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Seroprevalence of *Brucella ovis* in rams and associated flock level risk factors in the state of Rio Grande do Sul, Brazil

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ABSTRACT

A cross-sectional study based on a planned probabilistic sampling was carried out to estimate animal and flock prevalence of *Brucella ovis* in rams, as well as to determine risk factors at the flock level. Data regarding the flocks were collected by means of a questionnaire applied on 705 farms in the state of Rio Grande do Sul, Brazil, using one-stage cluster sampling. From the 705 flocks, 20 (2.5%, $CI_{95\%}$: 2.0–3.1%) had at least one positive ram. At the animal level, out of 1800 rams, 52 were positive (2.89%, $CI_{95\%}$: 0.4–5.3%). Statistical analysis identified the following as risk factors: average age of rams in the flocks (PR: 1.99, $CI_{95\%}$: 1.19–3.32); farms larger than 5 km^2 (500 ha) on extension area (PR: 7.46$CI_{95\%}$: 2.03–27.43); and the lack of lambing paddocks (PR: 5.56, $CI_{95\%}$: 1.70–18.11). This study provided relevant information for authorities to elaborate plans for the first Brazilian state based *B. ovis* disease control and eradication program. To the authors' knowledge, this is the first study that shows the importance of lambing paddocks in order to keep pre-lambing and lambing ewes away from the rest of the flock, the lack of this infrastructure was considered an important risk factor for *B. ovis*.

1. Introduction

Brucella ovis infection causes chronic epididymitis, which is characterized by pain, swelling, and induration of the epididymis. This disease is known to be an important cause of ram infertility around the world (Webb et al., 1980).

B. ovis is mostly carried by mature, sexually active rams. Transmission occurs via direct contact or passive venereal infection (Radostits et al., 2007). Ewes may carry *B. ovis* in the vagina for at least two months and can excrete the organism by vaginal discharges and milk. In Brazil, *B. ovis* was first isolated in 1972 in the state of Rio Grande do Sul (Blobel et al., 1972), and several studies based on non-probabilistic sampling reported prevalence among rams ranging from 3 to 35% (Poester et al., 2002).

The sheep industry is very important economically for the state of Rio Grande do Sul, which holds the major part of the sheep flocks in Brazil with approximately 3.5 million animals across 50,000 flocks (Santos et al., 2011). The aim of this study was to estimate the seroprevalence of *B. ovis* as well and to identify potential risk factors in ram flocks.

2. Material and methods

2.1. Study area and target population

Located in Brazilian's southern region, the state of Rio Grande do Sul has an area of 268,781,896 km^2 (3.16% of the country) (Fig. 1). It is divided into seven regions, which are subdivisions of Brazilian states (Fig. 1). The state holds 22.9% of Brazil's sheep population (IBGE, 2010).

The target population comprised rams more than six months of age in the state of Rio Grande do Sul. The exact number of rams was not known, so it was estimated using the mean number of ewes of reproductive age.

