DIEGO LEONARDO RODRIGUES

Situação epidemiológica da brucelose e tuberculose bovinas no estado do Paraná, Brasil

São Paulo

2022

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Tese apresentada ao Programa de Pós-Graduação em Epidemiologia Experimental Aplicada às Zoonoses da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo para a obtenção do título de Doutor em Ciências.

Departamento:
Medicina Veterinária Preventiva e Saúde Animal

Programa:
Epidemiologia Experimental Aplicada às Zoonoses

Orientador:
Prof. Dr. Prof. Dr. José Soares Ferreira Neto

Coorientador:
Prof. Dr. Christopher Compton

São Paulo

2022

DADOS INTERNACIONAIS DE CATALOGAÇÃO NA PUBLICAÇÃO

(Biblioteca Virginie Buff D'Ápice da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo)

4251
FMVZ

Rodrigues, Diego Leonardo
Situação epidemiológica da brucelose e tuberculose bovinas no estado do Paraná, Brasil / Diego Leonardo Rodrigues. – 2022.
86 f. : il.

Tese (Doutorado) – Universidade de São Paulo. Faculdade de Medicina Veterinária e Zootecnia. Departamento de Medicina Veterinária Preventiva e Saúde Animal, São Paulo, 2022.

Programa de Pós-Graduação: Epidemiologia Experimental Aplicada às Zoonoses.

Área de concentração: Epidemiologia Experimental Aplicada às Zoonoses.

Orientador: Prof. Dr. José Soares Ferreira Neto.
Coorientador: Prof. Dr. Christopher Compton.

1. Brucelose. 2. Tuberculose. 3. Prevalência. 4. Fatores de risco. 5. Análise de rede. I. Título.

Ficha catalográfica elaborada pela bibliotecária Maria Aparecida Laet, CRB 5673-8, da FMVZ/USP.

UNIVERSIDADE DE SÃO PAULO
FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA
Comissão de Ética no Uso de Animais

São Paulo, 29th April 2019

CERTIFIED

We certify that the Research "Epidemiological Situation of bovine brucelosis in the State of Paraná", protocol number CEUAx 3253011018 (ID 001104), under the responsibility José Soares Ferreira Neto, agree with Ethical Principles in Animal Research adopted by Ethic Committee in the Use of Animals of School of Veterinary Medicine and Animal Science (University of São Paulo), and was approved in the meeting of day April 17, 2019.

Certificamos que o protocolo do Projeto de Pesquisa intitulado "Situação epidemiológica da brucelose bovina no Estado do Paraná", protocolado sob o CEUAx nº 3253011018, sob a responsabilidade de José Soares Ferreira Neto, está de acordo com os princípios éticos de experimentação animal da Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, e foi aprovado na reunião de 17 de abril de 2019.

Anneliese Traldi

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Presidente da Comissão de Ética no Uso de Animais
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FOLHA DE AVALIAÇÃO

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Título: **Situação epidemiológica da brucelose e tuberculose bovina no Estado do Paraná, Brasil**

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Data: _____/_____/_____

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DEDICATÓRIA

Dedico a Michelle, Letícia, Anna Vitória, Isaac e Leonardo por me fazerem ver as alegrias espontâneas e gratuitas da vida.

AGRADECIMENTOS

Ao meu orientador, Prof. José Soares Ferreira Neto, pela disposição em mostrar os caminhos possíveis da jornada científica.

Aos demais professores do Laboratório de Epidemiologia e Bioestatística, por acompanhar e instigar o pensamento crítico em cada encontro nosso.

Aos amigos do LEB, por serem companheiros no caminho e compartilhar os bons momentos.

Aos professores e amigos na Massey University, pela acolhida e suporte durante meu tempo na Nova Zelândia.

Ao Ministério da Agricultura, Pecuária e Abastecimento por patrocinar esta pesquisa.

À Agência de Defesa Agropecuária do Estado do Paraná e seus servidores, pelo trabalho de campo e laboratório.

Aos meus pais e irmãos, que acompanharam cada partida e cada retorno meu, sem nunca deixar de fazer parte do que eu sou.

Ao criador, por nos presentear com a experiência majestosa da vida.

“O papel é curto.

Viver é comprido.”

Paulo Leminski

RESUMO

RODRIGUES, D. L Situação epidemiológica da brucelose e tuberculose bovina no estado do Paraná, Brasil. 2022. 86 f. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2022.

A prevalência aparente e os fatores de risco da brucelose e tuberculose bovina em rebanhos e em animais foram estimados por meio de estudo transversal no estado do Paraná, Brasil. A área foi dividida em sete regiões e uma amostragem aleatória em dois estágios foi realizada nas propriedades e nos bovinos de cada uma destas regiões no período de 2018 a 2019. Amostras de soro foram colhidas de 11.592 vacas para diagnóstico em série da brucelose utilizando AAT e TPF, enquanto 17.210 vacas foram testadas na prova de tuberculização comparativa em 1.757 propriedades. Um questionário foi aplicado visando estabelecer potenciais fatores de risco. A rede de movimentação de bovinos na região também foi analisada e os parâmetros de rede de propriedades infectadas e não infectadas por brucelose foram comparados. A soroprevalência de brucelose bovina em propriedades e em animais foi de 4,87% (IC 95%: 3,98-5,93%) e 2,24% (IC 95%: 1,47-3,41%), respectivamente e a doença está distribuída de forma desigual no estado. No caso da tuberculose bovina, a prevalência aparente em rebanhos foi de 2,5% (IC 95%: 1,87%-3%) e a prevalência aparente em animais foi de 0,35% (IC 95%: 0,21-0,59%) e foram detectados clusters na área de estudo. A regressão logística multivariada identificou que rebanhos maiores são um fator de risco importante nas duas doenças. Ainda, no caso da brucelose, a testagem de animais pelos produtores demonstrou ser um fator de proteção. Para a tuberculose bovina, os rebanhos leiteiros demonstram estar sob maior risco, bem como aquelas propriedades que fazem uso de soro de leite na alimentação do rebanho. Os resultados obtidos demonstram que não houve alteração na ocorrência das duas doenças em relação a estudos anteriores. A rede de comércio de gado do Paraná é do tipo livre de escala – os movimentos são altamente concentrados em uma proporção relativamente pequena de propriedades. Os rebanhos infectados por brucelose, em média, comercializam mais animais do que rebanhos não infectados e estão mais conectados entre si do que outros pares de rebanho da rede. Dados estes achados, recomenda-se que as políticas para controle da brucelose incluam um programa de vacinação amplo nas áreas de maior prevalência do estado e a estratégia de erradicação nas áreas de mais baixa prevalência. Recomenda-se que para o controle da tuberculose bovina inclua-se estratégias de vigilância baseada em risco e sacrifício de animais positivos. Os produtores devem estar cientes do risco de adquirir animais sem serem testados previamente.

Palavras-chave: Brucelose. Tuberculose. Prevalência. Fatores de risco. Zoonoses.

ABSTRACT

RODRIGUES, D. L. Epidemiological situation of bovine brucellosis and tuberculosis in state of Paraná, Brazil. 2022. 86 p. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2022.

Apparent prevalence and risk factors of bovine brucellosis and tuberculosis (bTB) in herds and cattle were estimated by a cross-sectional study in the state of Paraná, Brazil. The area was divided into seven regions and a random, two-stage sampling was performed on properties and cattle from each region between 2018 and 2019. Serum samples were collected from 11,592 cows over 24 months old for brucellosis testing in a serial protocol of BPAT and FPA, and 17,210 cows were tested with comparative tuberculin test in 1,757 properties. A questionnaire was applied to identify potential risk factors. Also, the network of cattle trade in the area was analysed and the parameters of brucellosis infected and non-infected farms were compared. The seroprevalence of bovine brucellosis on properties and in cattle was 4.87% (95% CI: 3.98–5.93%) and 2.24% (95% CI: 1.47–3.41%), respectively and the disease is unevenly distributed across the state. For bTB, apparent prevalence in herds was 2.5% (95% CI: 1.87-3%) and cattle prevalence was 0.35% (95% CI: 0.21-0.59) and clusters were identified in the area. Multiple logistic regression analysis identified larger herds as risk factor for both diseases. Also, for brucellosis, testing animals was found a protective factor. For bTB, dairy herds pose higher risk of infection, as well as feeding cows with whey. These results demonstrate no change in the prevalence when comparing previous studies. The network of cattle trade is a scale-free type, highly concentrated in a relatively small number of farms. Brucellosis-infected farms are more likely to trade animals than non-infected, and also, they are more closely connected among themselves than other pairs of farms in the network. Given our findings, it is recommended that policies for brucellosis control include a widespread vaccination program for higher prevalence areas and eradication approach to lower prevalence areas. It is recommended that policies for bTB control include risk-based surveillance and culling. Farmers should be advised about the risks of introducing animal into their herds without testing them for both diseases.

Keywords: Brucellosis. Tuberculosis. Prevalence. Risk factors. Zoonosis.

Sumário

1 INTRODUÇÃO GERAL

No Brasil, estima-se que 2,5 milhões de estabelecimentos rurais comportem 218 milhões de bovinos (IBGE, 2020). A cadeia produtiva da carne, do leite e demais produtos da bovinocultura confunde-se com a própria história do País e alicerçou a construção da sociedade brasileira, seja na sua função econômica, social ou cultural.

Mais recentemente, a tecnificação e mecanização do campo permitiu atingir níveis de produção que suprem a demanda interna do País e, no caso da carne bovina, alcança o mercado internacional com vigor, atingindo o recorde de 187.000 toneladas exportadas em um único mês, em setembro de 2021 (Brasil, 2022).

Entretanto, ao tempo em que a eficiência invade a agricultura, um olhar mais atento permite identificar lacunas na bovinocultura brasileira. Parte destas deficiências passam por questões sanitárias, doenças que minam a produtividade do setor.

O controle das doenças dos animais de produção no País é um objetivo compartilhado pelos produtores rurais, Governo e demais integrantes da cadeia produtiva agropecuária. A brucelose e a tuberculose nos bovinos estão entre as principais doenças endêmicas que têm sido combatidas de forma sistêmica e integrada por iniciativas públicas e privadas. Mais especificamente, em 2001 o governo brasileiro iniciou o Programa Nacional de Controle e Erradicação da Brucelose e Tuberculose (PNCEBT) em bovinos (Brasil, 2001).

O PNCEBT tem o objetivo de controlar e erradicar estas doenças no País. Não se pode controlar, entretanto, aquilo que não se conhece. Nesta esteira, proliferaram nos últimos anos pesquisas que visam expandir o conhecimento epidemiológico destas enfermidades (Ferreira Neto et al., 2016) – é nesse contexto amplo que nossa pesquisa tem suas raízes.

1.1 BRUCELOSE BOVINA

A história conhecida das bactérias do gênero Brucella iniciou-se em 1861, com a descrição do Dr Jeffrey Alan Marston sobre os diferentes tipos de febres que acometiam o exército real britânico e aliados durante a Guerra da Criméia e, em 1887, com a descoberta e cultivo do micrococcus causador da febre de Malta por David Bruce e pelo Dr. Giuseppe Caruana Scicluna (Wyatt, 2013).

Inicialmente, a diferenciação das espécies era baseada nas características fenotípicas e nos hospedeiros, sugerindo desde então a predileção do tipo espécie-específica. *B. melitenses*, *B. abortus*, *B.suis*, *B.ovis*, *B. neotomae* e *B. canis* foram, nesta ordem, as primeiras a serem descritas ainda no período que antecedeu a era das técnicas moleculares.

Devido à alta similaridade genética entre as espécies do gênero, considerou-se entre 1986 e 2003 que se tratavam de biovares de *B. melitenses*, porém por fim, o subcomitê de taxonomia da Brucella decidiu pelo retorno à nomenclatura com as 6 espécies, alegando em especial a distribuição ecológica das espécies nos hospedeiros e a diversidade de patogenicidade (Osterman e Moriyón, 2006).

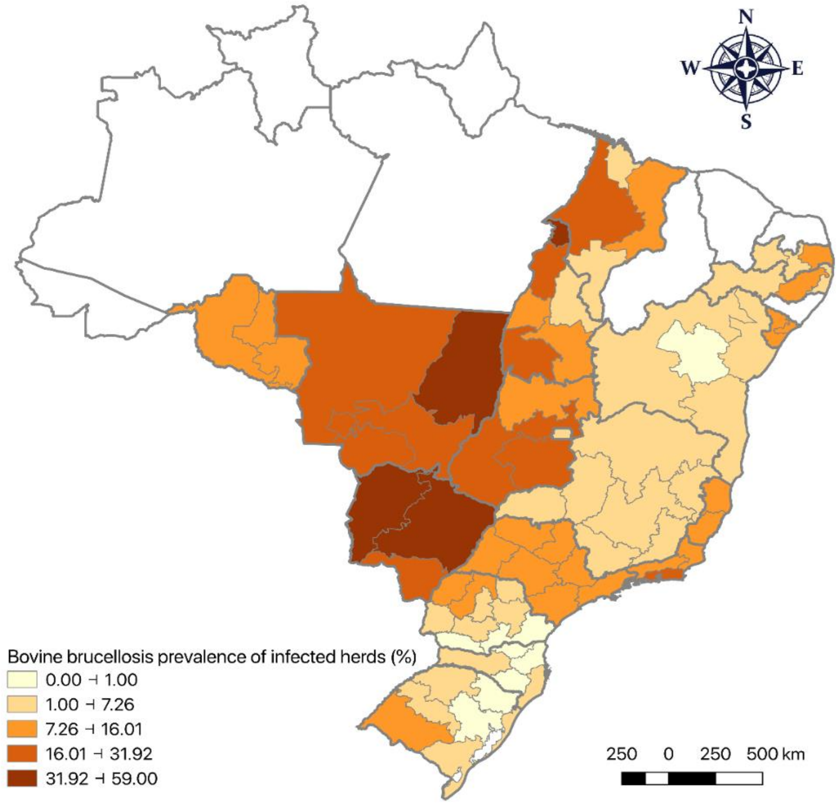
Com o advento das ferramentas moleculares, foram oficialmente reconhecidas as espécies *B. pinnipedialis* e *B. ceti* em 2007, *B.microti* em 2008 e *B. inopinata* em 2010. Técnicas mais recentes, como as análises em multilocus de repetições em tandem de número variável (MVLA) têm permitido uma melhor compreensão da taxonomia do gênero (SCHOLZ e VERGNAUD, 2016).

B. abortus representa especial interesse para a produção animal, em particular para a bovinocultura, devido às perdas econômicas decorrentes do aborto no terço de final de gestação e diminuição dos índices reprodutivos. Poester identificou que em 13 novilhas não vacinadas experimentalmente infectadas, 31% abortaram e 31% pariram bezerros fracos que morreram logo após o parto (Poester, 2006). Estima-se perdas de 25% na produção de leite e carne (BRASIL, 2006), sendo que Santos e colaboradores (Santos et al., 2013) apontaram perdas nacionais da ordem de $448 milhões.

Neste contexto, o conhecimento das condições de distribuição da doença torna-se imperativo para a adoção de medidas economicamente justificáveis, sob a perspectiva de implementar ações de maior impacto no controle desta zoonose ao considerar as especificidades de cada região brasileira.

Assim, diversos estudos transversais foram conduzidos na maior parte do território nacional. Ferreira Neto e colaboradores (Ferreira Neto et al., 2016) compilaram a prevalência de brucelose em propriedades em diversos estados brasileiros, conforme Figura 1.

Figura 1 – Prevalência aparente de propriedades positivas para brucelose bovina



Fonte: Ferreira Neto *et al,*2016

No Paraná, estudo conduzido em 2002 identificou prevalência de focos de 4%[IC 95%: 3,2-4,8%] e de animais de 1,7%[IC 95%: 1,1-2,4%], com importante diversidade entre as regiões do estado (Dias et al., 2009).

Estes mesmos estudos procuraram identificar os fatores de riscos para ocorrência da doença nas populações alvo. Dias e colaboradores relataram ainda que a compra de reprodutores e o aluguel de pastagens foram as práticas mais frequentemente relacionadas aos focos de brucelose no Paraná, assim como foram identificados mais focos da doença em gado de corte do que em rebanhos mistos ou leiteiros, em tese devido às diferenças de tamanho destes três tipos produtivos.

No período de 2001 a 2019, gradativamente o rebanho bovino do estado passou a ser vacinado e testado e os animais infectados foram eliminados do plantel. Assim, procura-se por meio desta pesquisa conhecer a prevalência atual da enfermidade, a mesmo tempo que se pretende identificar fatores de risco relevantes que subsidiam a tomada de decisão durante a modelagem do programa de controle da doença no estado.

