UNIVERSIDADE FEDERAL DE LAVRAS

ANNA CECÍLIA TROLESI REIS BORGES COSTA

RISK-BASED BOVINE BRUCELLOSIS SURVEILLANCE AND CONTROL: ASSESSMENT OF OUTBREAK, VACCINATION, AND CATTLE MOVEMENT IN MINAS GERAIS

LAVRAS - MG
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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós- Graduação em Ciência Veterinárias, área de concentração em Sanidade Animal e Saúde Coletiva, para a obtenção do título de Doutor.

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I dedicate this Thesis to my nephew Vinicius, my nice Alicia and my nephew Gabriel. Never underestimate what you can do. Always go beyond your limitations and do not fear new things and new knowledge. Love you!

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“There is nothing we can do that cannot be done with love” – author unknown.

RESUMO

A brucelose é uma doença de grande impacto para a pecuária e para a saúde humana em todo o mundo, afetando especialmente os países em desenvolvimento. No Brasil, o Programa Nacional de Controle e Erradicação da Brucelose e Tuberculose (PNCEBT) é responsável por regulamentar as medidas de controle e erradicação da doença, desde 2001. Entre os estados brasileiros, Minas Gerais é um dos mais importantes no que diz respeito à produção pecuária, sendo a brucelose bovina ainda prevalente entre os rebanhos, embora esteja diminuindo. Nesse sentido, o objetivo desta tese foi revisar a ocorrência da brucelose pecuária e a interface com a ocorrência humana em todo o mundo, combinada com o desenvolvimento de análises espaço-temporais e de rede como ferramentas para melhorar o controle da brucelose bovina em Minas Gerais, Brasil. Para tanto, foi realizada uma revisão da literatura sobre a ocorrência de brucelose pecuária em todo o mundo, seguida de uma análise espaço-temporal dos casos de brucelose bovina, testes de brucelose em bovinos, vacinação contra brucelose em bovinos e tamanho da população bovina em Minas Gerais, Brasil. Adicionalmente, foi realizada uma análise de rede com dados de movimentação de bovinos, também em Minas Gerais, Brasil. Os resultados desta tese mostraram a importância da brucelose para a pecuária em todos os países onde a doença é endêmica, especialmente em países onde não existiam programas de controle. A análise espaço temporal demonstrou as grandes conquistas do PNCEBT no estado de Minas Gerais e elucida regiões onde as medidas de controle não foram bem implementadas. Complementarmente, a análise da rede mostrou a formação de três grandes comunidades que seguem padrões semelhantes de movimentação de bovinos em cada ano analisado, sugerindo a necessidade de estratégias de controle diferentes em cada comunidade quando se considera a movimentação de bovinos. Portanto, esta tese atualiza o conhecimento sobre a ocorrência da brucelose na pecuária em todo o mundo e mostra alternativas de ferramentas analíticas para acessar as medidas de controle da brucelose bovina, em Minas Gerais, Brasil.

Palavras-chave: Epidemiologia. Zoonose. Vigilância. Doença. Modelo matemático.

ABSTRACT

Brucellosis is a disease of great burden for livestock and human health worldwide, affecting especially developing countries. In Brazil, the Programa Nacional de Controle e Erradicação de Tuberculose e Brucelose (PNCEBT) is responsible for regulate the control and eradication measures of the disease, since 2001. Among the Brazilian states, Minas Gerais is one of the most important considering cattle production, with bovine brucellosis still prevalent, although, the prevalence is decreasing. In this sense, the aim of this thesis was to review the occurrence of livestock brucellosis and the interface with human occurrence worldwide, combined with the development of spatiotemporal and network analysis as tools to improve the control of bovine brucellosis in Minas Gerais, Brazil. For that, it was conducted a literature review about the occurrence of livestock brucellosis worldwide, followed by a spatiotemporal analysis of bovine brucellosis cases, testing, vaccination, and bovine population size in Minas Gerais, Brazil. Additionally, a network analysis was performed with cattle movement data, also in Minas Gerais, Brazil. The results of this thesis showed the importance of livestock brucellosis in all countries where the disease is endemic, especially in countries where there were no control programs. The spatiotemporal analysis demonstrated the great achievements of the PNCEBT in the state and elucidates regions where the control measures were not well implemented. Complementarily, the network analysis showed the formation of three big communities that follow similar patterns of bovine movement in each analyzed years, suggesting the need for different control strategies in each community when considering cattle movement. In conclusion, this thesis apprises the knowledge on the occurrence of livestock brucellosis worldwide and showed alternatives analytical tools to access the control measures for bovine brucellosis, in Minas Gerais, Brazil.

Keywords: Epidemiology. Zoonosis. Surveillance. Disease. Mathematical model.

SUMMARY

PART ONE

GENERAL INTRODUCTION

Brucellosis is a very important disease worldwide, affecting many animals, including cattle and humans, being a zoonosis. The control of the disease in humans is directly related to the control in animals, since human vaccination for brucellosis is not available. The disease is responsible for great economic and health burden, compromising livestock production and the health of animals and humans. Due to this, many efforts have been made worldwide to control and eradicate animal brucellosis, with some countries being successful in eradication, especially the developed countries. However, most of the countries in development are still endemic to animal brucellosis, including Brazil. The control of brucellosis in Brazil is performed according to the Programa Nacional de Controle e Erradicação de Bucelose e Tuberculose (PNCEBT) (National Program of Control and Eradication of Brucellosis and Tuberculosis), which is implemented by official animal health authority of the states to attend the different epidemiological realities encounter in the country. In Minas Gerais, one of the protagonists of livestock production in Brazil, the PNCEBT is conducted by Instituto Mineiro de Agropecuária (IMA) (Minas Gerais official animal health authority). In this state, the control of animal brucellosis has been evolving throughout the years, with many achievements, albeit efforts must still be made to accomplish eradication. Among the several strategies that can be used to improve the control of animal brucellosis, mathematical tools have ultimate importance, since they could aid to assess and validate the control measures being applied, or to identify specific places (e.g., municipalities and livestock properties) to where directed control measures should be implemented according to its importance in the disease transmission chain.

Therefore, the main objective of this thesis was to evaluate the worldwide situation of animal brucellosis and perform a risk-based assessment of bovine brucellosis surveillance and control strategies by analyzing data on outbreaks, vaccination, and cattle movement in Minas Gerais in the last years.

In the first chapter, a review was conducted to assess the occurrence of brucellosis in livestock animals worldwide and the interface of the disease between animals and humans, especially in developing countries. Due to the importance of Minas Gerais in the livestock production of Brazil, the next chapters were all developed with data from this state. Therefore, the second chapter was a spatiotemporal analysis of bovine brucellosis cases notified from passive surveillance, bovine brucellosis tests (Rose Bengal test), and bovine population in the whole state, per municipality, from 2011 to 2018, to verify the evolution of PNCEBT in Minas Gerais, Brazil. The third chapter was a spatiotemporal analysis of vaccination rate, from 2011 to 2022, complemented by the investigation of the influence of sociodemographic factors of cattle farmers, to improve awareness of bovine brucellosis vaccination rate in the state and possible aspects related that could help improve the bovine brucellosis vaccination coverage in Minas Gerais, Brazil. Additionally, the fourth and fifth chapters were network analysis based on cattle movement in Minas Gerais, from January 2013 to August 2023 and January 2017 to August 2023, respectively. The fourth chapter was about the description of cattle movement in Minas Gerais and the characteristics of the network based on municipalities as nodes for interventions. Finally, the fifth chapter was a network analysis considering livestock properties as nodes, describing the attributes of this network and its application for disease control programs.

FINAL CONSIDERATIONS

The results found in this thesis elucidate that animal brucellosis is still a high risk among developing countries, especially in those where no control program is implemented. Additionally, it is of outmost importance the awareness of the human population regarding the professional safety practices when dealing with animals or inside laboratories, the security of food consumption avoiding raw animal products, the control of animal movement between free areas and endemic areas and the compliance to the control programs when it is present in the country.

Considering the assessment of the Brazilian brucellosis control program (PNCEBT) in Minas Gerais, the analysis of data on bovine brucellosis outbreaks, bovine population, bovine brucellosis tests and bovine brucellosis vaccination rate, demonstrated that the PNCEBT in the state is evolving and improving. However, it was identified regions where focused strategies should be implemented according to sociodemographic and cultural characteristics of cattle farmers, ensuring the successful and homogeneous application of the PNCEBT in Minas Gerais.

Additionally, the network description of cattle movement in Minas Gerais state with municipalities and livestock properties as nodes, showed the protagonist role of some municipalities in the state, where interventions should be conducted to avoid disease transmission. Furthermore, the livestock properties network demonstrated a more complex graph, being a robust tool to the direction of resources in diseases control, since it was designed with a more sensible unit of intervention, the livestock properties.

Overall, the results demonstrated the importance of animal brucellosis worldwide and showed that the evaluation of data using appropriate tools can reveal valuable information to improve control and eradication programs.

PART TWO

CHAPTER ONE

Prepared in accordance with Ciência Rural journal standards.

Interface between human and animal brucellosis worldwide

Interface entre brucelose humana e animal no mundo

ABSTRACT

The aim of this review was to present scientifically sound data regarding the occurrence of brucellosis in livestock animals and humans and its interface, especially in developing countries. Brucellosis is an infectious disease caused by members of the genus *Brucella* spp. The disease has been eradicated in many developed countries, however, in developing countries, brucellosis is still present and constitutes an important public health problem, additional to the serious economic issues. Brucellosis infection in humans occurs via direct contact with reproductive secretions and urine of infected animals or through consumption of animal products such as unpasteurized dairy products, raw and undercooked meat. Therefore, prevention and control measures for brucellosis in humans depend directly on the control and prevention in animals.

Keywords: *Brucella* spp., zoonosis; surveillance, animal health, One Health.

RESUMO

O objetivo desta revisão foi apresentar dados cientificamente robustos sobre a ocorrência de brucelose em animais de produção e humanos e sua interface, especialmente em países em desenvolvimento. A brucelose é uma doença infecciosa causada por membros do gênero *Brucella* spp. A doença já foi erradicada em muitos países desenvolvidos, porém, nos países em

desenvolvimento, a brucelose ainda está presente e constitui um importante problema de saúde pública, além dos graves problemas econômicos. A infecção por brucelose em humanos ocorre por contato direto com secreções reprodutivas e urina de animais infectados ou pelo consumo de produtos de origem animal, como laticínios não pasteurizados, carne crua e malcozida. Portanto, as medidas de prevenção e controle da brucelose em humanos dependem diretamente do controle e prevenção em animais.

Palavras-chave: *Brucella* spp., zoonose; vigilância sanitária, saúde animal, One Health.

INTRODUCTION

Animal origin food products such as milk, dairy products, meat, and meat products are important components of the food supply chain, being consumed worldwide. In most countries, the increase in income per capita is leading not only to greater food consumption, but also to changes in the composition of the diet with an increasing proportion of foods of animal origin (DELGADO, 2003). Between 1961 and 2007, milk consumption in developing countries almost doubled and meat consumption more than tripled (MUEHLHOFF et al., 2013). Complementarily, the number of cattle and buffalo is predicted to increase by 400 million animals by the year 2030 (FAO, 2015).

Considering food safety and food security, the control and prevention of foodborne pathogens are of primary importance to public health and to the economy sector. Farm animals represent a major reservoir of pathogens, such as *Brucella* spp. that cause brucellosis. This disease have a considerable impact, both for causing a chronic disease with a debilitating character in humans and for culminating in a reduction in animal production and reproduction rates, compromising the quantity of milk and meat produced (CORBEL, 2006).

Brucella spp. are frequently transmitted to humans through the consumption of contaminated animal products, through direct contact with tissues and secretions of infected animals, or inhalation (DADAR et al., 2019a). In fact, human brucellosis is an ancient condition linked more to the consumption of raw milk and milk products (KUPLULU & SARIMEHMETOGLU, 2004; TUMWINE et al., 2015; GARCELL et al., 2016; DADAR et al., 2019a), despite also having a strong occupational character. Veterinarians, farmers, personnel involved in the milk production and abattoir workers are frequently exposed to the pathogen by unprotected contact with infected animals or contaminated biological materials, besides accidental exposure to anti-*Brucella* spp. vaccines (KUTLU et al., 2014; HUNTER et al., 2015; LYTRAS et al., 2016; ZHAN et al., 2016; PEREIRA et al., 2020a). In addition, brucellosis transmission through inhalation is also reported among laboratory workers (BOUZA et al., 2005; TRAXLER et al., 2013; GONEN et al., 2014; PEREIRA et al., 2020b).

Currently, the genus *Brucella* includes fourteen species (WHATMORE & FOSTER, 2021; ABOUT et al., 2023; HERNÁNDEZ-MORA et al., 2023), not considering the 18 species of *Ochrobactrum* that were recently add to the genus *Brucella* (SCHOLZ et al., 2018) (Table 1), each one with a preferred animal host and different pathogenic potential for humans (LECLERCQ et al., 2020). Several *Brucella* isolates consider "atypical" still await formal taxonomic description (WHATMORE & FOSTER, 2021). The *Brucella* species with the greatest importance for animal and human health are listed in the Terrestrial Animal Health Code and their occurrence must be reported to the World Organization for Animal Health (WOAH): *B. abortus*, *B. melitensis* and *B. suis* (WORLD ORGANIZATION FOR ANIMAL HEALTH, 2022). These species cause abortion and infertility in their natural hosts, cattle, goats and sheep, and pigs, respectively (POESTER et al., 2013). In cattle, the disease is mainly caused by *Brucella abortus* but in regions where cattle are kept in close association with goats or sheep, infection can be caused by biovars of *Brucella melitensis* (SELEEM et al., 2010). Occasionally, *B. suis* may cause a chronic infection in the mammary gland of cattle, however it has not been reported to cause abortion or spread to other animals from cattle (FRETIN et al., 2008; WORLD ORGANIZATION FOR ANIMAL HEALTH, 2022). Following the infection, cows typically recover, and present mild can live offspring after the initial abortion, however, they may keep shedding the bacteria in milk and uterine discharges (XAVIER et al., 2009; CARVALHO NETA et al., 2010). In addition to the great damage caused by *Brucella* spp. in domestic animals due to abortion and reproductive problems, the agent can also infect a large number of wildlife species, including mammals, amphibians, and fishes (WHATMORE, 2009; EL-TRAS et al., 2010; WHATMORE et al., 2014; SCHOLZ et al., 2016b; MUHLDORFER et al., 2017). The recent expansion of the genus *Brucella,* since late 1990s with the discovery of new species and new hosts, such as *B. ceti, B. pinnipedialis, B. microti, B. inopinata* (NYMO et al., 2011a)*, B. papinions* (WHATMORE et al., 2014) and *B. vulpis* (SCHOLZ et al., 2016b), *B. amazoniensis* (ABOUT et al., 2023), *B. nosferati* (HERNÁNDEZ-MORA et al., 2023), indicate that there is still much to be understood about the agent's relationship with its hosts.

Table 1. *Brucella* species, preferred host, and pathogenicity for humans.

***Brucella* species**	**Biovars**	**Preferential animal host**	**Pathogenicity for humans**	**Reference**
B. melitensis	1-3	Sheep, goat, camels	High[a]	Corbel et al. (2006)
B. abortus	1-6, 9	Cattle	High[b]	Corbel et al. (2006)
B. suis	1-5	Pig, wild boar, feral swine, reindeer, caribou, rodents	High	Corbel et al. (2006)
B. ovis	NA	Sheep	No	Corbel et al. (2006)
B. canis	NA	Dog	Moderate[c]	Corbel et al. (2006)
B. neotomae	NA	Desert wood rat	Unknown[d]	Corbel et al. (2006); (Suarez-Esquivel et al., 2017)
B. ceti	NA	Porpoises, dolphins	Unknown[e]	Whatmore et al. (2008); Nymo et al. (2011b)
B. pinnipedialis	NA	Whales	Unknown[e]	Whatmore et al. (2008); Nymo et al. (2011b)

B. microti	NA	Wild boar, vole, red fox	Unknown	Rónai et al. (2015)
B. inopinata	NA	Frog, rodents	High	Kimura et al. (2017); Scholz et al. (2016a)
B. papionis	NA	Baboons	Unknown	Whatmore et al. (2014)
B. vulpis	NA	Red fox	Unknown	Scholz et al. (2016b)

[a] The most common cause of human brucellosis. Common sources are unpasteurized milk and milk products. [b] The second most common cause of human brucellosis. Common sources are unpasteurized milk and milk products. [c] Increasing reports of human brucellosis, particularly from South America, possibly understudied elsewhere. [d] It was isolated from cerebrospinal fluid of 2 men with neurobrucellosis in Costa Rica. [d] One human laboratory contamination has been described in the UK. Three naturally acquired cases have been described.

Studies have shown that the occurrence of brucellosis dates back to the 1st century BC, and since then this pathogen has had great economic importance in livestock farming and human health (AKPINAR, 2016). The disease is considered endemic in many regions of the world such as in the Mediterranean basin, Middle East, Asia, Africa, and Latin America (FRANC et al., 2018). In these endemic areas, it affects food production and has serious public health consequences. This article provides an overview and some considerations on prevention and control of brucellosis, especially in developing countries. Therefore, the aim was to draw a picture of brucellosis among the world's animal population and discuss the interface of animal brucellosis with brucellosis in humans.

ANIMAL BRUCELLOSIS

Animal brucellosis is clinically characterized by one or more of the following signs: abortion, stillbirth, birth of weak calves, retained placenta, orchitis and epididymitis, with elimination of the organisms in semen, uterine discharges and in milk (CARVALHO NETA et al., 2010). Additionally, articular disorders, as peri-articular hygromas, are common in wild-animal species chronically infected by *Brucella* spp. (GODFROID et al., 2013).

Control and eradication of brucellosis from livestock has been the goal of many countries and global institutions as Food and Agriculture Organization of the United Nations (FAO) and WOAH)

- having success in most developed countries, such as in many members of the European Union (Norway, Sweden, Denmark, Germany, Netherlands, Belgium, France, Austria, Czech Republic, Slovakia) and United Kingdom (EUROPEAN FOOD SAFETY AUTHORITY, 2016), United States, Canada, Australia, New Zealand, among others. Countries that eradicated the infection cannot afford to be complacent, as the threat of re-introduction is ever present, e.g. through the livestock movement, wildlife reservoirs, among others (CUTLER et al., 2005; MICK et al., 2014). On the other hand, for several emergent or developing countries, brucellosis remains a disease of great impact for livestock and for public health, due to lack of financial resources or not enough money allocation to the control and eradication programs (HERNÁNDEZ-MORA et al., 2017b; BUTTIGIEG et al., 2018; ZHANG et al., 2018; AVILA-GRANADOS et al., 2019).

Commonly, the approaches used to control and eradicate brucellosis from animals are compulsory vaccination and test-and-slaughter policy of positive-confirmed animals (DORNELES et al., 2017). After reaching low herd prevalence rates (< 1.00%) due to high vaccination coverage, usually more than a decade is needed to complete the brucellosis eradication program using a "test-and-slaughter" policy, being the success of this phase primarily related to the availability of sufficient financial compensation scheme for farmers for their culled livestock (GODFROID et al., 2013; ALVES et al., 2015; LEITE et al., 2017; ZHANG et al., 2018).

The most used vaccines against bovine brucellosis are S19 and RB51 (DORNELES et al., 2015). The first was developed in 1941 from a smooth attenuated *B. abortus* strain, that induces antibody response and can cause a misinterpretation on diagnosis tests for the disease (OLSEN & STOFFREGEN, 2005), if animals are vaccinated older than 8 months or tested before 24 months of age. RB51 was developed in 1982 and is a rough rifampicin resistant vaccine strain that does not express the O-chain of the lipopolysaccharide (LPS) on its membrane, therefore, it does not induce detectable antibodies in the routine diagnosis tests (DORNELES et al., 2015). For this reason, S19 vaccine is recommended for females from 3 to 8 months of age, while RB51 vaccine can be used in females at any age above 3 months (SCHURIG et al., 1991), although both are protective in cattle (OLIVEIRA et al., 2022). For small ruminants, a vaccine was developed in 1950 using a smooth strain of *B. melitensis* Rev. 1, resistant to streptomycin (BANAI, 2002), which is considered the best option for the prevention of brucellosis for these animals worldwide, especially in the standard dose of 10^9 colony forming units (CFU) for non-pregnant ewes by conjunctival route (CNEVA et al., 1995).

A country or a zone is considered free from bovine brucellosis when it fulfills the following requirements established by the WOAH: (*i*) bovine brucellosis is a compulsory notifiable disease and no case has been detected in the past three years; (*ii*) the entire cattle population of a country or zone is under official veterinary control and the rate of brucellosis infection does not exceed 0.2% of the cattle herds in the country or zone under consideration; (*iii*) serological tests for bovine brucellosis are periodically conducted in the herds; (*iv*) no animal was vaccinated against bovine brucellosis for at least the past three years; (*v*) all reactor animals must be slaughtered; and (*vi*) animals or their genetic material introduced into a free country or zone should come only from herds officially free from bovine brucellosis (WORLD ORGANIZATION FOR ANIMAL HEALTH, 2022).

To qualify as officially free from caprine and ovine brucellosis (*B. melitensis*), a country or zone must satisfy the following requirements: (*i*) the occurrence or suspected occurrence of caprine and ovine brucellosis are notifiable for at least five years; (*ii*) all flocks of sheep and goats in the country or zone are under official veterinary control; either (*iii*) 99.8% of these flocks are qualified as officially free from caprine and ovine brucellosis; or no case of brucellosis in sheep or goats has been reported for at least five years; and (*iv*) no sheep or goat were vaccinated against the disease for at least three years (WORLD ORGANIZATION FOR ANIMAL HEALTH, 2022)

BRUCELLOSIS IN LIVESTOCK IN DEVELOPING COUNTRIES

Latin America

Latin America is composed of all countries from South, Central America, Caribbean, and Mexico, having most of them already reported the occurrence of brucellosis caused by *B. abortus* and *B. melitensis*. Among the *Brucella* spp. natural hosts in Latin America, cattle are by far the most relevant, followed by goats, sheep, and pigs. In the following lines, we will discuss the epidemiological situation of the disease in Latin America countries where the occurrence of brucellosis has been better documented. In South American countries, the disease status in farm animals is well determined in some countries (e.g. Brazil, Chile, and Uruguay) (AZNAR et al., 2014; FERREIRA NETO, 2018). For the other countries, epidemiological data are scarce or do not represent the animal population in the region. The absence or the poor quality of information about the disease prevents the design of more assertive control and prevention measures, despite most of these countries have had a brucellosis control program established for a few years.

In Argentina, a National Brucellosis Control and Eradication Program was implemented in 1999 and *B. abortus*, the main causal agent of bovine brucellosis in the country, *B. melitensis* (predominantly in goat in central, western and northern regions of the country), *B. suis* (in pig farming in the Pampeana plain region) and *B. canis* (LUCERO et al., 2008b) have already been described. The result of a national brucellosis survey performed in 2004 indicated that 12.35% [95% Confidence Interval (CI): 10.89–14.00%] of Argentine beef farms were seropositive to *Brucella* spp. and that the apparent seroprevalence in cattle was 2.10% (95% CI: 1.90–2.40%)

(SOTA et al., 2006; AZNAR et al., 2014). The real epidemiological situation of the infection by *B. melitensis* in the country is unknown, although several studies confirmed the presence of the disease. An overall prevalence of 2.00%, with an intra-flock prevalence ranging between 1.00% and 40.00% (2010–2013), among sheep and goats was detected in four of the nine departments from the province of Formosa (RUSSO et al., 2016). Also, in the province of Mendoza, brucellosis caused by *B. melitensis* was observed in 28.10% goat farms (2005-2006) (ROBLES et al., 2007), which led to the implementation of mass vaccination program in this region using *B. melitensis* Rev.1 vaccine strain (ROBLES et al., 2020).

In Bolivia, the Programa Nacional de Control y Erradicación de Brucelosis - Tuberculosis Bovina y Bubalina (National Program for the Control and Eradication of Bovine Brucellosis and Tuberculosis) had its technical regulation approved in 2014 and established as objectives to reduce the prevalence of the disease, to certify herds under official control as free of the diseases and, to increase the supply animal products of low-risk for public health (BOLIVIA, 2014). The strategies of the Bolivian program are vaccination with S19 and RB51 and slaughter of test-positive animals. The seroprevalence of bovine brucellosis in cattle in four Bolivian provinces (Andrés Ibáñez, Warnes, Sara and Ñuflo de Chávez) was estimated to be 2.27% (95% CI: 1.70–2.83) (MANRRIQUE et al., 2005).

In Brazil, bovine brucellosis due to *B. abortus* is the most prevalent in the country and biovars 1, 2, 3, 4, and 6, were already identified (MINHARRO et al., 2013). In order to control the bovine brucellosis, in 2001, the Programa Nacional de Controle e Erradicação da Brucelose e Tuberculose Animal (PNCEBT – National Program of Control and Erradication of Brucellosis and Tubercullosis) was implemented to reduce the incidence and prevalence of these diseases in the national herd (BRASIL, 2001). Among the measures proposed by the PNCEBT are compulsory vaccination of bovine and bubaline heifers aged between 3 and 8months with S19 or RB51, testing for movement, breeding animals, and the slaughter of brucellosis-positive animals, besides voluntary actions, such as certification of brucellosis-free properties (BRASIL, 2017). Bovine brucellosis has heterogeneous distribution in Brazil, although it has already been diagnosed in all states (FERREIRA NETO et al., 2016). Santa Catarina, a state of the south region, has the lowest prevalence of seropositive herds (0.91%; 95% CI: 0.30–2.11) and it is the only one that has started the eradication of the disease (BAUMGARTEN et al., 2016); while Mato Grosso do Sul, a state in the midwest region, is the state with the highest seroprevalence (2009), showing 30.60% (95% CI: 27.40–34.00) of the cattle herds seropositives (LEAL-FILHO et al., 2016). In general terms, the midwest region of the country has the highest rates of seropositive animals and herds, followed by the northeast region. The south and southeast regions are among the regions with the lowest prevalences, however, marked differences are observed in the animal health situation among the states of these regions. Complementarily, it was estimated that for each infected cow, Brazil loses BR$ 420.12 (about US$84.02) and, considering the prevalence of brucellosis in the country, the total estimated losses were approximately BR$ 892 million (about US$ 448 million) in 2013 (SANTOS et al., 2013). *B. melitensis* and *B. neotomae* were never isolated in the country (POESTER et al., 2002; POESTER et al., 2009); *B. ovis* in sheep and *B. canis* in dogs have already been described (DORNELES et al., 2011; MINHARRO et al., 2013; DORNELES et al., 2014), despite their real epidemiological situation being unknown; whereas, *B. suis* seems to occur in low prevalence due to the production system of swine in the country (POESTER et al., 2002). Noteworthy, recently *B. ceti* and *B. pinnipedialis* were reported in Clymene dolphins in Brazil (ATTADEMO et al., 2017; SÁNCHEZ SARMIENTO et al., 2017).

In Colombia, the only species affecting livestock animals is *B. abortus,* being *B. melitensis* and *B. suis* never reported in the country (AVILA-GRANADOS et al., 2019). A national program for the control, prevention, and eradication of brucellosis in livestock animals, based mainly on vaccination of female cattle (using S19 and RB51) and epidemiological surveillance (notification of positive cases, control of animal movement, etc), has been in place since 2002 (HASSAN et al., 2020a). Data from the epidemiological surveillance of the disease, conducted between 2006 and 2012, showed an overall seroprevalence of positive herds of 22.00–23.00% (CÁRDENAS et al., 2018), whereas an epidemiological survey from 29,969 animals and 4,922 herds estimated a seroprevalence of 3.71% and 12.7%, respectively (HASSAN et al., 2020a).

Chile, in 1975, initiated the control program of bovine brucellosis in the southern and central part of the country, where 84.00% of their cattle herds were located. The initial strategy consisted only in vaccinating 3 to 8 month-old females with S19 (LOPETEGUI, 2004). In 1976, 7.00% of reactor animals were notified and, according to the first prevalence study, the notification rate decreased from 2.91%, in 1982, to 2.40%, in 1991 (LOPETEGUI, 2011). In 1992, in a program evaluation, it was verified that the prevalence had not lowered significantly in 9 years, probably because vaccination alone was not enough to reduce the disease occurrence to levels below those already observed. Such observation motivated the implementation of a more comprehensive strategy to achieve eradication (RIVERA et al., 2002; LOPETEGUI, 2011). The surveillance system involved (*i*) milk ring testing (MRT), in the milk-processing and dairy products industries, (*ii*) test animals at fairs, slaughterhouses and herds after notification of outbreaks, (*iii*) as well as the obligation to slaughter the test-positive animals. The program also established the brucellosis vaccination with RB51 in replacement to S19. In 2014, the national prevalence of brucellosis in cattle was 0.20% and the eradication of bovine brucellosis is thought to be reached in a short period of time

(LOPETEGUI, 2011). Chile is free of *B. melitensis* since 1975, situation that seems to remain considering more recent data (PÉREZ et al., 2006).

In Ecuador, the Ministry of Agriculture estimated prevalence of brucellosis in farm animals from 1979 to 2008 between 1.92% and 10.62% in cattle in highland provinces and from 4.12% to 10.62% in coastal provinces (POULSEN et al., 2014). In 2009, a program to control the disease was implemented and established different measures to be adopted according to the disease situation in the five epidemiological regions of the country (ECUADOR, 2016). More recent data obtained in the province of Manabí (2014–2015) showed a seropositivity of 1.99% of cattle in farms and 2.63% of slaughtered bovines (AGUAYO & PÉREZ RUANO, 2015). Moreover, the disease also affects goats (17.80%) as observed in a study conducted in 2011–2013 from a convenience sampling (POULSEN et al., 2014)

In Mexico, brucellosis is recognized in the country since the early 1990s and it is one of the main sanitary problems affecting livestock, with the largest number of reported outbreaks in herds in 2014 (5,514) (HULL & SCHUMAKER, 2018). Although good progress has been achieved in many areas, brucellosis is still present in cattle (dairy and beef), goats and sheep (MORALES-GARCIA et al., 2015). From classical *Brucella* species only *B. neotomae* have never been isolated in the country. The species already isolated include *B. melitensis* biovars 1–3; *B. abortus* biovars 1, 2, 4–6; *B. suis* biovar 1; *B. canis* and *B. ovis* (LUNA-MARTINEZ & MEJIA-TERAN, 2002). In 1971, Mexico started its program of control and prevention of brucellosis, based on the vaccination, identification and elimination of positive animals (LUNA-MARTINEZ & MEJIA-TERAN, 2002). Cattle vaccination using RB51 is officially performed since 1997 and vaccination of goats using Rev. 1 is applied in high-risk areas (LUNA-MARTINEZ & MEJIA-TERAN, 2002). A serological survey performed with 1,768 goats showed a high brucellosis apparent prevalence ranging from

11.00 to 38.00% depending on the country region and vaccination rate – 38.00% in Jalisco (poorly vaccinated) and 11.00% in Michoacán (intensively vaccinated) (OSEGUERA MONTIEL et al., 2013). To the best of our knowledge, no epidemiological study in cattle with a representative sampling has been conducted recently in the country, hindering the assessment of possible advances achieved in the control of the disease in this population.

In Paraguay, bovine brucellosis due to *B. abortus* has been detected for many years and its prevalence in cattle was estimated 1.43% in 2017 (MINISTERIO DE AGRICULTURA Y GANADERIA, 2017). Additionally to *B. abortus, B. melitensis* and *B. suis* were isolated and identified in the country (BAUMGARTEN, 2002). The Servicio Nacional de Calidad y Salud Animal (SENACSA – National Service of Quality and Animal Health) is the official organization in charge of the brucellosis control and eradication program in the country, which was approved in 1978. This program established the mandatory use of S19 vaccine in 3 to 8 months female calves, identification of vaccinated and positive animals, requirements for accrediting farms as '*Brucella*-free' and the control of animals prior their import or movements to markets or for reproduction purpose. In 1999, the RB51 vaccine was also released for cattle vaccination (AZNAR et al., 2014). In this country, economic losses due to bovine brucellosis were estimated at US$ 23.5 million per year (BAUMGARTEN, 2002).

In Peru, the technical regulation of the program for bovine brucellosis control, published in 2000, established the participation of veterinarians from the private sector, the use of Rose Bengal Test (RBT) and MRT for animal and herd screening, respectively, compulsory vaccination of young females cattle and certification of disease-free herds (PERU, 2000). A study on 5,439 cattle (2007) conducted in district of Codo del Pozuzo, in the region of Huanuco, showed a seroprevalence of 0.02% (95% CI: 0.00 to 0.06) (ZAVALA et al., 2011). Another study carried out in the same year but in another region, district of Puerto Inca, Huánuco, also revealed a very low seroprevalence of the brucellosis in cattle 0.03% (95% CI: 0.00 - 0.11%) (MEZA et al., 2012). Since 2004, Peru also adopts actions for prevention and control of caprine brucellosis (*B. melitensis*). Studies performed in Lima and Ancash departments, in 2004, showed a disease prevalence as follows: Lima (Canta Province 2.90%, Huaral 4.88%, Huaura 3.29%) and Ancash (Recuay Province 0.74%, Ocros 2.36%) (SERVICIO NACIONAL DE CALIDAD Y SEGURIDAD ALIMENTARIA, 2014). Another study carried out in 2003 showed 6.80% (95% CI: 6.60% 7.20%) of positive goats in the RBT in the district of Callao and 0.00% in Ventanilla (TABOADA et al., 2005). The program for the control and prevention of caprine brucellosis proposes among the interventions, vaccination of goats over 3 months of age with Rev. 1, which has been practiced since 1996, with the last campaign being carried out in 2003 (TABOADA et al., 2005).

In Venezuela, the most prevalent *Brucella* species is *B. abortus*, infecting cattle and buffaloes. Since 2003, the Programa de Prevención y Control de la Brucelosis Bovina (Bovine Brucellosis Prevention and Control Program) has been in place in the country (FRANCISCO & VARGAS, 2002). The vaccination with S19 against bovine brucellosis is mandatory throughout Venezuelan territory for all females between 3 and 8 months of age, with vaccines registered by the Instituto Nacional de Salud Agrícola Integral (National Institute of Agricultural Animal Health) (VENEZUELA, 2017). The program also establishes the control of cattle transit, slaughter of serologically positive animals and obligatory vaccination of female calves (VENEZUELA, 2017). In 2006–2007, a study in the country showed an average prevalence of 15.20% of seropositive cattle herds in the Buria County, Simón Planas Municipality Lara state (MOSQUERA et al., 2009). *B. melitensis* has been isolated in the country, however information on the infection in animals is scarce (LUCERO et al., 2007; FERREIRA NETO, 2018).

Uruguay is nowadays free of *B. melitensis* and *B. suis* and its bovine brucellosis program is well advanced towards the disease eradication, as bovine brucellosis caused by *B. abortus* also occurs at very low frequencies (AZNAR et al., 2014). The last cases of *B. suis* were reported in 2015, in a pig farm (41 cases) (OIE, 2015). Focusing on eradication strategies, bovine brucellosis vaccination with S19 had not been performed in the country since 1996 (GIL, 2002) and brucellosis-positive animals are slaughter within a program to support the producers (dairy and beef) (GARÍN, 2011; AZNAR et al., 2014; FERREIRA NETO, 2018).

