

PREVALENCE OF BOVINE BRUCELLOSIS AND TUBERCULOSIS IN DAIRY FARMING PROPERTIES IN FRANCISCO BELTRÃO, SOUTHWEST REGION OF PARANÁ

Adriano Ribeiro Machado

Prefeitura Municipal de Francisco Beltrão –
Agriculture Department
Francisco Beltrão, PR
<http://lattes.cnpq.br/1225417493020943>

Susana Regina de Mello Schlemper

Universidade Federal da Fronteira Sul
Realeza, PR
<http://lattes.cnpq.br/6515586356402204>

Wellinton Thiago Molinetti

Universidade Mater Dei
Pato Branco, PR
<http://lattes.cnpq.br/4477673700426898>

Tiago Machado

Clivet Clínica Veterinária
Francisco Beltrão, PR
<http://lattes.cnpq.br/7836450433065033>

Valfredo Schlemper

Universidade Federal da Fronteira Sul
Realeza, PR
<http://lattes.cnpq.br/2447428113787389>

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Abstract: Bovine brucellosis and tuberculosis are zoonoses, with worldwide distribution, which cause great socioeconomic damage. In this context, the objective of the study was to describe the current situation of these diseases in dairy herds in the Municipality of Francisco Beltrão, Southwest Region of the State of Paraná. The study carried out was cross-sectional, descriptive, retrospective and quantitative. Sampling covered a period of eight years, considering all tests that were positive for bovine brucellosis and tuberculosis, from 2014 to 2021. Data were collected from the database of the municipality's Rural Development Secretariat. The results were tabulated, organized in spreadsheets and presented in tables, where the prevalences were calculated. 93,727 animals were tested for bovine Brucellosis in 6,026 properties, with a prevalence of 0.11% of the animals tested and 10.84% in the properties, with 81% females and 19% males. Regarding tuberculosis, 131,570 females were examined, with a prevalence of 0.25% of the animals tested and 2.1% of the properties had some case. The study reveals that bovine brucellosis and bovine tuberculosis are present in the municipality of Francisco Beltrão, with a low prevalence compared to other studies and each year the number of infected animals has been decreasing, and in 2016 no positive animals were registered. For brucellosis, which may be the result of annual screening tests for both diseases, the sanitary slaughter of positive animals, vaccination of susceptible females and selection of breeding stock, which contributes to the disease not generating economic losses for producers in the region.

Keywords: Abortions. Granuloma. Hypersensitivity. single health.

INTRODUCTION

Bovine brucellosis is a cosmopolitan disease, widely distributed throughout Brazil. Because it is a zoonosis and causes socioeconomic problems, it has a particular focus among the diseases that affect production animals, in addition to causing harm to the health of the population. Infection in cattle is predominantly caused by *Brucella abortus* and is generally detected in pregnant females through abortions (OIE, 2022).

In all species, the main entry points for the bacteria are the skin and mucous membranes, mainly the digestive tract and the conjunctiva; dissemination occurs through fomites, food and water contaminated by secretions, fetal membranes, aborted fetuses or newborn calves affected by the disease (GŁOWACKA et al., 2018).

The diagnosis recommended by the National Program for the Control and Eradication of Brucellosis and Tuberculosis - PNCEBT includes a screening test, with acidified buffered antigen (AAT) and a confirmatory test, the 2-mercaptoethanol test (2-ME) (BRASIL, 2017).

The AAT test is a rapid, sensitive agglutination test using an antigen buffered to pH 3.65 and stained with rose bengal. The reading indicates the presence or absence of IgG being qualitative, as it does not indicate the antibody title of the tested serum. Cross reactions may occur with antibodies produced against other bacteria, such as enterobacteria (BRASIL, 2006). The chemical compound 2-ME destroys IgM macroglobulins, being inactive for immunoglobulins of the IgG class, which it detects, and the test must be performed with the slow test in tubes. The interpretation of the results is made by the difference between the titles of the untreated sera in the slow test in relation to the serum treated with 2-ME (BRASIL, 2017).

Brucellosis poses a major threat to public

health through consumption of unpasteurized milk and dairy products produced by unsanitary dairy farms in endemic areas. Regular and meticulous surveillance is necessary to determine the true picture of brucellosis, especially in areas with continued high prevalence (KHURANA et al., 2021).

Bovine tuberculosis is a chronic disease that leads to loss of productivity and poses a risk to public health. In cattle, it is caused by *Mycobacterium bovis*. It is characterized by its debilitating and progressive effect, and by the granulomas that can occur in any organ or tissue (FERLUGA et al., 2020). Transmission occurs by inhalation of aerosols, by ingestion or through skin lesions (ASSI; FRANCHI; RIBEIRO, 2021).

