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First study of *Brucella ovis* antibodies in purebred sheep flocks in the State of Parana, Brazil

[*Primeiro estudo de anticorpos para Brucella ovis em rebanhos de ovinos puros de Origem, no estado do Paraná, Brasil*]

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ABSTRACT

Brucella ovis, a non-zoonotic species, is the etiological agent of ovine brucellosis, an infectious disease of clinical or subclinical occurrence in sheep flocks. Until then, there is no serological study of anti-*Brucella ovis* antibodies in purebred sheep herds. This study aimed to determine the presence of anti-*Brucella ovis* antibodies in purebred sheep flocks with breeding purposes from Parana State. Blood samples from 728 animals, of which 563 were females and 165 males, between 8 and 56 months of age from the six major sheep producing mesoregions of Parana, were submitted to detection of anti-*Brucella ovis* antibodies by the Agar Gel Immunodiffusion technique using an antigen from the bacteria *Brucella ovis* (Reo 198). The results indicate the presence of this disease in purebred sheep from Parana State in a low occurrence of 0.27% (2/728). The only two positive animals were rams, Santa Inês breed, from the same flock in the East Center region of Parana, without clinical disease. In conclusion, *Brucella ovis* is present in purebred sheep in Parana State, Brazil, and this low occurrence may have occurred due to rigorous breeding systems that may contribute to reduce the transmission of this disease.

Keyword: ovine, AGID, *Brucella* spp., brucellosis

RESUMO

Brucella ovis, *espécie não zoonótica, é o agente etiológico da brucelose ovina, doença infecciosa de ocorrência clínica ou subclínica. Atualmente, não existe estudo sorológico de anticorpos anti-*Brucella ovis *em rebanhos de ovinos puros de origem. Este estudo teve como objetivo determinar a presença de anticorpos anti-*Brucella ovis *em rebanhos ovinos de raça pura de origem, com fins reprodutivos do estado do Paraná. Amostras de sangue de 728 animais, sendo 563 fêmeas e 165 machos, entre oito e 56 meses de idade, pertencentes a seis principais mesorregiões produtoras de ovinos no Paraná, foram submetidas à detecção de anticorpos anti-*Brucella ovis *pela técnica de imunodifusão em ágar gel usando-se um antígeno da bactéria* Brucella ovis *(Reo 198). Os resultados indicam a presença da doença em ovinos puros de origem do estado do Paraná em baixa ocorrência de 0,27% (2/728). Os dois únicos animais positivos foram reprodutores da raça Santa Inês, do mesmo rebanho da região Centro Leste do Paraná, sem manifestação clínica. Em conclusão,* Brucella ovis *está presente em ovinos puros de origem no estado do Paraná, e essa baixa ocorrência pode ter ocorrido devido a sistemas rigorosos de criação, que podem contribuir para a redução da transmissão dessa doença.*

Palavras-chave: ovinos, Brucella *spp., brucelose, IDGA*

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INTRODUCTION

The ovine herds have gowned significantly worldwide (Farias *et al.,* 2013), and Brazil, considered the 8th world's largest sheep producer, has reached 19.715 million heads, thereby the State of Parana represents 2.98% of the Brazilian herd, totalizing 588.996 heads (Efetivo…, 2019). Despite this growth, it is important to implement awareness around risk factors, control, and prevention of brucellosis dissemination in sheep flocks (Elderbrook *et al.,* 2019).

Brucella ovis (*B. ovis*), a non-zoonotic species (Poester *et al.,* 2013), is the causative agent of ovine brucellosis, an infectious disease of clinical or subclinical occurrence (Ovine…, 2015), that is sexually transmitted and introduced into flocks by infected rams or ewes, since the etiological agent may be excreted through semen or vaginal discharge, also occurring indirectly ram-to-ram via passive venereal contact with ewes when males share the same females during the breeding season (Hartley *et al.,*1955; Buddle, 1955; Blasco, 2010).

B. ovis is responsible for economic and reproductive losses (Poester *et al.,* 2013), reverberating in rams´ decreased fertility, ewes´ lower conception rates, and reduction in the birth of healthy lambs. Even if the most common clinical sign associated to *B. ovis* infection is epididymitis in rams, less than 50% of the infected rams develop epididymitis, revealing the possible silent character of ovine brucellosis (Van Metre *et al.,* 2012).

