STATEMENT OF EFSA

Bovine Besnoitiosis: An emerging disease in Europe[1]

European Food Safety Authority[2, 3,]

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ABSTRACT

The European Food Safety Authority (EFSA) asked the Panel on Animal Health and Welfare to deliver a scientific statement on bovine besnoitiosis. Recent epidemiological data confirm an increased number of cases and geographic expansion of besnoitiosis in cattle herds in some EU MS therefore bovine besnoitiosis should be considered an emerging disease in the EU. However many aspects of the epidemiology of bovine besnoitiosis remain uncertain including prevalence and incidence of infection and disease in endemic areas, routes of transmission and risk-factors associated to infection and disease. The infection caused by the cyst-forming apicomplexan parasite *Besnoitia besnoiti* can cause serious adverse effects both during the acute and chronic phases of disease. The Panel has highlighted the importance to raise awareness about this disease in particular with farmers and veterinarians regarding clinical signs and the known transmission routes. Epidemiological investigations in endemic areas in Europe are necessary to elucidate the importance of infected animals and the routes of transmission. Diagnostic tools need to be further developed and standardized to address unanswered questions related to the epidemiology and clinical progression of the disease. Appropriate measures and strategies to control of besnoitiosis need to be investigated using the available epidemiological information.

KEY WORDS

Bovine besnoitiosis, *Besnoitia besnoiti* geographical distribution, transmission, epidemiology, clinical signs, diagnosis.

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SUMMARY

The European Food Safety Authority (EFSA) asked the Panel on Animal Health and Welfare to deliver a scientific statement on Bovine Besnoitiosis.

Recent epidemiological data confirm an increased number of cases and geographic expansion of Besnoitiosis in cattle herds in some EU MS, therefore bovine Besnoitiosis should be considered an emerging disease in the EU. Many aspects of the epidemiology of bovine besnoitiosis remain uncertain including, prevalence and incidence of infection and disease in endemic areas, routes of transmission and risk-factors associated to infection and disease. It is suspected that *B. besnoiti* has a heteroxenous life cycle. The definitive host (DH) has not been identified. The relationship between *B. besnoiti* and other Besnoitia spp. from ungulates remains to be elucidated. Horizontal direct and indirect transmission seems to be responsible for the spread of the disease. Arthropods such as horseflies and deer flies may play a role by transmitting *B. besnoiti* mechanically from chronically or asymptomatic infected cattle. Wild ruminants and probably rodents should not be disregarded as reservoirs of the parasite.

The infection can cause serious adverse effects both during the acute and chronic phases that could compromise animal welfare. Bovine besnoitiosis has two distinct sequential clinical stages, namely, the acute anasarca stage, which is mainly associated with proliferation of endozoites in blood vessels, and the chronic scleroderma stage which is mainly associated with cyst formation. The severity of the disease may vary between mild and severe with possible deaths in seriously affected animals. Many infected animals remain asymptomatic and the only sign of the disease is the presence of cysts in sclera conjunctiva and/or vulval area in cows. A number of diagnostic tests such as cytology, histopathology, serology and PCR testing are available. There are not any effective drugs or vaccines available in Europe at present.

The Animal Health and Welfare Panel recommends that epidemiological investigations in endemic areas in Europe are necessary to elucidate the importance of infected animals and the routes of transmission. In this context, relevance of direct transmission through direct contact during natural mating should be evaluated. Moreover, entomological studies can be used to evaluate e.g. the abundance of stable flies (*Stomoxys calcitrans*) or tabanids during such surveys, addressing the potential role of these biting flies in transmission. Further studies are necessary to elucidate the role of wild ruminants and rodents as putative reservoirs of the parasite. The existence of a definitive host and its role in the epidemiology of bovine besnoitiosis should be studied. Furthermore, the relationship between *B. besnoiti* and other Besnoitia spp. isolated from ungulates (*B. tarandii* and *B. caprae*) should be investigated to assess the risk of infection for domesticated ruminants.Diagnostic tools need to be further developed and standardized.to address unanswered questions related to the epidemiology and clinical progression of the disease. Appropriate measures and strategies to control of Besnoitiosis need to be investigated using the available epidemiological information.