1.2 TUBERCULOSE BOVINA

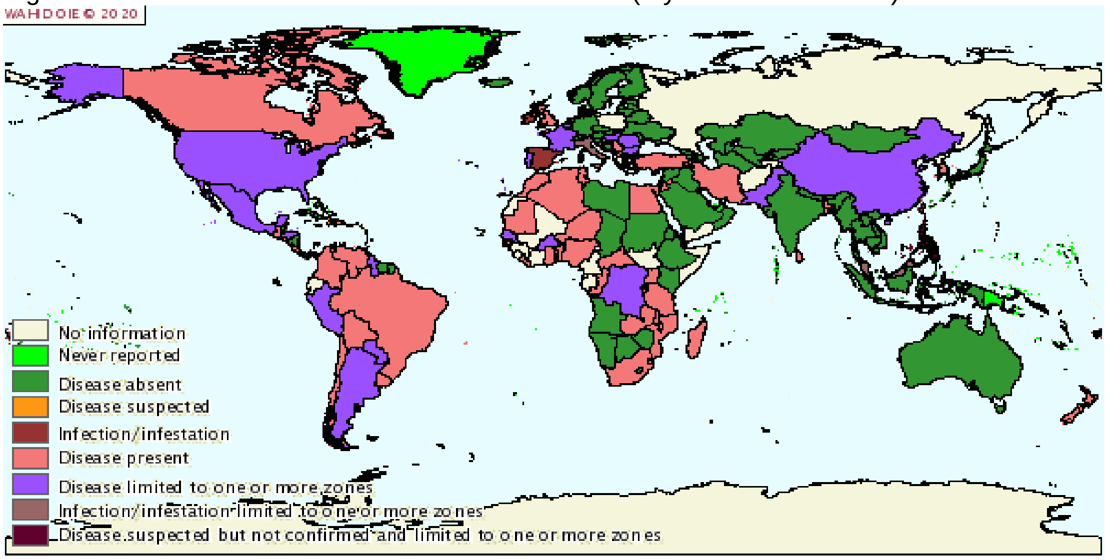
A tuberculose bovina é uma das principais doenças infecciosas dos rebanhos bovinos, e seu papel é relevante em saúde única ao responder por cerca de 140.000 casos anuais de tuberculose zoonótica em humanos (OMS, 2020).

As perdas causadas pela doença nos bovinos advêm da queda da produtividade animal, condenações no abatedouro e gastos relacionados ao seu controle nas propriedades, como exames e eliminação de animais infectados.

O agente etiológico da tuberculose, *Mycobacterium bovis*, tem sido reportado na maior parte dos países que mantém vigilância da doença. Entretanto, os países-membros da organização mundial de saúde animal (WOAH) se encontram em diferentes fases de seu controle.

Entre 2017 e 2018, apenas 44% (82/188) dos países-membros reportaram a doença (Fig. 2) e dentre estes, apenas 23% (19/82) implementaram todas as medidas indicadas pela OIE (OIE, 2020) . Parte dos países que não reportaram a doença podem ser considerados infectados pois, na maioria dos casos, o não relato a OIE deve-se a ausência de controles adequados das autoridades sanitárias.

Figura 2 - Ocorrência mundial da tuberculose bovina (Mycobacterium bovis) em 2018



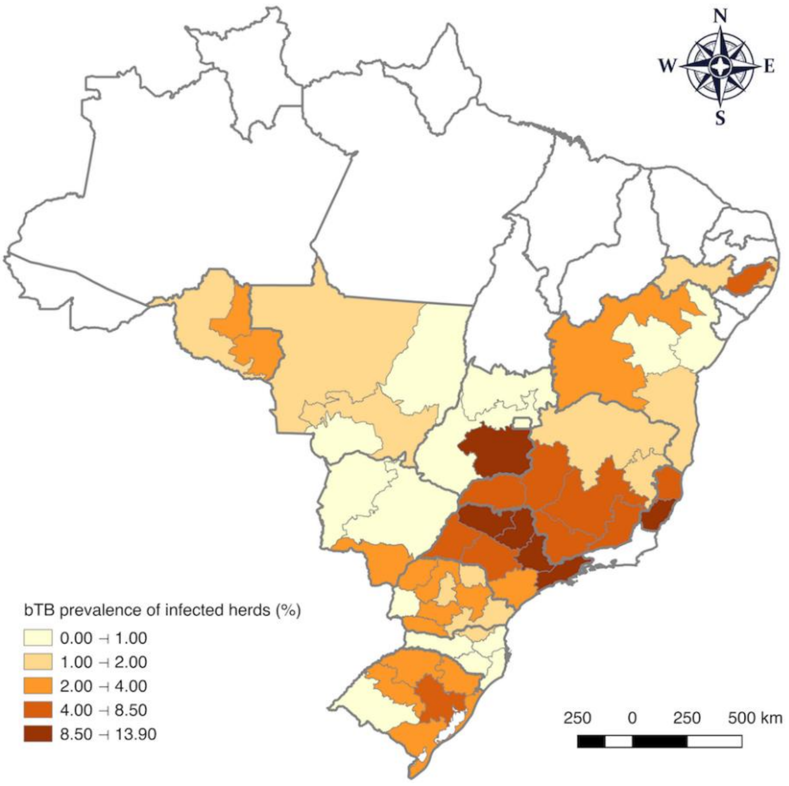
Fonte: OIE,2020

Poucos países obtiveram êxito na erradicação da doença. A Austrália a erradicou em 2002, após 27 anos da implementação de seu programa de controle de tuberculose bovina e, desde então, não registrou a doença nem em animais domésticos, nem em silvestres. Algumas das lições aprendidas na experiência australiana são: alinhamento de objetivos do setor privado e governamental, aplicação de modelos de negócios que incluem planejamento estratégico plurianual e planos operativos anuais, capacidade de rastreabilidade animal, uso intenso de vigilância em abatedouros, eliminação de focos residuais, transparência do processo com ganho de confiança entre os envolvidos e mensuração objetiva do progresso (More et al., 2015).

Alguns países têm registrado poucos focos de tuberculose bovina, resultado de frequentes reintroduções associadas ao contato próximo dos animais de produção com a fauna silvestre e a vigilância destas populações tem se mostrado desafiadora nas fases avançadas de erradicação (Réveillaud et al., 2018).

No Brasil, um panorama da distribuição da doença (Figura 3) e dos fatores de risco tem sido desenhado por meio de diversos estudos de campo (Ferreira Neto et al., 2016). Mais especificamente no estado do Paraná, a prevalência aparente da tuberculose bovina em propriedades foi estimada em 2,15% e em bovinos 0.42% em estudo realizado entre 2005 e 2007 (Silva et al., 2016).

Figura 3 – Prevalência aparente de propriedades positivas para tuberculose bovina



Fonte: Ferreira Neto *et al,*2016

Considerando a adoção de uma série de medidas no controle da doença no estado do Paraná durante os últimos anos, pretende-se como um dos objetivos desta pesquisa verificar a prevalência atual da doença e estabelecer fatores de risco que possam ser levados em conta no futuro no contexto das políticas de controle da doença.

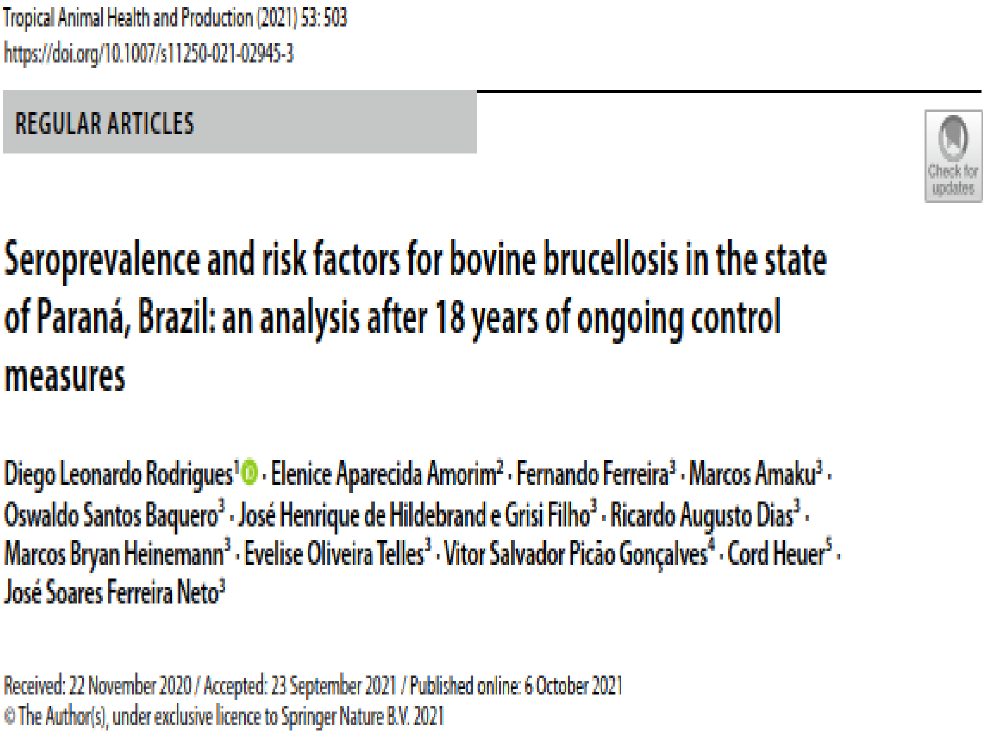
1.3 COMPOSIÇÃO DOS CAPÍTULOS DA TESE

Esta tese é composta por três capítulos principais. O primero deles é "*Seroprevalence and risk factors for bovine brucellosis in the state of Paraná, Brazil: an analysis after 18 years of ongoing control measures*", publicado conforme referência a seguir: Rodrigues, D.L., Amorim, E.A., Ferreira, F., Amaku, M., Baquero, O.S., de Hildebrand e Grisi Filho, J.H., Dias, R.A., Heinemann, M.B., Telles, E.O., Gonçalves, V.S.P., Heuer, C. and Neto, J.S.F., 2021. Seroprevalence and risk factors for bovine brucellosis in the state of Paraná, Brazil: an analysis after 18 years of ongoing control measures Tropical Animal Health and Production, 53, 503.

O segundo capítulo, relacionado a prevalência, análise espacial e fatores de risco da tuberculose bovina tem o título *"Apparent prevalence and risk factors for bovine tuberculosis in the state of Paraná, Brazil: an assessment after 18 years since the beginning of the Brazilian program",* e publicado em 24 de outubro de 2.022 para pela revista Tropical Animal Health and Production, disponível em acesso livre em https://doi.org/10.1007/s11250-022-03350-0

O terceiro capítulo trata da análise da rede de movimentação animal e como este trânsito animal pode influenciar a disseminação ou controle da brucelose no estado do Paraná. Seu título é "Trading network of bovines and its effect on the spread of brucellosis in Paraná, Brazil", e será oportunamente submetido para publicação.

2 SEROPREVALENCE AND RISK FACTORS FOR BOVINE BRUCELLOSIS IN THE STATE OF PARANÁ, BRAZIL: AN ANALYSIS AFTER 18 YEARS OF ONGOING CONTROL MEASURES



Tropical Animal Health and Production (2021) 53: 503
https://doi.org/10.1007/s11250-021-02945-3

REGULAR ARTICLES

Seroprevalence and risk factors for bovine brucellosis in the state of Paraná, Brazil: an analysis after 18 years of ongoing control measures

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Received: 22 November 2020 / Accepted: 23 September 2021 / Published online: 6 October 2021

2.1 ABSTRACT

Seroprevalence and risk factors of bovine brucellosis (*Brucella abortus*) in herds and cattle were estimated by a cross-sectional study in the state of Paraná, Brazil. The state was divided into seven regions and a random, two-stage sampling was performed on properties and cattle from each region between 2018 and 2019. Serum samples were collected from 11,592 cows over 24 months from 1,757 properties and a questionnaire was applied to identify potential risk factors. As recommended by the National Program for the Control and Eradication of Animal Brucellosis and Tuberculosis (PNCEBT), serological testing for the detection of anti-Brucella antibodies included the buffered plate agglutination test (screening test) and the fluorescence polarization assay (confirmatory test). The seroprevalence of bovine brucellosis on properties and in cattle was 4.87% (95% confidence interval [CI]: 3.98–5.93%) and 2.24% (95% CI: 1.47–3.41%), respectively. Multiple logistic regression analysis identified larger herd size and failure to test for brucellosis as risk factors for the presence of anti-*B. abortus* antibodies. These results demonstrate no change in the prevalence when comparing initial studies conducted in 2002. Given our findings, it is recommended that policies for brucellosis control include a widespread vaccination program for higher prevalence areas and eradication approach to lower prevalence areas. All steps related to correct immunization of the herds should be verified and improved by training and education. Health education action must be carried out informing farmers about the risks of introducing animals not tested for brucellosis into their herds and the benefits of testing their herds regularly.

Keywords Cattle · Brucella abortus · Prevalence · Risk factors · Brazil

2.2 INTRODUCTION

Bovine brucellosis is a zoonosis caused by Brucella abortus. The main clinical signs of bovine brucellosis are related to reproductive disorders in males and females (Neta et al., 2010; Poester et al., 2013). Other signs include interstitial mastitis and arthritis (Lage et al., 2008; Poester et al., 2013).

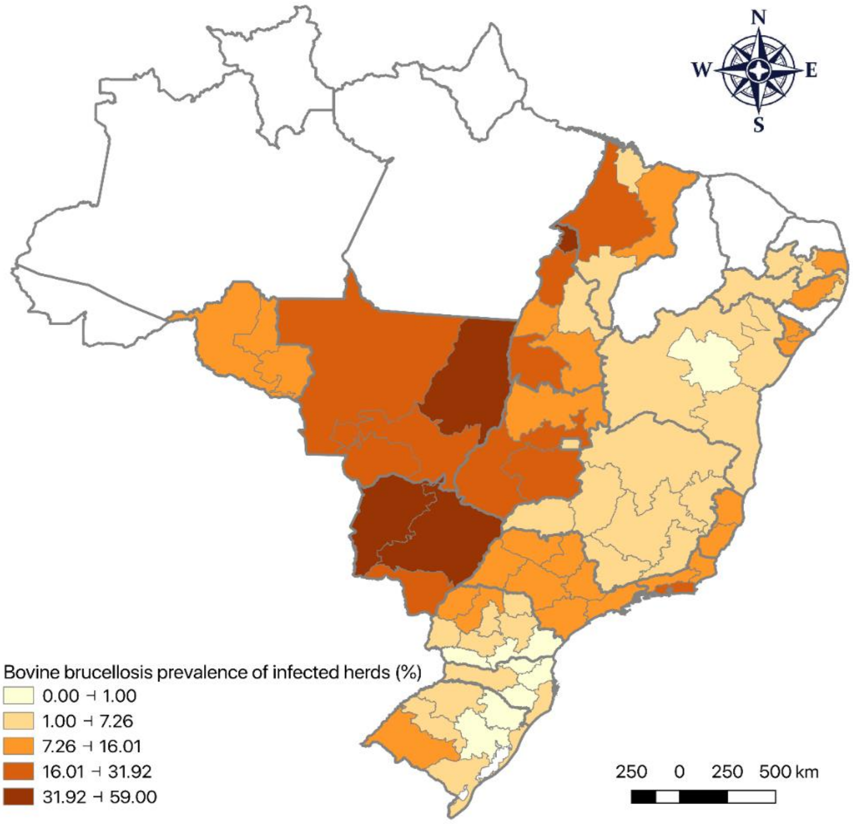
The disease has a worldwide distribution, even though several countries in Western and Northern Europe, Canada, Japan, Australia, and New Zealand are believed to be free from the causal agent (OIE 2020). This was achieved by animal health programs using different combinations of strategies (Zhang et al., 2018).

In Brazil, the mean economic impact of bovine brucellosis was estimated at US$206 per dairy cow infected and $108 per beef cow infected, taking into account costs associated with reproductive losses, decreased milk and beef production, and costs of veterinary interventions (Santos et al., 2013). According to that study, every 1% increase or decrease in prevalence in Brazil is expected to increase or decrease the economic burden of brucellosis by approximately $76 million per year. Economic impact of brucellosis has been described in detail by McDermott et al. (2013).

The Programa Nacional de Controle e Erradicação da Brucelose e Tuberculose Animal — PNCEBT (National Program for the Control and Eradication of Animal Brucellosis and Tuberculosis) was developed in 2001 in Brazil by the national animal health authority, Ministério da Agricultura Pecuária e Abastecimento — MAPA (Ministry of Agriculture, Livestock and Supply) (Brasil, 2001). The main objective of the PNCEBT is to reduce the prevalence and incidence of both diseases. To achieve this objective, sound evidence is necessary and thus many states performed brucellosis prevalence studies with a standard methodological approach. Reported prevalence of herds in the different states of Brazil varied between 0.32 and 41.5% and heterogenicity was evident throughout the country (Alves et al., 2009; Marvulo et al., 2009; Negreiros et al., 2009; Ogata et al., 2009; Rocha et al., 2009; Sikusawa et al., 2009; Silva et al., 2009; Villar et al., 2009; Azevedo et al., 2009; Chate et al., 2009; Dias et al., 2009; Gonçalves et al. 2009a; Gonçalves et al. 2009b; Klein-Gunnewiek et al., 2009; Borba et al., 2013; Almeida et al., 2016; Clementino et al., 2016).

Seven states of Brazil reported a second prevalence study. Of those, only Mato Grosso, Mato Grosso do Sul, and Rondônia described reduction of prevalence of seropositive herds due to vaccination (Anzai et al., 2016; Barddal et al., 2016; Baumgartem et al., 2016; Dias et al., 2016; Guedes et al., 2016; Inlamea et al., 2016; Silva et al., 2016). Herd prevalence in several states of Brazil was obtained between 2003 and 2014 and can be seen in Fig. 1.

Fig. 1 Prevalence of herds infected for bovine brucellosis carried out in the Brazilian states between 2003 and 2014 (data not available in white areas)



Dias et al. (2009) estimated brucellosis prevalence in Paraná in 2002. They reported a herd prevalence of 4% [CI 3.2–4.8%] and animal prevalence of 1.7% [CI 1.1–2.4%] and identified the purchase of breeding cattle and sharing of pastures as risk factors.

