Survey of sheep antibodies of smooth and rough strains of brucella in the North Patagonic area of La Pampa

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Abstract

Brucellosis is an infectious disease that affects domestic animals, producing heavily losses through abortions, and infertility in rams. The flock epidemiology situation in the north Patagonic area of Argentina (La Pampa) to the present disease is unknown; consequently the objective was to determine the prevalence of the disease. Out of 10.000 sheep, there were sampled 1.800 animals according to the application of the minimum sample size for estimating a rate with specified degree of precision in the Departments of Mara-co, Chapaleufu, Realico, Rancul, Conhelo and Quemu-Quemu representing an area of 22.072 km2, in the frame of reintroduction of sheep production in the province. Independently of the number of sheep per farm, 20-30% of the flock was sampled, that represent 15% (270) rams and 85% (1.530) ewes. After collecting serum from jugular blood, the samples were analysed either for the presence of antibodies against smooth strain through Buffer Plate Agglutination Test (BPA); or antibodies against rough strain of brucella through Agar Gel Immuno-diffusion Test (AGID). Bewilderment of all the animals was negative to both tests. Despite brucellosis is a high prevalent infectious di-sease in other part of the country, these results emphasise the condition of free areas of the disease.

Key words: ovine, brucellosis, La Pampa

Introduction

Brucelosis is an infectious disease of high economic impact affecting several animal species, particularly those of economic interest such as bovine and ovine (Samartino, 2002). The World Organisation for Animal Health (OIE) considers the disease as a treat of public health, and a boundary for international trade of animals (OIE, 2004). Brucelosis is caused by a GRAM (-) bacteria, classified by antigenic differences and host specificity in: Brucella ovis (ovine), Brucella canis (canines), Brucella suis (porcine), Brucella abortus (bovines) and Brucella melitensis (ovine and caprine). In humans the infections are basically produced by Brucella abortus or melitensis which show that host specificity is not strict (Young, 1995; Wallach et al., 1998, World Health Organization, 2008). In Argentina the disease is high prevalent in bovines, around 4-5% (Samartino, 2002), but there is not precise data that document the epidemiology situation of the disease in ovine. Brucella melitensis, and ovis in less proportion, are the more common subtypes found in sheep-rearing countries (Garin-Bastuji et al., 1998; Minas et al., 2004). Local data from Patagonia and Buenos Aires province, show the presence of Brucella ovis infection, represented by infertility in rams and abortus in sheep (Alton et al., 1988; Robles et al., 1998; Lopez et al., 2005).

It is well known the laborious situation to control and eradicate brucelosis from beef cattle, despite the execution of actions such as compulsory vaccination from 3 to 8 months female calf, and segregation of adult positive animals. Those actions, certainly effective, are taken without any concern about the bacteriological situation of the ovine flock (potential host of Brucella abortus subtype), that usually share paddocks with cattle. Consequently, it is highly relevant to generate basic information about the current bacteriological situation of La Pampa ovine flock. In the frame of the national ovine law, where the sheep industry is recovering through precise actions taken by the

local and national governments, it is important to originate information to favour the productive process and to generate knowledge of the epidemiology status of the disease. Therefore, the main objective of the project was to determine the prevalence of the disease in the ovine flock in the north of La Pampa province.

Methods

Animals and size of the sample

Adult Merino and Corriedale sheep (ewes and rams), belonging to flocks registered in La Pampa Ovine Plan, were blood sampled during a period of six months. At the initial time of the project, 10.000 sheep were distributed in 300 farms at the Departments of Mara-co, Chapaleufu, Realico, Rancul, Conhelo and Quemu-Quemu, representing an area of 22.072 km2, at the north of La Pampa province. Independently of the farm flock size, 20-30% of animals were sampled, and the total number to be sampled for the project (n) was estimated by the equation suggested by Smith (1995) with specified degree of freedom. As it was not known the historic record of prevalence of ovine brucelosis in the province, we suggested a 5% prevalence, taken as reference the province bovine information of the disease, so that, and applying the equation sited by Smith, the number of animals to be sampled was 1.800.

$$N_{inf} = \frac{P\,(1\text{-}P)\,Z^2}{d^2}$$

P = the estimated prevalence of infection (as a decimal)
Z = corresponds to the degree of confidence in our estimate (usually Z = 1.96 for 95% confidence in our estimate)
d = the maximun difference between observed and true prevalence that we are willing to accept (as a decimal)

Blood samples

Glass tubes without additives were used for sampling of serum, which were analysed for presence of antibodies against both smooth and rough strains of Brucella. The tubes were centrifuged at 2000 g for 15 minutes to obtain serum, which were frozen at -20 °C until analyses.

