¿Bovine besnoitiosis: present in Colombia?

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Summary

Bovine besnoitiosis has recently been classed as a re-emerging disease in Western and Central Europe, due to a recent increase in reported cases and a geographic expansion of the disease in cattle herds in continental Europe. The disease has been present in some parts of France, Spain and Portugal for many years, a fact which may be an indicative of the disease's expansion, and it is important to know about it because it has a lot of similar lessons if compared with diseases we have in Colombia. The infection can cause serious adverse effects, both during the acute and the 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 the proliferation of endozoites in blood vessels, and the chronic scleroderma stage, which is mainly associated with the formation of cysts. 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 the sclera conjunctiva and/or the vulvae area in cows.

Key words: bovine besnoitiosis, *besnoitia besnoiti*, anasarca, scleroderma, hyperkeratosis.

¿Besnoitiosis bovina en Colombia?

Resumen

La besnoitiosis ha sido recientemente clasificada como una enfermedad que resurge en Europa central y occidental, debido a un reciente aumento de casos reportados y una expansión geográfica de la enfermedad entre hatos ganaderos en Europa continental. Se han visto casos en algunos lugares de Franca, España y Portugal por años, lo cual puede indicar que hay expansión de la enfermedad y es importante saber sobre ello porque hay gran similitud de lecciones si se compara con algunas de las enfermedades que se tienen en Colombia. La infección puede causar serios efectos adversos, tanto sea en condición de aguda o crónica, comprometiendo el bienestar del animal. La besnoitiosis bovina tiene dos etapas clínicas consecutivas: la etapa anasarca, principalmente asociada con la proliferación de endozoítos en los vasos sanguíneos, y la esclerodermia crónica, relacionada con la formación de quistes. La gravedad de la enfermedad puede variar entre leve y severa, con posibles muertes en los animales seriamente afectados. Muchos animales infectados permanecen asintomáticos y los únicos signos de la enfermedad son quistes en la conjuntiva esclerótica y/o en el área vulvar de las vacas.

Palabras clave: Besnoitiosis bovina, Besnoitia besnoiti, anasarca, esclerodermia, hiperqueratosis.

Besnoitiosis bovina na Colômbia?

Resumo

A besnoitiosis foi recentemente classificada como uma doença que resurge em Europa central e ocidental, devido a um recente aumento de casos reportados e uma expansão geográfica da doença entre hatos de gado em Europa continental. Viram-se casos em alguns lugares de Franca, Espanha e Portugal por anos, o qual pode indicar que há expansão da doença e é importante saber sobre isso porque há grande similitude de lições se se compara com algumas das doenças que se têm em Colômbia. A infecção pode causar sérios efeitos adversos, tanto seja em condição de aguda ou crônica, comprometendo o bem-estar do animal. A besnoitiosis bovina tem duas etapas clínicas consecutivas: a etapa anasarca, principalmente associada com a proliferação de endozoítos nos copos sanguíneos, e a esclerodermia crônica, relacionada com a formação de

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quistos. A gravidade da doença pode variar entre leve e severa, com possíveis mortes nos animais seriamente afetados. Muitos animais infectados permanecem assintomáticos e os únicos signos da doença são quistos na conjuntiva esclerótica e/ou no área vulvar das vacas.

Palavras Importantes: Besnoitiosis bovina, Besnoitia besnoiti, anasarca, esclerodermia, hiperqueratosis.

Introduction

Bovine besnoitiosis (also referred as Elephant Skin disease and bovine anasarca) is a disease in cattle caused by an obligate intracellular protozoan parasite that belongs to the phylum apicomplexa species, called *Besnoitia besnoiti*[1-4] which is transmitted by blood sucking insects, or in a smaller proportion via skin contact with other cattle. It is responsible for significant losses in the cattle industry, due to the high morbidity rate (up to 10%[5, 6]) in affected farms, including mortality, weight loss, prolonged convalescence, definitive or transient infertility in males, (with atrophy, sclerosis and focal necrosis of the testes[7, 8]) and a decline of milk production, reduction of slaughter weight and abortions -especially in recently affected areas. The cutaneous lesions brings the rejection of hide leather production[5] and systemic manifestations that cause a severe but usually non-fatal disease[9]. Most infections are mild or subclinical, characterized by the formation of numerous coetaneous and sub-cutaneous microcysts, scleroderma, hyperkeratosis, and alopecia, lowering the quality of the skins for the leather industry. Male sterility or impaired fertility is a common sequel in breeding bulls, and is one of the most negative aspects of the disease in animals that survive infection[10].

