Risk factors associated with hair sheep brucellosis in an intensive system

Article *in* Veterinarija ir Zootechnika · April 2015

CITATION
1

READS
195

5 authors, including:

Miguel Mellado
Universidad Autónoma Agraria Antonio Narro (UAAAN)
229 PUBLICATIONS **1,785** CITATIONS
SEE PROFILE

Evaristo Carrillo
29 PUBLICATIONS **274** CITATIONS
SEE PROFILE

Beatriz Arellano
32 PUBLICATIONS **327** CITATIONS
SEE PROFILE

RISK FACTORS ASSOCIATED WITH HAIR SHEEP BRUCELLOSIS IN AN INTENSIVE SYSTEM

Victor Marin[1], Miguel Mellado[2], Efrén Díaz-Aparicio[3], Beatriz Arellano-Reynoso[4], Evaristo Carrillo[1]
[1]Department of Engineering, Torreon Institute of Technology, Torreon, Mexico
[2]Department of Animal Nutrition, Autonomous Agrarian University Antonio Narro, Saltillo, Mexico
[3]National Institute of Forestry, Agriculture, and Livestock Research,
National Center of Microbiology Research, Cuajimalpa, Mexico City 05110, Mexico
[4]Department of Microbiology and Immunology, College of Veterinary Medicine,
National Autonomous University of Mexico, Coyoacan 04510, Mexico

Correspondence to: Miguel Mellado
Autonomous Agrarian University Antonio Narro, Department of Animal Nutrition
Saltillo, Coah. 25315, Mexico
Tel: (844) 4-11-03-24; Fax: (844) 4-17-37-84; E-mail: mmellbosq@yahoo.com

Abstract. Data from 303 hair sheep kept in confinement in a hot desert environment were used to determine the incidence and some risk factors for *Brucella* seropositivity in hair sheep in northern Mexico. Serums were tested for antibodies to *B. melitensis* by the standard agglutination card test (3% cell concentration); *B. ovis* was tested by the double agar gel immuno diffusion test (AGID). Incidence was 7.26 % (95 % confidence interval, 4.6 to 10.8) for *B. melitensis* and 1.98 % for *B. ovis* (95 % confidence interval, 0.7 to 4.3). All ewes seropositive for *B. ovis* were also seropositive to *B. melitensis*. Logistic regressions were used for the evaluation of some risk factors for *Brucella* seropositivity. Body condition score, thoracic circumference, height to withers and height/thoracic circumference were not associated with seropositivity to *Brucella*. On the other hand, crossbred ewes (Dorper x Pelibuey) were 2.31 times more likely (10.4% vs. 4.8%; P=0.06) to be seropositive to *Brucella* compared with Dorper and Pelibuey ewes. The incidence of seropositive animals decreased markedly in Dorper ewes than in Pelibuey and crossbred combined (1.1 % vs 6 %; P = 0.02). The finding that Dorper ewes present a lower incidence of seropositive animals to *Brucella* compared with Pelibuey and crossbred ewes is novel. These results also highlight the fact that *Brucella melitensis* represents an important hazard for intensive sheep operations, which contradicts the earlier perception that brucellosis was only prevalent in traditional pastoral sheep flocks.

Keywords: *Brucella melitensis*, *Brucella ovis*, risk factors, Dorper sheep, Pelibuey sheep

Introduction. Ovine brucellosis is an insidious and chronic disease that occurs in a great deal of the sheep-producing areas of the world, including Mexico (Nuñes-Torres et al. 1997; Méndez-Nárez et al. 1999). The causative pathogens of ovine brucellosis are *B. ovis* and *B. melitensis* (Ridler and West, 2011). Ovine brucellosis is an important problem in small ruminants in developing nations where this reproductive disease can be widespread due to poor husbandry practices such as lambing or kidding in crowded insanitary enclosures which favours the spread of this Gram-negative coccobacillus. Additionally in some countries, like Mexico, it is not uncommon to keep goats and sheep in the same flock, which favour transspecies transmission of *Brucella melitensis*.

