UNIVERSIDADE FEDERAL DE CAMPINA GRANDE
CENTRO DE SAÚDE E TECNOLOGIA RURAL
UNIDADE ACADÊMICA DE MEDICINA VETERINÁRIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E SAÚDE ANIMAL

AMANDA RAFAELA ALVES MAIA

Toxoplasmose e neosporose em bovinos: revisão sistemática e meta-análise e indicadores epidemiológicos no estado da Paraíba.

Patos – PB

2022

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Tese submetida ao Programa de Pós-Graduação em Ciência e Saúde Animal, da Universidade Federal de Campina Grande, como requisito parcial para obtenção do grau de Doutora em Ciência e Saúde Animal.

Prof. Dr. Sérgio Santos de Azevedo
Orientador

Patos – PB
2022

MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DE CAMPINA GRANDE
POS-GRADUACAO EM CIENCIA E SAUDE ANIMAL
Rua Aprigio Veloso, 882, - Bairro Universitario, Campina Grande/PB, CEP 58429-900

FOLHA DE ASSINATURA PARA TESES E DISSERTAÇÕES

AMANDA RAFAELA ALVES MAIA

TOXOPLASMOSE E NEOSPOROSE EM BOVINOS: REVISÃO SISTEMÁTICA E META-ANÁLISE E INDICADORES EPIDEMIOLÓGICOS NO ESTADO DA PARAÍBA

Tese apresentada ao Programa de Pós-Graduação em Ciência e Saúde Animal como pré-requisito para obtenção do título de Doutor em Ciência e Saúde Animal.

Aprovada em: 22/02/2022

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M217t Maia, Amanda Rafaela Alves.

Toxoplasmose e neosporose em bovinos: revisão sistemática e meta-análise e indicadores epidemiológicos no estado da Paraíba / Amanda Rafaela Alves Maia. - Patos, 2022.

85 f. il. color.

Tese (Doutorado em Ciência e Saúde Animal) - Universidade Federal de Campina Grande, Centro de Saúde e Tecnologia Rural, 2022.

"Orientação: Prof. Dr. Sérgio Santos de Azevedo."

Referências.

1. Epidemiologia. 2 .*Toxoplasma gondii*. 3. *Neospora caninum*. 4. Georreferenciamento. 5. Medidas de Controle. 6. Bovinos. 7. Nordeste – Brasil. I. Azevedo, Sérgio Santos de. II. Título.

CDU 616-036.22:591.2(043)

CATALOGRÁFICA ELABORADA PELA BIBLIOTECÁRIA ITAPUANA SOARES DIAS GONÇALVES CRB-15/93

AGRADECIMENTOS

À Deus, por guiar meus passos, pelo dom da vida, pela saúde, sabedoria e forças para enfrentar todas as batalhas (e mesmo com elas seguir em busca dos meus sonhos). Cada degrau não é em vão! À minha família, meus Pais João e Fátima, irmãos Adele e Emerson, que por toda minha vida sempre me incentivaram em tudo. Acreditando que sou uma das pessoas mais inteligentes do mundo, essa conquista de fato, é NOSSA!

Ao meu orientador, Prof. Dr. Sérgio Santos de Azevedo, por toda paciência, aceitando e driblando todas as dificuldas impostas pela vida, pandemia e orientanda. Obrigada pela oportunidade oferecida, pela atenção, compromisso, ensinamentos e por todos os produtivos momentos que pudemos vivenciar nesse período. Realmente, é muito difícil encontrar orientadores assim, professores de fato, pesquisadores e humanos. O senhor é admirável!

Aos Professores Vinícius Vilela, Thais Feitosa e todos os seus orientandos do IFPB (Sousa-PB), pela disponibilidade de tempo e auxílio na RIFI para *Toxoplasma gondii* no famoso LIDIC. Vocês foram imprescindíveis ao desenvolvimento desse estudo e ao meu crescimento acadêmico e profissional, os dias de luta ficaram mais prazerosos com vocês! Obrigada por todo apoio e compromisso. Ao laboratório de doenças infecciosas dos animais domésticos da UFRPE (Recife-PE), em nome do Professor Rinaldo Mota e Renata Melo pela disponibiladade e compromisso nas análises para *Neospora caninum*, também através da RIFI, muito obrigada.

As minhas amigas "vets mestras" (Ana Isabel A. Santos e Renata Peixoto), que me acompanham desde a graduação, as amigas doutoras Beatriz Braz e Karla Priscila Garrido que tive o privilégio de conhecer na temporada pela UFCG (Patos-PB). Obrigada minhas amigas, pelo incentivo diário e trocas de conhecimentos, foi e é maravilhoso ter vocês para incentivar, lamentar, chorar e crescer internamente! Ao meu namorado, Caio Santos, pelas cobranças e incentivo para o término desse doutorado, para continuarmos caminhando juntos na jornada da vida.

Ao Programa de Pós-Graduação em Ciência e Saúde Animal e aos professores da UFCG, pela oportunidade. A todos que direta ou indiretamente contribuíram para a realização desse trabalho! Muito obrigada!

RESUMO

Os objetivos deste estudo foram determinar a soroprevalência de anticorpos anti-*Toxoplasma gondii* e *Neospora caninum*, assim como os fatores de risco e realizar geospacialização de rebanhos focos, em bovinos do Estado da Paraíba. Além de realizar revisão sistemática com meta-análise da prevalência de *T. gondii* em bovinos. O Estado foi dividido em três grupos amostrais: mesorregião do Sertão, Borborema e mesorregiões da Zona da Mata/Agreste. Foram amostradas, de forma planejada, 1.895 vacas para diagnóstico de *T. gondii* e 1.891 para *N. caninum*, oriundas de 434 propriedades (rebanhos). O diagnóstico sorológico foi realizado através da Reação de Imunofluorescência Indireta (RIFI), técnica considerada padrão ouro para diagnóstico desses agentes. Na infecção por *T. gondii*, 197/434 fazendas investigadas tinham pelo menos uma vaca positiva, obtendo-se prevalência total de 49,0% (IC 95% = 44,3–53,8), 60,3% (IC 95% = 52,1% –67,9%) na região do Sertão, 26,5% (IC 95% = 19,7% –34,6%) na Borborema e 48,3% (IC95% = 9,3% –20,2%) no Agreste / Zona da Mata. A prevalência animal foi 18% (IC 95% = 1,6% –3,3%) no Estado da Paraíba, 18,5% (IC 95% = 0,8% –2,5%) no Sertão, 15,7% (IC 95% = 1,7% –7,4%) na região da Borborema e 18,3% (IC95% = 40,2% –56,5%) no Agreste / Zona da Mata. Os títulos, variaram de 1:64 a 1:1024, sendo que os títulos mais frequentes foram 1:64 (10,81%) e 1:128 (3,7%). Após realizada análise univariável, através do modelo final de regressão logística, os fatores de risco identificados foram: propriedade localizada no Sertão (OR = 3,07), propriedade localizada no Agreste/Zona da Mata (OR = 2,00), compra de animais (OR = 2,68), rebanhos com 34-111 animais (OR = 2,91), rebanhos > 111 animais (OR = 6,97). Na infecção por *N. caninum*, das 1.891 amostras 306 (18,1%, IC 95% = 14,7% –22,1%) foram positivas e das 434 fazendas investigadas 67 (17,8%, IC 95% = 14,3–21,8) tinham pelo menos duas vacas positivas. Nas mesorregiões as prevalências encontradas por rebanho foram 17,8% na região do Sertão, 7,4% (IC 95% = 4,0% –13,2%) na Borborema e 7,5% (IC95% = 4,2% –13,1%) no Agreste / Zona da Mata. E a prevalência por animal foi 18,1% (no Estado da Paraíba, 24,8% (IC 95% = 20,83 –30,0%) no Sertão, 18,0% (IC 95% = 10,4% –29,3%) na região da Borborema e 8,3% (IC95% = 5,5% –12,3%) no Agreste / Zona da Mata. No modelo final de regressão robusta de Poisson, os fatores de risco identificados foram: propriedade localizada no Sertão (RP = 2,37), produção mista (RP = 1,64), rebanhos com 34-111 animais (RP = 35), rebanhos > 111 animais (RP = 6,14). Devido ao impacto à saúde pública, saúde animal e economia, o conhecimento da situação epidemiológica e dos fatores associados a infecção por *T. gondii* e *N. caninum* em bovinos, é imprescindível para o desenvolvimento e implementação de medidas de controle para a toxoplasmose e neosporose bovina. **Palavras-chave: *Toxoplasma gondii*; *Neospora caninum*; Epidemiologia; Georreferenciamento; Controle; Nordeste; Brasil**

ABSTRACT

The objectives of this study were to determine the seroprevalence of anti-*Toxoplasma gondii* and *Neospora caninum*, as well as the risk factors and to carry out geospatialization of focus herds in cattle from the State of Paraíba. In addition to a systematic review with meta-analysis of the prevalence of *T. gondii* in cattle. The state was divided into three sample groups: Sertão Mesoregion, Borborema and Zona da Mata/Agreste mesoregions. We safely sampled 1,895 vacancies for diagnosis of *T. gondii* and 1,891 for *N. caninum*. from 434 herds. Serological diagnosis was performed using the Indirect fluorescent antibody test (IFAT), a technique considered the gold standard for their diagnosis. For *T. gondii* infection, of the 434 farms investigated, 197 had a positive cow, obtaining at least a total prevalence of 49.0% (95% CI = 44.3–53.8% (95% CI = 52.1% –67.9%) in the Sertão region, 26.5% (95% CI = 19.7% –34.6%) in Borborema and 48.3% (95% CI = 9.3% –20.2%) in the Agreste / Zona da Mata. The animal prevalence was 18% (CI 95% = 1.6% –3.3%) in the State of Paraíba, 18.5% (CI 95% = 0.8% – 2 .5%) in the Sertão, 15.7% (95%CI = 1.7% -7.4%) in the Borborema region and 18.3% (95%CI = 40.2% -56.5%) in the Agreste / Zona da Mata. The titles ranged from 1:64 to 1:1024, with the most frequent titles being 1:64 (10.81%) and 1:128 (3.7%). Through the final univariate variable model, the identified risk factors were identified: property located in Sertão (OR = 3), property located in Agreste/Zona da Mata (OR = 2.00), purchased animals (OR = 2, 68), herds with 34-111 animals (OR = 2.91), herds > 11 animals (OR = 6.97). In *N. caninum* infection, of the 1,891 samples, 306 (18.1%, 95% CI = 14.7% -22.1%) were positive and of the 434 farms investigated, 67 (17.8%, 95% CI = 14 .3–21.8) had at least two positive cows. In the mesoregions, the prevalences found per herd were 17.8% in the Sertão region, 7.4% (95% CI = 4.0% -13.2%) in Borborema and 7.5% (95% CI = 4.2 % –13.1%) in the Agreste / Zona da Mata. And the prevalence per animal was 18.1% (in the State of Paraíba, 24.8% (CI 95% = 20.83 –30.0%) in the Sertão, 18.0% (CI 95% = 10.4% –29.3%) in the Borborema region and 8.3% (95%CI = 5.5% –12.3%) in the Agreste / Zona da Mata In the final robust Poisson regression model, the identified risk factors were: property located in Sertão (PR = 2.37), mixed production (PR = 1.64), herds with 34-111 animals (PR = 35), herds > 111 animals (PR = 6.14). impact on public health, animal health and economy, knowledge of the epidemiological situation and associated factors for infection by *T. gondii* and *N. caninum* in cattle is essential for the development and implementation of control measures for bovine toxoplasmosis and neosporosis **Keywords: *Toxoplasma gondii*; *Neospora caninum*; Epidemiology; Georeferencing; Control; Northeast;**

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LISTA DE ABREVIATURAS E SIGLAS

AESA	Agência Executiva de Gestão das Águas do estado da Paraíba
DAT	Direct agglutination test
ELISA	Ensaio imunoenzimático / Enzime-liked Immuno sorbent assay
IFAT	Indirect fluorescent antibody test
IBGE	Instituto Brasileiro de Geografia e Estatística
IC	Índice de confiança
IHA	Indirect haemagglutination test
LAT	Latex agglutination test
MAT	Modified agglutination test
OR	Odds ratio
POA	Produtos de Origem Animal
RIFI	Reação de Imunofluorescência Indireta
RP	Razão de prevalência
SEDAP	Secretaria de Defesa Agropecuária do Estado da Paraíba

SUMÁRIO

INTRODUÇÃO E JUSTIFICATIVA

Toxoplasma gondii e *Neospora caninum* são protozoários coccídios, intracelulares obrigatórios, com ciclo de vida heteroxênico e distribuição mundial. Parasitam seres humanos e outras espécies animais (mamíferos e aves), incluindo os bovinos, podendo causar abortamentos e alterações congênitas. Tem como hospedeiro definitivo para *T. gondii* e *N. caninum* os felídeos e várias espécies de canídeos, respectivamente, os quais eliminam oocistos que contaminam o ambiente, água e alimentos, causando transmissão horizontal em diversos animais, que atuam como hospedeiros intermediários (Wiengcharoen et al.,2011; Dubey et al., 2020).

Até o momento *N. caninum* não apresenta potencial zoonótico, porém tem importância para a saúde animal e economia, visto que, esse parasito é reconhecido como importante causa de distúrbios reprodutivos, abortos e nascimento de crias fracas e debilitadas ou cronicamente afetadas, a depender do momento da gestação em que ocorreu a infecção. Em estudo realizado por Andreotti et al. (2010), foi visto que a presença de novilhas soropositivas para *N. caninum* causou impacto significativo na reprodução, com redução de 21,88% na taxa de extração do rebanho.