As per PNCEBT, control of brucellosis in Brazil is based on vaccination of female calves between 3 and 8 months of age, testing of cattle prior to their movements when animals are destinated for reproduction or agglomeration, elimination of all positive animals in abattoirs, voluntary certification of free herds, and surveillance and culling in areas with low prevalence. Female calves are mainly vaccinated with *B. abortus* strain S19, although strain RB51 may be used instead according to PNCEBT (Brasil, 2017b). Also, the latter can be used in a revaccination program at the farmer will.

Considering the importance of understanding the results achieved under the PNCEBT actions in the state of Paraná, Brazil, we aimed in this study to assess current risk factors and the variation in prevalence of brucellosis in cattle herds in Paraná, Brazil. Furthermore, data obtained would allow the evaluation of the efficacy of control measures implemented since the inception of PNCEBT 18 years ago.

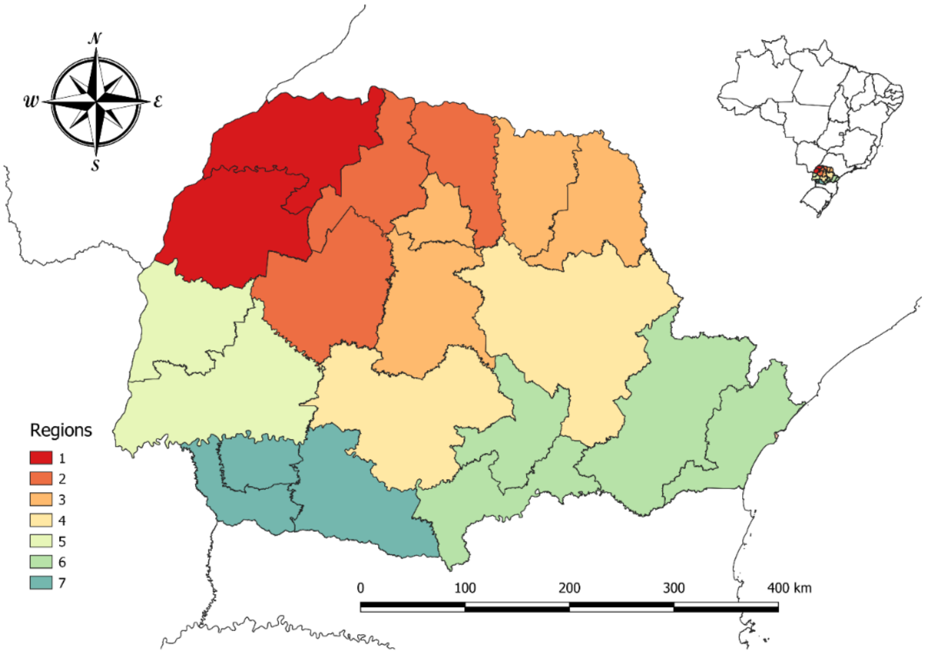
2.3 MATERIALS AND METHODS

2.3.1 Study area and population

This epidemiological study was performed in the state of Paraná, positioned in the south region of Brazil. The target population was all its domestic female bovines and buffalos (in this text generically referenced as cattle) over 24 months of age. The region has a geographical area of 199.305 k m^2 and a cattle population of 8,397,219 individuals (IBGE 2017), which represents 4.8% of the national herd. Its bovine population is the eighth largest in the nation. Paraná has the third highest milk production in the country (IBGE 2017).

The state is divided into seven regions to characterize the different regional production profiles. This regional partitioning is the same as was used in a previous study (Dias et al., 2009), to allow comparisons (Fig. 2).

Fig. 2 Map of the state of Paraná, showing the regions defined in the current study. The state was divided into seven regions: 1. Noroeste; 2. Centro-Oeste-Norte; 3. Norte pioneiro; 4. Centro-Sul; 5. Oeste; 6. Leste-Sul; and 7. Sudoeste



2.3.2 Sampling

A two-level sampling was applied to estimate seroprevalence of herds and cattle seropositive for anti-Brucella antibodies. The first step consisted of choosing a defined number of farms, followed by the random selection of cows over 24 months of age within those farms. We limited the scope of the study to this category in order to focus on reproductive age and reduce false positive due to immunization, considering that female calves are vaccinated between 3 and 8 months of age in Brazil, according to PNCEBT (Brasil, 2017b). Collection of samples and their analysis in the laboratory were accomplished from September 2018 to April 2019.

The sample size of properties to be accessed in each of the seven regions was estimated in accordance with the formula for simple random samples (Thrusfield, 2018):

$$\boldsymbol{n = \frac{Z_{\alpha}^{2} * P * (1 - P)}{d^{2}}}$$

where *n* denotes the sample size, *Z* is the normal distribution value at *α* level of confidence, *P* is expected prevalence, and *d* is the absolute error.

Thus, we defined the parameters to define sample size of properties as degree of confidence of 0.95, expected apparent prevalence of 5%, and absolute error of 0.03.

An exhaustive alphabetically sorted list of primary units was used to apply a systematic random sampling. When the selected unit needed to be replaced, it was substituted by the next available propriety in the list. To select the number of animals to be tested in each farm, we calculated the minimum number of cows that should have been tested to reveal the brucellosis status of the herd, considering the sensitivity and specificity of the test protocols (animal level) as 95% and 99.5%, respectively (Gall and Nielsen, 2004), 20% of intra-herd prevalence, and a sensitivity and specificity greater than or equal to 90% (herd level). Thus, 10 animals were sampled in properties with up to 99 cows older than 24 months and 15 animals were sampled in properties with 100 or more cows older than 24 months. Table 1 provides information regarding the target population and samples per region.

Table 1 – Population and sample data in the study per region and total

Region	Nº of municipalities	Total herds with reproductive activity	Total cows	Herds sampled	Sampled cows
1. Noroeste	57	18875	763643	251	2035
2 Centro-Oeste-Norte	78	15533	488045	250	1766
3. Norte pioneiro	80	28133	795964	253	1702
4. Centro-Sul	32	26011	729383	251	1702
5. Oeste	52	23153	583813	251	1528
6. Leste-Sul	56	18697	182131	251	1105
7. Sudoeste	44	32786	583796	250	1755
Total	399	163188	4126775	1757	11592

Within the properties, cows were selected randomly. Cows in the peripartum period, i.e., approximately 15 days before and after delivery, were excluded from the selection, since this can affect diagnosis results.

2.3.3 Serologic tests

A serial testing scheme was performed as described in national regulation (Brasil, 2017a). Each serum sample was submitted to buffered plate agglutination test (BPAT) and reactors (positive samples) were subsequently submitted for fluorescence polarization assay (FPA), both tests accomplished as described in OIE standards (OIE 2019). BPAT has sensitivity of 95.4% and 98.4%, while those values are 97.5% and 98.9% for FPA (Gall and Nielsen, 2004). Samples that reacted to both assays were considered seropositive. All tests were performed by the Centro de Diagnóstico Marcos Enrietti (Diagnosis Centre Marcos Enrietti) in 2019.

2.3.4 Apparent prevalence in herds and animals

Apparent prevalence in herds was calculated for each region as the direct proportion of tested herds that were seropositive. A herd was considered positive when one or more of its cows tested positive.

Herd prevalence in the state of Paraná was based on the relative weight of each region (HWS) as follows:

$$\mathbf{HWS} = \frac{\textbf{properties in the region}}{\textbf{properties sampled in the region}}$$

Thus, when calculating herd prevalence in Paraná, each farm weight was applied based on what region this farm is located in and regions with larger total number of properties contributed more to state level herd prevalence.

The weight of each animal in the calculation of the prevalence of infected animals within the regions (AWR) was given by the following equation:

$$\mathbf{AWR} = \frac{\textbf{cows} \geq \textbf{ 24 months in the property}}{\textbf{cows} \geq \textbf{ 24 months sampled in the property}}$$

To compute apparent animal prevalence within the state of Paraná, each sampled cow had its weight (AWS) calculated by the following expression (DOHOO et al., 2003):

$$\mathbf{AWS} = \frac{\textbf{cows} \geq \textbf{24 months in the property}}{\textbf{cows} \geq \textbf{24 months sampled in the property}} * \frac{\textbf{cows} \geq \textbf{24 months in the region}}{\textbf{cows} \geq \textbf{24 months sampled in the region}}$$

Therefore, herds contributed according to their size to animal prevalence in each region and in the state level.

The final weight of each farm and animal can be conferred in the data made available below in “Availability of data and material” section.

Also, a spatial analysis using a kernel density estimate map was produced to represent clustering of positive herds. The model was design to consider and correct the differences of sampling density across the state of Paraná.

2.3.5 Study of risk factors

A questionnaire to access potential risk factors was fulfilled based solely on the owner or farm manager answers. All herds sampled provided one set of answers each. Data were collected in loco, in a face-to-face interview during the same visit to collect samples, using an electronic device, and then uploaded into an electronic spreadsheet.

Questions included information regarding the number of bovines and buffalos reared by age and sex category, presence of other domestic and wild species, management and operation type, breeds, trade of animals, placental waste disposal, veterinary attention, vaccination, and history of abortions on the property.

2.3.6 Statistical analysis

Statistical analyses were performed using R Core Team (2018). Confidence intervals (CIs) were obtained by binomial logistic regression model.

Variables with more than two possible answers (not dichotomic) were regrouped in some cases for a more meaningful analysis. The number of cows was used as a measure of herd size and was categorized based on percentiles.

All potential risk factors were submitted to univariate analysis regarding whether the respective farm was classified as brucellosis infected herd or not.

Techniques used to achieve this were the chi-squared test or Fischer test. A conservative p value of ≤ 0.2 was used as criteria to select which variables would be used in a multivariate logistic regression (Silva Abreu et al., 2009). The construction of a statistical model was based in a backward elimination of variables until all the variables were statistically significant in the model ($p < 0.05$). When necessary, models fitting was compared using Akaike information criterion (AIC).

2.4 RESULTS

Apparent herd prevalence per region and for the entire state is presented in Table 2. Of 1,757 herds, 95 had one or more positive cows. Prevalence at state level was calculated applying weight as described in “Materials and methods”.

Table 2 Apparent prevalence of herds seropositive for brucellosis in cattle in seven regions and in the state of Paraná, 2019

Region	Herds Sampled	Herds positive	Prevalence (%)	CI (95%)
1. Noroeste	251	33	13.14	8.95 – 17.33
2. Centro-Oeste-Norte	250	24	9.6	5.94 – 13.25
3. Norte Pioneiro	253	9	3.55	1.27 – 5.84
4. Centro-Sul	251	10	3.98	1.55 – 6.40
5. Oeste	251	10	3.98	1.55 – 6.40
6. Leste-Sul	251	3	1.19	0 – 2.54
7. Sudoeste	250	6	2.4	0.4 – 4.3
Paraná	1757	95	4.87†	3.98 – 5.93†

†Calculated using weights as described

Table 3 shows the apparent prevalence in cows aged over 24 months. Of 11,592 animals, 137 were seropositive (2.24%).

Table 4 shows the apparent prevalence in herds stratified by type of production. Beef herds had higher prevalence, except in region 4, while mixed-type herds had lower levels, except in region 7; when considering the 95% confidence intervals, the only difference observed was between mixed type and beef herds in region 2.

Table 3 Apparent prevalence of cattle seropositive for brucellosis in seven regions and in the state of Paraná, 2019

Region	Cows Sampled	Cows Positive	Prevalence (%)	CI (95%)
1. Noroeste	2035	47	4.28	1.73 - 6.84
2. Centro-Oeste-Norte	1766	28	1.38	0.52 - 2.23
3. Norte Pioneiro	1701	22	1.15	0 - 2.68
4. Centro-Sul	1702	12	1.43	0.28 - 2.58
5. Oeste	1528	15	1.54	0.34 - 2.73
6. Leste-Sul	1105	5	1.14	0 - 2.65
7. Sudoeste	1755	8	2.06	0 - 4.63

Paraná	11592	137	2.24	1.47 – 3.41

Note: Calculated using weights as described

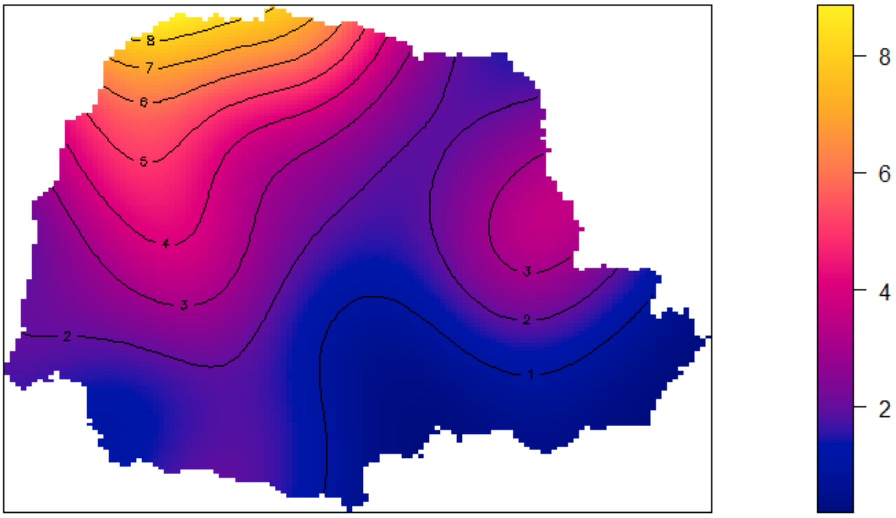
Table 4 Apparent prevalence of herds seropositive for brucellosis in cattle in seven regions stratified by type of production (beef, dairy and mixed), 2019

Region	Beef		Dairy		Mixed	
	Prevalence%	CI 95% (%)	Prevalence%	CI95 (%)	Prevalence%	CI 95% (%)
1	20.79 (21/101)	12.86 – 28.72	8.69(8/92)	2.90 - 14.48	6.89 (4/58)	0.31 - 13.47
2	20.00(15/75)	10.88 - 29.11*	6.73(7/104)	1.89 - 11.56	2.81(2/71)	0 - 6.69*
3	8.00(8/100)	2.65 - 13.34	1.04 (1/96)	0 - 3.08	0(0/57)	0 - 6.26†
4	4.10 (3/73)	0 - 8.69	5.94(6/101)	1.30 - 10.57	1.29(1/77)	0 - 3.84
5	11.36(5/44)	1.96 - 20.75	3.20 (4/125)	0.10 - 6.29	1.21 (1/82)	0 - 3.60
6	4.05(3/74)	0 - 8.57	0(0/72)	0 - 4.99†	0(0/105)	0 - 6.26†
7	5.26 (2/38)	0 - 12.45	1.11 (2/179)	0 - 2.66	6.06(2/33)	0 – 14.32

* Statistically different (p<0.05) †Calculated by exact binomial method.

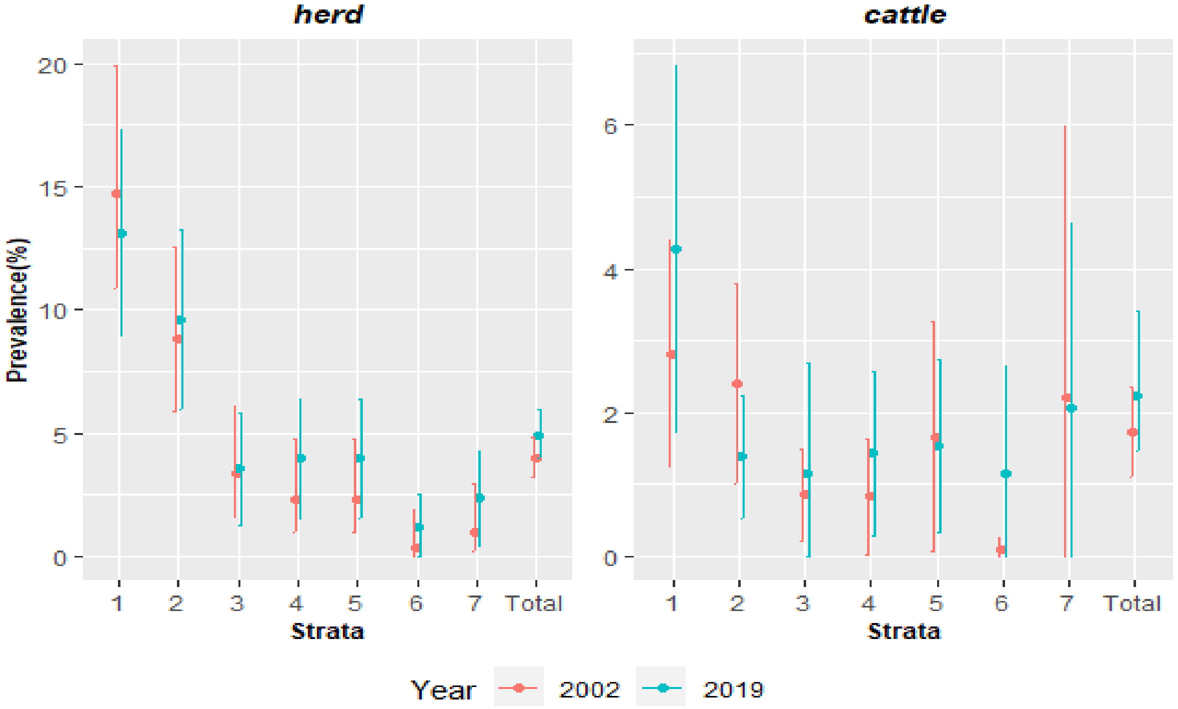
Based in the geolocation of each of the farms sampled, a kernel density estimate was produced for positive herds in Paraná (Fig. 3). It shows that seropositive farms are unevenly distributed across the area, with higher density in the north and northwest regions. The model considers the sampling density of the different areas of the state.