Knowledge of the clinical course of bovine besnoitiosis is important in order to identify infected animals as soon as possible and prevent the disease's spreading[11].

An etiology and life cycle

Besnoitia besnoiti is a cystforming coccidian protozoan parasite, classified within the *Toxoplasmatinae* subfamily, Sarcocystidae family, phlymyn Apicompleza and have nine different species: *Besnoitia besnoiti, Besnoitia benetti, Besnoitia jellisoni, Besnoitia wallacei, Besnoitia tarandii, Besnoitia darling, Besnoitia caprae, Besnoitia akadoni and Besnoitia oryctofelis*[2].

Molecular analyses based on large and small subunits of the nuclear ribosomal DNA have shown that *Besnoitia spp* is closely related to *Toxoplasma gondii* and *Neospora caninum*[11, 12] and there is a serological cross-reactivity between these related parasites[13]. There are besnoitia species infecting ungulates[14] and other species infecting rodents, lagomorphs and marsupials[15]. This result represents the knowledge of life cycles, suggesting an evolutionary split within the genus besnoitia towards two lineages, likely link to the intermediate host. This point requires further investigation. The relationship among species is also unclear, especially between *B. besnoiti* and *B. caprae*. No significant differences can be seen in the morphological description of bradizoites[12-16] but biological properties of *B. caprae* are different from *B. besnoiti*, infection in cattle or rodents occurs with *B. besnoiti* contrary to *B. caprae*[17].

The biology of B. *besnoiti*, the cause of bovine besnoitiosis, is poorly understood[9]. Its definitive host has not been identified, and information on potential intermediate hosts is scarce, but it is known that the intermediate host is the cow and another wild ruminant[10]. In order to investigate potential definitive and intermediate hosts for European isolates of *B. besnoiti*, domestic dogs, cats, rabbits, guinea pigs (*Cavia porcellus*), gerbils (*Meriones unguiculatus*), common voles (Microtus arvalis) and NMRI-mice were inoculated with *B. besnoiti* isolated from naturally infected German cattle[7].

Domestic dogs and cats could not be shown to be definitive hosts of *B. besnoiti*, but cats seroconverted after feeding on *B. besnoiti* tissue cysts indicating that *B. besnoiti* tachyzoites had invaded the cat's tissues[18]. Moreover, the cats play an important role in the transmission of other species, such as *Besnoitia caprae*[19]. A

molecular and serological study indicates that European *B. besnoiti* isolates may infect cats, rabbits, guinea pigs, gerbils, mice and voles. However, a persistence of the parasite could be demonstrated only in voles[18].

The transmission of the disease and the life cycle of this parasite are to date not fully understood[5, 6]. A possible route of transmission might be the mechanical, by biting flies such as *Tabanus* and *Stomoxys calcitrans*; by medical devices and a direct animal to animal transmission seems to be likely, and seroconversions occurred throughout the year, reaching their highest number in spring. In addition, many seroconversions were reported in the two months before turn-out and could be associated with a high indoors activity of *S. calcitrans* during this period[20]. Once the infection is introduced into a herd it can spread fast, in a way that a large proportion of the herd may seroconvert within two or three years[21].

These Toxoplasma-like organisms multiply in endothelial, histiocytic and other cells, and produce characteristic large, thick-walled cysts filled with bradyzoites. The cysts of *Besnoitia besnoiti* (200 – 600 µm in diameter) may appear as white pinhead-shaped nodules that are found in the conjunctive sclera of the eyes, subcutaneous tissue, fascia and mucous membranes of the respiratory and genital tract of infected animals and remain in the animal's body for years[11].

Epidemiology

Besnoitiosis is widespread in Africa, Asia and in the West and Central Europe[10-12]. It was first reported in Sub-Saharan Africa[22] and Asia[23]. In Europe, bovine besnoitiosis is enzootic only in three countries: Portugal[9], Spain[24] and the south of France[21]. Recent epidemiological data confirm an increased number of cases and a geographic expansion of besnoitiosis in cattle herds. Many recent cases have been described in different European countries, and for the first time in Germany[7] and Italy[25], which may be an indicative of expansion of the disease. Just recently, there was a success in the first detection of cattle besnoitiosis in Central Spain and the stages of this parasite were clearly described during the chronic phase of the disease in a cow that presented tissue cysts, cyst-stages and some of their molecular biological features[26]. Many infected animals remained asymptomatic and, therefore, serological tests are essential tools for diagnosis[22].

