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PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA VETERINÁRIA

EPIDEMIOLOGICAL AND SPATIAL CHARACTERIZATION OF BOVINE CYSTICERCOSIS IN PARAÍBA STATE, NORTHEASTERN BRAZIL

Dissertação apresentada ao Programa de Pós-Graduação em Medicina Veterinária do Centro de Saúde e Tecnologia Rural da Universidade Federal de Campina Grande, como parte das exigências para a obtenção do título de Mestre.

AMANDA RAFAELA ALVES MAIA

PATOS – PB

AGOSTO – 2016

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Orientador: Prof. Dr. Sérgio Santos de Azevedo

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EPIDEMIOLOGICAL AND SPATIAL CHARACTERIZATION OF BOVINE CYSTICERCOSIS IN PARAÍBA STATE, NORTHEASTERN BRAZIL

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Dissertação aprovada em 30/08/2016.

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PATOS

2016

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ABSTRACT

This study focused on estimating the herd-level and animal-level prevalences, and identifying herd-level spatial clustering and risk factors associated with herd-level prevalence for bovine cysticercosis 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). For each sampling stratum, herd-level and animal-level prevalences were estimated by 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). In total, 2382 animals were sampled from 474 herds. Serological diagnosis was initially performed by the indirect ELISA, and positive sera were confirmed by immunoblot. A herd was deemed positive if it included at least one positive animal in herds of up to 29 females, and two positive animals in herds with more than 29 females. The herd-level prevalence in the State of Paraíba was 10.8% (95% CI = 8.1–14.1), 10.3% (95% CI = 6.4%–16.1%) in the region of Sertão, 6.9% (95% CI = 3.9%–12.1%) in Borborema, and 13.8% (95% CI = 9.3%–20.2%) in Agreste/Zona da Mata. The animal-level prevalence was 2.3% (95% CI =1.6%–3.3%) in the State of Paraíba, 1.4% (95% CI = 0.8%–2.5%) in Sertão, 3.6% (95% CI = 1.7%–7.4%) in the region of Borborema, and 3.2% (95% CI = 1.9%–5.4%) in Agreste/Zona da Mata. The frequency of seropositive animals per herd ranged from 7.1% to 100% (median of 16.7%). The risk factors identified were as follows: animal purchasing (OR = 2.19) and presence of flooded pastures (OR = 1.99). A significant clustering of positive herds was detected in Southern part of Borborema mesoregion. Our findings suggest that bovine cysticercosis herd-level seroprevalence in the State of Paraíba, Northeastern Brazil, is high, and support the idea that prevention measures should be applied at herd level and farmers could restrict the access of their cattle to flooded pastures.

Key words: Cysticercosis; Bovine; Epidemiology; Spatial cluster analysis; Control; Northeastern Brazil.

RESUMO

Os objetivos deste trabalhos foram estimar as prevalências em nível de rebanho e nível animal, identificar agrupamentos espaciais em nível de rebanho e fatores de risco associados à prevalência de rebanhos positivos para cisticercose bovina no Estado da Paraíba, Nordeste do Brasil.O Estado foi dividido em três grupos amostrais: estrato amostral 1 (mesorregião do Sertão), estrato amostral 2 (mesorregião da Borborema) e estrato amostral 3 (mesorregiões da Zona da Mata e Agreste). Para cada estrato amostral, as prevalências de rebanhos positivos e de animais soropositivos foram estimadas por amostragem em dois estágios. No primeiro estágio, um número pré-estabelecido de rebanhos (unidades primárias de amostragem) foi selecionado aleatoriamente; no segundo estágio, um número pré-estabelecido de vacas com idade ≥ 24 meses (unidades secundárias de amostragem) foi selecionado aleatoriamente. No total, 2.382 animais foram amostrados de 474 propriedades.O diagnóstico sorológico foi inicialmente realizado com o teste de ELISA indireto e as amostras positivas foram confirmadas por immunoblot. Um rebanho foi considerado positivo se incluiu pelo menos um animal positivo em rebanhos de até 29 fêmeas, e dois animais positivos em rebanhos com mais de 29 fêmeas. A prevalência de rebanhos positivos no Estado da Paraíba foi de 10,8% (IC 95% = 8,1-14,1), 10,3% (IC 95% = 6,4% -16,1%) no Sertão, 6,9% (IC 95% = 3,9 % -12,1%) na Borborema, e 13,8% (IC 95% = 9,3% -20,2%) no Agreste/Zona da Mata. A prevalência de animais soropositivos foi de 2,3% (IC 95% = 1,6% -3,3%) no Estado da Paraíba, 1,4% (IC 95% = 0,8% -2,5%) no Sertão, 3,6% (IC 95% = 1,7 % -7,4%) na Borborema, e 3,2% (IC 95% = 1,9% -5,4%) no Agreste/Zona da Mata. A frequência de animais soropositivos por rebanho variou de 7,1% a 100% (mediana de 16,7%). Os fatores de risco identificados foram os seguintes: compra de animais (OR = 2,19) e presença de pastos alagados (OR = 1,99). Foi detectado um agrupamento significativo de rebanhos positivos na parte sul da mesorregião da Borborema. Os resultados sugerem que a soroprevalência de cisticercose bovina em nível de rebanho no Estado da Paraíba, Nordeste do Brasil, é alta, bem como recomenda-se que medidas de prevenção devem ser aplicadas em nível de rebanho e os produtores poderiam restringir o acesso dos animais à pastagens alagadas.

Palavras-chave: Cisticercose; Bovino; Epidemiologia; Análise de aglomerados espaciais; Controle; Nordeste Brasil.

SUMMARY

LIST OF TABLES

CHAPTER I

CHAPTER II

LIST OF FIGURES

CHAPTER I

CHAPTER II

LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
≡	Equal
<	Less than
>	Bigger than
≤	Menor ou igual
≥	Maior ou igual
°	Degree
°C	Degree Celsius
ELISA	Enzyme-linked immunosorbent assay
OR	Odds Ratio
TC	Taeniosis-cysticercosis
SEDAP	Agricultural and Livestock Defense Service of the State of Paraíba
OD	Optical Densites
Se	Sensitivity
Sp	Specifity
CNPq	National Counsel of Technological and Scientific Development
CSTR/UFCG	Health Center and Rural Technology/Centro de Saúde e Tecnologia Rural/ Federal University of Campina Grande
Km	Kilometers
IC	Confidence Interval
UFV	Federal University of Viçosa
OR	Odds ratio
sp.	Species
spp.	Subspecie

GENERAL INTRODUCTION

The taeniosis-cysticercosis (TC) complex caused by *T. saginata* is a tropical disease that causes economic losses to the beef supply chain and has a great public health importance in developing countries (ROSSI et al., 2016), particularly in Latin America, such as Guatemala, Honduras, Ecuador, Peru, Colombia, Venezuela, Haiti and Brazil, where it is endemic (WHO, 2011).Cattle become infected by consuming contaminated water or pasture with viable eggs of the parasite or by any other manner that leads to the intake of these eggs.

In Brazil, bovine cysticercosis is endemic in several states, with a significant prevalence in Midwest, Southeast and Southern regions, where the highest rates in slaughtered cattle have been identified by the Federal Inspection Service (DUTRA et al., 2012). Despite the limitations, postmortem inspection have been previously used to indicate the degree of bovine cysticercosis infection, therefore, a visual inspection of beef carcasses during slaughter is very important to reduce the risk for consumers (HILL et al., 2014).

Despite the economic and public health impacts of TC complex, the epidemiological situation of the disease in Brazil is unknown because taeniosis is not a reportable disease, so that data on bovine cysticercosis occurrence is available from veterinary inspection records at slaughterhouses; however, some cases may be unnoticed, especially in mild infections, which make it relevant the use of serological tests with greater sensitivity than the postmortem routine inspection (PAULAN et al., 2013; GUIMARÃES-PEIXOTO et al., 2015). Thus, immunodiagnostic testing alternatives, such as indirect ELISA and immunoblot have been recommended as an option for antemortem detection of bovine cysticercosis, allowing a more accurate early identification of infected animals (GIROTTO et al., 2009; DORNY et al., 2002).