A transmissão vertical também deve ser levada em consideração, por promover a manutenção do parasito no rebanho. Em infecção experimental que comparou a transmissão transplacentária de *N. caninum* e *T. gondii* em bovinos, atestou que fêmeas gestantes inoculadas com cepa RH abortaram ou pariram crias congenitamente infectadas (sem sinais clínicos aparentes). Fêmeas gestantes inoculadas com *N. caninum* pariram crias com infecção subclínica (Wiengcharoen et al., 2011).

Já a toxoplasmose é reconhecida por ser uma zoonose, apresentando risco principalmente para mulheres grávidas, imunodeprimidos e portadores de doenças crônicas. Diversos mamíferos podem ser afetados, porém os bovinos geralmente são resistentes à infecção (Albuquerque et al., 2011). As carnes que contenham bradizoítos e seus derivados malcozidos que contenham taquizoítos podem ser consideradas fonte de infecção. Como o que aconteceu em um surto de toxoplasmose associado ao consumo de queijo artesanal fresco produzido com leite de vaca (Da costa et al., 2020). Representando importância para a saúde pública, principalmente à exposição da mulher não imune à infecção por *T. gondii* durante a gestação, podendo ocorrer óbito, má formação ou retardo mental no feto humano (Bariel et al., 1999; Cook et al., 2000; Dubey, 1986; Dubey et al., 2020; Georgi, 2010).

É sabido que a região Nordeste é considerada endêmica para várias doenças infecciosas e parasitárias devido a baixas condições socioeconômicas encontradas na região, e que tal característica também pode favorecer a transmissão dessas enfermidades. Sendo assim, o esclarecimento da situação epidemiológica da toxoplasmose e neosporose alinhado ao seu controle, possui uma importância para a saúde pública, forncendo um panorama geral da dinâmica dessas doenças na região e contribui para a redução de perdas econômicas ligadas aos distúrbios reprodutivos. Considerando a escassez de trabalhos utilizando amostragem planejada em nível de rebanhos e animais, sendo originária de dados isolados, pouco ordenados e não comparáveis, depara-se atualmente com a necessidade de pesquisas sobre o mapeamento destas enfermidades com o propósito de nortear medidas de controle eficazes em cada localidade.

Desta forma, devido à falta de estudos sobre toxoplasmose e neosporose bovina no estado da Paraíba, o presente estudo tem como objetivo determinar a situação epidemiológica de toxoplasmose e neosporose em bovinos no estado da Paraíba, utilizando amostragem planejada, através da determinação da prevalência de anticorpos anti-*T. gondii* e anti-*N. caninum* de propriedades positivas e animais soropositivos, e identificar os possíveis fatores associados, bem como realizar revisão sistemática e meta-análise da soroprevalência da toxoplomase em bovinos.

Esta tese é composta por três artigos. Para o primeiro capítulo, a ser submetido à Revista Brasileira de Parasitologia Veterinária, o objetivo foi elaborar uma revisão sistemática com meta-análise acerca da soroprevalência da toxoplasmose bovina; o segundo, determinar a situação epidemiológica, fatores associados e a geoespacialização da infecção por *T. gondii* em bovinos no estado da Paraíba, Nordeste do Brasil, o terceiro, determinar a situação epidemiológica, os fatores associados e a geoespacialização da infecção por *N. caninum* em bovinos no estado da Paraíba, Nordeste do Brasil, a serem submetidos à Preventive Veterinary Medicine.

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OBJETIVOS

Objetivo Geral

Avaliar o perfil epidemiológico da infecção por *Toxoplasma gondii* e *Neospora caninum* em bovinos no estado da Paraíba, Nordeste do Brasil.

Objetivos Específicos

- Determinar a soroprevalência de anticorpos anti-*Toxoplasma gondii* em bovinos do Estado da Paraíba;
- Determinar a soroprevalência de anticorpos anti-*neospora caninum* em bovinos do Estado da Paraíba;
- Avaliar os fatores associados à infecção por *T. gondii* e *N. caninum* em propriedades rurais de bovinos no Estado da Paraíba;
- Realizar Geospacialização de rebanhos focos para infecção por *T. gondii* e *N. caninum* em bovinos no Estado da Paraíbada;
- Realizar revisão sistemática com meta-análise da prevalência de *Toxoplasma gondii* em bovinos.

CAPÍTULO I:

SOROPREVALÊNCIA DA TOXOPLASMOSE BOVINA: REVISÃO SISTEMÁTICA E META-ANÁLISE

Amanda Rafaela Alves Maia, Clécio Henrique Limeira, Nathanael Natércio da Costa Barnabé, Rafael Rodrigues Soares, Sérgio Santos de Azevedo

Trabalho a ser submetido à Brazilian Jounal of Veterinary Parasitology

(Qualis: A2)

Soroprevalência da toxoplasmose bovina: Revisão sistemática e meta-análise

Bovine toxoplasmosis seroprevalence: Systematic review and meta-analysis

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RESUMO

Objetivou-se com esta pesquisa realizar uma revisão sistemática de literatura com síntese quantitativa (meta-análise) sobre toxoplasmose bovina. Para isso foi realizada uma busca estruturada em quatro bases de dados, o que gerou um total de 675 registros, dos quais 25 atenderam aos critérios estabelecidos e foram considerados na presente revisão sistemática. A soroprevalência combinada por animal foi calculada por meio da meta-análise por subgrupo, de acordo com o continente de realização de cada estudo. Dessa forma, observa-se uma soroprevalência total combinada nos quatro continentes (Ásia, América, Europa e África) representada por 18%, sendo a maior encontrada na América (33%). Para o diagnóstico da toxoplasmose bovina foram utilizados diferentes pontos de corte e métodos de imunoensaio para a detecção de anticorpos, o que dificulta a comparação dos resultados. As técnicas mais utilizadas foram o ensaio imunoenzimático (ELISA) e reação de imunofluorescência indireta (RIFI). Foi observada heterogeneidade nos estudos, o que pode ser explicada devido a grande variabilidade de métodos utilizados para diagnóstico da toxoplasmose em bovinos. Sendo importante a padronização dos métodos de diagnósticos para não gerar resultados conflitantes e valores de prevalência que podem não ser fidedignos à situação local. **Palavras-chave: Bovinos, diagnóstico, *Toxoplasma gondii*, meta-análise, zoonose.**

ABSTRACT

The objective of this research was to carry out a systematic literature review with quantitative synthesis (meta-analysis) on bovine toxoplasmosis. For this, a structured search was carried out in four databases, which generated a total of 675 records, of which 25 met the established criteria and were considered in this systematic review. The combined seroprevalence per animal was calculated using meta-analysis by subgroup, according to the continent in which each study was performed. Thus, there is a combined total seroprevalence in the four continents (Asia, America, Europe and Africa) represented by 18%, the highest being found in America (33%). For the diagnosis of bovine toxoplasmosis, different cut-off points and immunoassay methods were used for the detection of antibodies, which makes it difficult to compare the results. The most used techniques were the enzyme immunoassay (ELISA) and Indirect fluorescent antibody test (IFAT). Heterogeneity was observed in the studies, which can be explained by the great variability of methods used for the diagnosis of toxoplasmosis in cattle. It is important to standardize diagnostic methods so as not to generate conflicting results and prevalence values that may not be reliable in the local situation. ***Keywords:*** **Cattle, diagnosis, *Toxoplasma gondii*, meta-analysis, zoonosis.**

INTRODUCTION

Toxoplasmosis is a zoonosis whose etiological agent is *Toxoplasma gondii*, a coccidian protozoan, obligate intracellular, with a heteroxenic life cycle and worldwide distribution. They infect humans and other animal species (mammals and birds). Having the felids, mainly the domestic cat as definitive hosts.

Horizontal transmission is the main form of introduction of the infection and occurs during the sexual phase of the parasite, when it is eliminated in the feces in the form of an oocyst (which gives it resistance to environmental conditions). food contaminated with oocysts. Contaminated Products of Animal Origin (POA) are the main form of infection for humans; from the point of view of Public Health, the problem occurs when it involves the ingestion of raw or undercooked meat and milk containing bradyzoites and tachyzoites, respectively. Immunosuppressed individuals and pregnant women represent the risk group, and death, malformation, or mental retardation in the human fetus may occur. (Dubey, 1986; Baril et al., 1999; Cook et al., 2000; Georgi, 2010).

Studies also demonstrate the importance of vertical transmission of *T. gondii*, being responsible for maintaining the parasite in the herd, in which Wiengcharoen et al. (2011), attested that pregnant females aborted or gave birth to congenitally infected offspring (with no apparent clinical signs). Although it presents resistance to clinical toxoplasmosis, the bovine species also presents individuals susceptible to the infection (Albuquerque et al., 2011; Dubey & Lindsay, 2006). According to Dubey (1983), *Toxoplasma* can remain encysted in cattle for up to 287 days after infection. Dong et al. (2018) and García-Bocanegra et al. (2013) reported seroprevalence of *T. gondii* between 1.93 in China and 83.33% in Spain per animal; at herd level, 13.08% to 100% (Schoonman et al., 2010; Garcia-Bocanegra et al., 2013).

Due to the variability and the large number of serological studies, it is necessary to determine the combined seroprevalence and to evaluate the heterogeneity of toxoplasmosis in cattle. Therefore, this research aimed to perform a systematic literature review with quantitative synthesis (meta-analysis) on bovine toxoplasmosis.

METHODS

Research design

The research consists of a systematic literature review with meta-analysis of quantitative data available in articles from indexed journals, nationally and internationally. To prepare the study, the recommendations of the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) methodology were observed (Moher et al., 2009).

Eligibility of articles / inclusion and exclusion criteria

We considered eligible full articles and short communications published in indexed journals with relevant information related to the theme (Toxoplasmosis in cattle), and published in the last 15 years (2006 to 2020), disregarding book chapters, technical manuals, congress abstracts and case reports. The selection was based on the methodology of the study and relevance of the information. There was no restriction on the language in which the article was published or the country in which the work was developed.

Suitable productions were retrieved in their entirety for reading, disregarding studies with limited access to information. After reading, studies addressing experimental infection, vertical transmission and serological data from animals sent to slaughter were excluded.

Information sources and search strategy

Considering the pre-established inclusion criteria, the article identification process occurred by searching the Web of Science, PubMed, ScienceDirect and Scopus databases, using combinations of the following terms: "*toxoplasma* OR *toxoplasmosis*" AND "*bovine* OR *cattle* OR *cow*" AND "*prevalence* OR *seroprevalence*". After the search process in each database, the information obtained was converted to "BibTex" format and then imported into Mendeley® software, in which duplicates were excluded, keeping only one of the files. The searches were conducted between January 13 and 31, 2020.

Study selection and data extraction

Two researchers independently performed a selection of the studies by analyzing the title and abstract, and then reading the full text. After full-text evaluation, other studies were excluded for not meeting the eligibility criteria. The divergent cases between the two researchers were resolved by consensus. We chose to perform a meta-analysis to obtain the combined seroprevalence for animals and for herds. The information collected (title; authors; country of origin; year of publication; material collected; diagnostic method; total sample and number of positives) was organized in a Microsoft Excel® spreadsheet to perform the meta-analysis.

Data analysis

The random effects model was applied to the quantitative data to estimate the combined seroprevalence of bovine toxoplasmosis. The presence of heterogeneity among studies was checked by Cochran's Q test, and through Higgins and Thompson's I^2 test the outcome was measured. The presence of publication bias was analyzed by viewing the inverted funnel plot and applying Egger's test. The analyses were performed using the statistical program R (version 3.5.1), RStudio interface (version 1.1.463).

RESULTS

The searches in the four electronic databases returned a total of 675 studies, 135 of which were excluded because they were duplicates, leaving 540 for the screening phase of titles and abstracts. In this phase, another 502 publications that did not meet the pre-established inclusion and exclusion criteria were excluded, electing 38 studies for full reading. After detailed analysis of these, 13 more articles were excluded. Finally, 25 studies met the established criteria and were considered in this systematic review (Figure 1).

The 25 selected articles included research conducted on four continents: Asia (9), America (6), Europe (6) and Africa (4). The countries represented were: Bangladesh, China, Iran, Mongolia, Pakistan and Thailand (Asia); Brazil (America); Spain, Estonia, France, Poland and Serbia (Europe); Algeria, Burkina Faso, Ethiopia and Tanzania (Africa). It is important to emphasize that some countries contributed with a larger number of publications, especially Brazil (6), China (3), Spain (2), and Bangladesh (2). ELISA is the diagnostic method most often used to diagnose bovine toxoplasmosis (11), while DAT was used in only one study in Estonia, and Brazil was the only country in which samples were analyzed by IFAT (5 articles) (Table 1).

Some studies have analyzed the seroprevalence of *T. gondii* in cattle both individually, per animal, and for the herd, where the presence of at least one seroprevalent animal was enough to classify the herd as seropositive. Thus, we chose to perform a meta-analysis to obtain the combined seroprevalence for animals (Figure 2) and another for herds (Figure 3).

The combined seroprevalence per animal was calculated through meta-analysis by subgroup, according to the continent where each study was conducted. Thus, we observed a total combined seroprevalence in the four continents represented by 18% (95% CI = 12 - 27%), being the highest found in America (33%; 95% CI = 18 - 53%), followed by Europe (27%; 95% CI = 8 - 60%), Africa (15%; 95% CI = 7 - 27%) and Asia (10%; 95% CI = 6 - 16%). Meta-analysis of animal-level seroprevalence further revealed the presence of high heterogeneity between studies (p=0, I2=100%), suggesting that variability of primary results occurs within and between continents themselves (Figure 2).

Nine studies also reported seroprevalence by herd, and the result of this meta-analysis showed that 72% (95% CI = 48 - 88%) of the analyzed properties had at least one positive animal. As with the animal-level assessment, herd results showed high heterogeneity among the primary studies (p<0.01; I2=96%), and subgroup meta-analysis was not possible in this case due to the low number of studies that reported herd seroprevalence (n = 9) (Figure 3).