Contrary to what was observed for several South America countries and Mexico, high quality information about animal brucellosis is not available for all Central America and Caribbean countries. However, it should be noted that the size and economic importance of the population of livestock animals in these countries is considerably lower compared with other Latin America countries. Hence, the impact of animal brucellosis and the lack of knowledge about its real epidemiological situation is also smaller in this region. The prevalence of bovine brucellosis in Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua and Panama was estimated between 4.00 and 8.00%, with higher prevalence in dairy herds, and losses calculated at US$ 25 million per year (MORENO, 2002). Bovine and swine brucellosis caused by *B. abortus* and *B. suis*, respectively, have been identified in all continental Central America countries, while ovine and caprine brucellosis caused by *B. melitensis* has been detected in Guatemala and Costa Rica (SUÁREZ-ESQUIVEL et al., 2020). Most Central America countries have initiated programs for brucellosis control, with the economic and technical help of international agencies. All the programs for the control of brucellosis have been based on calf vaccination and elimination of the seroreactors (MORENO, 2002).

In Belize, bovine brucellosis is under control by Autoridad de Sanidad Agropecuaria de Belice para la Prevención, Control y Erradicación de la Brucelosis Bovina (Belize Agricultural Health Authority for Prevention, Control and Eradication of Bovine Brucellosis), which is working towards self-declaration of free status for the disease. The program consists in the diagnosis, prevention, control and eradication of bovine brucellosis, being the vaccination prohibited in cattle (BELIZE, 2011). No information is available on brucellosis in other livestock animals in the country.

Brucellosis is endemic in humans and cattle in Costa Rica, since the beginning of XX century (QUIRÓS, 1915). The microorganism was already isolated from humans, pigs, dogs, horses, water buffalo and in striped dolphins (SEQUEIRA et al., 1984), being the last one the main host of *B. ceti* in Costa Rica, with symptoms that corresponds to neurobrucellosis. The latest founded survey (2012–2013) showed a herd seroprevalence of bovine brucellosis of 6.00%, ranging from 1.90 to 14.90% according to the region of the country (HERNÁNDEZ-MORA et al., 2017a). Similarly, an assessment of brucellosis in different host mammal species, from 1999–2016, showed a seroprevalence of 21.70% and 6.50% in water buffalo (2014–2016) and horses (2014–2016), respectively (HERNÁNDEZ-MORA et al., 2017a); whereas, the prevalence of brucellosis in goat and sheep was estimated in 0.98% and 0.70%, respectively, between 2012 and 2013 (HERNÁNDEZ-MORA et al., 2017a).

In El Salvador, studies conducted in sheep and goat population did not reveal anti-*Brucella* antibodies in these animals (MORENO, 2002; LINDEROT DE CARDONA et al., 2016) suggesting that *B. melitensis* is probably exotic in the country. Bovine brucellosis caused by *B. abortus,* albeit present, occurs at a low frequency. Indeed, the disease prevalence from 1975 to 1983 was estimated between 1.00 to 1.95% in cattle (GARCÍA CARRILLO, 1981). Similarly, in a properly designed epidemiological survey conducted in 1978, a low prevalence of bovine brucellosis was observed in both animals (0.93%) and herd level (1.46%) (KNOKE et al., 1984). In small ruminants, in 2016, CARDONA et al. (2016) found 1.00% of seroprevalence in sheep and no disease in goats, recommending the inclusion of these species in the national eradication program, to prevent the spread of brucellosis.

In Guatemala, the Programa de Control Progresivo de Brucelosis y Tuberculosis (Program for the Progressive Control of Brucellosis and Tuberculosis), has the objective of minimizing the prevalence and incidence of the disease until 2027, through certification of free properties. The control program was created as a request of the presidents of Federaciones de Carne y Leche de Centroamérica (Meat and Dairy Federations of Central America), in order to open international trade to the country, which is currently closed to meat and milk (MAGA, 2017). Retrospective analyzes of exams submitted to official laboratories between 2010 and 2015 (except 2014) showed an annual seroprevalence of bovine brucellosis in the country between 4.80 and 9.80% (MAGA, 2017).

The presence bovine brucellosis caused by *B. abortus* has also been reported in Haiti, an island of Central America, and Honduras (MORENO, 2002; LUCERO et al., 2008a). The seroprevalence of bovine brucellosis was estimated in 1.40% among slaughtered cattle between 1961 and 1963, and in 3.00 – 5.00%, in 1964 (GROSNIER, 1964) in Haiti. However more recent data on the real prevalence of the disease are not available for both countries in the searched literature.

In Nicaragua, the prevalence of bovine brucellosis in animals was estimated in 0.18% in 2020 (INSTITUTO DE PROTECCIÓN Y SANIDAD AGROPECUARIA, 2020). Since 2009, it is in place the Programa Nacional de Brucelosis y Tuberculosis (National Brucellosis and Tuberculosis

Program), which seeks the control and eradication of bovine brucellosis through the certification of free herds and areas, diagnosis, and animal movement control, being vaccination prohibited in the country (NICARAGUA, 2009). *B. suis* was also reported in the country (CASTAÑO et al., 2017).

Some regions of Panama are considered free from bovine brucellosis and data from the Ministerio de Desarrollo Agropecuario (Ministry of Agriculture) revealed a very low seroprevalence of the disease, between 0.02 and 0.20% (MIDA, 2020). Between 2006 and 2012, *B. abortus* caused 59 outbreaks in 7 different provinces, however other *Brucella* species, as *B. melitensis* and *B. suis*, are reported as absent in domestic animals (BERGER, 2018).

In the Central America countries, the challenge for the control of brucellosis, as well as to countries worldwide, involves the implementation of measures such as the identification and slaughter of positive animals in each property, associated with the vaccination of the healthy ones, which is the most effective set of measures. There are already strong control and prevention programs in some countries, but the collaboration of farmers is essential for the efficacy of them and to enable the elaboration and execution of an eradication program.

Considering all Latin American countries, the main issue is not the lack of control and eradication programs, but the lack of reliable data on the epidemiological situation of regions/countries. The lack or incompleteness of such information severely prevents these programs from moving forward to achieving their goals. Well-designed epidemiological surveys require a well-structured animal health defense service and financial resources to conduct a representative sampling, which are probably the reasons for not being conducted in most of the countries.

Africa

Ruminant brucellosis is an endemic disease in most part of African continent, having great impact on public and animal health in the region. Recent studies on bovine brucellosis revealed a seroprevalence ranging from 7.90% (95% CI: 4.40–11.40) in Lusaka province to 18.70% (95% CI: 7.50–29.90) in the Chibombo district of Zambia (CHIMANA et al., 2010); whereas the disease prevalence reported in small ruminants (sheep and goat) ranged from 0.40% (95% CI: 0.34–0.54) in Dahir Dar, Northwest of Ethiopia (HEGAZY et al., 2011) to 5.41% (95% CI: 5.81–6.91) and 3.55% (95% CI: 5.75–6.35) in sheep and goats from Egypt, respectively (SAMAHA et al., 2008).

Throughout the years, serological evidence of brucellosis in cattle population was found in many sub-Saharan African countries where investigations were performed, including Benin, Burkina Faso, Cameroon, Ethiopia, Gambia, Ghana, Guinea, Ivory Coast, Kenya, Mali, Niger, Nigeria, Senegal, Sierra Leone, South Africa, Tanzania, Togo, Uganda and Zambia (MANGEN et al., 2002; HESTERBERG et al., 2008; CHIMANA et al., 2010; SCOLAMACCHIA et al., 2010; MAKITA et al., 2011; SANOGO et al., 2013a; ASMARE et al., 2014; SINGH et al., 2015; ALONSO et al., 2016; TEREFE et al., 2017). Seroprevalence by RBT was estimated range between 10.20 and 25.70% in cattle population of sub-Saharan Africa (SANOGO et al., 2013a). The endemicity of brucellosis in this region may be attributed to the absence of sustainable efficient control programs. According to some authors, even currently, the surveillance and control of animal brucellosis in sub-Saharan Africa is rarely implemented outside of southern Africa (BERTU et al., 2012).

Indeed, in Ethiopia, several brucellosis studies have demonstrated the endemicity of animal brucellosis in the country, mainly in cattle and occasionally in small ruminants (goat and sheep) (YOHANNES et al., 2013). The seroprevalence of brucellosis in dairy cattle in the country was estimated to be 3.30% (95 % CI: 2.60–4.20%) (ASMARE et al., 2014). However, data from other studies reveals a great heterogeneity of animal seroprevalence among different regions, ranging from 1.00% to 18.70% (DINKA & CHALA, 2009; IBRAHIM et al., 2010; MEKONNEN et al., 2010; ADUGNA et al., 2013; TSCHOPP et al., 2013; ASMARE et al., 2014; TEREFE et al., 2017). The few published reports on the status of small ruminant brucellosis in Ethiopia reveal a seroprevalence of animals between 0.40 and 3.50% (FEREDE et al., 2011; DABASSA et al., 2013; TEKLUE et al., 2013). It is important to mention that there is no national program proposed for prevention and control of brucellosis in Ethiopia, nor for cattle or small ruminants. Likewise, at regional levels, no strategy is in place to control brucellosis, which is probably result of lack of facilities and budget to run such programs (YOHANNES et al., 2013).

As observed for Ethiopia, other countries in sub-Saharan Africa have already reported the occurrence of brucellosis in different animal populations. In Kenya, the seroprevalence of brucellosis in cattle ranged from 9.90 to 15.00% (NJERU et al., 2016). Low seroprevalence of brucellosis was found in goats and sheep, and vaccination is not conducted as part of a coordinated national program (NJERU et al., 2016). Moreover, the disease has already been described in other regions of the country, as in sheep and goats from Kiambu (AKOKO, 2010) and cattle from the Coast and upper Eastern regions. Additionally, in Zimbabwe, studies indicated that bovine brucellosis seroprevalence is between 5.60%–9.90%, being cows with a history of abortion more likely to be seropositive (MATOPE et al., 2011; GOMO et al., 2012). In addition, there is also evidence of transmission of the disease between cattle and wild animals in shared grazing areas in Zimbabwe (GOMO et al., 2012).

In Tanzania, a meta-analysis estimated the seroprevalence of bovine brucellosis in 8.20% (95% CI: 6.50–10.20) (ALONSO et al., 2016). Similar seroprevalence was also estimated in Zambia, where 5.70% (95% CI, 3.40–8.20%) of seropositivity was observed in cattle kept by smallholder dairy farmers from Lusaka and southern provinces. In these areas, the vaccination with S19 or RB51 was practiced, contributing to the low seroprevalence (MUMA et al., 2012).

Among the countries of northern Africa, Egypt is the one that has more information about the epidemiological situation of animal brucellosis. In Egypt, brucellosis, particularly by *B. melitensis*, is endemic, affecting many animals, as well as humans (FOOD AND AGRICULTURAL ORGANIZATION, 2009; MENSHAWY et al., 2014). Several attempts have been performed to control the disease by the national veterinary services, with assistance from development agencies and international organizations. A Spanish-Egyptian cooperation project for the control of ruminant brucellosis in the Upper Egypt area was funded by a Spanish Cooperation project from 2005-2009 (FOOD AND AGRICULTURAL ORGANIZATION, 2009), and this project included seven governorates and involved primarily smallholders. The project also sought to strengthen the Egyptian veterinary services' capabilities to control brucellosis by improving surveillance at both, field and laboratory levels, and by implementing a massive vaccination campaign, training veterinary personnel, implementing brucellosis public awareness campaigns and enforcing brucellosis control legislation (FOOD AND AGRICULTURAL ORGANIZATION, 2009). Brucellosis is widespread and heterogeneously distributed among different ruminant species in Upper Egypt, with the true prevalence of the disease estimated in 0.79% (95% CI: 0.71%–0.87%) for cattle, 0.13% (95% CI: 0.08%–0.18%) for buffaloes, 1.16% (95% CI: 1.05%–1.27%) for sheep and 0.44% (95% CI: 0.34%–0.54%) for goats. In addition, still in Upper Egypt, it was estimated that 0.20% of households keep seropositive ruminants (HEGAZY et al., 2011), which represents a great risk of spreading infection and the occurrence of outbreaks. Complementarily, keeping seropositive animals contributes to an increase in zoonotic risk for humans who have direct contact with these animals and consume their products.

In South Africa, a retrospective study with 963 *Brucella* spp. strains (2008 and 2018), showed a high frequency for *B. abortus* (n = 883; 91.60%), followed by *B. melitensis* (n = 42; 4.40%), *B. ovis* (n = 29; 3.00%) and *B. canis* (n = 9; 0.90%) (MATLE et al., 2021). Another study focusing on cattle that were slaughtered at Gauteng province (2016 to 2017) isolated *B. abortus* (5/11, 45.45%) and *B. melitensis* (6/11, 54.54%) from 11 different tissue samples (KOLO et al., 2019). Also, a study reported an outbreak of *B. melitensis* in KwaZulu-Natal province in goats, with a prevalence that varied between 17.00% and 100.00% from 6,266 tested goats (REICHEL et al., 1996). Curiously, dog serum was tested with 2-mercaptoethanol-tube agglutination test (2ME-TAT), from three provinces (Gauteng, Eastern Cape, and Western Cape) in South Africa, recovering 4.40% of positives from 1,191 tested dogs (OOSTHUIZEN et al., 2019) .

In Botswana a cross-sectional study (2018) found that a total of 0.80% (6/770) samples were positive in the tested cattle (PFUKENYI et al., 2020). Even more recently, the country of Burkina Faso, had it first study on seroprevalence and risk factors performed in the Province of Bam (2021), where it was estimated individual seroprevalence of 6.00% (18/300) in sheep and 4.30% (13/300) in goats, while a herd prevalence was estimated in 60.00% and 40.00% in sheep and goats, respectively (TIALLA, 2022). Regarding cattle population, in the Burkina Faso region of Ouagadougou, 2.80% (1/52) herds were found positive for *Brucella* spp. in Indirect Enzyme-Linked Immunosorbent Assay (iELISA) (2017 and 2018) (MUSALLAM et al., 2019), and 7.30% of 464 transhumant cattle (95% CI: 3.50–14.70%) were also positive in iELISA in another study performed in 2012 (DEAN et al., 2013).

Burundi is one of the smallest countries in Africa, it is located at West Africa sharing borders with Rwanda, Tanzania and Democratic republic of Congo. The only study found available for animal brucellosis in Burundi pointed a dairy herd seroprevalence of 14.70% (95% CI: 9.40–20.80) in the province of Bujumbura, from samples collected from 2017 to 2018 (MUSALLAM et al., 2019).

The official number of animals infected with brucellosis in Cameroon is unknown, mainly due to the absence of a structured surveillance system for animal brucellosis. However, there are various studies estimating the occurrence of animal brucellosis in this country, which reported 8.40% of seroprevalence in Holstein cattle (BAYEMI et al., 2009) and 16.00% of seroprevalence within-herd in *Bos indicus* cattle (SCOLAMACCHIA et al., 2010). Also, a review that gathered studies from 1982 to 2020 estimated a seroprevalence from 3.00% to 30.80% in livestock animals, and from 5.60% to 28.10% in abattoir workers (LAINE et al., 2020). Furthermore, a study carried out in 2013, found a seroprevalence in cattle varying from 1.10% (95% CI: 0.50%–2.40%) to 5.00% (95% CI: 0.00%–10.60%) in the Northwest region and Vina division, Cameroon (KELLY et al., 2021). More recently, a cross-sectional study performed at Southern region of Cameroon (2016 to 2018), found an overall seroprevalence of 6.35% (118/1873), being 9.12% (78/855) in cattle; 8.04% (30/373) in sheep, 6.06% (2/33) in dog, 1.87% (3/160) in pig and 1.10% (5/452) in goat (KAMGA et al., 2020).

Ivory Coast is a country localized in the West African region, where the isolation of *B. abortus* biovars 1, 3, and 6 from cattle was already reported (SANOGO et al., 2013a). Although few studies are available, the seroprevalence of bovine brucellosis in savannah-forest region of Ivory Coast was found as 10.30% (95% CI: 8.40–12.40) from samples of 2005 and 2009 (SANOGO et al., 2012); whilst another study (2005 and 2009), observed a true seroprevalence of bovine brucellosis of 4.60% (95% CI, 0.60–9.50) (SANOGO et al., 2013b).

In Eritrea, vaccination against brucellosis in animals was not practiced due to the low prevalence of disease in the country. However, in 2020 the Ministery of Agriculture (MoA) in face of the increased number of cases in the town Gindae, decided to apply vaccination of cattle with S19 as a control measure (TEKLOM, 2020). Previous studies conducted at the end of 1990 demonstrated a low seroprevalence of brucellosis among cattle, goat, sheep, and horses in the country [135/2427 (5.56%) cattle; 21/765 (2.74%) small ruminants; 0/765 (0.00%) horses] (OMER et al., 2000a). Another study conducted between 1997 and 1998 in the Asmara region with dairy cattle found 36.00% (23/64) of seropositive herds (OMER et al., 2000b). More recently, in 2009 the MoA conducted a study in cattle herds in all regions of Eritrea showing a seroprevalence of 2.80% (CI: 2.50%–3.00%) among the tested animals (SCACCHIA et al., 2013).

Information on animal brucellosis in Gambia is scarce, wherein the data found for cattle in Western Region revealed 24/35 (75.00%) positive animals on RBT and iELISA (BANKOLE et al., 2010). Another study performed in 2014 at 12 villages in Kiang West district, and in Abuko and Brikama (2014) found only 14/1123 (1.25%) small ruminants as positive in RBT, however all samples were negative on iELISA and PCR (from milk samples and vaginal swabs) (GERMERAAD et al., 2016), demonstrating the heterogeneous epidemiologic situation for animal brucellosis in these regions. Additionally, *B. abortus* biovar 3 was also isolated among cattle in the country (SANOGO et al., 2013a).

Animal brucellosis in Guinea Equatorial was investigated by a study that isolated *B. ovis* from sheep, between 2013 and 2014, showing a seroprevalence of 0.60% in the total animal tested and a seroprevalence of 42.80% in the isolated native animals. The results demonstrated the considerable presence of *B. ovis* was found in the native animals which is a sanitary problem for the country herd (LOUREIRO et al., 2017). Information about other livestock animals infected with *Brucella* spp. in Guinea Equatorial was not found in this review.

The first strain of *Brucella* spp. identified in Madagascar was reported in 1975, isolated from a female dog (VERGER et al., 1975). A more recent study from 2017, conducted in the municipality of Bemasoandro, found positive only one zebu cattle from the 214 sampled in qPCR performed from blood, even though serology and bacteriology was negative (BOONE et al., 2017) According to the reported cases in humans in this study, the authors infer that cattle brucellosis in Madagascar does not seems to contribute to human brucellosis and other routes needs to be considered (BOONE et al., 2017).

In Malawi, information on livestock animal brucellosis is scarce, however two studies were found on bovine brucellosis. One study was carried out in two districts at the northern region of Malawi, in Mzimba and Nkhata Bay, in 2011, found 12/251 (7.70%) animals with antibodies against *B. abortus* (TEBUG et al., 2014). Similarly, other study conducted in the southern region of Malawi, in 2020, sampled serum from 529 cattle, and no animal tested positive to brucellosis in this study (KOTHOWA et al., 2021).

In Mali, a study published in 1994 reported a 53.00% (n = 236) herd and an animal seroprevalence of 23.30% (n = 1,000) (TOUNKARA et al., 1994). More recently, another study conducted, between 2017 and 2018, found 32.50% (95% CI: 28.00–37.00) of positive herds from the 120 herds tested from Bamako (Musallam et al., 2019). Furthermore, a study carried out in 2016 in the major livestock producing regions of Mali observed a seroprevalence of brucellosis in cattle of 0.30% (1/304) (CI 95%;0.00–1.80), 0.63% (2/318) (CI 95%;0.10–2.20) in sheep, and 0.00% (0/290) in goat (DIONE et al., 2022), pointing to a low occurrence of animal brucellosis in the investigated regions.

In Namibia, a case of human brucellosis confirmed in 2009 lead to the investigation of cases in animals and other humans in the surrounding areas (MAGWEDERE et al., 2011). The seroprevalence found in the area varied from 0.14% (2008 to 2010) to 11.62% (38/327) in goats (MAGWEDERE et al., 2011; MADZINGIRA & MCCRINDLE, 2015). Additionally, another study found only one cow from the one sampled farm that was seropositive for brucellosis in 2013 (0.01%) (MADZINGIRA & SEZUNI, 2017). Finally, another retrospective study, assessed bovine brucellosis testing results from 2004 to 2018, and found a cattle and herd seroprevalence of 0.49% (244/49,718) (95% CI 0.43–0.56%) and 9.26% (78/842) (95%CI, 7.49–11.41%), respectively (MADZINGIRA et al., 2020). The available literature for Namibia suggest the presence of animal brucellosis, possibly influencing the occurrence of the disease in humans in the country.

In Niger, another West African country, a survey carried out in Niamey from 2007 to 2008, and that sampled 5,192 animals (cattle, sheep, and goats) from 681 herds, revealed a true brucellosis seroprevalence of 3.40% (CI: 2.10-7.50) in cattle, 0.80% (CI: 0.10-3.90) in sheep, and goats were seronegative (BOUKARY et al., 2013). The results also showed 13.70% of seropositive herds (BOUKARY et al., 2013). Another survey conducted in February 2007, at herd level using samples from a milk bulk tank from Niamey found 1.20% (95%CI: 0.08–5.30) of milk samples positive (MUSALLAM et al., 2019).

Animal brucellosis in Nigeria has been described since 1962, when 13 cattle were found positive from the 96 tested (ADAMS & MCKAY, 1966). Since then, innumerous studies had been conducted in Nigeria showing the presence of brucellosis in dogs (OKOH et al., 1978; CADMUS et al., 2011; AYOOLA et al., 2016), camels (OKOH, 1979; SALISU et al., 2017), indigenous breeds of goats (OLUFEMI et al., 2018), donkeys (TIJJANI et al., 2017; ADAMU et al., 2020) and horses (OCHOLI et al., 2004; EHIZIBOLO et al., 2011; ARDO & ABUBAKAR, 2016). Information on cattle, goats and sheep is also available and more recent findings in a study conducted in Sokoto state revealed a herd-level brucellosis seroprevalence of 23.20% (95% CI: 11.07–42.54%) and 42.00% (95% CI: 25.27–61.11%) by RBT and cELISA, respectively (CADMUS et al., 2021). Noteworthy, a study from 2018 at Lagos State also observed high prevalence of the disease, finding 38/221 (17.20%) seropositive cattle, 38/192 (17.20%) seropositive goat and 5/60 (8.30%) seropositive sheep in RBT (UKWUEZE et al., 2020). Complementarily, animal brucellosis have been described in other areas of Nigeria, a survey conducted in Enugu state revealed 14/340 positive (4.10%) goats and 12/484 positive cattle (2.50%) (EKERE et al., 2018).

Information on animal brucellosis in Central African Republic is scarce and the real impact of the disease on livestock production is unknown (NAKOUNÉ et al., 2004). However, between 1998 and 2000, the National Agency of Breeding Development collected and tested by RBT bovine serum samples, revealing a brucellosis seroprevalence of 3.40% (69/2,032) (NAKOUNÉ et al., 2004).

In Rwanda, a cross-sectional study conducted in Southern Province, Western Province and Eastern Province, in 2013, found a brucellosis seroprevalence of 33.00% (95% CI: 24.00-43.00%) in cattle at the farm level, and 14.00% (95% CI: 11.00-17.00%) at the animal level (KIIZA et al., 2021). Furthermore, another study published in 2021, investigated the presence of anti-*Brucella* spp. antibodies in 330 milk samples from bulk tanks across Rwanda and found 19.70% (95% CI: 15.50-24.40) of positive samples (DJANGWANI et al., 2021). Furthermore, *Brucella* spp. DNA was identified in cultures from aborted tissues (10.50%, [2/19]) from cattle and 100% of goats (10/10) were found brucellosis seropositive (DJANGWANI et al., 2021). Mixed infections caused by *B. melitensis* and *B. abortus* were also identified in cattle in the country (NTIVUGURUZWA et al., 2022), pointing for the presence of *B. melitensis* also in cattle.

In Senegal, animal brucellosis was investigated in cow milk and no antibody anti-*Brucella* was found in a study from 2010 (BREUREC et al., 2010) and 2019 (MUSALLAM et al., 2019). However, in another country in the opposite side of the African continent, anti-*Brucella* spp. antibodies were identified in 28/250 (11.20%) samples from goats of Mogadishu abattoir in Somalia (FALADE & HUSSEIN, 1979). Additionally, between 2017 and 2018, blood from 609 animals, 201 cattle, 203 goats and 205 sheep, from Afgoye and Jowhar districts of Somalia were sampled and a seroprevalence of 10.50 % (64/609) (95 % CI: 8.20–13.20 %) were found in all ruminants, being 19.40% (39/201) (95 % CI: 14.20–25.60 %) in cattle, 7.90% (16/203) (95 % CI: 4.60–12.50 %) in goats and 4.40% (9/205) (95 % CI: 2.00–8.20 %) in sheep (HASSAN-KADLE et al., 2021). Therefore, animal brucellosis in Somalia is still a concern, especially considering the unsafe practices regarding aborted materials from animals, contributing to the risk for human health for this neglected disease (HASSAN-KADLE et al., 2021).

In Sudan, animal brucellosis has been a concern for decades. A study published in 1974 reported the isolation *B. abortus* from a cow milk sample (IBRAHIM, 1974), as well as *B. abortus* biovar 6 strains were isolated in 20.48% (213/1040) of tested small ruminants at Nayala (year not reported) (MUSA et al., 1990a) Moving forward in time, *B. abortus* biovar 6 was again isolated from hygromas of three cattle and a camel (OMER et al., 2010), confirming the circulation of *Brucella* spp. among different animals around the country. In another study, brucellosis clinical signs were investigated in 131 zebu cattle in Darfur province, among which 85.5% were serologically positive and 89 also had hygroma (MUSA et al., 1990b). *B. melitensis* biovar 3 has also been isolated in the country from sheep (testicles) (MUSA & JAHANS, 1990). In 1999, sera samples from cattle, goats and sheep in an Kassala abattoir were tested and from 1,038 sera tested, 103 were positive (52 were cattle, 41 goats and 10 sheep), revealing a low seroprevalence of brucellosis in Kassala area, compared with other areas in Sudan (EL-ANSARY et al., 2001). The disease is also endemic in goats, as it was demonstrated by a cross-sectional study performed in 2012 in Khartoum state that found a seroprevalence of 11.40% (35/307) (95% CI: 7.80–15.00) (MOHAMED et al., 2018).

In South Sudan a study published in 2016 found an overall animal seroprevalence of bovine brucellosis in cattle was observed as 23.20% (95%CI: 18.4–28.8) and 19.20% (95%CI: 2.5–14.0), depending on the used test (RBT and cELISA respectively), in peri-urban Juba, and in rural Terekeka County, the individual seroprevalence was of 40.5% (95%CI: 34.5 – 46.4) and 39.3% (95%CI: 33.3–45.2) for RBT and cELISA, respectively (LITA et al., 2016). In addition, between 2015 and 2016, in the Greater Bahr el Ghazal region, brucellosis seroprevalence was 31.00% (95% CI: 28.00–34.20) among 893 cattle serum samples collected (MADUT et al., 2018).

Animal brucellosis in Togo has been demonstrated since 1984, with 30 *B. abortus* strains isolated from animals (VERGER & GRAYON, 1984). *B. abortus* strains (n = 3) were also isolated from cows of the northern region of Togo (DEAN et al., 2014). Antibodies against *Brucella* spp. in milk samples, indicating that the pathogens was circulating among dairy cattle were observed in 62% (62/100) of dairy herds sampled from 2017 to 2018 (MUSALLAM et al., 2019). In 25 different villages Savannah region, the presence of anti-*Brucella* spp. antibodies was not observed in sheep and goats samples and in 8.90% (95% CI: 7.00–10.70) of sampled cattle (n = 596) and 7.10% (95% CI: 5.00–9.50) of transhumant cattle (n = 464) (DEAN et al., 2013).

In Tunisia cases of animal brucellosis were also reported. A study conducted in central-eastern Tunisia showed a true animal prevalence of 23.50% for cattle and 13.50% for sheep and an adjusted herd level prevalence of 55.60% for cattle and 21.80% in sheep (BARKALLAH et al., 2017). Noteworthy, a survey carried out in 150 aborted tissues from cattle, found 31.30% of samples positive for *Brucella* spp. in PCR (BARKALLAH et al., 2014), demonstrating without doubt the presence of infection in these herds and also the occupational risks for those dealing with aborted material (PEREIRA et al., 2020b).

In Uganda animal brucellosis has been described in innumerous studies in cattle (MAKITA et al., 2011; NIZEYIMANA et al., 2013; MUGIZI et al., 2015a; MUGIZI et al., 2015b; ROCK et al., 2016; KAMWINE et al., 2017; WOLFF et al., 2017; BUGEZA et al., 2019) with prevalences varying from 1.20% (4/345) to 26.50% (49/185). The disease was also reported in goats (KABAGAMBE et al., 2001; LOLLI et al., 2016; MILLER et al., 2016; NGUNA et al., 2019) with prevalence of 0.30% (10/351), and sheep (LOLLI et al., 2016), with a prevalence of 2.60% (8/306). Moreover, brucellosis in other animal species have also been described, such as in swines [0.18% (4/1665)] (ERUME et al., 2016) and dogs [1.30% (1/80)] (KALULE et al., 2020).

Information on livestock brucellosis is better documented for some African countries, being not available for all countries in the continent that were thereby not discussed in the present review. In fact, countries such as Angola, Argelia, Algeria,Benim, Cape Green, Comores, Chade, Democratic Republic of Congo, Djibouti, Eswatini, Gabao, Gana, Guinea, Guinea Bissau, Lybia, Lesoto, Marrocos, Mozambique, Mauritama, Mauricia, Republic of Congo, Seychelles Isle and San Thome and Prince, have scarce literature about brucellosis occurrence in livestock animals.

Despite of that, the literature on animal brucellosis was available for many African countries, with several studies with different levels of representativeness been recorded. Overall, these studies point absent or punctual disease control strategies in most countries, as well as to no robust surveillance system or structured control program in place in many of them, which prevents monitoring the epidemiological situation over time in the countries. Broader information of livestock animal brucellosis is urgently needed for most of countries to guide implementation of tailored disease control measures. The common challenges for the establishment of control programs in these countries are probalby the lack of public and veterinary health services due to both, reduced government resources and the low interest by the private sector to support the internal control activities and require collaboration at the regional level.

Asia

In Asia, bovine brucellosis shows the major economic impact, caused by *B. abortus*. However, especially in the Western Asia, cattle is maintained in close association with small ruminants, and the infection can also be caused by *B. melitensis* (WORLD ORGANIZATION FOR ANIMAL HEALTH, 2000). The canine brucellosis is important, especially in China, since these animals are raised to serve as food, not as pets like in the western world, the information about dogs is only tangential considering that these animals are not the objective of this work.

Data from 2013 shown that the prevalence of *B. abortus* and *B. melitensis* among livestock animals in the countries of the Association of Southeast Asian Nations (ASEAN) (Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar, the Philippines, Singapore, Thailand and Vietnam), can be considered low (0.00 to 4.80%) and very low (0.00 to 1.00%), respectively (ZAMRI-SAAD and

KAMARUDIN (2016). Among ASEAN countries, Malaysia has one of highest bovine brucellosis prevalences, anti-*Brucella* antibodies were detected in 21.80% of sampled herds (95% CI: 21.01–22.59) and 2.50% (95% CI: 2.45–2.55) of sampled cattle, in Pahang, Malaysia (ANKA et al., 2013). In small ruminants, the first report of brucellosis in sheep was in 1987, in Terengganu, Kedah, Perlis and Pahang, all in Malaysia, resulting in a prevalence of 0.02%, in 1987, and 0.13%, in 1991 (MAHENDRAN, 1992). In goats, the infection by *B. melitensis* has increased during the period between 2000 and 2009, affecting all states in Malaysia (ANIMAL PRODUCTION AND HEALTH COMISSION FOR ASIA, 2010), being estimated in 14.50% in 2009 in small ruminants (KHOR et al., 2010). A decrease in the occurrence of brucellosis in cattle and small ruminants was described more recently, with a variation of 4.00–5.00% in cattle and 1.00% in goats (ZAMRI-SAAD & KAMARUDIN, 2016). Now the country faces the challenge of eradication by identifying the hotspots for the disease, controlling the movement of infected animals and restricting commerce of animals from non-free brucellosis countries (ZAMRI-SAAD & KAMARUDIN, 2016).

In Thailand, another ASEAN country, brucellosis caused by *B. abortus* was first reported in 1956 (EKGATAT et al., 2011). In 1970, brucellosis was considered endemic in Thailand, with huge economic losses on farms (SUB HARNGKASEN, 1970). More recent studies estimated, in 2013–2017, a prevalence of 2.60% (95% CI: 0.90–7.30) for bovine brucellosis and 13.30% (95% CI: 3.70–37.90) for small ruminants (COLOMBE et al., 2018). National control program is in place in Thailand and is based mainly in surveillance actions (PECK et al., 2018).

Serological studies have indicated the presence of bovine brucellosis in cattle in different islands from Indonesian archipelago including South Sulawesi, West Timo, and Eastern islands (GEONG & ROBERTSON, 2000; MUFLIHANAH et al., 2013), although infection in goats and sheep in Indonesia has not been reported (MUFLIHANAH et al., 2013). Around the same Asian area, reliable estimates of the occurrence of animal brucellosis in Cambodia, Laos and Vietnam are not available, but evidence of *Brucella* spp. infection has been reported in several animal species in these parts of Asia (BURNS et al., 2018; SIENGSANAN-LAMONT & BLACKSELL, 2021). No recent data on animal brucellosis in Brunei, Philippines and Singapore were published.

Brucellosis is a significant and increasing veterinary and public health problem also in India (VERMA, 2013), a country that has one of the largest ruminant (cattle and buffaloes) population in the world (LINDAHL et al., 2020). According to an estimate, the annual losses due to brucellosis in India are US$ 3.40 billion (95% CI: 2.80-4.20 billion), with bovines and buffalo being the main responsible (95.60%) for these losses (SINGH et al., 2015). The prevalence of the disease in both species is estimated in 23.51% and 10.20%, in bovines and buffalo respectively, with both prevalences considered high and worrisome (SHOME et al., 2019). Vaccination is the measure use to control brucellosis in India, since test and slaughter of positive animals for brucellosis is not a alternative because of religious culture and due to economic reasons (CHAND & CHHABRA, 2013). Brucellosis in goats and sheep by *B. melitensis* is also of high concern in India, since they are the main responsible cause of human cases (MANTUR & AMARNATH, 2008), besides the adverse economic impact on international trade for milk and meat. The national cumulative incidence of brucellosis in sheep and goat was estimated as 7.90%, and 2.20%, respectively (RENUKARADHYA et al., 2002).

