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BRIEF REPORT

Serological study of brucellosis in Argentine Creole sheep

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Abstract Ovine cattle was introduced into America during the Spanish conquest with the second journey of Columbus to the Antilles and was disseminated throughout the region. In 1587, sheep were introduced into Argentina, later developing into the ''Creole'' breed. We selected 486 animals from different Argentine provinces with the aim of determining the serological status of brucellosis caused by *Brucella melitensis* and *Brucella ovis*. For the detection of antibodies against smooth *Brucella* spp., the Rose Bengal test (RBT) was performed as screening test while the serum agglutination test (SAT) and 2 mercapto-ethanol (2ME) were run as a confirmatory technique. Moreover, for the detection of antibodies against rough *Brucella* spp., we used the rapid slide agglutination test (RSAT) for screening and an indirect ELISA (IELISA) as confirmatory assay. This study showed that the total positive percentage of brucellosis due to *B. ovis* was 2.9%. Excluding the animals mixed with the Suffolk breed; seropositivity would be 0.6%. All animals tested negative for brucellosis caused by *B. melitensis*.

PALABRAS CLAVE
Ovejas criollas de Argentina;
Brucelosis en ovejas;
Brucelosis

Estudio serológico de brucelosis en ovejas criollas de Argentina

Resumen El ganado ovino fue introducido en América durante el segundo viaje de Colón a las Antillas y rápidamente se diseminó por la región. En 1587 entraron a la Argentina las primeras ovejas, estas se adaptaron a las condiciones naturales y se desarrolló así la denominada «oveja criolla». Se seleccionaron 486 animales de diferentes provincias para conocer el estado serológico de la enfermedad producida por *Brucella melitensis* y *Brucella ovis*. Para detectar

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anticuerpos anti-*Brucella* en fase lisa se utilizó la prueba de rosa de Bengala (RBT) como tamiz y como confirmatorias seroaglutinación lenta en tubo (SAT) y 2-mercaptoetanol (2ME). En fase rugosa se realizó como tamiz la prueba rápida de aglutinación (RSAT) y ELISA indirecto como prueba confirmatoria (IELISA). Este estudio mostró un porcentaje de seropositividad de brucelosis causada por *B. ovis* del 2,9%. Excluyendo los animales cruzados con la raza Suffolk, la seropositividad sería del 0,6%. Todos los animales fueron negativos a brucelosis causada por *B. melitensis*.

Most of the ovines introduced into America during the conquest had been rejected from Iberian Churra, Mountain and Merino Spain sheep flocks. Because of their ability to live in the hot and humid tropical environment, they rapidly disseminated throughout the region, competing with domestic animals for plants and grass foods. They were gradually forced to move to Peru at the end of the 16th century, and in 1587 a significant number was introduced into Argentina by Juan Torres y Aragón.[10] These sheep found optimal nutritional conditions in the region and quickly expanded in the new environment. Zeballos in 1898 stated that in the course of time, their natural evolution developed into the ''Creole'' breed with special characteristics.[10]

The number of animals increased and in 1810 had grown to 3 000 000 ovines. In 1825 crossbreeding with Merino began, and in the following decades new crossbreeds were essayed in order to improve wool production. However, pure Creole sheep continued to develop in areas where the new breeds were unable to adapt.

Lynch et al.[6] stated that in spite of not having sanitary control, Creole flocks look healthy and with good adaptation to the environments. They are currently present in most Argentine provinces and should be considered a potential economic resource in areas where a population with low socio-economic status subsists, considering their adaptation to adverse soil and climatic conditions and consumption of low quality pastures.

Currently the ovine population in Argentina, including all breeds, is estimated to have reached 14–15 million animals, 8% of which belongs to the Creole breed [8]that is located mainly in the Northwestern and North Central regions.[6]

Brucellosis in sheep is mainly caused by *Brucella ovis* and *Brucella melitensis*. *B. ovis* produces a clinical or subclinical disease characterized by genital lesions in rams and placentitis in ewes. Therefore, the main consequences of the disease are reduced fertility in rams, frequent abortions in ewes, increased perinatal mortality and susceptibility of sheep breeds in the affected region.[9] However, *B. ovis* has not been conclusively implicated in human disease.

