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# Seroprevalence of *Babesia ovis* in Mouflon Sheep in Spain

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ABSTRACT: A serological survey detected antibodies against *Babesia ovis* in mouflon sheep (*Ovis musimon*) from two different reserves located in Catalonia in northeastern Spain. An indirect fluorescent antibody test (IFAT) was developed using a *B. ovis* isolate of ovine origin as the antigen. Of 50 sera tested, six (12%) showed titres between 1:160 and 1:640 and were considered positive. These results indicate that exposure of mouflon to *Babesia ovis* is common in this region.

*Key words:* *Babesia ovis*, indirect fluorescent antibody test, mouflon, *Ovis musimon*, serology, serosurvey.

*Babesia* spp. are intraerythrocytic protozoan parasites of domestic and wild animals causing anemia and hemoglobinuria. Babesiosis in domestic small ruminants is due to at least three species, namely *Babesia ovis*, *Babesia motasi*, and *Babesia crassa*. The taxonomy of the *Babesia* spp. of sheep and goats is not well-defined (Friedhoff, 1988). Sheep babesiosis caused by *B. ovis* is known to occur in the Mediterranean basin as well as in other areas where the tick vector is present (Yeruham et al., 1992). *Babesia ovis* is transmitted by the two-host tick *Rhipicephalus bursa*. This tick species is widespread between the latitudes 31° and 45°N (Yeruham et al., 1985). The aim of this work was to determine the seroprevalence of *Babesia ovis* in two different populations of mouflon sheep (*Ovis musimon*) in Catalonia (northeastern Spain). For this purpose, an indirect fluorescent antibody test (IFAT) was developed using a *B. ovis* isolate of ovine origin as the antigen.

Blood was collected from several domestic sheep suffering from acute babesiosis. Blood samples were scanned for the presence of *B. ovis*; positive samples were mixed and inoculated intravenously into a previously splenectomised sheep. The parasitaemia was monitored daily by examination of thin blood smears stained with Giemsa. Blood for indirect fluorescent antibody test (IFAT) antigen preparation was drawn at maximum parasitemia of 2%. Venous blood was drawn and diluted 1:20 with phosphate buffered saline (PBS), pH 7.2 immediately after bleeding and washed four times at 700 × g for 10 min with PBS. The final sediment of washed erythrocytes was restored to the initial blood volume. This final suspension was dispensed in drop preparations on 10-well, glass slides using a special applicator (Christensson, 1986). Once allowed to dry, they were fixed in ice-cold acetone and kept at −80C until used.

Sera were collected from two separated mouflon populations in Catalonia from 1991 to 1996. Twelve and 38 samples were collected in the Hunting Reserves of Tortosa-Beseit (40°45′N, 0°15′E), and Freser-Setcases (42°23′N, 2°15′E), respectively. Sera belonged to 34 females and 16 males. Animals were hunter-killed; immediately after death blood samples were obtained by intracardiac puncture.

Positive and negative control sheep sera for *B. ovis* were kindly provided by M. A. Habela (School of Veterinary Medicine, Cáceres, Spain). A standard IFAT procedure was developed as follows. Antigen slides were incubated overnight with 1% bovine serum albumin in PBS (pH 7.2) to avoid nonspecific antibody binding in the subsequent incubations. Slides were washed three times for 5 min each with 0.05% Tween-20 in PBS (PBS-T). Test sera were used at dilutions ranging from 1:160 to 1:2,560 for titration and at 1:160 for the determination of the prevalence of infection. This latter titre was chosen because it had given the best sensitivity and

specificity results in a previous checkerboard titration with positive and negative control sera. Serum drops were dispensed into each slide well. The slides were subsequently incubated at 37 C in a humid chamber for 30 min and then washed three times in PBS-T. Commercial fluorescein isothiocyanate-conjugated rabbit anti-sheep IgG (Sigma Chemical Co., St. Louis, USA) was used at a dilution of 1:160 in PBS. The slides were incubated and washed as before. They were dried, mounted in buffered glycerine (9 parts of glycerine and 1 part of PBS) and examined immediately with an Olympus fluorescence microscope at a magnification of ×400. The definitive titres were determined as the lowest dilution of sera giving a positive fluorescence pattern.

In order to avoid false positive results in the IFAT, antigen slides were tested for the presence of antibodies bound to the erythrocyte cell membranes. Several randomly chosen antigen slides were incubated with fluorescein isothiocyanate-conjugated rabbit anti-sheep IgG alone.

