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Review

Molecular pathology, taxonomy and epidemiology of *Besnoitia* species (Protozoa: Sarcocystidae)

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ABSTRACT

Until recently, besnoitiosis has been a neglected disease of domestic animals. Now, a geographic expansion of the causing protozoan parasite *Besnoitia besnoiti* in livestock has been recognized and the disease in cattle is considered emerging in Europe. Bovine besnoitiosis leads to significant economic losses by a decline in milk production, sterility, transient or permanent infertility of bulls, skin lesions and increase of mortality in affected cattle population. Phylogenetically, the *Besnoitia* genus is closest related to the well studied and medically important protozoans, *Toxoplasma gondii* and *Neospora caninum*. In contrast, discriminative molecular markers to type and subtype large mammalian *Besnoitia* species (*B. besnoiti*, *B. caprae*, *B. tarandi*, *B. bennetti*) on a relevant level of species and strains are lacking. Similarly, these cyst-forming parasites may use two hosts to fulfill their life cycle, but this has not been proven for all large mammalian *Besnoitia* species yet. Most important though, the final hosts and transmission routes of these *Besnoitia* species remain mysterious. Here, we review aspects of parasite's pathology, speciation, phylogeny, epidemiology and transmission with a special focus on recent molecular studies of all to date known *Besnoitia* species. Using an integrated approach, we have tried to highlight some promising directions for future research.

Contents

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1. Introduction

The cyst-forming parasite *Besnoitia* (*B.*) *besnoiti* causing bovine besnoitiosis is now known for a century, but despite intensive research the transmission mode and life cycle remains mysterious. Only recently, the disease in cattle has been recognized as emerging in Europe by the European Food Safety Authority (EFSA), since cases in France, Spain, Italy and Germany suggest a geographic expansion over the past decade (Ferrié, 1984; European Food Safety Authority, 2010). Bovine besnoitiosis is characterized by skin lesions, weight loss, transient or permanent sterility in males, and a decline in milk production and disturbance of general condition in highly affected animals. This may be the cause for significant economical losses. It can cause up to 10% mortality and more than 80% morbidity in an affected herd (Bigalke, 1968; Pols, 1960). It was first described in cattle in Southern France in 1912 (Besnoit and Robin, 1912), but probably existed in this area for a much longer time (Bigalke, 1968).

Phylogenetically, *Besnoitia* species form a sister taxon to other medically and veterinary important parasitic protozoa such as *Toxoplasma gondii* and *Neospora caninum*, the causative agents of toxoplasmosis and neosporosis, respectively (Dubey et al., 2007; Ellis et al., 2000). Despite *B. besnoiti*, nine other named *Besnoitia* species have been reported in domestic and wild large mammals (goats, donkeys, horses, reindeer and caribou) as well as in rodents, lagomorphs, marsupials and lizards. So far, there is no evidence that parasites of the genus *Besnoitia* are anthropozoonotic. It is suspected that all *Besnoitia* species use a two-host (heteroxenous) life-cyle with a predator as final host and a prey species as intermediate host in which cysts are formed, which has been demonstrated for small mammalian *Besnoitia* species. However, to date the final hosts and transmission pathways of *Besnoitia* species afflicting large mammals remain to be elucidated. The inner taxonomy of the genus is only partly understood and significant gene targets have not been established, yet. Further molecular analysis is crucial to understanding epidemiology and pathogenicity of these enigmatic parasites for controlling disease.

Here, we review aspects of pathology, taxonomy and epidemiology of all to date named *Besnoitia* species with special emphasis on medically and economically important species of large mammals. We do not provide an exhaustive review, but try to use an integrated approach (Tibayrenc, 1998) to gain insights into epidemiology and demonstrate future ways of research.