Bangladesh and India, before 1945, were the same country, and brucellosis was first recognized in India in 1942 (RENUKARADHYA et al., 2002). After the separation, brucellosis was identified in Bangladesh in 1967 (MIA & ISLAM, 1967). Almost 40 years later, brucellosis was diagnosed in humans, caprine and bovines (AMIN et al., 2005). In this country, 80.00% of rural population are directly or indirectly involved with livestock rearing, usually living in close proximity with their animals, and vaccination against bovine brucellosis has never been practiced (RAHMAN et al., 2018). In 1997, RAHMAN et al. (1997) found a prevalence in buffaloes of 6.90% and, in 2005, AMIN et al. (2005) reported a 5.00% of prevalence in bovines. A more recent study estimated in the Mymensingh district a brucellosis prevalence of 3.70% (95% CI: 2.10–66.00) and 4.00% (95% CI: 1.70–9.20) in bovines and buffaloes, respectively (ISLAM et al., 2013). Regarding the diagnostic methods, despite the pathogen isolation being recommended as best diagnostic test for identification of *Brucella* spp. (ALTON et al., 1988), in Bangladesh it is not a reality, due to the absence of biosecurity level 3 laboratories facilities that is required for isolation of *Brucella* spp. (AHASAN et al., 2017). Therefore, it is still valid to remember the public health relevance of brucellosis wherein nearly every human case has an animal origin, showing the importance of control measures.

In Pakistan, brucellosis is endemic in cattle, small ruminants and dogs, caused by *B. abortus*, *B. melitensis* and *B. canis*, respectively (DÍAZ, 2013). The average prevalence of the disease in small ruminants was estimated in 4.78% in district Quetta, Balochistan (ALI et al., 2017); whereas bovine brucellosis has been estimated as 15.00% in district Karak, Khyber Pakhtunkhwa, North west of Pakistan (KHAN et al., 2017). For the disease control, the vaccination is performed in cattle, with S19 and RB51, and in small ruminants, with Rev. 1, together with test-and-slaughter policy (JAMIL and KASI (2020). Even though animal brucellosis is still a problem in the country, requiring educational campaign for farmers and more policies to encourage adherence to control programs among them.

In Nepal, bovine and caprine brucellosis are endemic and considered a serious public health threat. More than 90.00% of the population live in villages and has direct contact with domestic animals (PYAKURAL & MISHRA, 1977). Some of the problems that Nepal faces to control the disease are inadequate laboratory facilities, financial constraints, religious restrictions, lack of compliance of farmers and abandonment of positive animals, contributing for the spreading of the disease. The major approach to improve livestock production is usually focused on animal genetics and not necessarily on animal health, which can be one of the reasons for brucellosis to be neglected in Nepal (ACHARYA et al., 2016). Furthermore, animals are usually housed in unhygienic sheds and in close association with other species. The close contact among different species represents a significant risk of brucellosis transmission between animals and to humans (ACHARYA et al., 2016). In the city of Pokhara, the prevalence of buffalo brucellosis was 22.60%, 8.70% in cattle, 3.60% in sheep and goats and 6.10% in humans (JOSHI, 1983). One control measure implemented is vaccination, which is performed in cattle, using S19 or RB51, and for small ruminants, Rev. 1. However, combining the fact that slaughter of animals is prohibited with the lack of awareness among farmers, control measures in Nepal are scarce (ACHARYA et al., 2016). Noteworthy, only vaccination of animals does not decrease fast enough the disease prevalence and a program of awareness directed to the farmers and the population about the risks related to brucellosis and its importance for public health should be implemented (DADAR et al., 2021).

In Iraq, the incidence risk of seroconversion for brucellosis was found as 10.60% (95% CI: 6.90–15.30) for small ruminants and the animals that seroconverted to *Brucella* were 2.90 times more likely to lose their pregnancy (95% CI: 1.60–5.50) than animals that remained seronegative, in Dohuk Province (AL HAMADA et al., 2021). A previously study conducted in the same area found a prevalence of 31.70% (95%CI: 26.10-36.30) of sheep and 34.00% (95% CI: 24.70-44.30) of goats (ALHAMADA et al., 2017). A systematic review conducted to access brucellosis in food-producing animals in Mosul, Iraq, found an overall seroprevalence of 14.14% of the animals positive, from which 14.50% was sheep, 13.00% was goats, 11.70% was cattle, and 22.6% was buffalo (DAHL, 2020). All these data demonstrate that animal brucellosis in Iraq is of great concern for animal and human health, considering that most human brucellosis cases originated from contact with animals and their products.

Brucellosis in Saudi Arabia is endemic for humans and animals (Memish, 2001), and has been described since 1984. Consequently, to evaluate a control program in Najdi, sheep were serologically tested three times at six-month intervals, showing a decrease in occurrence of serologically positives among the first test 387/2721 (14.20%), second test 31/2072 (1.50%) and third test nine/2963 (0.30%) (RADWAN et al., 1984). Nonetheless, another study found that occurrence of brucellosis in sheep was 7.70% of 665 tested, 8.80% of 228 tested goats, and 6.50% of 107 tested camel, in Medina region. Therefore, restricted control measures and vaccination of animals should be implemented to prevent the spread of animal brucellosis (SHABANA & KRIMLY, 2020). Furthermore, *Brucella* spp. contamination in raw cow milk in Saudi Arabia was found to be 3.00% in a meta-analysis (ABEDI et al., 2020).

In Oman, brucellosis was investigated in sera from goats, sheep, cattle, and camels, between 1985 and 1986, and a seroprevalence of 0.90% (118) was found for goats, 1.60% (23) for sheep, 2.90% (87) for cattle, and 3.6% (20) for camels for brucellosis (ISMAILY et al., 1988). More recently, a study identified 11.10% (36/324) of positive goats, in Al Jabal Al Akhdar, Sultanate in Oman (ELTAHIR et al., 2018). Due to the importance of camels in the Middle East, it is important to report about a study that was conducted to analyze isolated *B. melitensis* from these animals in relation to origin from the other animals such as goat, sheep, and cattle. The study described that *B. melitensis* affecting camels was originated likely from North Africa countries with animals entering in Oman by trade of infected livestock (FOSTER et al., 2017). Thereafter, besides vaccination and test and slaughter of positives, as control measures for small ruminants and bovines, surveillance of the trade of animals is another aspect to be importantly considering in the control of animal brucellosis in Oman.

In fact, a review on incidence and control of the disease in the East Region pointed out an increase of prevalence of animal brucellosis, explaining that in 1989, a prevalence of 2.86% was found in cows and 1.81% in sheep, and in rural areas of Damascus the incidence was 7.83% in cows. The review also identified a survey from 1990–1991 with an incidence of 2.00–7.80% in cattle and 1.40–5.00% in sheep and goats (REFAI, 2002). A national brucellosis survey from 1990/1991 found a seroprevalence of 3.57% (448/12,554) for cattle and 2.50% (672/26,755) in small ruminants, also, the survey described the identification of *Brucella* isolates from Syria, which included isolates from sheep (*B. melitensis* biovars 2 and 3), one isolate from a ram with orchitis (*B. melitensis* biovar 3), others isolated from sheep fetuses from Damascus and Aleppo (*B. melitensis* biovar 2), and cattle isolates that were typed as *B. abortus* biovar 9 (DARWISH & BENKIRANE, 2001). Regarding dairy products contaminated with *Brucella* spp., another review only found one study approaching isolation of this bacteria from bovine milk (AL-MARIRI, 2015; ABEDI et al., 2020), also *Brucella* was isolated from 677 milk samples in a study from 2011 that evaluated 2,375 milk samples from sheep (AL-MARIRI et al., 2011), demonstrating once more the importance of accessing the epidemiological status of the disease in animals and avoiding the consumption of row dairy products to prevent human cases.

Among the Near East region countries, animal brucellosis in Turkey is also a concern, with *B. melitensis* biovar 2 from sheep and *B. abortus* biovar 3 from cattle, being identified in the country. Additionally, biovars 1, 2, 4 and 6 for *B. abortus* and biovar 1 for *B. melitensis*, were identified in Turkey (REFAI, 2002). Furthermore, *B. melitensis* was isolated in 5 (14.20%) of 35 ewes' milk cheese samples from Kirikkale city (KASIMOĞLU, 2002) and *Brucella* spp. were isolated from

16 stomach content samples from aborted fetuses of sheep (CETINKAYA et al., 1999), indicating the circulation of *Brucella* spp. in different parts of the country including in animal products. There is an active surveillance program as part of a national control program for animal brucellosis in Turkey conducted by the Ministry of Agriculture and Rural Affairs, created since 1937. The program preconize vaccination with S19 for cattle, which has been applied since 1960, and Rev. 1 for goats and sheep since 1968 (YUMUK & O'CALLAGHAN, 2012). Even though there is a long-time standing control program, animal brucellosis still a concert in Turkey, therefore, it is necessary the implementation of other effective policies in controlling the disease in ruminants and their products.

In Israel, since 1984 and 1985 brucellosis caused by *B. abortus* in beef cattle and in dairy cattle, has been eradicated, however in small ruminants, brucellosis by *B. melitensis* it is still endemic since 1970. In 1997 a full program to eradicate the disease was implemented (BANAI, 2010)..

Throughout the years animal brucellosis has been a concern also in Jordan, with 34 *B. melitensis* strains isolated from samples of aborted, still born, or weak full-term animals and vaginal swabs of cattle and sheep (ALDOMY et al., 1992), being biovar 3 the most common isolated from animals (REFAI, 2002). In Northern Jordan it was found a brucellosis seroprevalence of 14.30% by RBT, 7.20% by ELISA and 2.20% using tests in series, in combination with isolation of *B. melitensis* biotype 3 from aborted fetuses and vaginal swabs of sheep (AL-TALAFHAH et al., 2003). In cattle, a seroprevalence of 6.50% of 671 cattle sampled was found which represented 23.00% of positive herds from 62 herds sampled (AL-MAJALI et al., 2009) showing a better scenario compared to small ruminants. In the Mafraq region of Jordan, 188 animals were sampled and a prevalence of *B. melitensis* of 27.10% (51/188) was reported in sheep (SAMADI et al., 2010). Additionally, a cross-sectional study found an estimated true seroprevalence of 18.10% (95% CI: 11.00–25.30%) for cattle herds, 22.20% (95% CI: 16.50–28.80%) for sheep flocks, 45.40% (95% CI: 30.30-61.60) for goat herds, 70.40% (95% CI: 55.50–84.90%) for mixed sheep-goat flocks, 34.30% (95% CI: 28.40– 40.40%) of small ruminant flocks and 38.50% (95% CI: 24.30–51.80%) of seroprevalence of mixed herds of cattle and small ruminants (MUSALLAM et al., 2015). Therefore, animal brucellosis in ruminants in Jordan has an alarming epidemiologic situation, especially for small ruminants, representing a problem for animal and public health.

Contrary to the previous country, in Lebanon, animal brucellosis is more important for cattle, where 7.93% (28/353) were positive in RBT and 10.48% (37/353) in iELISA test for identification of antibodies anti-*B. abortus* (HASSAN et al., 2020b). Additionally, in the Southern region of Lebanon, 15.30% (95% CI: 10.30–20.20%) of 121 milk samples were found positive using RBT, and all positive samples were from Holstein breed (HASSAN et al., 2020a).

Iran is one of the many countries that has not yet been successful in bovine brucellosis eradication, probably due to its variety of reservoirs and for not consider them as important components of disease epidemiology, for both animal and public health, as well as for the livestock industry (ASSADI et al., 2013). Additionally, the species and biovars are still under-reported because of the lack of laboratorial facilities and adequate diagnosis protocols. DADAR et al. (2019a) isolated *B. abortus* from bovines and *B. melitensis* from sheep, from samples of blood, cerebrospinal fluid, abomasum content and aborted fetus tissues (DADAR et al., 2019b), indicating bovines as important reservoirs. The first vaccination program in Iran against bovine brucellosis occurred in 1949, and from 1988 forward, vaccination only in calfhood started along with implementation of hygiene education programs. In 2007, the S19 vaccine was removed from the program of control and prevention of bovine brucellosis and RB51 was implemented for all adult bovines. (ESMAEILI & SALEHI, 2012). Despite vaccination, in 2007 new cases of bovine brucellosis were reported, for that reason, the Iranian Veterinary Organization implemented a program with quarantine and test-and-slaughter for eradication (YAZDI et al., 2009). The most prevalent biovars for *B. melitensis* is biovar 1 and for *B. abortus*, biovar 3, in different species and hosts, an important information for healthcare professionals and for public health (DADAR et al., 2019b). ASHRAFGANJOOYI et al. (2017) performed isolation of the *Brucella* microorganism from goat and sheep milk and 1.28% of the samples were contaminated with *B. melitensis* and only one with *B. ovis*. Regarding vaccination of small ruminants, brucellosis vaccine production and animals vaccination against the disease were initiated in 1963 (ASSADI et al., 2013), with Rev. 1 vaccine. Since then, tests on vaccine efficacy has proven that Rev. 1 vaccine can decrease the prevalence from 45.00% to 1.80% (ESMAEILI, 2015). From 2003, the control program was based in vaccination of lambs and goats from 4 to 7 months old, using the standard dose of Rev. 1 (1-3 x 10^9 CFU) and, in adults, using the reduced dose (0.5-2 x 10^6). Vaccination of animals combined with public education, sanitary practices, and microbiological assessment of herds with abortion outbreaks, allowed the human new cases to decrease in the (BEHROOZIKHAH et al., 2012).

The Pacific Island Countries and Territories (PICTs) were free of brucellosis until 2009, when an outbreak occurred in Fiji Islands. The outbreak occurred in cattle with a prevalence of 1.50% and evolved to 3.50% in 2010 and decreased to 0.12% in 2013. The start of cases in 2009 was hypothesized to be due to the lack of surveillance of brucellosis, since there were no cases until that year, combined with inadequate management (TUKANA et al., 2015). Some risk factors were associated with the occurrence of brucellosis in farms such as history of cattle that tested positive to brucellosis or bovine tuberculosis and sharing of water sources by animals, within and outside the properties. TUKANA et al. (2016) also describe that the lack of funds, low technical capacity, and equipment for collect and processsamples, a low number of veterinarians were factors that contribute to the poor control of the disease nowadays. The population and farmers awareness together with a good surveillance are essential for brucellosis control in Fiji (TUKANA et al., 2015; TUKANA et al., 2016).

In Korea, *B. abortus* is the main *Brucella* specie infecting humans and cattle (PARK et al., 2005). The first human cases were reported in 2002 with increased occurrence throughout the years which entailed the implantation of an aggressive eradication policy that contributed to the rapid decrease in prevalence of brucellosis in human and bovines (KIM et al., 2017). Control and prevention programs were described in Korea to be mostly based on test-and-slaughter (JUNG et al., 2010). The first *Brucella* species isolated in Korea was *B. canis*, in 1848 (KIM et al., 2003) but since then, the prevalence was not well stablished for this specie. Between March 2015 and December 2016 it was performed a prevalence study of canine brucellosis that found 1.30% of 1,825 animals as seropositive for the disease, being 0.90% companion dogs and 2.50%, stray dogs (JUNG et al., 2018). There is no national control program against canine brucellosis in Korea and although the prevalence is low; the circulating bacteria may affect humans (animal owners). Some authors suggests that periodical screening tests might be necessary to improve dogs heath and, consequently, to protect the public health in Korea (JUNG et al., 2018).Considering eradication of bovine and human brucellosis in Korea would be possible with animals' vaccination, government surveillance and better management conditions, as quarantine. According to a study published in 2017, if bovine brucellosis is controlled, the human cases will also disappear (KIM et al., 2017).

Since 1950, brucellosis has been studied in China, for being an endemic disease in the country (DEQIU et al., 2002). The S19 vaccine, against bovine brucellosis, was first used in 1958 with the dose of $5x10^{10}$ CFU (YAN et al., 1986), which had satisfactory results, however around 1970, its use was gradually interrupted (SHANG, 2000). During the years 1977 to 1988, brucellosis control programs were implemented, which included vaccination of domestic animals as a measure of disease control (JIANG et al., 2020). Later on, from 1990 until the present, brucellosis became known as reemergent (JIANG et al., 2020), affecting domestic animals and exposing the public health to a great risk (RAN et al., 2018). In the last decade the demand for dairy products has generated investments in dairy farming and, consequently increasing livestock and its transportation, accelerating the spread of brucellosis (MAMANI et al., 2018). In 2020, brucellosis remains a problem for human and animal health. Despite Rev. 1 vaccine has been used since 1990, in a dose of $2x10^9$ CFU (DEQIU et al., 2002), in 2018, goats from Anhuí Province, China, showed an average prevalence of 3.10% (RAHMAN et al., 2019) which is considered an worrying situation. There was an increase in the prevalence of brucellosis, both in sheep and goats, throughout the country, from the years 2000-2009 (1.00%; CI: 95%, 0.70-1.30) to the years 2010-2018 (3.20%; CI: 95%, 2.70-3.60) (RAN et al., 2018). In the same meta-analysis, the highest prevalence of brucellosis in small ruminants flocks was found in eastern China, with 7.00% (95% CI 0.00–17.40%), while the region with the highest rate of positivity was the province of Shandong, with an 18.70% (95% CI 15.80–21.60%) of disease. It should also be noted that the prevalence of caprine brucellosis is more expressive than that of sheep (RAN et al., 2018). The canine brucellosis is a source of brucellosis infection for humans and its relevance for public health remains under debate (LIU et al., 2020). *Brucella canis* is a bacteria with a limited host range, being already reported in Japan, United States, Argentina, Mexico and Brazil (LUCERO et al., 2005). Additionally, it is necessary to reiterate that more efforts on prevention and control actions are needed to improve public awareness and understanding the health risks caused by *B. canis* (LIU et al., 2020).

Therefore, considering animal brucellosis in Asia, knowledge of the disease seroprevalence, especially at a state level of the countries, contributes to the design of control strategies, which combined with a good surveillance approach may evolve to eradication programs. The evaluation of the economic impact and cost of control measures, such as vaccination and the correct management of the infected animals, are important and should be considered to reduce the prevalence of animal brucellosis in these regions. Noteworthy, the prevention also depends on public awareness through sanitarian education and secure practices in livestock, combine with reliable laboratories and veterinarians, awareness programs, and protection measures (SHOME et al., 2020).

Europe

In Europe, bovine brucellosis is a priority disease for many WOAH members, not only where the disease is endemic but also in the official free countries (WORLD ORGANIZATION FOR ANIMAL HEALTH, 2019). There are countries officially brucellosis-free (OBF) in Europe, such as Belgium, Bulgaria, Denmark, Estonia, Finland, France, Germany, Great Britain, Ireland, Lithuania, Malta, Poland, Netherlands, Northern Ireland, Norway, Romania, Slovakia, Slovenia, Sweden, and Switzerland, and countries that still have to implement more efficient control measures for the disease to achieve eradication, as Albania, Bosnia and Herzegovina, Croatia, Portugal, Serbia, and Spain, where prevalence of bovine brucellosis varying from 0.02 to 0.51% (HÉNAUX et al., 2018). The OBF status facilitates the access to export markets, though it do not prevent the possibility of re-emergence of brucellosis (EUROPEAN COMISSION, 2009), as happened in Belgic (from 2010 to 2013) (BRONNER et al., 2013) and France (in 2012) (MAILLES et al., 2012) where the wildlife was also affected (GARIN-BASTUJI et al., 2014). Among the measures to manage transmission of brucellosis from wildlife to domestic animals spatial-temporal segregation, culling of identified positive wild animals and even vaccination of these populations was considered (GODFROID, 2017). Complementarily, actions such as test-and-remove of target populations as female ibex, combine with targeting core areas was demonstrated by a model to be more promising for the control of brucellosis transmission from wild to domestic animals, offering more diversity of approaches due to target strategies (LAMBERT et al., 2021).

Bovine and small ruminant brucellosis-infected herds seem to be geographically concentrated in the southern European members states of the European Union (EU) (EUROPEAN FOOD SAFETY AUTHORITY, 2012b). The percentage of small ruminant farms presenting seropositive animals in Sicily, Italy, for example, in 2007, 2008 and 2009 was 7.00%, 11.00% and 7.00% respectively (CURRÒ et al., 2012). In the EU countries considered non-officially brucellosis free the proportion of existing cattle herds positive for brucellosis in 2010 was as follow: 0.84% in Italy, 0.36% in Portugal, 0.77% in Greece, 0.30% in Northern Ireland, 0.17% in Spain, and 0.00% in Malta, Cyprus, Bulgaria, Hungary, Latvia, Lithuania and Romania, all of them considered a low prevalence of the disease. Additionally, in the EU countries considered non-officially *B. melitensis* free, the proportion of existing sheep and goat positive for *Brucella* spp. was: 1.20% in Portugal, 1.06% in Italy, 0.79% in Spain, 0.20% in Greece and less than 0.10% in Cyprus (EUROPEAN FOOD SAFETY AUTHORITY, 2012b). Noteworthy, in Southeast Europe, animal brucellosis was identified in Kosovo where 6.26% (95% CI: 5.50–7.10%) of 3,548 sampled sheep, 7.24% (95% CI: 5.30–9.80%) of 511 sampled goats and 0.58% (95% CI: 0.43–0.77%) of 7,941 cattle sampled in 2001 were positive (JACKSON et al., 2004). In another country, the Republic of North Macedonia, it was found in 2008, that 2.80% (16,853/596,213) of positives from sheep and goats, and in 2009, sheep and goats were 1.80% (9,606/543,011) positive for brucellosis (KIRANDJISKI et al., 2010). Complementarily, a strain was isolated from aborted fetal of sheep in Ukraine (BOLOTIN et al., 2021).

In Albania, brucellosis is one of the most common zoonosis (MERSINI et al., 2019) and, according to the study published in 2020, the seroprevalence of herds in the country was 55.00% and the average of animal prevalence only in positive herds was 28.00% (95% CI: 40.00-71.00), being brucellosis prevalence greater in beef cattle, other than in dairy cattle (FERO et al., 2020). The test-and-slaughter program is not a reality in Albania, and the country should work with mass vaccination, preferably with S19, and restriction of movement of the animals, intending to control the disease (FERO et al., 2020) and to reduce the prevalence.

Bosnia and Herzegovina first cases of human brucellosis were reported in 2000 (ŠIŠIRAK, 2020) caused by *B. melitensisis*. However, only in 2009, that test-and-slaughter policy was implemented for sheep and goats, when the prevalence of brucellosis in these species was already 4.60% (OBRADOVIĆ & VELIĆ, 2010). After that, vaccination was implemented in the same species contributing to disease occurrence decrease in small ruminants and also in humans (ŠIŠIRAK, 2020). It has been suggested that the solution for Bosnia and Herzegovina brucellosis problems might be the continuous mass vaccination programs (OBRADOVIĆ & VELIĆ, 2010).

Croatia reported the first human brucellosis case in 2004 (PUNDA-POLIC & CVETNIĆ, 2006), a few years latter from Bosnia and Herzegovina, country bordered the south-west of Croatia. However, brucellosis has been a notifiable disease in humans since 1960 and no cases were documented previously. The breeding of goats and sheep in the border area is an important economic activity, reiterating the importance of animal surveillance (PUNDA-POLIC & CVETNIĆ, 2006). However, a study was conducted between 2009 and 2015, focusing on finding spread routes of *Brucella* spp. to guide the control program to possible new directions, and they found stability of housekeeping genes between samples from Bosnia and Herzegovina and South area of Croatia, indicating that the routs of disease spread is well stablished in the area (DUVNJAK et al., 2018). The same study found that *B. melitensis* in Croatia is affecting not only sheep and goats, but humans and bovines, with the endemic strains of this country being phylogenetically very close to Turkey, Greece, and Syria strains (DUVNJAK et al., 2018). These findings reinforce the importance of avoiding illegal movement of animals, additional to monitoring and understanding how the pathogens are transferred through the continents (DUVNJAK et al., 2018). Additionally, collaborations between clinicians and veterinarians are important to prevent human outbreaks (PUNDA-POLIC & CVETNIĆ, 2006). Complementarily, it is necessary to consider that these regions of Croatia had recent social and economic changes, turning the monitoring and control of diseases even harder.

According to the guidelines that helped eradicate brucellosis in the European Union, the first step is to implement good disease control program, lowering the percentage of prevalence in all herds through the organization of veterinary services, epidemiological evaluation, proper diagnosis and animal traffic control (EUROPEAN COMISSION, 2009). Apparently, even the disease control situation is far off for countries like Serbia, where the highest incidence of seropositive sheep and goats was 26.80% and 35.00% respectively, from 2003 to 2007 (DJURICIC, 2010). During the period from 2007 to 2009, it was observed an increased in the number of registered cases of animal brucellosis and a remarkable number of new affected areas (DJURICIC, 2010). A similar situation happen in Georgia, where the incidence of 6.79% was observed in cattle, sheep and goat, tested from 2008 to 2011 (MAMISASHVILI et al., 2013).

Among the control measures that were established in countries that have already eradicated brucellosis, careful surveillance of animal movement was the major one. There is a high risk of reintroduction of bovine brucellosis in the herd throughout animal movement. As stated by (STRINGER et al., 2008) the odds of seropositivity in animals that moved were 19.00 (95% CI: 7.80-46.40) times higher when compared to animals that did not moved. The OBF countries generally test their animals before and after moving, to avoid the introduction of the disease in the herd. Another strategy is to test the semen, perform the artificial reproduction in a strictly controlled manner, and annually subjected to a serological diagnostic the bulls that are kept in an approved semen collection center. Likewise, the surveillance of the slaughter houses is important to avoid the reintroduction of the disease in herds (HÉNAUX et al., 2018). Regarding the diagnostic tests, the recommendation is to start with Rose-Bengal test (RBT), since is a test with high sensitivity, followed by Complement Fixation Test (CFT), which has a high specificity (PRAUD et al., 2016). Therefore, the best control measure to avoid the reintroduction of the disease is the maintenance of strict surveillance of animal movement, despite of the great impact on the agricultural and state budgets (HÉNAUX et al. (2018).

HUMAN BRUCELLOSIS

Human brucellosis is a zoonotic disease with a major impact on public health, even though successful eradication and control programs for domestic animals have been established in several countries around the world (AL DAHOUK et al., 2013). Human brucellosis cases have been estimated in 2.1 million cases worldwide, with most cases in Africa and Asia (LAINE et al., 2023), with Syria been classified as one of the countries from the Middle East with greater prevalence of human brucellosis (PAPPAS et al., 2006). As stated, human brucellosis is directed related to animal brucellosis, even though, information on animal brucellosis in Syria is scarce. The disease in humans has mainly two origins, (i) foodborne: consumption of unpasteurized milk and milk products, and raw or undercooked meat, or (ii) occupational: unsafe working practices by farmer, butcher, veterinarian, microbiologists, etc. (MAGWEDERE et al., 2011; GODFROID et al., 2013; ACHARYA et al., 2016; TUKANA & GUMMOW, 2017; AWAH-NDUKUM et al., 2018; PEREIRA et al., 2020a). Nonetheless, other ways of human infection has happen, such as in 2019, when occurred a leak of a waste gas of a vaccine factory in China, contaminated with *Brucella*, that formed aerosols which drifted downwind, infecting more than 3,000 people (YEUNG & CHEUNG, 2020). Another accident that culminates in more than 10,000 human brucellosis cases in November 2020, occurred in Lanzhou, China by an inadequate sanitizing process in a biopharmaceutical plant during July and August 2019, which led aerosolization of *Brucella* that subsequently spread through wind to nearby settlements and academic institutes (PAPPAS, 2022). Additionally, human-to-human transmission has been described as well, occurring mainly in newborns and breastfeeding babies, by placental barrier and lactation, respectively, also through sexual contact, blood and bone marrow transfusion (TUON et al., 2017).

Three *Brucella* spp. species have major impact on public health, *B. melitensis*, *B. abortus*, and *B. suis* in order of their pathogenic significance (GODFROID et al., 2011). Nonetheless, *B. canis* has also emerged as important human pathogen in the last few years albeit its low pathogenic to man (KOLWIJCK et al., 2022). Indeed, the virulence of this species is low to moderated and it was suggested that healthy humans are resistant to it (HENSEL et al., 2018). However, human infection of *B. canis* was described to be generally associated with *Mycoplasma pneumoniae*, resulting in the decrease of the human immunity, and causing an opportunist brucellosis (LIU et al., 2020). Therefore, the canine brucellosis remains a threat for these animals and for humans that have close contact with dogs (LUCERO et al., 2010). Similarly, *Brucella* spp. of marine origin (*B. ceti* and *B.*

pinnipedialis) have also been recognized as causing infection in humans (SOHN et al., 2003; MCDONALD et al., 2006), a recently a new species, *B. inopinata*, was isolated from a patient with clinical symptoms of brucellosis (SCHOLZ et al., 2010) and another novel species called *B. amazonensis* isolated from two golden miner Brazilian man in French Guiana (ABOUT et al., 2023). Although brucellosis in human is rarely fatal, it can be severely debilitating and disabling (LAI et al., 2017). The first stage of the disease in humans is characterized by flu-like symptoms such as fever, headache and weakness of variable duration (WORLD HEALTH ORGANIZATION, 2020). Human brucellosis usually evolve to cause long-lasting or chronic symptoms, including recurrent fever, joint pain, arthritis and fatigue (EUROPEAN FOOD SAFETY AUTHORITY, 2012a), additional to epididymo-orchitis in man, arthralgia, myalgia and back pain, with complications such as endocarditis and neurological cases (DEAN et al., 2012). Indeed, severe infections of the central nervous system or endocarditis may also occur. In addition, the treatment is long, with several drugs being associated (MCDERMOTT et al., 2013) and the relapse rate is high in humans, reported to be around 10.00% (ÖGREDICI et al., 2010; ROUSHAN et al., 2015).

According to WHO, at least a quarter of brucellosis in humans are unreported worldwide (WORLD HEALTH ORGANIZATION, 2020). Additionally, it is known that some of the human cases in the European Union (EU) are related to travel. Indeed, 85.40% of the brucellosis infections acquired by traveling came from outside of EU and Iraq, Syria, Somalia, and Turkey were pointed as the most probable countries where the infection could come from (EUROPEAN FOOD SAFETY AUTHORITY, 2017).

A study from 2020 suggest that autochthonous infections of human brucellosis must be further investigated, though, imported dairy products seems to play a very important role in the epidemiology of the human brucellosis infection in the EU countries (ENKELMANN et al., 2020).

In addition, physicians should consider brucellosis as a differential diagnosis, especially when there is history of travel to endemic countries (ENKELMANN et al., 2020).

The number of human brucellosis cases has varied worldwide, being the disease still ranked as number 1 or 2 among the reported zoonotic diseases (WHO, 2020). The decreased number of human cases in some countries could presumably be due to ongoing control and eradication programs of animal brucellosis and the promotion of the pasteurization of dairy products, enhancing food security.

INTERFACE BETWEEN HUMAN AND ANIMAL BRUCELLOSIS

In humans, brucellosis is considered as anthropozoonosis and an occupational disease (PAPPAS et al., 2006) whose impact is not well known. Being a zoonosis, almost all human cases have animal origin and eradication of disease in humans is directly related to its eradication in animals. The transmission of brucellosis to humans can occur by different routes. Ingestion is the most common form of transmission between animals and humans due to the habit of consuming raw milk. Despite the knowledge of epidemiology and transmission of brucellosis, outbreaks continue to occur after the consumption of contaminated milk (MÉNDEZ MARTÍNEZ et al., 2003). Likewise, the consumption of raw meat with residues of blood and lymph tissues from infected animals may also contain viable microorganisms, which pose a risk to humans. However, it is important to note that milk and meat when subjected to adequate heat treatment does not pose risks to public health.

Infection by direct contact with the pathogen is more common for people who work with animals or their products in labor activities, such as farmers, veterinarians, agricultural workers, and butchers (PEREIRA et al., 2020b). The routes of infection are direct through conjunctival or respiratory mucosa, or penetration of the agent throughout skin lesions. Accidental inoculation can also occur during the handling of syringes with live microorganisms, especially during vaccination with live attenuated vaccines Rev.1, S19 and RB51 (PEREIRA et al., 2020a). Likewise, slaughterhouses are an enabling environment for the occurrence of occupational infection, since the workers are in direct contact with carcasses and large amounts of aerosols that could potentially transmit the agent to the workers (PEREIRA et al., 2020a).

Noteworthy, brucellosis is the most common bacterial infection acquired in laboratories by humans (YOUNG, 1995), its transmission in research and diagnostic laboratories is well documented in the literature (PEREIRA et al., 2020b), and outbreaks has been reported for laboratory workers (FIORI et al., 2000). In this sense, laboratory technicians may become infected by handling large masses of bacteria to produce vaccines and antigens or even in routine diagnostic. The bacteria can pass directly through breaks in the skin or by contact with mucous membranes, especially the conjunctiva and respiratory mucosa, as explained before. Special care should be given to aerosols formation during manipulation of *Brucella* spp., being transmission related not only to accidents (MARTIN-MAZUELOS et al., 1994). The fact that brucellosis is the most common infection in laboratory workers, it is essential to implement biosafety measures during handling of *Brucella* spp., mainly in research laboratories, where most of the infection occurs (PEREIRA et al., 2020b). The ability of *Brucella* spp. to spread by aerosols and the potential risk of lethal infections, call for the manipulation of the bacteria in a level 3 or higher biosafety laboratory when manipulating live cultures, clinical materials of suspected human or animals and products of abortions (WORLD ORGANIZATION FOR ANIMAL HEALTH, 2022).

The transmission from person to person is not common, being considered insignificant in epidemiological terms. Blood transfusion, tissue transplantation and sexual transmission are possible routes of infection, though rare (CORBEL et al., 2006). Nevertheless, the severity of the disease in humans and the lack of a vaccine protection, justify the eradication of brucellosis in animals worldwide.

Interestingly, the Center for Disease Control and Prevention (CDC) classifies *Brucella* spp. as a microorganism belonging to category B, which corresponds to the second group in order of priority as an agent of bioterrorism (CENTERS FOR DISEASE CONTROL AND PREVENTION, 2018). Interest in *Brucella* spp. as a biological weapon stem from the fact that the pathogen has an airborne transmission, which is highly contagious, with symptoms mostly nonspecific, with very low infectious dose, being only 10 to 100 organisms to establish infection by aerosols in humans (BOSSI et al., 2004).

Therefore, human brucellosis is directed related to the occurrence of brucellosis in animals, less frequently by laboratory exposure and rarely throughout human-to-human transmission. The host preference of *Brucella* spp. maintains the pathogen in domestic animals, which are the ones responsible for most human cases.

Even though wild animals can also be infected by *Brucella* spp. their significance in transmitting brucellosis to humans is low. However, considering that wild animals maintain *Brucella* spp., domestic animals can become infected when in contact with these animals and then transmitted to humans. Hence, it is necessary to consider the wild animal population and the prevalence of brucellosis in this population when implementing control and eradication measures for domestic animals, additional to vaccination, surveillance of cases, animal transportation control and slaughter of positives domestic animals. Therewith, Awareness programs could be implemented among farmers about the control measures available, combined with education about farm practices (TEBUG et al., 2014), safe consumption of animal products by general population and safety while dealing with suspect animals and samples, which can be of great importance to help decrease the burden caused by brucellosis in human and animal health.

FINAL CONSIDERATIONS

The animal brucellosis eradication/control programs in developing countries seem to be not quite efficient or not adequately implemented, since few countries or regions within a country demonstrated a low rate of animal brucellosis infection. The human infection typically results from the consumption of unpasteurized dairy products, raw or undercooked meat and from unsafe occupational exposure. For the protection of the population as a whole the control of brucellosis must be primarily based on control/eradication of the disease in the animal reservoirs. Additional to the effective heating of any potentially contaminated animal food product before consumption, as well as adoption of hygienic and safety precautions to limit exposure in occupational activities and effective disease surveillance. There are relative few studies on alternative methods to control *Brucella* spp. in food and more information on the survival and alternative control of the pathogen in milk and milk products, meat and meat products would be important for both developing and developed countries.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

EFA and EMSD conceptualized the manuscript. ACTRBC, EFA, MMO and EMSD write the manuscript. EMSD, CRP, and ASG revised the manuscript.