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BACKGROUND AS PROVIDED BY EFSA

Bovine besnoitiosis is a protozoal disease of cattle caused by the cyst-forming apicomplexan parasite *Besnoitia besnoiti*. Bovine besnoitiosis is either a severe but usually non-fatal disease of cattle, or a mild clinical disease. The disease is responsible for both cutaneous and systemic manifestations and may cause significant economic losses. The severity of the disease may vary between mild and severe cases and even death of seriously affected animals (normally less than 1%). Many infected animals remain subclinical and the only sign of the disease is the presence of parasitic cysts in the sclera and conjunctiva.

Initially the infection is characterised by hyperthermia and non-specific signs, such as depression, swelling of superficial lymph nodes and loss of weight. Oedema in joints may cause pain during movements and may lead to permanent posterior lameness. A progressive thickening, folding or wrinkling of the skin, alopecia, and hyperkeratosis and the typical feature of scleroderma (known as elephant skin) are noticed with the eventual shedding of epidermis. Sterility in males is caused by a necrotizing orchitis. Death may occur due to both anasarca and scleroderma in chronic stages of the disease and animals may be culled after severe body condition deterioration (Fernandez-Garcia et al., in press).

Besnoitia besnoiti (Apicomplexa, Sarcocystidae) has a heteroxenous life cycle, with both domestic (cattle) and wild bovids (antelopes) as intermediate hosts (IH). The definitive host (DH) has not been identified, although a role for cats has been suggested in other *Besnoitia* species.

Horizontal direct and indirect transmission seems to be responsible for the spread of the parasites between hosts. It was observed that the emergence of clinical signs coincides with summer periods, when mixed herds share pastures. Some authors have associated the disease with the period when blood-sucking arthropods, such as horseflies and deer flies, become active. These may play a role by transmitting *B. besnoiti* mechanically from chronic or asymptomatic infected cattle. In addition, wild ruminants and rodents should not be disregarded as reservoirs of the parasite.

Bovine besnoitiosis has been previously described in Africa, the Middle East and Europe, and was deemed to be an emergent disease since the 1990`s in European countries such as Spain, Portugal, France, Italy and Germany. Recent epidemiological data confirm the increased prevalence and geographic expansion of the disease.
In 1986, several outbreaks were observed in the south of Portugal (Cortes et al. 2006a). Bovine besnoitiosis has been observed along the Pyrenees Mountains and in the last decade, different outbreaks have been reported in non-endemic areas, such as southern Basque Country, central and southern Spain at the border with Portugal (Castillo et al., 2009). France was affected focally only in the eastern Pyrenees. Since 2001, several serious outbreaks have been reported in the French Alps, the Massif Central and in the western part of the country (Alzieu, 2007). Subsequently, a few positive animals have been found among cattle exported to Italy, and recently, a larger outbreak occurred in Germany upon importation of Charolais and Limousine cattle into a beef cattle herd in Bavaria (Schares, et al, in press).
It is important to avoid introduction of the infection into non-infected herds as a result of trade of infected animals. Clinical cases are easily detectable, whereas sub-clinically infected animals are more difficult to diagnose but they may play an important role in the transmission either iatrogenically or by insect vectors. At present, there are no effective drugs or vaccines against bovine besnoitiosis available.
A group of scientists from different European countries met last August in Calgary (Canada) in the context of the 22nd WAAVP Congress and agreed that bovine besnoitiosis is an emerging disease affecting cattle herds of western and central Europe (Gottstein, et al 2009). Unfortunately, many aspects of epidemiology and transmission of the infection are still unknown.