This dissertation consists of two chapters. In chapter I, submitted to Preventive Veterinary Medicine, herd-level and animal-level prevalences of bovine cysticercosis using serology were determined in cattle from the State of Paraíba, Northeastern Brazil, as well as risk factors associated with herd-level prevalence were identified. In chapter II, a spatial cluster analysis was performed aiming to determine the spatial distribution of the disease in the State of Paraíba, and the article was submitted to Brazilian Journal of Veterinary Parasitology.

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CHAPTER I

Herd-level prevalence and associated risk factors for bovine cysticercosis in the State of Paraíba, Northeastern Brazil

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Herd-level prevalence and associated risk factors for bovine cysticercosis in the State of Paraíba, Northeastern Brazil

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ABSTRACT

This study focused on estimating the herd-level and animal-level prevalences, and identifying the risk factors associated with herd-level prevalence for bovine cysticercosis 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). For each sampling stratum, herd-level and animal-level prevalences were estimated by 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). 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, 2382 animals were sampled from 474 herds. Serological diagnosis was initially performed by the indirect ELISA, and positive sera were confirmed by immunoblot. A herd was deemed positive if it included at least one positive animal in herds of up to 29 females, and two positive animals in herds with more than 29 females. The herd-level prevalence in the State of Paraíba was 10.8% (95% CI = 8.1–14.1), 10.3% (95% CI = 6.4%–16.1%) in the region of Sertão, 6.9% (95% CI = 3.9%–12.1%) in Borborema, and 13.8% (95% CI = 9.3%–20.2%) in Agreste/Zona da Mata. The animal-level prevalence was 2.3% (95% CI =1.6%–3.3%) in the State of Paraíba, 1.4% (95% CI = 0.8%–2.5%) in Sertão, 3.6% (95% CI = 1.7%–7.4%) in the region of Borborema, and 3.2% (95% CI = 1.9%–5.4%) in Agreste/Zona da Mata. The frequency of seropositive animals per herd ranged from 7.1% to 100% (median of 16.7%). The risk factors identified were as follows: animal purchasing (OR = 2.19) and presence of flooded pastures (OR = 1.99). Our findings suggest that bovine cysticercosis herd-level seroprevalence in the State of Paraíba, Northeastern Brazil, is high, and support the idea that prevention measures should be applied at herd level and farmers could restrict the access of their cattle to flooded pastures.

Keywords: Cysticercosis; Bovine; Epidemiology; Control; Northeastern Brazil

1. Introduction

Cysticercosis and taeniosis are foodborne zoonotic infections with larval and adult tapeworms, respectively. Bovine cysticercosis is a skeletal and cardiac muscle tissue infestation in cattle, involving the larvae *Cysticercus bovis* of the tapeworm *Taenia saginata* (Calvo-Artavia et al., 2013). The taeniosis-cysticercosis (TC) complex caused by *T. saginata* is a tropical disease that causes economical losses to the beef supply chain and has a great public health importance in developing countries (Rossi et al., 2016), particularly in Latin America, such as Guatemala, Honduras, Ecuador, Peru, Colombia, Venezuela, Haiti and Brazil, where it is endemic (WHO, 2011).

In bovine TC complex, humans are the only definitive hosts for *T. saginata*, which acquire taeniosis through the consumption of raw or underdone meat containing the larvae of the parasite, called cysticercus (Rossi et al., 2014). Cattle become infected by consuming contaminated water or pasture with viable eggs of the parasite or by any other manner that leads to the intake of these eggs. Despite the limitations, postmortem inspection have been previously used to indicate the degree of bovine cysticercosis infection, therefore, a visual inspection of beef carcasses during slaughter is very important to reduce the risk for consumers (Hill et al., 2014).

In Brazil, bovine cysticercosis is endemic in several states, with a significant prevalence in Midwest, Southeast and Southern regions, where the highest rates in slaughtered cattle have been identified by the Federal Inspection Service (Dutra et al., 2012). Prevention of the disease is achieved by proper disposal of carcasses and organs of infected cattle, resulting in condemnation of carcasses and significant economic losses. Estimates of annual economic losses due to bovine cysticercosis reach values close to US$ 164 million in Latin America (Schantz et al., 1994).In Brazil, these losses estimated for beef production chain is around US$ 11.5 million (Bavia et al., 2012).

Despite the economic and public health impacts of TC complex, the epidemiological situation of the disease in Brazil is unknown because taeniosis is not a reportable disease, so that data on bovine cysticercosis occurrence is available from veterinary inspection records at slaughterhouses; however, some cases may be unnoticed, especially in mild infections, which make it relevant the use of serological tests with greater sensitivity than the postmortem routine inspection (Paulan et al., 2013; Guimarães-Peixoto et al., 2015). Thus, immunodiagnostic testing alternatives, such as indirect ELISA and immunoblot have been recommended as an option for antemortem detection of bovine cysticercosis, allowing a more accurate early identification of infected animals (Girotto et al., 2009; Dorny et al., 2002).

During the last few decades, cattle raising have become significantly important within animal husbandry in the State of Paraíba, Northeastern Brazil. Except for the Zona da Mata region (where sugarcane crops prevail), small cattle-raising farms are widespread in the Agreste, Borborema and Sertão regions. Whereas cultivated grasses (mostly *Brachiaria* spp.) are the basis for Agreste livestock, cattle are usually reared extensively on native Caatinga in most of the Borborema and Sertão farms. Following the Brazilian scenario of milk production, in the State of Paraíba around 69% of milk was produced in small cattle-raising farms (IBGE, 2006). In this context, the performance of epidemiological studies to investigate bovine cysticercosisis important. Therefore, the aim of this study was to determine the herd-level and animal-level prevalence of bovine cysticercosis using serology in cattle from the State of Paraíba, Northeastern Brazil, as well as to identify risk factors associated with herd-level prevalence.

2. Material and methods

2.1. Characterization of the study area

The State of Paraíba, located in the Northeastern region of Brazil, is characterized by warm weather throughout the year. The state is geographically subdivided into the following four major regions, based mostly on vegetation type and rainfall: (i) Zona da Mata (Atlantic forest), (ii) Agreste, (iii) Borborema, and (iv) Sertão. The Zona da Mata and Agreste have relatively higher rainfall regimes (Cabrera and Willink, 1973). Both Borborema and Sertão (the semiarid region) are typically within the Caatinga biome, which encompasses an area of 900,000 km² (11% of Brazilian territory) and is the only major biome that occurs exclusively in Brazil. Caatinga is xeric shrubland and thorn forest, which consists primarily of small, thorny trees that shed their leaves seasonally. Cacti, thick-stemmed plants, thorny brush and arid-adapted grasses make up the ground layer; however, during the dry periods there is no ground foliage or undergrowth (Andrade-Lima, 1981). The weather is characterized by a hot and semiarid climate, with temperatures averaging 27 °C, and the mean annual rainfall is typically ≈500 mm. There are typically two seasons: a rainy season from February to May, and a long drought period from June to January. However, occurrences of droughts sometimes lasting for longer than one year is also a characteristic of the region (Batista et al., 2007).

2.2. Division of the State of Paraíba into stratified sampling groups

The State of Paraíba was divided into three sampling groups: sampling stratum 1 (mesoregion of Sertão), sampling stratum 2 (mesoregion of Borborema), and sampling stratum3 (mesoregions of Zona da Mata and Agreste) (Fig. 1). When creating this stratification scheme, the operational capacity of the Agricultural and Livestock Defense Service of the State of Paraíba (SEDAP) was considered based on the areas of action of its regional units in order to ensure that the agency could conduct the fieldwork.