Fig. 3 Kernel density of herd prevalence across the state of Paraná, 2019



Herd and cattle prevalence obtained in a similar cross-sectional study developed in 2002 (Dias et al., 2009) was compared to the present study (Fig. 4). No evident differences in terms of prevalence were noticed.

Fig. 4 (a) Herd apparent prevalence in seven regions of state of Paraná in 2002 and 2019 (b) Cattle apparent prevalence in seven regions of state of Paraná in 2002 and 2019



As result of univariate analysis, the following variables had $p \leq 0.2$ when confronted with the herd outcome for brucellosis and were included in a multivariate logistic regression analysis: purchase ($p < 0.001$) and sale ($p < 0.001$) of cattle, introduction of breeding cattle ($p = 0.02$), number of cows aged over 24 months ($p < 0.001$), brucellosis vaccination ($p = 0.01$), correct administration of vaccine ($p < 0.001$), slaughter of animals on the farm ($p < 0.001$), presence of equines ($p < 0.001$), breed ($p < 0.001$), presence of domestic birds ($p = 0.01$), presence of domestic feline ($p = 0.04$), presence of tapir ($p = 0.05$), presence of swine ($p = 0.05$), presence of wild feline ($p = 0.08$), presence of flooded areas on the farm ($p = 0.08$), presence of wild canine ($p = 0.12$), concentration of cattle in some areas of the farm ($p = 0.14$), presence of resting areas to other herds within the farm ($p = 0.15$), system intensity — confined, semi-confined, or extensive ($p = 0.15$) — and presence of caprine or ovine ($p = 0.18$).

In the multivariate logistic regression, only two variables had a significative result ($p \leq 0.05$) and were included in the final model: number of cows aged over 24 months (3 levels: small herds (≤ 8 cows — 50th percentile), medium herds (≥ 9 to ≤ 88 cows — 51st to 95th percentiles), and large herds (≥ 89 cows — 96th to 100th percentiles)) and if cattle were tested for brucellosis (Table 5).

Table 5 Final multivariate logistic regression model of the association of risk factors with bovine brucellosis in herds from the state of Paraná, 2019

Variable and levels	Estimate	Odds ratio	95% CI	P
Herd size (cows aged over 24m)				
Herd ≤ 8 cows (50th percentile)	base category			
Herd ≥ 9 to ≤ 88cows	1.51	4.56	2.54 – 8.19	<0.001
Herd > 88 cows (95th percentile)	2.87	17.62	8.69 – 37.76	<0.001
Diagnosis				
Brucellosis regularly tested	base category			
Brucellosis not regularly tested	0.82	2.28	1.40 – 3.69	<0.001

2.5 DISCUSSION

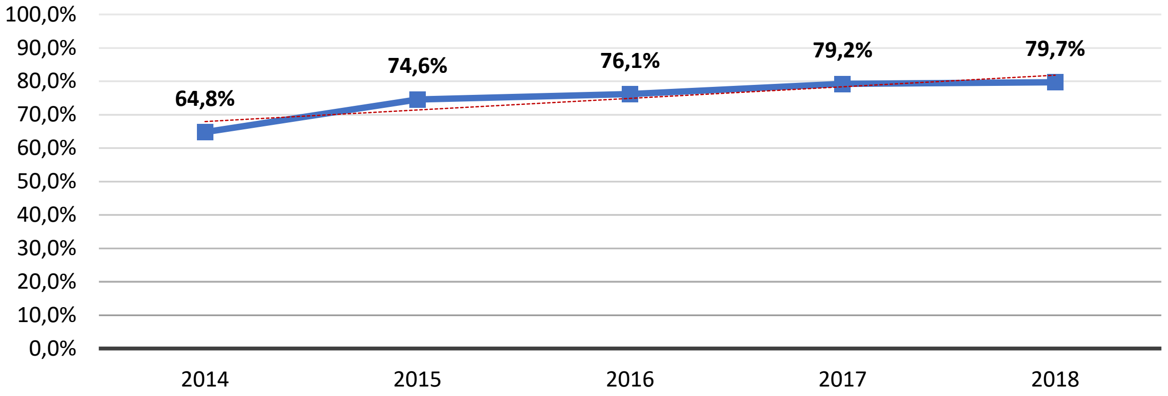
The results revealed an overall herd prevalence of 4.87% (CI 3.98–6.00), distributed heterogeneously in the state of Paraná. Regions 1. Noroeste (13.14%) and 2. Centro-OesteNorte (9.6%) had the highest prevalence in the area while regions 6. Leste-Sul (1.19%) and 7. Sudoeste (2.4%) had the lowest (Table 2). The differences can be partially attributed to different production systems and practices within each region, as discussed below. Also, a kernel density model (Fig. 3) has shown an intense variation of infected farms across the areas of the state of Paraná.

The geographical pattern of higher herd prevalence of brucellosis in the northern areas of the state of Paraná when compared to its southern areas mirrors the prevalence of their bordering states, that is, São Paulo and Santa Catarina, respectively. In São Paulo, the bordering state in the north, herd prevalence was estimated at 10.2% (Dias et al., 2016), comparable to regions 1. Noroeste and 2. CentroOeste-Norte of Paraná, while a herd prevalence of 1.2% was reported by the bordering state in the south, Santa Catarina (Baumgartem et al., 2016), comparable to regions 6. Leste-Sul and 7. Sudoeste of Paraná. Bordering regions share some features as same climate and types of production and hold a higher rate of animals’ trade within its area, which may explain those spatial patterns. Argentina and Paraguay reported similar overall cattle prevalence — 2.1% and 3.15%, respectively (Aznar et al., 2012), compared with 2.24% in Paraná.

Animal prevalence (Table 3) tends to follow the same distribution seen in herd prevalence (Table 2). However, its lower values do not allow confirmation that differences observed between regions are statistically significant.

Herd prevalence at the state level was 4.87 (CI 95% 3.98–5.93) and animal prevalence was 2.24 (CI 95% 1.47–3.41). Thus, no major change was detected when comparing that figures to those of a similar study conducted in 2002 (Dias et al., 2009) when those authors reported herd prevalence being 4.02 (CI 95% 3.23–4.80) and animal prevalence 1.73 (CI 95% 1.10–2.36), as shown in Fig. 4. Since 2001, the PNCEBT has introduced a range of measures to control cattle brucellosis in Brazil (Brasil, 2001), including mandatory vaccination of all female calves. Regardless of this, the vaccination coverage has grown slowly in most states of Brazil (Brasil, 2020). In Paraná, vaccination levels in female calves increased from 64.8 to 79.7% between 2014 and 2018 (Fig. 5). Even considering this increasing pattern, limited immunization at the population level may partially explain why no decrease in brucellosis prevalence was observed in this period. Different levels of vaccination will lead to different intensity in herd prevalence decrease (Amaku et al., 2009). This is of particular relevance to areas with high prevalence, in which successful immunoprotection tends to reduce prevalence levels (Barddal et al., 2016; Inlamea et al., 2016). To better understand the role of vaccination, further analysis of prevalence of brucellosis and vaccination coverage in each area of the state is recommended. Also, it is worthwhile to consider that higher levels of vaccination may produce a slightly higher number of false positive when using BPAT and FPA testing protocol (Nielsen et al., 1996; Gall and Nielsen, 2004).

Fig. 5 Brucellosis vaccination coverage of female calves in the state of Paraná between 2014 and 2018 (Brasil, 2020)



Conversely, the southern area of Paraná (regions 6 and 7) will benefit much less from a reliable vaccination program due its low prevalence of brucellosis. In these areas, prevalence is as low as that observed in the state of Santa Catarina (Baumgartem et al., 2016), where brucellosis vaccines are forbidden as a general rule and a surveillance program aimed at eradicating the disease is the current strategy. The network surveillance in this state includes slaughterhouse serum sampling, milk pool sampling in every dairy herd, and investigation of epidemiological links of infected herds. Each infected herd is fully tested twice, and positive animals are eliminated before restrictions may be lift. A test-and slaughter strategy has been used worldwide in countries that successfully eradicated the disease (Zhang et al., 2018).

Risk factors identified in multivariate logistic regression were herd size and frequency of testing animals for brucellosis (Table 4). Herd size seems to play an important role in brucellosis transmission as larger herds are concentrated in areas with higher prevalence. While herd size is a relative concept, its association with brucellosis has been demonstrated over a diverse range of situations (Crawford et al., 1990). Although animals within large herds are not intrinsically more susceptible to infection, there are aspects associated with large-sized herds which may facilitate the transmission of brucellosis. Farms running larger herds are more likely to source replacement animals externally and from different origin, which increases the risk of introducing *B. abortus.* Also, more complex and intense relationship between animals tends to occur within larger herd size, resulting in wider opportunities to transmission events occur (Ossada et al., 2013). Herd size has been described as a major risk factor in many other states in Brazil. Using massive datasets from different areas of Brazil, de Alencar Mota et al. (2016) concluded that larger herds that purchased replacement stock pose greater risk regarding brucellosis.

Routine testing of animals for brucellosis allows assessment of herd status and control of the disease. Our data indicated that of the 778 farms where brucellosis is tested regularly, 33 (4.29%) were classified as positive, while in the 979 farms where testing was not regularly performed, 60 (6.12%) were classified as positive. The state of Paraná accounts for 30% of all brucellosis tests in Brazil, which corresponds to roughly 3 million tests performed between 2014 and 2018 (Brasil, 2020). Nevertheless, it is recommended that further analysis is undertaken to ascertain how the current testing protocol impacts on disease control. The adequate identification of positive animals and their culling should be followed by a consistent surveillance program in their farm of origin. All dairy herds must be annually tested for brucellosis following a policy enforced by authorities (PARANA, 2017) and in our sample, 572/769 (74.38%) dairy herds reported regular animal testing, while only 54/505 (10.69%) beef herds reported regular testing for brucellosis. Accordingly, beef cattle had a higher herd prevalence (Table 4) and this is in line with reports from Argentina (Caione et al., 2010) and other states of Brazil (de Alencar Mota et al., 2016).

In a previous study of risk factors for brucellosis in Paraná, purchase of breeding cattle and pasture rental were described as risk factors (Dias et al., 2009), and in their univariate analysis, the authors reported that the herd size had a high correlation ($p < 0.001$) with the disease. However, the influence of brucellosis testing was not assessed in this previous study. Our data did not show any relationship to prevalence of brucellosis for the variable pasture rental ($p = 0.78$).

Given the limitation of cross-sectional studies to detect risk factors (DOHOO et al., 2003), it is our recommendation that longitudinal studies are conducted to further explore risk and causal effects. As well, consideration should be given to the fact that transversal studies will present limitations in detecting eventual reduction of brucellosis prevalence in regions 3, 4, 5, 6, and 7, due to their low level of disease prevalence and their respective confidence intervals.

In conclusion, it is recommended that policies for brucellosis control include a consistent vaccination program, especially in areas of high risk and/or higher prevalence, thus including beef and larger herds. All steps related to immunization of the herds should be verified and improved by training and education, from the adequate maintenance of cold chain to the correct inoculation of cattle. Furthermore, a strong health education action must be implemented, informing farmers about the risks of introducing animals into their herds without testing them. In areas exhibiting low levels of disease prevalence (regions 6 and 7), the state should assess the possibility of implementing eradication strategies. New approaches should be adapted to access eventual changes in the pattern of this, including those based on surveillance system.

2.6 ACKNOWLEDGEMENTS AND DECLARATIONS

The authors wish to thank all field workers from Paraná agribusiness defense agency (Adapar) who contribute to this research.

Author contribution Conceptualization: M.M., O.S.B., J.H.H.G.F., R.A.D., M.B.H., E.O.T., V.S.P.G., J.S.F.N., and F.F.; analyzed data: D.L.R.; performed research: D.L.R. and E.A.A..; wrote paper: D.L.R..; review and editing: J.S.F.N., V.S.P.G., and C.H. All authors have read and agreed to the published version of the manuscript.

Data availability The following raw data are available online at https:// data.mendeley.com/datasets/3ntpkdvscc/2,TableS1: Brucellosis_Parana_Animal_ Prevalence, Table S2: Brucellosis_Parana_Herd_Prevalence, Table S3: Risk Factors Brucellosis—NoGeo.xlsx, and Table S4: Variables key.pdf.

Declarations Ethics approval The manuscript does not contain clinical studies or patient data and the research was approved by the Ethic Committee in the Use of Animals of School of Veterinary Medicine and Animal Science under protocol number CEUA 3253011018.

Conflict of interest The authors declare no competing interests.

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3 APPARENT PREVALENCE AND RISK FACTORS FOR BOVINE TUBERCULOSIS IN THE STATE OF PARANÁ, BRAZIL: AN ASSESSMENT AFTER 18 YEARS SINCE THE BEGINNING OF THE BRAZILIAN PROGRAM

Tropical Animal Health and Production (2022) 54:360
https://doi.org/10.1007/s11250-022-03350-0

REGULAR ARTICLES

Apparent prevalence and risk factors for bovine tuberculosis in the state of Paraná, Brazil: an assessment after 18 years since the beginning of the Brazilian program

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Received: 16 May 2022 / Accepted: 12 October 2022 / Published online: 24 October 2022

3.1 ABSTRACT

Bovine tuberculosis (bTB) impacts considerably animal production and one health worldwide. To describe the prevalence,risk factors, and spatial pattern of the disease in the state of Parana, Brazil, a cross-sectional study was conducted from September 2018 to February 2019. The area was divided into seven regions. Within each region, farms were randomly selected,and a predetermined number of cows was selected and tested by a comparative cervical tuberculin test. 17,210 animals were tested across 1757 farms. Herd prevalence of bTB-infected herds in Parana was 2.5% [1.87–3.00%]. It has varied from 0.8 to 3.98% among seven regions, with clustering being detected in the west, central, and northeast areas. Animal prevalence was 0.35% [0.21–0.59%] and has varied from 0.08 to 0.6% among the pre-set regions. No major shifts in the prevalence of bTB were detected since 2007. Large-sized herds, dairy production, and feeding with whey were detected to be correlated with the presence of bTB. Exclusively among dairy herds, veterinary assistance from cooperatives, possession of self-owned equipment to cool milk, and feeding with whey were correlated with the disease. Considering these results, it is recommended that the state of Parana seek to implement a surveillance system for the detection of bTB-infected herds transforming them into free ones, if possible, incorporating elements of risk-based surveillance. Health education is also recommended to inform farmers about the risks of introducing animals without testing and of feeding raw whey to calves.

Keywords Tuberculosis. Prevalence. Risk factors. Paraná. Brazil. Cattle

3.2 INTRODUCTION

Bovine tuberculosis (bTB) is a bacterial zoonosis caused by *Mycobacterium bovis* that is responsible for considerable losses in the dairy and beef sectors of cattle industries. Direct economic losses from bTB in animal production can be quantified in terms of the reduction of milk and meat production, condemned carcasses, and organs at the slaughterhouses and, eventually, cattle mortality. Cows found positive for the intradermal test were reported to produce 10% less milk on average, ranging from a reduction of 0.5% and 14.6% (Boland et al., 2010). Additionally, the World Health Organization states that at least 140,000 cases of human tuberculosis caused by *M. bovis* are reported annually around the world (WHO, 2021). Therefore, in addition to the animal health costs and animal welfare impacts, the effect of bTB on international trade and human health should be further considered to provide a more precise assessment of the burden of the disease (Azami and Zinsstag, 2018). These reasons have led many countries to implement measures to control or eradicate bTB.

Few countries have managed to eradicate the disease. Australia was considered free in 1997 and the path followed was described by More (2015). Netherlands, Sweden, Denmark, Finland, Estonia, Czech Republic, Latvia, Lithuania, Luxembourg, Slovakia, and Canada are considered officially free of bTB in cattle (OTF), with no cases registered in cattle for many years, while France, Germany, Austria, Poland, Belgium, Hungary, and Slovenia, are also OTF, but with few cases reported annually (EFSA - European Food Safety Authority, 2019). According to the World Organisation for Animal Health (WOAH), a country can declare itself free of bTB if it demonstrates through a surveillance system that in the last three years 99.8% of the premises were tested and found not infected (OIE, 2019).

On the other hand, many other countries have implemented strategies but with poor or unreported results. In some of them, economic and political constraints still play an important role and can limit the efforts to control bTB. In other cases, wildlife reservoirs have proved to be an obstacle to sustainable free status. Badgers in the United Kingdom, wild boar in parts of the EU, white-tailed deer in the United States of America and brushtail possums in New Zealand are good examples of how those non-domestic bTB reservoir species are a barrier to control of bTB. Programs for control and eradication of bTB consist mainly of detecting infected herds and animals through surveillance systems, culling test-positive animals, and movement restrictions of infected herds. Some countries have additionally included control schemes for wildlife reservoir species.

The economic benefits of such programs should take into account the complexity of the direct and indirect impacts, both on animal and human health. A systematic review of the economic benefits of bTB control has shown many gaps of knowledge in this field but concluded that this classical approach using surveillance, culling and movement restrictions is still viable and worth doing in the long-term scenario (Caminiti et al., 2016).