There is no report of *Besnoitiosis* infection in any animal in South America, but *Besnoitia* sp. infections were reported in naturally infected rabbits, among which tissue cysts were seen in several tissues of five rabbits from a rabbit breeder in La Plata, Argentina[27]. The global warming generates climate changes that influence the structure and function of natural ecosystems, including host–parasite interactions, wildlife species and disease emergence[28]. This is why besnoitisis has been recently classified as a re-emerging disease in Europe by the European Food Safety Authority[3-5]. Another reason of the increase of bovine besnoitiosis is that the seroprevalence and clinical signs can be also be associated with the increasing age of the animals, suggesting a rapid horizontal transmission of the disease[29].

Pathogenesis, clinical signs and lesions

As obligate intracellular parasites, endozoites proliferating in cells of blood vessel walls causes degenerative and necrotic vascular lesions, vasculitis and thrombosis in mainly the medium and smaller veins and capillaries of the dermis, subcutis, nasal mucosa, larynx, trachea and testes[23]. These lesions, together with a toxic effect, apparently cause an increased permeability of blood vessels manifested as anasarca and subcutaneous edema[10].

The animals begin to develop clinical signs after approximately two weeks after theinfection. There are however some animals in which the incubation period can be extended to two months[1-10], and many infected cattle remain asymptomatic or show scleral-conjunctival cysts only[30].

Typical clinical cases of bovine besnoitiosis can appear in two stages: the acute anasarca stage, that is associated with the endozoites proliferation in the blood vessels, and the chronic scleroderma stage, associated with the cyst

formation[2]. The acute stage is associated with the proliferative forms (tachyzoites and endozoites). This is when the tachyzoites invade endothelia of blood vessels and the edema appears as the consequence of the vascular damage; the endozoites proliferate and cause degenerative and necrotic vascular lesions[30]. This stage is characterized by fever, edema, whimper, general weakness and swelling of the superficial lymph nodes. Other clinical signs in this stage are non-specific, such as depression, loss of weight, subcutaneous edema and a pain in joints during the movements that may progress to lameness[3-11].

The chronic phase has its origin in the formation of macroscopically cysts visible in the sclera conjunctiva three weeks after the start of the acute phase, and subsequently appears in the mucous membrane of the vestibule vagina and vulvae region. Visible cysts, which look like grains of sugar, may disappear after some months and can appear in a later phase of the disease[10-11]. This process is non-reversible and chronic besnoitiosis is characterized by hyper-sclerodermia (also called elephant skin), hyperkeratosis, and alopecia in bulls, atrophy, sclerosis and focal necrosis that cause irreversible lesions in the testis[2-5]. The typical chronic clinical signs in the cattle are progressive thickening and wrinkling of the skin, and the eventual shedding of epidermis affecting the eyelids and the area of the back; the skin signs are accompanied by poor general conditions of the infected animal[31.]

Death may occur in both phases, and the mortality rate is approximately 10%[9-32]. The most common postmortem changes found in necropsy are: inflammation of the pharynx, larynx and trachea, sand-like granules and cysts in the turbinates and nostrils, sand-like granules in the endothelium of large vessels and dermatitis. Histologically, there are epidermal hyperplasia, marked hyperemia, dermal edema and perivascular accumulations of lymphocytes, plasma cells and large histiocytes that will become hosts to parasites accompany the acute febrile stages[33].

Differential diagnosis

In tropical parts of the world like Colombia, *Besnoitia besnoiti* is responsible for skin problems and infertility in cattle. That is why it is often helpful to approach differential diagnoses with dose diseases that involve skin lesions characterized by painful swellings, alopecia and thickening of the skin, and those that shown infertility in males due to testes inflammation[34-36].