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CHAPTER TWO

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A spatiotemporal analysis of bovine brucellosis cases in Minas Gerais state, Brazil, from 2011 to 2018

Abstract

Our study explored the patterns of bovine brucellosis dissemination in Minas Gerais state, Brazil, by examining data on passive surveillance of bovine brucellosis cases from the Instituto Mineiro de Agropecuaria (IMA) (Animal Health Authority), as well as cattle population and bovine brucellosis testing, from 2011 to 2018 by means of a spatiotemporal analysis. We plotted cases, populations and testing distributions and performed spatial autocorrelation (Moran's I test) and local indicators of spatial autocorrelation (LISA) analyses. Moreover, we assessed the correlation of the spatial distribution and the compiled data (brucellosis cases, cattle populations, and brucellosis testing) by Lee's test. Our results showed that bovine brucellosis cases occurred mainly in the Triângulo Mineiro, Alto Paranaíba and Northwest regions, which reported cases in all analyzed years (2011 to 2018). The cattle population of Minas Gerais was concentrated in the same regions as bovine brucellosis cases, and the performed tests through the analyzed years (2011 to 2018). Moran's I test results of the case data showed significant spatial autocorrelation in 2011, 2015 and 2018 (p value < 0.05), and from 2011 to 2018, the population and testing data were also significant in Moran's I test (p value < 0.01). The results of cluster analysis (LISA) of cases showed clusters mainly in the Triângulo Mineiro, Alto Paranaíba, Northwest and South regions in 2011, 2015 and 2018. The local clusters for cattle populations and brucellosis testing were also observed in the same regions as bovine brucellosis cases in all years (2011 to 2018). The correlation results between clusters (Lee's test) were 0.22 (p value < 0.01) in 2011, 0.15 (p value < 0.01) in 2015 and 0.43 (p value <0.01) in 2018 between cases and populations, and 0.25 (p value <0.01) in 2011, 0.14 (p value <0.01) in 2015 and 0.38 (p value < 0.01) in 2018 for testing and cases. Therefore, our results showed that brucellosis cases were distributed together with cattle populations and brucellosis testing data, indicating that brucellosis in cattle in Minas Gerais state is being identified where there are more animals and where more tests are performed.

Keywords: *Brucella abortus*; epidemiology; GIS; LISA; Lee's test

Introduction

Brucellosis is a bacterial infectious disease that affects livestock (Corbel et al., 2006), humans (Pappas et al., 2006), and multiple wildlife species (Simpson et al., 2021). In cattle, the disease is primally due to *Brucella abortus* infection and results in economic losses from reproductive failure, decreased production of meat and milk, and the establishment of sanitary barriers to the international trade of animals and their products (McDermott et al., 2013).

Because of the economic losses and the public health risks of bovine brucellosis, the Programa Nacional de Controle e Erradicação de Brucelose e Tuberculose Animal (PNCEBT) (National Program for the Control and Eradication of Animal Brucellosis and Tuberculosis) was launched in Brazil in 2001 by the Ministério da Agricultura e Pecuária (MAPA, Ministry of Agriculture and Livestock Food). The goal of the program is to decrease the prevalence of brucellosis (and tuberculosis) in cattle and thereby to decrease the economic burden of the disease and the incidence of human brucellosis in the country (Ferreira Neto et al., 2016; Brasil, 2017). The control strategies proposed in the PNCEBT are mainly compulsory vaccination of heifer calves between 3 and 8 months of age with S19 or RB51 *B. abortus* strains, voluntary certification of brucellosis- and tuberculosis-free herds, test-and-slaughter procedures for positive animals, and testing policies prior to animal movement (Ferreira Neto et al., 2016; Brasil, 2017).

Among the Brazilian states, Minas Gerais has a prominent role as it is the leading producer of milk in Brazil (IBGE, 2023c) and the fifth in meat production (IBGE, 2023b). The state started compulsory bovine vaccination with S19 in 1998 (Minas Gerais, 1997) prior to the implementation of the PNCEBT. The control program in the state has achieved a considerable decrease in herd seroprevalence of bovine brucellosis from 6.00% [95% confidence interval (CI): 5.00-7.00] (Gonçalves et al., 2009) in 2002 to 3.59% (95% CI: 2.76-4.42%) in 2011 (Oliveira et al., 2016).

Since 2011, an epidemiological survey by systematic representative sampling has not been conducted in Minas Gerais to estimate the prevalence of *B. abortus* infection in cattle. However, passive surveillance data on bovine brucellosis cases (positive for antibodies against *B. abortus*) from the Instituto Mineiro de Agropecuária (IMA), the official animal health authority of the state, are available. It is possible to use these data combined with other datasets, such as bovine population and brucellosis testing data, to assess the success of control measures adopted by the PNCEBT in Minas Gerais.

The aim of this study was to describe the distribution of passive surveillance cases of bovine brucellosis in Minas Gerais state, Brazil, from 2011 to 2018 and to compare these data with cattle population and testing data in the same period, to assess the success of control measures adopted by the PNCEBT in Minas Gerais and to support the formulation of public policies that contribute to the eradication of the disease in the state.

Material and Methods

Study location

Minas Gerais state is in the southeast region of Brazil, at latitudes 14°13'58" and 22°54'00" South and longitudes 39°51'32" and 51°02'35" West, and it is divided into 853 municipalities, grouped into twelve regions: Northwest Minas, North Minas, Jequitinhonha, Vale do Mucuri, Triângulo Mineiro/Alto do Paranaíba, the Central Minas, the metropolitan area of Belo Horizonte, Vale do Rio Doce, West Minas, South/Southeast Minas, Campo das Vertentes and Zona da Mata (Figure 1). The state climate is classified as tropical, with subregional differences, such as high-altitude tropical, humid tropical, and semiarid (only in the extreme north of the state) (https://www.mg.gov.br/pagina/geografia). The state covers an area of 586,513,983 km^2, with a population of 20,538,718 people in 2022 (IBGE, 2023a) and 22,856,143 cattle heads in 2021 (IBGE, 2023d).

Data source

Passive surveillance data on bovine brucellosis cases in Minas Gerais from 2011 to 2018 were obtained from the IMA. The first dataset consisted of records of passive surveillance of bovine brucellosis outbreaks, the municipality and the farm code for the positive animal(s), the number of positive animals identified and the date of the beginning of the outbreak investigation. For the purpose of this study, a case was defined as cattle that were euthanized after positive result in the screening test [Rose Bengal Test (RBT)] or after positive result in the screening (RBT) and confirmatory test [2-mercaptoethanol (2-ME)], following the recommendations of the PNCEBT (Brasil, 2017). Information on the georeferencing of the farms was not available, therefore, cases were grouped by municipality for the spatial analysis.

The second dataset was about the cattle population size, counted in heads per municipality. The data was obtained from IMA and contained the municipality name and number, the properties with their codes and names, and the number of live cattle and species [*Bos indicus/Bos taurus* (cattle) or *Bubalus bubalis* (buffalo)] present in the farms. This second dataset came from the foot and mouth disease vaccination report (2011 to 2018).

A third dataset, about serological tests for brucellosis in Minas Gerais, was composed of bovine brucellosis RBT results, performed by officially accredited private veterinarians in Minas Gerais state (from 2011 to 2018). This dataset was also obtained from IMA and contained RBT results, year of animal sampling, municipality, tested species (cattle or buffalo), and number of positive and/or negative animals in the RBT.

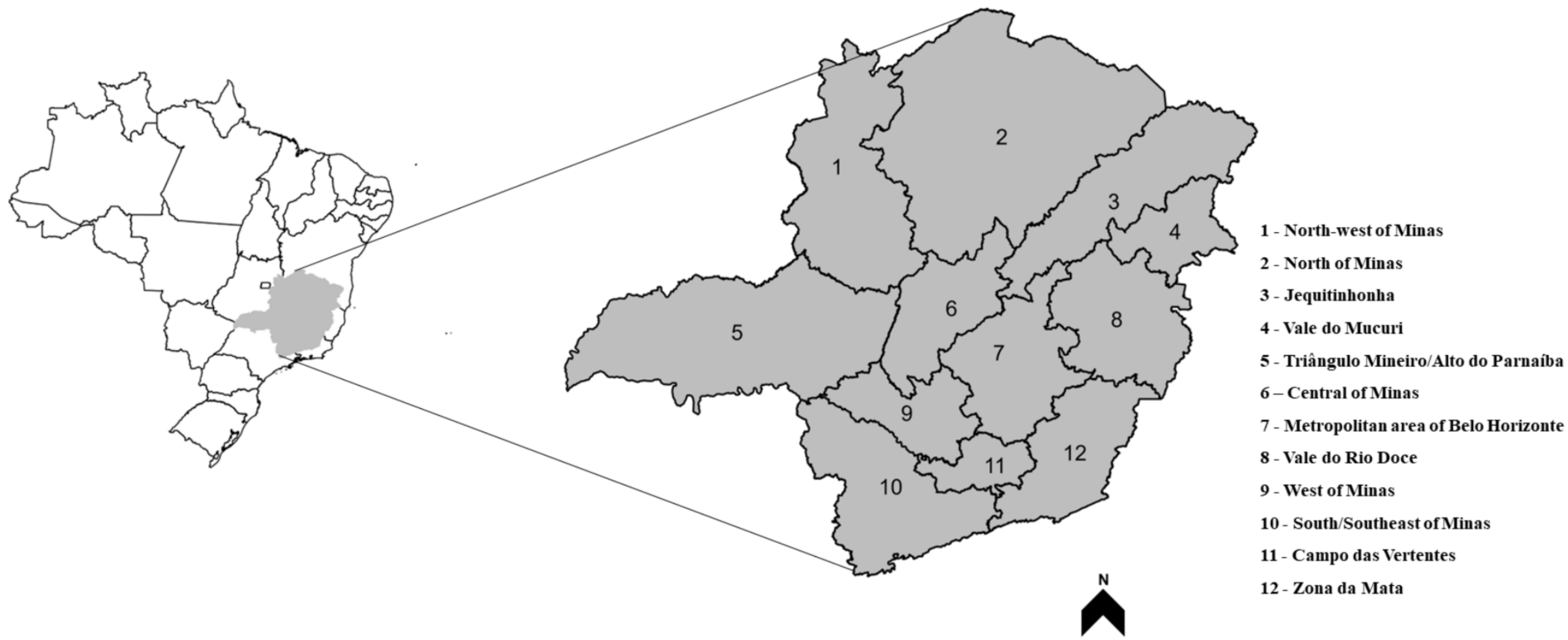

Figure 1: Map of the state of Minas Gerais, showing the regions defined in the current study. The state is divided into twelve regions: Northwest Minas, North Minas, Jequitinhonha, Vale do Mucuri, Triângulo Mineiro/Alto do Paranaíba, the Central Minas, the metropolitan area of Belo Horizonte, Vale do Rio Doce, West Minas, South/Southeast Minas, Campo das Vertentes and Zona da Mata.

Descriptive analysis

Datasets were imported into R software version 4.3.0 (R Core Team, 2023) using the package “readxl” version 1.4.2 (Wickham and Bryan, 2023) and were cleaned using the “tidyverse” package (Wickham et al., 2019). The number of cases, the number of animal populations and the number of tests performed in each municipality and each year across the study period (2011 to 2018) were assessed. Additionally, the mean, median and interquartile range (IQR) of brucellosis cases per year were calculated.

Spatiotemporal analysis

Statistical analysis was performed using R software version 4.3.0 (R Core Team, 2023). The distribution of brucellosis cases, cattle populations and animals tested for brucellosis were plotted in the Minas Gerais base map with the package “geobr” version 1.7.0 (Pereira and Goncalves, 2022) using the “tidyverse” package (Wickham et al., 2019). The positivity rate per municipality was determined by dividing the number of RBT-positive results by the number of animals tested in the municipality, in the test data.

Moran’s I test was used to assess the spatial autocorrelation of brucellosis cases, cattle populations and brucellosis tests (random or clustered) for each year of the study period (2011 to 2018). In the years with significant autocorrelation ($p < 0.05$ for Moran’s I test), Moran’s local test was used to estimate the local indicators of spatial autocorrelation (LISA) (0 was nonsignificant, 1 was low-low interaction, 2 was low-high, 3 was high-low and 4 was high-high interaction) for cases, populations and tests from 2011 to 2018 (Bivand et al., 2013).

Following cluster analysis, Lee’s correlation test was used to evaluate whether clustered cases were correlated with population clusters and/or with test clusters using the “spdep" package in R (Bivand et al., 2013). In all analysis, significance was defined as a p value ≤ 0.05 (Bivand et al., 2013).

Results

Descriptive analysis

The highest number of bovine brucellosis cases was observed in 2011 (392 cases), while 2016 had the fewest number of cases (69 cases) (Table 1). The largest cattle population was reported in 2013 (23,932,963 animals), while 2011 was the year with the fewest animals (17,082,032 animals) in the state. The highest number of brucellosis RBTs (398,734 tests) was performed in 2011, and 2016 had the fewest tests (173,358 tests). The complete descriptive statistics of the distribution of bovine brucellosis cases in Minas Gerais state from passive surveillance conducted by the IMA from 2011 and 2018 are shown in Table 1.

Table 1: Descriptive statistics (means, standard deviations, medians and interquartile ranges) of the distribution of bovine brucellosis cases, bovine populations and RBTs performed in Minas Gerais, Brazil, from 2011 and 2018.

Year	**Bovine brucellosis cases[a]**					**N of cattle**	**N of RBTs[d]**	**Positivity rate (%)**
	N[b]	**Mean**	**Standard deviation**	**Median**	**IQR[c]**			
2011	392	3.85	2.56	2.00	4.00	17,082,032	398,734	2.16
2012	201	3.33	2.88	2.00	2.25	23,898,392	371,918	2.02
2013	202	4.37	3.06	2.00	4.75	23,932,963	352,690	1.13
2014	231	6.36	10.43	2.00	2.50	23,516,524	299,526	0.59
2015	96	3.56	3.04	2.00	3.00	23,346,594	254,136	1.02
2016	69	4.31	4.98	2.00	3.00	23,317,639	173,358	0.32
2017	91	5.06	9.97	1.00	2.50	23,302,815	187,788	0.58
2018	90	3.46	2.00	2.00	2.00	21,039,612	255,913	2.49

[a]A case was defined as an animal that was positive in the screening test (RBT) or that was positive in the screening (RBT) and confirmatory test [2-mercaptoethanol (2-ME)] and that in either case was euthanized; [b]N: absolute number of cases; [c]IQR: interquartile range; [d]Number of RBT tests that were performed by officially accredited private veterinarians in Minas Gerais state.

Spatiotemporal analysis

The number of municipalities with cases of bovine brucellosis across the study period decreased from 2011 to 2018. In 2011, 98 different municipalities reported a case, while only 26 different municipalities reported a case in 2018. The Triângulo Mineiro/Alto do Paranaíba and Northwest regions had more municipalities with reported cases in all years (2011 to 2018) (Figure 2) and the largest cattle populations from 2011 to 2018 (Figure 3).

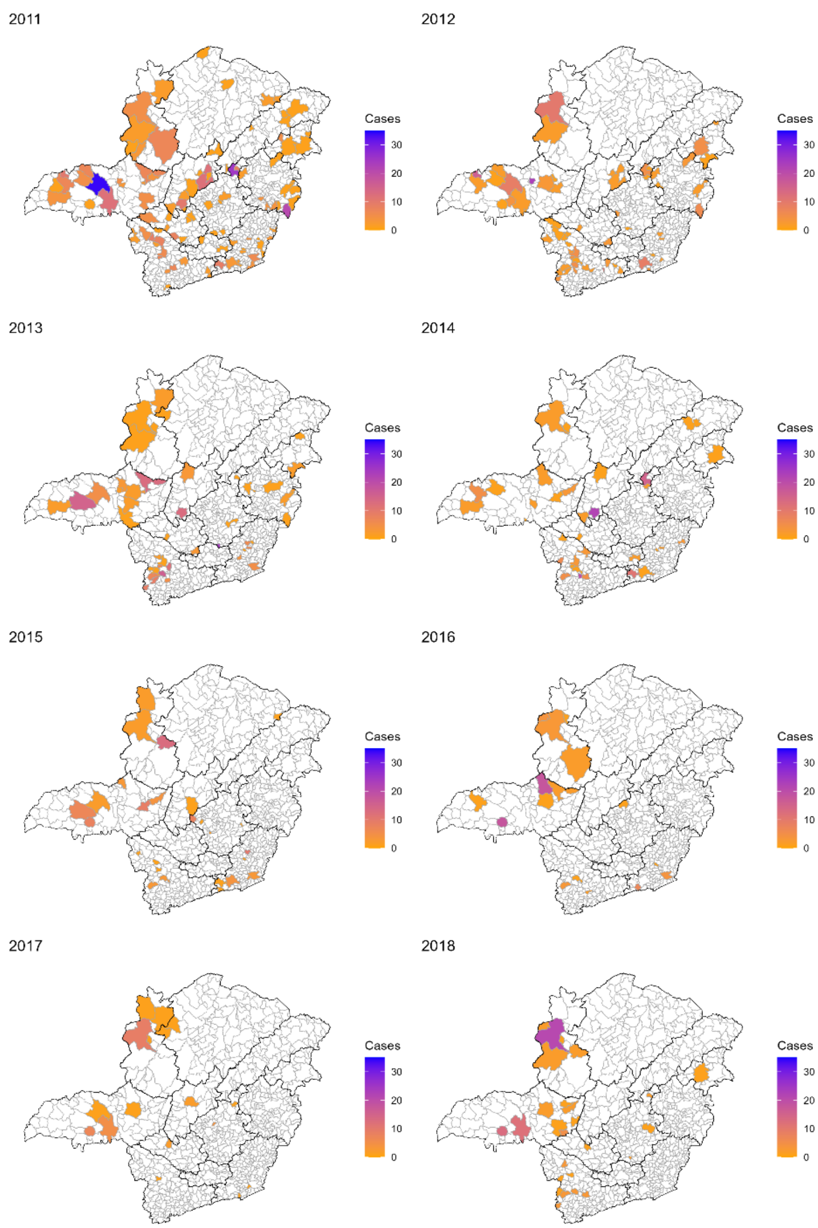

Figure 2: Distribution of bovine brucellosis cases defined as the samples that were positive in the screening test [Rose Bengal Test (RBT)] or samples that were positive in the screening and confirmatory test [2-mercaptoethanol (2-ME)] and those that were positive in the screening test and were euthanized] per year between 2011 and 2018 in Minas Gerais state, Brazil.

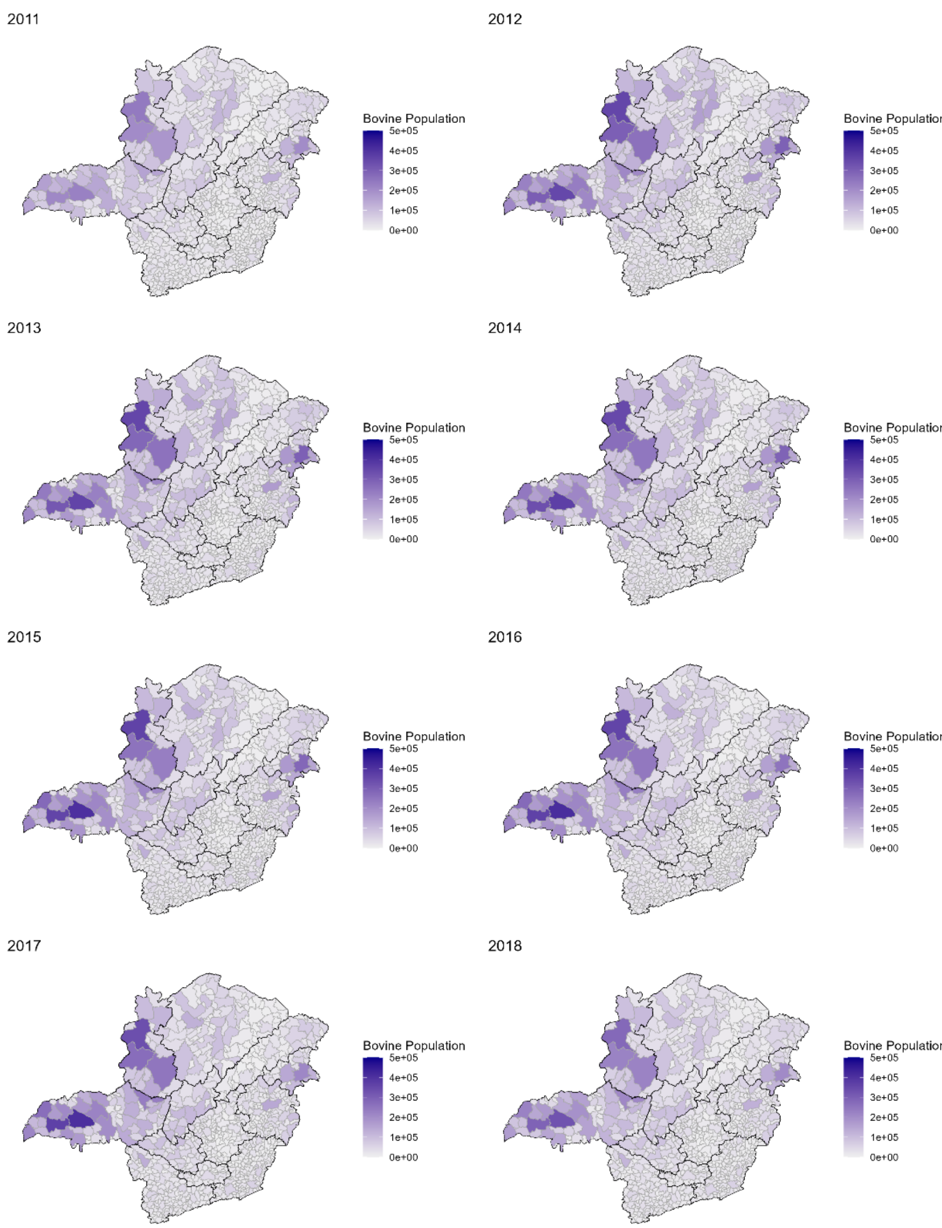

Figure 3: Geographic distribution of cattle populations - the number of live bovines and species [*Bos indicus/Bos taurus* (domestic cattle) or *Bubalus bubalis* (buffalo)] - per year between 2011 and 2018 in Minas Gerais state, Brazil.

The number of different municipalities where the tests were performed decreased over time, from 698 municipalities in 2011 to 586 municipalities in 2018, whereas the total number of tests was similar over that period. The state regions with greater numbers of tests in all years were the Triângulo Mineiro/Alto do Paranaíba and Northwest regions (Figure 4).

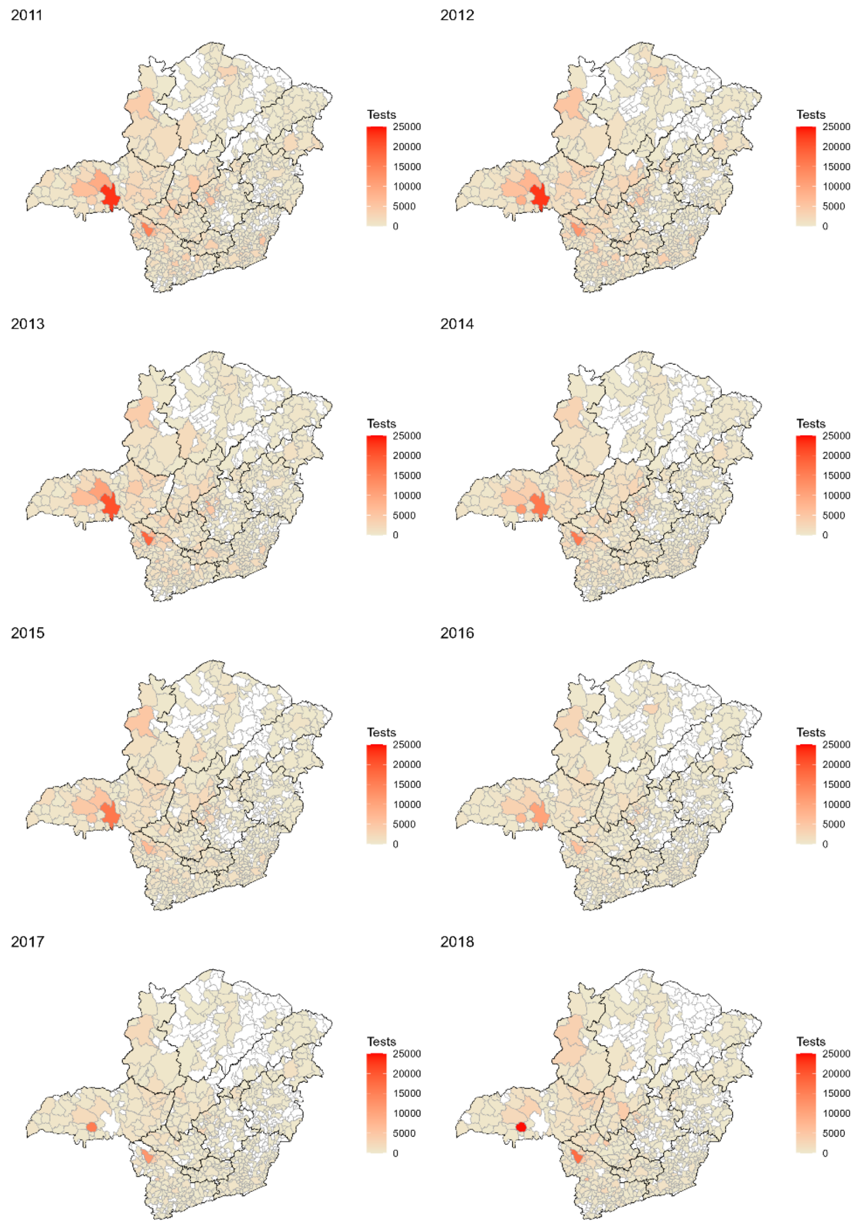

Figure 4: Geographic distribution of bovine brucellosis tests [number of bovine brucellosis Rose Bengal Tests (RBT) that were performed by officially accredited private veterinarians] per year between 2011 and 2018 in Minas Gerais state, Brazil. White municipalities had no data.

Between 2011 and 2018, the distribution of the positivity rate (number of positive tests/total number of tests) for bovine brucellosis serum samples collected by accredited veterinarians was different than the distribution of the number of brucellosis cases obtained from passive surveillance by the IMA in the state. Most municipalities had low positivity rates with similar geographical distributions over time (Figure 5).

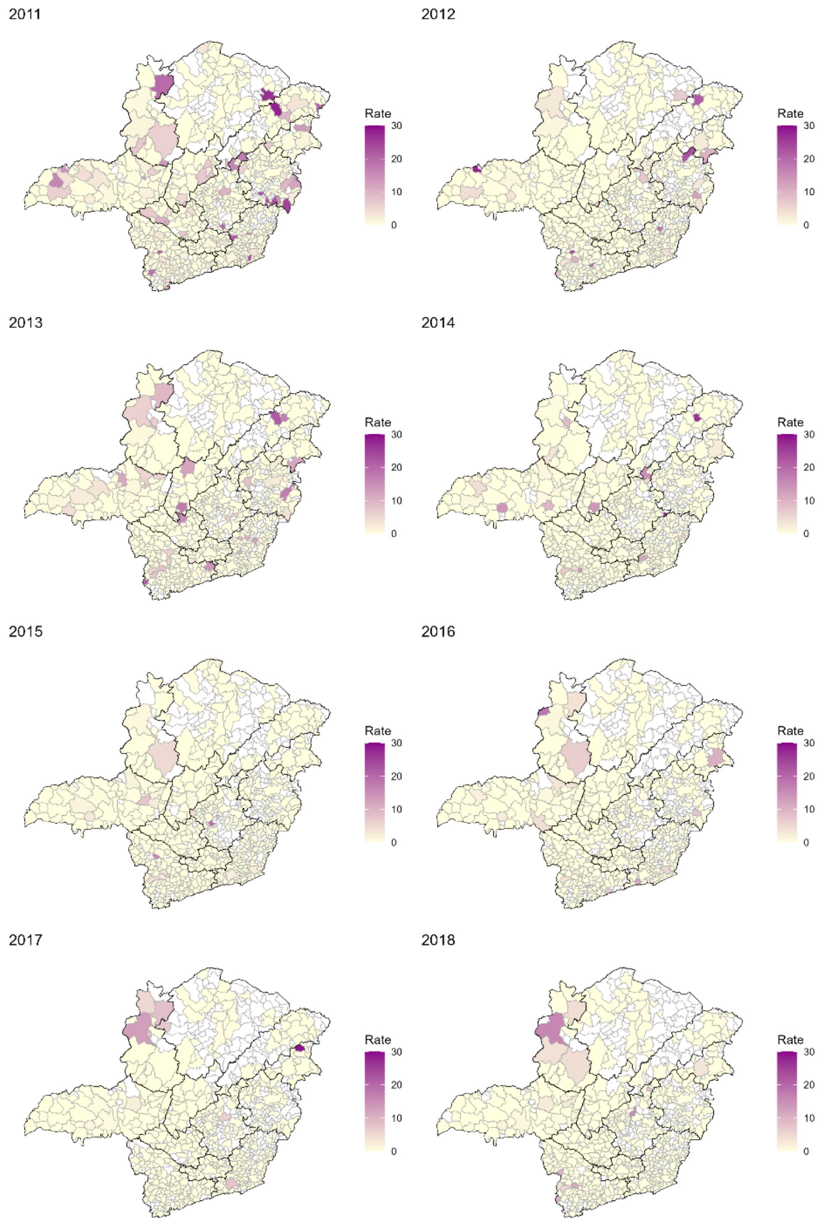

Figure 5: Positive rate [number of positive RBTs performed by officially accredited private veterinarians divided by the number of sampled animals that were tested by RBT] of the geographic distribution of bovine brucellosis between 2011 and 2018 in Minas Gerais state, Brazil.

Moran's I test identified spatial autocorrelation of cases in 2011 [0.10 (p value < 0.01)], 2015 [0.05 (p value < 0.01)] and 2018 [0.08 (p value < 0.01)]. Brucellosis cases were clustered in the Triângulo Mineiro/Alto Paranaíba, Northwest, Central, metropolitan area of Belo Horizonte, Jequitinhonha, South/Southeast, Zona da Mata and Vale do Rio Doce regions in 2011, Triângulo Mineiro/Alto Paranaíba, Northwest, North, Central, metropolitan area of Belo Horizonte, South/Southeast and Zona da Mata regions in 2015, and Triângulo Mineiro/Alto Paranaíba, West Minas, Northwest and South/Southeast regions in 2018 (Figure 6). The Northwest region of the state had clusters of high-high (places with a high number of cattle interacting with each other) interactions in the three years (2011, 2015 and 2018) (Figure 6). Across all these years, there were clusters of bovine populations, primarily in the Triângulo Mineiro/Alto Paranaíba, Northwest, North, Central and Vale do Mucuri regions, with the majority of clusters having high-high interactions (places with a high number of bovids interacting with each other) (Figure 7).

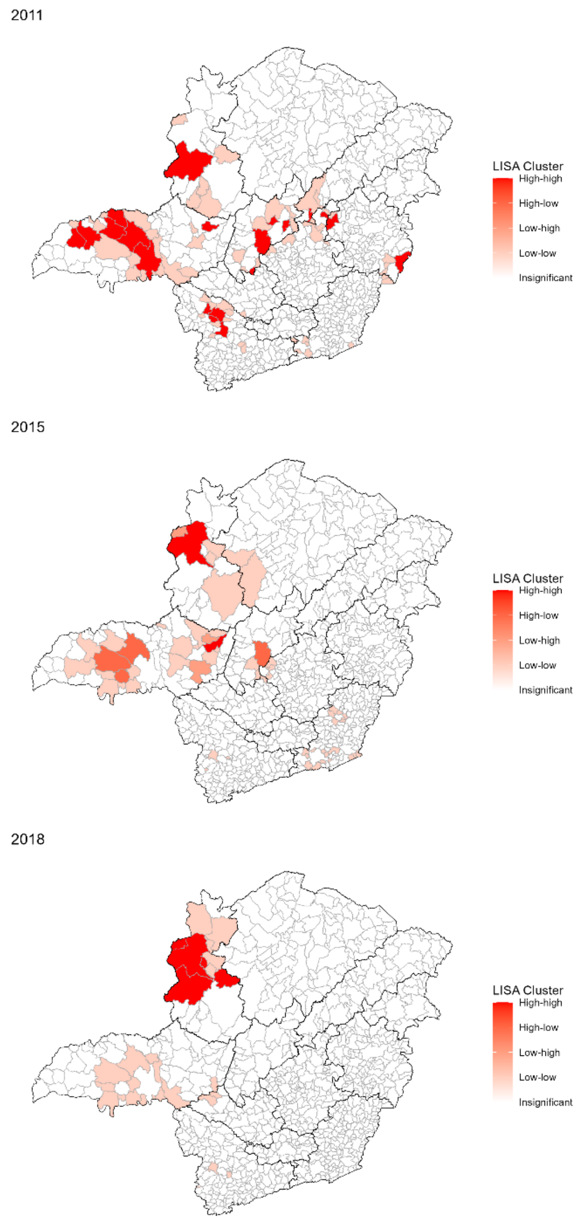

Figure 6: Clusters of bovine brucellosis cases [defined as the samples that were positive in the screening test, Rose Bengal Test (RBT), in samples that were positive in the screening and confirmatory test, 2-Mercaptoethanol (2-ME), and those that positive in the screening test and were euthanized] in 2011, 2015 and 2018, in Minas Gerais state, Brazil.

