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Spatial and temporal analyses of culls due to bovine tuberculosis in slaughterhouses of Minas Gerais state, Brazil, 2008 to 2012[1]

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ABSTRACT.- Baptista T.F.S., Alves M.C., Pereira-Dourado S.M., Costa G.M., Lopes E., Bruhn F.R.P., Braz M.S. & Rocha C.M.B.M. 2021. **Spatial and temporal analyses of culls due to bovine tuberculosis in slaughterhouses of Minas Gerais state, Brazil, 2008 to 2012.** *Pesquisa Veterinária Brasileira 41:e06933, 2021.* Laboratório de Epidemiologia, Faculdade de Zootecnia e Medicina Veterinária, Departamento de Medicina Veterinária, Universidade Federal de Lavras, Cx. Postal 3037, Lavras, MG 37200-900, Brazil. E-mail: rochac@ufla.br

The application of spatial and temporal analysis in epidemiology aims to understand the causes and consequences of spatial and temporal heterogeneity in studies of infectious diseases. Bovine tuberculosis (bTB) is a chronic and progressive infectious disease caused by *Mycobacterium bovis* and is an important zoonosis worldwide. The aim of this study was to conduct spatial and temporal analyses of a secondary database of bTB-positive cases registered by the Federal Inspection Service in two slaughterhouses from the West of Minas mesoregion of Minas Gerais state, Brazil, from 2008 to 2012 and to suggest its use in epidemiological surveillance. The culled cattle with bTB macroscopic lesions during *post mortem* inspection were considered positive in this study. The data used were the positive cases registered on the "Sistema de Informações Gerenciais do Serviço de Inspeção Federal" (SIG/SIF - Management Information System of the Federal Inspection Service) of the "Ministério da Agricultura, Pecuária e Abastecimento" (MAPA - Ministry of Agriculture, Livestock and Supplies), the number of animals slaughtered monthly and their municipalities of origin, per slaughterhouse. The prevalence (%) of cases per cattle slaughtered was calculated, and the relationship between these cases of bTB and cases of human tuberculosis (hTB) and the GDP *per capita* of the mesoregions comprising the municipalities surveyed was evaluated. The prevalence was 1,030 cases of bTB (0.28%). The lowest case numbers and prevalence rates were obtained in 2010, while the highest rates were observed in 2011. Slaughterhouse 1 showed stationarity, while Slaughterhouse 2 showed an increasing annual trend for data since October 2009. A statistical correlation between the numbers of cases of bovine and human TB (p=0.006, r=0.148) was observed. The prevalence for Slaughterhouses 1 and 2 were not correlated (p>0.05). The municipalities of origin demonstrated randomness in their spatial distribution. SIG/SIF-MAPA may be used for the analysis of spatial and temporal distribution to contribute to the monitoring of animal health services with information on

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the prevalence of culling for bTB in the West of Minas, South/Southwest of Minas, Campo das Vertentes and Zona da Mata mesoregions of Minas Gerais, Brazil.

INDEX TERMS: Spatial analysis, temporal analysis, bovine tuberculosis, monitoring, slaughterhouses, Brazil.