In the search to elucidate and identify the origin of the high heterogeneity among the studies included in the meta-analysis, an evaluation was performed according to the techniques used in the diagnosis of toxoplasmosis in bovines, observing the application of enzyme-linked Immuno sorbent assay (ELISA, n=11), indirect fluorescente antibody test (IFAT, n=5), modified agglutination technique (MAT, n=4), indirect haemagglutination test (IHA, n=2), latex agglutination (LAT, n=2) and direct agglutination test (DAT, n=1), as shown in table 2. The same table summarizes another meta-analysis, which sought to evaluate the seroprevalence of *T. gondii* in cattle according to their aptitude (milk and beef). For this, we considered only studies that made it clear in the methodology whether the collection of material for diagnosis occurred in dairy or beef cattle, excluding those that did not specify and those that evaluated animals of mixed aptitude. Thus, the seroprevalence of toxoplasmosis in beef cattle was 19.54% (95% CI = 10.63 - 33.15%), while 11.13% (95% CI = 3.26 - 31.76%) for dairy cattle, with high heterogeneity in the two groups (I2 = 99.1% and 99.8%, respectively).

Figure 4 shows the funnel plot generated from the meta-analysis data, in which the dots represent the included studies, the x-axis corresponds to the seroprevalence (with Logit transformation) and the y-axis the inverse of the standard error. Thus, a symmetrical distribution of the points on both sides of the dotted line (combined seroprevalence) is visually observed. Egger's test was also not significant (p=0.332), indicating no publication bias (Figure 4).

DISCUSSION

According to the number of articles included in this paper, it can be inferred that the topic is widely researched due to the zoonotic nature of toxoplasmosis.

Separating the articles by continent (Asia, America, Europe and Africa), Asia was represented mainly by China, with 3 papers, which currently represents a great technological power with much investment in research; America had Brazil (6) as the only representative of this continent, important results for Public Health, since beef is the second most consumed meat in Brazil, second only to chicken due to the price difference, as on the part of livestock (largest commercial cattle herd, with about 213. 523 million (IBGE, 2019); in Europe (6 works), a reference continent in development, with great investment in research, even though beef is not the most consumed, the European consumer is among the most demanding, having a strict health surveillance of POA; Africa: Low capacity for investment in research (due to the economic condition of countries), most nations have cattle herd little expressive and is unevenly distributed across the continent, where approximately 36 million head of cattle are kept for agricultural fertilizer, plowing, breeding and milk (FAO, 2006). Although Ethiopia has the largest cattle herd in Africa, it does not have favorable climatic and vegetation conditions for the expansion of cattle farming.

For the diagnosis of bovine toxoplasmosis, different cutoff points and immunoassay methods were used for the detection of antibodies, making it difficult to compare the results. The most used techniques were ELISA (n = 11) and IFAT (n = 5), the former is cost-effective, easy to perform and highly sensitive, the latter is considered the gold standard because of its high specificity (Remington et al., 2004). The other methods, MAT (n = 4), IHA (n = 2), LAT (n = 2) and DAT (n = 1) were used in fewer papers.

Regarding the heterogeneity analysis using the diagnostic techniques as a subgroup, all showed high heterogeneity, and this evaluation is not possible with the DAT, since only one study used this direct agglutination test as a form of diagnosis. According to the author, the test has a sensitivity of 96.22% and specificity of 98.80% mentioned by the DAT manufacturer for human samples. However, we do not know if these values can be extrapolated when used in other animal species. Also, according to the work, the DAT detects only one class of antibodies, immunoglobulin G, not detecting these antibodies in some stages of the animal's life (young animals, lactation) (Jokelainen et al., 2017).

In one of the studies analyzed here (Oliveira et al., 2018), the authors used two diagnostic methods in the same animals, ELISA and IFAT. Therefore, only the ELISA results were considered for the calculation of the meta-analysis, to avoid duplicating the number of positive animals and, consequently, overestimating the combined seroprevalence. Some methods, such as MAT, are cheaper, easier to perform, and do not require special equipment. However, indirect ELISA and IFAT are more sensitive and specific than agglutination tests (Garcia et al., 2006).

The estimated seroprevalence in cattle is 9% (Dubey, 2004), in this study the total seroprevalence (four continents) was twice as high, 18%, and among the four continents, America had the highest prevalence (33%), and all the studies were conducted in Brazil, which can be explained by the environmental conditions of this continent, where prevalence is higher in warm climates, favoring the sporulation and viability of oocysts and consequent contamination of cattle (Dubey and Beattie, 1998).

Through the funnel plot and application of Egger's test, no publication biases were observed, despite the different techniques used to diagnose toxoplasmosis in cattle. This absence of bias can be explained by the fact that the authors mostly used representative samples and sample size calculations to determine the number of animals to compose the study.

CONCLUSIONS

Through this systematic review with meta-analysis, it can be concluded that the combined seroprevalence in the four continents (Asia, America, Europe, and Africa) is high, and there is a great variability in the methods used to diagnose toxoplasmosis in cattle. Therefore, it is important to standardize the diagnostic methods in order to avoid generating conflicting results and prevalence values that may not be reliable for the local situation.

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APPENDIX

List of figures

Figure 1. Flowchart of the search process, selection and inclusion of studies in the systematic review.

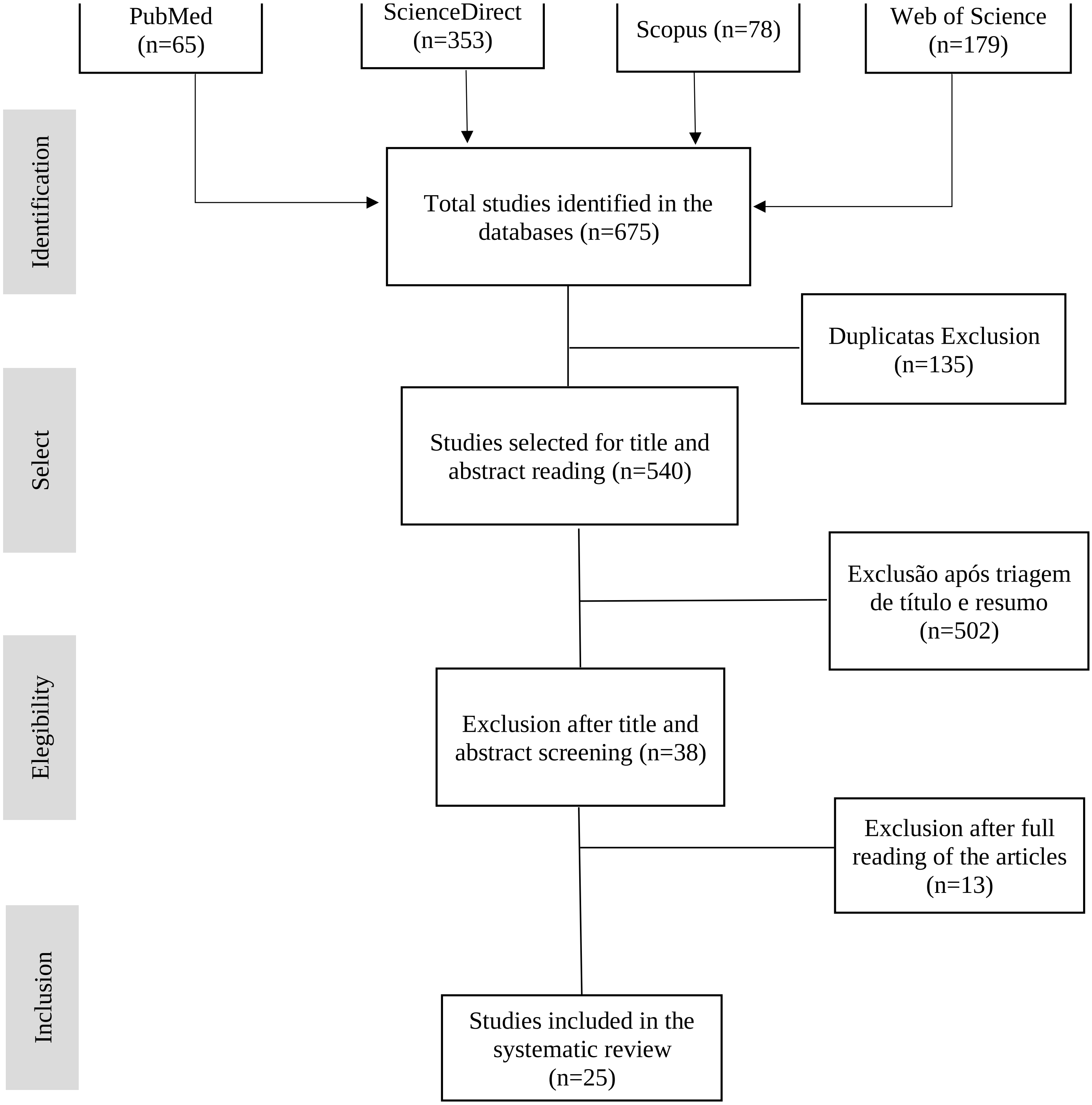

Figure 2. Forest plot representing the meta-analysis with random effects model for seroprevalence of *T. gondii* infection in cattle, per animal, divided into subgroups according to the continent where the studies were conducted, from 2006 to 2020.

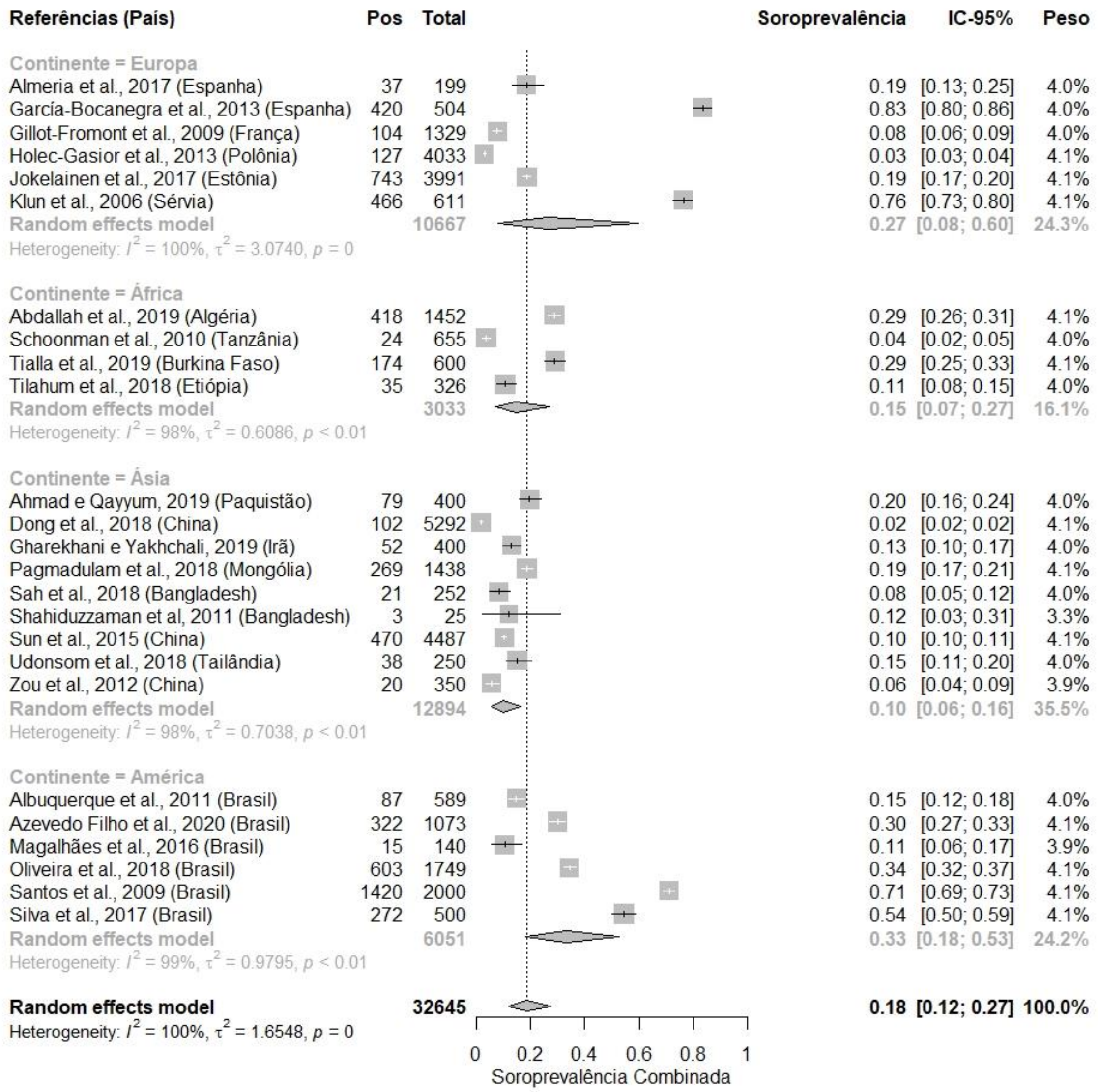

Figure 3. Forest plot representing the meta-analysis with random effects model for seroprevalence of *T. gondii* infection in cattle, by herd, from 2006 to 2020.