The diagnosis recommended by the PNCEBT routinely includes a screening test, which in dairy cattle is the simple cervical test (SCT) and in beef cattle, the caudal fold test (CPT). The confirmatory test is the comparative cervical test (CBT), which uses bovine and avian tuberculin simultaneously. This test allows eliminating false-positive reactions and distinguishing infections between tuberculosis and other environmental mycobacteria (BRASIL, 2017).

For animal production, it is a very important disease that can lead to great losses, also due to an increase in the mortality rate, carcass condemnation and a decrease in exports (ASSI; FRANCHI; RIBEIRO, 2021). With the aim of controlling the disease and preventing new cases, MAPA created the PNCEBT in 2001, with four fundamental axes, vaccination of female calves between three and eight months of age, control of the interstate transit of animals, diagnosis with notification of Brazilian authorities and euthanasia for positive animals and the certification of properties free of the disease (BRASIL, 2017).

According to the National Confederation

of Agriculture, only in direct financial losses, with the slaughter of infected animals, it is estimated more than eight million reais per year in Paraná (CNA, 2022). In order to reduce the losses of family farming producers, a program was instituted in the municipality of Francisco Beltrão that subsidizes 50% of the cost of the exam for the producer who performs the tests for brucellosis and tuberculosis in 100% of the animals, in addition to the obligation of periodic tests imposed by milk processing companies, in order to offer a better quality product to the consumer.

In this context, this study aimed to determine the occurrence of brucellosis and tuberculosis through the prevalence in dairy herds in the Municipality of Francisco Beltrão, officially registered in the last eight years, in the period between 2014 and 2021.

MATERIAL AND METHODS

The study area was the Municipality of Francisco Beltrão, whose territorial extension is 735,711 Km², altitude of 554 m, latitude 26°04'42" and longitude 53°03'22" (IBGE, 2021). One of the main sources of income in the municipality is related to milk production, being among the main dairy regions of the State of Paraná, with a herd of 50,594 head, 21,437 cows being milked with a production of 89,458 (x1000) liters of milk /year and a revenue of BRL 81,533,263 (x1000) (IBGE, 2020).

The study design was a cross-sectional, descriptive, retrospective and quantitative research, with determination of the occurrence of diseases by calculating the prevalence. The sampling covered a period of eight years, considering all the tests that resulted positive for bovine brucellosis and tuberculosis, from 2014 to 2021. The data were compiled from the database of the Municipal Secretary of Rural Development, prepared monthly by veterinarians qualified by Ministry of

Agriculture, Livestock and Supply - MAPA, through the program developed by the city hall in which examinations were carried out on the entire herd. The results were tabulated in spreadsheets, from which simple descriptive statistics were made and subsequent analysis of the results found, with presentation in tables.

Data on brucellosis-reactive animals came from those submitted to a serial test protocol, as recommended by the PNCEBT. As a screening test, the AAT test was used, performed in the municipal laboratory. Sera that reacted positively to this test were sent to the Marco Enrietti Diagnostic Center for the 2-ME confirmatory test. Tuberculosis data came from animals submitted to TCS. If it was considered positive or inconclusive,

it went through the confirmatory test, the TCC, which was performed by the qualified veterinarian. The reading and interpretation of the results were carried out in accordance with the standards described in the PNCEBT (BRASIL, 2017).

RESULTS AND DISCUSSION

The results demonstrated for brucellosis were obtained through the serological test with acidified buffered antigen (AAT), for screening, of 93,727 animals, in 6,026 dairy farms, between the years 2014 and 2021. Among these, all reactive or inconclusive sera for brucellosis underwent the 2-ME test, obtaining 110 positive sera from 51 different farms (Table 1).

Year	nP	nFe	Fe+2ME/ nP	PrPFe+ (%)	PrFe+ (%)	nMa	Ma+2ME/ nP	PrMa (%)	PrPMa+ (%)
2014	1.647	21.539	55/12	0,72	0,25	12	12/3	100,0	0,04
2015	1.141	18.487	13/10	0,87	0,07	9	9/4	100,0	0,06
2016	896	16.219	0	0	0	15	0	0	0
2017	610	8.101	3/3	0,49	0,03	40	0	0	0
2018	657	7.528	11/13	1,97	0,14	72	0	0	0
2019	526	8.660	4/4	0,76	0,04	70	0	0	0
2020/ 2021	549	12.726	3/2	0,36	0,01	249	0	0	0
TOTAL	6.026	93.260	89/44	0,73	0,09	467	21/7	4,49	0,11