The relative importance of *B. melitensis* for sheep varies depending on the geographic region, and can be influenced by husbandry practices where goats and sheep are in close contact. The predominant symptoms in naturally infected sheep and goats are abortions, stillbirths and the birth of weak offspring. Some infected animals carry the pregnancy to term, but shed the organism.[9] Most cases of human brucellosis in the world are caused by *B. melitensis* that is almost invariably transmitted by direct or indirect contact with infected animals or their products. It affects people of all age groups and both sexes and usually presents as an acute febrile illness that may persist as relapse, chronic localized infection or delayed convalescence.

In Argentina, brucellosis caused by *B. ovis* has been found in all the regions were sheep are located while *B. melitensis* is not a major problem although few cases have been described.[11] However, in all these studies, Creole sheep were never considered and there is no information about the infection in this breed.[14]

The objective of this observational cross sectional study was to determine the serological status of brucellosis caused by *B. melitensis* and *B. ovis* in Creole flocks. The 486 ovines, including males and females, belonging to nine Argentine provinces were sampled by convenience, selecting sexually mature animals in 10 areas (Fig. 1). All animal manipulations were performed using protocols previously authorized by the National University of Lomas de Zamora (protocol A123/06)

1. Rio Cuarto-Córdoba: 39 (35 females) of a flock of 150 animals that had no contact with other animal species.
2. 25 de Mayo-Buenos Aires: 75 (62 females) of 653 animals, in close contact with bovines.
3. La Junta-Catamarca: 22 (20 females) of 85 animals belonging to one family with low income and resources which exploited the herd for their own consumption, including wool that was used to make handcrafted garments for commercial purposes. During the day, animals shared communal grazing land with other flocks, including goats and swine, belonging to other families sharing similar socioeconomic conditions. Sheep were kept by their shepherd throughout the night.
4. Iruya-Salta: 39 (33 females) of 250 animals belonging to seven families with similar conditions to the former region, families worked in groups looking after the large flock.
5. Malargüe-Mendoza: 87 (77 females) of 380 animals in close contact with sheep of different breeds, goats and bovines. The peculiarity of this area is that Creole ewes were crossbred with the Suffolk breed from Chile, where they are predominant, because they were in close contact during the summer.
6. Caspi Corral-Santiago del Estero: 46 (31 females) of 462 animals belonging to five families who shared the land during the day, also with goats and bovines. Sheep were kept at night in each family's own corral for protection.
7. Tafí del Valle-Tucumán: 27 (24 females) of 250 animals. The main characteristic of these animals was that they

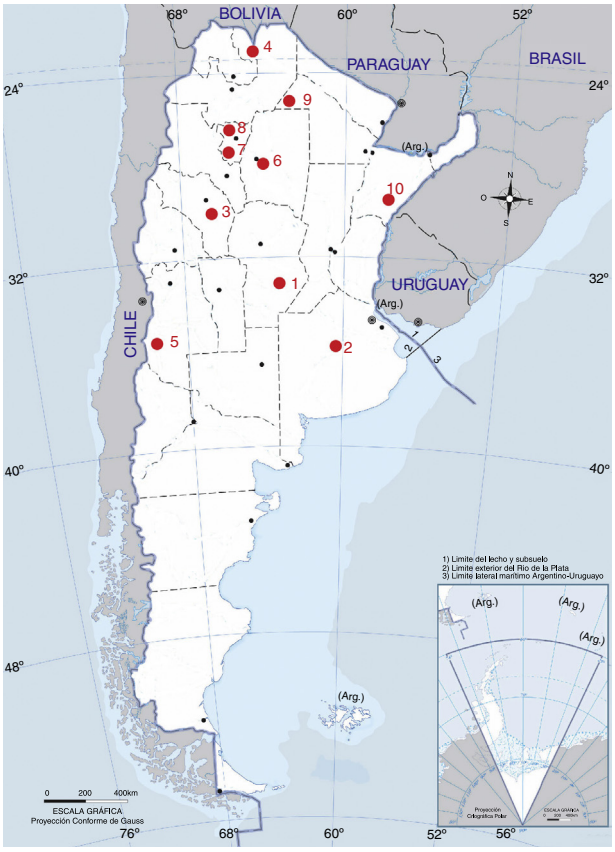

Figure 1 Argentine areas where Creole sheep were sampled. 1 – Río Cuarto; 2 – 25 de Mayo; 3 – La Junta; 4 – Iruya; 5 – Malargue; 6 – Caspi Corral; 7 – Tafí del Valle; 8 – Colalao del Valle; 9 – Taco Pozo; 10 – Sauce.

were living between 3000 and 3600 m above sea level, although their management was similar to area 6.