This organism is predominantly located in tissue-cysts in the skin, mucosal membranes and sclera conjunctiva. In most cases, the typical clinical signs of the disease are cutaneous lesions such as thickening and folding of the skin, dry seborrhea, and hypotrichia or alopecia[33, 37]. Skin diseases in cattle are relatively uncommon, but skin abnormalities are frequently seen during clinical examination. Some of those diseases are:

- **Bovine herpes mammillitis:** the lesions are superficial (involving only the epidermis) and occur predominantly on the cooler parts of the body such as teats and muzzle. Generalized skin lesions can occur accompanied by a transient fever (1 to 3 days). Resolution of the lesion is rapid and results in focal alopecia, but there is no hide damage[38].
- **Streptotrichosis (*Dermatophilus congolensis* infection)**: lesions are superficial (often moist and appear as crusts) scabs or 0.5- to 2-cm diameter accumulations of keratinized material. Lesions are common in the skin of the neck, axillary region, inguinal region and perineum. The presence of the organism can be demonstrated by Giemsa staining[38].
- **Ringworm**: The lesions of ringworm in cattle are grayish, raised, plaque-like and often pruritic. The presence of the organism can be demonstrated with a silver stain.
- **Hypoderma bovis infection**: The parasitic fly larvae of this parasite have a predilection to migrate to the dorsal skin of the back. They cause a nodule with a small central hole through which the larva exits the body, which results in significant hide damage[38].
- **Photosensitization:** Dry, flaky, inflamed areas are confined to the nonpigmented parts of the skin.
- **Bovine papular stomatitis (lumpy skin disease LSD)**: Pox-like lesions occur in the skin of the muzzle, oral cavity, and esophagus. There is no generalized disease.

- **Insect bites:** The trauma from insect bites causes local inflammation, edema, and pruritus. Insects seldom bite mucous membranes.
- **Urticaria:** Delayed hypersensitivity reactions can be confused with LSD. Such lesions generally resolve within 3 to 5 days. An example of this was described by Shimshony (1989) regarding allergic reactions that occurred after vaccination with a foot-and-mouth disease vaccine.

The difference from those skin diseases is that in Besnoitiosis the lesions and thick-walled cysts in the skin are caused by sporozoan parasites, which are transmitted mechanically by certain biting flies, and the histological sections will reveal the parasites. Histopathologically, the skins were thickened by hyperplasia of the epidermis, inflammatory cellular exudates, fibroplasia and empty or parasitic cysts. Apart from the *Besnoitia* cyst, the causes of the abnormal thickening of the skin were not ascertained. It was presumed that the lesions of dermatitis and dermatosis might be associated with any of the various causes such as physical trauma and chronic irritations, tick and mite infestations or bacterial and mycotic infections[39, 40].

Also besnoitia cysts and some lesions have been observed in the testes, epididymes and blood vessels of bull, causing inflammation of the testicles and sterility in those bulls[41] and here in Colombia there are some diseases that cause similar signs such as brucelosis, infectious bovine rhinotracheitis, bovine virus diarrhea, bovine genital campylobacteriosis, *Trichomona* infection, and other non-infectious diseases, such as tumors and traumas[42].

Brucellosis is caused by the bacterium *Brucella abortus*. The organism has an affinity for certain body tissues such as the udder, uterus, lymph nodes, testicles, and accessory sex glands. *B.abortus* is a very common cause of orchitis. In the bull the injury results in acute infection and it is difficult to cure. The lesion is a necrosis with a subsequent granuloma formation. Usually only one testicle is affected. However, the bull is sterile because the semen is mixed with the inflammatory exudate, or because the testicle suffers thermal degeneration[43]. Scrotal swelling occurs quickly, getting hot. This is due to inflammatory changes in the tunica and epididymis[44].

Infectious bovine rhinotracheitis (IBR) is an infectious disease caused by bovine herpesviruses type 1 (BHV 1). Depending on the subtype of viruses and animal age, the infection shows itself as pneumonia, conjunctivitis, rhinotracheitis, encephalitis, balanopostitis, and reproduction disorders. IBR is a highly contagious disease of the upper respiratory tract and can lead to serious primary or secondary pneumonia. The clinical signs of the disease are nasal discharge, fever and conjunctivitis. Acute disease in dairy cattle is usually accompanied by a severe and prolonged drop in milk production. Adult cows may also suffer from abortion and reduced fertility. Animals suffering from IBR are highly susceptible to secondary bacterial infections[45].

Campylobacteriosis is a major infectious cause of infertility in cattle herds[46]. The vast majority of problems associated with Campylobacter are linked to venereal infection. This disease is spread by infected bulls during service or through poor instrumentation hygiene during an artificial insemination programme[42]. A bull acquires the disease by mating with an infected cow. Once infected, a bull remains an asymptomatic carrier of the condition[46].