RESUMO.- [**Análise espacial e temporal das condenações por tuberculose bovina em frigoríficos do estado de Minas Gerais, Brasil, 2008 a 2012.**] A aplicação de análises espacial e temporal em epidemiologia visa compreender as causas e consequências da heterogeneidade espacial e temporal nos estudos de doenças infecciosas. A tuberculose bovina (TBb) é uma doença infecciosa crônica e progressiva causada pelo *Mycobacterium bovis* e uma importante zoonose em todo o mundo. O objetivo deste estudo foi realizar análises espaciais e temporais em um banco de dados secundário de casos positivos de TBb registrados pelo Serviço de Inspeção Federal em dois frigoríficos da mesorregião Oeste de Minas, estado de Minas Gerais, Brasil, de 2008 a 2012 e sugerir sua utilização na vigilância epidemiológica. Os bovinos abatidos com lesões macroscópicas de TBb durante a inspeção *post mortem* foram considerados positivos neste estudo. Os dados utilizados foram os casos positivos cadastrados no Sistema de Informações Gerenciais do Serviço de Inspeção Federal do Ministério da Agricultura, Pecuária e Abastecimento (SIGSIF/MAPA), o número de animais abatidos mensalmente e seus municípios de origem, por frigorífico. Foi calculada a prevalência (%) de casos por bovinos abatidos e avaliada a relação entre esses casos de TBb e os casos de tuberculose humana (TBh) e o PIB *per capita* das mesorregiões que compõem os municípios pesquisados. A prevalência foi de 1.030 casos de TBb (0,28%). Os menores números de casos e prevalências foram obtidos em 2010, enquanto as maiores prevalências foram observadas em 2011. O Frigorífico 1 apresentou estacionariedade, enquanto o Frigorífico 2 apresentou tendência anual crescente para os dados desde outubro de 2009. Correlação estatística entre o número de casos de TBb e TBh (p=0,006, r=0,148) foi observada. As prevalências nos Frigoríficos 1 e 2 não se correlacionaram (p>0,05). Os municípios de origem demonstraram aleatoriedade em sua distribuição espacial. O SIGSIF/MAPA pode ser utilizado para a análise da distribuição espacial e temporal a fim de contribuir com o monitoramento dos serviços de saúde animal com informações sobre a prevalência de condenações por TBb nas mesorregiões Oeste de Minas, Sul/Sudoeste de Minas, Campo das Vertentes e Zona da Mata de Minas Gerais, Brasil.

TERMOS DE INDEXAÇÃO: Análise espacial, análise temporal, tuberculose bovina, monitoramento, frigoríficos, Brasil.

INTRODUCTION

Bovine tuberculosis (bTB) is a chronic and progressive infectious disease caused by the bacillus *Mycobacterium bovis* and is considered an important zoonosis worldwide. This disease is characterized by the formation of typical granulomatous lesions with various degrees of necrosis, calcification and delimitation by connective tissue (Etter et al. 2006, Bayraktar et al. 2011, Domingo et al. 2014). According to the "Ministério da Agricultura, Pecuária e Abastecimento" (MAPA - Ministry of Agriculture, Livestock and Supply), from 1989 to 1998, the national average prevalence of bTB in the Brazil was 1.3% (Brasil 2006). On the last survey conducted in 2013 in the state of Minas Gerais (MG), and the prevalence for herds was estimated at 4.25% (Barbieri et al. 2016).

Spatial and temporal analyses techniques have been most recently applied in tuberculosis studies in humans in Brazil, China, and Taiwan (Rodrigues-Jr et al. 2006, Ng et al. 2012, Wang et al. 2012). However, in animal health, such spatial and temporal statistical tools were applied for the first time in this paper through the analysis of secondary data from slaughterhouses in the West of MG, Brazil. There have been several studies performed in the animal health literature to verify the distribution of bTB in different regions of the world, such as those conducted by Mwakapuja et al. (2013) in Tanzania, using the tuberculin test as a diagnostic method, and Kelly & More (2011) in the UK and Ireland.

The aim of this study was to conduct spatial and temporal analyses of the secondary database of positive cases of cattle with bTB lesions recorded by the "Serviço de Inspeção Federal" (SIF - Federal Inspection Service) in slaughterhouses. The catchment area this study is the West of Minas, South/Southwest of Minas, Campo das Vertentes and Zona da Mata mesoregions of MG from 2008 to 2012. The results obtained may subsidize efforts to control the disease by the animal health protection service. Thus, these results may contribute to the planning of services, monitoring and decision-making in the control actions of the "Programa Nacional de Controle e Erradicação de Brucelose e Tuberculose Animal" (PNCEBT - National Program on the Control and Eradication of Animal Brucellosis and Tuberculosis) (Brasil 2006, 2017), and may serve as a model for broader-scope analysis. Therefore, due to the importance of bTB for national livestock production and public health, the study suggests the use of this methodology in epidemiological surveillance in animal health care.