The *Brucella melitensis* Rev. 1 vaccine is a live strain vaccine that can stimulate immunity against *B. ovis*, harming serological tests, but it is totally forbidden in Brazil (Ovine…, 2015). Moreover, this vaccine can cause human infection by accidental self-inoculation (Estein *et al.,* 2009), and it is important to emphasize that there is no report of this *Brucella* species occurrence in Brazil.

Serological tests for antibodies detection are the most useful epidemiological tool for diagnosis of *Brucella* infection around the world (Erdenebaatar *et al.,* 2004). In Brazil, the Ministry of Agriculture, Livestock and Food Supply (MAPA) recommends Agarose Gel Immunodiffusion (AGID) as the standard test (Brazil, 2004), and it has been already used to identify seropositive sheep in the Parana State (Cunha Filho *et al*., 2007; Oliveira *et al*., 2016), but never in purebred sheep flocks.

Although the infection by *B. ovis* has already been identified in several Brazilian states using AGID as a serological diagnostic method, and it is included in the National Program for the Sanity of Goats and Sheep of Ministry of Agriculture, Livestock and Food Supply (MAPA) (Brazil, 2004), studies have demonstrated the occurrence of *B. ovis* antibodies only in commercial sheep herds from non-mechanized farms in Brazil through AGID test (Marinho and Mathias, 1996; Chiebao, 2011), there are even a few from Parana State (Cunha Filho *et al*., 2007; Oliveira *et al*., 2016), and most of all, there are no studies with data from purebred sheep herds that sell animals with genealogical records to properties all over the country with breeding purposes, which is of great importance since *B. ovis* is sexually transmitted (Hartley *et al.,*1955; Buddle, 1955; Blasco, 2010) and the infection may have silent dissemination in the flocks (Van Metre *et al.,* 2012). Thus, this is the first serological study that aimed to determine the occurrence of antibodies anti-*B. ovis* in naturally infected purebred sheep from Parana State, Brazil.

MATERIAL AND METHODS

The experiment was carried out in accordance with the guidelines of the Ethics Committee on the usage of animals in experiments and was approved by the scientific committee (CEA/UNOPAR 006/16).

The minimum number of serum samples (n = 728) to be tested was calculated using the EpiInfo7® program, using the following parameters: expected prevalence of 5% (95% confidence interval and 6% standard error), considering the 1.40% of prevalence observed in 2007 by Cunha Filho *et al.* (2007) in Parana State. The animals were randomly selected so that 10% from the total number of sheep were sampled on each farm.

Overall, this study covered farms located in the in six mesoregions of Parana State: Western Center (n= 119, municipality of Araruna), East Center (n= 241, municipalities of Castro, Piraí do Sul and Ventania), South Center (n= 49, municipalities of Candói and Pitanga), Metropolitan Curitiba (n=

101, municipalities of Colombo and São José dos Pinhais), North Central (n= 185, municipalities of Arapuã, Bom Sucesso, Itagujé, Mandaguari, Maringá and Rosário do Ivaí), and North Pioneer (n=33, municipalities of Congoinhas and Rancho Alegre). These regions comprehend 60% of the state herd (Paraná, 2017) and are located between latitudes 22 °S and 26 °S (Cartas..., 2017), and and also comprised a large part of purebred sheep with genealogical registration according to information obtained through the Brazilian Association of Sheep Breeders.

The samples were collected before the breeding season, in January and February of 2017. From the total of 728 animals, ewes comprised 77.33% (563/728) and rams comprised 22.66% (165/728), between 8 to 56 months old. The animals sampled belonged to Dorper (39.01%), Ille de France (16.62%), Lacaune (5.22%), Santa Ines (9.07%), Suffolk (8.24%), Texel (19.10), and White Dorper (2.74%) breeds, all with genealogical records in the Brazilian Association of Sheep Breeders.

The males were all negative in the individual evaluation for the epididymitis occurrence by observation and palpation of testicular structures. The history concerning abortion, acquisition of animals and participation in fairs in the last year were fully questioned in each farm at the blood collection moment for the purpose of an epidemiological characterization. From the 728 animals, 144 (19.78%) were kept totally confined in pens, and 584 (80.22%) were kept semi-confined in pens with access to collective paddocks, but all of them had exclusively natural breeding season.