2.3. Sampling, target condition and herd-level case definition

The samples used in this study were obtained from a study of bovine brucellosis in the State of Paraíba made by the National Program for Control and Eradication of Brucellosis and Tuberculosis, and sampling design was adjusted for bovine cysticercosis. For each sampling stratum, the prevalence of herds infected with bovine cysticercosis and the prevalence of seropositive animals were estimated bya 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).

In farms with more than one herd, the cattle herd of greater economic importance was chosen as the target of the study; the animals in the selected cattle herd were subjected to the same type of management system as the other herds, i.e., had the same risk factors as the other herds. The selection of the primary sampling units was random (random drawing), and was based on the records of farms of the SEDAP. If a herd that was selected could not be visited, the herd was replaced by another one in the vicinity with the same production characteristics. 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, 1.1%estimated herd-level prevalence (Santos et al., 2013), and 5% 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: 81.25% (Silva et al., 2015a) and 98.3% (Silva et al., 2015b) for the sensitivity and specificity, respectively, of the test protocol (indirect ELISA and immunoblot tests serially applied) and 31% (Asaava et al., 2009) for the intra-herd estimated prevalence. Herdacc version 3software (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.

The target condition was a sero-positive 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 31% (Asaava et al., 2009), and the sensitivity and specificity of the diagnostic tests serially used (indirect ELISA and immunoblot), with the goal of obtaining a herd sensitivity and specificity of ≥90%. After new simulations using Herdacc software, a herd was deemed positive for cysticercosis if it included at least one positive animal in herds of up to 29 females, and two positive animals in herds with more than 29 females.

The field activities included blood collection, provision of an epidemiological questionnaire, and sending the samples to the laboratory. The veterinarians and agricultural and livestock technicians of the SEDAP were involved in the fieldwork. Blood samples (10-mL volume) were collected from September 2012 to January 2013, from cows aged ≥ 24 months by jugular vein puncture with a disposable needle and a 15-mL capacity vacuum tube (without anticoagulant). An 11-digit code was used for identification of the tubes, of which the first nine digits referred to the herd code and the final two digits to the number sequence of the sampled cow. After draining, the serum was transferred to microtubes and was frozen.

2.4. Data collection

A structured questionnaire including closed-ended questions was designed to obtain information concerning (a) the identification and location of the herd; (b) management practices; (c) structure and composition of the herd; and (d) presence of other domestic and wildlife species in the farm. Questionnaires were administered to the owner or person in charge of the herd either by the primary author or by a veterinarian from the SEDAP at the same time of the visit to blood collection. The description of the questions included in the questionnaire can be found in the supplementary material.

2.5. Serological diagnosis

Serological diagnosis of bovine cysticercosis was initially performed by the indirect ELISA, and positive sera were confirmed by immunoblot. Both tests were carried-out according to methodologies previously described by Pinto et al. (2000), Silva et al. (2015a) and Silva et al. (2015b) using *T. crassiceps* larvae as antigens. For indirect ELISA, the positivity and negativity of each sample was determined by calculating the cut-off points, which were defined as the average of the optical densities (OD) of the reactions of the negative control sera, plus two or three standard deviations.

2.6. Prevalence calculations

A herd was deemed positive for cysticercosis if it included at least one positive animal in herds of up to 29 females, and two positive animals in herds with more than 29 females. EpiInfo 6.04 software was used to calculate the apparent prevalences and respective confidence intervals (Dean et al., 1996). Stratified random sampling was utilized to calculate the herd-level prevalence in the State of Paraíba (Thrusfield, 2007). The required parameters were as follows: (a) condition of the herd (positive or negative), (b) sampling stratum to which the herd belonged, and (c) statistical weight. The statistical weight was determined by applying the following formula (Dean et al., 1996):

$$Weight = \frac{\text{number of herds in the stratum}}{\text{number of herds sampled in the stratum}}$$

The calculation of the herd-level prevalence per sampling stratum employed the sampling design of a simple random sample by using the following parameters: (a) number of positive herds and (b) number of herds sampled in the stratum.

The sampling design for calculating the animal-level prevalence in the State of Paraíba employed a two-stage stratified cluster sampling, and a two-stage cluster sampling in each stratum (Thrusfield, 2007), where each herd was considered a cluster. The following parameters were used: (a) animal condition (seropositive or seronegative), (b) sampling stratum to which the animal belonged, (c) herd code (to identify each cluster), and (d) statistical weight. The statistical weight was calculated with the following formula (Dean et al., 1996):

$$Weight = \frac{\text{cows} \geq 24 \text{ months in the stratum}}{\text{cows} \geq 24 \text{ months in the sampled herds}} \times \frac{\text{cows} \geq 24 \text{ months in the herd}}{\text{cows} \geq 24 \text{ months sampled in the herd}}$$

In the above expression, the first term refers to the weight of each animal in the calculation of the animal-level prevalence within the stratum.

2.7. Risk factor analysis

Data obtained with the epidemiological questionnaires were used in the analysis of risk factors associated with the herd-level prevalence. The analyzed variables and respective categories were as follows: sampling stratum (Sertão/Borborema/Agreste and Zona da Mata), type of production (beef/milk/mixed), management system (intensive/semi-intensive/extensive), predominant breed (zebu/European dairy/crossbreed), local of animal slaughter (not slaughter/in slaughterhouses/on the farm), type of farm (classic rural/urban periphery), no. of cows aged ≥ 24 months (cut-off point: 3rd quartile), herd size (cut-off point: 3rd quartile), presence of poultry, wild animals, cervids and capybaras (no/yes), animal purchasing (no/yes), rental of pastures (no/yes), sharing of pastures (no/yes), sharing of water sources (no/yes), presence of flooded pastures (no/yes),use of maternity pens(no/yes), raw milk consumption (no/yes), and veterinary assistance (no/yes).

The variables were organized for presentation in ascending or descending order regarding scale of risk. When necessary, these variables were re-categorized. The lower-risk category was considered the basis for comparison for the other categories. An initial exploratory analysis of the data (univariable) was conducted for selection of variables with $P \leq 0.2$ by the chi-square test or Fisher's exact test; subsequently, the variables that passed this cut-off were utilized for logistic regression (Hosmer and Lemeshow, 2000). The fit of the final model was verified with the Hosmer and Lemeshow test, and collinearity between independent variables was verified by a correlation analysis; for those variables with a strong collinearity (correlation coefficient > 0.9), one of the two variables was excluded from the multiple analysis according to the biological plausibility (Dohoo et al., 1996). Confounding was assessed by monitoring the changes in the model parameters when adding new variables. If substantial changes (i.e., higher than 20%) were observed in the regression coefficients, this was considered as indicative of confusion. The calculations were performed by using SPSS software version 20.0.

3. Results

The census data and the sample studied in each sampling stratum are shown in Table 1. In total, 2382 animals were sampled from 474herds. Herd-level and animal-level prevalences are presented in Tables 2 and 3, respectively; further, the geographical distribution of positive and negative herds are shown in Fig.1. The herd-level prevalence in the State of Paraíba was 10.8% (95% CI = 8.1–14.1), 10.3% (95% CI = 6.4%–16.1%) in the region of Sertão, 6.9% (95% CI = 3.9%–12.1%) in Borborema, and 13.8% (95% CI = 9.3%–20.2%) in Agreste/Zona da Mata. The animal-level prevalence was 2.3%(95% CI =1.6%–3.3%) in the State of Paraíba, 1.4% (95% CI = 0.8%–2.5%) in Sertão, 3.6% (95% CI = 1.7%–7.4%) in the region of Borborema, and 3.2% (95% CI = 1.9%–5.4%) in Agreste/Zona da Mata. The frequency of seropositive animals per herdrangedfrom7.1% to 100% (median of 16.7%).