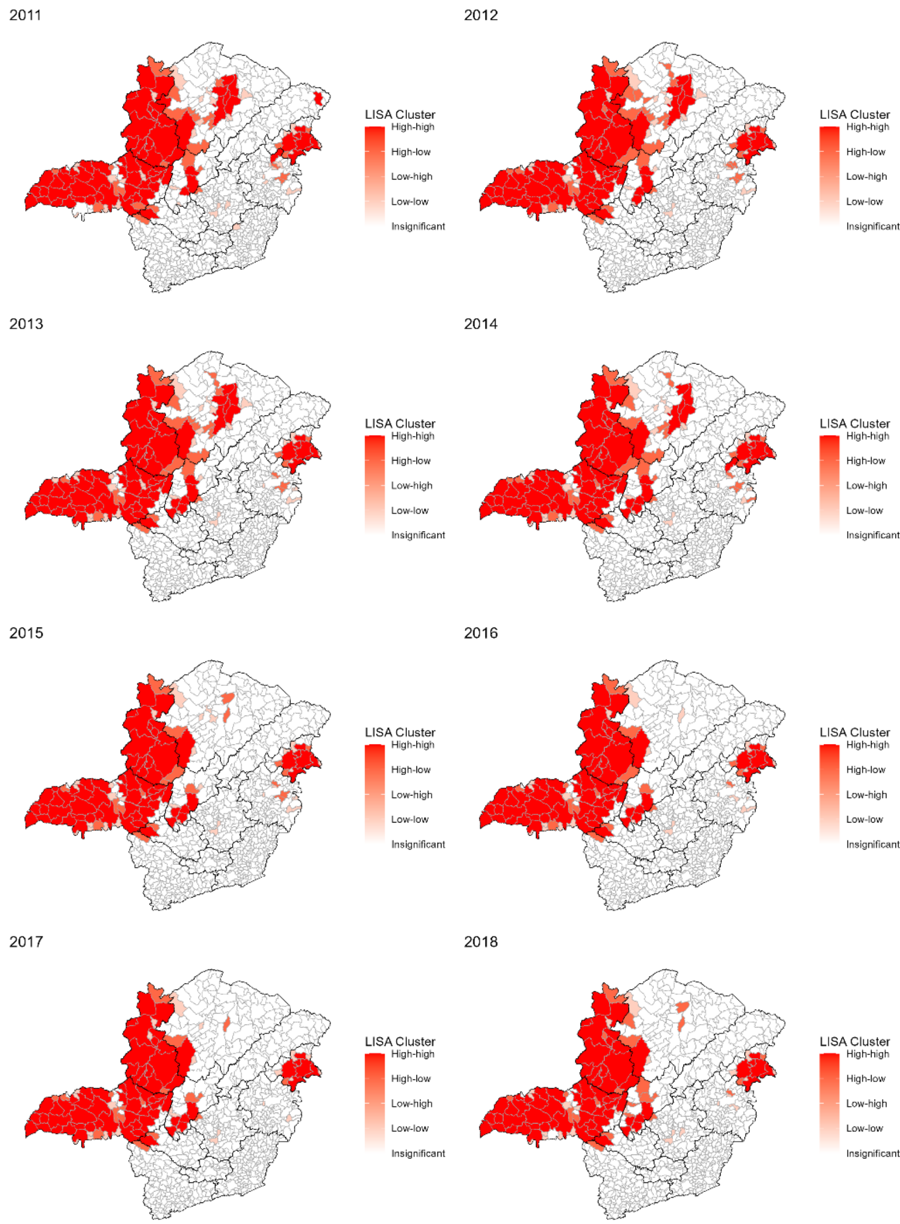

Figure 7: Clusters of bovine population (the number of live bovine, and species [*Bos indicus/Bos taurus* (cattle) or *Bubalus bubalis* (buffalo)]) from 2011 to 2018, in Minas Gerais state, Brazil.

Likewise, for the brucellosis testing data, clusters were identified in all years (2011 to 2018), with the LISA test highlighting the clusters mainly in the Triângulo Mineiro/Alto Paranaíba and South/Southeast regions, although other regions also exhibited clusters, such as the metropolitan area of Belo Horizonte, West Minas and Central Minas from 2011 to 2015 (high-high interactions)

(Figure 8). Details on the cluster distribution of bovine brucellosis cases, cattle populations and testing data in Minas Gerais can be found in Figures 6, 7 and 8.

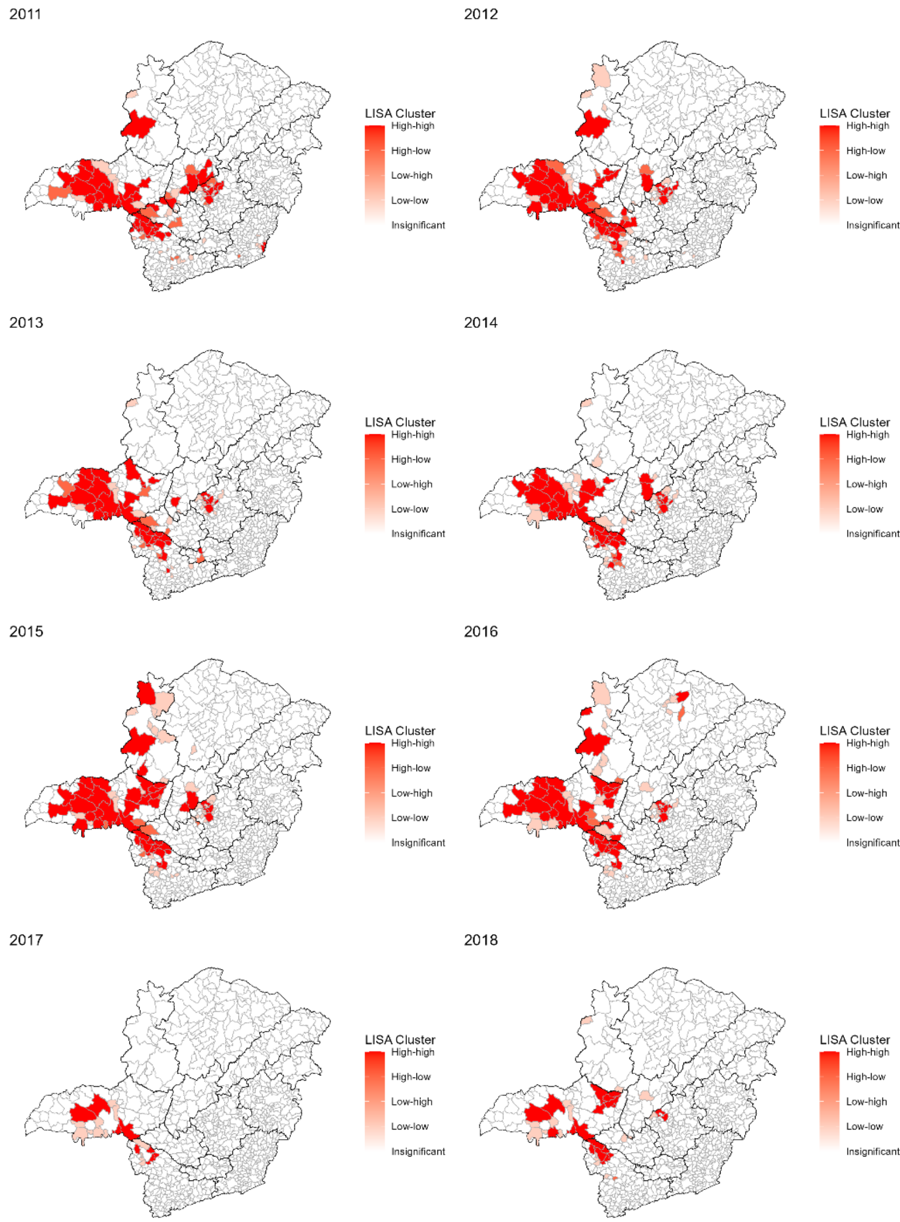

Figure 8: Clusters of bovine brucellosis test [number of Rose Bengal test (RBT) results performed by the officially accredited private veterinarians] from 2011 to 2018, in Minas Gerais state, Brazil.

Lee's test results showed a positive correlation between the clusters of brucellosis cases and cattle populations for 2011, 2015 and 2018, as well as a positive correlation between clusters of brucellosis cases and testing in the same years (Figure 9).

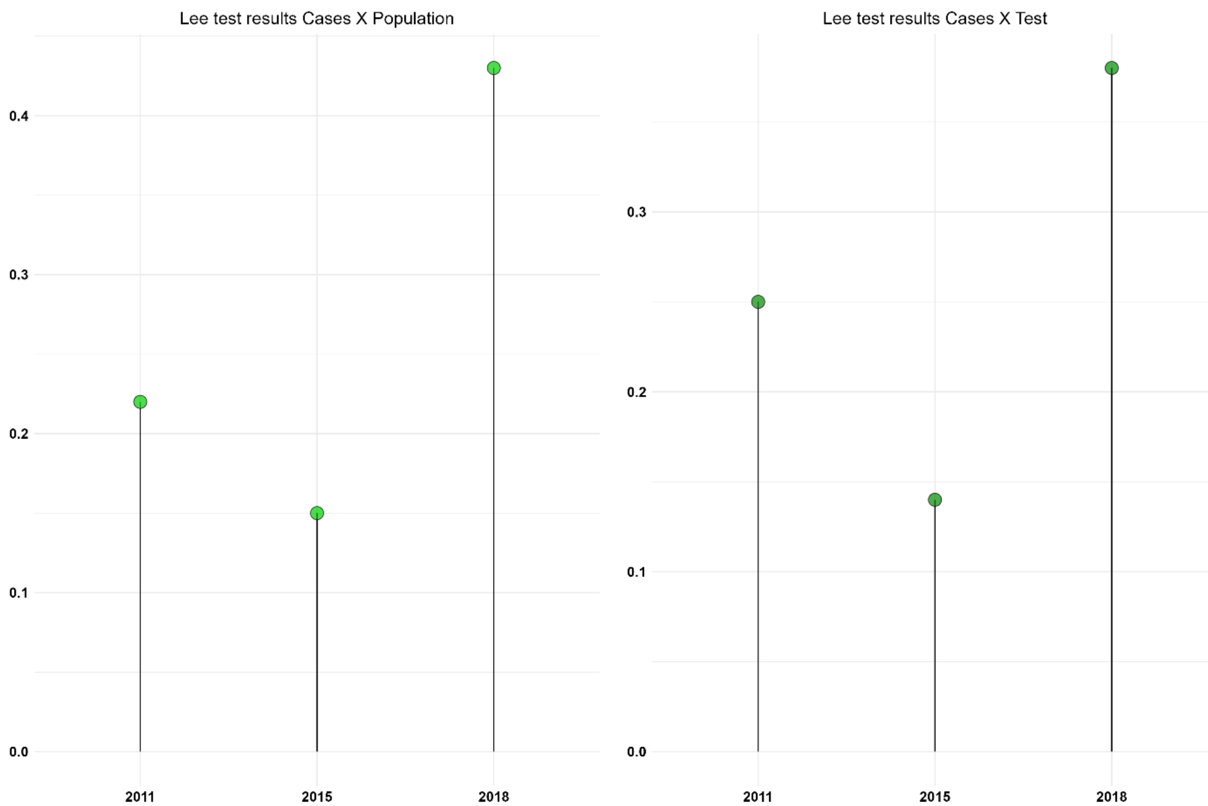

Figure 9: Lee's test results of correlation between clusters of cattle brucellosis cases [defined as the samples that were positive in the screening test, Rose Bengal Test (RBT)] or samples that were positive in the screening and confirmatory test, 2-mercaptoethanol (2-ME), and those that were positive in the screening test and were euthanized] and cattle populations - the number of live bovines and species [*Bos indicus/Bos taurus* (cattle) or *Bubalus bubalis* (buffalo)] - and between clusters of cattle brucellosis cases and cattle brucellosis testing, Rose Bengal Tests performed by accredited veterinarians, in 2011, 2015 and 2018.

Discussion

Minas Gerais is classified as a low-risk state for bovine brucellosis, according to the PNCEBT, because the prevalence of brucellosis is between two and five percent; in this situation, the state must achieve vaccination coverage of at least 80% of eligible breeding animals with an approved live vaccine (currently S19 or RB51), implement initial/medium-term quality surveillance measures, and perform mandatory follow-up of detected outbreaks and epidemiological surveillance in the state to detect new outbreaks (Brasil, 2017). Currently, Minas Gerais has achieved vaccination coverage of 80% in most of its municipalities of, and the follow-up of the outbreaks have been conducted, together with the control of cattle transit with the Guia de Transito Animal (GTA – Animal Transit Guide). In this scenario, the results of the present study are positive for improve the understanding of the epidemiological situation of the bovine brucellosis in the state, as they show that the disease in Minas Gerais is being identified where there are more animals and where more tests are performed. This suggest an effective implementation of the program in the state and the usefulness of analyzing data obtained through passive surveillance for directing control and eradication actions, although underrepresented areas, where active surveillance should be implemented, were also identified.

Indeed, in alignment with the aims of the PNCEBT, the IMA is working to decrease the incidence of brucellosis in cattle. The results from this analysis indicated a decrease in bovine brucellosis cases in the state throughout the analyzed years (2011 to 2018). The agreement between the bovine brucellosis cases and positivity rate suggests that the passive surveillance system provides a meaningful estimate of the total number of infections and a good representation of the epidemiological situation of the disease in Minas Gerais. The strong correlation between the clusters of animals in the municipalities and both the number of tests performed and the number of positive cases further support the validity of the IMA surveillance system. Clusters were found in the regions (Triângulo Mineiro/Alto Paranaíba and Northwest regions) previously described as having larger herds and a higher level of technological production (Alves et al., 2018). This concurs with previous work that indicated properties with a higher number of cows at higher risk for infection with *B. abortus* (Oliveira et al., 2016). Interestingly, the regions with larger herds and more intensive production also have many fairs, livestock auctions and other livestock events, which require testing to participate (due to cattle movement) in those events (Brasil, 2017). Conversely, there were areas where no tests were performed and therefore no cases were reported (Figure 2 and Figure 4), indicating a need to increase surveillance in those herds/municipalities (in the Jequitinhonha, Vale do Mucuri and Vale do Rio Doce regions), since their infection status is unknown. The Vale do Mucuri region showed a large cattle population and little to no reported tests, exemplifying one of the regions where surveillance should be increased to improve the PNCEBT in Minas Gerais state.

The present study has several limitations. First, some animals may have been counted more than once in the brucellosis testing database (retesting), which could artificially reduce the animal positivity rate, as the assumption was that one tested sample was equal to one sampled animal. On the other hand, despite the recommendation to use a confirmatory test after a positive screening test, at the farmer's discretion positive RBT animals can be euthanized. Slaughtering animals without confirmatory testing may lead to an overestimation of the number of cases, considering the lower specificity of this diagnostic strategy compared to the use of serial tests (Dohoo et al., 2014). In this sense, also, the exclusive use of 2ME as a confirmatory test observed in the analyzed data could be questionable, since it is a test that is not among those recommended by the World Organisation for Animal Health (WOAH) (WOAH, 2022). Nevertheless, a recent meta-analysis showed that this test has a high specificity [99.20% (CI 95%, 98.05 to 99.67)] (Andrade et al., 2023)thus being recommended as a confirmatory test for bovine brucellosis. Furthermore, although the association between the observed results and the real epidemiological scenario of bovine brucellosis in Minas Gerais state seems to be strong, passive surveillance data are often biased by overrepresenting animals from progressive producers who are willing to test and may have a different rate of infection than those from herds that are unwilling to test. Moreover, the absence of testing in some regions could underestimate the occurrence of brucellosis cases in cattle and the positivity rate of the disease in Minas Gerais state. Nonetheless, studies such as this one is fundamental in identifying these underrepresented areas. The degree of bias could be assessed by conducting systematic random sampling as part of a prevalence study, especially in the areas with less testing and larger cattle populations; however, this would be expensive and time consuming. Altogether, the low prevalence of positive municipalities in the state, the chronic nature of *B. abortus* infection, the lack of new control measures since the last survey and the desire to move toward eradication should be carefully considered before conducting a large-scale active sampling program, as the answers given by a new cross-sectional study may be not worthy (given time and resources as constraints). The use of advanced analytical tools, coupled with targeted active sampling of underrepresented areas in the state, would likely to produce a similar outcome at a lower cost than a state-wide active sampling program (Lee et al., 2021), assuming a continuation of a high rate of immunization in young female cattle (Kiiza et al., 2023).

Conclusions

Our study demonstrated that bovine brucellosis is mainly present in the Triângulo Mineiro/Alto Paranaíba and Northwest regions of Minas Gerais state and that the geographic distribution of brucellosis cases, cattle populations, and the number of RBTs are similar. These results indicate that the IMA's passive surveillance data are effective in identifying cases of brucellosis, albeit also identified targeting areas where the active surveillance should be performed for more precise information. Moreover, the decrease in the test positivity rate from 2011 to 2018 suggests a satisfactory implementation of the brucellosis control program in the state.

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CHAPTER THREE

Prepared in accordance with Vaccines journal standards.

Spatiotemporal analysis of bovine brucellosis vaccination in Minas Gerais state, Brazil, from 2011 to 2022

Abstract

The aim of this study was to perform a spatiotemporal analysis of the bovine brucellosis vaccination rate in Minas Gerais state, Brazil, 2011-2022, and identify if sociodemographic factors were associated with bovine brucellosis vaccination in the state. Data on bovine brucellosis vaccination was acquired from Instituto Mineiro de Agropecuária (IMA) and sociodemographic data of farmers was from Instituto Brasileiro de Geografia e Estatistica (IBGE). The bovine brucellosis vaccination rate distribution was described, evaluating the average, median, interquartile interval (IQR), standard deviation (SD) of the vaccination rate, per year. Also, a spatiotemporal analysis by means of Spatial Autocorrelation test (Moran's I test) and Local Spatial Autocorrelation Analysis (LISA) was conducted. Additionally, a generalized linear mixed effect model (GLMM) was developed to investigate the influence of farmers sociodemographic characteristics over the number of vaccinated cattle, in 2017, due to availability of farmers data. The bovine brucellosis vaccination rate in Minas Gerais increased over time, with an average of 0.48 (SD: 0.13) and median of 0.47 (IQR: 0.14) in 2011, whereas, in 2022 the average was 0.90 (SD: 0.28) and median of 0.87 (IQR: 0.28). The Moran's I test revealed clusters of vaccination rate in all analyzed years (2011-2022), with clusters low-high (vaccination rate below the average and autocorrelation with space above the average) and high-high (vaccination rate and autocorrelation with space above the average) more present over the years. The GLMM revealed a positive influence of technical assistance combined with disease and parasite control over the average of vaccinated cattle. Therefore, the bovine brucellosis vaccination rate increased over the twelve analyzed years (2011-2022), being positively influenced by technical assistance combined with disease and parasite control, considering 2017 data. Moreover, our results pointed regions with vaccination rate below the average and high spatial autocorrelation, which should be taking into account to directly address control measures according to particularities of each region.

Keywords: PNCEBT, epidemiology, “animal disease prevention”

1. Introduction

Brucellosis is a bacterial disease caused by bacteria of the genus *Brucella,* which are intracellular facultative, Gram negative, coccobacillus, aerobic and nonmotile bacteria [1]. *Brucella* spp. has been reported in many domestic animal species, including cattle [1], several wildlife species [2], as well as in humans [3]. Bovine brucellosis is mainly caused by *Brucella abortus*, with reproductive failures and decrease of production of milk and meat production being the main economic losses attributed to the disease [4, 5]. In addition to the damage to livestock, bovine brucellosis is also important from the public health point of view, since it is a zoonotic disease usually transmitted to humans by direct or indirect contact with infected animals or their products (unpasteurized milk and dairy products) [6].

Hence, considering the importance of bovine brucellosis to animal and human health, Brazil created in 2001 the Programa Nacional de Controle e Erradicação de Brucelose e Tuberculose Animal - PNCEBT (National Program for the Control and Eradication of Animal Brucellosis and Tuberculosis) to control and eradicate the disease from cattle [7]. One of the control measures of the PNCEBT is the mandatory vaccination of females from 3 to 8 months of age with S19 or RB51 vaccines [8]. These two vaccines are live attenuated strains worldwide used to protect cattle against abortion and infection caused by *B. abortus* [9]. Noteworthy, in Minas Gerais, the first state in milk production [10] and the fifth in meat production in Brazil [11], vaccination against brucellosis is performed as a control measure since 1998 for the whole state territory [12, 13]. The adoption of vaccination program in the state, even before the implementation of the PNCEBT, was probably largely responsible for the significant decrease in the prevalence of the disease in Minas Gerais, comparing the last two surveys carried out in the state (2002 and 2011). Indeed, the herd seroprevalence of bovine brucellosis in the Minas Gerais decreased from 6.00% [95% confidence interval (CI): 5.00-7.10] [14] in 2002 to 3.59% (95% CI: 2.76-4.42%) in 2011 [13].

Despite the undeniable advances that Minas Gerais has achieved in recent years in controlling bovine brucellosis, there is still much to be done until the state reaches the eradication phase. Prior to this moment (eradication phase), vaccination will continue to be the main measure to control the disease. Thus, a spatiotemporal analysis of vaccination rate (vaccination coverage) in Minas Gerais is an important tool to identify areas within the state where strategic vaccination actions should be strengthened, so that PNCEBT continues to advance. These actions would be further reinforced by understanding the socioeconomic factors associated with vaccination coverage [15, 16].

Therefore, the aims of this study were (i) to perform a spatiotemporal analysis of the bovine brucellosis vaccination rate in Minas Gerais state, Brazil, from 2011 to 2022, and (ii) to assess whether sociodemographic factors were associated with vaccination.

2. Material and Methods

2.1.Study location

Minas Gerais is a state in the Southeast of Brazil and has no access to the sea. In latitude, it is localized between the 14º13'58" e 22º54'00" south parallels, while the longitude location is between the 39º51'32" e 51º02'35" west meridians. The state has 853 municipalities divided in 12 regions: North-west of Minas, North of Minas, Jequitinhonha, Vale do Mucuri, Triângulo Mineiro/Alto do Paranaíba, Central of Minas, Metropolitan Area of Belo Horizonte, Vale do Rio Doce, West of Minas, South/Southeast of Minas, Campo da Vertentes and Zona da Mata (IBGE) (Figure 1). Minas Gerais has 586.513,983 km² of area, approximately 20 millions of habitants [17] and 23 millions of cattle, being the fourth state in the cattle herd of the country, with 212 thousand tons of meat

(2023) and 9.3 million liters of milk (2022) [18]. The Human Development Index of the state is 0.774 [19].

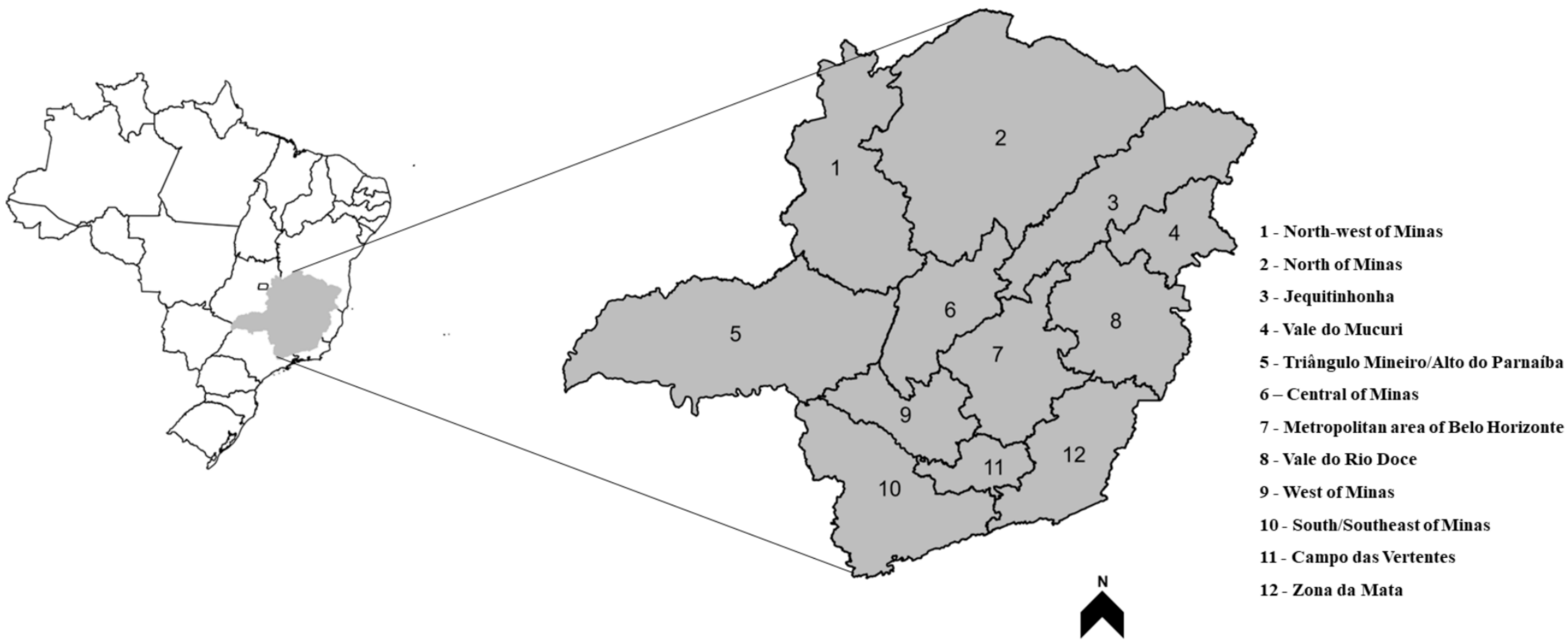


Figure 1: Map of the state of Minas Gerais, showing the regions defined in the current study. The state divided into twelve regions: Northwest Minas, North Minas, Jequitinhonha, Vale do Mucuri, Triângulo Mineiro/Alto do Paranaíba, the Central Minas, the metropolitan area of Belo Horizonte, Vale do Rio Doce, West Minas, South/Southeast Minas, Campo das Vertentes and Zona da Mata.

2.2.Data source

The bovine brucellosis vaccination data for Minas Gerais was provided by the Instituto Mineiro de Agropecuária (IMA), the official animal health authority of Minas Gerais, Brazil. Due to the organization of data in IMA, the vaccination datasets were provided in two different formats. The first vaccination dataset, from 2011 to 2014, contained the number of existing female calves from 0-12 months of age and the number of vaccinated females in each year grouped by IMA's sectional offices (ESECs) (a division of the state in microregions by IMA). Each ESEC was composed of approximately 4.08 (□ 2.29) municipalities. The vaccination data observed for each ESEC, from 2011 to 2014, was applied to all municipalities within its composition, from the list of municipalities per ESECs from 2015. The second vaccination dataset, from 2015 to 2022, contained the number of existing female calves from 0-12 months and the total number of vaccinated heifers grouped by municipality.

Five datasets on socioeconomics factors were used, all acquired from Brazilian Institute of Geography and Statistic (Instituto Brasileiro de Geografia e Estatistica - IBGE) (www.ibge.com.br). The sociodemographic datasets contained information on cattle producers in Minas Gerais state, per municipality, from the agricultural census from 2017 [20]. The first dataset consisted of information on literacy and had 19 different variables (Appendix A). The literacy variables were grouped into a new variable with six categories: never went to school, primary literacy, elementary school, high school, college, and graduation. The second dataset contained data on the specialized technical assistance and the variables in this dataset were grouped into four categories: private (people and companies), government, non-governmental organizations (non-profit organizations and autonomous social services) and cooperatives. Additionally, the third, fourth and fifth datasets had information on the farmers self-described race (white, black, yellow, brown, and indigenous) and sex (man or woman), and access of disease and parasite control (yes or no), respectively. All the variables used from these datasets are in Appendix A.

2.3.Descriptive analysis

Vaccination rate (vaccination coverage) was calculated dividing the total number of female calves vaccinated by the total number of existing female calves from 0 to 12 months of age, for each municipality of Minas Gerais state per year.

The median, interquartile range (IQR), average and standard deviation of the vaccination rate were also calculated per year, from 2011 to 2022, considering the missing values as zero. Additionally, vaccination rate was also calculated for each region (North-west of Minas, North of Minas, Jequitinhonha, Vale do Mucuri, Triângulo Mineiro/Alto do Paranaíba, Central of Minas, Metropolitan Area of Belo Horizonte, Vale do Rio Doce, West of Minas, South/Southeast of Minas, Campo da Vertentes and Zona da Mata) during the analyzed period (2011 to 2022). Additionally, a ranking of municipalities was created, considering the 10% municipalities that showed the highest vaccination rate and the lowest vaccination rate in at least 6 of the 12 analyzed years.

2.4.Spatiotemporal analysis

The vaccination rate was plotted per municipality in the Minas Gerais base map from the "geobr" package version 1.7.0 [21] using R software version 4.2.2 [22]. Additionally, the state regions were identified on the maps, using the same package previously described, having as reference the IBGE divisions (North-west of Minas, North of Minas, Jequitinhonha, Vale do Mucuri, Triângulo Mineiro/Alto do Paranaíba, Central of Minas, Metropolitan Area of Belo Horizonte, Vale do Rio

Doce, West of Minas, South/Southeast of Minas, Campo da Vertentes and Zona da Mata). The Moran's I test was performed to identify the presence of spatial autocorrelation of bovine brucellosis vaccination rate (random or clustered), in all the analyzed years (2011 to 2022). The significance of the autocorrelation was defined for the Moran's I test as p value ≤ 0.05. In the years with a significant Moran's I test, the Local Indicators of Spatial Autocorrelation (LISA) was calculated using the local Moran's I test. LISA values of 0 were classified as insignificant interactions, 1 as low-low interactions (vaccination rate and autocorrelation with space were below their averages), 2 as low-high interactions (vaccination rate was below the average and autocorrelation with space was above the average), 3 as high-low interactions (vaccination rate was above the average and autocorrelation with space was below the average) and 4 as high-high interactions (vaccination rate and autocorrelation with space were above their averages), from 2011 to 2022 [23].

All descriptive and spatiotemporal analyzes were conducted in R software version 4.2.2 [22], using the packages "tidyverse" version 2.0.0 (Wickham et al., 2019), and the "spdep" version 1.2-8 [23].

2.5.Assessment of sociodemographic factors associated with vaccination

A generalized linear mixed effects model was developed to assess whether sociodemographic factors were associated with bovine brucellosis vaccination in Minas Gerais. Only vaccination data from 2017 was used to build the model as the sociodemographic data available were from the same year (2017 agricultural census) [20]. A generalized linear mixed effect model (GLMM) was adjusted to counts of vaccinated animals per municipality assuming it has a Poisson distribution and canonical link. Fixed effects of the explanatory covariates were the last year count of animals in the municipality (that entered the model as a normalized local control), and five sets of sociodemographic variables (that entered the model as percentage to aggregate counts in each case). Those variables and respective number of levels used in the model were: literacy and ethnicity (with six classes each, although highly colinear, with two dimensions explaining about 95% of the variability in each information matrix), sex, disease and parasite control and specialized technical assistance (those are binary variables and had each only one dimension in the fixed effects model). Random variables were assumed to the region and municipality effects. Main effects and low order interactions were tested for all combinations in a stepwise backward procedure using the Akaike Information Criterion (AIC) [24]. All factors kept in the final model have p-values ≤ 0.05.

The models were conducted in R software version 4.2.2 [22], using the “tidyverse” package version 2.0.0 (Wickham et al., 2019) to manipulate the data, and “MASS” package [25] and “lme4” package [26] to build the models.

3. Results

3.1.Descriptive analysis

The bovine brucellosis vaccination rate in Minas Gerais increased over time, showing in 2011 the lowest global average [0.80 ± 0.18] and median (0.84; IQR = 0.22), and in 2022 the highest global average (0.90 ± 0.28) and a median (0.87; IQR= 0.28). There were missing values for vaccination rate in the years 2011 (6 municipalities), 2012 (7 municipalities) and 2014 (2 municipalities) (Table 1), from the 853 municipalities that compose the Minas Gerais state. The complete evolution of bovine brucellosis vaccination rate in Minas Gerais from 2011 and 2022 can be found in Table 1 and Figure 2.

Table 1: Descriptive analysis of bovine brucellosis vaccination in Minas Gerais, Brazil, per municipality per year, from 2011 to 2022.

Year	Total female[a]	Total vaccinated[b]	Total Average	NA.s[c]	Vaccination rate			
					Median	IQR[d]	Average	SD[e]
2011	2,310,263	1,947,640	0.84	6.00	0.84	0.22	0.80	0.18
2012	2,444,900	1,858,317	0.76	7.00	0.72	0.22	0.72	0.18
2013	2,429,321	1,783,030	0.73	0.00	0.72	0.21	0.72	0.16
2014	2,432,344	1,798,476	0.74	2.00	0.71	0.21	0.72	0.16
2015	2,363,908	1,574,709	0.67	0.00	0.65	0.20	0.64	0.17
2016	2,342,048	1,765,436	0.75	0.00	0.74	0.28	0.73	0.19
2017	2,252,671	1,737,557	0.77	0.00	0.77	0.26	0.79	0.23
2018	2,233,907	1,723,142	0.77	0.00	0.78	0.25	0.77	0.18
2019	2,255,988	1,761,315	0.78	0.00	0.79	0.24	0.78	0.16
2020	2,375,575	1,732,227	0.73	0.00	0.74	0.26	0.74	0.19
2021	2,453,408	1,901,610	0.77	0.00	0.80	0.27	0.80	0.16
2022	2,330,896	1,979,081	0.85	0.00	0.87	0.28	0.90	0.28

[a] Total of existing female calves with 0 to 12 months of age. [b] Total of vaccinated female calves with 3 to 8 months of age. [c] Missing municipalities with data on bovine brucellosis vaccination rate. [d] IQR: interquartile interval of cattle brucellosis vaccination rate. [e] SD: standard deviation of cattle brucellosis vaccination rate.

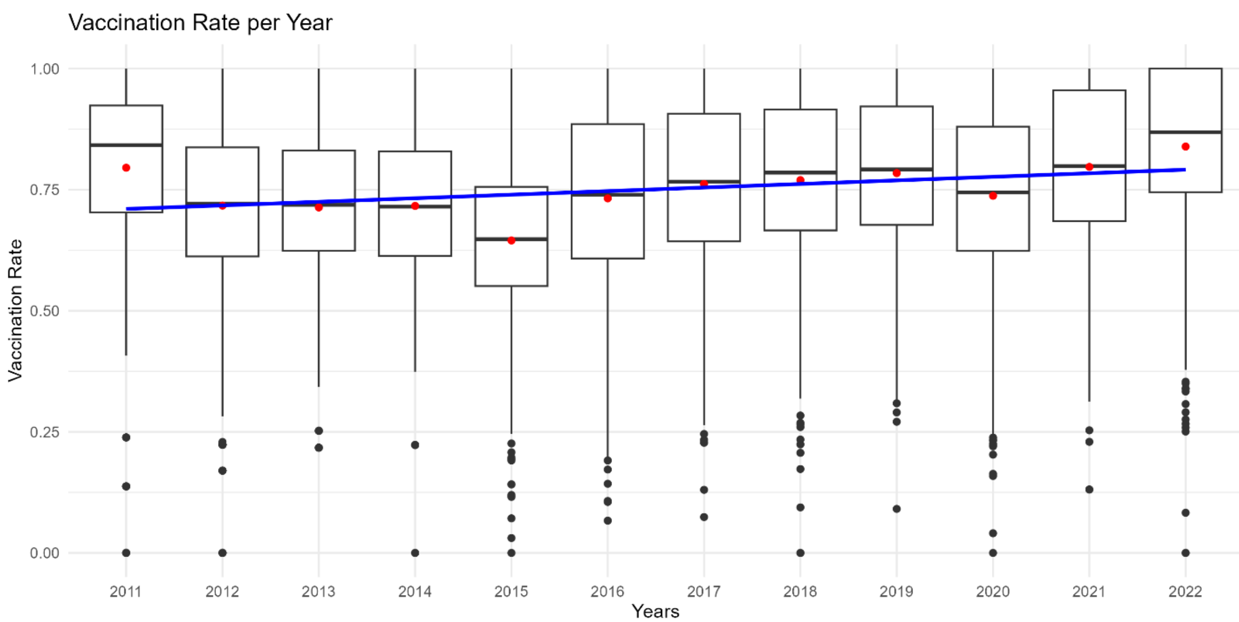


Figure 2: Boxplot of vaccination rate distribution per year, from 2011 to 2022 in Minas Gerais, Brazil. Red dot is the average of the year, and blue line is the tendency of vaccination rate over the analyzed years (2011 to 2022).

