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EXPERIMENTAL *BRUCELLA OVIS* INFECTION IN MOUFLON (*OVIS MUSIMON*)

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ABSTRACT: *Brucella ovis* was isolated for the first time in Italy in 1994 from the genital organs of two domestic rams. In subsequent years bacteriologic and serologic investigations demonstrated an increasing distribution of this disease in domestic sheep. Mouflon (*Ovis musimon*) occur in several hilly and mountainous areas of Italy where they can potentially contact domestic sheep. To determine if this species may have a role in the epidemiology of *B. ovis,* four male and four female mouflon, serologically negative for *B. ovis* and other *Brucella* spp., were infected intraconjunctivally with *B. ovis* strain BG1/94. Physical examinations, including collection of blood samples for serology and bacteriology, were performed weekly. The animals were euthanized 8 mo postinoculation (p.i.). Samples of retropharyngeal, parotid, and iliac lymph nodes; bone marrow; kidneys; spleen; epididymis; testicle; bulbourethral glands; seminal vesicles; uterus; and oviducts were collected from each animal as appropriate for histopathology and bacteriology. At the time of euthanasia none of the animals exhibited obvious clinical signs of brucellosis. The animals seroconverted 2 wk p.i. and became seronegative 24 wk p.i. Bacterial cultures, including hemocultures, were negative. No lesions due to *B. ovis* infection were revealed by histologic examinations. *Brucella ovis* probably did not infect mouflon and this wild sheep is not likely to play a role in the epidemiology of contagious epididymitis caused by *B. ovis.*

Key words: *Brucella ovis,* experimental infection, microbiology, mouflon, *Ovis musimon,* serology.

INTRODUCTION

The mouflon (*Ovis musimon*) is a wild and highly adaptable species. It is endemic in Sardinia (Italy) and Corsica (France) where it has been present from the Neolithic Period. In the last century, mouflon have been introduced throughout Europe, even though its introduction is not recommended because of competition with other species and the possibility of hybridization with domestic sheep. Mouflon and domestic sheep often graze the same pastures, which could expose them to mutual infections. Little is known about the susceptibility of mouflon to contagious epididymitis caused by *Brucella ovis,* which occurs in domestic sheep.

Brucella ovis causes a chronic disease characterized by granulomatous epididymitis, often unilateral, and testicular atrophy, with subsequent low fertility of rams. Abortion occurs occasionally in ewes, and perinatal mortality is frequently reported in infected flocks. *Brucella ovis* infection was first reported in domestic sheep in Australia and New Zealand (Buddle and Boyes, 1953) and it is widespread in South and North America, South Africa, and many European countries (Blasco, 1990). In 1994, *B. ovis* was isolated for the first time in Italy from epididymis and testicles of two domestic rams (Farina et al., 1995). The disease has spread since then as demonstrated by bacteriologic (Bassi et al., 1998) and serologic investigations (Ciuchini et al., 1998).

Passive venereal transmission from ram to ram via ewes is the primary route for spread of *B. ovis* infection, however ingestion of feed or water contaminated by aborted fetuses or vaginal discharge may also allow transmission of the disease (Bulgin, 1990a, b). *Brucella ovis* may localize in the kidneys and its presence in urine

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has been demonstrated in rams (Bassi et al., 1998).

Considering the absence of information about susceptibility of small wild ruminants to *B. ovis* infection, the purpose of this study was to investigate the response of mouflon to experimental *B. ovis* infection in order to verify whether this wild animal may play a role in the epidemiology of brucellosis.

MATERIALS AND METHODS

Four female (two had lambed some weeks earlier) and four 2–3 yr old male mouflon, were used. The animals were captured in a hilly area of Tuscany (Miemo, Pisa, Italy; 43°28′N,10°40′E); they were marked with ear tags and kept in a paddock for 32 wk. This area, of about 90,000 m^2, was fenced and covered by a metal net. The animals were fed alfalfa hay, fodder, and fresh water daily. For collection of samples, the mouflon were pushed into a containment cage. Two lambs were left with their dams as uninfected controls. All animals were serologically negative for *B. ovis* and *Brucella* spp.