In Brazil, a national program for the control and eradication of bTB (PNCEBT) was established in 2001 (Brasil, 2001) and during its first years, herd and animal prevalence of the disease was obtained in many states of the country, ranging from

1.3% to 9.0% and from 0.03% to 1.3%, respectively (Bahiense et al., 2016; Barbieri et al., 2016; Silva et al., 2016; Veloso et al., 2016; Vendrame et al., 2016; Dias et al., 2016; Galvis et al., 2016; Guedes et al., 2016; Lima et al., 2016; Néspoli et al., 2016; Queiroz et al., 2016; Ribeiro et al., 2016; Rocha et al., 2016). In the state of Paraná, a study between 2005 to 2007 revealed an infected herd prevalence of 2.15% and an animal prevalence of 0.42% (Silva et al., 2016). Those studies indicated that the introduction of new animals to a herd without testing was a major risk factor for bTB in herds in Brazil (Ferreira Neto et al., 2016), while the presence of wild animal species on farms has not been associated with the disease so far.

Understanding the epidemiology of bTB is fundamental for the development of its control strategies. The efficiency of such strategies should be verified continually (Thrusfield and Christley, 2018). Resources expended and their respective results should be assessed to maximize the benefit-cost ratio of any measures adopted and if necessary alternative approaches should be explored. Considering all the measures used to control bTB in Brazil for 18 years between 2001 and 2018, it is necessary to evaluate their effectiveness. Thus, this study aimed to (i) assess the herd and animal prevalence of bTB in the state of Paraná, Brazil after 18 years of the launch of PNCEBT (ii) assess those parameters in seven areas of the state, (iii) compare results/prevalence with previous studies and, (iv) undertake an analysis of risk factors for bTB in cattle in this state.

3.3 MATERIALS AND METHODS

This study was conducted in cooperation with Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA), the Collaborating Centre on Animal Health of the Faculty of Veterinary Medicine and Animal Science of the University of São Paulo (FMVZ/USP), Epicentre – Massey University, New Zealand and the Animal Health Agency of the State of Paraná (ADAPAR). Fieldwork was carried out from September 2018 to February 2019 by ADAPAR.

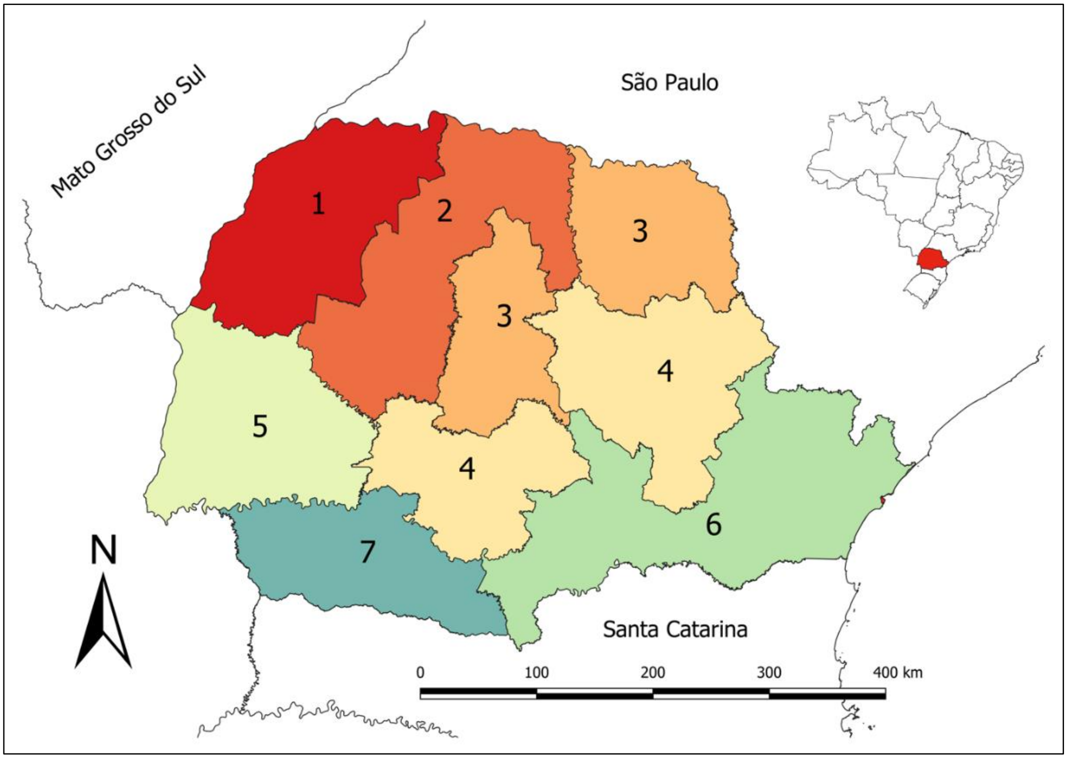
3.3.1 Study population

The target population was breeding female bovines and buffalos (for this text referred simply as cattle or bovine) over 24 months of age. The state has a cattle population of 8,397,219 individuals, from which 4,126,775 are cows (i.e., target population) (IBGE, 2017). It represents 4,8% of the cattle in the country and has the third largest dairy production by state.

3.3.2 Sampling

For sampling, the state was divided into seven administrative regions (Figure 1) to characterize differences between production systems, management practices, breeds, average herd size, marketing methods, and sanitary practices. A cross-sectional study was performed in each region to investigate the prevalence of bTB-infected herds and animals by using a two-stage sampling method (Thrusfield and Christley, 2018). In the first stage, a predetermined number of farms with cows over 24 months were randomly selected (primary sampling units). In the second stage, within each selected farm, a predetermined number of female cattle aged over 2 years were selected (secondary sampling units).

Figure 1 Map of the state of Paraná divided into seven regions: 1. Noroeste 2. Centro-Oeste-Norte; 3. Norte pioneiro; 4 Centro-Sul; 5 Oeste; 6 Leste-Sul; and 7.Sudoeste, 2019



The sample size of properties to be accessed in each region was estimated in accordance with the formula for simple random samples for large populations (Thrusfield, 2018):

$$\boldsymbol{n = \frac{Z_{\alpha}^{2} * P * (1 - P)}{d^{2}}}$$

n being the sample size, *Z* the normal distribution value at α level of confidence, *P* the expected prevalence and *d* being the absolute error.

Sample size parameters were: 0.95 confidence level, 0.2 of expected apparent prevalence and 0.05 absolute error.

An exhaustive alphabetically sorted list of farms in each region was produced for the first stage of the sampling scheme. Then, systematic random sampling was applied. When the selected unit needed to be replaced, the next available propriety in the list was sampled.

In each randomly selected farm, a minimum number of cows over 24 months was tested to classify it as an infected or not infected herd. Freedom from disease formula was applied (Sergeant, 2018) to estimate if a farm was infected. The following parameters were used: the value of sensitivity and specificity of the test protocols (animal level) were 0.775 and 0.995 respectively (Lôbo, 2008), 15% of intra-herd prevalence and an aggregate sensitivity and specificity greater than or equal to 0.90 (herd level). Considering those values, all cows were sampled in properties with up to 20 cows, 20 animals were tested on farms with 21 to 99 cows and 40 cows were sampled in properties with over 100 cows. The cut-off number of cows to define a property as infected for properties up to 99 cows was 1 infected animal (i.e., farms with 1 or more positives were considered infected) and for properties over 100 cows, the cut-off was 2 infected animals.

3.3.3 Test protocol

A delayed hypersensitivity skin test using purified protein derivative (PPD) was used to test the selected cows. A comparative cervical skin test was undertaken using bovine and avian tuberculin simultaneously, with the test read after 72 hours of inoculation, as described by OIE and PNCEBT (Brasil, 2017; OIE, 2019).

3.3.4 *Calculation of apparent prevalence in herds and animals*

The apparent prevalence of infected herds was calculated for each region as the proportion of positive herds divided by the number of tested herds. The apparent herd prevalence in the state of Paraná was based on the relative weight of each region as follows:

$$Herd\ weight\ = \frac{FR}{FSR}$$

where FR is the number of farms within the region and FSR is the number of farms sampled in the region.

When calculating the animal prevalence in each region, we applied the following expression as the weight of each animal within that region:

$$Animal\ weight = \frac{CF}{CSF}$$

where CF is the number of cows in the farm and CSF is the number of cows sampled in the same farm.

In order to calculate apparent animal prevalence in the Paraná, each sampled cow has its weight given by the following expression (Dohoo et al., 2003):

$$Animal\ weight\ for\ state\ level = \frac{CF}{CSF} * \frac{CR}{CSR}$$

where: CF = number of cows on the farm; CSF = number of cows sampled on the same farm; CR = number of cows in the region; CSR = number of cows sampled in the region.

Herd prevalence in previous studies was compared with current values using a proportion test. Statistical analyses were performed using R (R Core Team, 2019). Confidence intervals (CI) were obtained by the binomial logistic regression model.

3.3.5 *Spatial analysis*

A point pattern analysis to investigate density variation of positive farms was performed applying kernel density estimation applying the *density* function of the *stats* package of R (R Core Team, 2019) and bandwidth was selected according to the sampling density. Considering that sampling density was different among the 7 regions, a weighting process was applied to allow comparison across the whole state of Paraná. Sampling density in each of the seven regions was calculated and applied accordingly to undertake a pattern analysis across the whole state of Paraná (Wang et al., 2013). Next, a hypothesis test of significantly increased prevalence at a confidence level of 90% was conducted (Hazelton and Davies, 2009).

3.3.6 Study of risk factors

Farm owners or farm managers answered a questionnaire. Questions included the number of bovines and buffalos by age and sex category, management, and operation type, production practices, breeds, purchase, and sale of animals, use of calving pasture, indirect contact with other properties, presence of other domestic and wild species and veterinary care. All herds sampled provided one set of answers each. Data were collected in a face-to-face interview during the same visit to collect samples, using an electronic device and then uploaded into an electronic spreadsheet.

Some variables with more than two possible answers were regrouped in some cases for a more meaningful analysis. The number of cows over 24 months in a herd was used as a measure of herd size, and the 3rd quartile was the cutoff point for categorization in two groups (small and large-sized herds).

All potential risk factors were submitted to univariate analysis regarding the farm bTB status applying the Chi-squared test or Fischer’s exact test. A conservative *p-value* of 0.2 or less was used as criteria to select which variables would be used in multivariate logistic regression, then a backward process was used to eliminate the variables statistically not significant (Silva Abreu et al., 2009) and the Akaike Information Criterion (AIC) was applied to determine the best fitness. Considering the results obtained, we also applied the same methodology exclusively to the dairy farms in the sample. Risk factors analyses were performed using R (R Core Team, 2019).

3.4 RESULTS

Table 1 provides information regarding the target population and samples per region. Table 2 shows the apparent prevalence of bTB-infected herds in the state of Paraná.

Table 1 – Population and sample data of the study per region in the state of Paraná, Brazil, 2019

Region	Nº of municipalities	Total herds with reproductive activity	Total cows	Herds sampled	Sampled cows
1. Noroeste	57	18875	763643	251	3406
2 Centro-Oeste-Norte	78	15533	488045	250	2673
3. Norte pioneiro	80	28133	795964	253	2352
4. Centro-Sul	32	26011	729383	251	2692
5. Oeste	52	23153	583813	251	2147
6. Leste-Sul	56	18697	182131	251	1450
7. Sudoeste	44	32786	583796	250	2490
Total	399	163188	4126775	1757	17210

Table 2 – Apparent prevalence of bTB-infected herds in the state of Paraná, 2019

Region	Herds Sampled	Herds positive	Prevalence (%)	CI (95%)
1. Noroeste	251	6	2.39	[0.49 – 4.28]
2. Centro-Oeste-Norte	250	9	3.6	[1.28 – 5.91]
3. Norte Pioneiro	253	6	2.37	[0.49 – 4.25]
4. Centro-Sul	251	10	3.98	[1.55 – 6.40]
5. Oeste	251	8	3.18	[1.01 – 5.36]
6. Leste-Sul	251	5	1.99	[0.25 -3.72]
7. Sudoeste	250	2	0.8	[0 – 1.90]
Paraná State	1757	46	2.5	[1.87 – 3.0]

The spatial density of infected farms in the state varied between 0.5 to 2.5 for the bandwidth selected and was higher in the east and western regions of Paraná

(Figure 2). Also, it was detected some areas with significantly higher prevalence at a 90% confidence level (Figure 3).

Figure 2 Spatial density of the bTB-infected farms across the state of Paraná, Brazil,2019

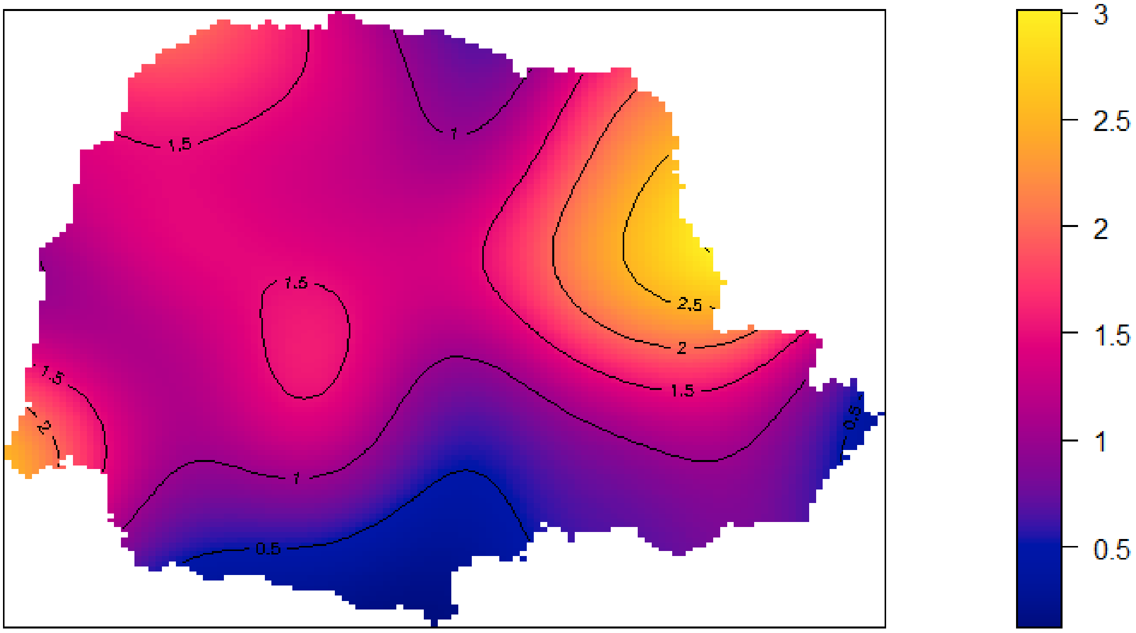


Figure 3 Areas with a significantly higher prevalence of bTB at a 90% confidence level across the state of Paraná, Brazil, 2019

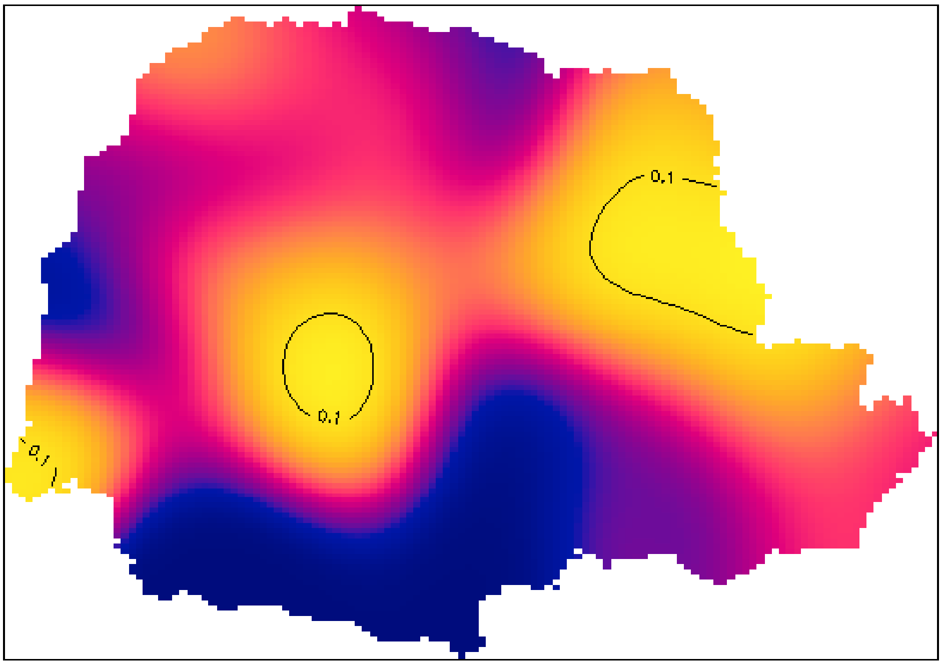


Table 3 shows the apparent prevalence of bTB-infected herds in the regions, stratified by production type, and Table 4 reports the apparent prevalence of bTB in cows in the state of Paraná.