Of the 50 sera tested, six (12%) were considered positive. Two and four positive results were found in the Hunting Reserves of Freser-Setcases and Tortosa-Beseit, respectively. The highest titre, 1:640, was found in two animals, while titres 1:320 and 1:160 were detected in one and three animals, respectively. All positive sera belonged to females. No fluorescence was detected in the antigen slides incubated with fluorescein isothiocyanate-conjugated rabbit anti-sheep IgG.

Our results of seroprevalence show that exposure of mouflon sheep to *Babesia ovis* is common in the studied areas. *Babesia ovis* has been recorded in blood smears from a mouflon from the Reserve of Tortosa-Beseit (S. Lavín, unpublished data). However, clinical cases of babesiosis due to *B. ovis* are regularly found in sheep which share pastures with this wild ruminant. *Rhipicephalus bursa* is the most common tick found on both domestic and wild ruminants in this area. *Babesia ovis* is transmitted transstadially and transovarially in this vector tick. *Rhipicephalus turanicus* also is found regularly on ruminants in this area, but its role in the transmission of *B. ovis* is not well-determined.

*Babesia capreoli* has been reported in several species of Cervidae in Europe, but not in Spain. Moreover, it has never been reported in Bovidae. The vector tick for *B. capreoli, Ixodes ricinus,* can be regularly found on domestic and wild ruminants in the Hunting Reserve of Freser-Setcases, but never in Tortosa-Beseit.

Serological cross-reactivity between species of *Babesia* in small ruminants was studied by Papadopoulos et al. (1996) in Greece. These authors reported that cross-reactions between *Theileria ovis* and the three *Babesia* spp. of small ruminants are non-existing or weak. They also concluded that there are common epitopes between *B. motasi* and *B. crassa,* and to some extent also between them and *B. ovis.* According to these cross-reactions, found only at low dilutions, they fixed the specificity level of the test at 1:160 in order to avoid false-positive results. This is the cut-off titre we fixed for our IFAT. Additionally, in another study carried out in Spain Habela et al. (1990) found no cross-reaction between *B. ovis* antigens and *B. motasi, B. crassa,* or *T. ovis.*

Considering the above studies we herein conclude that the test used in our serological survey was specific for *B. ovis* and that this mouflon population might be acting as reservoir of this parasite.

In our IFAT, antigen slides should be prepared from donor animals before they have the chance to form enough of their own antibodies to adhere to the red cell membrane and thus give false positive results. As we have indicated, we did not find any reaction when the slides were incubated with the conjugated antibody alone.

*Babesia* spp. have been documented in several wild ruminant species. In the USA *Babesia odocoilei* has been isolated from white-tailed deer (*Odocoileus virginianus*)

in Texas (Emerson and Wright, 1968) and Oklahoma (Waldrup et al., 1989) and other *Babesia* spp. have been isolated from bighorn sheep (*Ovis canadensis*) in California (Goff et al., 1993; Thomford et al., 1993), mule deer (*Odocoileus hemionus*) in California (Thomford et al., 1993), caribou (*Rangifer tarandus caribou*) in Minnesota (Holman et al., 1994a) and elk (*Cervus elaphus*) in Texas (Holman et al., 1994b). In Europe, *Babesia capreoli* was first described by Enigk and Friedhoff (1962) in roe deer (*Capreolus capreolus*), and this species has been documented subsequently in red deer (*Cervus elaphus*) in Scotland (Blewett and Adam, 1978) and sika deer (*Cervus nippon*) in Ireland (Gray et al., 1991). Hinaydi (1987) reported latent asymptomatic *Babesia* spp. infections in red deer and roe deer in Austria as well as acute fatal cases of babesiosis in Père David's deer (*Elaphurus davidianus*). However, *Babesia* spp. have not been reported previously in wild ruminants in Spain, although 33% positive titres to *B. ovis* have been recently reported in a large population of Spanish ibex (*Capra pyrenaica*) from the Reserve of Tortosa-Beseit (Ferrer et al., 1998).

## LITERATURE CITED


BLEWETT, D. A., AND K. M. G. ADAM. 1978. *Babesia capreoli* (Enigk and Friedhoff, 1962), in red deer in Scotland. *In* Tick-borne diseases and their vectors, J. K. H. Wilde (ed.). University Press, Edinburgh, UK, pp. 377–378.

