0.6</td>
<td>0.27</td>
<td>1.8</td>
<td>[1.1–3.1]</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Dairy/Mixed</td>
<td>0.5</td>
<td>0.26</td>
<td>1.6</td>
<td>[1.0–2.7]</td>
<td>0.04</td>
</tr>
<tr>
<td>Use of rented pasture to feed cattle</td>
<td>Yes</td>
<td>0.6</td>
<td>0.27</td>
<td>1.8</td>
<td>[1.1–3.1]</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0.5</td>
<td>0.26</td>
<td>1.6</td>
<td>[1.0–2.7]</td>
<td>0.04</td>
</tr>
<tr>
<td>Presence of wetlands on the home farm</td>
<td>Yes</td>
<td>0.6</td>
<td>0.27</td>
<td>1.8</td>
<td>[1.1–3.1]</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0.5</td>
<td>0.26</td>
<td>1.6</td>
<td>[1.0–2.7]</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Intercept = −2.8, P=0.00; −2 log likelihood = 439.7; Hosmer and Lemeshow x² = 5.25 (d.f. = 8, P=0.73).

* 4th quartile.

b 1st, 2nd, and 3rd quartile.
(which represents larger herds) were identified as a risk factor for the disease in the state. The association between herd size, stocking density, and the presence of bovine brucellosis has been demonstrated in other studies (Crawford et al., 1990; Kadohira et al., 1997; Omer et al., 2000; Lee et al., 2009). Although these findings might simply reflect the number of tested animals (Martin et al., 1992), they may also be explained by: (1) a positive association between herd size and the purchase of replacement cattle from outside sources, which increases the probability of introducing infected cattle, and (2) enhanced disease transmission as a result of higher opportunity for complex interactions among the population at risk, particularly in areas with greater animal concentration (Nicoletti, 1980; Salman and Meyer, 1984; Crawford et al., 1990). The hypothesis that brucellosis transmission is directly influenced by cattle movements has been supported by other studies, which report that the introduction of either infected or susceptible animals from outside the herd is a risk for disease (Nicoletti, 1980; Crawford et al., 1990; Stringer et al., 2008).

The routine use of rented pasture to feed cattle and the presence of wetlands on the home farm increased the risk of a herd being brucellosis positive. The practice of renting pasture may facilitate contact with infected cattle or environments contaminated from bovine abortions (Crawford et al., 1990). Furthermore, this practice may facilitate contact between infected and uninfected stock, although the movement of cattle away from the home farm for the purpose of breeding is not commonly practiced in Maranhão. The presence of wetlands (that is, swamps, streams and rivers) is likely to increase the survival of Brucella abortus in the environment. Cattle are also likely to concentrate around water sources, providing the opportunity for close contact and subsequent disease spread (Radostits et al., 2002). Beef herds had 0.4 (95% CI 0.2–0.7) times the odds of being brucellosis positive compared with dairy and mixed herds. The lower risk of brucellosis in beef herds was most likely due to their lower stocking rates, compared with dairy and mixed herds. The AUC value (0.73) shows that the final model adequately distinguished between diseased and non-diseased herds in Maranhão, indicating that even if brucellosis risk truly increases with the presence of the predictors, factors other than those present in the PNCBET questionnaire and included in the risk factors analysis also explain the disease occurrence in Maranhão.

The descriptive spatial analyses show that brucellosis positive farms were located all over the state (Fig. 1). Fig. 2, which shows the distribution of brucellosis positive herds corrected for the spatial distribution of sampled herds (that is, a proxy for the population of cattle herds at risk), reinforce the descriptive result described above that the prevalence of the disease varied by production region (Table 1). The Hazelton and Davies (2009) Z-test analyses identified a single area of significant disease excess ($P < 0.05$) adjacent to the northwest boundary of Maranhão.
State, where three out of four cattle herds were brucellosis positive. Although this could be a spurious finding arising from the way herds were selected for sampling, it could also reflect some similar factor among the three herds related to brucellosis occurrence. According to the information gathered from the three herd managers, herd size ranged from 30 to 1346 cattle. Two of the three herd managers stated that they used rented pasture to feed cattle and all of them had wetlands on the home farm. All three herd managers reported that they routinely purchased animals directly from other farms. Given the lack of additional information it was not possible to determine if cattle trade occurred among the three farms. The presence of disease clusters has been documented in other studies of bovine brucellosis (Kellar et al., 1976; Abernethy et al., 2011; Hegazy et al., 2011).

