Spatial cluster analysis for bovine paratuberculosis in Paraíba State, Northeastern Brazil


The aim of this survey was to identify spatial clustering of bovine paratuberculosis positive herds in the State of Paraíba, Northeastern Brazil. The state was divided into three sampling groups: sampling stratum 1 (mesoregion of Sertão), sampling stratum 2 (mesoregion of Borborema), and sampling stratum 3 (mesoregions of Zona da Mata and Agreste). Ten animals were sampled in herds with up to 99 cows aged over 24 months; 15 animals were sampled in herds with 100 or more cows aged over 24 months; and all animals were sampled in those with up to 10 cows aged over 24 months. In total, 2504 cows aged ≥ 24 months were sampled from 480 herds. Indirect enzyme-linked immunosorbant assay (ELISA) test kits were used for Mycobacterium avium subsp. paratuberculosis (MAP) antibody detection. A herd was deemed positive for paratuberculosis if it included at least one positive animal in herds of up to 24 females, and two positive animals in herds with more than 24 females. Spatial clustering was assessed using the Cuzick-Edwards’ $k$-nearest neighbor method and spatial scan statistics. Two significant clustering of positive herds were detected in Northern part of Borborema mesoregion, a border region with the State of Rio Grande do Norte, in which there is a large animal movement from different locations without knowing the sanitary condition of animals. As serological tests for MAP diagnosis are not widely available and are very expensive, as well as replacement or maintenance of livestock by animal purchasing is common in the region, it is concluded that prevention measures should be applied at herd level.

INDEX TERMS: Paratuberculosis, cattle, epidemiology, cluster analysis, control.
Paratuberculosis is a chronic intestinal infection of global importance in mainly domestic and wild ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Paratuberculosis, also known as Johne’s disease, can cause significant economic losses in cattle primarily related to reduced milk production and premature culling (Raizman et al. 2009), decreased value at slaughter (Kudahl & Nielsen 2009), and eventual death (Kudahl et al. 2007), reduced feed efficiency, decreased fat and protein content in the milk, decreased fertility, and increased incidence of mastitis (Nielsen et al. 2008).

MAP can be transmitted via milk and colostrum from infected animals and intrauterine route (Streeter et al. 1995). Infected animals shed MAP in faeces and can lead to widespread contamination of environment, including the presence of viable MAP in settled dust particles suggesting potential transmission of MAP infection through bio-aerosols (Eisenberg et al., 2010). In cattle, clinical cases can be diagnosed without difficulty because chronic diarrhea in adult animals is indicative of the disease. In the laboratory, a diagnosis can be made by isolating the agent from feces or necropsy material, by histological study of the lesions, and polymerase chain reaction (PCR) assays. Subclinical cases can be diagnosed by isolating the bacteria from the feces, serological tests, or allergy tests (Lilenbaum et al. 2007). However, the assessment of MAP infection status is subject to misclassification, especially low sensitivity of the diagnostic test used in the control programme (Nielsen & Toft 2011).

In the State of Paraíba, a cross-sectional study based on a planned sampling was carried out to determine the epidemiological situation of the disease (Vilar et al. 2015). The herd-level prevalence in the State of Paraíba was 34.5% (95% CI = 30.2%-39.1%), 26.6% (95% CI = 20.2%-34.2%) in the region of Borborema, 30.5% (95% CI = 23.9%-38.0%) in Agreste/Mata, and 41.4% (95% CI = 34.0%-49.1%) in Sertão (Table 1). In understanding risk and controlling disease it is important to know the spatial distribution of the disease in the environment. To date, there is no survey on spatial clustering analysis for bovine paratuberculosis in Brazil. Spatial clustering analysis is a useful tool to study the spread of infectious diseases in animal populations, and the identification of clusters might yield important information about the transmission and/or control of such diseases (Carpenter 2001). Thus, in the present study a spatial cluster analysis was performed aiming to determine the spatial distribution of the disease in the State of Paraíba.