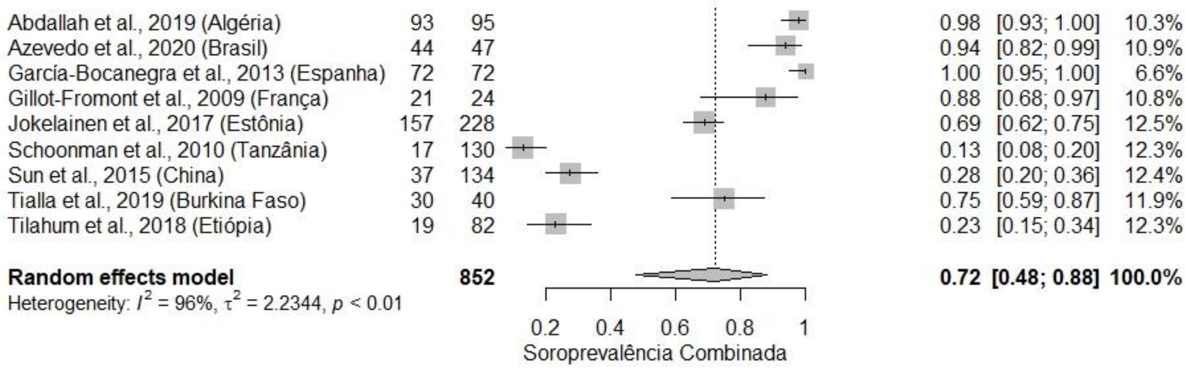

Figure 4. Funnel plot demonstrating the 25 studies included in the meta-analysis, distributed according to seroprevalence (Logit transformed) and the standard error of each study, for analysis of the presence of publication bias, from 2006 to 2020.

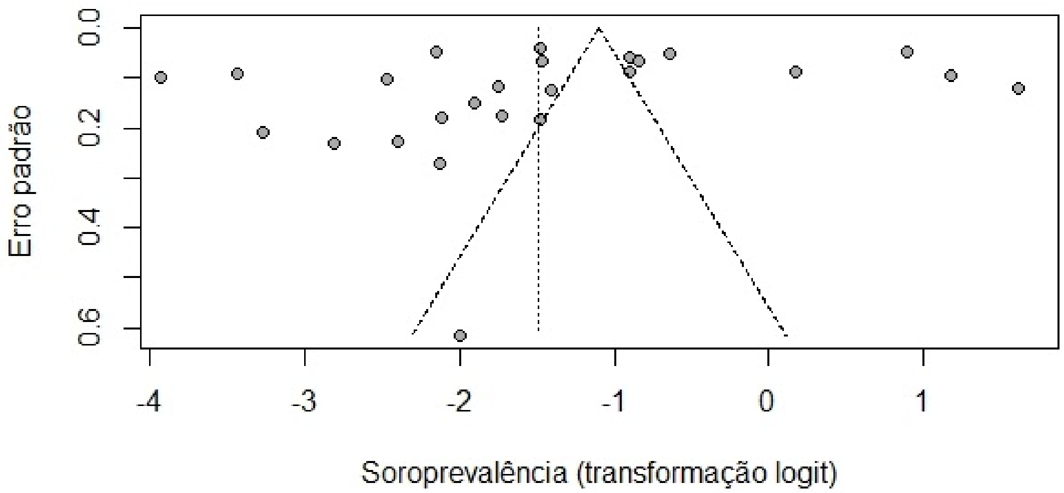

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Table 1. Main characteristics of seroprevalence studies of *T. gondii* infection in cattle included in the meta-analysis, from 2006 to 2020.

References	Continents	Country	Analysis level	Total	Positives	Seroprevalence	Type of production	Diagnostic methods
Klun et al., 2006	Europe	Serbia	Animal	611	466	76.27	Mixed	MAT
Gillot-Fromont et al., 2009	Europe	France	Animal	1329	104	7.83	Beef	MAT
Gillot-Fromont et al., 2009	Europe	France	Herd	24	21	87.50	Beef	MAT
Santos et al., 2009	America	Brazil	Animal	2000	1420	71.00	Milk	IFAT
Schoonman et al., 2010	Africa	Tanzania	Animal	655	24	3.66	Milk	LAT
Schoonman et al., 2010	Africa	Tanzania	Herd	130	17	13.08	Milk	LAT
Albuquerque et al., 2011	America	Brazil	Animal	589	87	14.77	Milk	IFAT
Shahiduzzaman et al., 2011	Asia	Bangladesh	Animal	25	3	12.00	Mixed	LAT
Zhou et al., 2012	Asia	China	Animal	350	20	5.71	Milk	IHA

García-Bocanegra et al., 2013	Europe	Spain	Animal	504	420	83.33	Mixed	ELISA
García-Bocanegra et al., 2013	Europe	Spain	Herd	72	72	100.00	Mixed	ELISA
Holec-Gasior et al., 2013	Europe	Poland	Animal	4033	127	3.15	Mixed	ELISA
Sun et al., 2015	Ásia	China	Animal	2340	293	12.52	Beef	IHA
Sun et al., 2015	Ásia	China	Animal	2147	177	8.24	Milk	IHA
Sun et al., 2015	Ásia	China	Animal	134	37	27.61	Mixed	IHA
Magalhães et at., 2016	América	Brazil	Animal	140	15	10.71	Mixed	IFAT
Almeria et al., 2017	Europe	Spain	Animal	199	37	18.59	Mixed	MAT
Jokelainen et al., 2017	Europe	Estonia	Animal	1506	232	15.41	Beef	DAT
Jokelainen et al., 2017	Europe	Estonia	Herd	84	54	64.29	Beef	DAT
Jokelainen et al., 2017	Europe	Estonia	Animal	1659	308	18.57	Milk	DAT
Jokelainen et al., 2017	Europe	Estonia	Herd	92	70	76.09	Milk	DAT

Jokelainen et al., 2017	Europe	Estonia	Animal	826	203	24.58	Mixed	DAT
Jokelainen et al., 2017	Europe	Estonia	Herd	52	33	63.46	Mixed	DAT
Silva et al., 2017	América	Brazil	Animal	500	272	54.40	Beef	IFAT
Dong et al., 2018	Ásia	China	Animal	5292	102	1.93	Milk	MAT
Oliveira et al., 2018	America	Brazil	Animal	1749	603	34.48	Mixed	ELISA
Oliveira et al., 2018	America	Brazil	Animal	1749	772	44.14	Mixed	IFAT
Pagmadulam et al., 2018	Asia	Mongolia	Animal	1438	269	18.71	Mixed	ELISA
Sah et al., 2018	Asia	Bangladesh	Animal	252	21	8.33	Mixed	ELISA
Tilahum et al., 2018	Africa	Ethiopia	Animal	326	35	10.74	Mixed	ELISA
Tilahum et al., 2018	Africa	Ethiopia	Herd	82	19	23.17	Mixed	ELISA
Udonsom et al., 2018	Asia	Thailand	Animal	250	38	15.20	Beef	ELISA, LAT, IFAT
Abdallah et al., 2019	Africa	Algeria	Animal	1452	418	28.79	Mixed	ELISA

Abdallah et al., 2019	Africa	Algeria	Herd	95	93	97.89	Mixed	ELISA
Ahmad e Qayyum, 2019	Asia	Pakistan	Animal	400	79	19.75	Mixed	ELISA
Gharekhani e Yakhchali, 2019	Asia	Iran	Animal	400	52	13.00	Milk	ELISA
Tialla et al., 2019	Africa	Burkina Faso	Animal	600	174	29.00	Milk	ELISA
Tialla et al., 2019	Africa	Burkina Faso	Herd	40	30	75.00	Mixed	ELISA
Azevedo Filho et al., 2020	America	Brazil	Animal	1073	322	30.01	Beef	IFAT
Azevedo Filho et al., 2020	America	Brazil	Herd	47	44	93.62	Beef	IFAT

ELISA – Enzyme-Linked Immuno Sorbent Assay; DAT – Direct agglutination test; IHA – Indirect haemagglutination test; LAT – Latex agglutination test; MAT – Modified agglutination test; IFAT – Indirect fluorescent antibody test;

Table 2. Summary of the meta-analysis of seroprevalence of *T. gondii* infection in cattle, subdivided according to the technique used for diagnosis and the fitness of the animals, from 2006 to 2020.

	Nº of studies	Sample size	Positives	Seroprevalence (IC 95%)	Heterogeneity		
Método de Diagnóstico					Q	p-Value	I^2 (%)
ELISA	11	11404	2236	20.05% (11.58 – 32.45%)	1418.95	<0.01	99%
IFAT	5	4302	2116	32.86% (14.79 – 57.98%)	781.6	<0.01	99.50%
MAT	4	7431	709	15.76% (16.40 – 67.71%)	1462.11	<0.01	99.80%
IHA	2	4837	490	8.05% (4.41 -14.24%)	7.81	<0.01	87.20%
LAT	2	680	27	5.94% (1.82 – 17.69%)	3.86	0.05	74.10%
DAT	1	3991	743	18.62% (17.44 – 19.85%)		Not applied	
Combined effect	25	32645	6321	18.50% (12.01 – 27.41%)	5584.42	0	99.60%
Type of production							
Milk	8	13092	2190	11.13% (3.26 – 31.76%)	2975.32	0	99.80%
Beef	6	6998	1261	19.54% (10.63 – 33.15%)	583.27	<0,01	99.1
Combined effect	14	20090	3451	14.28% (7.35 - 26%)	3619.83	0	99.60%

ELISA – Enzyme-Linked Immuno Sorbent Assay; DAT – Direct agglutination test; IHA – Indirect haemagglutination test; LAT – Latex agglutination test; MAT – Modified agglutination test; IFAT – Indirect fluorescent antibody test;

CAPITULO II

SOROREVALÊNCIA E FATORES ASSOCIADOS À INFECÇÃO POR TOXOPLASMA GONDII EM BOVINOS, NO ESTADO DA PARAÍBA, NORDESTE DO BRASIL

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Trabalho a ser submetido à Preventive Veterinary Medicine

(Qualis A2)

Soroprevalência e fatores associados à infecção por *Toxoplasma gondii* em bovinos no estado da Paraíba, Nordeste do Brasil

Herd-level prevalence and associated factors for *Toxoplasma gondii* infection in cattle, in the the state of Paraíba, Northeastern Brazil

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RESUMO

Toxoplasmose é uma zoonose parasitária, causada pelo protozoário *Toxoplasma gondii*, sendo capaz de infectar a maioria das espécies homeotérmicas, incluindo humanos. As pessoas são infectadas com o parasito mais comumente por via oral, por meio do consumo de carne crua ou malpassada contendo cistos teciduais, ou vegetais e frutas contaminados com oocistos eliminados por felídeos. Devido à carne bovina ser uma das carnes mais consumidas em todo o mundo, a importância desse alimento na cadeia epidemiológica da toxoplasmose não pode ser ignorada. Desta forma, esse estudo teve por objetivo determinar a soroprevalência de anticorpos anti-*T. gondi* em fêmeas bovinas do Estado da Paraíba, Nordeste do Brasil, bem como identificar os fatores de risco associados à prevalência da infecção e realizar geospacialização de propriedades focos. Foram amostradas, de forma planejada, 1.895 vacas oriundas de 434 fazendas de todo o estado da Paraíba, os soros obtidos foram submetidos à Reação de Imunofluorescência Indireta (RIFI). Das 434 fazendas investigadas, 197 tinham pelo menos uma vaca positiva, obtendo-se prevalência total de 49,0% (IC 95% = 44,3–53,8), sendo 60,3% (IC 95% = 52,1% –67,9%) na região do Sertão, 26,5% (IC 95% = 19,7% –34,6%) na Borborema e 48,3% (IC95% = 9,3% –20,2%) no Agreste / Zona da Mata. A prevalência animal foi 18% (IC 95% = 1,6% –3,3%) no Estado da Paraíba, 18,5% (IC 95% = 0,8% –2,5%) no Sertão, 15,7% (IC 95% = 1,7% –7,4%) na região da Borborema e 18,3% (IC95% = 40,2% –56,5%) no Agreste / Zona da Mata. Os títulos variaram de 1:64 a 1:1024, sendo que os

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títulos mais frequentes foram 1:64 (10,81%; 205/320) e 1:128 (3,7%; 70/320). Após realizada análise univariável, através do modelo final de regressão logística, os fatores de risco identificados foram: propriedade localizada no Sertão (OR = 3,07), propriedade localizada no Agreste/Zona da Mata (OR = 2,00), compra de animais (OR = 2,68), rebanhos com 34-111 animais (OR = 2,91), rebanhos > 111 animais (OR = 6,97). O modelo final teve um bom ajuste (teste de Hosmer e Lemeshow: qui-quadrado = 1,498; P = 0,983). De acordo com os resultados, pode-se observar que o parasito tem ampla distribuição em todo o estado, e sua manutenção no rebanho pode ser favorecida devido à possibilidade de transmissão vertical (transplacentária). Servindo de alerta à população, onde não se pode ignorar o risco de ingestão de carne bovina (crua ou malpassada), com cistos teciduais e consequente infecção por *T. gondii*. Desta forma, estudos sobre a soroprevalência de *T. gondii* em bovinos e diversos outros animais, é de suma importância para a saúde pública. **Palavras-chave: Zoonose, Nordeste brasileiro, coccídio, epidemiologia**

ABSTRACT

Toxoplasmosis is a parasitic zoonosis, caused by the protozoan *Toxoplasma gondii*, and is capable of infecting most homeothermic species, including humans. People are infected with the parasite most commonly by the oral route, through consumption of raw or undercooked meat containing tissue cysts, or vegetables and fruits contaminated with oocysts shed by cats. Because beef is one of the most consumed meats worldwide, the importance of this food in the epidemiological chain of toxoplasmosis cannot be ignored. Thus, this study aimed to determine the seroprevalence of anti-T. gondi antibodies in female cattle in the state of Paraíba, northeastern Brazil, as well as to identify the risk factors associated with the prevalence of infection and to perform a geospatialization of focal properties. A total of 1,895 cows from 434 farms in the state of Paraíba were sampled in a planned sampling, and the sera obtained were submitted to the Indirect Immunofluorescence Reaction (IIDR). Of the 434 farms investigated, 197 had at least one positive cow, obtaining a total prevalence of 49.0% (95% CI = 44.3-53.8), with 60.3% (95% CI = 52.1% -67.9%) in the Sertão region, 26.5% (95% CI = 19.7% -34.6%) in Borborema and 48.3% (95% CI = 9.3% -20.2%) in Agreste / Zona da Mata. The animal prevalence was 18% (95% CI = 1.6% -3.3%) in Paraíba State, 18.5% (95% CI = 0.8% -2.5%) in Sertão, 15.7% (95% CI = 1.7% -7.4%) in Borborema region and 18.3% (95%