Subtitle: nP=number of properties tested; nFe=number of females tested for brucellosis (AAT); Fe+2ME/nP= number of females positive for brucellosis by the 2-ME confirmatory test/number of focus properties; PrPFe+(%)=prevalence of brucellosis in positive females; PrPFe+(%)= prevalence of properties with positive females; nMa= number of males tested for brucellosis; Ma+2ME/nP=number of males positive for brucellosis by the 2ME confirmatory test/number of affected properties; PrMa(%) =prevalence of brucellosis in males; PrPMa+(%)= prevalence of properties with positive males.

Table 1 - Testing and prevalence of brucellosis in dairy cattle in the city of Francisco Beltrão, PR, from July 2014 to December 2021.

The control and prevention of diseases that can affect herds are of great importance for animal production. Diseases common to men and animals, zoonoses, are of constant relevance in production chains, making it necessary to preserve the health of herds, essential for production, depending on the management conditions that are practiced on the properties (CUNHA et al., 2012).

Dias et al. (2009), in a study carried out on 65 properties in the Castro and Curitiba region, tested 797 animals raised in a semi-intensive system, detecting 63 positive animals, which corresponds to 7.9% of the herd. In Castro, they verified a very high rate of infection, with 29.92% of positive confined animals, when compared to animals in an extensive system, in the same region, which was 9.35%. A great difference in the infection installed in the herd can be noticed, which can be explained by the differences in the breeding systems, since in the intensive system the animals end up having more contact with each other, establishing a higher rate of transmission of the disease among the herd.

Geographical, social and economic conditions contribute to the existence of different production systems in Paraná. The present study covered properties with both intensive and semi-intensive rearing systems, with a much lower prevalence, 0.09% for females; for males, the prevalence was higher, 4.49% for males, in relation to the herd tested.

Between 2001 and 2002, the official veterinary service of Paraná carried out examinations for brucellosis in all geographic regions of the state. In the Southwest Region, dairy herds from 300 properties were sampled, totaling 1,891 females aged over 24 months. The prevalence for brucellosis was 0.85% in dairy herds in the municipalities of Francisco Beltrão and Pato Branco and 1% in properties considered positive, with the presence of a focus of the disease (DIAS

et al., 2009). Possibly, the program carried out by the Secretary of Rural Development of the Municipality, which subsidizes 50% of the value of the exams for family farming producers, managed to reach a greater number of animals examined.

Magalhães et al. (2018) evaluated the epidemiological profile of bovine brucellosis in the Northwest Region of Paraná between 2014 and 2016, using data from examinations performed by a licensed veterinarian. Among male and female bovines, 12,640 animals were tested by the AAT test, and 26 animals were positive for brucellosis, making up a percentage of 0.2% of the analyzed herd.

The lowest prevalence of outbreaks in the State was found, among others, in the region of Francisco Beltrão and Pato Branco, with 0.34% and 1%, respectively. According to DIAS et al. (2009), the two municipalities are characterized by properties with a small number of animals and a predominance of mixed and dairy farming.

The results obtained in this study reveal a decrease in the prevalence of the disease, which suggests that the municipal program was efficient, as it was able to test 100% of those susceptible. Each year it was possible to track and test every dairy cattle in the municipality. The greater sanitary requirement in dairy products by agencies linked to animal health and public health, the vaccination of females between three and eight months and the sanitary slaughter of positive animals were determinant for the results found.

In the period considered, between 2014 and 2021, only in 2016 was no animal positive for the disease registered. All dairy farms that had at least one case of a positive animal both by AAT and by 2-ME were considered outbreaks, and treated as such by animal health agencies.

Kanashiro, Filho and Tirado (2014) reported a survey carried out at the Nossa Senhora Aparecida Settlement in the

municipality of Mariluz, PR, to characterize the epidemiological situation of bovine brucellosis, in addition to meeting the criteria of the dairy to continue delivering the milk to be marketed. 141 dairy farms were sampled, with all females over 24 months old and bulls being tested, totaling 1,264 animals, with 23 farms having at least one positive animal, the prevalence of outbreaks and infected animals respectively being 16.31% and 2.6%.

The adherence of dairy farmers to control norms such as vaccination of susceptible females, annual routine examinations in females older than 24 months and carrying out examinations before introducing new animals into the herd, also contributed to the reduction of outbreaks in milk producing properties.