**MATERIALS AND METHODS**

Data used in the present study were originated from the epidemiological survey for bovine paratuberculosis in the State of Paraíba (Vilar et al. 2015). The state was divided into three sampling groups: sampling stratum 1 (mesoregion of Sertão), sampling stratum 2 (mesoregion of Borborema), and sampling stratum 3 (mesoregions of Zona da Mata and Agreste) (Fig.1). For each sampling stratum, a two-stage sampling survey. In the first stage, a pre-established number of herds (primary sampling units) were randomly selected; in the second stage, a pre-established number of cows aged ≥24 months were randomly selected (secondary sampling units).

The number of selected herds per sampling stratum was determined by using the formula for simple random samples proposed by Thrusfield (2007). The parameters adopted for the calculation were as follows: 95% confidence level, 47.4% estimated prevalence (Sá et al. 2013), and 8% error. Further, the operational and financial capacity of the SEDAP was taken into consideration when determining the sample size of the sampling stratum.

For the secondary units, the minimum number of animals to be examined within each herd was estimated in order to allow its classification as positive herd. For this purpose, the concept of aggregate sensitivity and specificity was used (Dohoo et al. 2003). For the calculations, the following values were adopted: 73.6% (Hendrick et al. 2005) and 98% (Sweeney et al. 1995) for the sensitivity and specificity, respectively, of the test protocol and 37.9% (Fonseca et al. 2000) for the intra-herd estimated prevalence. Herdacc version 3 software (Jordan 1995) was used during this process, and the sample size was selected so that the herd sensitivity and specificity values would be ≥ 90%. Therefore, 10 animals were sampled in herds with up to 99 cows aged over 24 months; 15 animals were sampled in herds with 100 or more cows aged over 24 months; and all animals were sampled in those with up to 10 cows aged over 24 months. The selection of the cows within the herds was systematic. In total, 2504 animals were sampled from 480 cattle herds.

The target condition (Gardner et al. 2011) was a seropositive animal within an infected herd. The herd-level case definition was based on the size of the population (cows aged ≥ 24 months), number of females sampled, an intra-herd apparent prevalence of 37.9% (Fonseca et al., 2000), and the sensitivity and specificity of the diagnostic test used (indirect ELISA), with the goal of obtaining a herd sensitivity and specificity of ≥90%. After new simulations using Herdacc software, a herd was deemed positive for the presence of MAP if it included at least one positive animal in herds of up to 24 females, and two positive animals in herds with more than 24 females.

The serological examination was performed according to the protocol recommended by the kit for detection of antibodies to MAP (Pourquier-IDEXX ELISA Paratuberculosis Screening Ab Test). To calculate the results, the reactions were considered valid when the average of the positive control (PCs) had a minimum OD450 mean value of 0.350 and the coefficient between the average of the PCs and the negative control (NC OD450) was ≥ 3.00. Samples with percentages ≥70% were considered positive for the presence of antibodies against MAP.
Spatial clustering of bovine paratuberculosis positive herds was assessed using two methods (Ward & Carpenter 2000). First, the Cuzick-Edwards' $k$-nearest neighbor method (Cuzick & Edwards 1990) was used to detect the possibility of global spatial clustering at herd level using the ClusterSeer 2.5.1 software (BioMedware, Ann Arbor, MI, United States). Existence of potential spatial clustering was analyzed at each of the first 10 neighborhood levels, and the overall p-value was adjusted for multiple comparisons with the Simes approach. Further, scan statistics by the SatScan software version 9.0 (Kulldorff & Nagarwalla 1995) was used to identify local clusters of positive herds. A Bernoulli model was applied, the scanning window was circular, and the spatial size of scan window was limited to 25% of the total population. Because of the large proportion of positive herds (Table 1), analysis was not run on herd-level, and then considering within-herd prevalence.

RESULTS AND DISCUSSION
Significant clusters were not identified (Simes p>0.05) by the Cuzick and Edwards' method for the entire Paraíba State. However, when considering the state division into separate strata a significant global clustering (Simes p<0.05) of positive herds was detected by the Cuzick and Edwards' method at $k=3$ neighborhood level in Borborema mesoregion. The results of the SatScan cluster analysis is shown in Table 2 and Figure 1. Using the Bernoulli model, two spatial clusters of positive herds with high within-herd prevalence were detected in Northern part of Borborema mesoregion. In the primary cluster, the number of herds was 4, the radius of the cluster was 14.57 km, and the number of observed and expected cases (positive animals) were 11 and 2.60, respectively, where the risk for infection was 4.91 (relative risk =4.91; p=0.014) times higher in herds located inside cluster than in those located elsewhere. In the secondary cluster, the number of herds was 4, the radius of the cluster was 19.20 km, and the number of observed and expected cases (positive animals) were 7 and 1.17, respectively, and the risk for infection was 6.58 (relative risk =6.58; p=0.022).