TERMS OF REFERENCE AS PROVIDED BY EFSA

1. Provide a general overview of the geographical distribution of Bovine besnoitiosis in Europe.

2. Provide a review of the current information on the pathogenesis, epidemiology and transmission of the disease

3. Identify current knowledge gaps and future research needs

CONSIDERATION

1. Introduction

Bovine besnoitiosis (also referred to as bovine elephantiasis and bovine anasarque) is a protozoal disease of cattle. Bovines act as the intermediate host in the life cycle of the causative agent: the cyst-forming apicomplexan parasite *Besnoitia besnoiti* (Marotel, 1912). Bovine besnoitiosis is either a severe but usually non-fatal disease of cattle, or a mild clinical disease. The severe form of the disease is characterized during the acute phase by fever, inappetence, hyperaemia of the skin and orchitis, which is associated with parasite proliferation (endozoytes or tachyzoites) in endothelial cells of blood vessels. This is followed by a chronic phase, characterized by scleroderma, hyperkeratosis, alopecia, loss of necrotic epidermis, and atrophy and induration of the testes of bulls. This phase is associated with the development of cystozoites (bradyzoites) in tissue cysts in the skin, mucosal membranes and the sclera and conjunctiva (Pols, 1960; Bigalke, 1981). The mortality rate[4] is low (less than 10%) but a significant percentage of affected animals suffer loss of productivity and bulls are frequently rendered permanently infertile (Pols, 1960; Kumi-Diaka et al., 1981; Fernandez-García et al., 2009a). Approximately half of the animals in a herd are sub-clinically infected and the rest remain clinically healthy (Fernandez-Garcia et al., 2009a). The complete life cycle of the parasite remains unknown, although seasonal presentation of the disease (mainly in summer and in beef cattle while they are on pastures) suggests an important role for horizontal transmission by either direct contact, through natural mating or through mechanical transmission by blood-suckling arthropods (Bigalke, 1968; Castillo et al., 2009). In the last decade, the disease has extended to new areas in Europe (Gottstein et al., 2009). At present, bovine besnoitiosis has not been reported to infect humans.

2. Geographical distribution of Bovine Besnoitiosis

Bovine besnoitiosis has been described in Sub-Saharan Africa (Shout Africa, Swaziland, Botswana, Namibia, Zimbabwe, Angola, Congo, Kenya, Tanzania, Uganda, Sudan, Cameroon and Nigeria) , Asia (Israel, Russia and South Korea) and western and central Europe (Bigalke, 1981, Juste et al., 1990; Cortes et al., 2004; Cortes et al., 2005; Cortes et al., 2006b,c; Alzieu, 2007; Castillo et al., 2009; Fernandez-Garcia et al 2009; Gottstein et al., 2009; Zacarias, 2009; Fernandez-Garcia et al., 2009a). For decades, bovine besnoitiosis in Europe was restricted to a few areas of south-western Europe (Fig. 1). The first recorded cases of bovine besnoitiosis were described in southern France by Cadéac (1884) and by Besnoit and Robin (1912) (both cited by Pols, 1960) in cattle from the Pyrenees. Four years later, the disease was reported in the Alentejo region of Portugal (Franco and Borges, 1915, cited by Pols, 1960). Until approximately 20 years ago, the disease received little attention in Europe. In the early 1990’s it was reported in northern Spain (Juste et al., 1990), in the Alentejo region of Portugal (Cortes et al., 2004, 2005, 2006c) and widely around the French Pyrenees and in some areas of Massif Central (Alzieu, 2007; Jacquiet, personal communication). Recently, besnoitiosis has been recognized as an emerging disease in European countries such as France, Portugal, Spain, Germany and Italy (Gottstein et al., 2009) and there is evidence of an increased number of cases and geographic expansion of the disease based on epidemiological data (Fig. 1). In France, several serious outbreaks have been reported in the French Alps, the Massif Central and in the western part of the country (Alzieu et al., 2007; Jacquiet, personal communication) and a suspect case has been recorded close to the Belgium border (Jacquiet, personal communication). Countries neighbouring France, such as Switzerland and Belgium expect similar events, and presently prepare for it. In Italy, a few positive animals have been found among imported cattle from France (Agosti et al., 1994) and very recently the disease has been confirmed in Northern Italy in two herds (Gentile, personal communication). In

[4] From the original reference Pols, 1960 it is not possible to tell whether the author refers to overall death rate (due to that disease) in the affected herd (over a given period of time (mortality rate) or to the proportion of diseased animals that eventually die of the disease (lethality or case fatality rate).