Brucella melitensis is highly pathogenic for humans, constituting one of the gravest zoonosis in the world, causing considerable economic losses to small ruminant producers (Benkirane 2006). *B. ovis* has a predilection for replication in the genital tract of rams, where it causes unilateral or bilateral chronic epididymitis, which impairs their fertility (Searson 1987; Tsolis et al. 2009). It is not completely known why *B. ovis* preferently colonize the epididymis; it has been proposed that this affinity is due to adhesion to mucosal epithelial cell of this duct by roughed *Brucella*, which is essential for the initiation of the pathogenesis of theses bacteria (Paolicchi, 2000). Even in the absence of noteworthy clinical changes, rams can begin shedding *B. ovis* in semen as early as 28 days after exposure and lesions of epididymitis develop as early as 36 days after exposure (Ridler et al. 2014), which has a detrimental effect on ejaculate quality (Carvalho-Júnior et al. 2012).

Ewes rarely carry the infection for more than one or two oestrus cycles (Grilló et al. 1999), but it has been postulated that ewes could play a role in keeping the infection in flocks (Afzal and Kimberling 1986; Marco et al. 1994) by transmitting *Brucella* at joining when a healthy ram serves a ewe that has recently been served by an infected ram in the same cycle. Moreover, these bacteria can alter blood circulation of sheep placenta, entailing placentitis, abortion as well as neonatal death (Ficapal et al. 1998).

This disease causes large economic loss in flocks due to increased culling of rams, as a consequence of infertility in these animals (*Brucella ovis* is recognized as the most important cause of contagious ovine epididymitis), reduced occasionally abortions and the birth of weak unviable offsprings (Libal and Kirkbride 1983). The present status of ovine brucellosis in Mexico is not well defined, due to the limited studies on this disease in sheep and the fact that the surveillance and control of ovine brucellosis is rarely implemented. In fact, vaccination against *Brucella* is rare in ewes in all zones of

Mexico (Díaz-Aparicio et al. 1996). Therefore, the aim of this study was to estimate the incidence of brucellosis in a hair sheep flock under intensive conditions. An additional objective was to identify some risk factors associated with the occurrence of seropositive sheep to *Brucella*.

Material and Methods. All animal care and experimental procedures were conducted in accordance with institutional policies for animal health and well-being and approved by the Autonomous Agrarian University Antonio Narro Animal Care and Use Committee.

Animals and housing. This study was carried out on a well-managed herd of hair sheep kept in confinement in open pens in a hot area of northern Mexico (26° 23′ N, 104°47′ W, about 1000 m above sea level). Long- term mean annual rainfall is 230 mm and average year temperature is 27°C. Ewes were Dorper, Pelibuey and crosses of Dorper x Pelibuey.

Ewes were vaccinated against several *Clostridium* and *Pasteurella* (Bayovac Blacklegol Triple® Bayer Mexico, Ecatepec, Edo. de México, Mexico) and ivermectine-based parasiticide was applied I.M. regularly. Diets were formulated based on NRC (1985) containing energy levels of 2.50 Mcal EM/kg DM and 120 g/kg crude protein on DM basis. The bulk was composed of corn silage (60 %) and a concentrate composed of soybean meal, corn, and minerals. Ewes and lambs had free access to food and water. Feed was provided twice daily at 0730 and 1600 in quantities sufficient to insure 10 % orts.

Ewes were exposed to rams year-round, in groups of approximately 40 ewes per pen. These animals were joined with mixed age rams of their respective breed or cross, at a ram/ewe ratio of 1:10. Upon weaning of their lambs (average age 70 days), ewes were immediately rebred. On the day of blood collection, the following information was recorded for each ewe: breed, height to withers, body condition score (estimated on a 0 to 5 scale), thoracic circumference and height/thoracic circumference.

Blood sampling and testing. Screened ewes (n=303 average BW = 36.3 kg) had not been vaccinated against brucellosis. Also, ewes had no history of having been tested for brucellosis. Blood was obtained in December 2013 by jugular venipuncture using 21-gauge sterile needles and disposable 5-ml plastic syringes. Blood was collected in vacuum plastic tubes (Vacutainer®) and left for 30 min at ambient temperature to obtain the serum. These serums were tested for antibodies to *B. melitensis* by the standard agglutination card test (3 % cell concentration; 98 % sensitivity and 100 % specificity; Díaz-Aparicio et al. 1999). *B. ovis* was tested by the double agar gel immunodiffusion (AGID) test using *B. ovis* Reo 198 as stock of reference.