Considering the bovine brucellosis vaccination rate by region and year, the Campo das Vertentes region showed the greater average of vaccination rate in 2012 to 2015, 2017 and 2019, and the West region had the highest average of vaccination rate in 2011, 2017, 2020 and 2021 (Figure 3). In 2022, the region with highest average of vaccination rate was Zona da Mata. The regions with lowest bovine brucellosis vaccination rate were Jequitinhonha from 2015 to 2022, followed by Zona da Mata in 2011 and 2012 and North of Minas in 2013 and 2014 (Figure 3).

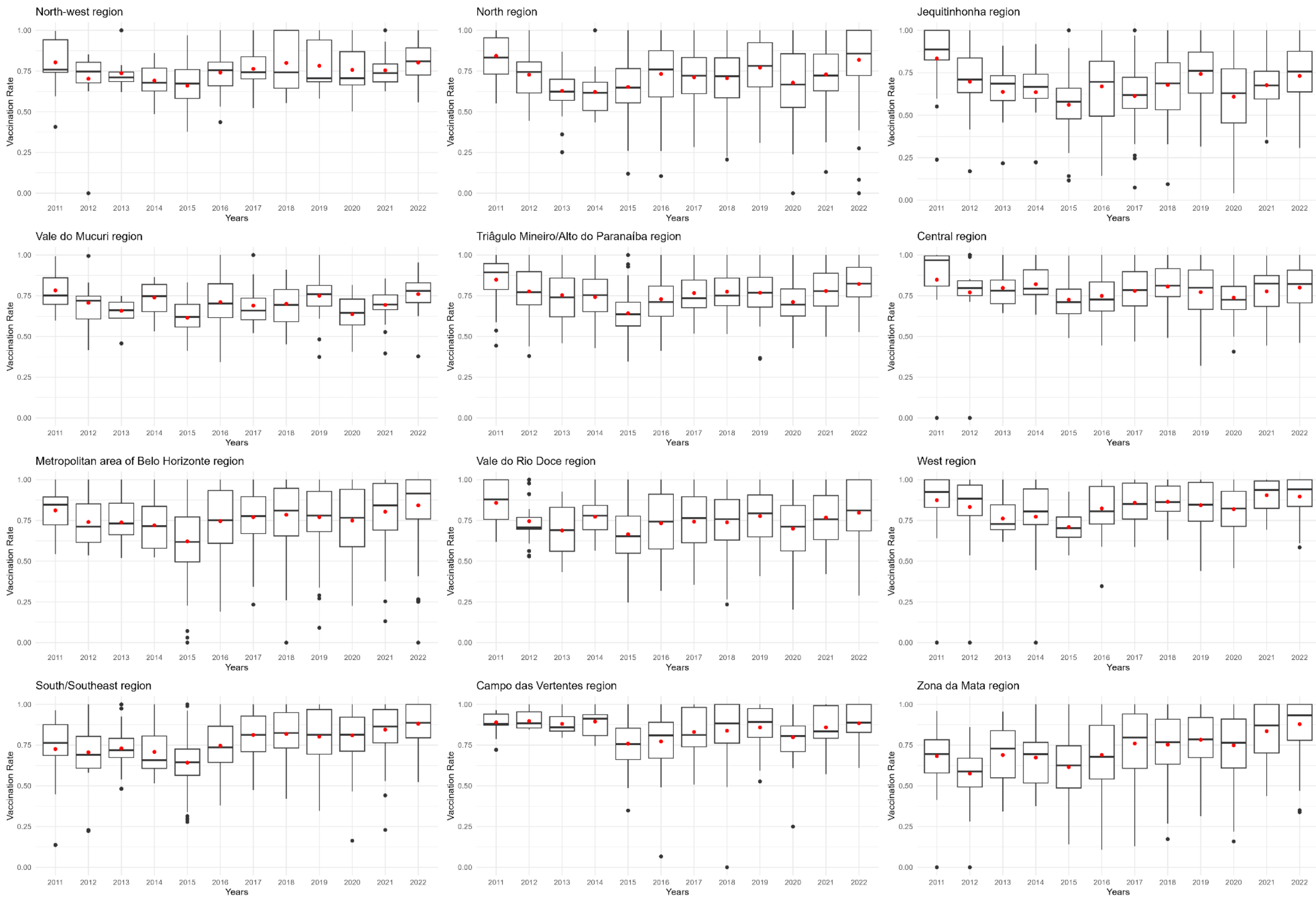

North-west region
North region
Jequitinhonha region
Vale do Mucuri region
Triâgulo Mineiro/Alto do Paranaíba region
Central region
Metropolitan area of Belo Horizonte region
Vale do Rio Doce region
West region
South/Southeast region
Campo das Vertentes region
Zona da Mata region
Vaccination Rate
Years

Figure 3: Boxplot of vaccination rate distribution per region per year, from 2011 to 2022 in Minas Gerais, Brazil. Red dot is the average of the year.

The ranking of municipalities (10%) with the best and worst brucellosis vaccination rates in at least 50% of the evaluated years in Minas Gerais is shown in the Figure 4. Considering the first municipalities with the highest vaccination rate, according to the defined criteria, 9 municipalities (Capitão Andrade, Pitangui, Cana Verde, Ibupiúna, Mercês, Natércia, Piedade dos Gerais, Santa Barbara do Tugúrio and Senhora dos Remédios) appeared 7-9 years (times) considering the analyzed period (2011-2022) in the ranking (Figure 4A). The average of vaccination rate of the municipalities with the highest values (n = 24) was 0.99 (±0.002). Among the municipalities with the lowest vaccination rates according to the defined criteria, 6 municipalities (Minas Novas, Indaiabira, Botumirin, Coluna, Leme do Prado and Rio Pardo de Minas) were present in 9-11 of the 12 analyzed years with lowest vaccination rate in the state (Figure 4B). The average vaccination rate of the municipalities with the lowest values was 0.38 (±0.07) (n = 44).

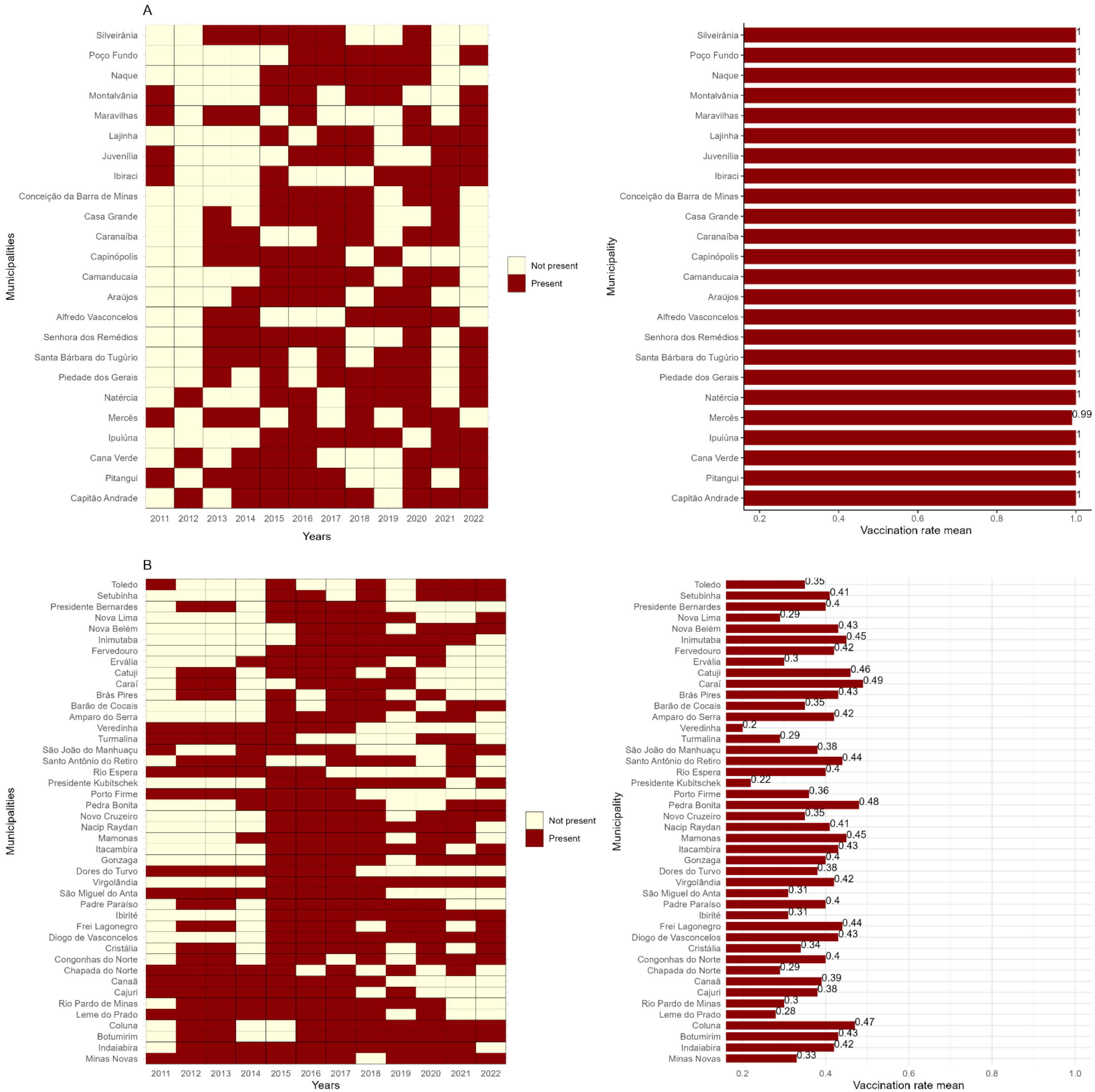
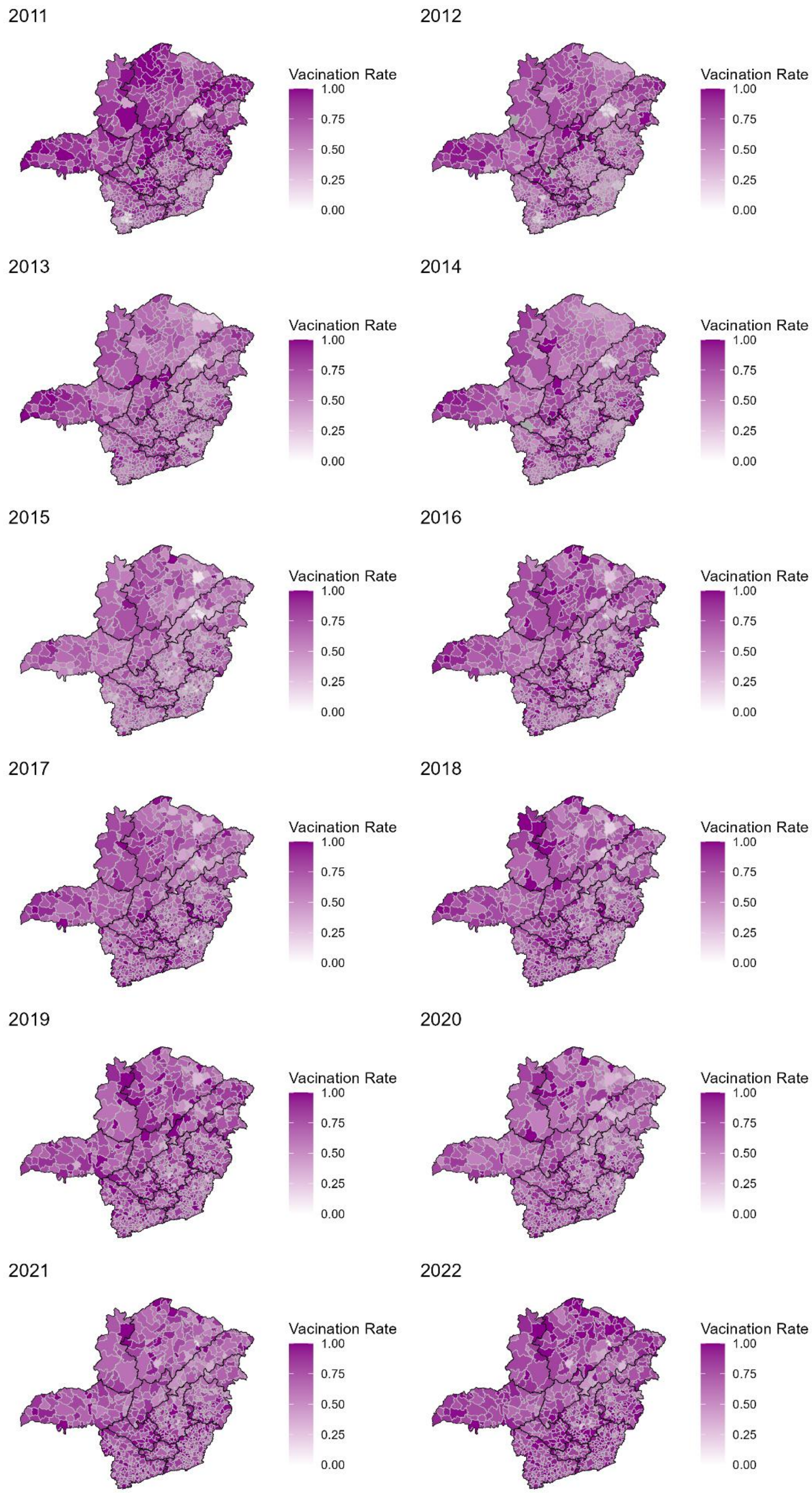

A
Silveirânia
Poço Fundo
Naque
Montalvânia
Maravilhas
Lajinha
Juvenília
Ibiraci
Conceição da Barra de Minas
Casa Grande
Caranaíba
Capinópolis
Camanducaia
Araújos
Alfredo Vasconcelos
Senhora dos Remédios
Santa Bárbara do Tugúrio
Piedade dos Gerais
Natércia
Mercês
Ipuiúna
Cana Verde
Pitangui
Capitão Andrade
Municipalities
2011 2012 2013 2014 2015 2016 2017 2018 2019 2020 2021 2022
Years
Not present
Present
Municipality
1
0.99
0.2 0.4 0.6 0.8 1.0
Vaccination rate mean
B
Toledo 0.35
Setubinha 0.41
Presidente Bernardes 0.4
Nova Lima 0.29
Nova Belém 0.43
Inimutaba 0.45
Fervedouro 0.42
Ervália 0.3
Catuji 0.46
Caraí 0.49
Brás Pires 0.43
Barão de Cocais 0.35
Amparo do Serra 0.42
Veredinha 0.2
Turmalina 0.29
São João do Manhuaçu 0.38
Santo Antônio do Retiro 0.44
Rio Espera 0.4
Presidente Kubitschek 0.22
Porto Firme 0.36
Pedra Bonita 0.48
Novo Cruzeiro 0.35
Nacip Raydan 0.41
Mamonas 0.45
Itacambira 0.43
Gonzaga 0.4
Dores do Turvo 0.38
Virgolândia 0.42
São Miguel do Anta 0.31
Padre Paraíso 0.4
Ibirité 0.31
Frei Lagonegro 0.44
Diogo de Vasconcelos 0.43
Cristália 0.34
Congonhas do Norte 0.4
Chapada do Norte 0.29
Canaã 0.39
Cajuri 0.38
Rio Pardo de Minas 0.3
Leme do Prado 0.28
Coluna 0.47
Botumirim 0.43
Indaiabira 0.42
Minas Novas 0.33
Municipalities
2011 2012 2013 2014 2015 2016 2017 2018 2019 2020 2021 2022
Years
Not present
Present
Municipality
0.2 0.4 0.6 0.8 1.0
Vaccination rate mean

Figure 4: Rank of the municipalities that appeared in the top 24 municipalities with greater vaccination rate and bottom 44 municipalities with lowest vaccination rate, from 2011 to 2022, in Minas Gerais, Brazil. A: Rank of the municipalities that appeared in half of the analyzed years as top vaccination rate and the average of vaccination rate of these municipalities, from 2011 to 2022. B: Rank of the municipalities that appeared in half of the analyzed years as bottom of vaccination rate and the average of vaccination rate of these municipalities, from 2011 to 2022.

3.2.Spatiotemporal analysis

The bovine brucellosis vaccination rate plotted by municipality shows an increase of this rate throughout the years (Figure 5). The Moran's I test revealed cluster formation of vaccination rate in all analyzed years (2011 to 2022). The regions that exhibited more cluster formation over the years were Jequitinhonha, Triângulo Mineiro, North, Zona da Mata and Metropolitana de Belo Horizonte. In Jequitinhonha region, high-high clusters (vaccination rate and autocorrelation with space above their average) were observed in 2011 and low-high clusters (vaccination rate below the average and autocorrelation with space above the average) from 2012 to 2022. In the Triângulo Mineiro region, high-high clusters were observed from 2011 to 2018, being absent from 2019 to 2022. The complete evolution of the bovine brucellosis vaccination rate clusters can be seen in Figure 6.



2011
Vacination Rate
1.00
0.75
0.50
0.25
0.00
2012
Vacination Rate
1.00
0.75
0.50
0.25
0.00
2013
Vacination Rate
1.00
0.75
0.50
0.25
0.00
2014
Vacination Rate
1.00
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2015
Vacination Rate
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2019
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2020
Vacination Rate
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0.00
2021
Vacination Rate
1.00
0.75
0.50
0.25
0.00
2022
Vacination Rate
1.00
0.75
0.50
0.25
0.00

Figure 5: Distribution of cattle brucellosis vaccination rate (number of vaccinated female cattle divided by the total number of female cattle with 0 to 12 months old) per municipality between 2011 and 2022 in Minas Gerais state, Brazil.

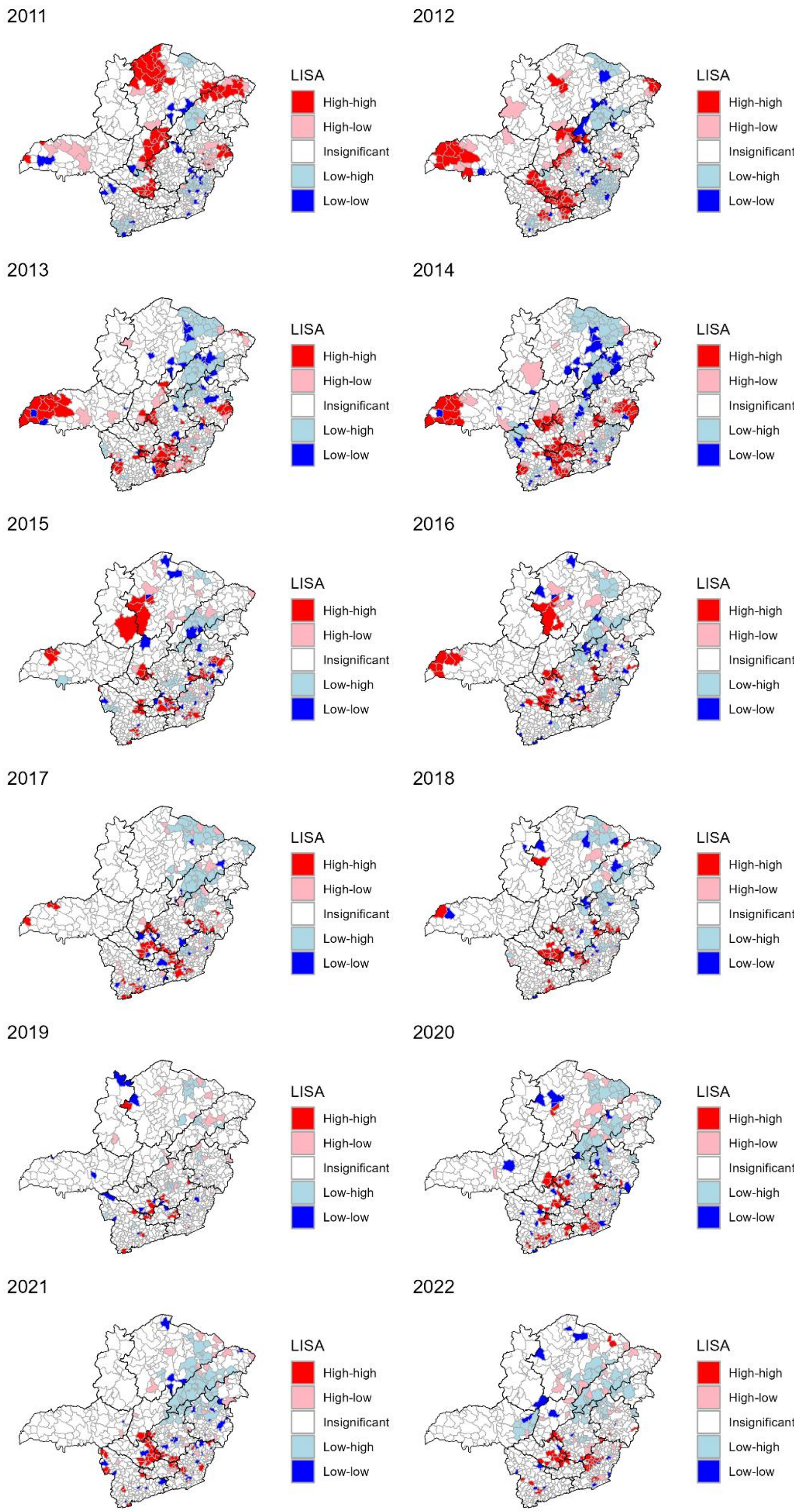
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
2021
2022
LISA
High-high
High-low
Insignificant
Low-high
Low-low

Figure 6: LISA Clusters [LISA was classified as 0 in insignificant interactions, 1 in low-low (vaccination rate and autocorrelation with space were below their averages), 2 in low-high (vaccination rate was below the average and autocorrelation with space was above the average), 3 in high-low (vaccination rate was above the average and autocorrelation with space was below the average) and 4 in high-high (vaccination rate and autocorrelation with space was above their averages) interactions] of cattle brucellosis vaccination rate (number of vaccinated female cattle divided by the total number of female cattle with 0 to 12 months old) from 2011 to 2022, in Minas Gerais state, Brazil.

3.3.Sociodemographic factors associated with vaccination

The GLMM with best fit was the one with the interaction between technical assistance and disease and parasite control. The farmers self-described race (white, black, yellow, brown, and indigenous) and sex (man or woman) were tested but with no fitting into de model. The model demonstrated that the technical assistance and disease and parasite control alone had a negative influence over the average of vaccinated animals. However, all the interactions between technical assistance and disease and parasite control positively influenced the average of vaccinated animals Furthermore, the greater positive influence over the average of vaccinated animals was due the combination between technical assistance given by cooperatives and the presence of disease and parasites control in the property. The detailed description of the GLMM can be found in Table 2.

Table 2: Generalized linear mixed effect final model for the assessment of sociodemographic factors associated with bovine brucellosis vaccination in Minas Gerais, Brazil, in 2017.

Variables	Estimate (log)[b]	Std. Error[c]	P-value
Intercept	21.51	5.89	0.00
Btotn[a]	0.69	0.03	0.00
Technical assistance			
Government	-16.89	5.95	0.00
Cooperative	-23.96	7.90	0.00
Private	-19.13	6.00	0.00
Disease and parasite control (DPC)	-17.84	6.45	0.00
Government assistance * DPC	20.14	6.51	0.00
Cooperative assistance * DPC	27.79	8.53	0.00
Private assistance * DPC	23.00	6.55	0.00

[a]Btotn: standardize number of animals per municipality (the total number of cattle minus its mean, divided by its standard deviation). [b] Estimate (log): results shown as logarithmic. [c]Std. Error: standard error.

4. Discussion

Vaccination of cattle against brucellosis is one of the major measures recommended by PNCEBT in Brazil [8] and worldwide to control the disease [27] which should be focused on increase the vaccination coverage year by year. In this sense, the aim of this study was to analyze the bovine brucellosis vaccination rate evolution in Minas Gerais, Brazil, from 2011 to 2022, as well as to investigate the farmers' sociodemographic factors that could influence the vaccination. Overall, our results showed an increase in the vaccination rate in the whole state throughout the years (Figure 1 and Figure 4), being the control of disease and parasite along with technical assistance positively associated to the increase of the average of vaccinated cattle (Table 2).

It is tempting to speculate that this positive evolution of bovine brucellosis vaccination rate in the last twelve years possibly contributed towards the decrease of bovine brucellosis cases in Minas Gerais, Brazil, as showed elsewhere (2011-2018) [28]. Indeed, the significant efficacy of S19 and

RB51 [29], the cost effective of bovine brucellosis vaccination campaigns [30] and the long history of vaccination in the state, was indicated as responsible for the decrease in the herd seroprevalence of the disease between 2002 and 2011[13], reinforces this statement. Taking together these data demonstrate the success of bovine brucellosis control in Minas Gerais, Brazil, in the last decades; however, it should be mentioned that considering the actual low prevalence/occurrence of the disease in the state [13, 28], the brucellosis vaccination must be complemented by other policies (e. g., test-and-slaughter, active surveillance and movement control) in pursuit of eradication [31, 32].

Moreover, considering the bovine brucellosis vaccination rate per region in 2022 (Figure 3), the Vale do Mucuri and Jequitinhonha regions were the only ones identified with a vaccination rate below 80%, which is the recommended rate for the states as Minas Gerais classified as score B (low risk - herd prevalence $\geq 2 < 5\%$) in the risk assessment for the disease [8]. These regions were also identified to have little or no information about bovine brucellosis testing (Rose Bengal Test) [28] and thereby previously indicated as important regions for interventions by IMA (the official animal health authority) for the control of bovine brucellosis in Minas Gerais, as also observed in the present study. Additionally, the results of the ranking of the municipalities with the lowest vaccination rate also point to IMA the exact places where the awareness of farmers, regarding the importance of this practice, should be raised.

Furthermore, the cluster analysis (Figure 5) identified significative local spatial autocorrelation of bovine brucellosis vaccination rate (clusters) in most regions and in all years, with clusters low-high and high-high more frequent over the years, which indicated a high local spatial autocorrelation of the vaccination rate, with coverages below and above the average of the state, respectively. Albeit no pattern of clusters, comparing the type, number and regions, was observed considering the whole period analyzed, the findings for the last three years point to a great number of clusters low-high (vaccination rate below the average and autocorrelation with space above the average), especially in Jequitinhonha region but also in some municipalities of the North, Vale do Mucuri, Vale do Rio Doce and Metropolitan area of Belo Horizonte. These areas should be priorities for the official animal health authority aiming to increase the bovine brucellosis vaccination coverage in the state. Indeed, the formation of clusters low-high was present in all years (2011 to 2022) in the Jequitinhonha region, suggesting that some characteristics of this region (e. g., social, cultural or animal husbandry) could be affecting (local autocorrelation above the average) the vaccination rate, which was below the average. Supporting this hypothesis, the Jequitinhonha region appeared among the first four regions with more farmers with no education, and farms with no technical assistance and no disease and parasite control, according to the 2017 agricultural census [20].

Additionally, the GLMM developed confirmed that disease and parasite control combined with technical assistance significantly influenced (positively) the number of vaccinated cattle against brucellosis, suggesting that the technical assistance along with disease and parasite control was probably focused on animal health. In fact, these two variables (disease and parasite control and technical assistance) individually negatively affected the average of vaccinated cattle in the model. Moreover, it is important to notice that technical assistance given by cooperative combined with disease and parasite control was the technical assistance with more influence over the average of vaccinated cattle, which could be justified by the positive effect that being part of a cooperative have over agricultural production in Brazil. In fact, it has been demonstrated that being part of cooperative in Brazil decreases costs of production, including the costs with veterinary and animal health [33]. Therefore, the investment in technical assistance that prioritize disease and parasite control should be the effort of IMA to improve brucellosis vaccination in the state, with the help of Empresa de Assistência Técnica e Extensão Rural do Estado de Minas Gerais (EMATER- Technical Assistance and Rural Extension Company of the State of Minas Gerais), particularly in the regions that exhibited low-high clusters in recent years (Figure 6), as this is fundamental for the control of the disease and thereby to move towards to eradication of bovine brucellosis in Minas Gerais state, Brazil.

There were some minimal limitations in this study, including the vaccination data used for 2011 to 2014, which had some municipalities with the same vaccination rate, as explained in Material and Methods. Albeit this limitation could compromise the assessment of vaccination rate (Figure 5) and the cluster analysis (Figure 6) in this period (2011 to 2014), this did not occur since changes in cluster patterns and increase in the vaccination rate were observed throughout these years (Figure 1 and Figure 2). Another limitation was that sociodemographic data on bovine producers, for the studied period (2011 to 2022), was only available for 2017, which prevented the temporal analysis of the influence of this data over bovine brucellosis vaccination. Nonetheless, the sociodemographic data was from the agricultural census, which truly represent the entire population and can be used to estimate data for the period between census [34]. These two limitations were the reason for the model, regarding the influence of sociodemographic factors over bovine brucellosis vaccination, be performed only with data for 2017. In addition, as the model was built using secondary data (not collected for the purpose of this study), conclusions must be kept as initial clues for epidemiological control, not asserting any known degree of evidence.

5. Conclusion

The bovine brucellosis vaccination rate increased over the twelve analyzed years (2011 to 2022), achieving almost 90% in 2022, being Jequitinhonha and Vale do Mucuri the regions where the vaccination rate was below the average in most of the years, except for 2011 (Jequitinhonha), 2014 and 2016 (Vale do Mucuri). Moreover, our results pointed regions with vaccination rate below the average and high spatial autocorrelation, which should be considered to directly address control measures according to particularities of each region. Additionally, the data also showed that technical assistance combined with disease and parasite control positively influenced the increase of the average of vaccinated cattle.

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Supplementary materials

Appendix A: Original names of the variables, in Portuguese, in the sociodemographic datasets of bovine producers in Minas Gerais, from 2017, used for the Generalized Linear mixed model, acquired from Instituto Brasileiro de Geografia e Estatística (IBGE)

Literacy	**Specialized assistance**	**Race**	**Sex**	**Disease and parasite control**
Município	Município	Município	Município	Município
Total	Total	Total	Total	Total
Não lê	Assistência especializada	Branco	Homem	Sem controle de doenças e parasitas
Lê	Sem assistência especializada	Preto	Mulher	Com controle de doenças e parasitas
Não se aplica	Assistência governamental	Amarelo	Não se aplica	
Nunca foi a escola	Privada	Pardo		
Classe de alfabetização CA	Cooperativas	Indígena		
Alfabetização de jovens adultos AJA	Empresas integradoras	Não se aplica		
Antigo primário	Empresas de planejamento privado			
Antigo ginasial médio 1 ciclo	ONGs			
Ensino fundamental regular 1 grau	Sistema S			
EJA educação de jovens adultos supletivo ensino fundamental 1 grau	Outras			
Antigo científico classico medio 2 ciclo				
Regular ensino medio 2 grau				

Técnico ensino medio 2 grau
EJA Educação de jovens adultos supletivo ensino medio 2 grau
Superior graduação
Mestrado e doutorado
Não se aplica

CHAPTER FOUR

Prepared in accordance with Preventive Veterinary Medicine standards.

Network analysis of cattle movement among municipalities in Minas Gerais State, Brazil, from 2013-2023

Abstract

The aim of this study was to characterize the cattle movement among municipalities in Minas Gerais state, Brazil and perform a network analysis based on this movement. Data on cattle movement in Minas Gerais state, Brazil, from January 2013 to August 2023, was acquired from Guia de Trânsito Animal (GTA) (Animal Transit Guide), mandatory for all livestock movements, provided by Instituto Mineiro de Agropecuária (IMA) (official animal health authority in the state). Descriptive analysis was performed accessing the five most common municipalities from origin and destination of movements, as well as by calculating the average and standard deviation (SD), of the number of GTAs for all years. Municipalities were considered as the nodes and the movements were the edges in the network analysis, which also considers a direct graph, with origin and destination. The most municipalities of origin and destination of movements were in Triângulo Mineiro / Alto do Paranaíba region, and that the transportation between farms [2,829,104/6,801,953 (41.59%)] were more frequent, followed by farm to slaughterhouse [1,771,704/6,801,953 (26.05%)], livestock event to farm [1,389,883/6,801,953 (20.43%)] and farm to livestock event [782,121/6,801,953 (11.50%)]. The analysis cattle movements (GTAs) among the years showed an average of 1,042,153.27 (SD: 2.889.595.96). Additionally, the network analysis results described static and high-connected networks, with great values of municipalities into the strong component, and the presence of three large communities, covering most of the state, in all years (2013 to 2023). In general, our results demonstrated a highly connected network throughout the analyzed years, with the movements particularly concentrated in the Triângulo Mineiro / Alto Paranaíba region.

Keywords: livestock, “animal transportation”, “communities”, “data science”.

Introduction

Food security is defined as food availability, food access, food utilization and food stability (FAO, 2016). This concept was created to fully guarantee secure, safe, and nutritious food worldwide, especially considering the actual world human population, estimated in more than 8 billion people in 2023 (The World Bank, 2023) Additionally, it is also important to taken into account the different availability of safe and secure food between developed and developing countries (FAO, 2021). In this context, production, and access to animal products, such as milk and meat, are of great benefice to assure access to nutritious food (Salter, 2018; Adesogan and Dahl, 2020), being both rich in protein, vitamins, and mineral salts, especially vitamin B12 (meat) and calcium (milk) (Murphy and Allen, 2003).

The world average cattle meat production was approximately 68 million tons in 2020, being 48.4% from the Americas, which was also responsible for 28% of the 72 million tons of whole fresh milk produced in the world (FAOSTAT, 2023). In this scenario, Brazil was responsible for the production of more than 212 million tons of meat in 2023 and 3.4 million tons of milk in 2022 (IBGE, 2024a). Brazil has also the second greater cattle production, with approximately 218 million heads (2022), whereas for buffalos is the eighth in the ranking, with almost 1.5 million heads (2022) (IBGE, 2024a). Composing the animal production chain, transportation of cattle is fundamental to guarantee animal's commerce, reproduction, and slaughter; however, it can also pose a risk, considering the potential dissemination of infection diseases, such as foot and mouth disease, brucellosis and tuberculosis. Indeed, among the main imposed risks for disease transmission, related to animal transportation, are the introduction of new animals in a herd from traders or other farms and intra- and inter-herds contact among the animals (Cowie et al., 2014; Alencar Mota et al., 2016; Tulu, 2022; Souley Kouato et al., 2018). Therefore, comprehension of patterns in animal transportation and the assessment of the network of movements may reveals areas where direct interventions could be performed to control and contain disease spread (Chaters et al., 2019).

In Brazil, the control of all movements of livestock animals is mandatory and performed throughout the Guia de Trânsito Animal (GTA) (Animal Transit Guide), which contain data on traceability of the transportation (origin, destination, reason, number of animals, etc), being its emission conditioned to the health status of animals, according to the species and transportation reason (Brasil, 2023). In Minas Gerais state, the Instituto Mineiro de Agropecuária (IMA) is the official animal health authority, responsible for GTA emission, administration, and analysis (IMA, 2023). Accordingly, the analysis of data acquired from GTA would allow the comprehension of cattle movement and where are the locations with more entrance and exit of animals, leading strategic interventions to prevent and control disease spread by building a network model associating movement and disease data (Cardenas et al., 2021a). In addition, network analysis is a powerful tool to comprehend the structure of complex systems, as cattle movements, allowing the identification of patterns of relationships among the nodes, as well as the understanding of the influence of these relationships on the cattle movement behavior (Luke and Harris, 2007). Therefore, the aim of this study was to characterize the cattle movement among municipalities in Minas Gerais state, Brazil, and to perform a network analysis based on this movement, from 2013 to 2023.