8. Colalao del Valle-Tucumán: 76 (62 females) of 190 animals from six families.
9. Taco Pozo-Chaco: 45 (32 females) of 423 animals belonging to six families living in different areas of the *Chaco Impenetrable* Forest, a remarkably flat and arid land covered in thorny bushes and trees, with very scanty resources; the animals were looked after by the farm families.
10. Sauce-Corrientes: 30 (17 females) of a flock of 312 animals. In this area, located in the Northeast of Argentina, sheep shared the land with bovines, sheep of other breeds and goats. The peculiarity of this area was that farms ranged in size from 2000 to 3000 ha.

Blood samples taken from the animals were collected without anticoagulant by jugular venipuncture and transferred to laboratory at room temperature (RT) the same day. Sera were separated by centrifugation at 1500 rpm during 7 min and immediately stored at −20 °C until assayed.

For antibody detection against smooth *Brucella* spp. (*B. melitensis*), the Rose Bengal test (RBT) was performed as screening test while serum agglutination test (SAT) and 2 mercapto-ethanol (2MET) were run as confirmatory assay in accordance with SENASA regulations.[12] The procedures were described by Alton et al.[1] and the antigens were prepared at the National Laboratories and Institutes of Health Administration ''Dr. C. G. Malbrán'' (ANLIS) using the *B. abortus* 1119-3 strain.

Table 1 Detection of anti-R- and anti-S-*Brucella* spp. antibodies in 486 ovines from 10 Argentine areas

Region	n F/M	RBT F/M	RSAT F/M	IELISA F/M
1	35/4	0	0	0
2	62/13	0	0/4	0
3	20/2	0	0	0
4	33/6	0	12/3	0
5	77/10	1/0	18/4	9/2
6	31/15	0	6/4	1/0
7	24/3	0	1/0	0
8	62/14	0	4/0	2/0
9	32/13	0	1/3	0
10	17/13	0	6/11	0
Total	393/93	1	48/29	12/2

F: female; M: male.
RBT: Rose Bengal test (screening test, for detection of antibodies against smooth *Brucella* spp. (*B. melitensis*).
RSAT: rapid slide agglutination test (screening test, for detection of antibodies against rough *Brucella* spp. (*B. ovis*).
IELISA: indirect ELISA, cut off ≥39 [12](confirmatory test, for detection of antibodies against rough *Brucella* spp. (*B. ovis*).

For antibody detection against rough *Brucella* spp. (*B. ovis*), the rapid slide agglutination test (RSAT) was used for screening as previously described.[7] The antigen was prepared at ANLIS using the strain (M-) variant of *B. canis*.

An indirect ELISA (IELISA) test was performed as confirmatory test; the antigen was obtained from the (M-) variant of *B. canis* as previously reported [7]and the lyophilized protein A/G, horseradish peroxidase conjugated from ImmunoPure, Pierce Lab was used. Optical density (OD) values were compared to those for the control serum included in each 96-well plate and a relative percent positivity value (%P) was calculated: (%P) = (OD test sample/OD control serum) × 100. The control serum was obtained from an experimentally-infected ram. The cut off value is ≥39.[7] Comparison of these tests with SENASA methods were previously described.[7,13]

Over 486 animals screened by RSAT and RBT, 77 (29 males) were positive by RSAT and one female from area 5 also had anti-S-*Brucella* spp. antibodies. The latter was studied by SAT and 2MET and was weakly positive by SAT (1:25) and negative by 2MET.