CI = 40.2% -56.5%) in Agreste / Zona da Mata. Titers ranged from 1:64 to 1:1024, and the most frequent titers were 1:64 (10.81%; 205/320) and 1:128 (3.7%; 70/320). After univariable analysis, through the final logistic regression model, the risk factors identified were: property located in the Sertão (OR = 3.07), property located in the Agreste/Zona da Mata (OR = 2.00), purchase of animals (OR = 2.68), herds with 34-111 animals (OR = 2.91), herds > 111 animals (OR = 6.97). The final model had a good fit (Hosmer and Lemeshow test: chi-square = 1.498; P = 0.983). According to the results, it can be observed that the parasite has a wide distribution throughout the state, and its maintenance in the herd may be favored due to the possibility of vertical transmission (transplacental). Serving as a warning to the population, where the risk of ingesting bovine meat (raw or undercooked), with tissue cysts and consequent infection by *T. gondii* cannot be ignored. Thus, studies on the seroprevalence of *T. gondii* in cattle and several other animals are of utmost importance for public health. **Keywords: Zoonosis, Northeast Brazil, coccidia, epidemiology**

INTRODUCTION

Toxoplasmosis is a parasitic zoonosis, caused by the obligate intracellular protozoan *Toxoplasma gondii*, which is capable of infecting most homeothermic species, including humans, by forming tissue cysts. The infection is generally asymptomatic and does not cause clinical disease in several animal species, but in some, it causes acute life-threatening illness (OIE, 2018). Carnivores are infected with the parasite, most commonly orally (more than 80% of cases), through consumption of raw or undercooked meat containing tissue cysts, or vegetables and fruits contaminated with oocysts shed by felids (Hill and Dubey, 2013; Jones and Dubey, 2012; Tenter, et al. 2000).

Felids are important in the life cycle of *T. gondii* because they are definitive hosts and, therefore, the only ones that can contaminate the environment with oocysts, which after sporulating, become infective, spreading through soil, water and vegetation, and because they are resistant they can survive in humid environments for several months (Dubey et al., 2004; Dubey, 2002). The risk factors associated with *T. gondii* infection in animals is similar to that in humans, and depends on the type of geographical region, the sanitary conditions and management of the farms, and the animal's diet (origin of the water and food offered). The most common source of infection in animals is through the ingestion of sporulated oocysts, either through food or water. Hay, straw and grain, both a source of food and/or waste, often become contaminated with cat feces containing oocysts and are the primary source of infection for many farm animals (Ferra et al., 2020).

In veterinary medicine, toxoplasmosis represents a serious problem and is considered a major cause of reproductive losses in sheep, goats and pigs (Ferra et al., 2020). Although it is considered a poor host for *T. gondii*, natural and experimental infections in cattle have been reported (Dubey, 2010; Costa et al., 2011). Vertical transmission should also be considered, since in experimental infection, inoculated pregnant females aborted or gave birth to congenitally infected offspring (without apparent clinical signs), favoring the maintenance of the agent in the herd (Wiengcharoen et al., 2011).

Due to the resistance to the development of toxoplasmosis symptoms in cattle and the difficulty in isolating this parasite from tissues of infected cattle, the ability and role of the protozoan in beef and its transmission to humans has been questioned (Dubey & Jones, 2008; Tenter et al., 2000). However, *T. gondii* has been isolated from bovine tissues and unpasteurized milk (Dubey, 1986) and outbreaks such as the one that occurred in 2006 in São Paulo, associated with the consumption of raw beef (steak tartar) demonstrate an important source of infection for toxoplasmosis in humans (Eduardo et al., 2007).

The national cattle herd has reached 218.2 million heads and the data from the Brazilian Institute of Geography and Statistics report that by the third quarter of 2021, 20,614,976 million cattle were slaughtered, taking into account establishments under federal, state or municipal health inspection, in which 40,420 of these cattle correspond to the state of Paraíba (IBGE, 2021), which shows the importance of this food. Due to the isolation of this protozoan in bovine tissues, the importance of this infection in the epidemiological chain of toxoplasmosis cannot be neglected (Gomes et al., 2020).

Studies on bovine toxoplasmosis are of utmost importance due to the reproductive and economic losses and the zoonotic potential. Therefore, the objective of this study was to determine the seroprevalence of anti-Toxoplasma gondi antibodies in bovine females in the State of Paraíba, Northeastern Brazil, through planned sampling, as well as to identify the risk factors associated with infection and to perform geospatialization of focal properties.

MATERIAL AND METHODS

Characterization of the study area

The study was carried out in establishments in Paraíba, located in the Northeast region of Brazil, characterized by a warm climate throughout the year. The stratification was performed according to the operational capacity of the Secretariat for Agricultural Defense of the State of Paraíba (SEDAP) based on the areas of operation of its regional offices to ensure that the agency could perform the field work. It was divided into three sample groups: sampling stratum 1 (meso-region of Sertão), sampling stratum 2 (meso-region of Borborema) and sampling stratum 3 (meso-regions of Zona da Mata and Agreste) (Figure 1). The maps were made using the GIS software QGIS.

Sampling

The samples used in this study were obtained from a bovine brucellosis study in the State of Paraíba conducted by the National Program for the Control and Eradication of Brucellosis and Tuberculosis, and the sampling design was adjusted for bovine toxoplasmosis. For each sampling stratum, the prevalence of herds infected with bovine toxoplasmosis and the prevalence of seropositive animals were estimated by two-stage sample survey. In the first stage, a predetermined number of herds (primary sampling units) were randomly selected; in the second stage, a predetermined number of cows aged ≥ 24 months were randomly selected (secondary sampling units).

On farms with more than one herd, the cattle herd with the greatest economic importance was chosen as the target of the study; the animals in the selected herd were subjected to the same type of management system as the other herds, i.e., they had similar conditions to the other herds. The selection of the primary sampling units was random (random draw) and was based on the SEDAP farm registers. If a selected herd could not be visited, the herd was replaced by another nearby herd with the same production characteristics. The number of herds selected per sampling stratum was determined using the formula for simple random sampling proposed by Thrusfield (2007). The parameters adopted for the calculation were as follows: confidence level of 95%, estimated intra-herd prevalence 71% (Santos et al., 2009) and 5% error. In addition, the operational and financial capacity of SEDAP was taken into account in determining the sample size of the sampling stratum.

For the secondary units, we estimated the minimum number of animals to be examined within each herd, in order to allow their classification as positive herds. For this, the concept of aggregate sensitivity and specificity was used (Dohoo et al., 2003). For the calculations, we adopted 100% for the sensitivity and specificity of the test protocol (Sunanta et al., 2009), Indirect Immunofluorescence Reaction (IIDR), and 71% for the estimated intra-herd prevalence (Santos et al., 2009). 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 were ≥ 90%. Therefore, 10 animals were sampled in herds with up to 99 cows older than 24 months; 15 animals were sampled in herds with 100 or more cows older than 24 months; and all animals were sampled in those with up to 10 cows older than 24 months. The selection of cows within herds was systematic.

The target condition was a seropositive animal in an infected herd. The herd-level case definition was based on population size (cows aged ≥ 24 months), number of females sampled, an apparent intra-herd prevalence of 71.0% (Santos et al., 2009), and the sensitivity and specificity of IFT, with the goal of obtaining herd sensitivity and specificity ≥90%. After further simulations with the Herdacc software, a herd was considered positive for toxoplasmosis if it had at least one seropositive animal.

Field activites

The field activities included blood collection, application of an epidemiological questionnaire, and sending the samples to the laboratory. SEDAP veterinarians and agricultural and livestock technicians were involved in the field work. Blood samples (10 mL volume) were collected from September 2012 to January 2013, from cows aged ≥ 24 months by puncture of the jugular vein with disposable needle and vacuum tube with 15 mL capacity (without anticoagulant).

Indirect fluorescente antibody test (IFAT)

Serological analyses were performed in the Laboratory of Immunology and Infectious Diseases (LIDIC) of the Adílio Santos Azevedo Veterinary Hospital (ASA) of the Federal Institute of Education, Science and Technology of Paraíba (IFPB), Sousa-PB. For detection of anti-*T. gondii* antibodies, blood serum samples were submitted to IFAT, considering the dilution of 1:64 as cutoff point (Santos et al., 2009). Tachyzoites of *T.*

gondii strain ME-49, maintained in mice, were used as antigen. Positive and negative control sera of bovine origin were included in each slide. The conjugate used in the reactions was anti-bovine IgG (whole molecule with FITC, produced in rabbits, Sigma/F-7887), at a dilution of 1:400 in PBS 7.2 solution containing 10.0% Evans Blue. Reactions were considered positive when tachyzoites showed full peripheral fluorescence. Reactive serum samples were titrated in serial dilutions until the highest positive dilution was obtained in the IFAT.

Calculation of prevalence and risk factors

A herd was considered positive for toxoplasmosis if it included from one positive animal in herds of up to 29 females. EpiInfo 6.04 software was used to calculate apparent prevalences and their confidence intervals (Dean et al., 1996). Stratified random sampling was used to calculate herd-level prevalence in Paraíba State (Thrusfield, 2007). Required parameters were as follows: (a) herd condition (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 herd-level prevalence per sampling stratum employed a simple random sample design using the following parameters: (a) number of positive herds and (b) number of herds sampled in the stratum. The sampling design for the calculation of animal-level prevalence in Paraíba State used a two-stage stratified conglomerate sampling and a two-stage conglomerate sampling in each stratum (Thrusfield, 2007), where each herd was considered a conglomerate. The following parameters were used: (a) animal status (seropositive or seronegative), (b) sample 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 expression above, the first term refers to the statistical weight of each animal in the prevalence calculation at the animal level within the stratum.

Pre-structured questionnaires were applied to assess the risk factors associated with *T. gondii* infection, the variables included in the final analysis model were: region, production type, breeding system, insemination, herd size, presence of cats, presence of wild animals, abortion, animal purchase, animal sale, animal slaughter, pasture rental, shared pasture, shared water source, presence of flooded pastures, use of maternity paddocks, raw milk consumption, veterinary care, and property type.

The variables were organized for presentation in increasing or decreasing order as to the scale of risk. When necessary, these variables were recategorized. The lowest risk category was considered the basis of comparison for the other categories. An initial exploratory data analysis (univariable) was performed to select the variables with $P \leq 0.2$ by the chi-square test or Fisher's exact test, when necessary; The final model fit was verified with the Hosmer and Lemeshow test and collinearity between the independent variables was verified by correlation analysis; for variables with strong collinearity (correlation coefficient> 0.9), one of the two variables was excluded from the multiple analysis according to biological plausibility (Dohoo et al., 1996).

The check for confounding variables was assessed by monitoring changes in model parameters when adding new variables. If substantial changes (i.e. greater than 20%) were observed in the regression coefficients, this was considered an indication of confounding. The independent or explanatory variables considered in the final model were those with statistical significance <0.05. Calculations were performed in SPSS software version 20.0.

RESULTS

The census data and the sample studied in each sampling stratum are presented in Table 1. In total, 1,895 animals were sampled in 434 farms. Herd- and animal-level prevalences are presented in Tables 2 and 3, respectively; in addition, the geographic distribution of positive and negative herds is shown in figure 1. Of the 434 farms investigated, 197 had at least one positive cow, and a prevalence of 49.0% (95% CI = 44.3-53.8) was observed, 60.3% (95% CI = 52.1% -67.9%) in the Sertão region, 26.5% (95% CI = 19.7% -34.6%) in Borborema, and 48.3% (95% CI = 9.3% -20.2%) in Agreste / Zona da Mata. The prevalence at animal level was 18% (95% CI = 1.6% -3.3%) in

Paraíba State, 18.5% (95% CI = 0.8% -2.5%) in Sertão, 15.7% (95% CI = 1.7% -7.4%) in Borborema region and 18.3% (95% CI = 40.2% -56.5%) in Agreste / Zona da Mata. The titers of anti-*T. gondii* antibodies (table 4) ranged from 1:64 to 1:1024, with the most frequent titers being 1:64 (10.81%; 205/320) and 1:128 (3.7%; 70/320).

The results of the univariable analysis for the risk factors are presented in Table 5. The variables selected ($P \leq 0.2$) for the multiple analysis were the following: region, type of production, breeding system, insemination, herd size, presence of dog, presence of cat, presence of wild animals, abortion, purchase of animals, sale of animals, slaughter of animals, renting of pastures, sharing of pastures, sharing of water source, presence of flooded pastures, use of maternity paddock, consumption of raw milk, veterinary care, and type of property. In the final logistic regression model (Table 6), the risk factors identified were: property located in the Sertão (OR = 3.07), property located in Agreste/Zona da Mata (OR = 2.00), mixed production (OR = 2.68), herds with 34-111 animals (OR = 2.91), herds > 111 animals (OR = 6.97). The final model had a good fit (Hosmer and Lemeshow test: chi-square = 1.498; P = 0.983).

DISCUSSION

In this study, a comprehensive epidemiological survey was performed with planned sampling to detect anti-*T. gondii* antibodies in cattle. Only samples from female bovines were used because there is a pre-existing serum bank, females are animals that remain longer in the herd and can provide a more accurate profile of infection, and are animals that contribute to the maintenance of the agent circulating in herds, due to the possibility of vertical transmission.