Material and methods

Study location

Minas Gerais state is in the southeast region of Brazil, at latitudes 14°13'58" and 22°54'00" south and longitudes 39°51'32" and 51°02'35" west, divided into 853 municipalities, grouped into twelve regions: Northwest Minas, North Minas, Jequitinhonha, Vale do Mucuri, Triângulo Mineiro/Alto do Paranaíba, the Central Minas, the metropolitan area of Belo Horizonte, Vale do Rio Doce, West Minas, South/Southeast Minas, Campo das Vertentes and Zona da Mata (Figure 1). The state climate is classified as Aw (tropical savannah climate with dry winter season), Cwa (humid temperate climate with dry winter and hot summer), and Cwb (humid temperate climate with dry winter and moderately hot summer) (Reboita et al., 2015). The state covers an area of 586,513,983 km^2, with a population of 20,538,718 people in 2022 (IBGE, 2023a) and 22,993,105 cattle heads in 2022 (IBGE, 2024b).

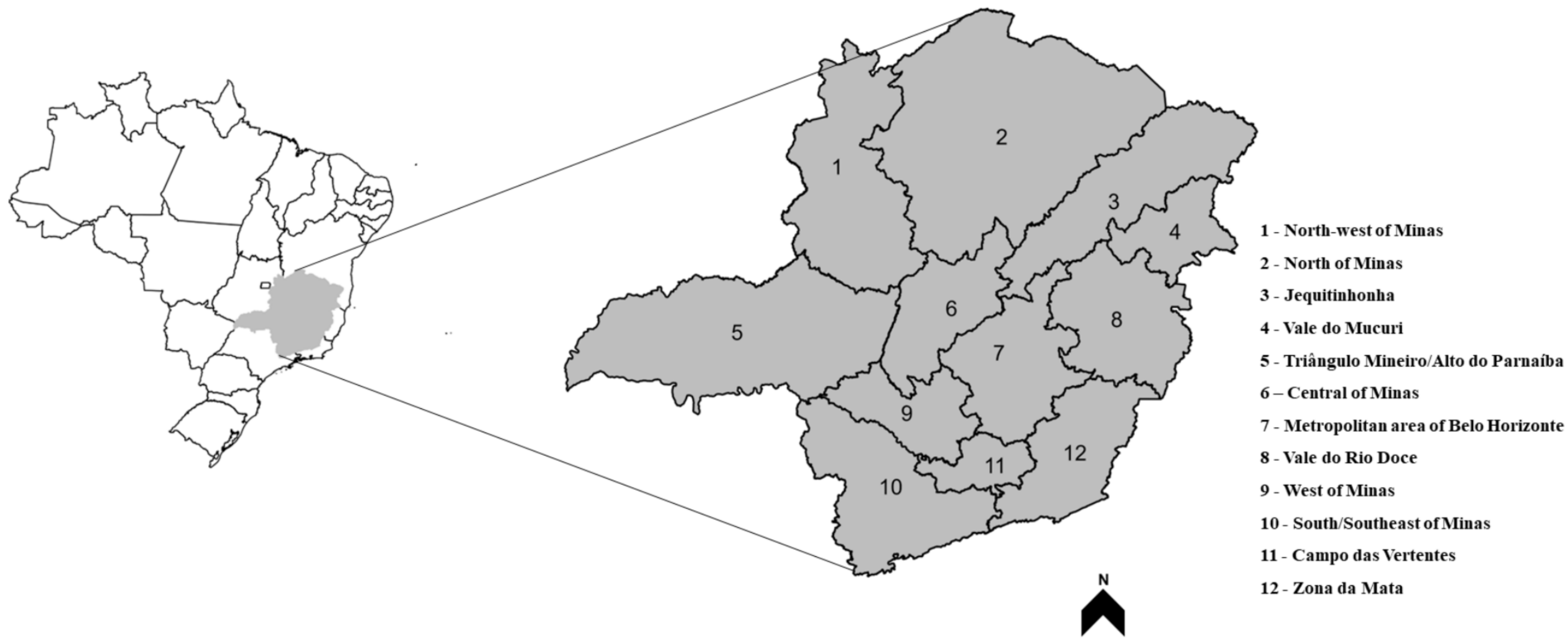

Figure 1: Map of the state of Minas Gerais, showing the regions defined in the current study. The state was divided into twelve regions: Northwest Minas, North Minas, Jequitinhonha, Vale do Mucuri, Triângulo Mineiro/Alto do Paranaíba, the Central Minas, the metropolitan area of Belo Horizonte, Vale do Rio Doce, West Minas, South/Southeast Minas, Campo das Vertentes and Zona da Mata.

Data source and description

Information about cattle movement (GTA) in Minas Gerais state, Brazil, from January 2013 to August 2023, was acquired from IMA. The GTA database contained information on series and number, year of emission, total number of animals transported, transported species (cattle and buffalo), reason for transportation, type of transportation, origin of animals [state, code and name of the municipalities, local identification (name and code)], and destination of animals [state, code and name for the municipalities, local (name and code) identification]. Sensitive information about the animal owners' (name and document) and situation of GTAs (e. g., printed, emitted by the producer, manual) were excluded.

The variables were reorganized or excluded, in case it was not necessary to the network analysis (detailed below), therefore every yearly dataset presented the same format and composition. Information on number of animals transported by age and sex was summed to obtain the total of animals transported by GTA. The variable reason for transportation were grouped into the categories: farm (rising, fattening, and reproduction), livestock event [agglomeration with and without commercial purposes and return from agglomeration (e.g., fairs, exhibitions, auction)], slaughterhouse (slaughter, sanitary slaughter and return to origin) and others (e.g., veterinary care, research, exportation and weighing).

All data were organized into R software version 4.3.0 (R Core Team, 2023), with the packages “readxl” version 1.4.2 (Wickham and Bryan, 2023), “forecast” (Wickham, 2021), and “tidyverse” (Wickham et al., 2019b).

Descriptive analysis of cattle movement

Descriptive analysis of cattle movement from January 2013 to August 2023, was performed accessing the five most common municipalities from origin and destination of movements, per year. Additionally, average, standard deviation (SD), median, interquartile range (IQR) and maximum and minimum values of the numbers of transported animals were also calculated. Also, the average and SD of the number of emitted GTA was calculated by month and per year, from 2013 to 2023. The number of emitted GTA was additionally used to analyze the reason for transportation (recategorized as previously described) throughout the years (2013 to 2023).

All descriptive analyses were performed in R software, version 4.3.0. (R Core Team, 2023), using the packages “tidyverse” (Wickham et al., 2019b), "stringi" (Gagolewski, 2021) and “circlize” (Gu et al., 2014).

Network analysis

The cattle movement data, from January 2013 to August 2023, was used as base to develop the static network for each year. For this, municipalities were considered as the nodes and the movements were the edges. The approach for the network analysis was carried out considering a direct graph, with origin and destination, since each movement had a specific direction. The description of the network was performed identifying the following measures: degree centrality, closeness, betweenness, presence of bridges, alpha of power-law distribution, average path length, cluster coefficient, diameter, reciprocity, edges density, transitivity, strong connected component (giant component), weak connected component, minimum cut and community by modularity score (Cardenas et al., 2021b; Kolaczyk and Csárdi, 2020; Luke, 2015; Vinueza et al., 2022; Barabási and Albert, 1999; Cairo et al., 2021). The definition of each measure and its application for the network analysis of municipalities in the present study is shown in Table 1. The fitting of cattle movement data from 2013 to 2023, considering municipalities as nodes, into the power law distribution was tested by Kolmogorov-Smirnov test (Clauset et al., 2009), with p-values ≤ 0.05 indicating that the test rejected the hypothesis of data following a power-law.

All measures of the network were calculated in R software version 4.3.0. (R Core Team, 2023), with the “igraph” package (Csardi and Nepusz, 2006), and the community plotting was performed using the base Minas Gerais map from “geobr” package (Pereira and Goncalves, 2022) and the “tidyverse” package for plotting (Wickham et al., 2019a).

Table 1: Definitions and applications of network measures used for this paper.

Measure	Definition	Reference	Application
Degree centrality	The number of nodes that a node is connected	(Vinueza et al., 2022)	It identifies municipalities (nodes) more connected and thereby that has more trades with other municipalities
Closeness	The inverse of the sum of the shortest path between one node and all nodes	(Vinueza et al., 2022)	Measures how quickly information/disease moves from one node to another by measuring how short the shortest path is between a node and all other nodes. In other words, when this centrality is smaller, it means that the municipality is closer connected, and the disease can spread more quickly in the network
Betweenness	Frequency with which a node appears on the shortest path between other pair of nodes	(Vinueza et al., 2022)	Measures the amount of influence that a node has on the flow of information within the network. It is used to find bridges between one part of the network and another. The nodes with the highest betweenness are those that connect groups, clusters of the network. Good places for interventions.
Bridges	It is an edge that removed increase the number of strongly connected components of the graph	(Cairo et al., 2021)	Complementary to betweenness, bridges identify the right edges to intervene to break the connections between municipalities to avoid movement and disease transmission
Power law distribution	Classification of the degree distribution as presenting a right-skewed distribution, free of scale, for large *k* (edges) with an exponent α between 2.1 and 4.	(Barabási and Albert, 1999)	When a network has a power law distribution there is a small number of municipalities that concentrate the majority of movements, being the municipalities of more interest in interventions
Average path length	Average number of links along the shortest paths for all possible pairs of network nodes. The average path length changes proportionally to the number of nodes and in a small world	(Vinueza et al., 2022)	It is the average length of movements between municipalities and how connected they are. It indicates how many movements (steps) on average are needed for a municipality to reach any other municipality.

	the change is proportionally to the log of number of nodes.		
Diameter	The most extensive shortest path among all the shortest paths in the network	(Vinueza et al., 2022)	How far can a disease move in the network considering the shortest movements that occur in the network. The larger the diameter, the further the disease will spread
Small world graph	It is given by the joint analysis clustering coefficient and average path length. A small world graph network represents a situation in which most nodes have close neighbors, but few nodes have very distant neighbors	(Vinueza et al., 2022)	Whether the network has a high clustering coefficient, this means that the municipality in the same group have many contacts with each other, which can increase the chance of disease spread. In addition, whether the network has a low average distance between nodes, this means that any municipality can be reached in a few movements (steps)
Reciprocity	A measure of the likelihood of vertices in a directed network to be mutually linked	(Vinueza et al., 2022)	It describes whether, on average, the movements among municipalities within the network, influences a specific farm as much as this farm influences the whole network
Edges density	Density is the proportion of observed ties in a network to the maximum number of possible ties	(Luke, 2015)	Indicates how dense are the movements among all municipalities in the network
Transitivity or Cluster Coefficient	The proportion of closed triangles (triads where all three ties are observed) to the total number of open and closed triangles (triads where either two or all three ties are observed)	(Luke, 2015)	It is the probability of municipalities to be clustered together by movements within the network. The clustering coefficient is between zero and one.
Strong component	Component that every node v is reachable from every edge by a directed walk	(Kolaczyk and Csárdi, 2020)	A large group of municipalities that transport intensively among themselves which facilitates the spread of diseases in this strong connected municipalities. It means that all municipalities in this group will be reached by the disease if there is no intervention

Weak component	Subgraph in which any two or more nodes are connected to each other	(Cardenas et al., 2021)	The largest group of municipalities of a network that transport animals with other municipalities by short and directed movements. It defines the maximum of affected municipalities in an epidemic if there is no intervention
Minimum cut	Calculates the minimum cut between two vertices in a graph or the minimum cut of the graph to separate the graph in at least two others	(Kolaczyk and Csárdi, 2020)	The number of movement restrictions to separate the network in at least two graphs
Community	Spatial connectivity among different nodes, including pairs that shared common direct and indirect connections within the network	(Cardenas et al., 2021)	Communities are groups of municipalities that has similar characteristics of movements among them

Results

Descriptive analysis of movement

The analysis cattle movements (GTAs) among the years (2013 to 2023) showed 2021 with the highest number of movements [1,048,781 (average: 87,398.42; SD: 12,834.88)] and 2016 the lowest [883,904 (average: 73,658.67; SD: 15,544.67)]. Among the months, April was the month with more movements [1,121,786 (average: 101,980.54; SD: 13,721.89)] and November with less [568,480 (average: 51,680.00; SD: 19,091.41)] (Table 2).

Table 2: Description statistic of cattle movement between municipalities (number of movements) per month from January 2013 to August 2023, in Minas Gerais state, Brazil.

Month	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	Total	Average	SD
January	65,188	78,058	81,138	68,449	65,826	77,525	82,722	86,108	83,963	78,256	79,486	846,719	76,974.45	7,266.02
February	63,900	80,487	71,716	78,440	60,189	67,287	78,195	82,682	82,749	76,008	78,253	819,906	74,536.91	7,702.589
March	71,738	82,767	93,107	84,434	82,111	82,118	79,954	81,897	93,569	99,754	101,603	953,052	86,641.09	9,142.19
April	110,124	107,673	110,810	105,374	86,456	109,073	113,527	69,497	114,948	97,383	96,921	1,121,786	101,980.54	13,721.89
May	64,713	69,464	63,332	59,192	65,112	53,343	74,528	58,239	82,312	86,982	105,948	783,165	71,196.82	15,382.34
June	89,765	91,258	94,007	90,418	89,151	96,017	93,774	97,136	101,310	101,751	109,028	1,053,615	95,783.18	6,165.46
July	91,957	91,446	90,730	78,902	77,405	88,194	101,353	101,336	89,281	101,998	104,729	1,017,331	92,484.64	9,178.32
August	79,870	81,535	77,196	71,115	83,102	84,407	89,588	87,523	85,767	92,972	98,174	931,249	84,659.00	7,482.01
September	73,782	86,875	77,562	60,734	74,678	73,336	88,314	88,289	74,220	80,940	-	778,730	70,793.64	24,850.71
October	86,501	90,602	87,062	69,531	85,123	91,670	109,758	91,906	74,842	83,941	-	870,936	79,176.00	28,175.39
November	48,508	51,968	53,771	45,856	49,159	52,229	66,989	64,418	69,047	66,535	-	568,480	51,680.00	19,091.41
December	72,768	86,359	83,191	71,459	79,640	83,702	92,580	87,894	96,773	87,450	-	841,816	76,528.73	26,485.02
Total	918,814	998,492	983,622	883,904	897,952	958,901	1,071,282	996,925	1,048,781	1,053,970	774,142	1,058,6785	-	-
Average	76,567.83	83,207.67	81,968.50	73,658.67	74,829.33	79,908.42	89,273.50	83,077.08	87,398.42	87,830.83	64,511.83	882,232.08	-	-
SD	1,6264.78	13,521.04	15,061.82	15,544.67	12,233.71	16,614.90	13,988.65	12,931.18	12,834.88	11,292.66	48,556.87	147,186.38	-	-

Regarding the origin of cattle movement, Frutal, Uberlândia, Unaí, Patos de Minas and Prata, appeared in all analyzed years (2013 to 2023) as the five municipalities from where more movement originated. On the other hand, Araguari, Ituitaba and Uberlândia were present in all years among the five municipalities that received more movements. More details of municipalities of origin and destination, according to the number of movements (GTAs) can be found in Table 3.

Table 3: The five municipalities from where more cattle movement originated and were destinated, from January 2013 to August 2023, in Minas Gerais state, Brazil.

Year	Cattle Movement			
	Origin	N movement	Destination	N movement
2013	Frutal	29,453	Ituiutaba	27,556
	Uberlândia	21,068	Frutal	24,970
	Patos de Minas	20,606	Araguari	23,618
	Unaí	18,397	Uberlândia	19,927
	Prata	16,588	Campina Verde	19,075
2014	Frutal	30,568	Ituiutaba	27,865
	Patos de Minas	21,751	Frutal	26,028
	Uberlândia	21,004	Araguari	24,008
	Unaí	17,908	Campina Verde	22,733
	Prata	17,144	Uberlândia	21,168
2015	Frutal	30,672	Frutal	27,665
	Patos De Minas	23,719	Ituiutaba	26,139
	Uberlândia	21,107	Araguari	22,213
	Unaí	18,710	Campina Verde	19,892
	Prata	18,325	Uberlândia	19,674
2016	Frutal	49,874	Ituiutaba	45,118
	Patos de Minas	39,118	Frutal	43,589
	Uberlândia	38,390	Uberlândia	38,443
	Prata	36,086	Araguari	38,415
	Unaí	33,151	Prata	34,700
2017	Frutal	26,545	Araguari	27,024
	Uberlândia	20,337	Ituiutaba	23,040
	Prata	18,579	Uberlândia	21,722
	Patos de Minas	17,373	Frutal	21,082
	Unaí	16,932	Itajubá	17,446

	Frutal	27,252	Araguari	26,288
	Uberlândia	20,037	Ituiutaba	24,868
2018	Prata	19,893	Frutal	23,803
	Unaí	18,965	Uberlândia	21,064
	Patos de Minas	17,662	Prata	19,366
	Frutal	31,532	Araguari	29,304
	Uberlândia	22,022	Frutal	29,057
2019	Prata	22,000	Ituiutaba	26,539
	Patos de Minas	21,632	Prata	23,000
	Unaí	20,199	Uberlândia	21,021
	Frutal	23,487	Araguari	31,287
	Uberlândia	23,183	Uberlândia	23,865
2020	Unaí	21,504	Ituiutaba	22,843
	Prata	19,732	Frutal	21,757
	Araguari	16,884	Unaí	21,513
	Uberlândia	27,845	Araguari	28,902
	Frutal	25,134	Uberlândia	25,876
2021	Unaí	23,080	Prata	23,140
	Prata	21,441	Unaí	22,990
	Uberaba	17,258	Ituiutaba	22,742
	Uberlândia	28,478	Araguari	27,917
	Frutal	26,701	Uberlândia	27,043
2022	Unaí	23,950	Ituiutaba	24,931
	Prata	20,313	Frutal	24,058
	Uberaba	18,998	Unaí	22,874
	Uberlândia	20,398	Uberlândia	19,808
	Frutal	20,079	Frutal	19,716
2023	Unaí	18,269	Araguari	19,374
	Uberaba	14,047	Ituiutaba	17,671
	Prata	13,553	Unaí	16,712

N movements: Number of movements.

Considering the number of animals transported per year, the year when more animals were transported was 2016 with a total of 30,585,452 transported animals, average of 17.37 (SD: 27.33) animals per GTA (median: 11.00; IQR: 18.00). Additionally, until August 2023 (eight months) 12,983,349 animals were transported, with an average of 16.77 (SD: 27.43) animals per GTA (median: 10.00; IQR: 18.00). More details on the annual number of animals transported are in Table 4.

Table 4: Descriptive statistic of the number of transported animals between municipalities per year, from January 2013 to August 2023, in Minas Gerais state, Brazil.

Year	**Mean**	**SD**	**Median**	**IQR**	**Minimum**	**Maximum**	**Total transported animals**
2013	16.95	25.79	12.00	17.00	1.00	3,093.00	15,575,381.00
2014	17.04	25.03	12.00	17.00	1.00	2,580.00	17,012,431.00
2015	17.46	29.16	11.00	17.00	1.00	4,365.00	17,178,482.00
2016	17.38	27.32	11.00	18.00	1.00	2,601.00	15,361,904.00
2017	17.06	26.66	11.00	17.00	1.00	2,690.00	15,318,235.00
2018	16.51	26.39	10.00	17.00	1.00	3,000.00	15,830,013.00
2019	16.06	26.10	10.00	17.00	1.00	4,911.00	17,205,555.00
2020	16.68	25.21	10.00	18.00	1.00	2,683.00	16,628,883.00
2021	16.57	26.66	10.00	18.00	1.00	6,722.00	17,374,056.00
2022	16.70	16.22	10.00	18.00	1.00	4,041.00	17,599,060.00
2023	16.77	27.43	10.00	18.00	1.00	4,775.00	12,983,349.00

SD: Standard Deviation. IQR: Interquartile Range.

In all analyzed years, movement between farms (farm-to-farm) [2,829,104/6,801,953 (41.59%)] was the most observed considering the reason of movement, followed by farm to slaughterhouses [1,771,704/6,801,953 (26.05%)], livestock event to farm [1,389,883/6,801,953 (20.43%)] and farm to livestock event [782,121/6,801,953 (11.50%)]. Movement from livestock events to slaughterhouses [15,225/6,801,953 (0.22%)], from farms to other locations [6,404/6,801,953 (0.09%)] and slaughterhouses back to farms [3,573/6,801,953 (0.05%)] appeared in less frequency throughout the years (Figure 2 and S1 Table).

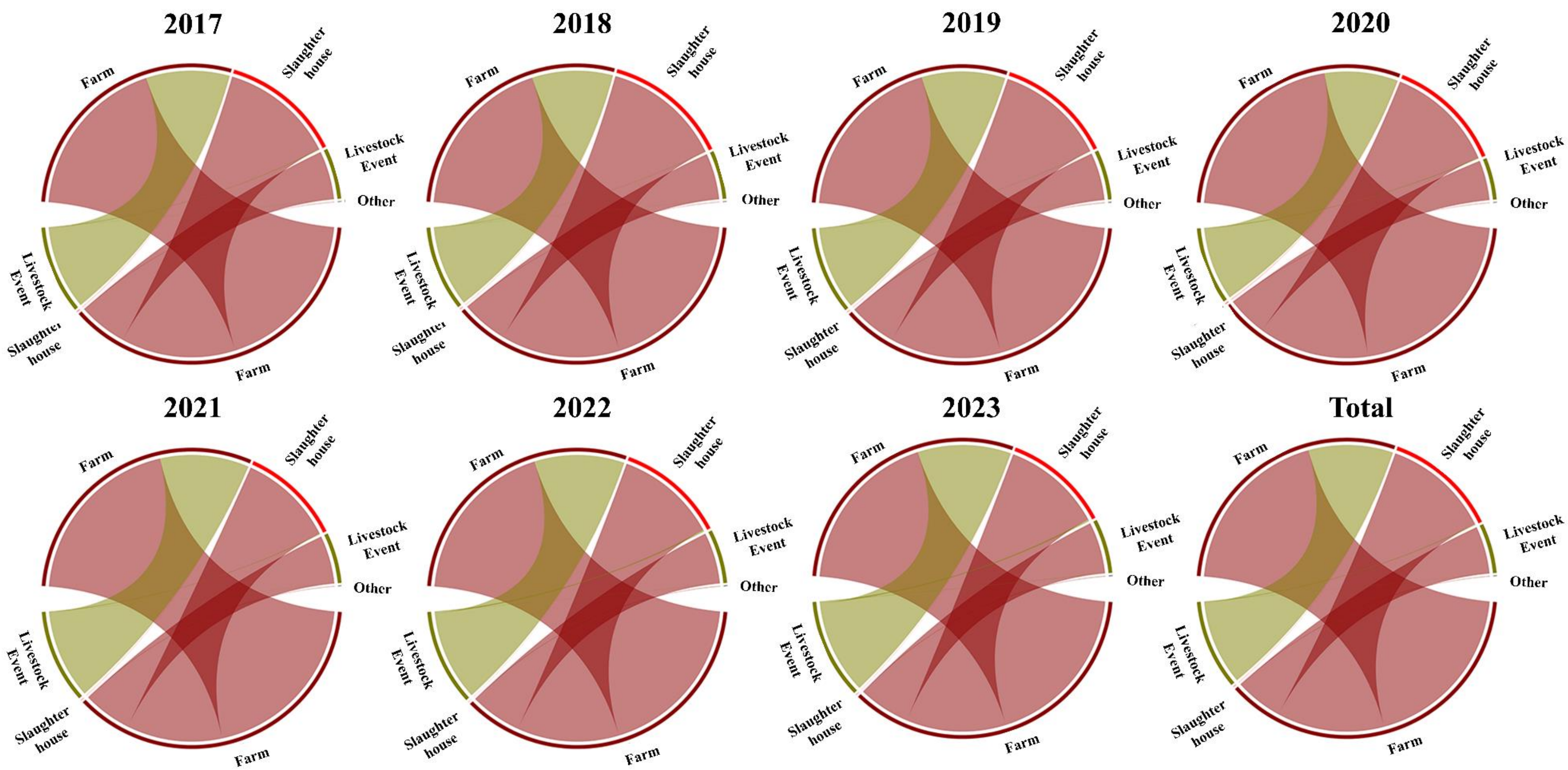

Figure 2: Chord Diagram of reason for cattle movement in Minas Gerais state, Brazil, from January 2017 to August 2023, grouped into the categories: farm (rising, fattening, and reproduction), livestock event [agglomeration with and without commercial purposes and

return from agglomeration (e.g., fairs, exhibitions, auction)], slaughterhouse (slaughter, sanitary slaughter and return to origin) and others (e.g., veterinary care, research, exportation and weighing). Above in the figure are the destination and bellow the origin of the movements.

Network analysis

The static network analysis of each year (2013 to 2023) demonstrated a similarity of the measures used to characterize the network. However, the year with the highest number of edges (movements) (44,924) and the higher mean degree centrality (105.46) was 2021; whereas 2017 was the year with less edges (33,969) and lower mean degree centrality (79.65), among the analyzed years. Also, throughout the years, the degree centrality analysis demonstrated the presence of highly connected municipalities (Figure 3). Additionally, the greater mean betweenness was observed in 2013 (1,635.90), and the year with the minor value was 2021 (1,395.34) (Table 5 and Figure 4). Considering that Minas Gerais has 853 municipalities (nodes), the strong component analysis showed that alongside the years, most nodes were part of the strong component, varying from 848 nodes (2020) to 852 nodes (2014, 2015, 2016). Complete annual information of the network measures is available in Table 5. Additionally, the results of the Kolmogorov-Smirnov test are shown in Table 6.

Table 5: Measures of the network of cattle movement between municipalities, from January 2013 to August 2023, in Minas Gerais state, Brazil.

Measure	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
Number of nodes	852	852	853	853	853	853	853	853	852	852	852
Number of edges	34,260	35,715	36,755	35,847	33,969	35,696	40,165	44,624	44,924	41,723	35,856
Mean Degree centrality	80.42	83.84	86.18	84.05	79.65	83.70	94.17	104.60	105.46	97.94	84.17
Mean In-degree centrality	40.21	41.92	43.09	42.02	39.82	41.85	47.09	52.31	52.73	48.97	42.08
Mean Out-degree centrality	40.21	41.92	43.09	42.02	39.82	41.85	47.09	52.31	52.73	48.97	42.08
SD Degree centrality	61.20	63.42	66.49	65.31	61.10	64.86	71.28	79.39	78.96	73.33	65.39
SD In-degree centrality	36.18	38.26	40.24	38.53	36.88	39.99	43.94	47.73	45.91	43.64	40.01
SD Out-degree centrality	29.30	29.45	30.35	31.29	28.29	29.77	32.16	36.84	38.10	34.72	30.04
Median Degree centrality	66.00	68.00	71.00	68.00	66.00	69.00	36.00	88.00	89.50	81.00	68.00
Median In-degree centrality	31.00	32.00	32.00	32.00	31.00	31.00	36.00	40.00	42.00	37.00	31.00
Median Out-degree centrality	34.00	35.00	37.00	35.00	33.00	36.00	40.00	44.00	45.00	42.00	35.00
IQR Degree centrality	60.25	63.00	66.00	66.00	61.00	62.00	35.00	82.00	80.50	76.00	64.00
IQR In-degree centrality	30.00	32.00	13.00	34.00	29.00	31.00	35.00	41.00	42.25	39.00	44.00
IQR Out-degree centrality	31.00	31.00	33.00	34.00	31.00	32.00	34.00	42.00	41.25	37.00	33.00
Mean Closeness	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean In-degree closeness	0.00	0.00	NaN	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean Out-degree closeness	0.00	NaN	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Mean Betweenness	1,635.90	1,597.19	1,581.40	1,591.29	1,613.59	1,579.44	1,497.38	1,408.04	1,392.34	1,445.63	1,539.88
Number of Bridges	1.00	0.00	0.00	1.00	1.00	1.00	2.00	2.00	2.00	1.00	2.00
Average path length	3.00	2.94	2.92	5.74	2.96	2.92	2.81	2.70	2.68	2.75	2.86
Number of Cluster	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Diameter	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Reciprocity	0.54	0.55	0.54	0.54	0.55	0.53	0.53	0.49	0.50	0.52	0.51
Edges density	0.05	0.05	0.05	0.05	0.05	0.05	0.06	0.06	0.06	0.06	0.05
Transitivity or Cluster Coefficient (C)	0.28	0.28	0.28	0.27	0.28	0.28	0.29	0.30	0.29	0.29	0.28
Strong component	851 nodes	852 nodes	852 nodes	852 nodes	851 nodes	850 nodes	850 nodes	848 nodes	849 nodes	851 nodes	849 nodes
Weak component	852 nodes	852 nodes	853 nodes	853 nodes	853 nodes	853 nodes	853 nodes	853 nodes	852 nodes	852 nodes	852 nodes
Diameter Strong component	16.00	9.00	10.00	46.00	16.00	8.00	8.00	7.00	8.00	18.00	9.00
Average path length Strong component	3.00	2.94	2.92	5.74	2.96	2.92	2.81	2.70	2.68	2.75	2.86
Transitivity (C) Strong Component	0.28	0.28	0.28	0.27	0.28	0.28	0.29	0.30	0.30	0.30	0.28
Minimum cut	842	844	838	809	819	698	853	853	689	852	844
Number of Communities	5.00	7.00	5.00	5.00	7.00	5.00	7.00	4.00	5.00	4.00	6.00

SD: Standard Deviation. IQR: Interquartile Range.

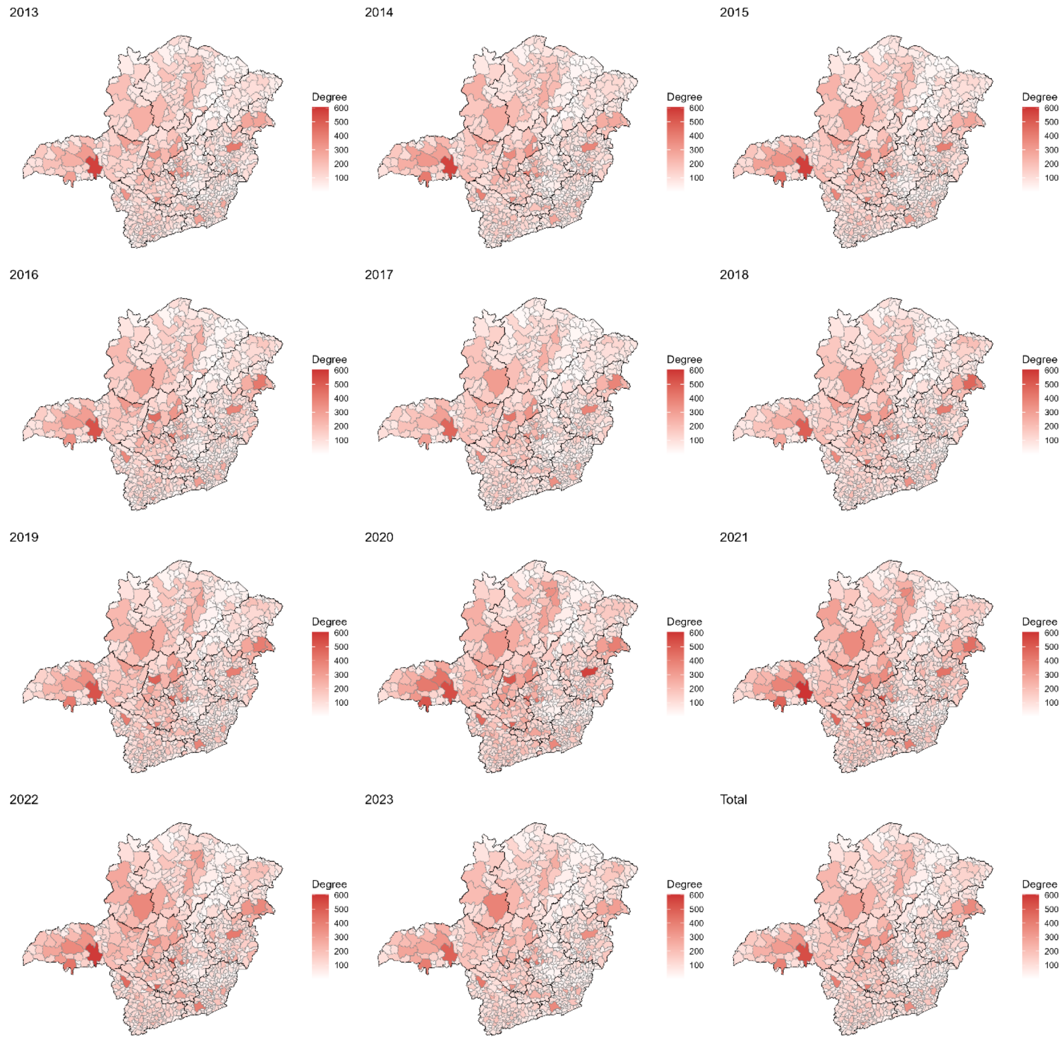

Figure 3: Degree centrality of cattle movement between municipalities in Minas Gerais state, Brazil, from January 2013 to August 2023.

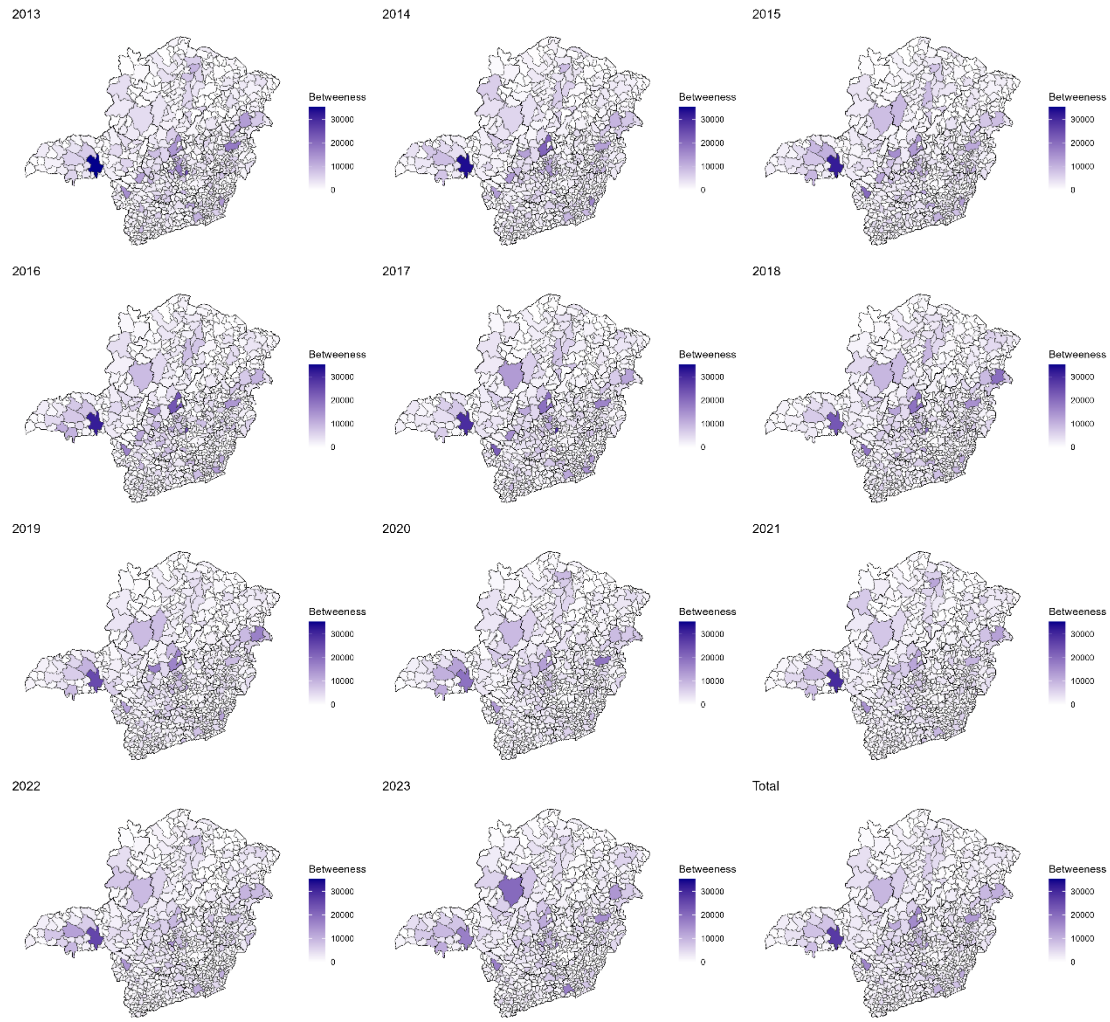

Figure 4: Betweenness centrality of cattle movement between municipalities in Minas Gerais state, Brazil, from January 2013 to August 2023.