MATERIALS AND METHODS

This ecological study analyzed the number of cases of bTB diagnosed during *post mortem* inspection in slaughterhouses inspected by the "Serviço de Inspeção Federal" (SIF - Federal Inspection Service). Month and municipality of origin were used to characterize the cases. Data from the two slaughterhouses located in Campo Belo (20°53'50" S and 45°16'38" W), West of Minas mesoregion, Minas Gerais, Brazil (Fig.1) were collected. The slaughterhouses were referenced as Slaughterhouses 1 and 2 and performed the slaughter of cattle from four mesoregions of Minas Gerais, Brazil: West of Minas, South/Southwest of Minas, Campo das Vertentes and Zona da Mata (Fig.1). These slaughterhouses represent 100% of the slaughterhouses from the West of Minas and 67% of the South/Southwest of Minas inspected by SIF.

The bTB-positive cases were defined by *post mortem* examination performed according to the SIF's routine, characterized by the identification of macroscopic lesions suggestive of bTB. Thus, the number of culls for bTB in the slaughterhouses studied was considered positive. Bacteriological and serological tests are not

performed according to the routine of *post mortem* examinations of the SIF. All cases that were identified and culled by the inspection service were considered in this study. The animals' municipalities of origin were determined through the "Guia de Trânsito Animal" (Animal Transport Paperwork). The attributes used (socioeconomic information regarding the municipalities of origin), such as the annual cattle yield and the gross domestic product (GDP) *per capita* (2010), were obtained from the "Instituto Brasileiro de Geografia e Estatística" (IBGE - Brazilian Institute of Geography and Statistics) (IBGE 2020). The number of hTB cases was determined by the rate of disease incidence obtained from the DATASUS (Brasil 2014), by municipality, from 2008 to 2012. Specific prevalence rates (no. of cases/cattle slaughtered x 100) were calculated per slaughterhouse, month, year, and municipality. All the statistical analyses were performed using the software Statistical Package for the Social Sciences (SPSS) 20.0. A database created and structured in Excel 2013 was used to evaluate the occurrence of stationarity, trend, or seasonality. The temporal series was analyzed using Gretl1.9.12 software (GNU Regression, Econometric and Time-series Library of 2013). The maps were constructed, and spatial analyses were performed using the commercial geographic information system ArcGis10.2 (ESRI Inc., Redlands/CA, USA).

For the preparation of thematic maps, the municipalities were geocoded with the aid of information from the latitude-longitude projection system and the city codes, which were obtained from IBGE records. Annual maps were constructed to verify the distribution patterns of the municipalities where the cases originated. The criterion for defining classes of cases was the method of quantile in five classes. The High/Low Clustering (Getis-ord General G) method was used to test the hypothesis of distribution patterns present in the spatial data. This method uses the inverse of the distance (Euclidean) as a concept of spatial relationship. The threshold of distance applied was set at 10^9. This threshold was also tested using Moran's method.

RESULTS

A total of 134 municipalities of origin were identified for animals culled due to bTB in Slaughterhouses 1 and 2. Among the 1,030 cases identified, n=531 (51.55%) were in the South/Southwest of Minas, distributed in 79 municipalities (58.96%). The West of Minas mesoregion had the second highest number of positive cases (n=240, 23.30%), distributed in 20 municipalities (14.93%). The Campo das Vertentes mesoregion had n=166 positive cases (16.12%) distributed in 19 municipalities (14.18%). The Zona da Mata mesoregion had n=93 (9.03%) positive cases distributed in 16 municipalities (11.94%). In this study we found a prevalence rate of 0.28% cattle culls due to bTB identified in slaughterhouses inspected by the SIF. These cases were distributed in 134 municipalities of origin (Fig.2) and among 371,285 cattle slaughtered. The annual distribution of the number of animals slaughtered in the period analyzed revealed that there were no significant annual case trends (Fig.3). The results of the temporal series analysis, which included the prevalence rates, showed stationarity in the distribution of cases from Slaughterhouse 1 and a trend in Slaughterhouse 2 since 2009/2010 (Fig.4). The prevalence rates of bTB calculated for animals slaughtered were 0.34% at Slaughterhouse 1 and 0.20% at Slaughterhouse 2. The prevalence rates were higher in Slaughterhouse 1 for all years evaluated. The prevalence rates of Slaughterhouses 1 and 2 were not statistically correlated (p>0.05). The monthly distributions of the prevalence rates of bTB dropped steeply on both slaughterhouses in June (0.19% and 0.18%, respectively) (Fig.5). The only variables that were statistically correlated (p<0.05) when testing the relationships between the studied attributes were the number of cases of bTB and hTB (p=0.006, r=0.148). No statistically significant difference (p>0.05) was observed between the mesoregions regarding the occurrence of bTB and hTB.