The eight adult mouflon were infected intraconjunctivally with 0.5 ml (0.25 ml/eye) of a solution containing 3.4×10^9 colony forming units/ml of *B. ovis* strain BG1/94 (Farina et al., 1995) prepared as previously described (Alton et al., 1988). Briefly, *B. ovis* strain BG1/94 was cultured on *Brucella* medium base (BMB, Difco, Detroit, Michigan, USA) with 10% decomplemented horse serum. Microorganisms were harvested with sterile normal saline solution and passed through a sterile gauze. Serial dilutions (from 10^{-5} to 10^{-9}) were passed onto BMB plates. After incubation at 37 C for 4 days colonies were counted and the number of viable bacteria/ml of suspension was calculated. After instillation, the eyelids were manually closed for about 60 sec to increase conjunctival adsorption.

Blood for serology was collected from the jugular vein (Vacutainer system, Belliver Industrial Estate, Plymouth, UK) before infection and weekly for 8 wk postinoculation (p.i.), and at 10, 12, 14, 16, 18, 24, and 32 wk p.i. Blood was allowed to clot, centrifuged, and the serum harvested and stored at −20 C. Hemocultures were done on blood samples collected weekly from 1–8 wk and at 10, 12, and 14 wk p.i. Bacteriology was performed on milk samples manually collected at 4, 6, 7, and 8 wk p.i. from the two females with lambs. At 32 wk p.i., after deep anesthesia was induced, the inoculated animals were euthanized (intravenous Tanax® injection; 5 ml containing embutramide 200 mg/ml, mebezone iodure 50 mg/ml, and tetracaine chloridrate 5 mg/ml; Hoechst Roussel Vet Gmbh, Munich, Germany) and immediately necropsied. The lambs remained serologically and bacteriologically negative and were not sacrificed. Tissues used for microbiology and pathology were selected on the basis of *B. ovis* experimentally and naturally infected domestic sheep (Alton et al., 1988; Blasco, 1990; Cerri et al., 1999). Representative portions of epididymis, testicles, bulbourethral glands, seminal vesicles, uterus, oviducts, spleen, kidneys, and bone marrow, and retropharyngeal, parotid, and iliac lymph nodes were collected for bacterial culture and histopathology.

Serum samples were tested using the complement fixation (CF) test for the detection of specific antibodies against *B. ovis* (Alton et al., 1988). The antigen was a hot saline extract of *B. ovis* strain 63/290 titered with standard Weybridge serum (Central Veterinary Laboratory, Weybridge, UK). Titers of ≥1:4 (corresponding to ≥50 CF test international units/ml), were considered positive.

To verify the presence of bacteremia, three drops of whole blood, aseptically collected with heparin, were cultured in tubes containing BMB with 10% decomplemented horse serum. One ml of milk was cultured in tubes containing the same medium. All tubes were incubated at 37 C with 10% CO_2 for 3–5 days. Culture of the organs sampled was carried out as previously described (Cerri et al., 1999).

Representative portions of each organ were fixed in buffered formalin at pH 7.2 and embedded in paraffin. Five μm-thick sections were stained with hematoxylin and eosin and specific stains for reticular tissue (Gomori's stain) and connective tissue (Masson's thricrome). Periodic acid-Schiff and Ziehl-Neelsen stains were also performed.

RESULTS

None of the animals exhibited obvious clinical signs of brucellosis during the study. Tests on sera collected prior to the study to detect the presence of specific anti-*B. ovis* antibodies were negative. All the inoculated animals seroconverted on the CF test at 2 wk p.i.; then the specific antibody titers declined (Table 1). The two uninfected lambs remained seronegative. Cultures of blood and milk samples and tissues collected at necropsy were consistently negative in all animals.

At necropsy, all the mouflon were in

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TABLE 1. Complement fixation titers in adult mouflon experimentally inoculated with *Brucella ovis.* The results are expressed as reciprocal of the highest positive serum dilution or as negative (−).