2. The disease

2.1. The clinical course of disease

Bovine besnoitiosis is a severe, but usually non-fatal disease of cattle. All breeds seem to be susceptible. The mortality rate in severely afflicted herds is under 10%, but economic losses due to significant reduction of productivity can be high (Bigalke, 1968; European Food Safety Authority, 2010; Pols, 1960). In the acute stage of the disease (anasarca stage), bovine besnoitiosis is characterized by edema, pyrexia, hyperaemia and orchitis, while *B. besnoiti* proliferates in macrophages, fibroblasts and endothelial cells of blood vessels (Bigalke, 1981). Transient or definitive infertility occurs and abortion has also been reported. The latter may lead to transient or definitive infertility of males. Abortions have also been reported (Cortes et al., 2005; Kumi-Diaka et al., 1981). Photophobia, epiphora, ocular and nasal discharge, arrest in rumination, anorexia, increased heart and respiratory rates are common (Jacquiet et al., 2010).These and non-specific clinical signs may be confused with other infectious diseases such as blue tongue or malignant catarrhal fever (Alzieu et al., 2007b). In the following chronic stage (scleroderma stage), skin becomes alopecic and severely lichenified and hyperpigmented; testes of bulls may become permanently atrophic and indurated (Bigalke, 1981; Jacquiet et al., 2010). Myriads of tissue cysts containing bradyzoites are formed in the same tissues where the initial proliferation occurred: dermis, subcutis, upper respiratory tract, scleral conjunctiva, vestibulum vaginae and vagina, testis, connective tissues, muscles and sometimes spleen, liver, lung and heart (Basson et al., 1970; Nobel et al., 1977; Rommel, 1978; Rostaher et al., 2010). Death may occur in either the anasarca or scleroderma stage. The majority of cases, however, are subclinical and go unnoticed (Bigalke, 1981). In enzootic areas seroprevalence is around 50% (Goldman and Pipano, 1983; Janitschke et al., 1984; Neuman, 1972), but can reach over 80% in herds of non-enzootic areas (Fernández-García et al., 2010; Schares et al., 2009).

Besnoitiosis of other large mammals such as goats, reindeer, caribou, antilopes and equids is very similar to bovine besnoitiosis, as anasarca and scleroderma stages both do occur and cysts develop mostly in the same organs as in cattle (Ayroud et al., 1995; Bennett, 1933; Choquette et al., 1967; Dubey et al., 2004, 2005b; Oryan et al., in press). Seroprevalence in goats and histological detection of cysts in some caribou herds can, reach more than 18% and 30%, respectively (Ducrocq, personal communication; Oryan et al., 2008; Oryan and Sadeghi, 1997). The pathomorphology of small mammalian besnoitiosis in their natural hosts has barely been examined. However, cyst development in the skin as well as on serosal layers in body cavities and reproductive organs has been reported for *B. darlingi*, *B. jellisoni*, *B. oryctofelisi*, *B. akodoni* and *B. neotomofelis* (Charles et al., in press ; Chobotar et al., 1970; Grisard et al., 1997; Smith and Frenkel, 1977; Venturini et al., 2002). *B. jellisoni* can cause severe disease in naturally infected kangaroo rats with cysts developing in the subcutis, reproductive organs, lung, heart, oesophagus, trachea, spleen, skull and body cavities (Chobotar et al., 1970). Domestic cats, so far established as final hosts for four small mammalian *Besnoitia* species, have not been reported to show significant clinical signs caused by the parasites (Dubey et al., 2002b, 2003b; Dubey and Yabsley, 2010; Wallace and Frenkel, 1975).

2.2. The parasite within its host

Similar to *Toxoplasma*, the *Besnoitia* genus is suggested having a facultative two-host (heteroxenous) life cycle with a multiplication in two phases. Transmission by forming of intestinal sexual stages (oocysts) as a first phase has been found in the domestic cat, identified as final host for *B darlingi*, *B wallacei*, *B. oryctofelisi* and *B. neotomofelis* (Fig. 1; Dubey and Lindsay, 2003; Dubey and Yabsley, 2010; Smith and Frenkel, 1977; Wallace and Frenkel, 1975). No other final host species are known and a heteroxenous transmission has not yet been identified for *B. jellisoni* and all large mammalian *Besnoitia* species. The second, asexual phase of the life cycle is induced in intermediate hosts after ingestion of oocysts shed by the final host. Oocysts release sporozoites in the digestive tract, which differentiate into tachyzoites and invade multiple tissue cells (Bigalke, 1981; Schares et al., 2009). In the intermediate host, the parasite undergoes an asexual intracellular development as fast multiplicating tachyzoites. During this febrile period of illness (anasarca stage) tachyzoites can also be detected extracellular and intracellular in monocytes and neutrophilic granulocytes of the blood (Pols, 1954; Schares et al., 2009). Finally, in the chronic stage of the disease tissue cysts evolve, containing myriads of bradyzoites (syn. cyst-merozoites, cystozoites). Remarkably, *Besnoitia* cysts of all species have a high affinity for dermal, mucosal and serosal surfaces. Horizontal transmission of bradyzoites and tachyzoites between intermediate hosts has been shown experimentally (Bigalke, 1968; Cuillé et al., 1936; Jellison et al., 1956; Pols, 1960).