DISCUSSION

It is already clear in the literature that the prevalence of bTB calculated in slaughterhouses is underestimated compared to its prevalence in the general population, given that bTB

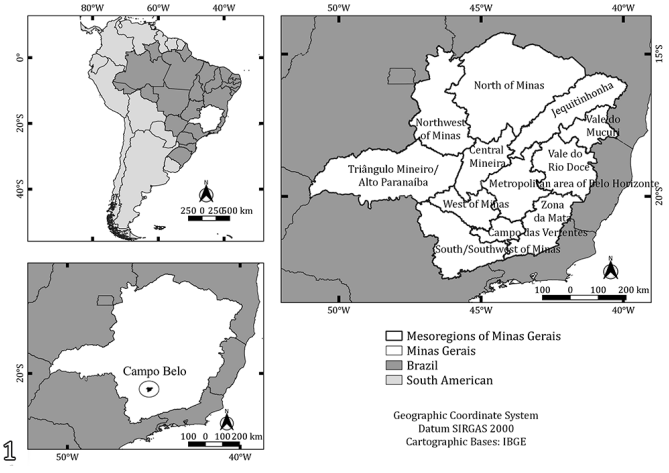


Fig.1. Campo Belo and Mesoregions of Minas Gerais state, Brazil.

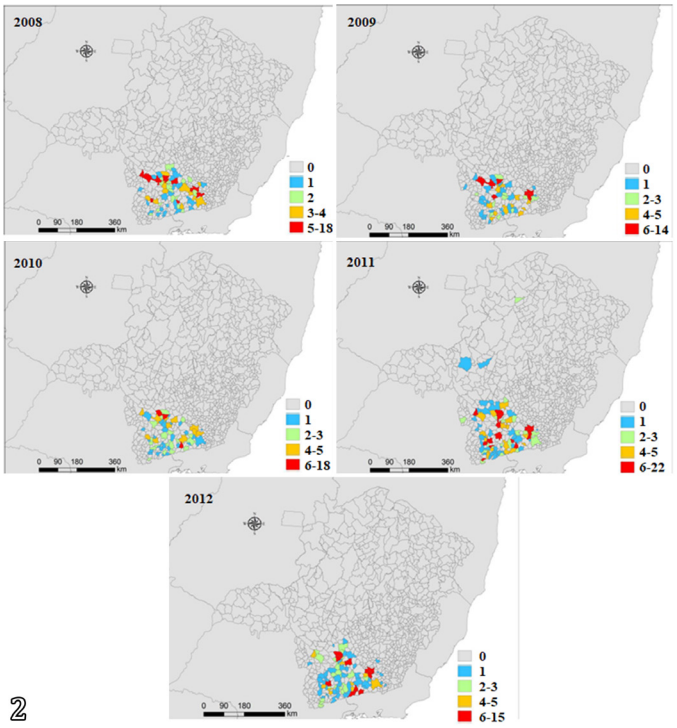


Fig.2. Distribution of cattle culls due to bovine tuberculosis in slaughterhouses according to the Federal Inspection Service from the West of Minas mesoregion of Minas Gerais, Brazil, 2008-2012.

cases without macroscopic lesions, which are not detectable during a *post mortem* examination, should be added to this prevalence to come close to the reality (Corner 1994, Baptista et al. 2004, Teklul et al. 2004). However, this form of diagnosis may be considered as an indicator to provide information regarding the presence of herds infected by the disease, as the animals discarded and sent to slaughterhouses do not constitute a random sample (Asseged et al. 2004, Furlanetto et al. 2012). Therefore, the information from this study is applicable to the analysis of cattle populations for slaughter.