In a survey to describe the spatial pattern of MAP prevalence throughout Denmark it was found a number of significant clusters, identifying geographical areas with higher apparent within-herd prevalence (Bihrmann et al. 2012). This study found consistency between kriging and scan statistics results with respect to location of areas with high apparent within-herd prevalence of MAP. However, these authors did not take any covariate information into account. Recently, Bihrmann et al. (2016) identified the spatial pattern in infection prevalence in Denmark and found a significant spatial component, suggesting that the estimated range of influence and the overall location of areas with increased prevalence are not very sensitive to diagnostic misclassification.

In the present study there was a lack of spatial cluster of bovine paratuberculosis positive herds throughout the Paraíba State, but spatial clusters were identified when considering the separate mesoregions. However, it can be inferred that these clusters cannot be explained by spatially structured factors as referred by Avila et al. (2013), which
detected cluster for bovine tuberculosis in Bahia State only when analyzed regions separately. The geographic division (Sertão, Borborema, Agreste/Zona da Mata) created in this study for analysis purposes is not subject to real parameters occurrence of paratuberculosis, and does not respect geographical boundaries. Thus, the clusters found in the Borborema region can be explained by being a border region with the State of Rio Grande do Norte, in which there is a large movement of animals from different locations without knowing the sanitary condition of the animals, which may result in a greater number of traded animals subclinically infected with MAP.

The detection of spatial clustering is a complex methodology and has limitations, however, the obtaining of more accurate results and security for decision-making lead to a greater efficiency of sanitary defense actions (Ávila et al. 2013). In this context, it is not plausible to suggest measures based on animal testing prior to purchasing because serological tests for MAP diagnosis are not widely available and are very expensive, as well as replacement or maintenance of livestock by animal purchasing is common in the region. Furthermore, in general, the design quality, implementation, and reporting of test results for paratuberculosis have been generally poor (Nielsen & Toft 2008).

Therefore, measures should be based on the prevention of the disease at herd level, such as keep calves in areas free of manure and raised separate from adults until at least one year old, reducing fecal contamination in animal housing areas by elevating food and water sources, and using colostrum only from the dam of the calf (Nielsen et al. 2008, OIE 2016).

**CONCLUSION**

We detected two spatial clusters of cattle herds with a high within-herd seroprevalence of paratuberculosis in the State of Paraíba, in a border region with the State of Rio Grande do Norte, which suggests a between-states trade of infected animals.

<table>
<thead>
<tr>
<th>Radius (km)</th>
<th>No. of herds in cluster</th>
<th>No. of cases in cluster</th>
<th>RR &lt;sup&gt;a&lt;/sup&gt;</th>
<th>p-value</th>
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<tr>
<td>14.57&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>19.20</td>
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<td>1.77</td>
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<sup>a</sup>Relative risk, <sup>b</sup>Primary cluster.

It is also suggested that paratuberculosis prevention measures should be applied at herd level.

**REFERENCES**


**Table 1. Census data of the cattle population in the State of Paraíba, Northeastern Brazil, according to sampling stratum, and herd-level prevalence for bovine paratuberculosis**

| Sertão | 24,356 | 162 | 67 | 41.4 | [34.0 – 49.1] |
| Borborema | 11,603 | 154 | 41 | 26.6 | [20.2 – 34.2] |
| Agreste/Zona da Mata | 18,398 | 164 | 50 | 30.5 | [23.9 – 38.0] |
| State of Paraíba | 54,357 | 480 | 158 | 34.5 | [30.2 – 39.1] |

Source: Vilar et al. (2015).

**Table 2. Statistically significant clusters of herds with a high within-herd prevalence of paratuberculosis in the State of Paraíba**

<table>
<thead>
<tr>
<th>Radius (km)</th>
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