Mouflon number	Sex[a]	Weeks postinoculation															
		0	1	2	3	4	5	6	7	8	10	12	14	16	18	24	32
1	M	−	−	32	16	64	32	32	16	8	4	−	−	−	−	−	−
2	M	−	−	64	16	64	16	16	8	4	−	−	−	−	−	−	−
3	F	−	−	32	32	32	8	8	−	−	−	−	−	−	−	−	−
4	M	−	−	256	128	64	32	16	8	4	−	−	−	−	−	−	−
5	M	−	−	256	128	64	32	16	16	16	16	16	4	4	−	−	−
6	F	−	−	256	256	128	64	32	32	32	16	16	4	4	4	−	−
7	F	−	−	128	128	32	16	8	−	−	4	4	−	−	−	−	−
8	F	−	−	64	256	128	32	32	16	16	4	4	−	−	−	−	−

[a] M = male, F = female.

good body condition. No significant gross lesions were present in lymph nodes, spleen, or male and female genital organs. Parasitic pneumonia, caused by *Muellerius capillaris,* was observed in one male mouflon. No specific histologic lesions due to *B. ovis* infection were found.

DISCUSSION

Both male and female mouflon had low susceptibility to experimental *B. ovis* infection, even though the inoculum used was 1.5 times higher than that previously used to induce an experimental infection in domestic rams (Cerri et al., 1999). However, all the wild sheep seroconverted with antibodies evident in five of eight *B. ovis*-infected animals at 10 wk p.i. The antibody titers detected in the experimentally infected mouflon were similar to those previously observed in domestic rams experimentally infected with *B. ovis* (Cerri et al., 1999).

Because all blood cultures were negative, it was not clear if bacteremia did not occur or if it lasted only for a short time. However, high antibody titers and duration of antibodies in serum suggest occurrence of transient bacteremia with consequent involvement of the immune system. In contrast, domestic ram lambs intraconjunctivally infected with the same *B. ovis* strain BG1/94 developed bacteremia and mounted a strong specific immune response at 3, 4, and 5 wk p.i. (Cerri et al., 1999). These animals also showed macroscopic and microscopic lesions in genital organs and *B. ovis* was isolated from urine and genital organs (Cerri et al., 1999).

Antibodies to *B. abortus* and *B. melitensis* have been sporadically demonstrated in wild sheep. Only one of 31 mouflon shot in Ecrins Massif in France (Gourreau et al., 1993) and one of seven mouflon from Jaén in Spain (Leon-Vizcaino et al., 1985) were positive for specific anti-*Brucella* antibodies. Suspected serologic reactions occurred in three of 619 (0.5%) desert bighorn sheep (*Ovis canadensis nelsoni*) (Drew et al., 1992).

In contrast, other wild ruminants play an important role in the epidemiology of brucellosis both in Paleartic and Neoartic regions. Brucellosis has been reported from roe deer (*Capreolus capreolus*) (Guiraud et al., 1984), fallow deer (*Cervus dama*) (McDiarmid, 1951; Leon-Vizcaino et al., 1985), red deer (*Cervus elaphus hispanicus*) (Leon-Vizcaino et al., 1985), and reindeer (*Rangifer tarandus tarandus*) (Golosov and Zabrodin, 1959). Cases of brucellosis in wild ruminants are sporadic in Europe. Clinical cases of *Brucella melitensis* infections have been reported in two roe deer from the Pyrenees in the south of France (Guiraud et al., 1984), in four chamois (*Rupicapra rupicrapa*) from the central and southern French Alps (Garin-Bastuji et al., 1990), and one alpine ibex (*Capra ibex*) from Gran Paradiso Na-

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tional Park in the north of Italy (Ferroglio et al., 1998). In contrast, in North America brucellosis is endemic in some populations of bison (*Bison bison*), elk (*Cervus elaphus nelsoni*), and caribou (*Rangifer tarandus tarandus*) (Davis, 1990).

Our study demonstrated that mouflon are resistant to *B. ovis* strain BG1/94. It does not appear that mouflon play an important role in the epidemiology of brucellosis caused by *B. ovis.*

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