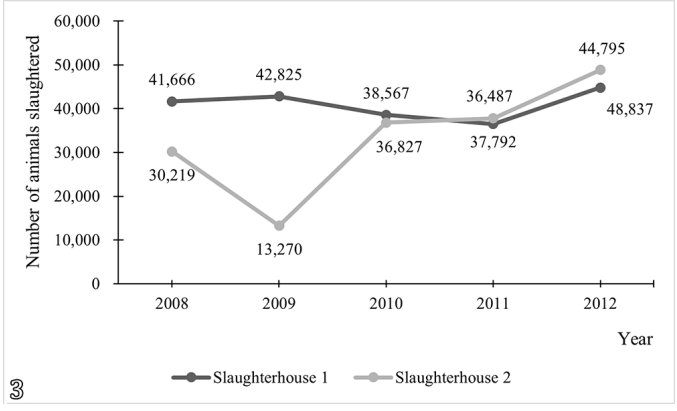
The prevalence identified in this study was like that presented by PNCEBT (Brasil 2006) and lower than that found by Barbieri et al. (2016), although these studies used another diagnostic method (the tuberculin test). Oliveira et al. (1986) and Baptista et al. (2004), who conducted similar studies in slaughterhouses, reported similar prevalence rates in the state of MG. This similarity demonstrates that the use of the inspection by the SIF as an indicator of prevalence in certain regions covered by the slaughterhouses can evolve as an auxiliary tool for epidemiological surveillance.

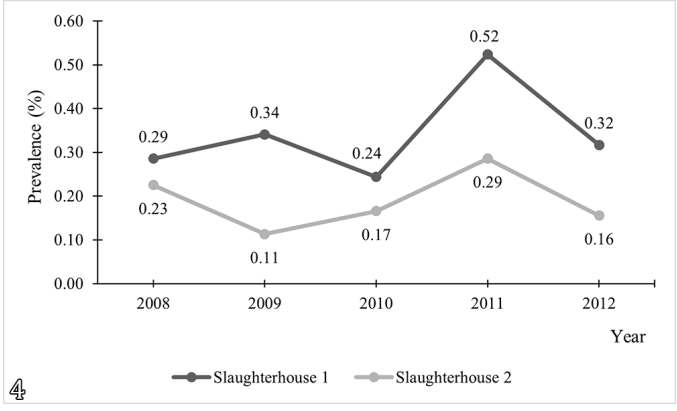
The sudden June drop in prevalence in terms of the monthly distribution of bTB cases may be correlated to the onset of drought and, hence, reduced pasture availability. In Brazil, this phenomenon is known as the "safra do boi" ("cattle harvest"). At this time of year, the slaughterhouses have an increased availability of animals raised on pasture or that were confined and raised with the specific purpose of being killed (Paciulo et al. 2008). Because bTB has a higher prevalence in dairy herds, the slaughter of specialized animals should decrease the bTB incidence in slaughtered cattle (Fig.5).

This increased risk of bTB in dairy herds compared to beef herds is related to the type of farming, such as density, stress, breeds created and other factors (Ameniet al. 2007, Firdessa et al. 2012). These findings corroborate with Vordermeier et al. (2012), who reported a lower susceptibility of Zebu cattle to bTB infection when compared to specialized breeds for dairy production, such as Holstein-Friesian.

Faced with the facts cited, regarding the data used in this study, it should also be considered that the South/Southwest of Minas is a region with a significant proportion of milk production systems. According to the Agricultural Census for the year 2006, out of the total number of farms in the state of MG, 39.87% were dedicated to milk production (IBGE 2006), with annual production estimated at 8,905,984 liters in 2012 (IBGE 2020). Therefore, many of the animals sent to slaughter in these slaughterhouses are the result of culling from milk production systems. Thus, it is interesting to conduct systematic studies with representative samples of herds to provide estimates of more realistic frequencies for the region so that the relationship with the data from the slaughterhouse can be studied. In this sense, the slaughterhouse can best contribute to the function of monitoring and tracking the actions of the bTB control and eradication programs, serving as markers with known validity.