The study was carried out in all regions of the state of Paraíba, conferring relevant impact for this state. The presence of anti-*T. gondii* antibodies in 18% (320/1,895) of cattle (15.3%-21.1%, 95% CI) demonstrates that the prevalence is close to those observed in other states in Brazil, such as 11.8% in Bahia (Spagnol et al., 2009), 16.6% in Pernambuco (Guerra et al., 2014), 18% and 17.4% in São Paulo (Costa et al., 2011; Santos et al., 2013).

The map of *T. gondii* infection in cattle herds in Paraíba shows that these are distributed in all sample strata (Figure 1). With a prevalence of 49% (44.3%-53.8%, 95% CI), that is, almost half of the herds have anti-*T. gondii* antibodies, due to the parasite's capacity of dissemination and adaptability (Tenter et al., 2010), we hypothesize environmental contamination by the parasite. The occurrence of infection due to *T. gondii* in the Brazilian cattle herd is variable with frequencies from 1.0% (Gondim et al., 1999) to 89.1% (Santin et al., 2017), however studies should be compared with caution due to the use of different diagnostic techniques with different cutoff bridges.

In Brazil, studies on the frequency of anti- *T. gondii* antibodies in cattle have been based mainly on IFAT (Dubey et al., 2012), in a review conducted by (Gomes et al., 2020) of the 35 studies conducted in Brazil, 24 (68.5%) used IFAT to make the diagnosis, among which 22 (91.6%) established 64 as the cutoff point, as in this study. The most frequent antibody titer obtained from this study was 64, corresponding to 10.81% (205/320), followed by 1:128 (3.7%; 70/320), results similar to those obtained by Gomes et al. (2020). Dubey and Thulliez (1993) support that because of resistance to toxoplasmosis, antibody titers lower than 1,024, frequent in the species, are indicative of chronic infection, suggesting the presence of tissue cysts.

Although the role of beef in the transmission of the parasite to humans is not known for sure, beef is often eaten undercooked and may pose a risk to the population. Cattle can be readily infected with *T. gondii*, but are considered poor hosts because they develop a more effective immune response to *T. gondii* infection than other animals (Esteban-Redondo & Innes, 1997; Munday et al., 1979). However, *T. gondii* has been isolated from bovine tissues and unpasteurized milk (Dubey, 1986), indicating that meat and milk can be a source of *T. gondii* infections. The ingestion of beef of questionable provenance and that has not undergone prior inspection increases the risk of *T. gondii* infection to the consumer (Millar et al., 2008). A worrisome factor, due to the informality of slaughterhouses and food markets in our country, especially to Paraíba where there is no federal inspection for bovines (Maia, 2017), clandestine slaughter corresponds to an important part of the national market.

T. gondii infection is often more common in areas with warm, humid climates and lower altitudes (Dubey, 2010), characteristics of the entire state of Paraíba. The average temperatures of the Northeast region are between 26º and 28ºC, with low annual variability, the state of Paraíba due to its location within the Equatorial belt, is subjected to the incidence of high solar radiation with a large number of hours of insolation. Such condition determines a warm climate and average annual temperature of 26ºC, also with little intra-annual variation and spatial distribution of temperature dependent on the relief (AESA, 2016). In this study, the Sertão mesoregion (OR=3.07) followed by the

Agreste/Zona da Mata region (OR=2.00) showed a higher chance of having *T. gondii* infection in cattle.

It is known that *T. gondii* infection in herbivores is more prevalent in humid areas, which favor sporulation conditions and maintenance of oocyst viability in vegetation (Dubey, 2010). Brejo Paraibano, a microregion that belongs to Agreste, has characteristics with the presence of Atlantic Forest fragments, high rainfall, and a wide variety of fauna, which may favor the viability of oocysts in this region.

Multivariate analysis showed that a higher frequency of seropositive animals for anti-*T. gondii* antibodies was associated with the purchase of animals, herds with 34 to 111 cattle, and more than 111 cattle. Breeding in the Northeast, is mostly extensive; in this breeding system, cattle live in close proximity to other species of domestic animals, in addition to wild animals. This practice allows animals to come into contact with oocysts, the main source of infection for ruminants, and which are released into the vegetation by domestic and wild felines (Ahmad et al., 2015).

Animal purchase is a commonly found risk factor for several bovine diseases in Brazil, such as toxoplasmosis itself (Gomes et al., 2021) in the Brazilian cerrado, leptospirosis (Hashimoto et al., 2012) and neosporosis (Gindri et al., 2018) in Rio Grande do Sul; in the Brazilian Northeast diseases such as brucellosis (Silva et al., 2009), neosporosis (Silva et al., 2008), bovine viral diarrhea (Fernandes et al., 2016) and cysticercosis (Maia et al., 2017) have also been found. The producer often purchases animals without knowing the origin, and some diseases are not previously diagnosed due to the high cost and difficult accessibility of diagnostic tests.

According to the results, it can be observed that the parasite has a wide distribution throughout the state, and its maintenance in the herd may be favored due to the possibility of vertical transmission (transplacental). Serving as a warning to the population, where the risk of ingesting bovine meat (raw or undercooked), with tissue cysts and consequent infection by *T. gondii* cannot be ignored. Thus, studies on the seroprevalence of *T. gondii* in cattle and several other animals are of utmost importance for public health.

Conflict of interest declaration

The authors declare that there is no conflict of interest.

Acknowledgmente

This study was funded by the National Council for Scientific and Technological Development (CNPq), under codes 471571 / 2011-3, 302131 / 2012-4 and 476596/2013 -0.

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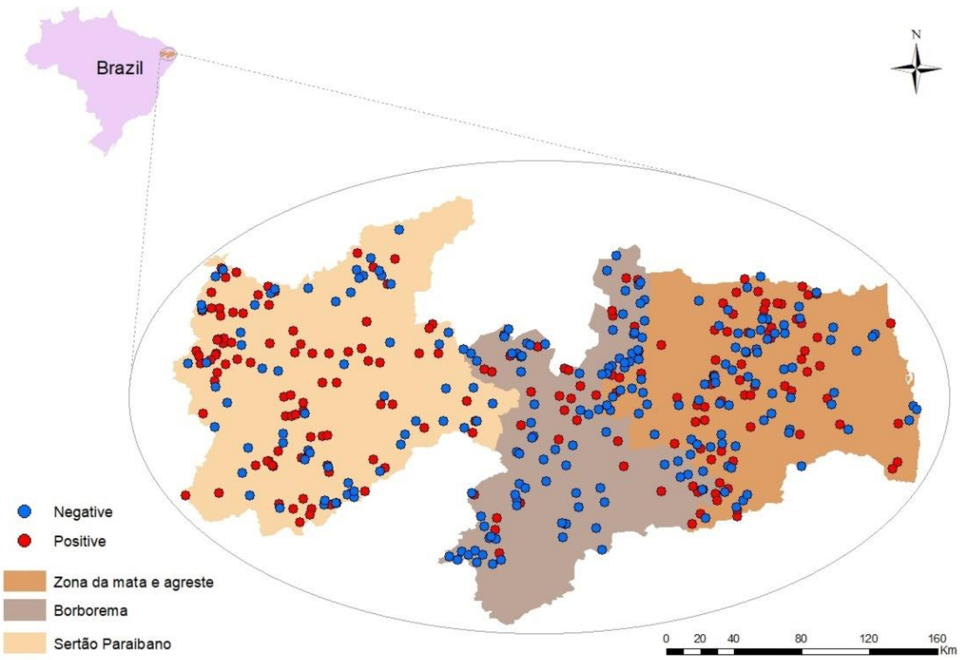
APPENDIX

List of figure

Figure 1. Division of Paraíba state into three sample strata (Sertão, Borborema, and Agreste/Zona da Mata) and distribution of positive and negative herds for *Toxoplasma gondii* infection.



List of table

Table 1

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

Sampling stratum	No. of herds		No. of cows ≥ 24 months of age	
	Total	Sampled	Total	Amostrados
Sertão	24,356	151	288,764	854
Borborema	11,603	136	83,428	356
Agreste/Zona da Mata	18,398	147	192,320	685
State of Paraíba	54,357	434	564,512	1,895

Table 2

Herd-level prevalence of *Toxoplasma gondii* infection in the State of Paraíba, Northeastern Brazil, according to sampling stratum.

Sampling stratum	No. of herds		Prevalence (%)	95% CI
	Tested	Positive		
Sertão	151	91	60.3	[52.1-67.9]
Borborema	136	36	26.5	19.7-34.6]
Agreste/Zona da Mata	147	70	48.3	40.2-56.5]
State of Paraiba	**434**	**197**	**49.0**	**[44.3-53.8]**

Table 3

Animal-level prevalence of *Toxoplasma gondii* infection in the State of Paraiba, Northeastern Brazil, according to sampling stratum.

Sampling stratum	Animals (cows)		Prevalence (%)	95% CI
	Tested	Positive		
Sertão	854	158	18.5	[15.2-22.5]
Borborema	356	47	15.7	[7.6-29.5]
Agreste/Zona da Mata	685	115	18.3	[14.0-23.4]
State of Paraíba	**1,895**	**320**	**18.0**	**[5.3-21.1]**

Table 4

Titration of anti-*Toxoplasma gondii* antibodies by Indirect fluorescente antibody test (IFAT) in cows in the state of Paraíba.

Sampling stratum	Animals		Positive Antibody titers				
	Tested	**Positive**	**64**	**128**	**256**	**512**	**1024**
Sertão	854	158	96	47	13	1	1
Borborema	356	47	31	4	8	2	2
Agreste/Zona da Mata	685	115	78	19	10	6	2
Estado da Paraíba	**1,895**	**320**	**205**	**70**	**31**	**9**	**5**
%		**16.9%**	**10.81%**	**3.7%**	**1.63 %**	**0.47%**	**0.26%**

Table 5

Univariable analysis for factors associated with the herd-level prevalence of *Toxoplasma gondii* infection in cattle, in the State of Paraiba, Northeastern Brazil.

Variables	Categories	No. of herds sampled	No. of positive herds (%)	*P*
Sampling stratum*	Sertão	151	91 (60.3)	
	Borborema	136	36 (26.5)	
	Agreste/Zona da Mata	147	71 (48.3)	< 0.001
Type of production	Beef	59	21 (35.6)	
	Milk	123	57 (46.3)	
	Mixed	252	120 (47.6)	0.244
Management system*	Intensive	29	7 (24.1)	
	Semi-intensive	257	111 (43.2)	
	Extensive	148	80 (54.1)	0.006
Insemination	Not use	432	197 (45.6)	
	Use only insemination	2	1 (50.0)	0.705
Herd size*	Up to 33 animals	218	58 (26.6)	
	34 – 111 animals	108	59 (54.6)	
	> 111 animals	108	81 (75.0)	< 0.001
Presence of cats	No	255	121 (47.5)	
	Yes	179	77 (43.0)	0.208
Presence of wild animals*	No	264	126 (47.7)	
	Yes	170	72 (42.4)	0.159
Abort*	No	398	178 (44.7)	
	Yes	36	20 (55.6)	0.141
Animals purchasing*	No	391	171 (43.7)	
	Yes	43	27 (62.8)	0.013
Animal sale	No	317	142 (44.8)	
	Yes	117	56 (47.9)	0.322
Local of animal slaughter	Not slaughter	196	86 (43.9)	
	In slaughterhouses	154	74 (48.1)	
	In establishment not inspection	81	35 (43.2)	
	In own farm	3	3 (100.0)	0.224
Rental of pastures	No	341	154 (42.5)	
	Yes	93	44 (47.3)	0.400

Sharing of pastures*	No	370	165 (44.6)	
	Yes	64	33 (51.6)	0.185
Sharing of water sources*	No	371	163 (43.9)	
	Yes	63	35 (55.6)	0.058
Presence of flooded pastures	No	288	134 (46.5)	
	Yes	146	64 (43.8)	0.334
Use of maternity pens*	No	322	138 (42.9)	
	Yes	112	60 (53.6)	0.032
Raw milk consumption	No	366	166 (45.4)	
	Yes	68	32 (47.1)	0.449
Veterinary assistance*	No	364	156 (42.9)	
	Yes	70	42 (60.0)	0.006
Property type	Rural	218	58 (26.6)	
	Indian vilage	108	59 (54.6)	
	Rural settlement	108	81 (75.0)	0.000

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

Table 6

Factors associated with the prevalence of herds positive for *T. gondii* infection.