Regarding the sex of the animals, it was found that among the 110 positive animals, 81% were females and 19% were males. In the considered period, of the 467 males used for reproduction, only 21 were confirmed with the disease, in seven properties; among the 93,260 females tested, 89 animals were positive during the study period.

The difference between the sexes in terms of prevalence (females = 0.09% and males = 4.49%) can be attributed to the number of females tested, much higher than that of males, and to the fact that infected pregnant females are a source of infection of the disease within a property. *Brucella* spp. makes tropism for the gravid uterus, where they predominate. The entry points are the skin, digestive mucosa and conjunctiva, and transmission occurs through pastures, food troughs and water contaminated by secretions, fetal attachments, aborted fetuses or newborn calves of infected females. Milk and semen can be sources of infection (MEIRELLES-BARTOLI, SOUSA AND MATHIAS, 2014).

Breeders reactive to AAT and positive for 2-ME were discarded from the respective

herds in view of current legislation (BRASIL, 2006) and the risk of maintaining the disease on the property.

Junqueira Junior et al. (2013) carried out a study with the objective of knowing the incidence of brucellosis in male cattle aged over eight months. 220 males were tested, of which seven were positive for AAT and three were positive for 2-ME. They concluded that bulls classified positive could be really sick or were mistakenly vaccinated with a B19 vaccine sample.

The antibodies produced in vaccination with a B19 sample, when performed up to the eighth month of life, disappear quickly and animals older than 24 months are negative in serological tests. In case the vaccine is performed in older animals, there is a great chance of interference of antibodies against the vaccine in the diagnostic tests (MARTÍNEZ-HERRERA et al., 2012). Although the use of the vaccine in males at risk of infecting them and developing the clinical form is prohibited, it may have occurred and the animals are false-positive since they are within the period where there is interference of vaccine antibodies (BRASIL, 2017).

Studies have shown that the acquisition of breeding stock is a risk factor for brucellosis (DIAS et al., 2009; JUNQUEIRA JÚNIOR et al., 2013) and Brazilian legislation requires a negative certificate for brucellosis of animals intended for reproduction when transported between states (BRASIL, 2017). Despite the knowledge of the importance of breeders in the epidemiology of brucellosis, there is little awareness among producers, as their commercialization within the same municipality or state is not subject to any inspection (JUNQUEIRA JÚNIOR et al., 2013).

Auxiliary prophylaxis methods can be used to reduce the prevalence, such as the training of breeders and handlers, the improvement of

sanitary management and the use of animal reproduction biotechniques (OLIVEIRA; SARAPIÃO; QUINTÃO, 2014), such as the artificial insemination in time fixed, embryo transfer and in vitro embryo production. They are tools that reduce the entry of breeders into the herd and, therefore, reduce the sexual spread of the disease in the susceptible population (KHURANA et al., 2021).

However, despite great efforts, the prevalence of brucellosis has fluctuated over the years without any downward trend. The limited budget allocated to brucellosis control is a limiting factor for the success of the program. For example, the absence of compensation for farmers results in infected animals remaining on farms, which potentially increases the risk of spreading the disease (ÁVILA-GRANADOS et al., 2019).

In Brazil, brucellosis is enzootic and, despite improvements in the structure of official services, a lack of resources still prevails, aggravated by economic crises (MEGID; MATHIAS; ROBLES, 2010; SANTOS et al., 2013).

Tuberculosis is a chronic bacterial disease related to the susceptibility of the affected hosts, being economically important due to the decrease in milk production and weight

gain, early culling and death, elimination of animals with high zootechnical value and condemnation of carcasses (BRASIL, 2006).

Tuberculosis has a direct impact on human and animal health, including conserved wild species, and also on the well-being of the population that lives from livestock, causing several economic repercussions due to the ban on the trade in livestock, the slaughter of positive animals and the reduction in the production of products of animal origin that become unfit for consumption and are sources of contagion (OIE, 2022).

The diagnosis of tuberculosis followed the official protocol recommended by the PNCEBT (BRASIL, 2017). The intradermal tuberculin test, which evaluates the cellular immune response, is the standard method for diagnosing bovine tuberculosis (OIE, 2022). The PNCEBT indicates the performance of intra-dermal tests in the suspected animals and, when a positive reaction occurs, the animals are slaughtered; in addition, their traffic is controlled (BRASIL, 2017).

Of the total of 131,570 females tested in the period, 340 animals were positive in the confirmatory test (TCC), with a prevalence of tuberculosis of 0.25% of the animals (Table 2).