The results of the univariable analysis for the risk factors are presented in Table 4. The variables selected ($P \leq 0.2$) for the multiple analysis were as follows: sampling stratum, predominant breed, local of animal slaughter, type of farm, no. of cows aged ≥ 24 months, herd size, presence of poultry, animal purchasing, presence of flooded pastures, and veterinary assistance. In the final logistic regression model (Table 5), the risk factors identified were as follows: animal purchasing (OR = 2.19) and presence of flooded pastures (OR = 1.99). Final model had a good fit (Hosmer and Lemeshow test: chi-square = 0.391; P = 0.983).

4. Discussion

The present study is the first one in Brazil to determine the prevalence of bovine cysticercosis at herd-level by using random sampling of herds and animals. In Brazil, most of the bovine cysticercosis data are originated from routine inspection and just a few studies reported results based on other diagnostic techniques such as serological tests or detailed meat inspection (Iasbik et al., 2010; Thomaz-Soccol et al. 2010; Acevedo-Nieto et al., 2012; Felippe et al., 2014; Santos et al., 2013; Garro et al., 2015). In a systematic review of bovine cysticercosis in Europe, Laranjo-González et al. (2016) also referred that available prevalence data for bovine cysticercosis are scarce (most of them originated from routine inspection) and of low quality, and suggested that in order to know the current epidemiological context of bovine cysticercosis the use of more sensitive surveillance strategies is needed and data collection and reporting throughout the years for all of the countries is essential. In our survey, we used serological methods (indirect ELISA and immunoblot) and we performed corrections for sensitivity (Se) and specificity (Sp) of the serological tests prior to classification of the herd, and herd-level case definition was based on the size of the population (cows aged ≥24 months), number of females sampled, intra-herd apparent prevalence and the Se and Sp of the diagnostic test used, which was important to minimize misclassification bias. It is well-known that meat inspection sensitivity has been estimated to be between 10 and 30% (Dorny et al., 2000; Murrell et al., 2005; Eichenberger et al., 2013), therefore, the data collected from routine inspection, although it may generate important information, underestimate the real prevalence (Laranjo-González et al., 2016).

The herd-level (10.8%; 95% CI = 8.1%-14.1%) and animal-level (2.3%; 95% CI= 1.6%-3.3%) prevalences found in the State of Paraíba, especially in the Agreste/Zona da Mata and Sertão mesoregions, where herd-level prevalences were 13.8% (95% CI = 9.3%-20.2%) and 10.3% (95% CI = 6.4% - 16.1%), respectively, indicate that bovine cysticercosis is spread in cattle herds in the region. Data on bovine cysticercosis prevalence using serological methods as diagnostic tests in Brazil are scarce and limited to local surveys. Seropositivities rates at animal level have been referred to range from 0.4% to 4.1% in surveys conducted in the state of Minas Gerais (Iasbik et al., 2010; Acevedo-Nieto et al. 2012; Santos et al., 2013; Felippe et al., 2014; Garro et al., 2015). It is believed that the animal-level prevalence could be even higher in Paraíba, once for this study only cows aged ≥ 24 months were used. Nevertheless, within-herd prevalence ranged from 7.1% to 100% (median of 16.7%).

A matter of concern is the public health impact of the high herd-level and within-herd prevalences found in this survey. In Brazil, the main intervention to control bovine cysticercosis is the detection of infected carcasses by meat inspection, followed by condemnation or freezing/heat treatment when necessary; however, this technique is time consuming, and lightly infected carcasses can be easily missed and passed for human consumption (Walther and Koske, 1980). In Belgium, Dorny et al. (2000) found that 36 serum samples (3.09%) were found positive in the antigen ELISA, while by meat inspection on the same animals cysticerci were detected in only three carcasses (0.26%). Likewise, in Catalonia region (North-Eastern Spain), Allepuz et al. (2012) referred that 23 (1.11%) of 2,073 animals were seropositive using antigen ELISA, i.e. the seroprevalence was about 50 times higher than the prevalence obtained by visual inspection within the same period: 19 positive animals of 90,891 slaughtered animals (0.02%) in the same slaughterhouses. None of the animals with positive result in the antigen ELISA was detected by meat inspection. In Paraíba State, where there is no cattle slaughterhouse with federal inspection and many animals are clandestinely slaughtered, this concern is even greater.

Because of the samples used in this study were obtained from a study of bovine brucellosis in the State of Paraíba made by the National Program for Control and Eradication of Brucellosis and Tuberculosis some risk factors for bovine cysticercosis were not addressed in the epidemiological questionnaires, such as the presence of fishermen in the surroundings of the farm (Rossi et al., 2015), the use of urban sewage sludge on pastures (Cabaret et al., 2002); the presence of roads or car parking lots adjacent to pastures as well as recreational sites (Flütsch et al., 2008), and contaminated food (Jenkins et al., 2013). Nevertheless, it was possible to identify important conditions which possibly are playing a role in the dissemination of the infection in the herds.

Animal purchasing was identified as risk factors for herd-level prevalence in this study. This variable is a classic risk factor for the occurrence of infectious diseases, and has been found for several cattle diseases in Brazil, such as neosporosis (Silva et al., 2008), brucellosis (Silva et al., 2009), leptospirosis (Hashimoto et al., 2012), and bovine viral diarrhea (Fernandes et al., 2016). In the case of bovine cysticercosis, it is not plausible to suggest measures based on animal testing prior to purchasing because serological tests for bovine cysticercosis are not widely available, as well as replacement or maintenance of livestock by animal purchasing is common in the region, so that measures should be based on the prevention of the disease at herd level, such as to avoid contact of cattle with human feces, and contaminated water and pasture (Murrell et al., 2005).

Presence of flooded pastures was also identified as risk factor for bovine cysticercosis. This variable was also referred by Boone et al. (2007) for Belgian dairy and mixed cattle herds, and indicates the hypothesis that water plays an important role in transmission of *T. saginata* eggs. Water can also carry eggs over long distances (Barbosa et al., 2001) and is one of the main routes of transmission of the disease (Allepuz et al., 2009). In the present survey, the presence of flooded pastures was referred in 36.3% (n = 172; Table 4) of the herds, which raises concern because often farmers cannot prevent their pastures to be accidentally flooded with wastewater containing *T. saginata* eggs (Boone et al., 2007). Furthermore, in general, bovine cysticercosis is an unknown disease for most farmers in Paraíba State, and although the high herd-level prevalence found in this state, most farmers are not aware of the public health impact of the infection and the economic losses that it can cause. Therefore, farmers should be fully supported and informed of the life cycle of *T. saginata* and potential risk factors for cattle to become infected.

5. Conclusions

The results presented here suggest that bovine cysticercosis herd-level seroprevalence in the State of Paraíba, Northeastern Brazil, is high. Based on the risk factor analysis, our findings further support the idea that prevention measures should be applied at herd level and farmers could restrict the access of their cattle to flooded pastures. This knowledge might be useful for design of future effective control programmes. It would be interesting and important the conduction of educative activities to farmers on the public health and economic impacts of the disease, as well as on its epidemiological aspects.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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Figure caption

Fig. 1. Division of the State of Paraíba into three sampling groups, and geographical distribution of positive and negative herds. Detail shows the State of Paraíba within Brazil.

Table1

Census data of the cattle population in the State of Paraíba, Northeastern Brazil, according to sampling stratum.

Sampling stratum	No. of herds		No. of cows ≥ 24 months of age	
	Total	Sampled	Total	Sampled
Sertão	24,356	156	288,764	962
Borborema	11,603	159	83,428	717
Agreste/Zona da Mata	18,398	159	192,320	703
State of Paraíba	54,357	474	564,512	2,382

Table 2

Herd-level prevalence for bovine cysticercosis in the State of Paraíba, Northeastern Brazil, according to sampling stratum.