Germany, a large outbreak occurred upon importation of Charolais and Limousine cattle from France into a beef cattle herd in Bavaria, where 80% of the adult herd was affected (Schares et al., 2009). In Portugal, historically Franco and Borges in 1915 (cited in Pols, 1960) stated that, since 1885, the disease was detected frequently in slaughter cattle at the abattoir in Lisbon, particularly in animals derived from the Alentejo province. In recent years, several outbreaks have been reported in the south of Portugal (Cortes et al., 2006a,b), mostly related to the purchase of breeding males. In Spain, bovine besnoitiosis has been observed in the Basque Country and Navarre and other areas of the Spanish Pyrenees (Juste et al., 1990; Castillo et al., 2009). However, the disease seems to be expanding and cases have been recorded in areas out of the Pyrenees such as the Iberic System, Castille and Leon, Cantabria and La Rioja (Castillo et al., 2009). A recent outbreak has been reported in a mountain area in central Spain (Fernandez-Garcia et al., 2009a) and the disease has been diagnosed as far as southern Spain (Alvarez-García, personal communication).

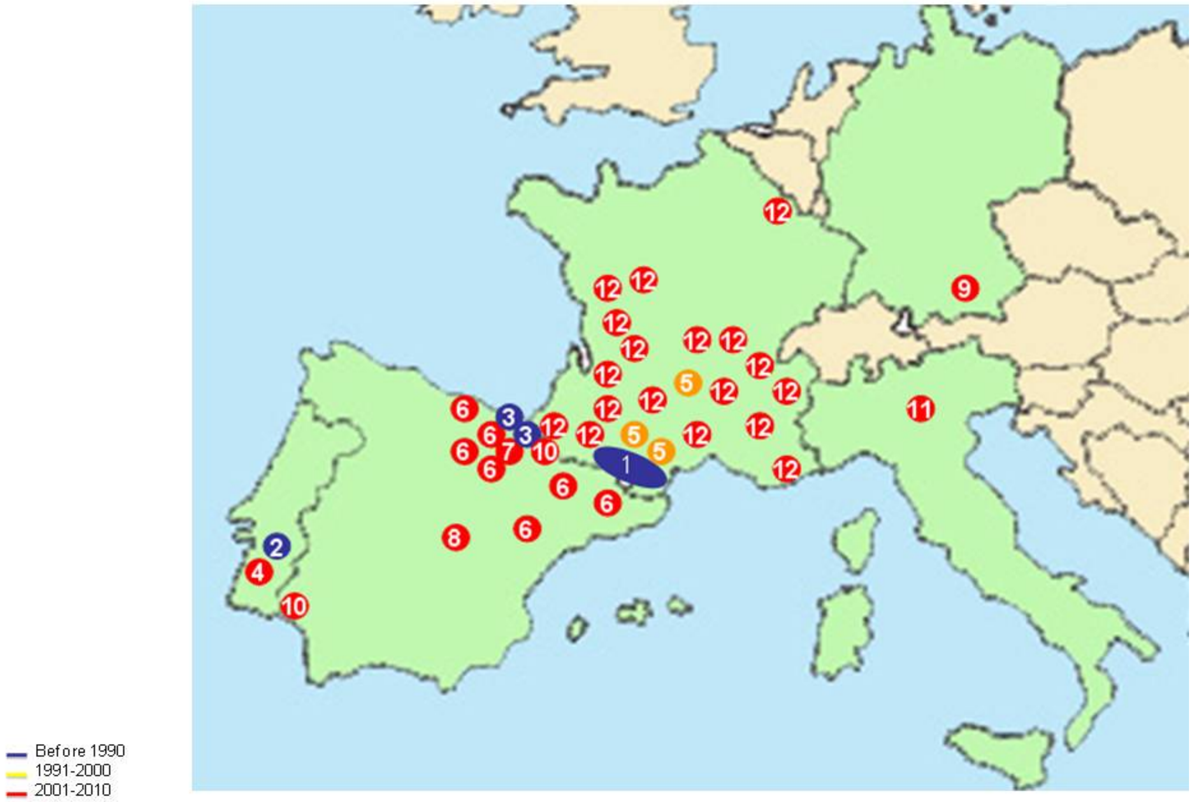

Figure 1: Geographical distribution of bovine besnoitiosis in western Europe.