Factor associated	Logistic regression coefficiente	Standard error	Wald	Odds ratio	(95% CI)	*P*-valor
Property located in the Sertão	1.122	0.278	16.222	3.07	(1.78 – 5.30)	< 0.001
Property located in the Agreste/Zona da Mata	0.692	0.276	6.276	2.00	(1.16 – 3.44)	0.012
Animal purchase	0.984	0.372	6.995	2.68	(1.29 – 5.55)	0.008
Herds of 34 – 111 animais	1.067	0.257	17.286	2.91	(1.76 – 4.81)	< 0.001
Herds of > 111 animais	1.942	0.279	48.429	6.97	(4.04 – 12.05)	< 0.001

CAPÍTULO III:

SOROPREVALÊNCIA E FATORES ASSOCIADOS A INFECÇÃO POR NEOSPORA CANINUM NO ESTADO DA PARAÍBA, NORDESTE DO BRASIL

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Trabalho a ser submetido à Preventive Veterinary Medicine
(Qualis A2)

Soroprevalência e fatores associados à infecção por *Neospora caninum* em bovinos, no estado da Paraíba, Nordeste do Brasil

Herd-level prevalence and associated factors for *Neospora caninum* infection in cattle in the State of Paraíba, Northeastern Brazil

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RESUMO

Objetivou-se com este estudo determinar a soroprevalência de anticorpos anti-*Neospora caninum* em fêmeas bovinas do Estado da Paraíba, Nordeste do Brasil, assim como identificar os fatores associados à soroprevalência de neosporose em propriedades rurais de bovinos no Estado da Paraíba, além de realizar geospacialização de propriedades focos para a doença. O estado foi divido em três mesorregiões, Sertão, Borborema e Zona da mata/Agreste. Um total de 1.891 amostras foram analisadas, oriundas de 434 fazendas, através de diagnóstico sorológico, a reação de imunofluorescência indireta (RIFI), considerada padrão ouro para diagnóstico de neosporose. Destas amostras, 306 (18,1%, IC 95% = 14,7% –22,1%) foram positivas e, das 434 fazendas investigadas, 67 (17,8%, IC 95% = 14,3–21,8) tinham pelo menos duas vacas positivas. Nas mesorregiões as prevalências encontradas por rebanho foram 17,8% na região do Sertão, 7,4% (IC 95% = 4,0% –13,2%) na Borborema e 7,5% (IC95% = 4,2% –13,1%) no Agreste / Zona da Mata. E a prevalência por animal foi 18,1% no Estado da Paraíba, 24,8% (IC 95% = 20,83 – 30,0%) no Sertão, 18,0% (IC 95% = 10,4% –29,3%) na região da Borborema e 8,3% (IC95% = 5,5% –12,3%) no Agreste / Zona da Mata. No modelo final de regressão robusta de Poisson, os fatores associados à infecção por *N. caninum* identificados foram: propriedade localizada no Sertão (RP = 2,37), produção mista (RP = 1,64), rebanhos com 34-111 animais (RP = 35), rebanhos > 111 animais (RP = 6,14). O modelo final da regressão de Poisson teve bom ajuste (Pearson Chi-Square value:335,940; degress of

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freedom- df:425; value/df: 0,790). A associação, aumento de prevalência e aumento do tamanho do rebanho, possivelmente se dá devido a medidas higiênicas deficientes e práticas inadequadas de eliminação de resíduos, aumentando a probabilidade de mais cães nas proximidades da fazenda. O conhecimento da situação epidemiológica e dos fatores associados a rebanhos adquirirem infecção por *N. caninum* é importante para o desenvolvimento e implementação de medidas de controle para a neosporose bovina. **Palavras-chave: Epidemiologia, perda reprodutiva, bovinos, Nordeste.**

ABSTRACT

The objective of this study was to determine the seroprevalence of anti-*Neospora caninum* antibodies in female bovine animals in the State of Paraíba, Northeastern Brazil, as well as to identify the factors associated with the seroprevalence of neosporosis in rural properties of cattle in the State of Paraíba, and to geospatialize the properties that are foci of the disease. The state was divided into three mesoregions, Sertão, Borborema and Zona da Mata/Agreste. A total of 1,891 samples were analyzed, from 434 farms, through serological diagnosis, the inndirect fluorescent antibody test (IFAT), considered the gold standard for diagnosing neosporosis. Of these samples, 306 (18.1%, 95% CI = 14.7% - 22.1%) were positive and, of the 434 farms investigated, 67 (17.8%, 95% CI = 14.3-21.8) had at least two positive cows. In the mesoregions, the prevalences found per herd were 17.8% in the Sertão region, 7.4% (95% CI = 4.0% -13.2%) in Borborema and 7.5% (95% CI = 4.2% -13.1%) in Agreste / Zona da Mata. And the prevalence per animal was 18.1% in Paraíba State, 24.8% (95% CI = 20.83 -30.0%) in Sertão, 18.0% (95% CI = 10.4% - 29.3%) in Borborema region and 8.3% (95% CI = 5.5% -12.3%) in Agreste / Zona da Mata. In the final robust Poisson regression model, the factors associated with N. caninum infection identified were: property located in Sertão (RP = 2.37), mixed production (RP = 1.64), herds with 34-111 animals (RP = 35), herds > 111 animals (RP = 6.14). The final Poisson regression model had good fit (Pearson Chi-Square value:335.940; degress of freedom- df:425; value/df: 0.790). The association, increased prevalence and increased herd size, is possibly due to poor hygienic measures and inadequate waste disposal practices, increasing the likelihood of more dogs in the vicinity of the farm. Knowledge of the epidemiological situation and factors associated with herds acquiring *N. caninum* infection is important for the development and implementation of control measures for bovine neosporosis. **Key words: Epidemiology, reproductive loss, cattle, Northeast.**

INTRODUCTION

Neospora caninum is a coccidial protozoan, microscopic parasite with worldwide distribution, many domestic animals can be infected, dogs, the definitive hosts and cattle, sheep, goats, buffaloes, horses, chickens and wild and captive animals, intermediate hosts. Since 1990, *N. caninum* has emerged as one of the most common causes of reproductive disorders and abortion in cattle worldwide, including in Brazil. In a study conducted in the Pantanal region (Brazil), the reproduction of *N. caninum* seropositive primiparous cows had an impact of 44% compared to seronegative heifers, demonstrating the impact of this agent in the herd (Andreotti et al., 2010).

Neosporosis abortion also occurs in sheep, goats, buffalo, and camelids, although they may be less susceptible than cattle, which has a major economic impact on cattle farming (Cerqueira-Cézar et al., 2017). Worldwide, the estimated average losses due to abortions caused by *N. caninum* have been estimated at over $1,298.3 million. In Brazil, losses to dairy farmers have been estimated at a national level of $51.3 million/year. In the Brazilian beef industry, losses totaled $101.0 million, ranging from $63.6 million to $111.7 million (Reichel et al., 2013).

Epidemiological studies to estimate the prevalence of *N. caninum* infection in cattle herds and its association with the occurrence of abortions in different parts of the world, have been carried out by means of antibody testing, being considered a method, mainly, of screening and tracking of the disease on the property (Dubey, 2003; Silva et al., 2003). Serological evaluation can be performed by different techniques, with differences in specificity, sensitivity and practicality (Guido et al., 2016).

The indirect fluorescent antibody test (IFAT) detects fluorescent antibodies indicative of protozoan infection and is considered the gold standard for the serological diagnosis of neosporosis (Anderson et al., 2000; Paré et al., 1995), together with the enzyme-linked immunosorbent assay (ELISA) represent the two most widely used techniques in serological research in Brazil as diagnostic screening (Faria et al., 2007; Guido et al., 2016).

The objective of this study was to determine the seroprevalence of anti-*Neospora caninum* antibodies in female bovines in the State of Paraíba, Northeastern Brazil, as well as to identify the factors associated with the seroprevalence of neosporosis in rural cattle farms in the State of Paraíba, and to geospatialize the focal properties for the disease.

MATERIAL AND METHODS

Characterization of the study area and Division of the State of Paraíba

The State of Paraíba, located in the Northeast region of Brazil, is characterized by a warm climate throughout the year. The state is geographically subdivided into the following four main regions, based mainly on the type of vegetation and precipitation: (i) Zona da Mata (Atlantic Forest), has a humid climate belt that follows the coast, in which the forest that once existed was substituted by sugarcane. It is the most populated and urbanized part of the state; (ii) Agreste, a transition region between the Zona da Mata and the traditional sertão region, the climate is semi-arid, although it rains more than in Borborema and the sertão; (iii) Borborema, located on the plateau of Borborema, between the sertão and the agreste, is the region where rainfall is scarcer, especially in Borborema where the phenomenon of droughts occurs and (iv) Sertão is the region of the caatinga vegetation, with a less dry climate than Borborema, of temporary rivers, of extensive cattle raising and cotton cultivation, the main product grown in the region. Due to the operational capacity of the Secretariat of Agricultural Defense of the State of Paraíba (SEDAP) based on the areas of operation of its regional offices to ensure that the agency could perform the field work. 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).

Sampling, target condition and case definition at the herd level

The samples used in this study were obtained from a bovine brucellosis study in the State of Paraíba conducted by the National Program for the Control and Eradication of Brucellosis and Tuberculosis, and the sampling design was adjusted for bovine neosporosis. For each sampling stratum, the prevalence of herds infected with *N. caninum* and the prevalence of seropositive animals were estimated by two-stage sample survey. In the first stage, a predetermined number of herds (primary sampling units) were randomly selected; in the second stage, a predetermined number of cows aged ≥ 24 months were randomly selected (secondary sampling units).

On farms with more than one herd, the cattle herd with the greatest economic importance was chosen as the target of the study; the animals in the selected herd were subjected to the same type of management system as the other herds, therefore., they presented similar conditions to the other herds. The selection of the primary sampling units was random (random draw), and was based on the SEDAP farm registers. If a selected herd could not be visited, the herd was replaced by another nearby herd with the same production characteristics. The number of herds selected per sampling stratum was determined using the formula for simple random sampling proposed by Thrusfield (2007). The parameters adopted for the calculation were as follows: confidence level of 95%, estimated prevalence at herd level of 1.1% (Santos et al., 2013) and error of 5%. In addition, the operational and financial capacity of SEDAP was taken into account in determining the sample size of the sampling stratum.

For the secondary units, we estimated the minimum number of animals to be examined within each herd, in order to allow their classification as positive herds. For this, the concept of aggregate sensitivity and specificity was used (Dohoo et al., 2003). For the calculations, the following values were adopted: 100% and 95.87% (Campero et al., 2015) for the sensitivity and specificity, respectively, of the test protocol (IFAT) and 53.5% (Benetti et al., 2009) for the estimated intra-herd 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 were ≥ 90%. Therefore, 10 animals were sampled in herds with up to 99 cows older than 24 months; 15 animals were sampled in herds with 100 or more cows older than 24 months; and all animals were sampled in those with up to 10 cows older than 24 months. The selection of cows within herds was systematic.

The target condition was a seropositive animal in an infected herd. The herd-level case definition was based on population size (cows aged ≥ 24 months), number of females sampled, an apparent intra-herd prevalence of 53.5% (Benetti et al., 2009), and the sensitivity and specificity of the indirect immunofluorescence reaction (IFT), aiming for herd sensitivity and specificity ≥90%. After further simulations with the Herdacc software, a herd was considered positive for neosporosis if it included at least two positive animals.

Field activities (blood and data collection)

Field activities included collecting blood, providing an epidemiological questionnaire, and sending the samples to the laboratory. SEDAP veterinarians and agricultural and livestock technicians were involved in the field work. Blood samples (10 mL volume) were collected from September 2012 to January 2013, from cows aged ≥ 24 months by puncture of the jugular vein with a disposable needle and a vacuum tube with a capacity of 15 mL (without anticoagulant). After drainage, serum was transferred to microtubes and frozen. A structured questionnaire with closed-ended questions was designed to obtain information on (a) herd identification and location; (b) management practices; (c) herd structure and composition; and (d) presence of other domestic and wild species on the farm. Questionnaires were administered to the owner or person in charge of the herd by the lead author or a SEDAP veterinarian at the time of the blood collection visit.

Serological diagnosis

The analyses were performed in the Laboratory of Infectious Diseases of Domestic Animals at the Universidade Federal Rural de Pernambuco, Recife-PE. For detection of IgG anti-*N. caninum* antibodies, the Indirect fluorescente atibody test (IFAT) technique was used, using as antigen suspensions of tachyzoites of *N. caninum*, NC-1 strain (107 tachyzoites/mL). The sera of the animals were diluted in phosphate buffered saline solution (PBS), considering 1:200 as the cut-off point (Dubey and Lindsay, 1996), in addition, a fluorescein-conjugated secondary anti-bovine IgG antibody (Sigma-Aldrich, Saint Louis, MO, USA) was used at a dilution of 1:800. Samples were considered positive if they showed total peripheral fluorescence of tachyzoites in 50% or more of the tachyzoites present in each well (Dubey and Lindsay. 1996; Paré et al., 1995). In this process, reference sera were used, identified as positive and negative controls on each slide.

Calculation of prevalence and associated factors

A herd was considered positive for neosporosis if it included at least two positive animals in herds of up to 29 females. EpiInfo 6.04 software was used to calculate apparent prevalences and respective confidence intervals (Dean et al., 1996). Stratified random sampling was used to calculate herd-level prevalence in Paraíba State (Thrusfield, 2007). The parameters required were as follows: (a) herd condition (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):

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

O cálculo da prevalência em nível de rebanho por estrato amostral empregou o desenho amostral de uma amostra aleatória simples, utilizando os seguintes parâmetros: (a) número de rebanhos positivos e (b) número de rebanhos amostrados no estrato. O delineamento amostral para o cálculo da prevalência em nível animal no Estado da Paraíba utilizou uma amostragem por conglomerados estratificada em dois estágios e uma amostragem por conglomerados em dois estágios em cada estrato (Thrusfield, 2007), onde cada rebanho foi considerado um conglomerado. Foram utilizados os seguintes parâmetros: (a) condição do animal (soropositivo ou soronegativo), (b) estrato amostral ao qual o animal pertencia, (c) código do rebanho (para identificar cada cluster) e (d) peso estatístico. O peso estatístico foi calculado com a seguinte fórmula (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 expression above, the first term refers to the statistical weight of each animal in the prevalence calculation at the animal level within the stratum. Apparent and actual prevalences were calculated according to Noordhuizen et al. (1997). To identify factors associated with seropositivity, a univariable analysis was initially conducted, each independent variable was subjected to an association analysis with a dependent variable (seropositivity on IFAT serology). Variables with $P \leq 0.2$ on the chi-square test were selected for multivariable analysis using robust Poisson regression.

A colinearidade entre variáveis independentes foi verificada por uma análise de correlação. Para as variáveis com forte colinearidade (coeficiente de correlação> 0,9), uma das duas variáveis foi excluída na análise de concordância múltipla com plausibilidade biológica. Para avaliar o ajuste do modelo de regressão, o teste qui-quadrado de Pearson foi usado. O nível de significância adotado no a análise múltipla foi de 5%, e as análises foram conduzidas em R (R CORE TEAM, 2019) usando o “Epi” (Carstensen et al., 2019), “Sandwich” (Berger et al., 2017), e pacotes “lmtest” (Zeileis e Hothorn, 2002).

