Epidemiological analysis of bovine tuberculosis in the southern region of Rio Grande do Sul from 2000 to 2015

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Bovine tuberculosis (BTB) is an infectious and zoonotic disease with socioeconomic importance, responsible for economic losses in livestock due to the decrease in animal productivity, mortality, bovine carcass condemnation and restriction on exportations. The objective of the present study was to evaluate the epidemiological aspects of this disease through a descriptive analysis and time series of cases of bovine tuberculosis diagnosed at the Laboratório Regional de Diagnóstico (LRD) of the Faculdade de Medicina Veterinária, Universidade Federal de Pelotas (UFPel) from 2000 to 2015. A survey was carried out in the LRD database of cases diagnosed as tuberculosis in cattle during this period. The incidence rate of BTB ([cases/population] x 100000) was calculated. Qualitative variables (sex, age, race, origin, type of material and presumptive diagnosis) were analyzed in a descriptive way, aiming to establish the profile of affected animals, characterize the municipalities in which the disease is originated and also fix the main referral organs for diagnosis. The time series analysis was performed using the Gretl statistical software 1.9.12 (GNU Regression, Econometric and Time-series Library). To verify the existence of the stationary distribution, the test of Dickey-Fuller was used, considering a value of p<0.05. During this period, 331 cases of BTB were diagnosed in the LRD. The present study identified that the predominant profile of the bovines affected by tuberculosis was female bovine aged more than two years old and crossbreed; the main organ sent for histopathological examination was lymph nodes. The microregion of Pelotas presented the highest incidence of the disease. There was also a tendency of a decrease in the incidence rate of this disease over the years, absence of seasonal influence in the occurrence of the disease and no cases of epidemics occurred from 2002 to 2015 in the area covered by the LRD-UFPel.

INDEX TERMS: Epidemiology, bovine tuberculosis, Rio Grande do Sul, Mycobacterium spp., cattle, tuberculosis, post mortem inspection, histopathological examination.
Bovine tuberculosis (BTB) is an infectious and zoonotic disease caused by Mycobacterium bovis, a highly pathogenic bacterium for cattle and humans (López 2013). The disease is characterized by the progressive development of nodular lesions, called "tubers", in any organ or tissue (Riet-Correa & García 2007). In livestock, this disease is responsible for significant economic losses due to the decrease in animal productivity and owing to interfere in the trade in animals and related products (Perez et al. 2002, Rocha et al. 2012).

BTB is a common disease that poses a public health risk, mainly in developing countries (Humblet et al. 2009). It is estimated that 5% to 10% of human tuberculosis cases are caused by M. bovis (Prasad et al. 2005, Schiller et al. 2011, Rocha et al. 2012). Consumption of raw milk and dairy products, produced without thermal processing, as well as direct contact with infected animals, constitute the primary forms of transmission of this bacterium to humans. The inspection service represents an essential tool for the control of this zoonosis (Pinto 2003).

The distribution of BTB across the national territory varies from the different production systems to the sanitary and technological practices employed in each region (Kantor & Ritacco 2006, Furlanetto et al. 2012). The prevalence of the disease in Brazil is higher in dairy farms, seeing that latest technologies are applied (Acha & Szyfres 2003, Ferreira Neto et al. 2016). The state of Espírito Santo and the northern regions of São Paulo, southern Minas Gerais, and southern Goiás have the highest BTB prevalence rates in the country, as they concentrate the largest dairy farming (Ferreira Neto et al. 2016). The prevalence of M. bovis infecting cattle herds in the state of Rio Grande do Sul (RS) is 2.8%. The highest rates in this state are also observed in the main milk producing regions (metropolitan area 5.2%, north region 3.9%, and mountain range 3.3%) (Queiroz et al. 2016). Due to the great socioeconomic impact of this disease, the objective of the present study was to carry out an epidemiological analysis of BTB cases, as well as evaluating the temporal distribution of this disease diagnosed at LRD of the Faculdade de Veterinária (FV-UFPel) from 2000 to 2015.

**MATERIALS AND METHODS**

A survey was carried out in the LRD-UFPel database regarding cases of BTB diagnosed from 2000 to 2015. Necropsy protocols and materials submitted for diagnosis were reviewed. Information regarding breed, sex, and age of animals, as well as presumptive diagnoses, type of material referred for diagnosis, the origin of animals, and month/year of disease occurrence were recorded in Excel spreadsheets and subsequently evaluated. The profile of the affected animals was established through a descriptive statistical analysis of the qualitative variables. In addition, the municipalities of disease occurrence were characterized, it was also checked the efficiency of post mortem examination in identifying macroscopic lesions and the main organs referred for histopathological examination. Incidence rates (cases/population x 100000) were calculated by municipality, month and year, after time series analysis from 2002 to 2015.

**Time series.** Data were analyzed by evaluating the distribution of BTB cases over time, in order to evaluate possible changes in the behavior of the disease over the years, especially about the incidence rate of tuberculosis and the sensitivity of the information system. LRD-UFPel. The temporal statistical analyses were done by using the Gretl 1.9.12 statistical software (GNU Regression, Econometricand Time-series Library), and an analysis of the existence of the trend and seasonality components was performed by verifying the series and their respective correlograms. Once verified the existence of these components, the series were differentiated and subsequently statistically tested for detecting analysis of stationarity, through increased Dickey-Fuller tests (Dickey & Fuller 1979), considering a value of p<0.05 (Morettin & Toloi 2006, Alberti et al. 2018). Once analysis of stationarity was observed in the differentiated series, the presence or absence of the trend and seasonality components in the monthly series considered was inferred. Ordinary Least Squares (OLS) model calculations were performed to estimate the statistically significant trend (p<0.05), and the coefficient of determination (R²) was calculated based on indicators time-series over time.