Statistical analysis. Descriptive statistics were used to determine percentage of ewes seropositive to Brucella. 95% confidence intervals were calculated with the SAS program (Proc Freq/binomial; SAS Inst. Inc., Cary, NC, USA). To analyze factors contributing to the probability of positive reaction to brucellosis (binary outcome), a multiple logistic regression model of SAS (LOGISTIC procedure) was used. The model included the following potentially explanatory variables of interest: breed (Dorper, Pelibuey and crossbreed), body condition score, and thoracic circumference, height to withers and height /thoracic circumference. Serum antibody status was the dependent variable. Body condition score was classified as being ≤3.3 or >3.3 units. Height to withers was categorized as lower or greater than 64 cm. Thoracic circumference was coded as less or higher than 85 cm. Height/thoracic circumference was classed as lower or greater than 0.75 units.

Results. Out of a total 303 ewes tested, 22 were positive (7.26 %; 95 % CI= 4.6-10.8) to *Brucella melitensis* on the standard card test. Six of the ewes seropositive to *B. melitensis* (1.98 %; CI= 0.7-4.3) also resulted positive to antibodies of *B. ovis* on the AGID test, with no ewes testing positive for only *B. ovis*. Body condition score, thoracic circumference, height to withers and height/thoracic circumference had no significant (P>0.05) influence on seropositivity to brucellosis (Table 1). Listed in Table 1 are factors which significantly affected the likelihood of ewes becoming seropositive to brucellosis. Crossbred ewes were 2.3 times more likely (P = 0.06) to be *Brucella*-positive than ewes of other breeds. On the other hand, the Dorper breed was identified as a protective factor for seropositivity for this disease, compared to all other breeds.

Table 1. **Risk factors associated with *Brucella* seropositivity in hair sheep in an intensive system in a hot arid-environment in Mexico**

Variable	n	Incidence	OR	CI OR	P
Genotype					
Crossbred	135	0.104	2.31	0.94 – 5.69	0.06
Pelibuey + Dorper	168	0.048	Reference		
Pelibuey	78	0.089	1.38	0.54 – 3.52	0.49
crossbred + Dorper	225	0.06	Reference		
Dorper	90	0.011	0.10	0.01 – 0.77	0.02
Pelibuey + crossbred	213	0.985	Reference		
Results presented as odds ratio (OR) and 95% confidence intervals (CI).					

Discussion. Because *B. ovis* is a rough strain that lacks O-LPS chain and *B. melitensis* is a smooth strain, they do not cross-react serologically with each other, thus, few ewes had simultaneously antigens against both strains

of *Brucella*, which indicates that possibly *B. melitensis* infection co-exists with *B. ovis*. These results reaffirm that sheep brucellosis is a zoonosis mainly due to *B. melitensis* with a minor involvement of *B. ovis* in ewes. This is one of the few studies to examine the incidence of *Brucella* serum antibody status in ewes in intensive systems. Prior to this study there was the perception that there is little brucellosis in commercial sheep flocks in Mexico, particularly in intensive systems. In fact, Carrera-Chavez et al. (2013) detected reactor rams to *B. ovis* antigens in semi-intensive and extensive systems, but not in intensive systems in Mexico. Lack of adequate sheep and goat *Brucella* control program in Mexico may contribute to the occurrence of ovine brucellosis in this particular intensive ovine operation.

The 7.26 % incidence of *Brucella*-seropositive ewes in the present study is very close to the 7.2 value found by Al-Talafhah et al. (2003) for native sheep of Jordan (ELISA test), 6.9–7.5 % for native sheep of Brazil (Agar gel immunodiffusion) observed by Alves et al. (2010) and Araujo et al. (2013) and 5.41 % by the BAPA test in native sheep of Egypt under pastoral condition (Samaha et al. 2008). The incidence of positive reactors to *Brucella* in the present study is more than twice the national figures reported to be 2.4 % in previous studies (Nuñes-Torres et al. 1997). This higher incidence is of major concern because it highlights the fact that the good management practices applied to this particular flock, including thorough hygiene practices, did not avoid the spread of positive reactors among ewes. In Mexico the brucellosis problem largely centres on goats, which gives rise to widespread infections of man, but this study demonstrate that sheep could also represent a reservoir of this infection.

Underfed sheep are expected to have a decreased immunity response that is manifested by animals in poor body condition (Lacetera et al., 2001). This response was not observed in the present study, which is agreement with other studies in camels (Hadush et al., 2013) and cattle (Kebede et al., 2008).