Antibodies detection

All serum samples were analysed for the presence of antibodies against the smooth strain of Brucella spp. through the Buffer Plate Agglutination Test (BPA) (potential positives to Brucella melitensis and abortus). Eighty µl of serum and 30 µl of antigen (B.P.A. Agropharma, Argentina) were mixed in a 3 x 3 cm2 glass plaque during 8 minutes and any sign of agglutination was considered positive thereafter Angus and Burton, 1984; Alton et al., 1988).

Agar gel inmunodifusion test (AGID) was used to determine the presence of serum antibodies against the rough strain of Brucella ovis. The gel was prepared dissolving 1,2 g of noble agar, 8.5 g of NaCl and merthiolate 1:10.000 in 100 ml of distilled water pH 8,2, in a boiling water bath. A plaque was covered with approximately 15 ml of melted gel, and then it was solidified, a hole was cut using a gel puncher. The hole was 3 mm in diameter and 3 mm apart, organised in a hexagonal pattern around a central hole, also 3 mm in diameter. Control and test sera were placed in wells with the antigen in the central well. The result was read after the incubation at room temperature during 48 hours in a humid chamber (Lopez et al., 2005). The antigen, precipitates with positive sera of rough strain of Brucella, was supplied by SENASA (National Animal Health Service) Argentina, which also provides the control standard serum.

Five percent of the AGID negative samples were sent to a referent laboratory (Laboratorio Central, INTA Castelar, Buenos Aires, Argentina) to be analysed for Complement

fixation and ELISA in order to corroborate the AGID results.

Results

Out of 1.800 sampled, 270 represented rams (15%) and 1.530 ewes (85%). All 1.800 serum sampled were negative to the presence of antibodies at both tests, BPA and AGID. Fifty percent of the rams (135) were clinically palpated, to detect genital abnormality at the time of blood sampling, but none of them were diagnosed with any lesion. Despite it is an unspecific diagnosis, in the present trial correlates positively with the antibodies tests.

Discussion

In many areas of Argentina, and in contrast to other sheep-rearing countries such as Spain or Italy, the predominant strain of infection is Brucella ovis (Robles et al., 1998; Lopez et al., 2005), which has many advantages in control and eradication programmes to Brucella melitensis infections. The ovis strain of Brucella lacks the lipopolissacaride responsible for the exacerbated virulence, and it has high host specificity restricted to the ovine population, relevant characteristics that make this bacterium feasible to control and eradicate (Marin et al., 1989).

The north-patagonic flock, with especial reference to La Pampa, has developed considerable during the last five years. The farmers, taking the governmental benefit, bought sheep without considering the health status as a priority, nevertheless and bewilderment of the results, the presence of the disease in the defined area is nil.

Different laboratories suggest the AGID test as a subjective assay, and are replaced by more sensitive and objective test such as enzime linked inmunoabsorband assay (ELISA). On the other hand, the World Organization for Animal Health ensures that the sensitibity of the AGID is similar to the ELISA test, and because of the low cost and simplicity it is still the recommended technique for Brucella ovis diagnose (Young, 1995). With a reduced number of samples (5%), we compared AGID with Complement Fixation and ELISA test (data not showed). All the negative samples to AGID were also negative to Complement Fixation, and just 7% were positive to ELISA with the optic density value at the limit of the cut point, suggested as a false positive reaction.

Conclusions

Despite the high increased in the number of sheep in the north patagonic area of La Pampa, the activity of brucelosis in the flock was nil. The epidemiology situation of the disease is relevant, and at the same time caution must be taken by the animal health authorities and farmers in order to avoid the introduction of the disease in the defined area.

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