Table 3 – Apparent prevalence of bTB-infected herds in the regions of the state of Paraná, stratified by type of production, 2019

Region	Beef		Dairy		Mixed	
	Prevalence%	**CI (95%)**	**Prevalence%**	**CI (95%)**	**Prevalence%**	**CI(95%)**
1	0.99 (1/101)	[0 – 2.92]	3.26 (3/92)	[0 – 6.89]	3.44 (2/58)	[0 – 8.15]
2	4 (3/75)	[0 – 8.44]	5.76 (6/104)	[1.27 – 10.25]	0 (0/71)	[0 – 4.08]*
3	0 (0/100)	[0 – 2.92]*	4.16 (4/96)	[0.16 – 8.17]	3.5 (2/57)	[0 – 8.29]
4	4.10 (3/73)	[0 – 8.67]	4.95 (5/101)	[0.7 – 9.18]	2.59 (2/77)	[0 – 6.15]
5	2.27 (1/44)	[0 – 6.68]	4.8 (6/125)	[1.04 – 8.55]	1.21 (1/82)	[0 – 3.59]
6	2.70 (2/74)	[0 – 6.40]	4.16 (3/72)	[0 – 8.79]	0 (0/105)	[0 – 2.79]*
7	0 (0/38)	[0 – 7.39]*	1.11 (2/179)	[0 – 2.66]	0 (0/33)	[0 – 8.43]*

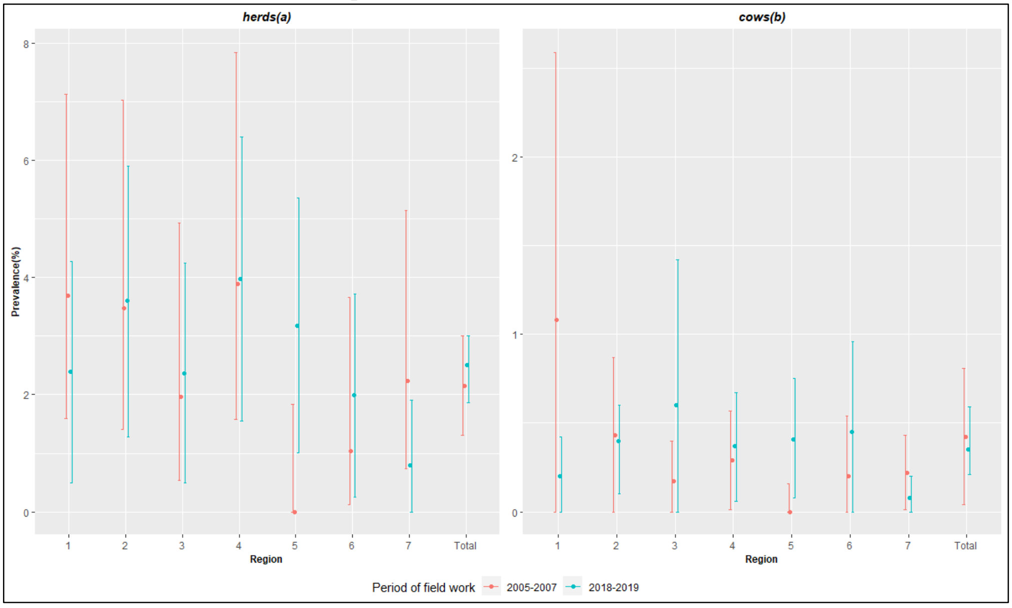
*Using beta distribution and Monte Carlo simulation

Table 4 – Apparent prevalence of bTB in cows in the state of Paraná, 2019

Region	Cows Sampled	Cows Positive	Prevalence (%)	CI (95%)
1. Noroeste	3,406	15	0.2	[0 – 0.42]
2. Centro-Oeste-Norte	2,673	14	0.4	[0.11 – 0.69]
3. Norte Pioneiro	2,352	21	0.6	[0 – 1.42]
4. Centro-Sul	2,692	13	0.37	[0.06 – 0.67]
5. Oeste	2,147	9	0.41	[0.08 – 0.75]
6. Leste-Sul	1,450	6	0.45	[0 – 0.96]
7. Sudoeste	2,490	4	0.08	[0.04 – 0.21]
Paraná	17,210	82	0.35	[0.21 – 0.59]

Figure 4 compares the results of the present study with the previous one, whose fieldwork was conducted between 2005 and 2007 (Silva et al., 2016).

Figure 4 Apparent prevalence of bTB-infected herds (a) and cows (b) in the state of Paraná in the studies of 2005-2007 and 2018-2019 (present one)



The variables selected by the univariate analysis (p≤0,20) were the type of farm, herd size (number of cows over 24 m), presence of any wild species, bTB diagnosis, the introduction of breeder in the last 2 years, slaughter bovines in the own farm, share only equipment with another farm, bovines concentrated in the shed, bovines concentrated in the salt trough, veterinary assistance, feeding with whey and milking technique. Table 5 the final multivariate logistic regression model for the state.

Table 5 –Results of final multivariate logistic regression model for bTB risk factors for farms from the state of Paraná, Brazil, 2019.

Variable and levels	**Odds ratio**	**95% CI**	***p-value***
Herd size (number of cows over 24 m)			
≤ 19 (3rd quartile)	base category		
> 19	2.11	1.14 – 3.91	0.01
Type of herd			
Meat and mixed type herd	base category		
Dairy herd	2.03	1.08 – 3.80	0.04
Use of whey to feed			
No	base category		
Yes	5.31	1.85 – 15.29	0.00

Considering the risks identified above and that bTB is mainly found in dairy herds, we further explored risk factors for dairy farms only (n=769; positive herds=29). Thus, we established production characteristics that were more like (p<0.2) to be seen in positive dairy farms: herd size (number of cows over 24 m), if their milk is sold to industry, veterinary assistance, feeding with whey, sort of milking and if the milk cooling equipment is self-owned. Next, it was fitted with a logistic regression model that better suited our dataset (Table 6), following the same methodology described previously.

Table 6 – Results of final multivariate logistic regression model for bTB risk factors for dairy farms from the state of Paraná, Brazil, 2019.

Variable and levels	Odds ratio	95% CI	*p-value*
Veterinary Assistance			
- No assistance or eventual private assistance	base category		
- Permanent veterinary assistance from Cooperatives	3.55	1.30 – 9.67	0.01

Milk cooling			
- No cooling or community coolers	base category		
- Using self-owned equipment	3.06	1.07 – 8.75	0.03
Use of whey to feed			
- No	base category		
- Yes	5.68	1.56 – 20.63	0.00

3.5 DISCUSSION

The apparent prevalence of infected herds among regions has ranged from 0.8 (region 7) to 3.98 (region 4) (Table 2), and there was a significant difference between regions 7 and 4 (p=0.02) and 7 and 2 (p=0.03). There is a tendency for lower values in regions 6 and 7, bordering the state of Santa Catarina (Table 2).

The overall apparent prevalence of bTB among herds in Paraná was 2.5% [1.87; 3.0] (Table 2), equal to that in the states of Bahia (Bahiense et al., 2016), Rio Grande do Sul (Queiroz et al., 2016), Mato Grosso do Sul (Guedes et al., 2016), Mato Grosso (Néspoli et al., 2016), Rondônia (Vendrame et al., 2016), Goiás (Rocha et al., 2016), Pernambuco (Lima et al., 2016) and Distrito Federal (Ribeiro et al., 2016), higher than that in the states of Santa Catarina (Veloso et al., 2016) and Tocantins (Ferreira Neto et al., 2021) and lower than that reported for São Paulo (Dias et al., 2016), Minas Gerais (Barbieri et al., 2016) and Espírito Santo (Galvis et al., 2016).

No significant changes occurred in the herd-level prevalence of bTB in the state of Paraná between 2007 (Silva et al., 2016) and 2019 (present study). However, when looking at each of the seven regions individually, a significant increase in positive herd prevalence from 0 to 3.18% was detected in region 5 (*p*=0.02). Considering the stability in the prevalence of bTB seen in the last 12 years, it is recommended that strategies adopted to control it should be assessed and reviewed.

It is useful to view those figures geographically and compare them with other states bordering with Paraná. Thus, in the Southern state of Santa Catarina herd prevalence ranges from 0 to 1.30% (Veloso et al., 2016), close to those found in southern areas of Parana: 0.8% (region 7) and 1.99% (regions 6) (Table 2). Conversely, in the Northeast areas (regions 2, 3 and 4) higher prevalence was detected (3.6%, 2.3% and 3.9%, respectively), and all of them bordering the state of

São Paulo which has the highest herd prevalence in Brazil – 9% (Dias et al., 2016). Thereby, the influence of adjacent administrative areas should be taken into account when defining strategies of control, considering especially the network of cattle trade in the area (Amaku et al., 2015).

Spatial analysis of the data suggests heterogeneity across the region, with the extreme West, Center and Northeast areas of Paraná showing a higher density of bTB-positive farms area (Figures 2 and 3). Those clustering areas tend to be an important source of bTB to other areas of the state and their cattle trade should be considered a higher risk.

Larger-sized farms, dairy-type herds and feeding whey to cattle were identified as risk factors for infection with bTB (Table 5). Herd size has been consistently identified as an important risk factor using different methodologies in different countries (Dias et al., 2016; Galvis et al., 2016; Néspoli et al., 2016). Production practices in larger-sized herds differ from the smaller ones and may explain this relationship. They are more likely to replace animals with purchased stock which increases the risk to introduce *M. bovis* (Gilbert et al., 2005)*.* According to our sample, from 822 smaller herds only 298 (36.25%) reported the introduction of bovines, while in the 935 larger herds, 458 (48.98%) did. This is in line with outbreaks investigations in Northern Ireland, which reported that 15% to 30% of them were caused by the purchase of infected animals (Menzies and Neil, 2000). In bTB, this risk factor is key to understanding the dynamics of the disease, considering that once infected, animals can potentially be a source of infection for their remaining life (Conlan, 2018). Producers may reduce that risk by carefully testing any bovines before introduction onto their properties.

The type of cattle farming enterprise is also a risk factor for herd bTB infection status. Cows in dairy farms are frequently reared in confined areas compared with beef farms which are more commonly managed outdoors. Milking parlours and sheds are areas within farms with high rates of contact between cows and direct transmission of *M. bovis* between cows occurs (Chopra et al., 2020). The main route of transmission of bTB is via the respiratory system (Cassidy, 2006) and therefore infected cows kept in high-density groups are more likely to infect other cows than those that are reared in extensive grass-fed beef systems. Accordingly, 3.8% (29/769) of dairy herds were bTB positive against only 1.7% (17/971) of beef or mixed ones. The data in Table 3 corroborate the importance of dairy farms in bTB transmission. In Brazil, dairy herds also presented an increased risk for bTB in the state of Rio Grande do Sul, Mato Grosso São Paulo and Santa Catarina (Dias et al., 2016; Néspoli et al., 2016; Queiroz et al., 2016; Veloso et al., 2016) and the same was verified in New Zealand (Porphyre et al., 2008).

A third factor found to be relevant was the use of whey to feed cattle. Milk derivates thermically unprocessed may be a route of infection for *M. bovis* (Conlan and Wood, 2018). It would be useful to investigate the source of this feed component. It is expected that the oral route of infection will produce lesions in the abdominal cavity (Neill et al., 1994), and while mesenteric lymph node bTB lesion is considered rare in developed countries, it still plays a considerable role in Brazil (Grisi-Filho et al., 2011; Ramos et al., 2018).

To better inform a risk-based surveillance programme for the state, the risk factors for bTB infection were analysed among dairy farms only. Previous evidence is that dairy farms that are more technically developed pose a greater risk to bTB (Ferreira Neto et al., 2016). Similarly, our model (Table 6) has shown that having their own cooling equipment at the farm, more intense assistance from veterinary services and the use of whey are characteristics associated with positive status for bTB. More intensively managed dairy farms are drivers for bTB as they tend to reduce the space available per animal on the farm and, as discussed previously, allow greater contact among animals (Chopra et al., 2020).

About veterinary care, it is important to highlight that our results show that properties belonging to cooperatives had a greater chance of being infected with tuberculosis, indicating that the disease is more concentrated in dairy cooperatives. Those production sets have different practices when compared with smaller producers, including a more intense trading pattern, which may explain this difference.

Given the limitation of cross-sectional studies to detect risks factors for diseases (Dohoo et al., 2003), especially in low prevalence scenarios, it is recommended to apply classic case-control studies to better individualize the risk factors for bTB in Paraná, aiming to provide greater certainty for the incorporation of risk-based surveillance elements into future strategies to fight the disease.

Considering these results, it is recommended that the state of Paraná seek to implement a surveillance system for the detection of bTB-infected herds transforming them into free ones, if possible, incorporating elements of risk-based surveillance.

Health education is also recommended to inform farmers about the risks of introducing animals without testing and of feeding raw whey to calves.

3.6 STATEMENT & DECLARATIONS

Acknowledgments The authors wish to thank all field workers from Paraná Agribusiness Defense Agency (ADAPAR) who contribute to this research.

Availability of data and material: The datasets generated during and/or analysed during the current study are not publicly available due to personal content but may be made available from the corresponding author at reasonable request.

Author Contributions Conceptualization, M.M., O.S.B, J.H.H.G.F, R.A.D, M.B.H, E.O.T., V.S.P.G., J.S.F.N., and F.F.; analyzed data, D.L.R.; performed research, D.L.R. and E.A.A..; Wrote paper D.L.R..; review and editing, J.S.F.N. and C.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding

Compliance with ethical standards The manuscript does not contain clinical studies or patient data and the research was approved by the Ethics Committee in the Use of Animals of the School of Veterinary Medicine and Animal Science under protocol number CEUA 3253011018.

Conflicts of Interest The authors declare no conflict of interest.

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4 TRADING NETWORK OF BOVINES AND ITS EFFECT ON THE SPREAD OF BRUCELLOSIS IN PARANÁ, BRAZIL

4.1 ABSTRACT

The network of cattle in Paraná in 2018 and 2019 was analysed to identify patterns that may influence in infectious disease dynamics and the status of the herd for brucellosis in 1,757 herds (nodes) was compared to their parameters in the network to understand their role as drivers of the transmission of the disease. The dataset of 608.807 shipments and their origin, destination, and number of bovines per shipment originates a network and allowed to describe the parameters of each node in the network and explore the formation of communities of traders. The parameters of nodes(farms) infected and non-infected by brucellosis were statistically compared using traditional techniques and quadratic assignment procedure (QAP). A multilinear regression model (MLR) was drawn to take in account risk factors previously described in the literature along with the network parameters. The network of cattle trade in Paraná in the period of study was formed by 115,296 nodes and is characterized by a high concentration of movements in a small proportion of farms – a scale-free network. All the parameters assessed were right-skewed, supplying evidence that hubs (more connected nodes) play a key role as a potential source of communicable diseases that rely on the movement of animals. The trading communities in Paraná follow a spatial pattern – closer farms are more like to trade. Brucellosis infected farms trade more frequently that non-infected farms ($p<0.001$), sourced cattle to others farms more frequently than expected (OR 2.12) and received more cattle than expected (OR 2.78). In-degree and out-degree were associated with the farm being infected by brucellosis. The mean shortest pathway between infected farms was 4.14 and for other pair of nodes was 4.49, being significatively shorter according QAP ($p=0.004$, OR 1.39). In the MLR, out-degree was associated with infected farms after accounting for herd size. This novel information provides insights into the drivers of the current endemic situation in the study area and better inform animal health policies for the future.

4.2 INTRODUCTION

Bovine brucellosis is a major reproductive disease, worldwide distributed. Some countries in Western and Northern Europe, Canada, Japan, Australia, and New Zealand are believed to be free from the causal agent (OIE 2020). This was achieved by animal health programs using different combinations of strategies, including measures to control animal movement (Zhang et al., 2018).

In Brazil, the disease is endemic in cattle and the prevalence was described in the main productive areas of the country (Ferreira Neto et al., 2016). More specifically, in the state of Paraná, the herd prevalence is 4.87% [CI 3.98–5.93%] and the animal prevalence is 2.24% [CI 1.47–3.41%] (Rodrigues et al., 2021).

Different productive practices of the cattle farms have been assessed in Brazil (de Alencar Mota et al., 2016) to understand the risk factors for brucellosis by applying cross-sectional studies. Farms that have sourced cattle outside their premisses and larger herds have been pointed as more prone to be infected according to questionnaires applied to farmers, but no evidence regarding the pattern of movement of cattle across the area was effectively explored.

Social network analysis (SNA) is a range of techniques that allows quantifying and qualifying the connection between entities (nodes) by the frequency or quality of their contact (links). It has been applied in veterinary epidemiology to explore the contact chain of animals – a central theme for the transmission of many infectious diseases (House and Keeling, 2011). Mapping the movement of potential sources of infection allows stakeholders to make more informed decisions regarding the risks of the spread of disease and enhanced risk-based surveillance (Amaku et al., 2015). Also, parameters that describe the connection between nodes may inform the main pathways of transmission of disease in a specific area and allow temporal comparison to verify if the network is changing over time (Cárdenas et al., 2020). Up to date, the network of cattle in most areas of Brazil, including the state of Paraná, was not described.