The spatial distribution indicated that the West of Minas and South/Southeast of Minas had 20 and 79 municipalities with animals killed at slaughterhouses, respectively representing 14.93% and 58.96% of all municipalities of origin for bTB cases. Together, these regions are the source of 73.88% of cases of cattle with bTB lesions. This result may be related to the proximity of these counties to the slaughterhouses





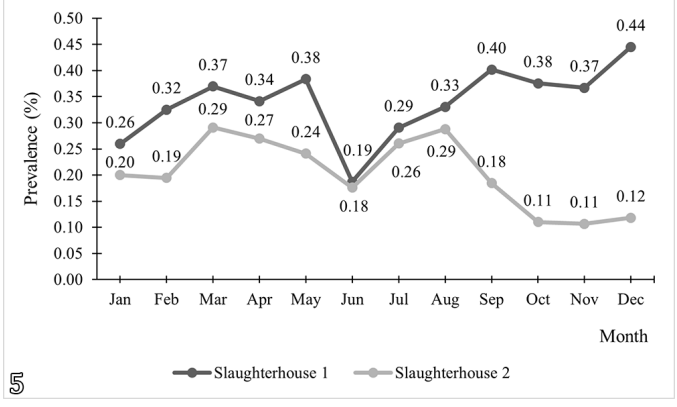


Fig.3. Annual distribution of the numbers of animals slaughtered in slaughterhouses according to the Federal Inspection Service from the West of Minas mesoregion of Minas Gerais, Brazil, 2008-2012.

Fig.4. Annual distribution of prevalence (%) of cattle with bovine tuberculosis lesions in slaughterhouses according to the Federal Inspection Service from the West of Minas mesoregion of Minas Gerais, Brazil, 2008-2012.

Fig.5. Monthly distribution of prevalence (%) of cattle with bovine tuberculosis lesions in slaughterhouses according to the Federal Inspection Service from the West of Minas mesoregion of Minas Gerais, Brazil, 2008-2012.

belonging to the municipality of Campo Belo (West of Minas). Furthermore, the higher frequency of bTB in the South/Southwest of Minas may be due to the predominant activity of dairy cattle; animals from this industry are disposed of in these slaughterhouses (Oliveira et al. 1986, Baptista et al. 2004).

The Campo das Vertentes and Zona da Mata mesoregions showed considerable frequencies of bTB cases, likely for the same reasons (Fig.2). The evaluation of the average cattle yield between the mesoregions contributes to relate the cases of bTB with the profile of the herds in the region. This relationship is due to the smaller sizes and the specialization of the herds in dairy production, with greater lengths of stay for animals in the herd; additionally, because bTB is a chronic disease, macroscopic lesions can occur (Ameni et al. 2007). These authors evaluated the prevalence of bTB in Ethiopia and found the highest prevalence of bTB in Holstein animals; this breed also experienced more severe injuries. In addition, these authors reported that animals between five and nine years of age have a higher risk of developing bTB than young animals (up to two years).

The evaluation revealed that the spatial distribution of bTB in the South/Southwest of Minas was random, except in 2008 (Fig.2). According to the maps, the distribution of the municipalities of origin was well dispersed; additionally, most municipalities exhibited only one case. Few municipalities had more than five cases. These findings confirm that bTB is an endemic disease that is fairly dispersed in the South/Southwest of MG, with a low prevalence of cases (Belchior et al. 2001). The fact that there are isolated cases per municipality also indicates that these cases may represent culled animals from dairy farms with more severe chronic infections. These data demonstrate the need to perform further studies with analysis per points, considering the farms of origin (Martínez et al. 2007, Carpenter 2011). The prevalence of cattle culling due to bTB verified our study is lower than the values of animal prevalence found by Barbieri et al. (2016) observed for the region South and Southwest, Zona da Mata region and Central region in the state of Minas Gerais. Our results are useful to help the official veterinary service, as the verified cases suggestive of bTB may be underestimated.