In green, countries where the disease has been reported. Areas with suspected non-confirmed cases.
1. Besnoit and Robin, 1912 (cited by Pols, 1960); 2. Franco and Borges,1916 (cited by Pols, 1960); 3. Juste et al. 1990; 4: Cortes et al., 2004, Cortes et al., 2005; Cortes et al., 2006c; 5. Alzieu, 2007; 6. Castillo et al., 2009; 7. Zacarias , 2009; 8. Fernández-García et al. in press; 9. Rostehaf et al., in press. 10. Álvarez-García, G.; personal communication.11: Gentile. A. personal communication. 2010; 12: Jacquiet. P. F. personal communication.

3. Aetiology and life cycle

Bovine besnoitiosis is caused by the cyst-forming apicomplexan parasite *Besnoitia besnoiti*. Parasites of the genus *Besnoitia* are classified in the subfamily Toxoplasmatinae, family Sarcocystidae, phylum Apicomplexa and comprises nine species (*B. besnoiti*, *Besnoitia benetti*, *Besnoitia jellisoni*, *Besnoitia wallacei*, *Besnoitia tarandii, Besnoitia darling, Besnoitia caprae, Besnoitia akadoni and Besnoitia oryctofelis*). However, uncertainty exists regarding the differentiation of some of these species because only the life cycles of *B. darlingi, B. wallacei* and *B. oryctofelis* are known (Dubey et al., 2003).

Phylogenetic analyses showed *Besnoitia* to be reproducibly the sister group to a clade containing *Hammondia*, *Neospora* and *Toxoplasma* (Ellis et al., 2000). *B. besnoiti* host range includes all breeds of cattle. Infection by *Besnoitia besnoiti*-like organisms has also been described in two species of antelopes (blue wildebeest and impala), although their isolates seem to be viscerotropic rather than dermatotropic, and are relatively non-pathogenic (Bilgake, 1968). The relationship of the *Besnoitia* sp. found in goats and named *B. caprae* (Bwangamoi, 1967; Cheema and Toofanian, 1979) to *B. besnoiti* has not been determined, although *Besnoitia* of cattle, wildebeest and goats had identical ITS1 rDNA sequences which questions the use of the taxon *Besnoitia caprae* to describe the *Besnoitia* found in goats (Ellis et al., 2000). The relationship between *Besnoitia tarandii* found in reindeer, caribou, mule-deer and roe deer in North America and Finland (Dubey et al, 2005) and *B. besnoiti* remains unexplored. A variety of laboratory rodents such as rabbits, gerbils, hamster and mice and some domestic and wild bovids (sheep, goats and black wildebeest) have been found to be susceptible to artificial infection with bovine strains of *B. besnoiti* (Pols, 1960; Bigalke, 1968; Dubey, 1977).

It is suspected that *B. besnoiti* has a heteroxenous life cycle. The definitive host (DH) has not been identified, although for other Besnoitia spp. a role for a carnivorous DH has been suggested (Wallace and Frenkel, 1975; Dubey, 1977; Rommel, 1978). Peteshev et al. (1974) (cited by Bigalke, 1981) suggested that both, domestic cats and a wild cat (*Felis lybica*), shed oocysts after ingestion of cyst-containing tissues. However, these observations have not been reproducible by others (Diesing et al., 1998) and the DH remains unknown. Two asexual and infective stages of this parasite develop in cattle, the fast-replicating merozoite-like endozoites (tachyzoites) that multiply by endodyogeny in endothelial cells of blood vessels and the slow-dividing cystozoites (bradyzoites), which gather into macroscopic cysts located inside cells of the subcutaneous connective tissue. Cyst formation starts approximately 1 week after the initial cycle of proliferation (Basson et al., 1970). The characteristic thick-walled cysts reach a size of up to about 400 µm and contain approximately 200.000 cystozoites each.

4. Epidemiology

Many aspects of the epidemiology of bovine besnoitiosis remain uncertain including prevalence and incidence of infection and disease in endemic areas, routes of transmission and risk-factors associated to infection and disease.