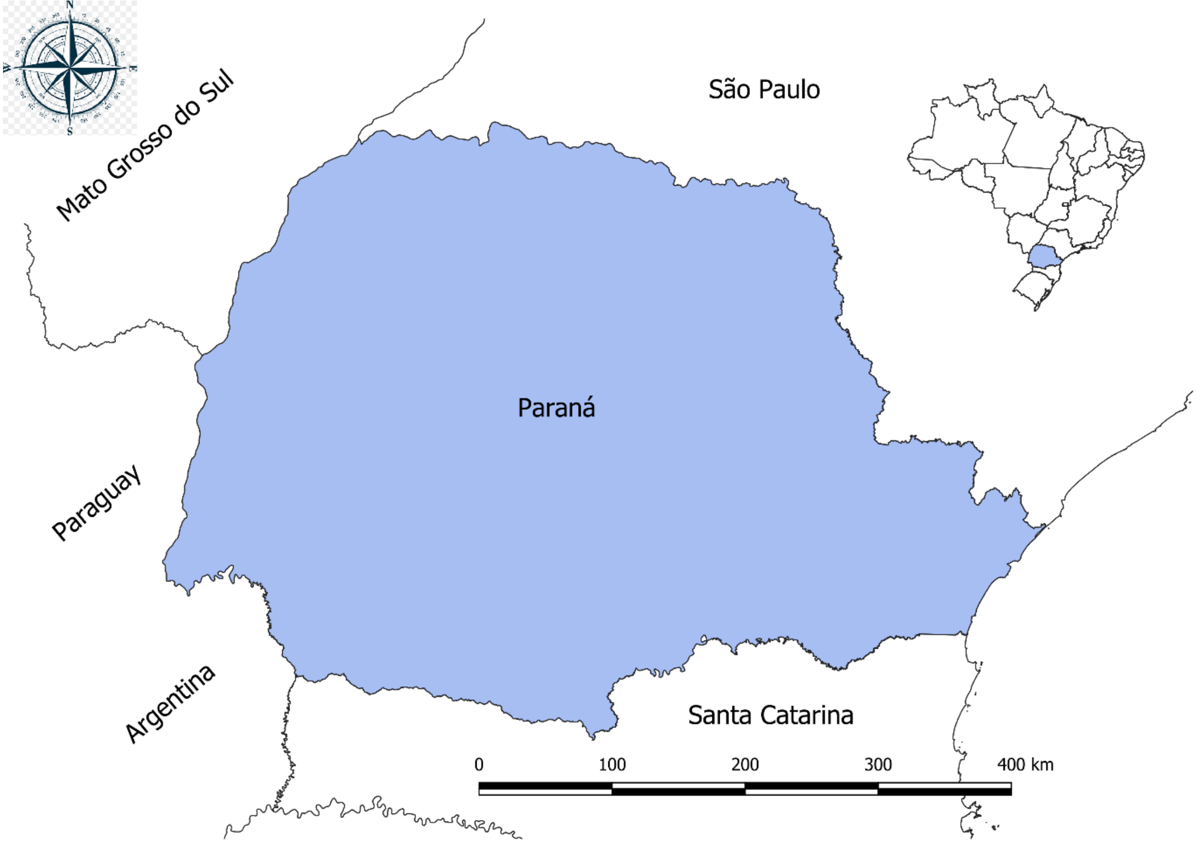
Considering this, we analysed the network of cattle trading in the state of Paraná in 2018 and 2019, and then investigate the spread of Brucellosis in the farms (nodes) with known status of the disease and the pattern of cattle movement.

4.3 MATERIALS AND METHODS

4.3.1 Study area and population

The area aimed was the state of Paraná, south region of Brazil (Figure 1). The target population was all its bovines and buffalos (in this text generically referenced as cattle) over 24 months of age. The region has a geographical area of 199,305 km^2 and a cattle population of 8,397,219 individuals, representing 4.8% of the national herd. Its bovine population is the eighth largest in the country and holds the third highest milk production in the country (IBGE 2017).

Figure 1 – Map of the state of Paraná, Brazil



4.3.2 Sampling to detect brucellosis status

In 2019, a random sampling was applied to detect herds seropositive for anti-Brucella antibodies. Among 1757 farms, 95 were found infected (Rodrigues et al., 2021). Their data was applied accordingly to explore Brucellosis dynamics in the network.

4.3.3 Database and network modelling

Cattle movements are subject to a mandatory registry in Brazil. Each shipment of cattle must be accompanied by one animal transit form (*Guia de Trânsito Animal* - GTA). The Official Veterinary Service of the state (Agência de Defesa Agropecuária do Paraná - ADAPAR) provided the database of all cattle movements originated in the state of Paraná from Jan 1, 2018, to Dec 31, 2019. This includes the information on the number of animals per shipment, specie, origin, destination, and purpose of the movement. Using this data, the static directed network of cattle movement was created– each farm in the dataset received a unique identifier and was considered a node in the network and each animal transported was considered a directed link between the origin and destination nodes.

4.3.4 Analysis of full network

Initially, we explored the full network (FNet), i.e. regardless of the status of brucellosis on the farm, calculating its parameters, verifying if the total degree fits a power-law distribution, and assessing the community's arrangement across the studied area. Power-law was assessed by calculating its α value and applying the Kolmogorov-Smirnof hypothesis test (KS.p), where a p-value <0.05 means the data doesn`t fit in power-law distribution (Clauset et al., 2009). As measures of the centrality, the following parameters were calculated: in-degree, out-degree, inward clustering coefficient, outward clustering coefficient, PageRank, betweenness, shortest pathway length, closeness in and closeness out (Table 1). R software was used for all analysis (R Core Team, 2022) unless otherwise stated.

Table 1 – Description of the network metrics

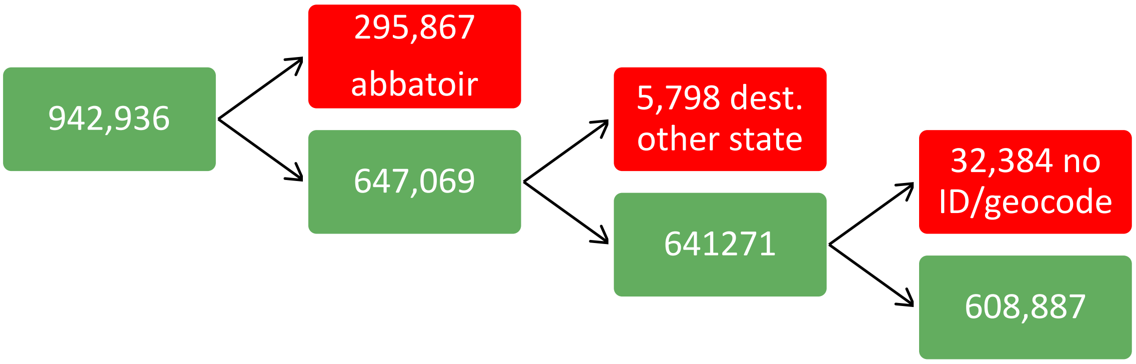
Metric	Description	Reference
In Degree	Assigns an importance score based on the number of inbound links by each node.	NA
Out Degree	Assigns an importance score based on the number of outbound links held by each node.	NA
Inward clustering coefficient	The local inward clustering coefficient of a vertex (node) in a graph quantifies how close its neighbours are considering inbound links of a directed network.	(Watts and Strogatz, 1998)
Outward clustering coefficient	The local outward clustering coefficient of a vertex (node) in a graph quantifies how close its neighbours are considering outbound links of a directed network.	(Watts and Strogatz, 1998)
PageRank	A link analysis algorithm that produces a ranking of importance for all nodes in a network with a range of values between zero and one. The PageRank calculation considers the indegree of a given premises and the indegree of its neighbors.	(Page and Brin, 1998)
Betweenness	A coefficient that indicates, for each node in the network, the number of shortest pathways between every pair of vertices that includes that node.	(Freeman, 1977)

Closeness in	Coefficient that represent how close a node is in relation to all other nodes of the network regarding the inbound links	(Freeman, 1978)
Closeness out	Coefficient that represent how close a node is in relation to all other nodes of the network regarding the outbound links	(Freeman, 1978)

4.3.5 Data Management

The original database has registered 942,936 shipments of cattle from Jan 1, 2018, to Dec 31, 2019, in the state of Parana, corresponding to 13,434,561 animals, an average of 14.24 animals per movement. Movements in which the destination was an abattoir were excluded (295,867). Were also excluded 5,798 movements destinated to other states. Finally, 32,384 movements destinated to events (i.e., temporary admission) with no geocoding nor ID available, were also excluded. Thus, the final database for the network modelling has 608,887 movements (Figure 2).

Figure 2 – Number of cattle trades in the state of Paraná from 2018 to 2019 and rationale of data filtering



4.3.6 Detection of communities

Strongly connected nodes in a network constitute a community. For the modelling of communities in the FNet, we used an algorithm that seeks to maximize the modularity of the network (i.e. the quality of the network partition) at a reasonable timeframe of computational processing as proposed by Blondel et al (Blondel et al., 2008).

We also have used the geocoding information of the farms to produce a visual presentation of the communities using Gephi software (Bastian M., Heymann S., Jacomy M. (2009).

4.3.7 Network of infected and non-infected farms and movement description

Network`s metrics of the brucellosis infected and non-infected farms were compared by Mann-Whitney test (conf. level=0.95, two-tailed test adjusted by Bonferroni method). Then, the movements of cattle on farms with known status for brucellosis were described and compared by one proportion Z-test (α 0.05) and odds ratio. Providing the network analysis was limited to farm-to-farm trades, movements towards temporary precincts (events, markets, and fairs) were described and analysed separately.

The shortest pathway length (Dijkstra, 1959) of infected and non-infected farms was analysed under the Quadratic Assignment Procedure (QAP) (Krackhardt, 1988). Standard tests of statistical significance rely on the assumption that observations are independent, but the dyadic observations in a network are inherently dependent and present some degree of autocorrelation. The QAP handle the issue, performing a more robust hypothesis test. The analysis was implemented by the *netlogit* function of the *SNA* package in R, applying 1,000 Monte Carlo simulations.

4.3.8 Multilinear logistic regression including previously detected risk factors

Finally, it was developed a logistic regression model of the status of farms regarding brucellosis as a function of three independent variables: network parameter of centrality, size of the herd, and if the farm regularly tests cattle for brucellosis. The last two were previously reported to be risk factors for brucellosis (Rodrigues et al., 2021). A backward process was used to exclude the non-significate (*p-value* > 0.05) variables and the pseudo R^2 criterion was applied to evaluate the best-fitted model.

4.4 RESULTS

4.4.1 Full network metrics

The network has 115,296 nodes, and their metrics are summarized in Table 2.

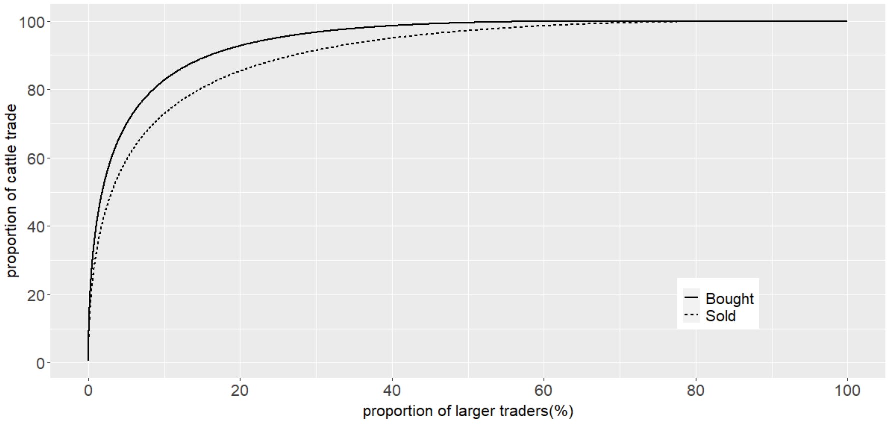
Table 2 – Network metrics of the cattle trade in the state of Parana, 2018 to 2019.

	1st Quartile	Median	Mean	3rd Quartile	Max. value
In Degree	0	5	79.71	30	45,379
Out Degree	4	14	79.71	48	46,221
Clustering In	0	0	0.08	0.07	1
Clustering Out	0	0	0.13	0.17	1
Page Rank	$1.791e^{-06}$	$2.380e^{-06}$	$8.673e^{-06}$	$4.811e^{-06}$	$4.545e^{-03}$
Betweenness	0	0	528,563	92,477	$7.3e^{8}$
Closeness In	$7.52273\ e^{-11}$	$7.52299e^{-11}$	$2.22424e^{-10}$	$3.79517e^{-10}$	$3.81499e^{-10}$
Closeness out	$1.45671e^{-10}$	$1.45712e^{-10}$	$1.31759e^{-10}$	$1.45722e^{-10}$	$1.45918e^{-10}$

The parameters are strongly asymmetric, right-skewed, except for the closeness out, which is left-skewed. The total degree follows a power-law distribution (α=2.69, p=0.73 - Kolmogorov-Smirnov test).

The trade of cattle is highly concentrated in a small proportion of farms. Taking the 10% larger sellers of cattle accounts for 73% of all sales and the 10% larger buyers account for 83% of all purchases of cattle (Figure 3).

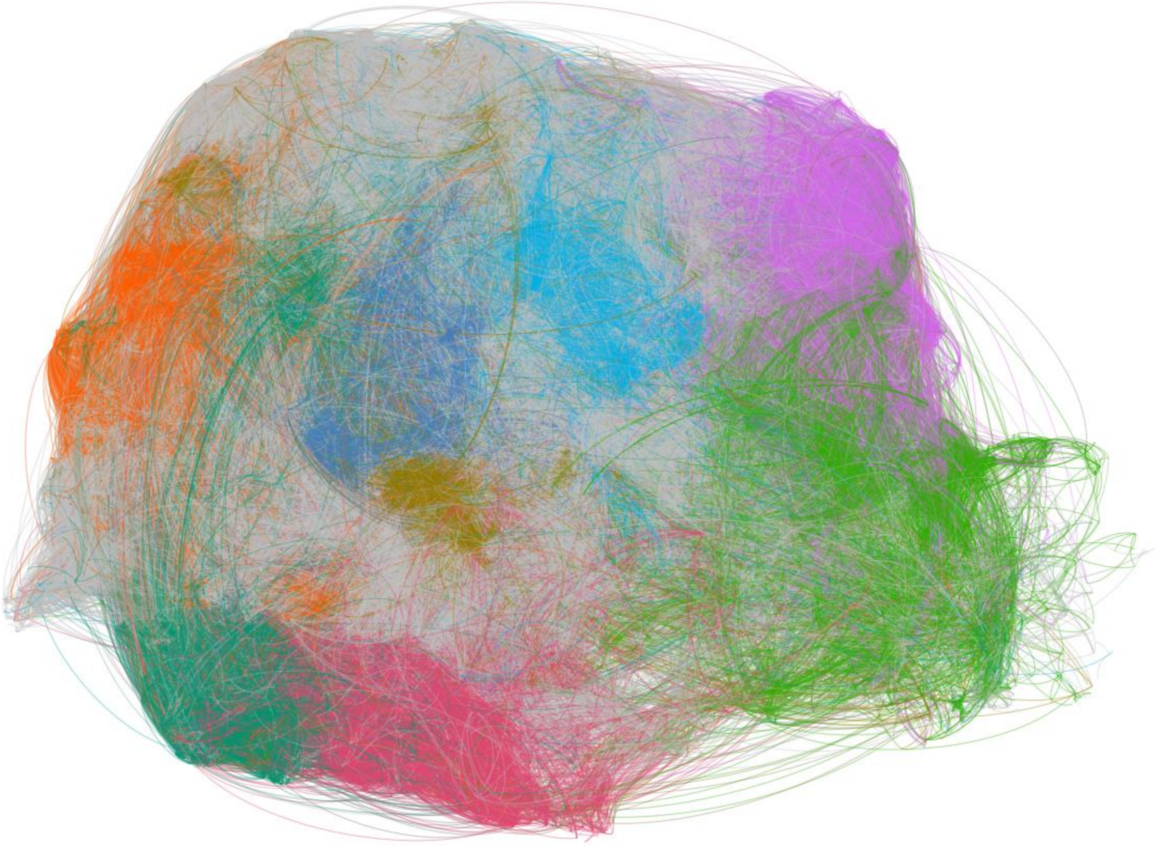
Figure 3 – Market proportion of the larger traders in cattle network in the state of Parana



4.4.2 Community detection

We have detected 2,272 communities of cattle trade under a modularity of 0,722. We have highlighted the eight larger communities, which include 55,36% of all nodes in the network (Figure 4).

Figure 4 – Eight larger communities for cattle trade network in the state of Parana in 2018 to 2019



4.4.3 Analysis of movements of cattle among farms with known status of brucellosis

The sum of all shipments involving at least one farm with known status for brucellosis was 20,143 for a total of 227,705 animals traded, an average of 11.30 animals per trade. Those 20,143 trades represent 3.30% of the total of 608,887 movements in the FNet in the relevant period.

The data reveals that 86 out of 95 (90,52%) farms infected with brucellosis had at least one trade of cattle in 2018 and 2019. Among the non-infected ones (1,662), this proportion was 72,56% in the same period (Figure 5). Thus, the proportion of traders among infected farms is higher than among non-infected (*p* <0.001).

Figure 5 – Proportion of traders among farms with known status brucellosis in the state of Parana, Brazil, in 2018 and 2019

Considering the estimated prevalence of brucellosis in herds (4.87%, (Rodrigues et al., 2021) it was then tested a hypothesis that infected farms send cattle to other farms more frequently than would be expected, based on the current prevalence in the region (Table 3). The null hypothesis was reproved (p < 0.001), thus infected farms trade more frequently than expected (OR =2.12).

Similarly, the hypothesis that infected farms get cattle more frequently than non-infected farms was tested (Table 3). Again, the infected farms have gotten more bovines than expected (p<0.001, OR=2.78).