Sampling stratum	No. of herds		Prevalence (%)	95% CI
	Tested	Positive		
Sertão	156	16	10.3	[6.4 – 16.1]
Borborema	159	11	6.9	[3.9 – 12.1]
Agreste/Zona da Mata	159	22	13.8	[9.3 – 20.2]
State of Paraíba	474	49	10.8	[8.1 – 14.1]

Table 3

Animal-level prevalence for bovine cysticercosis in the State of Paraíba, Northeastern Brazil, according to sampling stratum.

Sampling stratum	Animals		Prevalence (%)	95% CI
	Tested	Positive		
Sertão	962	19	1.4	[0.8 – 2.5]
Borborema	717	16	3.6	[1.7 – 7.4]
Agreste/Zona da Mata	703	30	3.2	[1.9 – 5.4]
State of Paraíba	2,382	65	2.3	[1.6 – 3.3]

Table 4

Univariable analysis for risk factors associated with the herd-level prevalence of bovine cysticercosis in the State of Paraíba, Northeastern Brazil.

Variables	Categories	No. of herds sampled	No. of positive herds (%)	*P*
Sampling stratum	Borborema	159	11 (6.9)	
	Sertão	156	16 (10.3)	
	Agreste/Zona da Mata	159	22 (13.8)	0.128*
Type of production	Beef	59	5 (8.5)	
	Milk	137	17 (12.4)	
	Mixed	278	27 (9.7)	0.615
Management system	Intensive	27	3 (11.1)	
	Semi-intensive	269	26 (9.7)	
	Extensive	178	20 (11.2)	0.859
Predominant breed	Zebu	25	1 (4.0)	
	European (dairy)	42	10 (23.8)	
	Crossbreed	407	38 (9.3)	0.008*
Local of animal slaughter	Not slaughter	212	17 (8.0)	
	In slaughterhouses	259	31 (12.0)	
	On the farm	3	1 (33.3)	0.158*
Type of farm	Classic rural	457	45(9.8)	
	Urban periphery	17	4(23.5)	0.087*
No. of cows aged ≥ 24 months	0 – 9	362	32 (8.8)	
	> 9	112	17 (15.2)	0.080*
Herd size	0 – 23 animals	358	31 (8.7)	
	> 23 animals	116	18 (15.5)	0,053*
Presence of poultry	No	167	24 (14.4)	
	Yes	307	25 (8.1)	0.049*
Presence of wild animals	No	299	35 (11.7)	
	Yes	175	14 (8.0)	0.262
Presence of cervids	No	467	49 (10.5)	
	Yes	7	0 (0.0)	1.000
Presence of capybaras	No	470	49 (10.4)	
	Yes	4	0 (0.0)	1.000
Animal purchasing	No	381	34(8.9)	
	Yes	93	15(16.1)	0.063*

Rental of pastures	No	364	36 (9.9)	
	Yes	110	13 (11.8)	0.687
Sharing of pastures	No	396	41(10.4)	
	Yes	78	8(10.3)	1.000
Sharing of water sources	No	402	42 (10.4)	
	Yes	72	7 (9.7)	1.000
Presence of flooded pastures	No	302	23 (7.6)	
	Yes	172	26 (15.1)	0.015*
Use of maternity pens	No	352	38 (10.8)	
	Yes	122	11 (9.0)	0.701
Raw milk consumption	No	394	41 (10.4)	
	Yes	80	8 (10.0)	1.000
Veterinary assistance	No	400	37 (9.2)	
	Yes	74	12 (16.2)	0.110*

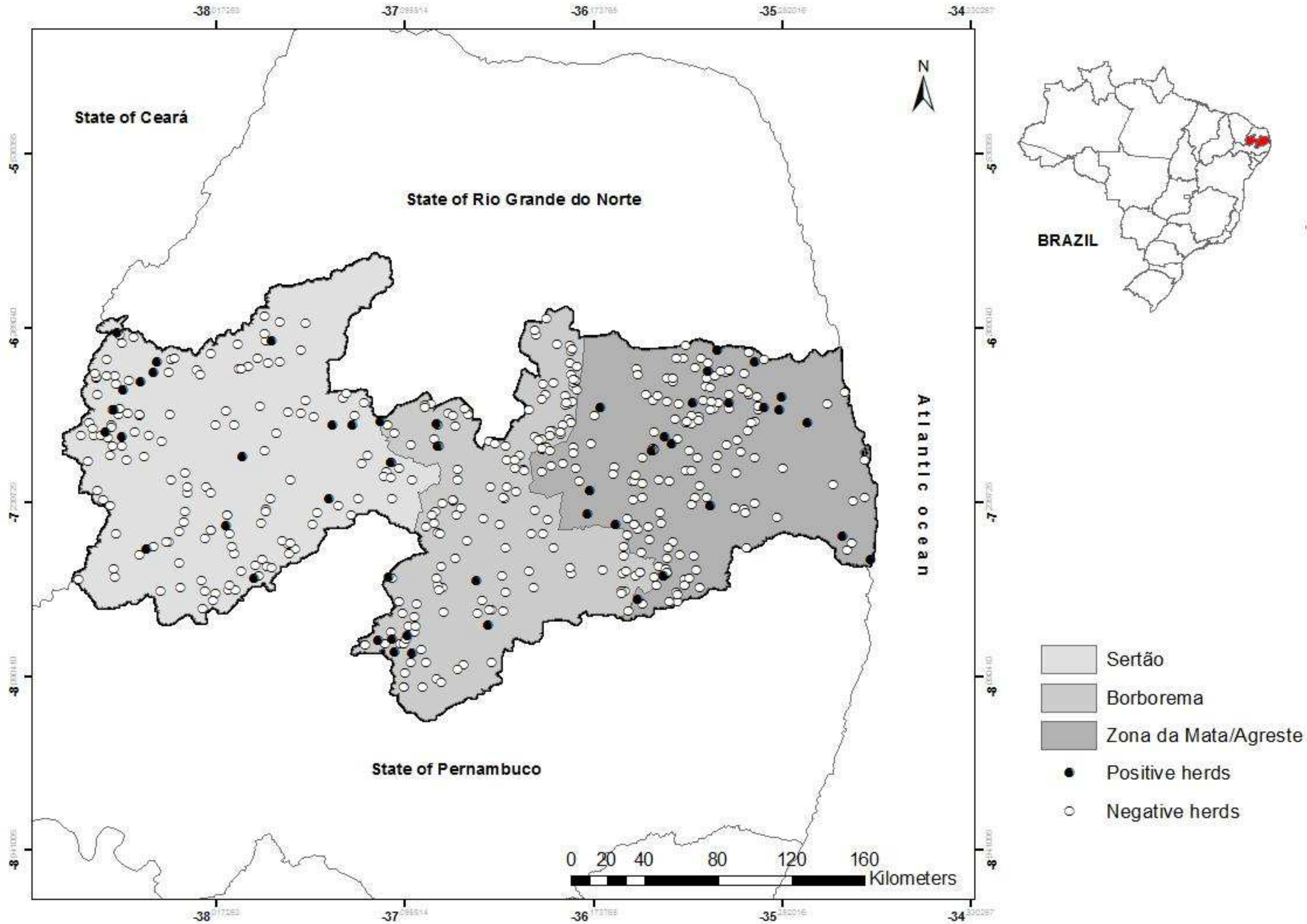
* Variables selected and used in the multiple analysis ($P \leq 0.2$)

Table 5

Risk factors associated with herd-level prevalence of bovine cysticercosis in the State of Paraíba, Northeastern Brazil.