4.1. Prevalence

Seroprevalence has mainly been studied in South Africa and Israel using serological techniques such as indirect immunofluorescence tests (IFATs) (Bigalke, 1968; Neuman, 1972; Frank et al., 1977; Goldman and Pipano, 1983). These studies reported higher seroprevalence rates in beef herds than in dairy herds, probably due to husbandry differences (beef cattle are more often raised under extensive management conditions). Janitschke et al. (1984) reported seropositivity of 46% with ELISA and 50% with immunofluorescence tests in animals without clinical signs. Neuman (1972), Frank et al., (1977), and Goldman and Pipano (1983) reported seroprevalence exceeding 50% by IFAT in beef cattle.

There is a lack of well-designed cross-sectional studies investigating the prevalence and incidence in endemic areas of western Europe. Recent work has been carried out in the Sierra de Urbasa Andia (Navarra, North Spain), an area located close to the Pyrenees (Zacarias, 2009). In this area, individual prevalence vary between 48.6% (95% CI 45.4- 51.9%) and 44.5% (41.3 – 47.7%) depending on the serological technique used, IFAT and ELISA, respectively. In non-endemic areas, in an outbreak recently reported in central Spain (Fernandez-García et al., 2009a) 90.8% (319/351) of females and 71.4% (5/7) of males were seropositive. Nevertheless, only 43.2% (154/358) of examined animals showed at least one clinical sign. In addition, only 5 out of 358 animals that showed clinical signs were seronegative by ELISA. In these animals, clinical signs consisted of mild oedema and skin lesions in eyes, on the udder and on the feet. The results showed a notably increased seroprevalence after the first appearance of the disease three years earlier. These results agree with a study on a beef cattle farm in Portugal, where a 36% seroprevalence increased to 70% over the course of 18 months (Cortes et al., 2006b).

4.2. Transmission

Horizontal transmission is probably the main method of transmission of infection, given the significant association between the disease prevalence and the animals' age (Bigalke, 1981; Fernandez-Garcia et al., 2009a). It is likely that horizontal transmission occurs as a consequence of direct contact among animals with wounds or lacerations, since subcutaneous tissue cysts can be located very superficially. A role for transmission through infected bulls in natural mating herds has been suggested (Castillo et al., 2009). The emergence of clinical signs coincides with summer, when mixed herds share pastures. However, some authors have associated this phenomenon with the period when blood-sucking arthropods, such as horseflies and deer flies, become active, and they may play a role by transmitting *B. besnoiti* mechanically from chronic or asymptomatic infected cattle. The role of a carnivorous definitive host in the epidemiology of bovine besnoitiosis remains to be elucidated. Wild ruminants and probably rodents should not be disregarded as reservoirs of the parasite (Castillo et al., 2009; Melhorn et al., 2009).

4.3. Risk factors

<u>Seasonality</u>. In South Africa the majority of new cases occur during the warmer, moister months of the year (Bigalke, 1981). Based on limited observations from two outbreaks in Europe, it was noted that the emergence of clinical signs coincided with the summer period, when mixed herds shared pastures (Alzieu, 2007; Fernandez-Garcia et al., 2009a) and blood-sucking arthropods, such as horseflies become active.

<u>Breed</u>. All breeds of cattle seem to be susceptible to besnoitiosis. In South Africa most cases occur in the Africander, a *Bos indicus* breed, which is the most common in the endemic region (Bilgake, 1981). The infection has been described both in dairy and beef cattle and in a high variety of breeds.

<u>Age</u>. Bigalke (1981) reported that there is a relationship between the age of the animal and the epidemiology of the disease. In this report, a statistically significant increase in seroprevalence and morbidity, evidenced by typical clinical signs, was associated with age. The highest incidence of infection was detected in adult animals on a farm where the disease was present, but was rarely encountered in calves under 6 months of age. A significant association has been found between the prevalence of the disease and the age of the animal in a recent outbreak in central Spain (Fernandez-Garcia et al., 2009a).

<u>Sub-clinical carriers</u>. A higher seroprevalence has been observed in subclinical animals in some outbreaks in Spain (Fernandez-Garcia et al., 2009a); this agrees with observations made by other authors in South Africa (Bigalke, 1968).