The fact that crossbred ewes presented higher risk for brucellosis seropositivity indicates that no apparent advantage for heterozygosity to brucellosis resistance exists in crossbred ewes. The observation that Dorper ewes compared with other hair breeds of ewes were less likely to be *Brucella* seropositive is in line with the data of Araujo et al. (2013), who also reported lower seroprevalence of *Brucella* in Dorper sheep compared to Santa Ines and crossbred ewes. Natural resistance against brucellosis has been demonstrated in bovines, particularly in cattle adapted to harsh environments, and it is linked with the ability of macrophages to prevent intracellular replication of *Brucella abortus* (Macedo et al. 2013) and the Nramp1 gene, which enhances innate and adaptive immunity favouring bacterial killing by macrophages (Paixão et al. 2006). Thus, Dorper sheep apparently have developed adaptive traits to resist *Brucella* infection.

Conclusions. The obtained results indicate that the incidence of *Brucella*-seropositive in hair sheep in this intensive sheep operation in north-eastern Mexico is not greater than seroprevalence among sheep in other countries. The study highlights mainly the presence of antibodies to *Brucella melitensis* in sheep. Sheep producers in intensive systems also are provided with evidence that strong variation in breed susceptibility to this infectious disease exists, with evidence that Dorper sheep are less susceptible to present serum antibodies for *Brucella* than Pelibuey and crosses between Dorper and Pelibuey ewes.

Acknowledgements

Homero Gonzalez is acknowledged for allowing data collection in the sheep flock and participating in the study.

Conflict of interest

None of the authors have any conflict of interest to declare.

References

1. Afzal M., Kimberling C. How to control *Brucella ovis*-induced epididymitis in rams. Veterinary Medicine., 1986. 81. P. 364–371.

2. Al-Talafhah A. H., Lafi S. Q., Al-Tarazi Y. Epidemiology of ovine brucellosis in Awassi sheep in Northern Jordan. Preventive Veterinary Medicine. 2003. 60. P. 297–306.

3. Alves C. J., Figueiredo S. M., Azevedo S. S., Clementino I. J., Keid L. B., Vasconcelos, S. A., Batista, C. S. A., Rocha, V. C. M., Higino, S. S. Detection of *Brucella ovis* in ovine from Paraíba State, in the Northeast Region of Brazil. Brazilian Journal of Microbiology. 2010. 41. P. 365–367.

4. Araujo B. R., Nunez-Costa J. N., Sampaio-de Souza T. S., Valenca de Lima C. C. V., Leite M. D. X., Oliveira-Costa-Neto A., Magnavita-Anunciacao V., Avila-Ribeiro-Almeida M. G., Barbosa de lima E. Seroepidemiology of sheep brucellosis in the microregion of Feira de Santana, BA, Brazil. Brazilian Journal of Veterinary Research and Animal Science. 2013. 50. P. 129–135.

5. Benkirane A. Ovine and caprine brucellosis: World distribution and control/ eradication strategies in West Asia/North Africa region. Small Ruminant Research. 2006. 62. P. 19–25.

6. Carrera J. M., Echavarría F. G., Aréchiga C. F., Bañuelos R., Tórtora J. L. Consideraciones epidemiológicas en la prevalencia serológica de *Brucella ovis* en Zacatecas, México (Possible risk factors for serological prevalence of *Brucella ovis* in Zacatecas, Mexico). Revista Mexicana de Ciencias Pecuarias. 2013. 4. P. 61–74.

7. Carvalho-Júnior C. A., Moustacas V. S., Xavier M. N., Costa E. A., Costa L. F., Silva T. M. A., Paixão T. A., Borges A. M., Gouveia A. M. G., Santos R. L. Andrological, pathologic, morphometric, and ultrasonographic findings in rams experimentally infected with *Brucella ovis*. Small Ruminant Research. 2012. 102.

P. 213–222.

8. Díaz-Aparicio E., Blasco-Martínez J. M. Suárez-Güemes F. Prueba de tarjeta modificada para el diagnóstico de la brucelosis caprina (Modified card test for diagnosis of brucelosis in goats). Técnica Pecuaria México. 1999. 30. P. 307–311.

9. Díaz-Aparicio E., Moriyon-Uria I., Blasco-Martínez J. M., Marin-Alcala C., Díaz-García R. Diagnóstico de *brucella melitensis* en ovinos usando inmunodifusion radial con hapteno nativo (Radial inmunodiffusion with native hapten for diagnosis of *brucella melitensis* in sheep). Técnica Pecuaria México. 1996. 34. P. 99–103.