Risk factors	Logistic regression coefficient	Standard error	Wald	Degrees of freedom	Odds ratio	95% CI	*P*
Animal purchasing	0.782	0.352	4.953	1	2.19	1.10 – 4.36	0.026
Presence of flooded pastures	0.691	0.308	5.021	1	1.99	1.09 – 3.65	0.025
Intercept	-2.964	0.289	104.935	1	0.052	...	<0.001

Hosmer and Lemeshow chi-square = 0.391; degrees of freedom = 4; P = 0.983



State of Ceará
State of Rio Grande do Norte
State of Pernambuco
Atlantic ocean
N
BRAZIL
Sertão
Borborema
Zona da Mata/Agreste
Positive herds
Negative herds
0 20 40 80 120 160 Kilometers
-38
-37
-36
-35
-34
-5
-6
-7
-8

Fig. 1

CHAPTER II

Herd-level spatial cluster analysis for bovine cyscicercosis in Paraíba State, Northeastern Brazil

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Herd-level spatial cluster analysis for bovine cysticercosis in Paraiba State, Northeastern Brazil

Análise de aglomerados espaciais de propriedades positivas para cisticercose bovina no Estado da Paraíba, Nordeste do Brasil

RUNNING TITLE: Cluster analysis for bovine cysticercosis, Paraíba

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Abstract

The aim of this survey was to identify spatial clustering of bovine cysticercosis positive herds in the State of Paraíba. 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), and 2382 cows aged ≥ 24 months were sampled from 474 herds. Serological diagnosis of bovine cysticercosis was initially performed by the indirect ELISA, and positive sera were confirmed by immunoblot. A herd was deemed positive for cysticercosis if it included at least one positive animal in herds of up to 29 females, and two positive animals in herds with more than 29 females. Spatial clustering was assessed using the Cuzick-Edwards' *k*-nearest neighbor method and spatial scan statistics. A significant clustering of positive herds was detected in Southern part of Borborema mesoregion. As serological tests for bovine cysticercosis are not widely available, 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.

Keywords: cattle, epidemiology, cluster analysis, bovine cysticercosis.

Resumo

O objetivo deste estudo foi identificar agrupamentos espaciais de rebanhos positivos para cisticercose bovina no Estado da Paraíba. O estado foi dividido em três grupos amostrais: estrato amostral 1 (mesorregião do Sertão), estrato amostral 2 (mesorregião da Borborema), e estrato amostral 3 (mesorregiões da Zona da Mata e Agreste), e 2.382 vacas com idade ≥ 24 meses foram amostradas a partir de 474 propriedades. O diagnóstico sorológico da cisticercose bovina foi inicialmente realizado pelo ELISA indireto, e os soros positivos foram confirmados por immunoblot. Um rebanho foi considerado positivo para cisticercose se apresentasse pelo menos um animal positivo em rebanhos de até 29 fêmeas, e dois animais positivos em rebanhos com mais de 29 fêmeas. Os agrupamentos espaciais foram avaliados com o uso da metodologia *k*-vizinhos mais próximos de Cuzick-Edwards e estatística espacial de varredura. Um agrupamento significativo de rebanhos positivos foi detectado na parte sul da mesorregião da Borborema. Tendo em vista que os testes sorológicos para diagnóstico de cisticercose bovina não são amplamente disponíveis, bem como é

comum na região a reposição e manutenção dos rebanhos por compra de animais, conclui-se que medidas de prevenção devem ser aplicadas em nível de rebanho.

Palavras-chave: epidemiologia, análise de cluster, cisticercose bovina.

Introduction

Bovine cysticercosis is a tropical zoonotic disease caused by the larval stage of *Taenia saginata* in cattle and the adult phase causes taeniosis in humans (CALVO-ARTAVIA et al., 2013). Cattle become infected by consuming contaminated water or pasture with viable eggs of the parasite or by any other manner that leads to the intake of these eggs. Despite the limitations, postmortem inspection have been previously used to indicate the degree of bovine cysticercosis infection, therefore, a visual inspection of beef carcasses during slaughter is very important to reduce the risk for consumers (COSTA et al., 2012), as it causes economic losses to the beef supply chain and has a great public health importance in developing countries (ROSSI et al., 2016)

The epidemiological situation of bovine cysticercosis in Brazil is unknown because the data of its occurrence is available only from veterinary inspection records at slaughterhouses, and some cases may be unnoticed, especially in mild infections, which make it relevant the use of serological tests with greater sensitivity than the postmortem routine inspection (PAULAN et al., 2013; GUIMARÃES-PEIXOTO et al., 2015). Positivity of bovine cysticercosis, based on absolute numbers of occurrence, enables the misinterpretation of the spatial distribution of the disease, as regions with high concentrations of these events do not always represent the areas of highest risk (BAVIA et al., 2012). Therefore, epidemiological maps of disease risk have been produced to relate disease data among environmental features at known infected sites of bovine cysticercosis. However, studies on the distribution of bovine cysticercosis in Brazil considered only post-mortem inspection, and not serological tests (BAVIA et al., 2012; DUTRA et al 2012; ROSSI et al., 2016). So, to date there is no survey on herd-level spatial clustering analysis for bovine cysticercosis seroprevalence in Brazil.

Spatial clustering analysis is a useful tool to study the spread of infectious diseases in animal populations. The identification of clusters might yield important information about the transmission and/or control of such diseases (CARPENTER, 2001). 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 (MAIA, 2016). The herd-level prevalence in the State of Paraíba was 10.8% (95% CI = 8.1% – 14.1%), 10.3% (95% CI = 6.4% – 16.1%) in the region of Sertão, 6.9% (95% CI = 3.9% - 12.1%) in Borborema, and 13.8% (95% CI = 9.3% - 20.2%) in Agreste/Zona da Mata (Table 1). Thus, in the present study a spatial cluster analysis was performed aiming to determine the spatial distribution of the disease in Paraíba State.

Materials and Methods

Data source

Data used in the present study were originated from the epidemiological survey for bovine cysticercosis in the State of Paraíba (MAIA, 2016). The State of Paraíba 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) (Figure 1). For each sampling stratum, a pre-established number of herds were randomly selected (primary sampling units) and then, 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, 1.1% estimated herd-level prevalence (SANTOS et al., 2013), and 5% 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, using the concept of aggregate sensitivity and specificity (DOHOO et al., 2003). For the calculations, the following values were adopted: 81.25% (SILVA et al., 2015a) and 98.3% (SILVA et al., 2015b) for the sensitivity and specificity, respectively, of the test protocol (indirect ELISA and immunoblot tests serially applied) and 31% (ASAAVA et al., 2009) 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, 2382 animals were sampled from 474 cattle herds.

The target condition 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 31% (ASAAVA et al., 2009), and the sensitivity and specificity of the diagnostic tests serially used (indirect ELISA and immunoblot), with the goal of obtaining a herd sensitivity and specificity of ≥ 90%. After new simulations using Herdacc software, a herd was deemed positive for cysticercosis if it included at least one positive animal in herds of up to 29 females, and two positive animals in herds with more than 29 females.

Serological diagnosis

Serological diagnosis of bovine cysticercosis was initially performed by the indirect ELISA, and positive sera were confirmed by immunoblot. Both tests were carried-out according to methodologies previously described by Pinto et al. (2000), Silva et al. (2015a) and Silva et al. (2015b) using *T. crassiceps* larvae as antigens. For indirect ELISA, the positivity and negativity of each sample was determined by calculating the cut-off points, which were defined as the average of the optical densities (OD) of the reactions of the negative control sera, plus two standard deviations.

Statistical analysis

Spatial clustering of bovine cysticercosis 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 analysed 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.

