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Serological evidence of *Besnoitia* spp. infection in Canadian wild ruminants and strong cross-reaction between *Besnoitia besnoiti* and *Besnoitia tarandi*

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ABSTRACT

Bovine besnoitiosis, caused by *Besnoitia besnoiti*, is considered to be emergent in Europe and responsible for severe economic losses due to the chronic and debilitating course of the disease but has not been reported in North America. *Besnoitia tarandi* is a related species and it has been reported in reindeer and caribou from different locations of the Arctic Pole, including North America. Diagnosis of clinical besnoitiosis is largely based on the recognition of dermal grossly visible tissue cysts of *Besnoitia*. Nothing is known of cross reactivity between *B. besnoiti* and *B. tarandi* species. Here, we evaluated the use of serological tests employed in the diagnosis of bovine besnoitiosis for the detection of *Besnoitia* spp. infections in different wild ruminant species (caribou, elk, mule-deer, white-tailed deer, moose, muskox and bison) from Canada and investigated cross-reactivity between *B. besnoiti* and *B. tarandi* species by indirect immunofluorescence antibody test and Western blot. For this, species-specific antibodies were obtained in rabbits experimentally infected with *B. besnoiti* and *B. tarandi*. Marked cross reactivity was found between *B. besnoiti* and *B. tarandi*. For the first time, antibodies to *Besnoitia* spp. infection were found in 16 of 20 caribou (*Ranginfer tarandus*), seven of 18 muskox (*Ovibos moschatus*), one of three bison (*Bison bison*), but not in 20 elk (*Cervus canadensis*), 20 white tailed deer (*Odocoileus virginianus*), and 20 moose (*Alces alces*) in Canada; results were similar using *B. besnoiti* and *B. tarandi* as antigen. There was no cross reactivity between the two *Besnoitia* species, *Neospora caninum* and *Toxoplasma gondii* with the cut-offs applied that prevented to observe it. The present study provides evidence that the serological assays can be useful to accomplish large scale prevalence studies in caribou and other wildlife species. Further studies are needed to study sylvatic and domestic cycle of *B tarandi* and *B. besnoiti*.

1. Introduction

The genus *Besnoitia* is a cyst forming coccidian of the Family Sarcocystidae and 10 species are currently recognized: *B. akadoni*, *B. bennetti*, *B. besnoiti*, *B. caprae*, *B. darlingi*, *B. jellisoni*, *B. neotomofelis*, *B. oryctofelisi*, *B. tarandi*, and *B. wallacei* (Nganga et al., 1994; Dubey and Lindsay, 2003;

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Dubey et al., 2003a,b, 2004, 2005; Oryan and Azizi, 2008; Dubey and Yabsley, 2010). The domestic cat is the only known definitive host, and it has been described in the life cycle of *B. darlingi*, *B. wallacei*, *B. oryctofelisi* and *B. neotomofelis*. In the intermediate host, tachyzoites are found in many organs during the acute phase of the infection, which in cattle is known as anasarca stage and characterized by hyperthermia, subcutaneous oedema and orchitis among others. Bradyzoites then develop within microscopic and macroscopic tissue cysts in fibroblasts at various locations, but mainly in the subcutaneous connective tissue, scleral conjunctiva and the vagina. Subsequently, dermal lesions typical of the chronic phase of infection develop, and are known as scleroderma stage which is characterized by a progressive thickening, hardening and folding of the skin, hyperkeratosis and alopecia (Bigalke, 1981).

Bovine besnoitiosis caused by *B. besnoiti* was first reported by Besnoit and Robin (1912) in cattle from France and later in blue wildebeest and impala from South Africa (McCully et al., 1966). Currently the disease is widely distributed in Africa, Asia and in the south-western Europe. In Europe, bovine besnoitiosis has recently emerged from traditional endemic areas (Pyrenees and Alentejo region of Portugal) and is expanding to previously free neighboring regions and countries where it is responsible for severe economic losses (EFSA, 2010). Concerning the other species affecting large ruminants, *B. tarandi* was originally described in reindeer and caribou from Alaska (USA) by Hadween (1922) but the parasite has not been seen in mainland USA. Similar infections characterized by the presence of macroscopic and microscopic tissue cysts in wild caribou, reindeer, mule deer, and muskox in Canada (Choquette et al., 1967; Wobeser, 1976; Ayroud et al., 1995; Leighton and Gajadhar, 2001), and in reindeer and roe deer in Sweden, Finland, Russia and Spain have been attributed to *B. tarandi* species (Leighton and Gajadhar, 2001; Dubey et al., 2004). *B. tarandi* was recently re-described from naturally infected reindeer from Finland using molecular and *in vitro* cultivation (Dubey et al., 2004). Many aspects of the epidemiology of this disease remain unknown, such us the prevalence and incidence of the infection in endemic areas, transmission routes and putative wild reservoirs. It is uncertain whether the *Besnoitia* species infecting different wild ruminants in Canada and other countries are same as *B. tarandi* from reindeer from Finland.