<u>Vectors and reservoirs</u>. The existence of blood-sucking flies could be a risk factor for the rapid spread of the disease (Zacarias, 2009). Mechanical transmission by *Glossina*, *Stomoxys* and tabanids has been demonstrated (Bigalke, 1968). The potential role of wildlife reservoirs of disease, e.g. wild ruminants and rodents, has also been suggested (Bigalke, 1981; Castillo et al., 2009; Mehlhorn et al., 2009).

5. Pathogenesis, clinical signs and lesions

Typical clinical cases of bovine besnoitiosis appear in two distinct sequential stages: the acute anasarca stage, which is mainly associated with proliferation of endozoites in blood vessels, and the chronic scleroderma stage, which is mainly associated with cyst formation. The severity of the disease may vary between mild and severe and even death of seriously affected animals. Many infected animals remain asymptomatic and the only sign of the disease is the presence of cysts in the sclera and conjunctiva and/or the vulval area in cows (Pols, 1960; Bigalke, 1968; Bigalke, 1981; Fernandez-Garcia et al., 2009a).

Initially the infection is characterised by hyperthermia and non-specific clinical signs, such as depression, swelling of superficial lymph nodes and loss of weight. Simultaneously, tachyzoites invade endothelia of blood vessels and subsequently oedema appears as a consequence of vascular damage. As obligate intracellular parasites, endozoites proliferating in cells of blood vessels cause degenerative and necrotic vascular lesions, vasculitis and thrombosis, mainly in the medium and smaller veins and capillaries. Oedema in joints causes pain during movement and may progress to permanent posterior lameness. This phase of the infection is known as the acute anasarca stage, and it is followed by chronic scleroderma stage. Very characteristic, macroscopically visible tissue cysts develop inside cells of the subcutaneous connective tissue and may be visualized by clinical inspection in the sclera and conjunctiva and vulval regions. With the disappearance of oedema, a progressive thickening, folding or wrinkling of the skin, alopecia, hyperkeratosis and the typical feature of scleroderma (known as elephant skin) are noticed, with the eventual shedding of epidermis. Sterility in males is caused by a necrotizing orchitis. Death may occur in both the anasarca and scleroderma stages of the disease, regardless of the sex of the animal. Animals may be culled after severe deterioration of body condition and loss of commercial value.
A herd study on clinical cases of bovine besnoitiosis showed that the mortality rate[5] is usually about 10% (Pols, 1960) even though that 70%–90% of animals within this herd had specific antibodies against *B. besnoiti* (Bigalke, 1968). Thus, most animals die during the chronic stage of infection. Only a few studies have been carried out on the early stage mechanisms of *B. besnoiti* infection and immune pathways (Basson et al., 1970; Bigalke et al., 1974). In an outbreak studied in central Spain, clinical signs observed in animals included oedema in the jaws and limbs (25.4%; 91/358), skin lesions such as scleroderma and hyperkeratosis (13.4%; 48/358), alopecia (5.6%; 20/358) and crusting on the feet and udder (20.7%; 74/358), as well as lameness and weight loss in severe cases (1.4%; 5/358). One male also developed orchitis (1/7). Tissue cysts were observed in scleral and palpebral conjunctiva (31.6%; 113/358) and in vulval region (18.4%; 66/358). Most animals that exhibited oedema also had cysts in their scleral and palpebral conjunctiva and/or in vulval region (84.6%; 77/ 91). As much as 6.14% (22/358) of the animals, including 1 male, showed cysts without having *Besnoitia*-related clinical signs (Fernandez-Garcia et al., 2009a).