Results and Discussion

Significant clusters were not identified (Simes $p > 0.05$) by the Cuzick and Edwards' method for the entire Paraiba 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. Using the Bernoulli model, a spatial cluster of positive herds was detected in Southern part of Borborema mesoregion (Figure 1). In this cluster, the number of herds was 7, the radius of the cluster was 8.02 km, and the number of observed and expected cases (positive herds) were 5 and 0.53, respectively, where the risk for infection was 15.4 (Relative Risk = 15.4; $p = 0.008$) times higher in herds located inside cluster than in those located elsewhere. Allepuz et al. (2009) identified two statistically significant cluster of bovine cysticercosis in the region of Catalonia (North-Eastern Spain), concluding that the location of the farm may also have an influence on the risk of bovine cysticercosis. These authors suggested the large number of animals infected and the fact that the animals originated from different regions in Spain and different countries in Europe practically discard the possibility of the animals being infected in origin, and there was a possibility of these animals getting infected at the same farm before being transferred to the others farms.

In the present study there was a lack of spatial cluster of bovine cysticercosis throughout the Paraíba State, but a spatial cluster was identified when considering the separate mesoregions. However, it can be inferred that this cluster cannot be explained by spatially structured factors as referred by Ávila 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 cysticercosis, and does not respect geographical boundaries. Thus, the cluster found in the Borborema region can be explained by being a border region with the State of Pernambuco, more precisely close to an animal fair in the county of Tabira, the second largest cattle fair in the state, 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 with cysticercosis.

In Paraiba State, most farms are family or subsistence, with predominantly mixed production and semi-intensive farming (CLEMENTINO et al., 2015), leading to inappropriate practices as meat self-consumption or for sale within the community, without any sanitary inspection (ARAGÃO et al., 2010). Thus, the cattle can be exposed to important environmental risk factors for bovine cysticercosis, such as surface water, flooded pastures and grazing on pastures contaminated with *T. saginata* eggs from human faeces, which favor persistence of the taeniosis-cysticercosis complex (BARBOSA et al., 2001; BOONE et al., 2007).

The detection of spatial clustering is a complex methodology and has limitations, however, the obtainment 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 bovine cysticercosis are not widely available, as well as replacement or maintenance of livestock by animal purchasing is common in the region, so that measures should be based on the prevention of the disease at herd level, such as to avoid contact of cattle with human feces, and contaminated water and food (MURRELL et al., 2005).

Taking into account the multiplicity of factors that are involved in the transmission of bovine cysticercosis, such as environmental, economic, sociocultural, hygienic and sanitary aspects of animal farming systems (BAVIA et al., 2012), and the high prevalence of bovine cysticercosis in Paraiba State, it is suggested the conduction of epidemiological surveys, both in humans and cattle, aiming to identify possible conditions that could act as risk factors for the occurrence and distribution of bovine cysticercosis in the region.

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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 cysticercosis.

Sampling stratum	Total no. of herds	No. of herds		Prevalence (%)	95% CI
		Tested	Positive		
Sertão	24,356	156	16	10.3	[6.4 – 16.1]
Borborema	11,603	159	11	6.9	[3.9 – 12.1]
Agreste/Zona da Mata	18,398	159	22	13.8	[9.3 – 20.2]
State of Paraíba	54,357	474	49	10.8	[8.1 – 14.1]

Source: MAIA (2016)

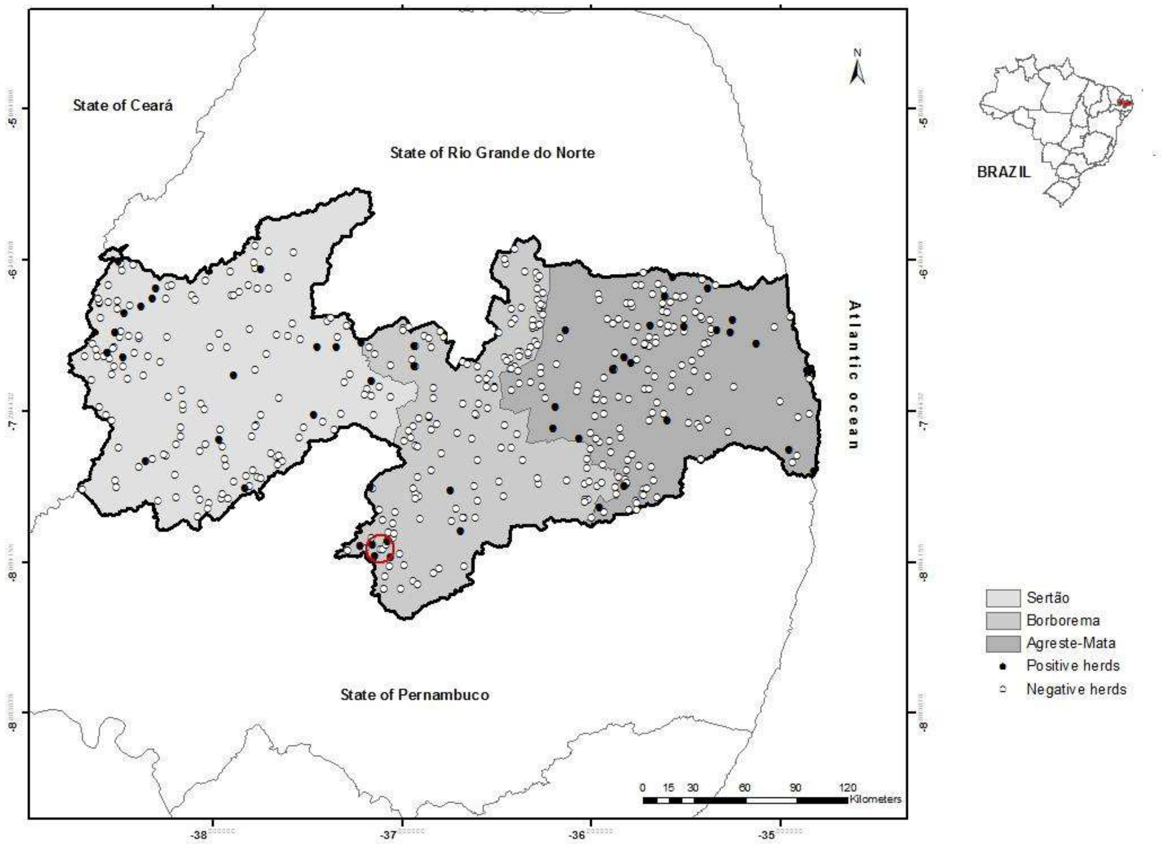

Figure 1. Significant cluster (red line) of bovine cysticercosis positive herds in the State of Paraíba. Detail shows Paraíba State within Brazil.

GENERAL CONCLUSIONS

In the present survey, it was possible to determine important epidemiological indicators for bovine cysticercosis in the State of Paraiba, Northeastern Brazil. The high herd-level seroprevalence found points out to a public health concern, once serology presents higher sensitivity that meat inspection, and then infected carcasses could not be detected by meat inspection in case of mild or moderate infections. According to risk factor analysis results, prevention measures applied at herd level and to avoid the access of cattle to flooded pastures could be important to prevent disease dissemination.

By spatial cluster analysis it was possible to identify a border area in the State of Paraíba with high risk of disease spread, which suggests that animal purchasing without knowing the sanitary conditions of the animals is acting as risk factor. So, it is suggested that the conduction of educative activities to farmers on the public health and economic impacts of the disease, as well as on its epidemiological aspects, could increase the education level of farmers on bovine cysticercosis and would be important for design of future effective control programmes.

ATTACHMENT I

Preventive Medicine Veterinary – Author's guidelines

GUIDE FOR AUTHORS

Preventive Veterinary Medicine's Editors and reviewers use several published guidelines for reporting standards; the websites are listed in the Appendix to this Guide for Authors. Conformation to these reporting standards allows our Editors and reviewers to judge the quality and originality of your work; conformation also offers readers sufficient information to judge the relevance of your study to the readers' own situations. Omission of substantive items from relevant guidelines for reporting standards is sufficient reason to reject your manuscript.