In addition, control diagrams were constructed based on the distribution of cases from 2002 to 2015, following the methodology of geometric mean, more appropriate when observing the asymmetric distribution of cases over the years, as described by Bortman (1999). The logarithmic transformation (log x) of the original data was performed. Regarding the transformed values, the geometric mean and 95% confidence intervals (95% CI) were calculated, which are used as the lower and epidemic endemic limits in the endemic curve (Bortman 1999).

**RESULTS**

From 2000 to 2015, 392 samples were sent to the LRD-UFPel with a presumptive diagnosis of tuberculosis, including 305 cases confirmed. In the same period, 23 samples were sent to the
Epidemiological analysis of bovine tuberculosis in the southern region of Rio Grande do Sul from 2000 to 2015

In RS, 57.48% of the cattle herd was classified as beef, and 43.11% as dairy animals, and this population consists mainly of female bovines over 36 months (38.95%) (Silva et al. 2014). The predominant profile of the bovines was adult females aged older than two years, crossbred, followed by dairy animals (Holstein and Jersey), and this result corroborated these findings with those observed by Grisi Filho et al. (2011). In a study conducted in the state of Mato do Grosso, the disease was also more frequent in animals older than two years (97.6%). However, lesions were more prevalent in male bovines (76.2%) than in female bovines.

To assess the time series of the incidence of BTB from 2002 to 2015, there was a downward trend in the number of cases of this disease (p<0.05) and absence of seasonality (p=0.05) (Fig.1A). The trend graph showed a higher number of cases from 2002 to 2006, with a sharp drop in the occurrence of this disease from 2003 to 2004 (Fig.1B). After comparing the monthly incidence rates obtained over the years concerning the epidemic limit observed in the endemic curve (Fig.2), it was possible to assess that there were no epidemics of BTB in the LRD region, indicating any incidence regarding the epidemic limit proposed by the curve.

DISCUSSION

In RS, 57.48% of the cattle herd was classified as beef, and 43.11% as dairy animals, and this population consists mainly of female bovines over 36 months (38.95%) (Silva et al. 2014). The predominant profile of the bovines was adult females aged older than two years, crossbred, followed by dairy animals (Holstein and Jersey), and this result corroborated these findings with those observed by Grisi Filho et al. (2011). In a study conducted in the state of Mato do Grosso, the disease was also more frequent in animals older than two years (97.6%). However, lesions were more prevalent in male bovines (76.2%) than in female bovines.

Fig.1 (A-B) Time series and equation of the trend line of the incidence rate of bovine tuberculosis (BTB) (cases/100000 cattle), in the area of “Laboratório Regional de Diagnóstico” (LRD) from the “Faculdade de Veterinária” (FV), “Universidade Federal de Pelotas” (UFPel), Rio Grande do Sul, from 2002 to 2015. (A) Original time series showing a downward trend in the number of cases diagnosed as tuberculosis in cattle in the LRD-FV-UFPel. (B) Differentiated time series (trend free), showing a higher incidence of tuberculosis from 2002 to 2006.

Fig.2. Endemic curve of the incidence rate of bovine tuberculosis (BTB) (cases/10000 cattle), in the area of “Laboratório Regional de Diagnóstico” (LRD) from the “Faculdade de Veterinária” (FV), “Universidade Federal de Pelotas” (UFPel) from 2002 to 2015.
the geographic location of the LRD-UFPel, which facilitates
the referral of samples for testing by veterinarians of this
region, and to the partnership with the municipal inspection
service of Pelotas-RS.

According to Ferreira Neto et al. (2016), there was a
significant increase (52%) in the number of BTB in 2015
when compared to 2014 in Brazil. Over the analyzed period,
there was a downward trend in the frequency of BTB cases
diagnosed in the LRD-UFPel. This downward trend in the
number of cases in the LRD-UFPel may be related to an
efficiency of the municipal inspection service in recognizing
the lesions found during the slaughter. In most cases, a macroscopic diagnosis is
made, reducing the total of samples sent to the laboratory for
histopathological examination. Another factor that may have
influenced this downward trend was the implementation of the
"Programa Nacional de Controle e Erradicação da Brucelose
e da Tuberculose Animal" (PNCEBT) in 2001, which provided
an improvement in the health status of the cattle.

In a study conducted in a slaughterhouse in Nigeria,
tuberculosis showed a cyclical behavior (seasonality), with
greater occurrence in July and August, differing from the result
found in the present study. The authors related this seasonality
to stressful factors that occur in this period (off-season) and
the increase in the number of religious slaughters (Okeke et al.
2016). The absence of seasonal influence on the occurrence
of tuberculosis, observed in the present study, can be
explained by the variables, such as: incubation period of the
microbiological agent and chronic character of the disease.
Thus, the transmission cycle of disease is certain to detect,
seeing that the disease is diagnosed, in most cases, during the
slaughter of the animals. The incidence rate of BTB, according
to diagnoses made on the base of LRD-UFPel, did not show
an epidemic behavior in this region; since the frequency of
the cases remained within the estimated safety zone for the
diagnosis same. In the evaluation of the endemic curve, it was
also observed that the epidemic limit determined for June
was higher and lower in July.

CONCLUSIONS

The prevalence of tuberculosis diagnosed in the LRD-UFPel
from 2000 to 2015 decreased significantly and the profile of
affected animals is female bovine, aged over 36 months of
age, and dairy cattle, especially crossbred.

Macroscopic identification of lesions during slaughter is
efficient since 77.8% of suspected post mortem examination
cases were histologically confirmed as tuberculosis.

During the study period, there were no seasonal and
epidemic cycles of the disease in the region.

Conflict of interest statement.- We have no conflict of interest to declare.

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