Year	nP	nP+	PrP (%)	nFe	nFe+	PrFe+(%)
2014	1.647	35	2,12	31.756	171	0,53
2015	1.141	20	1,75	24.385	113	0,46
2016	896	30	3,34	23.066	21	0,09
2017	610	12	1,96	11.619	12	0,10
2018	657	17	2,58	10.867	10	0,09
2019	526	9	1,71	12.545	8	0,06
2020/2021	549	16	2,91	17.332	5	0,02
TOTAL	6.026	127	2,10	131.570	340	0,25

Subtitle: nP=number of properties tested for tuberculosis; nP+=number of properties positive for tuberculosis; PrP(%)=prevalence of properties with animals reactive to tuberculosis; nFe=number of females tested for tuberculosis (TCS); nFe+=number of females reactive for tuberculosis by the confirmatory test (TCC); PrFe+(%)=prevalence of females reactive for tuberculosis.

Table 2 – Tuberculosis testing and prevalence in dairy cattle in the city of Francisco Beltrão, PR, from July 2014 to December 2021.

In Paraná, in 2008, 220,095 animals were tested and 225 properties were considered infected, totaling 496 positive reagent cattle and 491 slaughter (NÁPOLI; SARTOR; MARTINS, 2010).

In 2012, in a cross-sectional epidemiological study, the prevalence of tuberculosis in cattle herds and in adult animals in the reproduction phase in Paraná was calculated. The state was stratified into seven regions, and the Southwest Region was identified as region 7, with 16,045 animals being examined in 1,419 properties, with only five being positive. The test used was comparative cervical intradermal tuberculinization. The prevalence in properties and animals was estimated at 2.15% and in region 7, it was 2.24% (SILVA et al., 2016). The prevalence found is similar to that found in this study, which was 2.1%.

Another study carried out in the Southwest Region showed a much lower prevalence, when 23,421 animals were examined during four years. A total of 3,198 properties located in four municipalities were considered for the study, and the prevalence was 0.098% (SABEDOT et al, 2009).

Silva et al. (2016) detected a prevalence of herds with tuberculosis in Paraná of 2.25% [CI 95%: 1.31% - 3.00%]. They concluded that the prevalence of tuberculosis is low, although with a heterogeneous epidemiological profile throughout the state.

The Paraná Agricultural Defense Agency published a report on the sero-epidemiological survey of bovine brucellosis and tuberculosis, showing a prevalence of brucellosis of 4.87% in properties and 2.24% in animals. Tuberculosis were 2.5% in properties, and 0.35% in animals. The results by region considered similarity in structure and size of cattle herds, breeding system, insertion in the market and production characteristics. The survey was carried out in 2018 and involved 1,757 properties and about seventeen thousand animals in the study

(ADAPAR, 2018).

For Garcia et al. (2021), when the prevalence of the focus is above 1%, it indicates the spread of the disease in the studied location, and in these cases, the implementation of immediate and effective actions by health defense agencies is necessary, aiming at the control and prevention of the disease.

Aires, Coelho and Silveira Neto (2018), stated that the ability to identify problems in rural properties depends on the knowledge of the particular conditions under which livestock farms are developed. The health of herds in animal production is crucial, especially the prevention and control of diseases.

Disease control requires identification of infected animals through tuberculinization tests followed by the elimination of these animals and, for this reason, make their routine use complex (FERLUGA et al., 2020).

There is no vaccine or treatment for bovine tuberculosis, so preventing the entry of the disease into a property is the key to control (ARAÚJO, 2014).

CONCLUSION

The study demonstrated that bovine brucellosis and tuberculosis are present in the municipality of Francisco Beltrão, both with a low prevalence compared to studies in neighboring municipalities in the Southwest Region and even with other states. It also shows that the prevention work has had the desired effects, as each year the number of infected animals decreases, and the eradication of diseases can now become a reality. This is due to the tests carried out annually, the sanitary slaughter of positive animals, vaccination of susceptible females in the case of brucellosis, which contributes to reducing economic losses for producers.

Both diseases constitute a recurrent problem in the world, therefore, the aspects

presented place it in a position of strategic importance for conducting studies that bring relevant information about the disease, thus contributing to the adoption of correct measures to maintain animal and food health.

Appropriate sanitary management is essential on rural properties, as in addition to influencing production and productivity rates, it interferes with maintaining the health

status of herds, and requires raising awareness among producers in relation to diseases.

Thus, the implementation of public policies with actions aimed at animal health, taking into account the regular vaccination of herds, constant monitoring of properties and attention to those at health risk are effective measures for the control and even the eradication of these infectious diseases.

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