Bovine trichomoniasis is a reproductive disease of cattle which can have significant economic impact to cow-calf operations and other cattle enterprises[47]. Venereal transmission of the causative organism, *Trichomonas foetus*, can cause one of the most commonly recognized diseases leading to decreased reproductive efficiency in cattle[42]. There is no consistent observable sign to help with the diagnosis of the disease. The confirmation of the disease requires a demonstration of the organism in the tissue or, most frequently, in culture[47].

And the difference is that in besnoitiosis, one or both testes may become permanently athrophic and indurated. Bulls invariably develop an aspermatogenesis, which is usually permanent on account of the severe testicular lesions which usually develop two weeks after the initial clinical reaction[41].

Diagnosis

During the first weeks of infection, acutely affected animals may be difficult to diagnose as

clinical signs are non-specific[48]. Characteristic clinical signs tend to be seen with the chronic stage of the disease, primarily thickening of the skin, sclerodermia and the cysts in the sclera, conjunctiva and vulvar regions, and with the development of tissue cysts[1]. 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[49].

The technique of a skin biopsy was based on the demonstration of *Besnoitia besnoiti* bradyzoites (cystic stages), which appeared stumpy, each organism 6.2 µ by 3.1 µm in size, banana-shaped 7.7 µm by 1.5 µm in affected skin smears. A more rapid non-surgical technique, scleral conjunctival scraping, revealed similar bradyzoites, thus enhancing the diagnostic value of conjuctival cysts in more chronic infections[50].

However, there are a number of diagnostic test such as cytology, histopathology, serology (ELISA, Western blot) and PCR testing available, and they can be useful tools to detect asymptomatic/ sub-clinical cattle for control purposes[49, 51]. As a simple and inexpensive technique, the modified agglutination test (B-MAT) represents a valuable tool for the diagnosis and study of the epidemiology of bovine besnoitiosis[50].

While identification of clinical cases and their histopathological confirmation is relatively simple to carry out, finding subclinical forms of infection is more difficult. Therefore, a more sensitive test for the identification of the etiological agent may be an appropriate diagnostic tool. For this reason, the ITS1 rDNA-sequence-based conventional and real-time PCR have been developed, they are highly sensitive and specific for the detection of *B. besnoiti* infection in cattle[51].

Control

Concerning the control of this disease there are not many effective drugs or vaccines available, although in South Africa there is a live-attenuated vaccine that has been used, and the cattle could be protected from the clinical form of the disease[3]. *B. besnoiti* antigens may help to develop new specific and sensitive serological tests based on individual antigens and to identify possible vaccine candidates[47]. In Europe, at present, only reliable diagnosis together with herd-management measures are available to avoid that non-infected herds acquire the infection in the trade with infected animals. Serological identification of infected cattle is important because the introduction of these animals into naive herds seems to play a major role in the transmission of the parasite[2, 52, 53].

Given that the route of transmission of besnoitioisis is not yet fully known, the early detection of infected cattle and the control of their commercial movement might be crucial to improve our knowledge on the biology and the epidemiology of the disease[53, 54].

Conclusions

Typical clinical cases of bovine besnoitiosis appear in two distinct sequential stages: The acute anasarca stage, which is mainly associated with a proliferation of endozoites in blood vessels, and the chronic scleroderma stage, which is mainly associated with cyst formation. Affected animals show cutaneous and systemic manifestations and the disease may lead to considerable economic losses. Although generally associated to tropical and subtropical areas, bovine besnoitiosis is now considered an emergent disease in Europe, due to the increasing number of new cases and its apparent geographical expansion. The severity of the disease may vary between mild and severe, or even the 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 the present time.

Although this is an exotic disease in Colombia, it should be considered for having presentations in tropical countries where the climatic and environmental conditions are similar. Also, the free trade agreements that Colombia already has with other countries allow the entry of animals and animal products that could be potential carriers of this parasite. Another pos-

sibility is that the disease is already established in the country and is simply confused with similar lesions.

Domestic animals are often afflicted with various skin problems, some easy to cure, others more complicated, and even some of them can be highly contagious for the human handlers. The effect of skin problems on animal productivity also varies from mild irritations to rapid death, with all kinds of in between stages affecting the productivity and comfort of the animals. It really pays to pay close attention to the health and comfort of domestic animals.

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