The samples were collected by jugular venipuncture and serum were separated by centrifugation and stored in sterile microtubes at -20º C until further analyses. Serum samples were tested by the AGID technique with sensitivity and specificity of 70% and 100%, respectively (Xavier *et al.*, 2011), at the UNOPAR Laboratory of Infectious Diseases, using the diagnostic kit produced by the Parana Technology Institute (TECPAR, Curitiba, Brazil). The antigen consists of soluble proteins and lipopolysaccharides, extracted from the bacterium *Brucella. ovis*, sample Reo 198.

The agar preparation was carried out according to the manufacturer's specifications. Four samples were evaluated in each petri plate (55x15mm), where the positive control serum was applied in the center of the plate. Plates were read 72 h after sample application, and samples were considered positive when a clear line of precipitation was observed between the sample and the positive control.

The data obtained for AGID test of ovine brucellosis allowed only descriptive statistics analysis and the low occurrence obtained did not allow the study of risk factors associated to *B. ovis* infection.

RESULTS

From the 728 serum samples tested, only two (0.27%) reacted positively in the AGID test anti-*Brucella ovis*. The only two positive animals were rams without epididymitis, Santa Inês breed, at 12 and 24 months of age, from the same flock in Ventania municipality, belonging to the East Center region of Parana, the largest region in number of sheep analyzed, representing 33.10% of the sampled animals in this study.

Although the seroreactivity results did not allow to study the risk factors associated with *B. ovis* infection in the present study, the epidemiological characterization of the animals is presented in Table 1.

Table 1. Epidemiological characterization of the animals studied according to the occurrence of abortion, acquisition of animals and participation in fairs

Variable	Number	Percentage (%)
Abortion history		
Yes	668	91.76
No	60	8.24
Animal acquisition		
Yes	617	84.75
No	111	15.25
Participation in fairs		
Yes	679	93.27
No	49	6.73

DISCUSSION

In Brazil, the flock seroprevalence of *B. ovis* in the studied populations was between 0% (Marinho and Mathias, 1996; Chiebao, 2011) and 34% (Silva *et al.*, 2003). Our study seroprevalence

estimate, based on serologic testing, is much lower than other prevalence estimates published in Brazil with commercial non-mechanized sheep flocks (Magalhães Neto and Gil-Turnes, 1996; Schafer *et al.*, 1997; Coleto *et al.*, 2003; Silva *et al.*, 2003; Pinheiro Junior *et al.*, 2009; Rizzo *et al.*, 2009; Silva *et al.*, 2009; Alves *et al.*, 2010; Souza *et al.*, 2012; Araujo *et al.*, 2013; Martins *et al.*, 2013; Azevedo *et al.*, 2014; Rizzo *et al.*, 2014; Manhezzo *et al.*, 2015; Lima *et al.*, 2020; Teixeira *et al.*, 2021), including 1.40% (Cunha Filho *et al.*, 2007) and 18.26% of AGID seropositive sheep (Oliveira *et al.*, 2016) in the Parana State.

It is important to emphasize that this is the first serological detection of anti-*B. ovis* antibodies performed in sheep herds with genealogical records from the state of Parana, Brazil. In Parana State, studying commercial flocks, detection of *B. ovis* were recorded an incidence of 1.40% by Cunha Filho *et al.* (2007) in the north central mesoregion of Parana, and 18,26% by Oliveira *et al.* (2016) in the northwestern microregion of Parana.

Other states in southern Brazil reported higher occurrence of *B. ovis* antibodies in sheep flocks, with 18.84% of seropositive animals in Santa Catarina State (Schafer *et al.*, 1997), 13.4% State (Magalhães Neto and Gil-Turnes, 1996), and 2.89% (Machado *et al.*, 2015) in Rio Grande do Sul, but both studies analyzed intensive half-bred herds, not purebred animals, maybe justifying the higher occurrence when compared to this study, as more intensive systems can contribute to the introduction and persistence of *B. ovis* infection in sheep flocks (Elderbrook *et al.*, 2019) and rams from large flocks were 14 times more likely to become infected than rams from small flocks (Chávez *et al.*, 2013). It is also important to stress that molecular characterization showed a high genetic diversity among *B. ovis* field isolates from Rio Grande do Sul State, Brazil (Dorneles *et al.*, 2014), reinforcing the importance of studies on *B. ovis* survey.