6. Diagnosis and control

A number of diagnostic tests such as cytology (Sanussi, 1991), histopathology (Bigalke, 1968), serology (Cortes et al., 2006a; Fernandez-Garcia et al., 2009; Fernandez-Garcia et al., 2009a; Alvarez-Garcia et al., 2009) and PCR testing (Cortes et al., 2007) are available. During the first weeks following infection, acutely infected animals may be difficult to be accurately clinically diagnosed due to non-specific signs. The characteristic clinical signs appear with the development of tissue cysts during the chronic stage; when the clinical inspection is very useful to detect affected animals. Skin biopsies to confirm the existence of tissue cysts, examining the sample by trichinelloscopy plates or even histopathology are good methods to confirm the disease. However they are not suitable for the detection of chronic and subclinical infections, where the number of cysts in cattle overall may still be relatively high, but too low to detect cysts on a histological skin slide. Serological tests – ELISA and Western blot – could be useful tools to detect asymptomatic/sub-clinical cattle for control purposes.
Concerning control, there are not any effective drugs or vaccines available, although in South Africa and Israel a live-attenuated vaccine has been used (Basson et al., 1970). In South Africa, in an extensive field trial on farms where the disease was severe, Bigalke et al. (1974) found that 100% of the cattle were protected from the clinical form of the disease over an observation period of 1 to 4 years when inoculated with a blue wildebeest strain of *B. besnoiti* vaccine. In Europe, at present, only

[5] From the original reference Pols, 1960 it is not possible to tell whether the author refers to overall death rate (due to the disease) in the affected herd over a given period of time (mortality rate) or to the proportion of diseased animals that eventually die of the disease (lethality or case fatality rate).

reliable diagnosis together with herd-management measures are available to avoid that non-infected herds acquire the infection due to trade with infected animals.

7. Economic and welfare impact

The disease causes a serious illness both during the acute and chronic phases that could compromise animal welfare. Economic impact of bovine besnoitiosis in Europe remains undetermined.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Recent epidemiological data confirm an increased number of cases and geographic expansion of Besnoitiosis in cattle herds in some EU MS. Bovine besnoitiosis should be considered an emerging disease in the EU.

Many aspects of the epidemiology of bovine besnoitiosis remain uncertain including prevalence and incidence of infection and disease in endemic areas, routes of transmission and risk-factors associated to infection and disease.

It is suspected that *B. besnoiti* has a heteroxenous life cycle. The definitive host (DH) has not been identified. The relationship between *B. besnoiti* and other Besnoitia spp. from ungulates remains to be elucidated.

Horizontal direct and indirect transmission seems to be responsible for the spread of the disease. Arthropods such as horseflies and deer flies may play a role by transmitting *B. besnoiti* mechanically from chronically or asymptomatic infected cattle. Wild ruminants and probably rodents may play a role as reservoirs of the parasite although conclusive evidence is currently not available.

The infection can cause serious adverse effects both during the acute and chronic phases that could compromise animal welfare.

Typical clinical cases of bovine besnoitiosis appear in two distinct sequential stages, namely, the acute anasarca stage, which is mainly associated with proliferation of endozoites in blood vessels, and the chronic scleroderma stage which is mainly associated with cyst formation. The severity of the disease may vary between mild and severe, and even death of seriously affected animals. Many infected animals remain asymptomatic and the only sign of the disease is the presence of cysts in sclera conjunctiva and/or vulvar area in cows.

A number of diagnostic tests such as cytology, histopathology, serology and PCR testing are available. There are not any effective drugs or vaccines available in Europe at present.

RECOMMENDATIONS

To raise awareness about this disease in particular with farmers and veterinarians regarding clinical signs and the known transmission routes.

Epidemiological investigations in endemic areas in Europe are necessary to elucidate the importance of infected animals and the routes of transmission. In this context, relevance of direct transmission through direct contact during natural mating should be evaluated. Moreover, entomological studies can be used to evaluate e.g. the abundance of stable flies (*Stomoxys calcitrans*) or tabanids during such surveys, addressing the potential role of these biting flies in transmission. Further studies are necessary to elucidate the role of wild ruminants and rodents as putative reservoirs of the parasite. The existence of a definitive host and its role in the epidemiology of bovine besnoitiosis should be studied.

The relationship between *B. besnoiti* and other Besnoitia spp. isolated from ungulates (*B. tarandii* and *B. caprae*) should be investigated to assess the risk of infection for domesticated ruminants.

Diagnostic tools need to be further developed and standardized to address unanswered questions related to the epidemiology and clinical progression of the disease

Appropriate measures and strategies to control of Besnoitiosis need to be investigated using the available epidemiological information.

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