A total of 14 animals, 12 females and two males, were positive for both RSAT and IELISA tests, 11/14 were from area 5, 1/14 was from area 6 and 2/14 from area 8 (Table 1).

As shown in Table 1, while testing by IELISA led to differences in positive results only between areas 2 and 5, RSAT led to differences in many areas.

In Argentina, *B. melitensis* in ovines is not a major problem while *B. ovis* has been detected in most of the regions where ovines are bred. In the South of the country, a prevalence ranging from 4% to 10% was reported in Neuquén, Río Negro and Chubut provinces, while a prevalence ranging between 8 and 20% was found in Santa Cruz and Tierra del Fuego provinces.[11] In the Northeast region, a prevalence between 11% and 21% was reported and in the Humid Pampa

a survey of over 2652 animals found a prevalence of 12.9%[14]; however, there is no information about the infection in the Creole breed.

Different researchers had agreed about the rustic character of this breed and its capacity to adapt to harsh environmental conditions. The Creole breed became popular among small farmers who until their introduction had owned no livestock. They are animals that can be kept with minimum maintenance and supply the family needs for meat and wool in areas where deprived communities live. Ewes and rams are small size with hair of different colors and thin and hairless legs and were preserved in pure breed in environments where the other races did not adapt.[10]

In the present survey we have selected animals from different Argentine provinces where Creole sheep were located.

RBT detected only one ewe from area 5; however, when confirmatory tests were run this serum was weakly positive by SAT and negative by 2MET, probably due to some overlapping in the detection of antibodies against rough and smooth *Brucella* strains (data not shown).

The total positive percentage of brucellosis caused by *B. ovis* in this survey was 2.9%. RSAT is a sensitive test used as screening technique that showed 15.8% of positive results but IELISA, the confirmatory test, detected 2.9% (Table 1). As shown in Table 1, while testing by IELISA differences in positive results were observed only between areas 2 and 5 while RSAT resulted in differences among many areas. Consideration must be given to alternative risk factors for disease between the groups such as differences in local husbandry. Animals from area 5 were mixed with sheep from other flocks of Suffolk breed brought from Chile during the summer for sharing the land, using precarious husbandry practices.

Considering that the positive percentage of *B. ovis* brucellosis in other sheep breeds in Buenos Aires was 12.9% [14]and in Corrientes was 11–21%, a tentative explanation for our results could indicate that Creole sheep have some natural resistance to brucellosis. This should even be tested by a further statistically significant sample size because this is only a preliminary study.

The link between resistance to brucellosis and the race have been largely studied in different breeds; however, no differences were found between epididymitis in rams from Suffolk, Hampshire, Merino, Karakul, Persian and Dorper. Nevertheless, Hajdu in 1962 reported a brucellosis prevalence of 30% due to *B. ovis* in Tsugai and Valachian and 14% in Merinos.[5] Furthermore, when the transmission of *B. ovis* infection in rams was studied, more resistance was observed in the Rambouillet than in the Targhee and Columbia breeds.[3]

Other studies with animals produced by the crossing of resistant with susceptible breeds have demonstrated that the degree of resistance of the hybrids can vary as a function of the breeds evaluated, their age and natural or artificial infections.[2]

However, an evaluation over two years of physiological variables such as rectal temperature, cardiac and respiratory frequency, packed cell volume, white cell count, total protein, albumin, calcium, phosphorus and glycemia showed no significant differences between Creole sheep and the Texel breed used for comparison.[10]

The conservation and sustainable use of resources has become a priority, mainly because native breeds are affected by crossbreeding with foreign breeds in the interest of an increase in meat production. As a result, a loss of local genotypes that for long periods of natural selection and evolution have formed a cluster of genes related to their adaptation could have occurred.[4,15]

We can conclude from this preliminary study that the total positive percentage of brucellosis due to *B. ovis* was 2.9%, but the prevalence would only be 0.6%, if animals from area 5 where sheep were mixed with Suffolk breed are excluded.

The production of meat and wool represents an important source of income for a large number of family farms in different areas of Argentina where these genetic resources are sustainable.

Creole sheep should be considered a potential economic resource considering their adaptation to consumption of low quality pastures, adverse soil and harsh climatic conditions.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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