The low occurrence observed in this study can be explained by the fact that purebred sheep with genealogical record are constantly undergoing clinical exams, health management and are kept in low demographic concentrations. According to Lone *et al.* (2013), the prevalence of brucellosis was higher in farms without zootechnical bookkeeping and sanitary controlling (14.14%) than those who carry out the zootechnical and sanitary management of the herd (3.23%), and this may be due to the good management practices of organized farms, such as the selection of healthy animals for reproduction, which could justify the result obtained in the present study.

Since less than 50% of rams infected with *B. ovis* develop a palpable epididymitis (Van Metre *et al.*, 2012), the disease is introduced into a flock after the introduction of an infected ram, the most common route of disease spread is indirect ram-to-ram transmission via passive venereal contact with ewes during the breeding season and transition also occurs directly from ram-to-ram via oral and mucosal routes outside the breeding season (Hartley *et al.*, 1955; Buddle, 1955; Blasco, 2010), it is epidemiologically interesting to highlight that the only two seropositive animals were rams (n = 2/165) without epididymitis, reinforcing the subclinical character of the disease.

Data concerning *B. ovis* in Brazil are conflicting, which requires future clarifying studies. About this, in Sao Paulo State, infected sheep with *B. ovis* were not found in tests of AGID, indirect enzyme immunoassay (ELISA-I) and the complement fixation test (FC) (Marinho and Mathias, 1996; Chiebao, 2011), whereas Rizzo and collaborators demonstrated an incidence of *B. ovis* in sheep flocks from Sao Paulo State of 1.96% (2009) and 1.7% (2014), respectively, but studies demonstrated a frequent non-agreement of different serological test to detect *B. ovis* infection in sheep herds (Praud *et al*., 2012; Elderbrook *et al*., 2020).

Elderbrook *et al.* (2019) hypothesized that seroprevalence would be significantly higher after the breeding season compared to before the breeding season because *B. ovis* is sexually transmitted (Hartley *et al.*, 1955; Buddle, 1955; Blasco, 2010). In contrast, the authors observed higher apparent seroprevalence before the breeding season than after the breeding season, similar to this study, carried out previously to the breeding season. Explanations for this finding include: 1) infection and transmission of *B. ovis* is occurring more often directly from ram-to-ram before the breeding season, 2) there is a lack of seroconversion in animals after *B. ovis* exposure (Elderbrook *et al.*, 2019). To the best of our knowledge, no previous studies have considered

time of sample collection relative to breeding season as a possible risk factor for seropositivity in Brazil, but a more comprehensive and controlled investigation into this may be useful in the future.

The positive animals belonged to a semi-confined farm with a history of abortion, acquisition of animals in the last two years and participations in fairs. After obtaining the positive result, the sheep breeder reported that had acquired animals from different herds and participated in fairs in the states of Parana, Rio Grande do Sul, and São Paulo. The breeder also reported that he had never tested the herd for *B. ovis*, even less had required testing for the acquisition of new animals, which could have contributed to the obtained seropositivity. Santos *et al*. (2013) observed that animal acquisition is a potential risk factor for disease incidence, since 27.6% of the properties that acquired animals showed seropositivity, in comparison to the 12.8% seropositivity observed in the properties that did not acquire animals.

The low antibody detection herein described may have occurred even if AGID is the standard test according to MAPA for *B. ovis* antibodies detection (Brazil, 2004), since ELISA has been more promising as diagnostic tool (Elderbrook *et al.*, 2020), representing this study limitation, but it does not exclude the fact that serologically negative rams can excrete the organism whereas serologically positive rams may not excrete the organism (Elderbrook *et al.*, 2020), demonstrating that the diagnosis of B. ovis is still a challenge.

Concerning the inexistence of consistent epidemiological studies on risk factors, and the varying occurrence levels of *B. ovis* infection in the country, more studies are necessary around the infection profile, as well as also comparing different diagnostic tools, which should lead to improved management plans of sheep flocks worldwide.

CONCLUSION

It is concluded that ovine brucellosis is present in purebred sheep in the Parana State, Brazil, in a low occurrence under the conditions of the present study.

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