Little evidence of spatial autocorrelation in the Pearson residuals from the logistic regression model was observed over short distance ranges (0–10 km), which means that even if brucellosis has been diagnosed at one farm location, the likelihood of cases being identified on neighboring farms, at that scale, was not increased. Although Brucella abortus survives under certain conditions in pasture and water, the absence of direct contact between susceptible and infected animals or infected biological material (carcasses, uterine secretions, aborted fetuses, and semen for artificial insemination) almost eliminate the risk of disease spread (Nicoletti, 1980). According to Crawford et al. (1990) the tendency of infection to spread from infected herds to neighboring, uninfected herds has been described. For this to occur there is a need for cattle to make contact over fence lines or share pasture (Crawford et al., 1990).

The kernel-smoothed plot showing the spatial variation in the Pearson residuals produced from the logistic regression model (Fig. 3) identified a single area of disease excess in the center of the state not entirely explained by the predictors included in the final logistic regression model. A logical action point arising from this finding would be to carry out a targeted investigation of brucellosis in this area in an effort to identify the reasons for the unexplained disease excess. This process might uncover previously unrecognized risk factors for brucellosis in Maranhão.

A possible limitation of this study was selection bias, arising from sampled herds that refused to participate on the study. Missing data are a common problem in many investigations (Raghunathan, 2004). Selection can be biased when sampled subjects refuse to take part on the study (called unit nonresponse) and the replacement subjects who are included in the analysis are systematically different from those who were excluded in terms of one or more variables (Raghunathan, 2004). To minimize the impact of selection bias on the study results, herd managers from sampled herds were contacted by the Animal Health State Agency staff before farm visit. If a selected herd refused to participate on the study, another farm was randomly selected and contacted for visit. Also, the herd sampling weight calculation allowed compensating selection bias in the prevalence estimate. Since excluding unit nonresponse is a distortion of the representation in the original sample, weights were attached to herds included in the analysis to restore the representation and to compensate for unit nonresponse (Holt and Elliot, 1991; Raghunathan, 2004). The herd refusal rate was not recorded, but according to the State Agency it was minimal.

Other potential limitation was misclassification bias, arising from incorrect responses to questions posed to herd managers by the staff of the Animal Health State Agency of Maranhão. This might have occurred because herd managers did not have the required information available to them at the time the questionnaire was administered, or because the way the questionnaire was carried out encouraged herd managers to provide answers that were consistent with what they thought the person administering the questionnaire wanted to hear, rather than a description of the true situation. Two characteristics of the study design and conduct minimized the impact of misclassification bias on our results. The first was that the questionnaire had already been used in 15 Brazilian states by the time the study in Maranhão was started. This meant that most (if not all) of the issues around ambiguous questions and the way to record responses to questions had already been addressed. The second was that the Animal Health State Agency field staff that administered the questionnaire underwent a period of training at the start of the study, specifically designed to familiarize them with appropriate data collection methods. The impact of misclassification bias, if it was present, was judged to be small.

5. Conclusions

According to the results of this study, the practice of renting pasture should be discouraged in the state and the presence of wetlands on the home farm ought to be considered a risk factor in the planning of control measures. The results suggest that there is a case for strengthening the mandatory vaccination of heifers, especially in the center of Maranhão, where herds are larger and prevalence is higher. Since one single area of unexplained disease excess was identified in the center of the state, it is recommended that targeted investigations be carried out in this area to determine more precisely the reasons for the unexplained disease excess. This process might uncover previously unrecognized risk factors for brucellosis.

We expect that the methods described here could assist animal health policy-makers and field veterinarians who plan to conduct studies to control and eradicate brucellosis.

Conflict of interest

None.

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