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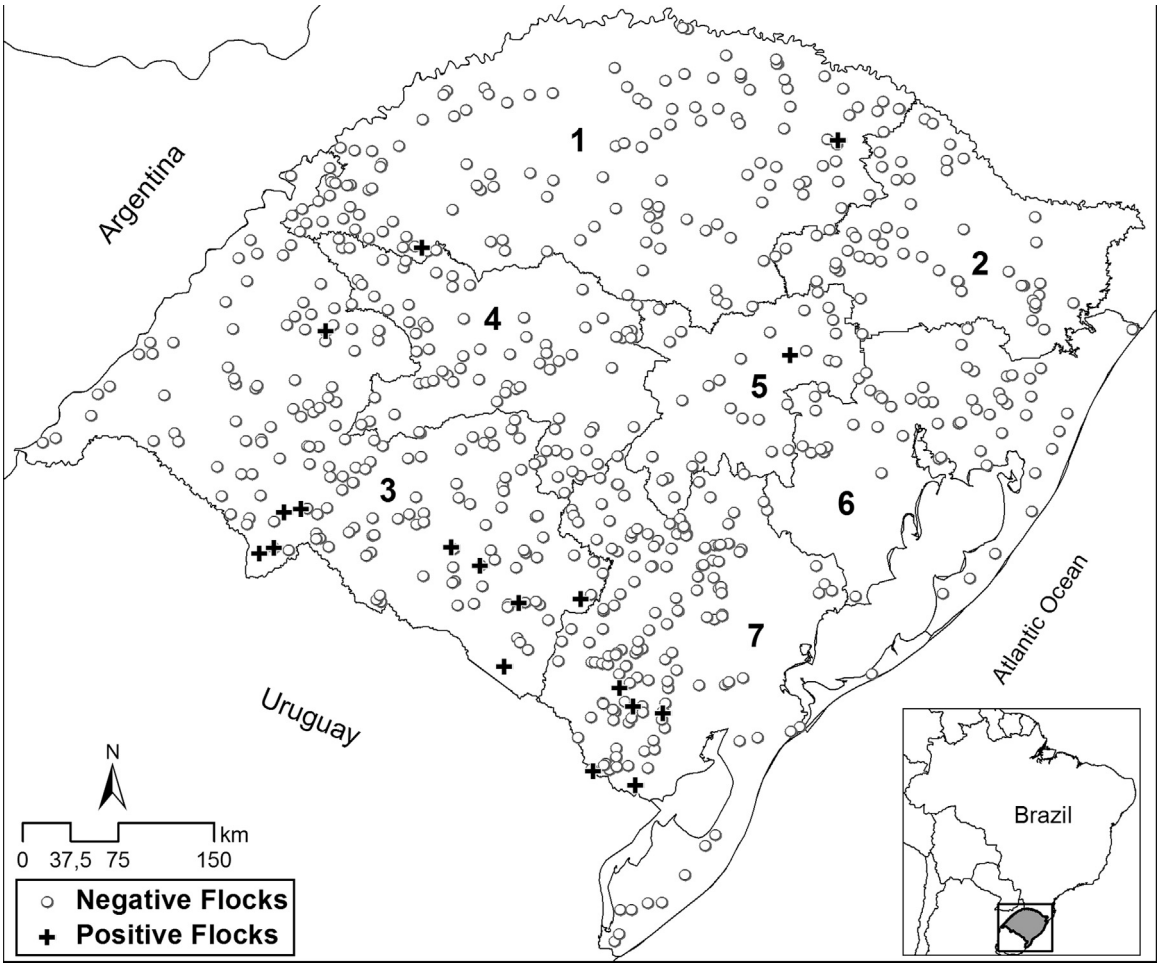

Fig. 1. Location of 705 sampled and 18 positive flocks in Rio Grande do Sul state (Brazil) examined for *Brucella ovis*. The names of the regions are indicated (1: Northwest Region, 2: Northeast Region, 3: Southwest Region, 4: Western central Region, 5: Eastern central Region, 6: Metropolitan Region, 7: Southeast Region).

2.2. Survey design and sample collection

A one-stage cluster sampling was performed, with all the rams more than six months of age (rams considered for breeding or for sale reasons) within a flock considered a cluster. The clusters were selected by stratified random methods. The number of clusters (nc) was obtained using the formulae:

$$\mathrm{nc} = \frac{1.96^2 \times (\mathrm{nr} \times \mathrm{Vc}) + P \times (1 - P)}{\mathrm{nr} \times d^2} \quad (1)$$

where nr is the predicted average number of rams per cluster, i.e., 2.8, Vc is the between-cluster variance, P the expected prevalence of 10% (Magalhães Neto and Gil-Turnes, 1996), and d is the desired absolute precision, which was 5%. Since no information about the Vc was available, a value of 0.42 was used in order maximize the sample size up to the limit of the resources available. A minimum of 695 clusters was required to accomplish the sampling parameters used. A sampling frame containing the producer's name (N = 46,874), location, and number of sheep in the flock was available from the Secretary of Agriculture, Livestock and Agribusiness of the state of Rio Grande do Sul (SEAPA-RS). The flock containing rams was considered the sampling unit for selection purposes (i.e., the clusters). A stratified random sample proportional to the flock population present within each seven region (strata) was performed and each flock was randomly sampled from all the individual strata (Fig. 1). All the rams more than six months of age were collected from the selected flock. If a sampled flock had no rams more than six months of age, the nearest flock was chosen.

Blood was collected, animals were ear tagged and general individual information were collected (age, breed). Serum samples were tested by the AGID technique with sensitivity and specificity of 96.4% and 100%, respectively (Marin et al., 1989). The mean prevalence adjustment for test accuracy was made according to Thrusfield, 2007.

A structured questionnaire was designed to gather information about potential risk factors associated with the occurrence of *B. ovis* infection in the studied flocks.

2.3. Statistical analysis

2.3.1. Estimation of seroprevalence

The seroprevalence of the rams was estimated using SAS SURVEYFREQ. This procedure estimates the frequencies for levels of the categorical variables (regions and clusters), together with their standard errors and confidence limits in order to fit the sampling design. Details about the SAS code and the standard error are provided in the Appendix A. A flock was classified as positive when at least one ram was found to be positive. The flock's seroprevalence and the standard error were estimated.

2.3.2. Univariate analysis

A zero-inflated negative binomial regression (ZINB) using the SAS GENMOD procedure was used in this study. A ZINB models