CHRISTENSSON, D. 1986. Improvement of the teflonized slides used in the immunofluorescent antibody technique. Acta Veterinaria Scandinavica 27: 296–298.

EMERSON, H. R., AND W. T. WRIGHT. 1968. The isolation of a *Babesia* in white-tailed deer. Bulletin of the Wildlife Diseases Association 4: 142–143.

ENIGK, K., AND K. FRIEDHOFF. 1962. *Babesia capreoli* n. sp. beim reh (*Capreolus capreolus* L.). Zeitschrift für Tropenmedizin und Parasitologie 13: 8–20.

FERRER, D., J. CASTELLÀ, J. F. GUTIÉRREZ, S. LAVÍN, AND I. MARCO. 1998. Seroprevalence of *Babesia ovis* in Spanish ibex (*Capra pyrenaica*) in Catalonia, northeastern Spain. Veterinary Parasitology 75: 91–96.

FRIEDHOFF, K. T. 1988. Transmission of *Babesia*. *In* Babesiosis of domestic animals and man, M. Ristic (ed.). CRC Press, Boca Raton, Florida, pp. 23–52.

GOFF, W. L., D. A. JESSUP, K. A. WALDRUP, J. W. THOMFORD, P. A. CONRAD, W. M. BOYCE, J. R. GORHAM, AND G. G. WAGNER. 1993. The isolation and partial characterization of a *Babesia* sp. from desert bighorn sheep (*Ovis canadensis nelsoni*). Journal of Eukariotic Microbiology 40: 237–243.

GRAY, J. S., T. M. MURPHY, K. A. WALDRUP, G. G. WAGNER, D. A. BLEWETT, AND R. HARRINGTON. 1991. Comparative studies of *Babesia* spp. from white-tailed and sika deer. Journal of Wildlife Diseases 27: 86–91.

HABELA, M., D. REINA, C. NIETO, AND I. NAVARRETE. 1990. Isolation and identification of *Babesia ovis* in Extremadura (Spain). Veterinary Parasitology 35: 233–238.

HINAIDY, H. K. 1987. Blutparasiten der wildlebenden wiederkäuer Österreichs. Journal of Veterinary Medicine B 34: 81–97.

HOLMAN, P. J., K. PETRINI, J. RHYAN, AND G. G. WAGNER. 1994a. In vitro isolation and cultivation of a *Babesia* sp. from an American woodland caribou (*Rangifer tarandus caribou*). Journal of Wildlife Diseases 30: 195–200.

———, T. M. CRAIG, D. L. D. CRIDER, K. R. PETRINI, J. RHYAN, AND G. G. WAGNER. 1994b. Culture, isolation and partial characterization of a *Babesia* sp. from a North American elk (*Cervus elaphus*). Journal of Wildlife Diseases 30: 460–465.

PAPADOPOULOS, B., N. M. PERIÉ, AND G. UILENBERG. 1996. Piroplasms of domestic animals in the Macedonia region of Greece. 1. Serological cross-reactions. Veterinary Parasitology 63: 41–56.

THOMFORD, J. W., P. A. CONRAD, W. M. BOYCE, P. J. HOLMAN, AND D. A. JESSUP. 1993. Isolation and in vitro cultivation of *Babesia* parasites from free-ranging desert bighorn sheep (*Ovis canadensis nelsoni*) and mule deer (*Odocoileus hemionus*) in California. The Journal of Parasitology 79: 77–84.

WALDRUP, K. A., A. A. KOCAN, T. QURESHI, D. S. DAVIS, D. BAGGETT, AND G. G. WAGNER. 1989. Serological prevalence and isolation of *Babesia odocoilei* among white-tailed deer (*Odocoileus virginianus*) in Texas and Oklahoma. Journal of Wildlife Diseases 25: 194–201.

YERUHAM, I., A. HADANI, F. GALKER, E. MAUER, M. RUBINA, AND S. ROSEN. 1985. The geographical distribution and animal hosts of *Rhipicephalus bursa* (Canestrini and Fanzago, 1877) in Israel. Revue d'Elevage et de Médecine Vétérinaire des Pays Tropicaux 38: 173–179.

———, ———, ———, S. ROSEN, AND J. SCHLIEN. 1992. A field study of haemoparasites in two flocks of sheep in Israel. Israel Journal of Veterinary Medicine 47: 107–111.


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