DIAGNOSTIC SURVEY ON CONTAGIOUS EPIDIDYMITIS OF RAMS IN PIEDMONT (ITALY)

Gennero M.S.*; Grattarola C.*; Zoppi S.*; Goria M.*; Di Giannatale E.°; Dondo A.*
**Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle D'Aosta*
° Centro di Referenza Nazionale per la Brucellosi (Teramo)

Introduction

Brucella ovis is the causative agent of contagious epididymitis of rams. It produces a clinical or subclinical disease in sheep that is characterised by genital lesions in rams and the main consequence of the disease is reduced fertility.

The disease is world-wide distributed and in Europe has been reported in France, Germany, Hungary, Romania, Russia, the Slovak Republic, Spain, but probably occurs in most sheep-raising countries (1) and in Northern Italy.

The existence of clinical lesions (unilateral or, occasionally, bilateral epididymitis) in rams may be indicative of the existence of infection, but laboratory examinations are necessary to confirm the disease. Laboratory confirmation may be based on direct (bacteriological isolation of *B. ovis* from infected tissues) or indirect methods (Complement Fixation Test, CFT). Molecular biological methods, such as polymerase chain reaction is being developed.

In 1998, after having done researches in order to establish the prevalence of epididymitis in rams on national territory, we pointed out in 2003, many positive serologic reactions to *B. ovis*.

Flocks on which we have diagnosed the infection, were officially brucellosis free since 1996 and usually go to mountain pasture during summer in regional territories.

Material and Methods

We have tested 634 animals by serological test (CFT).

Two positive rams were slaughtered and their samples were further processed with other laboratories researches: anatomo-pathological testing to elicit the presence, on these two animals, of the purulent orchids-epididymitis. Further more we used bacteriology and molecular biology techniques on tissue samples (spleen, testicles, epididymus) considering the difficulties in isolating *Brucella ovis*.

Serology - We used technique according to bibliography (5) and optimised by National Health Superior Institute.

Bacteriology – Tissue were macerated in sterile saline in a Stomacher. The whole material was inoculated onto selective medium developed by Farrell (7), with the addition of 10% horse serum and incubated at 37°C in an atmosphere containing 5% CO_2 for 15 days. All colonies resembling Brucella were sub-cultured onto blood agar medium with 5% sterile ovine blood and incubated for a further 2 days before re-examination. If Brucella was suspected using Stamp's staining (3), then the colonies were identified to species by classical techniques (2)

Molecular Biology (PCR) – We used primers according to bibliography (4,6), while the amplification protocol was optimised by our Laboratories.

Results

28 animals among 634 were resulted seropositive, both males and females. Bilateral orchid-epididymitis (ascessual form) was detected at necropsy.

Bacteriological testing has elicited the presence of *Corynebacterium* spp in both rams and the presence of *Brucella* spp in one of two rams. Species identification by PCR done in Brucellosis Reference Centre Laboratories, confirmed our diagnosis and the presence of *B. ovis*.

Molecular biology has detected *Brucella* spp on two examined rams.

Biochemical tests	*B.* spp (other than *B. ovis*)	*B. ovis*
Catalase	+	+
Oxidase	variable	-
Urease	variable	-
Mobility to 37°C	-	-
Mobility to 20°C	-	-
Lactose fermentation	-	-
Haemolysis	-	-
Nitrate reduction	+	-
Indole production	-	-
H_2S production	variable	-
CO_2 requirement	variable	+

Table n. 1: Biochemical differences between B. spp and B. ovis

Discussion

However, indirect diagnosis based on serological tests is preferred for routine diagnosis.

The demonstration of the existence of genital lesions (bilateral orchid-epididymitis) by palpating the testicles of rams was indicative of the presence of *B. ovis* infection in this flock. However, this clinical diagnosis is not sensitive enough because only about 50% of rams infected with *B. ovis* present epididymitis (5).

Moreover, the clinical diagnosis is extremely unspecific due to the existence of many other bacteria causing clinical epididymitis, e.g. *Corynebacterium* spp.

Conclusion

Bacteriological and molecular biology methods let us to confirm the presence of B. *ovis* on two seropositive rams. This case-report is very important for evaluation of *B. ovis* presence in Italy.

References

1. AA.VV., 2000. Ovine Epididymitis (*Brucella ovis*) in Manual of standards Diagnostic Tests and Vaccines, OIE.
2. Alton, G. G., Jones, L.M., 1967. Laboratory Techniques in *Brucella*, WHO.
3. Alton G.G., Jones L.M., Angus R.D. & Verger J.M., 1988. Techniques for the Brucellosis Laboratory. INRA, Paris, France.
4. Baily, G.G. et al., 1992. Detection of Brucella melitensis and Brucella abortus by DNA Amplification. J. Of Trop. Med. And Hyg. 95, 271-75.
5. Blasco J.M., 1990. *Brucella ovis*. in Animal Brucellosis, Nielsen K. & Duncan J.R., eds. CRC Press, Boca Raton, Florida, USA, 351-378.
6. Casanas, M.C. et al., 2001. Specificity of a Polymerase Chain Reaction Assay of a Terget sequence on the 31 Kd Brucella Antigen DNA used to diagnose Human Brucellosis. Enr. J. Of Clin. Microbiol & Inf. Dis. 20 (2), 127-31.
7. Farrell, I.D., 1974. The development of a new selective Medium for the isolation of *Brucella abortus* from contaminated sources. Res. Vet. Sci. 16, 280-86.