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BEFORE YOU BEGIN

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c. Description of numbers, training, experience, and representativeness of any **"experts"** used to provide opinions
d. Declaration of the **stakeholders** for any risk assessment
e. Distinction between assumptions, input data, calculations from intermediate steps in the modeling process, and model predictions

f. Descriptions of the assumed chance variation and assumed knowledge uncertainty in the inputs, and methods used to deal with those sources of total uncertainty
g. **Sensitivity analyses** of key assumptions and of the input variables that had the greatest uncertainty
h. Descriptions of the **variability in the "outputs"** from stochastic models

5. For **statistical-hypothesis tests:**
a. Declarations of the unit of statistical analysis and of the dependent ("outcome") variable
b. **Alpha** and **tails**, and any methods used to adjust for multiple comparisons (to protect experiment wise alpha from the problem of **multiplicity**)
c. **Methods used to adjust for clustering within the data**
d. Methods used to determine that the **statistical assumptions were met** (e.g., that the data were
Gaussian or that the odds ratio or hazards ratio was constant across the observed range of the risk factor)
e. Methods used to look for **collinearities** or other interrelationships among the risk factors being tested
f. Methods used to select or to retain risk factors within multivariable models (including the **test criterion**)
g. Clear declaration of any variables "forced into" the model (not allowed to drop out; this implies a need to account for that factor) or offered to the model on a priori grounds despite any screening results (this implies that the factor was part of a major hypothesis)
h. Description of the **goodness-of-fit** of any models
i. How **missing data** were handled

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ATTACHMENT II

Brazilian Journal of Veterinary Parasitology – Author's guidelines

Paper submission:
The articles submitted must undergo English-language revision, done by reviewers accredited by the RBPV (http://cbpv.org.br/rbpv/revisoes_traducoes.php). Likewise, the certificate of English-language revision should be sent together with the submitted article. The authors will be expected to bear the costs of the revision.

Publication fee:
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The manuscript review process will follow the journal's Editorial Guidelines and consider the editors' and/or the ad hoc reviewer's opinions. Articles that are submitted for publication will be reviewed by at least three anonymous reviewers, selected by the editor-in-chief and assistant scientific editors.

The reviewer should fill out the RBPV's evaluation form, which is available in the online submission system (http://mc04.manuscriptcentral.com/rbpv-scielo). The author will receive evaluations from at least two of the reviewers selected and will receive the evaluation forms and possible corrections made directly in the text. The reviewer may then correct the article again, if necessary.

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After the layout and editing processes, the assistant scientific editors and editor-in-chief of the journal will make any final corrections.

Transfer of author's rights:

At the time of submission, the article must be accompanied by a formal letter signed by all the authors, in which they all agree with the submission and, if approved, publication of the article only in the RBPV.

Ethics

Experiments using animals should be conducted following the Brazilian College of Animal Experimentation guidelines (http://www.cobea.org.br). Articles should include the protocol number approved by the Animal Ethics Committee.

Manuscript Preparation

The following guidelines should be followed during manuscript preparation:

All articles should be submitted in United States English. Always use concise and impersonal language. Footnotes should be placed at the bottom of the corresponding page and numbered with Arabic numerals in an ascending order.

All manuscripts should be typed in Times New Roman font, size 12, page setup with 2.5-cm top and bottom margins, 3-cm left and right margins, and 1.5-cm line spacing.

All pages should be numbered. Full Articles should have a maximum of 15 pages and Research Notes should have a maximum of 5 pages in the final layout. All tables and illustrations should be presented separately from the main text body and attached to the final manuscript without captions. The related captions should be included in the text after the References. When submitting your article, please send an e-mail with the deposit slip attached: http://www.scielo.br/rbpv. It is the authors' responsibility to make sure that accepted papers are reviewed by one of the English language reviewers certified by RBPV. Full Articles should be structured as follows: Original Title, Translated Title, Author(s), Affiliations, Abstract (Keywords), Introduction, Materials and Methods, Results, Discussion, Conclusions (or a combination of the last three), Acknowledgements (optional), and References. Research Notes should follow the same structure as described above but they can be presented as a continuous stream of body text with no need to include headings. Novelty and originality that bring to light new significant findings are expected.

Description of each item of the manuscript

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The full title and subtitle, if any, should not exceed 15 words. The title should not include any abbreviations, and species names and Latin words should be italicized. Titles that start with "Preliminary studies," "Notes about," and the like should be avoided. Do not use the author's name and date of citation in scientific names.

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List all authors' full name (with no abbreviations). Affiliations should include the original institution names, not their English translations, in the following order: laboratory, department, college or school, institute, university, city, state and country. Include at the bottom of the page the corresponding author information: full address, telephone number, and current e-mail.

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Abstracts are limited to 200 words and should be structured in a single paragraph with no indentation. The abstract should not include references. Acronyms or abbreviations should be written out in full and the abbreviation given in brackets the first time they are used in abstract, for example, indirect fluorescence assay (IFA). The abstract should be informative and present the objectives, a brief description of methods, the main results, and a conclusion.

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Should have a clear and concise justification of the study including its relevance and objectives and should keep the number of citations to a minimum.

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A concise description including core information for the understanding and reproduction of the study. Well-established methods and techniques should be cited and referenced but not described. Statistical analyses should be described at the end of the section.

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Its content should be interpretative and based on the study results only. The discussion can be a single section or it can be presented together with the results and conclusions. It should emphasize the relevance of new findings and new hypotheses clearly supported by the results.

Tables

Tables must be in editable format (e.g., Excel list format) and supplied in separate files. The word "Table" should precede the table title. Tables should be numbered consecutively with Arabic numerals and have a concise and descriptive title placed above them. They should be typed using double spacing and should have horizontal rules separating the header and the last row. The number of tables in the manuscript should be limited to a minimum.

Figures

Figures consist of drawings, photographs, boards, charts, flow charts, and diagrams and should be supplied in TIF, GIF, or JPG format with a minimum resolution of 300 dpi. They should be numbered consecutively with Arabic numerals and the word "Figure" should precede the legend placed below them. List all numbered legends with their symbols and standard icons in a separate file with double spacing. Figures should be limited to a minimum. Digital pictures should be supplied in separate files. A graphic bar scale instead of a numerical one should be used in all illustrations, as it can be adjusted with size reduction.

Conclusions
All conclusions may be presented in the Discussion section or in the Results and the Discussion sections when presented together, at the authors' choice. If this is the case, there is no need for a separate Conclusions section.

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References should be listed alphabetically and then sorted chronologically, if necessary. More than one reference by the same author(s) in the same year must be identified by the letters "a," "b," "c," etc., placed after the year of publication. Titles of journals should be abbreviated according to Index Medicus, http://www2.bg.am.poznan. pl/czasopisma/medicus.php?lang=eng.

Reference to book
Levine JD. Veterinary protozoology. Ames: ISU Press; 1985.

Reference to book chapter
Menzies PI. Abortion in sheep: diagnosis and control. In: Youngquist RS, Threlfall WR. Current therapy in large animal theriogenology. 2nded. Philadelphia: Saunders; 2007. p. 667-680.

Reference to full article
Paim F, Souza AP, Bellato V, Sartor AA. Selective control of Rhipicephalus (Boophilus) microplus in fipronil-treated cattle raised on natural pastures in Lages, State of Santa Catarina, Brazil. Rev Bras Parasitol Vet 2011; 20(1): 13-16.

Reference to thesis or dissertation
Araujo MM. Aspectos ecológicos dos helmintos gastrintestinais de caprinos do município de patos, Paraíba – Brasil[Dissertação]. Rio de Janeiro: Universidade Federal Rural do Rio de Janeiro; 2002.

Reference to internet URLs
Centers for Disease Control and Prevention. Epi Info [online]. 2002 [cited 2003 Jan 10]. Available from: http://www.cdc.gov/epiinfo/ei2002.htm.

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