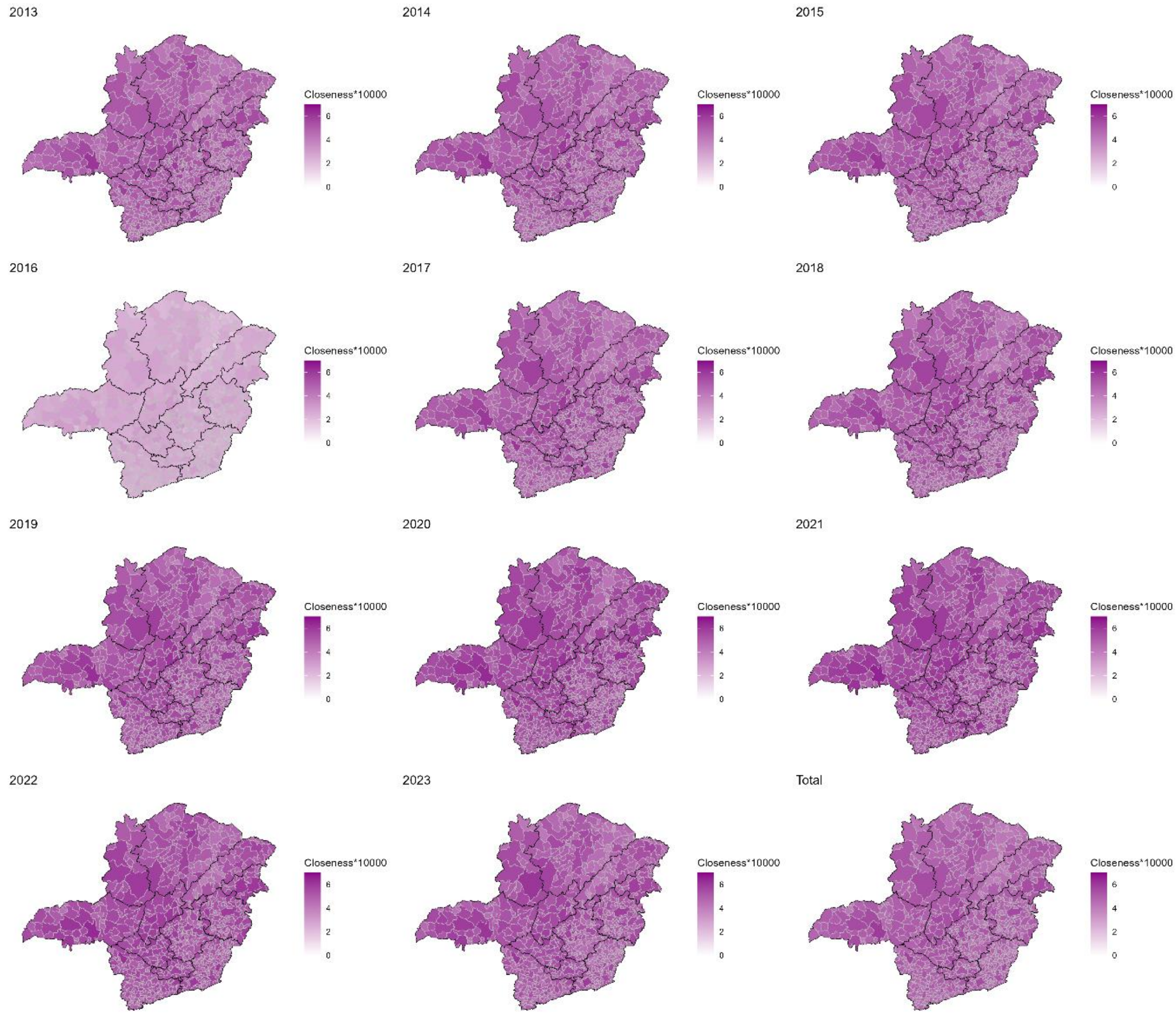

Figure 5: Closeness centrality of cattle movement between municipalities in Minas Gerais state, Brazil, from January 2013 to August 2023.

Complementarily, the community analysis showed the formation of 4-7 communities among the analyzed years (2013 to 2023) (Table 5 Figure 6), highlighting the formation of three major communities in all years, that covered most of the Minas Gerais state (Figure 5). Besides these three major communities, other smaller communities were also present in all years (Figure 5).

Table 6: Results of the Kolmogorov-Smirnov test for the fitting into a power-law distribution of the cattle movement between municipalities, from January 2013 to August 2023, in Minas Gerais state, Brazil.

Year	**Alpha**	**Log-likelihood**	**Kolmogorov-Smirnov**	**p-value**
2013	3.64	-933.67	0.04	0.89
2014	3.51	-1164.97	0.05	0.62
2015	3.51	-1186.19	0.04	0.74
2016	3.51	-1127.62	0.05	0.54
2017	3.17	-1834.7	0.06	0.15
2018	3.38	-1182.12	0.05	0.52
2019	3.19	-1806.37	0.05	0.28
2020	4.29	-1721.99	0.05	0.38
2021	3.29	-502.19	0.05	0.97
2022	3.43	-1593.61	0.05	0.46
2023	3.18	-1750.88	0.05	0.36

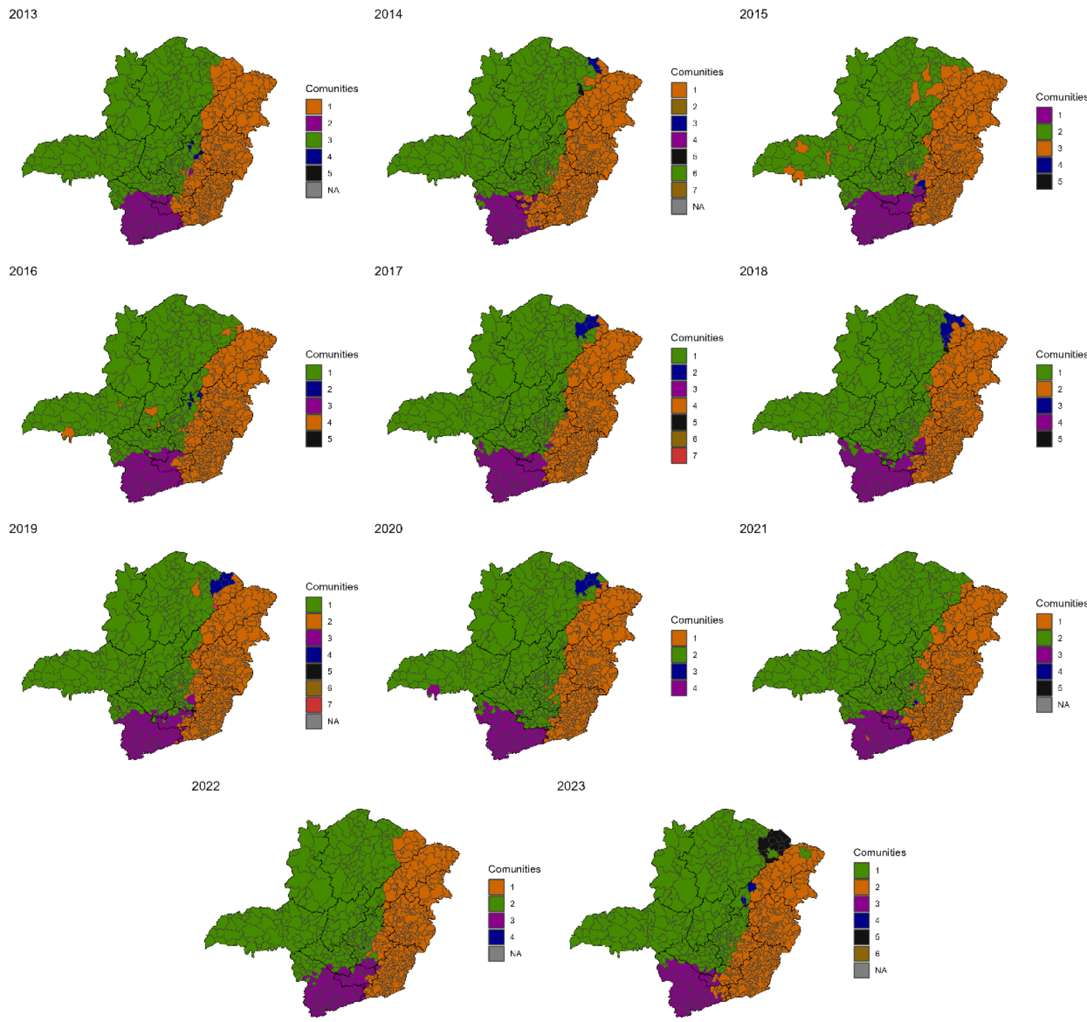

Figure 6: Network communities of cattle movement between municipalities in Minas Gerais state, Brazil, from January 2013 to August 2023.

Discussion

Network analysis based on animal movement is an important tool to help the efficacy of disease control programs to guarantee food safety and security. This analysis is especially relevant to the identification of highly connected premises that might act like hubs, considering origin and destination of movements, which could be exposed to control measures to reduce diseases transmission (Cardenas et al., 2021a). Hence, the aim of this paper was to describe the cattle movement among municipalities in Minas Gerais, Brazil, from 2013 to 2023, and to perform a network analysis of this movement. In general, our results demonstrated a highly connected network throughout the analyzed years, with the movements particularly concentrated in the Triângulo Mineiro/Alto Paranaíba region.

In fact, the results of clustering coefficient, the average path length and the strong component taken together, considering all analyzed years, suggest a highly connected network, possibility classified as a small world network (Watts and Strogatz, 1998). This network is characterized by lower values of average path length and higher values of clustering coefficient, which means that most nodes have close neighbors, but few nodes have very distant neighbors (Watts and Strogatz, 1998). Nevertheless, in opposite, the results also showed that the cattle movement data from Minas Gerais fitted into a power-law distribution (Table 6), which is reinforced by the presence of municipalities with high degree distribution (Figure 4), suggesting a preferential attachment behavior, which is one of the requirements to classify a graph as scale-free network (Barabási and Albert, 1999). Although, as the network was based on inter-municipalities cattle movement in Minas Gerais state, which allow only the increase in the number of edges (movements) but not in number of nodes (municipalities), the graph is not open (does not allow new nodes into the graph), preventing its truly classification as a scale-free network (Barabási and Albert, 1999). The classification of a graph into a small world or a scale free network is important to orientate the development of other models, such as disease transmission models, regarding the planning of the control measures, which could be focused on specific nodes (scale-free), or more omnibus, considering groups of nodes or the whole network (small word) (Luke and Harris, 2007).

Despite the inconclusion of adopting a single classification for the present network, the identification of highly connected municipalities through the years (Figure 3), point to preferential targets for control measures, since these municipalities greatly influences the movement system (those that moved more cattle) (Chaters et al., 2019; Cardenas et al., 2021b, Savini etal., 2017). Also, the high values of betweenness, observed for the whole state and for some municipalities (Table 5 and Figure 4), indicates, respectively, high connectivity among the nodes and ideal places for interventions (Vinueza et al., 2022), isolating areas of disease spread and avoiding diseases dissemination through the state. Complementarily, the community analysis (Figure 3) showed three major communities geographically connected in all the analyzed years (2013 to 2023), which should also be considered to design tailored disease control strategies (considering the different characteristics of cattle movement in each of these groups), improving the use of public resources. However, it is important to mention that as we performed static network analysis, this approach could overestimate the connectivity of the graph, negatively influencing risk assessment and control measures (Cardenas et al., 2021b). Albeit for diseases with low R0 (basic reproduction number of a disease), e. g. bovine brucellosis, static network analysis could be sufficient to explain and predict the disease transmission (Holme and Saramäki, 2012; Cardenas et al., 2021b).

The descriptive analysis of cattle movement in Minas Gerais, from 2013 to 2023, showed that the movements were especially concentrated in the municipalities from Triângulo Mineiro / Alto do Paranaíba region, in all years (Table 2). This region was also identified as belonging to the largest communities elucidated in the analysis, from 2013 to 2023 (Figure 1 and 3), in addition to having previously been described as the one with the large cattle population in the state (Costa et al., 2023). Among the main reasons for cattle transportation in Minas Gerais (2013-2023), movements that had farms as origin and destination were largely observed in all years, which was also the most common reason for transportation of cattle in two other Brazilian states (Menezes et al., 2020; Azevedo Júnior et al., 2022). The cattle movement between farms is frequently performed for rising, fattening, and reproduction, which are activities usually carried out in different properties, in accordance with the common cattle breeding system in the country (Ferraz and Felício, 2010). Additionally, Minas Gerais is among the Brazilian states that produces and slaughter more cattle in Brazil (IBGE, 2023b, 2024b), which explain the other reasons for movement in the state and the enormous number of transported animals per year.

This paper has some limitations, first, as we worked exclusively with intra state cattle movement data, this restriction prevented the assessment if the network was in fact a scale-free, since no new municipality (node) could be added to the graph at any moment (Barabási and Albert, 1999). A possible solution would be to conduct the same analysis considering the network as open, accounting for the movements to other states (new municipalities); or perform a different approach by taken properties instead of municipalities as nodes, which would allow more variability in the components of graph (nodes and edges) and therefore could reveal a scale-free network. These approaches were not possible at this time due to restrictions in available databases but will be tested in future studies. Another limitation was the chosen by a static analysis of the network, which precludes the use of this network in disease spread models for acute outcomes with high R0. Nevertheless, the present static network could be applied to modeling diseases with low R0 (Holme and Saramäki, 2012), since their dissemination are usually not time sensitive. Furthermore, the constant data collection on cattle movement in Minas Gerais, due to compulsory traceability of livestock movement, could also contributes to the development and improvement of network analysis and disease spread models for the state (Chaters et al., 2019), reassuring food safety. Additionally, the implementation of a permanent whole-of-life system that allows individual identification of animals and temporal analysis of the network would contribute even more to the robustness of these models and their application for the disease control (Savini et al., 2017).

Conclusion

The network analyzes, considering municipalities as nodes and cattle movements as edges, demonstrated a very connected graph with large communities occurring annually (2013 to 2023), suggesting places for target interventions to control diseases and ensure cattle production. Moreover, our results also described the cattle movement in Minas Gerais from 2013 to 2023, showing that the municipalities with more movement were in the Triângulo Mineiro / Alto do Paranaíba region.

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Supplementary Materials

S1 Table: Description of the number of cattle movements (Guia de Transito Animal – GTA) per reason of transportation in Minas Gerais, Brazil, from January 2013 to August 2023.

Year	Destination/Origen	Livestock event	Slaughterhouse	Farm
2017	Slaughterhouse	1,472	30	249,525
	Livestock event	428	0	105,431
	Farm	181,268	567	358,399
	Others	78	0	754
2018	Slaughterhouse	1,045	75	276,872
	Livestock event	443	0	107,233
	Farm	187,023	445	384,907
	Others	60	0	798
2019	Slaughterhouse	1,059	21	287,394
	Livestock event	408	0	123,376
	Farm	216,194	555	441,105
	Others	57	0	1,113
2020	Slaughterhouse	1,900	30	266,787
	Livestock event	128	0	964,22
	Farm	175,975	823	453,975
	Others	50	0	835
2021	Slaughterhouse	2,043	0	243,366
	Livestock event	400	0	122,451
	Farm	225,111	579	453,796
	Others	86	0	949
2022	Slaughterhouse	4,346	1	260,374
	Livestock event	617	0	130,758
	Farm	229,418	314	426,956
	Others	61	0	1,125
2023	Slaughterhouse	3,360	0	187,386
	Livestock event	895	0	96,450
	Farm	174,894	290	309,966
	Others	71	0	830

CHAPTER FIVE

Prepared in accordance with Preventive Veterinary Medicine standards.

Network analysis of cattle movement among properties in Minas Gerais State, Brazil, from 2017-2023

Abstract

The aim of this paper was to conduct a network analysis of cattle movements having properties as nodes, in Minas Gerais State, Brazil, from January 2017 to August 2023, to identify points of interventions for improvement of disease control strategies. The analysis was performed by calculating the number of nodes and edges, degree, closeness and betweenness distribution, the presence of strong and week component, the average path length, the cluster coefficient, and other measures to characterize the network. The results point to a scale free network in all analyzed years, suggesting the presence of livestock properties where disease control measures would greatly affect the network. Therefore, this paper classified as scale free the cattle movements network in Minas Gerais State, Brazil, allowing more strategic planning for disease control.

Introduction

Food security contributes significantly to peacebuilding efforts, by lowering the food prices, increasing food access, and improving rural livelihoods, which could prevent conflicts, reassuring peace (FAO, 2016). Additionally, food security is directed related to the improvement of human development index (HDI) (Gani and Prasad, 2007), and livestock products are among the options of nutritious food (Murphy and Allen, 2003) that can help this enhancement, especially in developing countries, where a diverse diet could be scarce for the majority of the population.

Complementarily, livestock production contributes around 40% to the world's agricultural gross domestic product (GPD) and 30% for the GPD in developing counties (World Bank, 2009). One of the important developing countries in amount of livestock production is Brazil, with 23.89 billion dollars production in 2022 (IBGE, 2023a), having Minas Gerais state as one of the protagonists of animal production in the country (IBGE, 2023). In Brazil, livestock production is encouraged and regulated by Minesterio da Agricultura e Pecuária (MAPA) (Ministery of Agriculture and Livestock). Among MAPA policies, the national animal health programs contribute greatly to the security of animal production in the country by performing actions of surveillance, prevention, control and eradication of diseases.

Indeed, among the factors that decrease animal production, infectious diseases play a important role, and the control of these diseases are fundamental to keep livestock production and its benefits (Huntington et al., 2021). In this regard, many efforts are made to create tools for disease control in livestock animals, including network models based on animal movement (Acosta et al., 2022; Cardenas et al., 2021a; Chaters et al., 2019). These models can be applied to investigate the success of control measures, to identify points of interventions, to support the targeting use of resources, improving the efficacy of tools to the decrease of disease burden (Luke and Harris, 2007). Combined with data from animal movement, the identification of epidemiologic characteristics of diseases, such as prevalence / incidence, vaccination status and outbreaks, can also aggregate to the development of models and to its robustness for the control of diseases (Huppert and Katriel, 2013).

Therefore, the aim of this paper was to perform a network analysis of cattle movements among properties in Minas Gerais State, Brazil, from January 2017 to August 2023, to identify points of interventions for improvement of disease control strategies.

Material and methods

Study location

Minas Gerais state is in the southeast region of Brazil, at latitudes 14°13'58" and 22°54'00" south and longitudes 39°51'32" and 51°02'35" west, divided into 853 municipalities, grouped into twelve regions: Northwest Minas, North Minas, Jequitinhonha, Vale do Mucuri, Triângulo Mineiro / Alto do Paranaíba, the Central Minas, the metropolitan area of Belo Horizonte, Vale do Rio Doce, West Minas, South/Southeast Minas, Campo das Vertentes and Zona da Mata (Figure 1). The state covers an area of 586,513,983 km^2, with a population of 20,538,718 people in 2022 (IBGE, 2023c) and 22,993,105 cattle heads in 2022 (IBGE, 2024). The climate was classified as Aw (tropical savannah climate with dry winter season), Cwa (humid temperate climate with dry winter and hot summer), and Cwb (humid temperate climate with dry winter and moderately hot summer) (Reboita et al., 2015).

Figure 1: Map of the state of Minas Gerais, showing the regions defined in the current study. The state was divided into twelve regions: Northwest Minas, North Minas, Jequitinhonha, Vale do Mucuri, Triângulo Mineiro/Alto do Paranaíba, the Central Minas, the metropolitan area of Belo Horizonte, Vale do Rio Doce, West Minas, South/Southeast Minas, Campo das Vertentes and Zona da Mata.

Data source and description

Information about cattle movement in Minas Gerais state, Brazil, from January 2017 to August 2023 (GTA), was acquired from IMA. The GTA database contained information on series and number, year of emission, total number of animals transported, transported species (cattle and buffalo), reason for transportation, type of transportation, origin of animals [state, code and name of the municipalities, local identification (name and code)], and destination of animals [state, code and name for the municipalities, local (name and code) identification]. The sensitive information about the animal owners' (name and document) and situation of GTAs (e. g., printed, emitted by the producer, manual) were excluded. The variables were reorganized or excluded in case it was not necessary to the network analysis therefore every yearly dataset presented the same format and composition. Information on number of animals transported by age and sex was summed to obtain the total of animals transported by GTA.

All data were organized into R software version 4.3.0 (R Core Team, 2023), with the packages "readxl" version 1.4.2 (Wickham and Bryan, 2023), "forecast" (Wickham, 2021), and "tidyverse" (Wickham et al., 2019b).

Network analysis

The cattle movement data, from January 2017 to August 2023, was used as base to develop the static network for each year. For the purpose of this study, property was defined as any unit of origin or destination of movement, including farms (rising, fattening, and reproduction), slaughterhouses, livestock events [agglomeration with and without commercial purposes (e.g., fairs, exhibitions, auction)], and others (e.g., veterinary care, research, exportation and weighing). In the network analysis, properties were considered as the nodes and the movements were the edges. The approach for the network analysis was carried out considering a direct graph, with origin and destination, since each movement had a specific direction. The description of the network was performed identifying the following measures: degree centrality, closeness, betweenness, presence of bridges, alpha of power-law distribution, average path length, cluster coefficient, diameter, reciprocity, edges density, transitivity, strong component, weak component, minimum cut and community (Cardenas et al., 2021b; Kolaczyk and Csárdi, 2020; Luke, 2015; Vinueza et al., 2022; Barabási and Albert, 1999; Cairo et al., 2021). The fitting of cattle movement data, from January 2017 to August 2023, considering properties as nodes, into the power law distribution was tested by Kolmogorov-Smirnov test (Clauset et al., 2009), with p-values ≤ 0.05 indicating that the test rejected the hypothesis of data following a power-law.

All measures of the network were calculated in R software version 4.3.0. (R Core Team, 2023), with the "igraph" package (Csardi and Nepusz, 2006), and the community plotting was performed using the base Minas Gerais map from "geobr" package (Pereira and Goncalves, 2022) and the "tidyverse" package for plotting (Wickham et al., 2019a).

Results

The static network analysis results of properties in Minas Gerais state, from January 2017 to August 2023, showed 2019 as the year with more nodes (196,542) and more edges (485,049), and 2020 with less nodes (184,206) and edges (458,386), considering the years with data available for all months (2017 to 2022) (Table 1).

Tables

Table 1: Measures of the network for cattle movement among livestock properties, including farms, slaughterhouses, livestock events and others (e.g., veterinary care, research, exportation and weighing), from January 2017 to August 2023, in Minas Gerais state, Brazil.

Measure	2017	2018	2019	2020	2021	2022	2023
Number of nodes	184,659	186,204	196,542	184,206	188,475	188,090	160,346
Number of edges	412,169	436,771	485,049	458,386	466,191	463,711	354,263
Mean Degree centrality	4.46	4.7	4.94	4.98	4.95	4.93	4.42
Mean In-degree centrality	2.23	2.35	2.47	2.49	2.47	2.46	2.21
Mean Out-degree centrality	2.23	2.35	2.47	2.89	2.47	2.46	2.21
SD Degree centrality	34.6	32.52	34.75	30.19	31.31	31.1	27.32
SD In-degree centrality	30.59	28.1	29.77	25.22	24.78	24.8	21.57
SD Out-degree centrality	8.21	8.1	8.65	8.49	9.84	9.72	8.75
Median Degree centrality	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Median In-degree centrality	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Median Out-degree centrality	1.00	1.00	1.00	1.00	1.00	1.00	1.00
IQR Degree centrality	3.00	3.00	3.00	3.00	3.00	3.00	3.00
IQR In-degree centrality	2.00	2.00	2.00	2.00	2.00	2.00	2.00
IQR Out-degree centrality	1.00	1.00	2.00	2.00	1.00	2.00	1.00
Mean Closeness	0.00	0.06	0.06	0.05	0.06	0.06	0.07
Mean In-degree closeness	0.01	0.14	0.13	0.13	0.13	0.13	0.14
Mean Out-degree closeness	0.12	0.001	0.09	0.09	0.09	0.09	0.11
Mean Betweenness	2,999,391.30	306,080.50	361,907.40	314,200.60	344,359.10	359,344.70	261,319.70
Number of Bridges	81,564	72,228	80,894	76,327	78,367	76,811	70,744
Average path length	9.59	9.2	9.1	8.81	8.97	9.77	9.45
Cluster coefficient	0.006	0.006	0.007	0.01	0.009	0.009	0.008
Diameter	38.00	29.00	34.00	29.00	32.00	37.00	36.00
Reciprocity	0.19	0.18	0.17	0.15	0.17	0.2	0.19

Edges density	$1.21*10^{-05}$	$1.26*10^{-05}$	$1.25*10^{-05}$	$1.35*10^{-05}$	$1.31*10^{-05}$	$1.31*10^{-05}$	$1.37*10^{-05}$
Strong component (giant component)	130,506	130,573	133,977	127,830	127,732	124,565	112,221
Weak connected component	164,483	169,952	181,512	171,021	173,112	172,106	144,378
Diameter Strong component	314	682	840	835	756	1,415	521
Average path length Strong component	9.59	9.2	9.1	8.81	8.97	9.77	9.45
Cluster Coefficient Strong Component	0.006	0.006	0.007	0.009	0.009	0.009	0.008
Minimum cut	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Number of Communities	10,484	8,635	9,068	7,439	8,254	9,551	8,277

SD: Standard Deviation. IQR: Interquartile Range.

The results of degree centrality demonstrated few properties with a high amount of connectivity in all the analyzed years (Figure 2A). Complementarily, the closeness distribution demonstrated many nodes with low values of this centrality and some nodes achieving the value of 1 (maximum value) in all years (Figure 2B). Additionally, considering only data from 2017 to 2022, the mean betweenness was high from January 2017 to August 2023, with the greater value in 2017 (2,999,391.3) and lower value in the next year (2018 – 306,080.5), with the majority of nodes with lower values compared to the mean (Figure 2C).

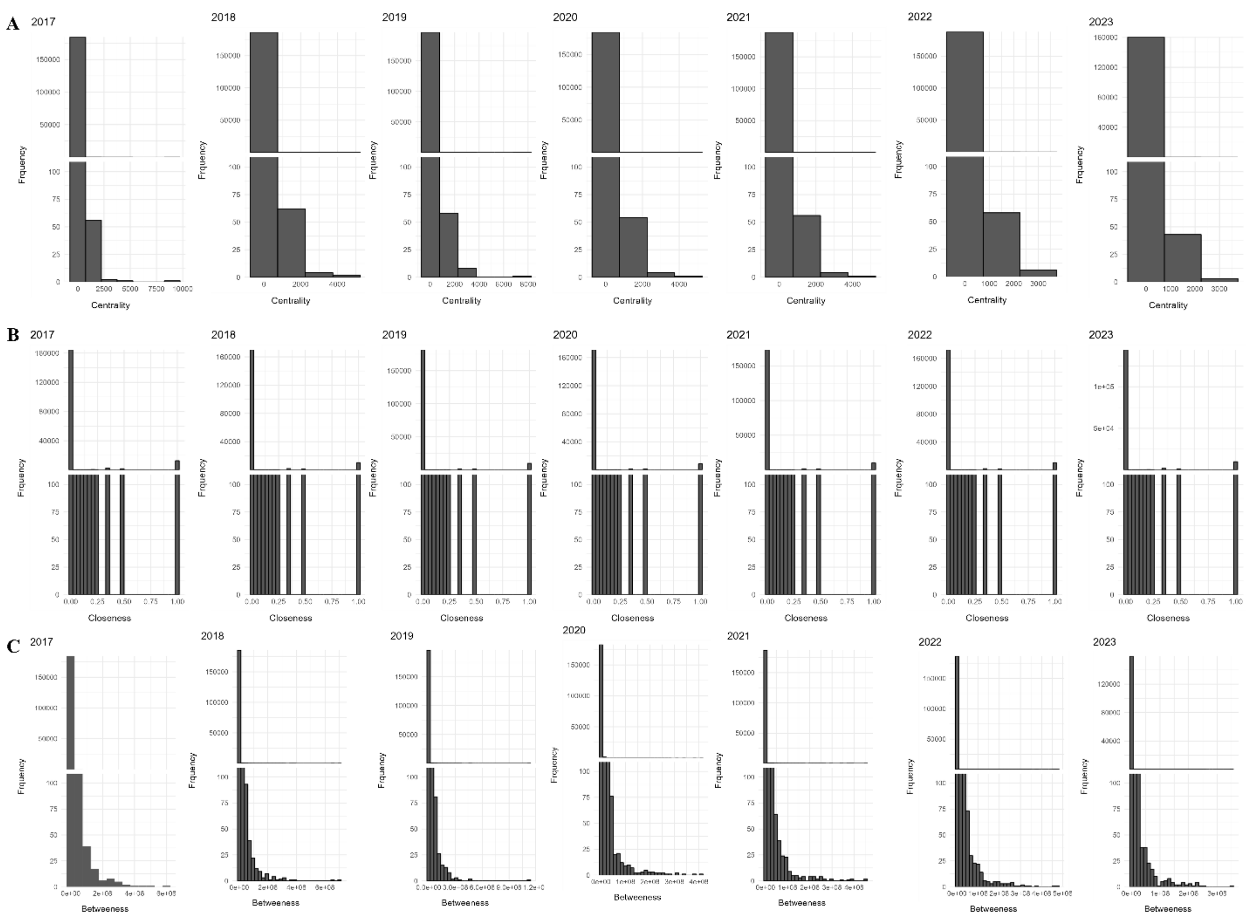

Figure 2: (A) Degree Centrality distribution, (B) Closeness distribution, and (C) Betweenness of cattle movement network, with livestock properties as nodes, in Minas Gerais, Brazil, from January 2017 to August 2023.

The average path length was high in all years, contrary to what was observed for the cluster coefficient (Table 1). The strong component (giant component) represented around 70% of the nodes in all years, and the weak connected component varied from 89 to 92% of the nodes in the analyzed years (2017 to 2023) (Table 1). More measures describing the network of properties in Minas Gerais, Brazil, from January 2017 to August 2023 are available in Table 1. The results also revealed that data on cattle movement of properties in Minas Gerais, January 2017 to August 2023, followed a power law distribution, with all p-values above 0.05 (Table 2).

Table 2: Results of the Kolmogorov-Smirnov test for the fitting into a power-law distribution of the cattle movement data, among livestock properties of from January 2017 to August 2023, in Minas Gerais state, Brazil.

Year	Alpha	Log Likelihood	Kolmogorov-Smirnov	p-value
2017	2.43	-69,934.63	0.006	0.41
2018	2.46	-57,439.8	0.007	0.3
2019	2.43	-66,499.16	0.008	0.21
2020	2.41	-57,522.22	0.005	0.71
2021	2.44	-57,449.82	0.005	0.81
2022	2.47	-63,067.61	0.008	0.16
2023	2.46	-71,325.87	0.007	0.19

Discussion

Network analysis is a very useful tool for the comprehension of complex systems (Luke and Harris, 2007), such as cattle movements, since identifies nodes of outmost importance to implementation of strategic measures of disease control (Cardenas et al., 2021a), improving livestock production and thus assuring food safety (Huntington et al., 2021). In this context, the aim of this study was to characterize the network of cattle movements, using livestock properties as nodes, in Minas Gerais, from January 2017 to August 2023, to advance disease control measures. Overall, the results pointed to a scale-free network with the presence of some livestock properties that highly influenced the network, which should be the focus of interventions for disease control.

Indeed, the static network analysis, with livestock properties as nodes, classified the graph as a scale free network in all years. This classification is sustained by the degree centrality distribution fitting a power law, which indicates preferential attachment, and by the different number of nodes throughout the years, which suggest an open graph (Barabási and Albert, 1999). Complementarily to the degree centrality, the closeness and the betweenness centralities (Figure 2 A, B, C) also indicates the preferential attachment of some livestock properties, by demonstrating the presence of few properties with enormous number of connections and the majority with less connections. In contrast, the network performed with cattle movement taking the municipalities as nodes did not reveal a real scale-free network, since the number of municipalities did not change throughout the years, which represented a less complex graph compared to the one analyzed in this paper. The high number of livestock properties in Minas Gerais allow the network to change over time, with nodes entering or living the graph, which add to the complexity of the network (constant change of number of nodes) and its use to orientate disease control measures. The scale free network classification is crucial for the strategic use of resources to control diseases, allowing the application of tailored measures to the livestock properties that greatly influence the network of cattle movement in Minas Gerais.

Regarding the number of movements in each year, 2019 was the year with more movements between livestock properties and 2020 was the year with less movements. This change between these two years could be explained by the SARS-Cov-19 pandemic (Rahman et al., 2022), which decreased the number of livestock events and therefore decreasing the number of movements in 2020. The Covid-19 pandemic negatively influenced the livestock production worldwide (Rahman et al., 2022), and in Minas Gerais was not different, except for the fact that in the state the reason for transportation with more prejudice was livestock events, that stopped occurring during the pandemic to avoid human agglomeration.

The limitations of this paper included the absence of georeferenced information of the properties, preventing better visualization of results in maps, which could show the places with higher degree centrality. However, it is important to state that the unique code of each node allows the identification of these properties for targeted control measures. The years of 2013 to 2016 was absent of this paper due to some missing unique identification data, which compromised the network analysis by livestock properties in these years. Although, the present years used in this paper (2017 to 2023), were sufficient to identify that there were properties important in the network. Finally, another limitation was the static network analysis conducted, since the changes in the network due to the passage of time were not measured, which impairs the use of the present network for modeling the spread of diseases with high attack rate. Nevertheless, this network from cattle movement in Minas Gerais state, can be particularly useful for diseases of low spread and the overall comprehension of network and its characteristics.

Conclusion

Our paper described the network of cattle movement, with properties as nodes, in Minas Gerais, Brazil, from January 2017 to August 2023, finding a complex and open graph with preferential attachment, which suggest a scale-free network in all analyzed years. This classification revealed the presence of hubs (livestock properties) where specific measures should be used to improve disease control.

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