RESULTS

The geographical distribution of positive and negative herds can be seen in Figure 1. Census data and the sample studied in each sampling stratum are presented in Table 1. The results of this study reveal that of the 1,891 samples analyzed, 306 (18.1%) were positive (Table 3) and 1,581 were negative. Of the 434 farms investigated, 67 (17.8%) had at least two positive cows (Table 2) and prevalence of 17.8% (95% CI = 14.3-21.8), 30.5% (95% CI = 23.6% -38.3%) in the Sertão region, 7.4% (95% CI = 4.0% -13.2%) in Borborema and 7.5% (95% CI = 4.2% -13.1%) in Agreste / Zona da Mata. The prevalence at animal level (Table 3) was 18.1% (95% CI = 14.7% -22.1%) in the state of Paraíba, 24.8% (95% CI = 20.83 -30.0%) in Sertão, 18.0% (95% CI = 10.4% -29.3%) in Borborema region and 8.3% (95% CI = 5.5% -12.3%) in Agreste / Zona da Mata.

The results of the univariable analysis for the factors associated with *N. caninum* infection are presented in Table 4. The variables selected ($P \leq 0.2$) for multiple analysis were the following: region, type of production, breeding system, insemination, herd size, presence of dogs, presence of cats, presence of wild animals, abortion, purchase of animals, sale of animals, place of animal slaughter, pasture rental, shared pasture, shared water source, presence of flooded pastures, use of maternity paddocks, consumption of raw milk, veterinary care, and type of property.

In the final robust Poisson regression model (Table 5), the factors associated with *N. caninum* infection identified were: farm located in the Sertão (PR = 2.37), mixed production (PR = 1.64), herds with 34-111 animals (PR = 35), herds > 111 animals (PR = 6.14). The final Poisson regression model had a good fit (Pearson Chi-Square value:335.940; degress of freedom- df:425; value/df: 0.790).

DISCUSSION

Neospora caninum is one of the main causes of abortion in cows in many countries, and is considered a significant problem in animal production. A study conducted in Paraná, Brazil, with herd samples collected over a nine year period, demonstrated that the proportion of abortions was 20% for seropositive cows with specific antibodies to *N. caninum* and 8% for seronegative cows, indicating that cows with a history of abortion that seroconverted to this agent had approximately 76% risk of abortion (Locatelli-Dittrich et al., 2001).

In this research, IFAT was used, a serological method considered the gold standard for neosporosis diagnosis. Sensitivity (Se) and specificity (Sp) corrections of serological tests were performed before herd classification, and the case definition at herd level was based on population size (cows aged ≥24 months), number of females sampled, apparent intra-herd prevalence, Se and Sp of the diagnostic test used, which is important to minimize misclassification bias.

The percentage of positive animals and risk factors are quite varied, the results, however, are not comparable due to the use of different serological assays, different cut-off points employed and antigens used, study design and sample size (Dubey et al., 2007; Cerqueira-Cézar et al., 2017).

Some studies that used IFAT as methodology had the following prevalence results: 91.2% (510/559) in Minas Gerais (Guedes et al., 2008); 53.5% (499/932) in Mato Grosso (Benetti et al., 2009); 9.1% (43/392) in Mato grosso do Sul (Mello et al., 2008); 14.7% (22/150) in Rio de Janeiro (Ragozo and Paula, 2003). Some Northeastern states, such as Bahia presented 14% (63/447) (Gondim, et al. 1999), Pernambuco 19.6% (31/158) (Ramos et al., 2016), both used as cutoff point 1:200. Similar results to that found in the present study, which obtained a prevalence of 18.1% for animals per herd in Paraíba. It is believed that the prevalence at the animal level may be even higher in Paraíba, since only cows aged ≥ 24 months were used for this study. However, within-herd prevalence ranged from 14.1% to 21.8% (median 17.8%).

Through the epidemiological questionnaires, it was possible to identify important conditions that possibly participate in the dissemination of infection in herds. The Sertão region was identified as a risk factor for prevalence at the herd level in this study, as well as mixed production (beef and milk), herds with 34 to 111 animals, and herds with more than 111 animals. Neosporosis in cattle is responsible for several cases of abortion in herds, Reichel et al. (2013) described the average specific risk of abortion due to *N. caninum* being higher in dairy cattle at 14.3% (range: 0.6-39.4%) than in beef cattle at 9.1%. The size of the herd and its aptitude are also considered important, as it has been observed a tendency to increase the prevalence of *N. caninum* in herds with larger numbers of animals and in herds with dairy aptitude (Asmare et al., 2013; Moore et al., 2009) possibly due to the higher concentration of females, these being the most studied herds, restricting the information on beef herds.

In this study, larger herds, with 34 to 111 animals and with more than 111 animals, presented as a factor associated with *N. caninum* infection with prevalence ratio 3.50 (95% CI = 1.64% - 7.48%) and 6.14 (CI = 2.96% - 12.74%), respectively. This association, increased prevalence and increased herd size possibly occur due to poor hygienic measures and in maintenance of proper waste disposal practices, increasing the likelihood of more dogs in the vicinity of the farm and/or a tendency to raise replacement heifers from infected dams.

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Thus, knowledge of the epidemiological situation and the factors associated with herds acquiring *N. caninum* infection is important for the development and implementation of control measures for bovine neosporosis (Dubey et al., 2007), in order to decrease the economic, production and animal health impact.

Conflict of interest declaration

The authors declare that there is no conflict of interest.

Acknowledgmente

This study was funded by the National Council for Scientific and Technological Development (CNPq), under codes 471571 / 2011-3, 302131 / 2012-4 and 476596/2013 -0.

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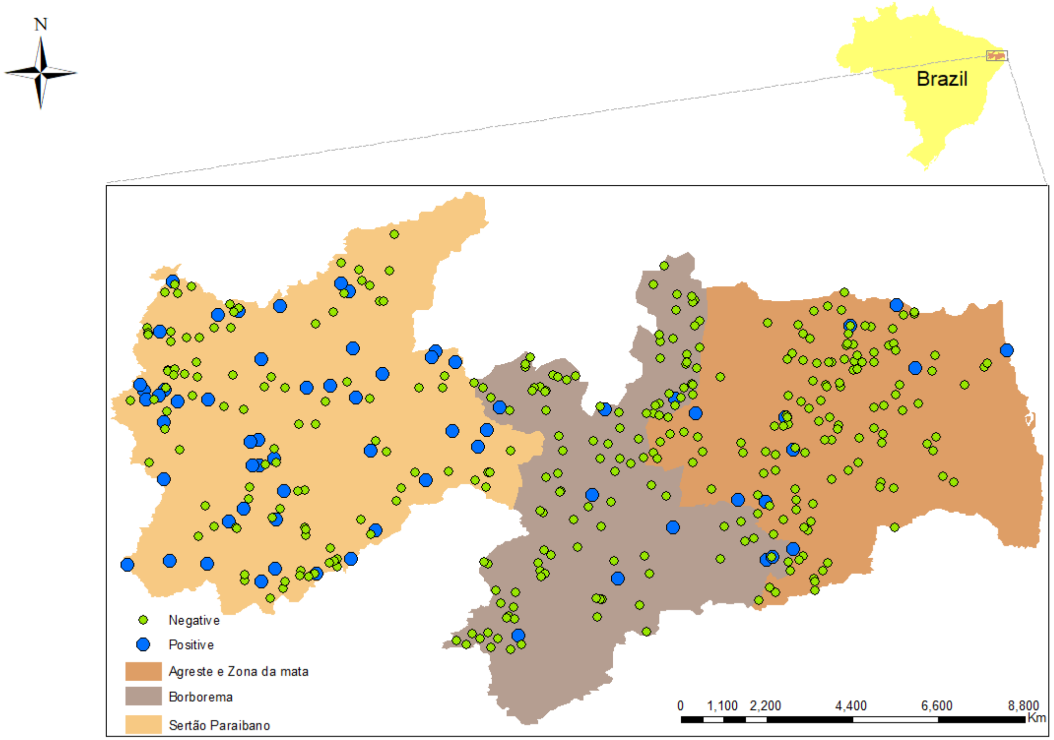
APPENDIX

List of figures

Figure 1 - Division of Paraíba State into three sample strata (Sertão, Borborema, and Agreste/Zona da Mata) and geographical distribution of positiveand negative herds for Neospora caninum infection.



List of tables

Table 1

Census data on the cattle population in Paraíba State, 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	151	288,764	852
Borborema	11,603	136	83,428	356
Agreste/Zona da Mata	18,398	147	192,320	683
Estado da Paraíba	54,357	434	564,512	1,891

Table 2

Herd-level prevalence of *Neospora caninum* infection in the State of Paraíba, Northeastern Brazil, according to sampling stratum.

Estrato amostral	No. de rebanhos		Prevalência (%)	95% CI
	Testados	Positivos		
Sertão	151	46	30.5	[23,6-38,3]
Borborema	136	10	7.4	[4,0-13,2]
Agreste/Zona da Mata	147	11	7.5	[4,2-13,1]
State of Paraíba	434	67	17.8	[14,3-21,8]

Tabela 3

Animal-level prevalence of *Neospora caninum* infection in the State of Paraíba, Northeastern Brazil, according to sampling stratum.

Sampling stratum	Animal (cows)		Prevalence (%)	95% CI
	Testados	Positive		
Sertão	852	199	24.8	[20.3-30.0]
Borborema	356	56	18.0	[10.4-29.3]
Agreste/Zona da Mata	683	51	8.3	[5.5-12.3]
State of Paraíba	1,891	306	18.1	[14.7-22.1]

Table 4

Univariable analysis for factors associated with the herd-level prevalence of *Neospora caninum* infection in cattle, in the State of Paraiba, Northeastern Brazil.

Variables	Categories	No. of herds sampled	No. of positive herds (%)	*P*
Sampling stratum*	Sertão	151	46 (30.5)	
	Borborema	136	10 (7.4)	
	Agreste/Zona da Mata	147	11 (7.5)	< 0.001
Type of production*	Beeff/milk	182	18 (9.9)	
	Mixed	252	49 (19.4)	0.004
Management system	Intensive/semi-intensive	286	41 (14.3)	
	Extensive	148	26 (17.6)	0.227
Insemination	Not use	432	67 (15.5)	0.715
	Use only isemination	2	0 (0.0)	
Herd size*	Up to 33 animals	218	9 (4.1)	
	34 – 111 animals	108	21 (19.4)	
	> 111 animals	108	37 (34.3)	< 0.001
Presence of dogs	No	143	22 (15.4)	
	Yes	291	45 (15.5)	0.552

Presence of wild animals	No	264	41 (15.5)	
	Yes	170	26 (15.3)	0.530
Abort*	No	398	59 (14.8)	
	Yes	36	8 (22.2)	0.173
Animals purchasing	No	287	41 (14.3)	
	Yes	147	26 (17.7)	0.214
Animal sale*	No	382	54 (14.1)	
	Yes	52	13 (25.0)	0.039
Local of animal slaughter	Not slalughter	196	25 (12.8)	
	At a slaughterhouse	235	41 (17.4)	
	In own farm	3	1 (33.3)	0.280
Rental of pastures	No	341	51(15.0)	
	Yes	93	16(17.2)	0.628
Shares of pastures	Não	370	56 (15.1)	
	Sim	64	11 (17.2)	0.397
Sharing of water sources*	No	371	54 (14.6)	
	Yes	63	13 (20.6)	0.148
Presence of flooded pastures	No	288	41 (14.2)	
	Yes	146	26 (17.8)	0.202
Use of maternity pens	No	322	43 (13.4)	

	Yes	112	24 (21.4)	0.320
Raw milk consumption	No	366	59 (16.1)	
	Yes	68	8 (11.8)	0.237
Veterinary assistance*	Não	364	52 (14.3)	
	Sim	70	15 (21.4)	0.094
Property type	Rural	218	9 (4.1)	
	Indian vilage	108	21 (19.4)	
	Rural settlement	108	37 (34.3)	0.000

Table 5

Factors associated with the prevalence of herds positive for *N. caninum* infection, in the State of Paraiba, Northeastern Brazil.

Factor associated	Logistic regression coefficiente	Standard error	Wald	Odds ratio	(95% CI)	*P*-valor
Property located in the Sertão	0.863	0.338	2.937	2.37	(1.22 – 4.60)	0.011
Mixed production	0.495	0.244	4.122	1.64	(1.02 – 2.65)	0.042
Herds of 34 – 111 animais	1.253	0.387	10.466	3.50	(1.64 – 7.48)	0.015
Herds > 111 animais	1.815	0.372	23.800	6.14	(2.96 – 12.74)	< 0.001

CONCLUSÃO GERAL

Estudos de revisão sistemática com meta-análise fornecem dados e informações da situação atual da patologia em questão. Através desse trabalho, pode-se concluir que a soroprevalência combinada de toxoplasmose bovina nos quatro continentes (Ásia, América, Europra e África) é alta e existe uma grande variabilidade de métodos utilizados para diagnóstico, sendo importante a padronização desses métodos, para não gerar resultados conflitantes e valores de prevalência que podem não ser fidedignos à situação local. Também foi possível determinar importantes indicadores epidemiológicos para infecções por *T. gondii* e *N. caninum* em vacas, no estado da Paraíba, Nordeste do Brasil, sendo identificado a disseminação desses parasitos em todo o território paraibano. Devido ao impacto da toxoplasmose para a saúde pública e da neosporose bovina para a saúde animal e economia, o conhecimento da situação epidemiológica, assim como seus fatores associados são importantes para implementação de medidas de prevenção e controle desses agentes.