In the period studied, 24 municipalities with high bTB prevalence rates were identified, considering five cases or more per municipality per year (Fig.2). This information can contribute to the monitoring of the epidemiological situation of bTB. The identification of municipalities with cases, preferably from farms, and particularly those with larger numbers of cases, may aid the state health protection services by influencing control actions and verifying the consistency of the epidemiological surveillance records.

Municipalities with the highest case frequencies may require health education measures, including the implementation of tuberculin testing and the slaughter of reactive animals, along with intensified monitoring and mentoring of animal buying to conduct prior tests and quarantine and to provide guidance and to monitor the health of rural workers. In addition, monitoring actions on domestic, wild and synanthropic reservoirs in these municipalities may be directed. Because these regions have milk production systems, the periodic cleaning and disinfection of facilities and equipment should be considered (Brasil 2006, Collins 2006, Allepuz et al. 2011, Skuce et al. 2012). Thus, this method already represents an indirect form of surveillance of the monitored herds. Therefore, it would be interesting to perform an analysis by property (i.e., spatial analysis by points), which would allow a more thorough risk analysis.

Some authors cite positive correlations for cases of hTB and bTB, referring to developing countries (Michel et al. 2010). In their work with countries in Latin America, Kantor et al. (2008) related the low percentage of *Mycobacterium bovis* infection in humans with reduced access to molecular diagnostic methods, contributing to the underestimation of these events. Although these authors confirmed the existence of these infections, they reported low frequencies. Thus, they recommend sanitary control, milk pasteurization and meat inspection in slaughterhouses as measures to contribute to human health. Studies with larger coverage areas should seek to use data from the SIF to contribute to the monitoring and evaluation of epidemiological surveillance data for bTB with greater validation of the epidemiological tools used. However, these spatial and temporal analyses are a way to accomplish these objectives.

According to the observations, this work provides initial evaluation steps that may be useful to animal health protection services and the SIF. New perspectives for analysis should be developed, incorporating information such as gender, age and others; improvements in *post mortem* diagnosis, with confirmatory testing; and verification of ways to obtain notifications of partial carcass condemnations, which were not considered in the reports generated by the SIG. A larger-scope spatial and temporal study would also be interesting. Therefore, the use of complementary methods in diagnosis (i.e., molecular methods such as PCR) in the routine of veterinary inspection services is suggested to perform monitoring with more consistent data (Etter et al. 2006, Rodrigues & Vadwai 2012, Seagar et al. 2012), as is conducted in the state of Mato Grosso (MT). To expand bTB surveillance to animals supplied to the industry, the state of MT signed Law no. 10,149 on July 11th, 2014, creating the Surveillance Plan for the eradication of bTB in the state of MT (Mato Grosso 2014). In this law, a single case will make the affected property a disease focus, and compulsory confirmatory tests must be performed; additional mandatory sanitation actions must also be implemented under the supervision of the state defense, INDEA/MT. Moreover, slaughterhouses subjected to municipal and state inspections will be required to perform the collection and analysis of material indicative of bTB.

CONCLUSIONS

The prevalence of cattle culling due to bovine tuberculosis (bTB) in slaughterhouses with the SIF from the West of Minas, South/Southwest of Minas, Campo das Vertentes and Zona da Mata mesoregions of Minas Gerais (MG) was 0.28%.

Temporal series analysis suggested seasonality, with decreased of detection of macroscopic lesions suggestive of bTB in June.

Spatial analysis revealed a random distribution over the years.

This study demonstrated that the SIG/SIF-MAPA database for the analysis of spatial and temporal distribution can be used to contribute to the monitoring of animal health services with information on bTB prevalence in the regions of MG.

There is need for improved data collected from slaughterhouses to contribute more effectively to animal health protection in Brazil.

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Conflict of interest statement.- The authors declare that there is no conflict of interests regarding the publication of this paper. We are responsible for the content and writing of the paper.

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