10. Ficapal A., Jordanab J., Blasco J. M., Moriyónd I. Diagnosis and epidemiology of *Brucella ovis* infection in rams. Small Ruminant Research. 1998. 29. P. 13–19.

11. Grilló M., Marín M., Barberan M., Blasco J. Experimental *Brucella ovis* infection in pregnant ewes. Veterinary Record. 1999. 144. P. 555–558.

12. Hadush A., Pal M., KassaT., Zeru K. F. Sero-epidemiology of camel brucellosis in the Afar region of Northeast Ethiopia. Journal of Veterinary Medicine and Animal Health. 2013. 5. P. 269–275.

13. Kebede T., Ejeta G., Ameni G. Seroprevalence of bovine brucellosis in

smallholder farms in central Ethiopia (Wuchale-Jida district). Revue de Médecine. Véterinaire. 2008. 159. P. 3–9.

14. Lacetera N., Bernabucci, U., Ronchi, B., Nardone, A. Effects of subclinical pregnancy toxemia on immune responses in sheep. American Journal of Veterinary Research., 2001. 62. P. 1020–1024.

15. Libal M. C., Kirkbride C. A. *Brucella ovis*-induced abortion in ewes. Journal of the American Veterinary Medical Association. 1983. 183. P. 553–554.

16. Macedo A. A., Costa E. A., Silva A .P., Paixão T. A., Santos R. L. Monocyte-derived macrophages from Zebu (*Bos taurus indicus*) are more efficient to control *Brucella abortus* intracellular survival than macrophages from European cattle (*Bos taurus taurus*). Veterinary Immunology and Immunopathology. 2013. 151. P. 294–302.

17. Marco J., Gonzalez L., Cuervo L., de Heredia F. B., Barberan M., Marin C., Blasco J. *Brucella ovis* infection in two flocks of sheep. Veterinary Record. 1994. 135. P. 254–256

18. Méndez-Nárez G., Díaz-Aparicio E., Morales-Álvarez J. F. Aguilar-Romero F., Suárez-Güemes F. Epidimitis ovina: Estudios bacteriológico y serológico (Ovine epidedymitis: bacteriological and serological studies). Técnica Pecuaria México. 1999. 30. P. 329–336.

19. Nuñes-Torres E. D., Díaz-Aparicio E., Velazquez-Quezasa F., Trigo-Tavera F., Suárez-Güemes F. Presencia de anticuerpos contra diferentes especies de Brucella en sementales ovinos jóvenes (Presence of antibodies against different species of *Brucella* in young rams). Técnica Pecuaria México. 1997. 28. P. 241–245.

20. Paixão T. A., Ferreira C., Borges A. M., Oliveira D. A., Lage A. P., Santos R. L. Frequency of bovine Nramp1 (Slc11a1) alleles in Holstein and Zebu breeds. Veterinary Immunology and Immunopathology. 2006. 109. P. 37–42.

21. Paolicchi F. A., Casaro P. A., Gimeno E. J., Kortebani L. G., Mazzolli A. B. Antisperm response in rams experimentally infected with *Brucella ovis*. Small Ruminant Research. 2000. 36. P. 7–15.

22. Ridler A. L., Smith S. L., West D. M. Seroconversion and semen shedding in rams experimentally infected with *Brucella ovis*. New Zealand Veterinary Journal. 2014. 62. P. 47–50.

23. Ridler A. L., West D. M. Control of *Brucella ovis* infection in sheep. Veterinary Clinics of North America: Food Animal Practice. 2011. 27. P. 61–66.

24. Samaha H., Al-Rowaily M., Khoudair R. M., Ashour H. M. Multicenter study of brucellosis in Egypt. Emerging Infectious Diseases. 2008. 14. P. 1916–1918.

25. Searson J. E. The distribution of histopathological lesions in rams reacting in a complement fixation test for *Brucella ovis*. Australian Veterinary Journal. 1987. 64. P. 108–109.

26. Tsolis R. M., Seshadri R., Santos R. L., Sangari F. J., Lobo J. M., de Jong M. F., Ren Q., Myers G., Brinkac L. M., Nelson W. C., Deboy R. T., Angiuoli S., Khouri H., Dimitrov G., Robinson J. R., Mulligan S., Walker R. L., Elzer P. E., Hassan K. A., Paulsen I. T. Genome degradation in *Brucella ovis* corresponds with narrowing of its host range and tissue tropism. PLoS One. 2009. 4. P. 1–9.

Received 17 March 2014
Accepted 24 October 2014