Recent molecular characterization studies demonstrated a close relationship among *Besnoitia* species (*B. caprae*, *B. besnoiti, B. bennetti* and *B. tarandi*) that affect ungulates (goats, cattle, equids and wild ruminants, respectively) (Kiehl et al., 2010; Namazi et al., 2011). Ellis et al. (2000) reported an identical ITS1 sequence in both *B. besnoiti* and *B. caprae* in agreement with Schares et al. (2011b), who also compared *B. tarandi* and *B. bennetti*. In addition, these *Besnoitia* species cause similar clinical signs in all affected ungulates, at least during the chronic phase of the disease with the characteristic *Besnoitia* spp. tissue cysts and lesions in the skin. Thus, the similarities and differences among *B. besnoiti* and these species should be investigated to clarify the epidemiology of the various infections and, in particular, to assess the risk of infection for cattle. Little is known of serological cross reactivity among different species of *Besnoitia* that cause dermal lesions in large animals.

In cattle, *B. besnoiti* infection has been traditionally diagnosed mainly by clinical inspection. Recently, attempts have been made to develop and validate sensitive and specific serological tests for the diagnosis of bovine besnoitiosis. These serological tests include the indirect fluorescent antibody test (IFAT), commercial and in-house enzyme linked immunoabsobent assays (ELISA), modified agglutination test (MAT) and Western blots (Shkap et al., 1984, 2002; Cortes et al., 2006b; Fernández-García et al., 2009a, 2010; Schares et al., 2010, 2011a; Waap et al., 2011; García-Lunar et al., 2012).

The aim of the present work was to evaluate the currently used IFAT and Western blot tests in bovines for the detection of *Besnoitia* spp. infection in wild ruminant species and to determine the level of cross-reactivity between *B. besnoiti* and *B. tarandi* in sera from wild ruminants and cattle.

2. Material and methods

2.1. Serum samples

All serum samples of wild ruminants were from across northern regions of western Canada were collected from various sources between 1995 and 2009 and kept frozen at the Canadian Food Inspection Agency Centre for Foodborne and Animal Parasitology, Saskatoon, Canada. A total of 94 samples used in this study were from caribou (*Ranginfer tarandus*, $n = 20$), elk (*Cervus canadensis*, $n = 20$), mule-deer (*Odocoileus hemionus*, $n = 1$), white-tailed deer (*Odocoileus virginianus*, $n = 20$), moose (*Alces alces*, $n = 12$), muskox (*Ovibos moschatus*, $n = 18$) and bison (*Bison bison*, $n = 3$). The samples were analyzed here by SALUVET research group at Complutense University of Madrid. Age and sex data of the animals sampled were unavailable.

All cattle sera analyzed ($n = 48$) were from a herd in Navarra, Spain, with a history of endemic besnoitiosis. These samples were obtained from animals with clinical signs consistent with clinical besnoitiosis, either acute (fever, swelling of superficial lymph nodes and oedema), or chronic infection scleroderma, hyperkeratosis, alopecia or orchitis in males and tissue-cysts in scleral conjunctiva), or asymptomatic but seropositive animals. These animals came from an area where farms share grazing pastures and are exposed to wild ruminants (*e.g.* red deer) and blood-sucking arthropods which are putative vectors for *Besnoitia* species in summer (Bigalke, 1968).

All serum samples were tested for evidence of infection with *B. besnoiti* and *B. tarandi* using IFAT and Western blot was used to confirm IFAT positive results. Additionally, cross reactivity with *Neospora caninum* and *Toxoplasma gondii* infection was also investigated.

2.2. Parasite production for serological procedures

Culture-derived tachyzoites of *Besnoitia* species, *N. caninum* and *T. gondii* were used to prepare antigens. The *B. besnoiti* strain Bb-Spain1 was the one originally isolated from a naturally infected cow in Spain (Fernández-García