Table 3 – Observed and expected shipments of cattle from and destinated to brucellosis infected farms in the state of Parana, Brazil, in 2018 and 2019

	Number of shipments from and to infected farms			
	Observed	Expected*	Odds Ratio	*p* value
From infected farms	1222	606	2.12	0.001
Destinated to infected farms	1147	447	2.78	<0.001

* Based in a herd prevalence of 4,87%

Regarding the behaviour of farms with known status of brucellosis when sending cattle to temporary precincts (markets, fairs, sports, and entertainment), infected farms also had a significant (p<0.001, OR 3.03) higher number of shipments than expected (Table 4).

Table 4 – Number of shipments of cattle from infected and non-infected farms towards temporary precincts (markets, fairs, sports, and entertainment)

Brucellosis status of farm of origin	Number of shipments towards temporary precincts	
	Observed	Expected*
Infected	47	17
Non-infected	309	339
Total	356	356

*Based in a herd prevalence of 4,87%

Then, when comparing the shortest pathway length between infected farms and the remaining pair of farms (Table 5), the QAP model revealed a significative difference (*p*=0.004) in those groups and an odds ratio of 1.39.

Table 5 – Shortest pathway statistics of infected and non-infected farms in the state of Parana, Brazil, in 2018 and 2019

	Shortest Pathway Length		
	Mean	SD	*p* value
Links between infected farms	4.14	0.87	0.004
Remaining links	4.49	1.06	

4.4.4 Network parameters of infected and non-infected farms

Parameters of the network for infected and non-infected farms are presented below (Table 6). Higher In-degree and Out-degree scores were strongly correlated with infected farms. Other parameters were not significatively different between the two groups.

Table 6 – Network metrics of brucellosis infected and non-infected farms in the network of cattle trade in the state of Parana

	Infected median	Non-infected median	Infected mean	Non-infected mean	*p* value
In Degree	11	3	180.5	76.94	0.006
Out Degree	46	15	245.3	92.43	0.000
Clustering In	0.00298	0	0.07318	0.05810	0.431
Clustering Out	0.06714	0.04590	0.13154	0.1143	0.121
Page Rank	$2.433e^{-06}$	$2.147e^{-06}$	$1.500\ e^{-05}$	$8.521e^{-06}$	0.166
Betweenness	6	0	1,200,582	1,090,916	0.159
Closeness In	$3.792e^{-10}$	$7.523e^{-11}$	$2.556e^{-10}$	$2.160e^{-10}$	0.061
Closeness out	$1.457e^{-10}$	$1.457e^{-10}$	$1.383e^{-10}$	$1.353e^{-10}$	0.999

4.4.5 Multilinear logistic regression for brucellosis infected farms including previously detected risk factors

When herd size effect and the regular testing of brucellosis in the farm are included in the model, the only network metrics that holds was out-degree (Table 7).

Table 7 – Multivariate logistic regression model for brucellosis infected farms in the state of Paraná, Brazil.

Variable and levels	Odds ratio	95% CI	P
Herd size (cows aged over 24m)			
Herd ≤ 8 cows (50th percentile)	base		
Herd ≥ 9 to ≤ 88cows	2.83	1.53-5.23	<0.01
Herd > 88 cows (95th percentile)	6.74	3.10-14.62	<0.01
Diagnosis			
Brucellosis regularly tested	base		
Brucellosis not regularly tested	2.77	1.72-4.48	<0.01
Out degree (categorized by median)			
Low out degree (≤17)	base		
High out degree (>17)	2.20	1.29-3.75	<0.01

4.5 DISCUSSION

4.5.1 Network of cattle trade in the state of Paraná

The network of cattle trade in the state of Parana is characterized by a high concentration of movements in a small proportion of farms – a scale-free network. All the parameters assessed were right-skewed, supplying evidence that hubs (more connected nodes) play a key role as a potential source of communicable diseases that rely on the movement of animals. Conversely, most nodes of the network trade a few cattle and, when they are eventually infected, it is reasonable to consider they pose a lower risk of transmission of the agent (Dubé et al., 2011). This pattern is commonly seen in other networks of animal trade, like in Northern Ireland (Brown et al., 2019), Italy (Natale et al., 2009), Uruguay (VanderWaal et al., 2016), and Denmark (Bigras-Poulin et al., 2007), but in Chile, the network follows a beta distribution (Alocilla and Monti, 2022). The meaning of this finding should not be taken lightly though: in a resourced-limited environment, the prioritisation of actions demands well-informed decisions and detecting hotspots for surveillance systems is becoming increasingly important.

It was detected that trading communities in Paraná follow a spatial pattern – closer farms are more like to trade, similar to findings of cattle network in Chile (Alocilla and Monti, 2022). More importantly, the main communities here presented contribute to understanding the variation of brucellosis prevalence within the state of Paraná (Rodrigues et al., 2021) and they may provide useful insights about compartmentalization or zoning areas toward free areas recognition in the future. The underlying background is that the chain of transmission of infectious agents exists within space - whom an individual spatially overlaps with defines who they can socially interact with, and more specifically, who can be infected when the host releases an infectious agent to the environment. Simultaneous spatial and network analysis have been only recently developed (Albery et al., 2021), but controversial evidence in cattle shows either that their movement between farms may be more important than the local spread between neighbours, as described for *Mycobacterium* subs. *Paratuberculosis* (MAP) in New Zealand (Marquetoux et al., 2016) or that introducing animals may be less important than other risk factors for brucellosis in Sicily, Italy (Calistri et al., 2013). More likely, those features overlap each other to a varying degree, as reported in a study of simultaneous risk factors for brucellosis and tuberculosis in Spain (Cowie et al., 2014). While the calf birth or abortion is crucial to *B. bovis* contaminating the environment within farms and surrounding areas, it is biologically plausible that the trading of infected cattle is the most important path to other farms. Thus, this mixed effect should be further explored. Meanwhile, endemic areas are primarily concerned about reducing the routes of the infectious agent between infected and non-infected farms. Here we introduced novel evidence that traders' communities in Paraná are geographically clustered, and this knowledge may be applied to developed local, personalised actions and policies. While this is the general rule for this network, we also detected that a few communities have a long-distance connection, creating a second centre geographically displaced from the main one. This potentially represents important commercial routes that follow a regional rationale and should be considered as well.

The static, two-year, network is a picture of the timeframe selected, 2018 and 2019 in this study. It is useful as a reference for future analysis, especially for chronic, endemic diseases. Since 2020, new policies regarding animal transit regulation have been put in place in Brazil, following its strategy to control foot and mouth disease. As a side effect, other disease dynamics, such as brucellosis, should be affected. Once those changes in the animal trade are consolidated, it is recommended to reassess the network and check for changes, if any. Also is recommended that temporal network analysis be performed, since the sequence of movements along the time cannot be detected properly in the static network and may over or underestimate the differences within it (Cárdenas et al., 2020; Lentz et al., 2016).

4.5.2 Movements of cattle in brucellosis-infected and non-infected farms

Our data revealed that infected herds proportionally purchased cattle more frequently than non-infected ones (OR of 2.78) when considering the current prevalence of brucellosis of 4,84%. It is hypothesized that each additional animal introduced from an infected area raises the probability of bringing a source of infection into the destination farm. It is a plausible explanation for the risk of brucellosis among farms. In Italy, a model to describe the vulnerability of its regions detected that the trade of cattle was decisive to the ongoing occurrence of brucellosis in the southern of the country (Darbon et al., 2018), and that is the case in the state of Paraná, where its prevalence remains the same after many years of implementation of a program of control (Rodrigues et al., 2021). Complementarily, it was detected that those infected herds have sold cattle more frequently (OR 2.1) than expected, which contributed to explaining the current situation in the area. When the surveillance system in place is not efficient in detecting the infected herds or measures of control are not implemented during the outbreak, the potential of the infected herd to source brucellosis to the next farm is enhanced by a high volume of cattle being sold. Evidence is that no compliance following outbreaks and a higher trading profile may contribute significantly to the development of brucellosis across any given region (Darbon et al., 2018). This picture may then be used to enhance the sensibility of the surveillance system by selecting more connected farms. Ultimately, a risk-based surveillance system in a scale-free network, like the one here described, is more like to identify infected farms by focusing on those more connected in the network (Amaku et al., 2015; VanderWaal et al., 2016). Markets and fairs are, in some sense, temporary hubs of the cattle trade. The high volume of animals in a short period in those events impacts some measures of the network, including the shortest pathway length between pairs of farms and the average shortest pathway length of the network, i.e., increasing the spread of sources of infection. We have detected that, proportionally, brucellosis premises sent cattle to those events more frequently than non-infected ones. Under certain circumstances, those events may be considered the main hubs of disease distribution (Alocilla and Monti, 2022), and in the network of the first outbreak of brucellosis in Sicily, the markets and staging points were frequently in the pathway between infected holdings (Savini et al., 2017). Thus, sanitary controls in the admission of animals in those places effectively reduce the risk of spreading brucellosis.

4.5.3 Comparison of network parameters

Here we have explored a range of node-level parameters in this network. The incoming and outgoing degrees were significantly correlated ($p<0.05$) with the holding being infected with brucellosis. Epidemiologically, a higher *degree* is a plausible explanation for holdings being more frequently infected by brucellosis and this relationship was similar to that found in Italy (Darbon et al., 2018).

Meanwhile, the out-degree parameter is proportional to the potential of amplifying the infection across the network (Brown et al., 2019). Betweenness, clustering in, clustering out, page rank, closeness in and closeness out were not significantly associated with brucellosis detected on the farm. These and many other parameters have been explored as predictors of risk for infectious disease, but frequently evidence points otherwise. Savini et al. reported that to fragment efficiently the cattle network, maximizing the isolation of brucellosis outbreaks with minimal impact on the network, in degree is a better parameter than out-degree, degree, betweenness, hub and authority (Savini et al., 2017) when selecting more connected nodes to be removed of the network. In their study, the six networks produced after the fragmentation were very different among themselves. For example, in-degree reduced its original network by 37% while limiting the reach of outbreaks in the fragmented network to only 4% of the original number of outbreaks, meanwhile, betweenness reduced it by 80% but its fragmented network still reaches 12% of the outbreaks. In Mato Grosso, Brazil, degree and out degree were associated with brucellosis, but ingoing contact chain, outgoing contact chain, clustering in, clustering out, closeness in, closeness out, page rank and betweenness were not (Cipullo et al., 2016). This highlights that parameters of centrality should be chosen wisely, considering their intrinsic features and any models or policies should be applied only after validation with real, empirical data. As SNA becomes more popular, the range of its methodologies and their respective application (and limitations) should be clarified (Sosa et al., 2021).

Examining the shortest pathway length between farms with known status of brucellosis in the network via QAP shows that this parameter is inversely proportional to the presence of brucellosis. Infected farms are topologically closer (shorter pathway) than other pairs of farms in the network. It is in agreement with findings in New Zealand, where the shortest pathway length between farms is positively correlated with them sharing the same strain of MAP (Marquetoux et al., 2016). This finding also highlights that investigating farms that connect two infected farms may also enhance the field investigation of outbreaks. In field conditions in Brazil, veterinary services normally only investigate direct contacts of the outbreaks, similarly to the Italian outbreak investigations (Savini et al., 2017). Further research may explore if this relationship between the shortest pathway and disease has an effective biological meaning or if it is an indirect effect of the infected holdings being more connected. Methodologically, the QAP here applied is a more robust hypothesis test for SNA (Krackhardt, 1988) , but its result agreed with the other classical hypothesis tests applied in our dataset, as presented in the previous sections.

Other risk factors have been reported for brucellosis in cattle. Our logistic regression indicated that the out-degree is positively correlated to infected herds, after considering the effect of two risk factors previously reported for brucellosis in the state of Paraná – herd size and regular testing of the herd. Bovines in large herds are not intrinsically more susceptible to infection, but this feature has been reported worldwide as correlated with infected herds (Calistri et al., 2013; Crawford et al., 1990; de Alencar Mota et al., 2016; Ogugua et al., 2018) – this relative concept of herd size needs though further clarification, once it contains a range of features within itself. For example, it is expecting a high correlation between herd size and trading frequency (Cipullo et al., 2016), and ultimately trading more may be an intrinsic part of larger herds. From a different perspective, it also means that other aspects must be considered beyond the cattle movement features when developing policies to control brucellosis; other pathways of transmission should also be explored accordingly.

Finally, in a practical sense, knowing that hubs are high-risk holdings is a source of risk-based surveillance (Cameron, 2012; Cárdenas et al., 2020; VanderWaal et al., 2016), enhancing the sensibility of the system. It is also a way to orient the outbreak analyses, especially when assessing data of movements into and from the farm under investigation. A scale-free network is particularly sensible when more connected nodes are affected (Brown et al., 2019), thus applying measures to those nodes as vaccination and movement restrictions are likely to produce better results (Dubé et al., 2011).

4.5.4 Limitations

The present study has some limitations that are worthy to be listed. First, the correct identification of the infected and non-infected farms is dependent on the features of the testing protocol used and its performance affects the analysis here produced. Namely, the sensibility and specificity of the protocol implicate some level of imprecision, even though the authors do not expect that to affect the conclusions presented. Secondly, the use of a static network over two years may introduce some level of bias, as the time flow is not considered, and some nodes may have their connectivity overestimated.

4.6 CONCLUSION

Networks aggregated over longer time scales are relevant for endemic, chronic diseases, such as bovine brucellosis. The present study concluded that the cattle trade in Parana, Brazil forms a scale-free network, highly concentrated in a relatively small number of farms and its communities are geographically aggregated. Higher levels of both global and farm-level connectivity are, generically, expected to be important indicators of the vulnerability of trade networks to infectious diseases (Keeling and Eames, 2005). Here we provided novel, empiric information that supports this assumption under certain conditions. In-degree, out-degree and shortest pathways are good candidates as parameters to identify brucellosis-infected farms, improving risk-based surveillance, but not every network parameter behaviour similarly. Any parameter should be considered alongside with further epidemiological information available and be validated with real data of disease occurrence. The information provided both gives insights into the drivers of the current endemic situation in the study area and better informs animal health policies for the future.

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5 CONCLUSÃO GERAL

A bovinocultura paranaense está distribuída em todo seu território, e a brucelose e tuberculose estão endemicamente associadas com esta atividade produtiva. Todavia, concluímos pelos dados aqui apresentados que esta população animal não está igualmente sujeita a ocorrência destas doenças. De fato, suas prevalências são heterogêneas tanto espacialmente como em função de tipos e práticas produtivas. Esta diferença pode e deve ser explorada por aqueles que procurem entender ou controlar estas enfermidades.

O tamanho do rebanho destaca-se como um fator importante para a ocorrência tanto de brucelose como de tuberculose. Em termos gerais, os maiores rebanhos estão sob maior risco e, portanto, servem como ponto de partida para a atuação assertiva no controle de ambas as doenças. Não se deve, entretanto, racionalizar de forma tão simplista estes graves problemas sanitários. Demonstramos que os tipos produtivos (leite, carne e misto) se comportam de forma diferente nas duas doenças, com rebanhos de leite mais associados a tuberculose e os rebanhos de corte mais associados a brucelose. Em seguida, continuamos a explorar diferenças de práticas produtivas e evidenciamos que as propriedades que testam seus animais para brucelose têm significativamente menos brucelose, após considerar o efeito de tamanho de rebanho e, no caso da tuberculose, o uso de soro de leite e maior tecnificação foram identificados como fatores de risco.

Ao explorar o trânsito bovino na região, descrevemos uma rede altamente concentrada, com hubs importantes tanto no envio como na aquisição de animais. As comunidades de comercialização de bovinos apresentam agrupamento espacial que confirma a tendência dos produtores em comercializar animais com propriedades mais próximas. Este padrão é significativo para entendermos o padrão de distribuição histórica de brucelose e tuberculose na região – observar e comparar o mapa de comunidades de comércio de bovinos e os mapas de densidade de prevalência de brucelose e tuberculose é um exercício estimulante para futuras pesquisas.

Ao longo dos capítulos 2 e 3 descrevemos as prevalências, tanto no nível estadual como nas sete regiões pesquisadas. Com base nas diferenças entre regiões, aprendemos que a comparação de prevalência em nível estadual (e seus respectivos intervalos de confiança) é limitada para descrever o avanço, estabilidade ou retrocesso deste indicador, pois mudanças de padrão da ocorrência das doenças em sentidos opostos em diferentes regiões podem mascarar a prevalência geral do estado e, doravante, as comparações regionais podem contribuir melhor para identificar variações, com uma melhor resolução da magnitude da mudança da ocorrência destas doenças.

A movimentação de bovinos é genericamente entendida como uma das principais formas de transmissão de doenças infecciosas entre rebanhos, e apresentamos evidências de que isto se aplica a brucelose no estado do Paraná ao analisar todas as movimentações de propriedades infectadas e não infectadas pela doença entre 2018 e 2019. As propriedades infectadas tendem a ser mais conectadas e esta diferença é significativa para a quantidade de animais enviados por estas propriedades (grau de saída), mesmo após considerar o efeito de tamanho de rebanho.

Por fim, ao entender que o presente trabalho compõe um panorama maior que é o esforço sistemático para controlar estas duas enfermidades no Brasil, temos esperança de que as políticas públicas e as práticas sanitárias individuais possam assimilar com sucesso o conhecimento científico que tem sido produzido em torno do tema, para benefício de toda a sociedade brasileira.

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