Parasitology Research (2021) 120:3547–3553
https://doi.org/10.1007/s00436-021-07298-2

PROTOZOOLOGY - ORIGINAL PAPER

Bovine besnoitiosis in a cattle herd in Sicily: an isolated outbreak or the acknowledgment of an endemicity?

Ettore Napoli[1] · Susana Remesar[2] · Jairo Mendoza-Roldan[3] · Giovanni De Benedetto[1] · Stefania Di Giorgio[1] · Alessandra Sfacteria[1] · Gabriele Marino[1] · Francesca Arfuso[1] · Giuseppe Catone[1] · Emanuele Brianti[1]

Received: 9 June 2021 / Accepted: 17 August 2021 / Published online: 3 September 2021

Abstract

Bovine besnoitiosis is a debilitating infectious disease caused by *Besnoitia besnoiti* (Apicomplexa; Sarcocystidae). The disease is mainly characterized by cutaneous and systemic signs, infertility in bulls, and abortion in cows. The current study describes an autochthonous outbreak of bovine besnoitiosis in Sicily, Southern Italy, being the first report of *B. besnoiti* infection in the island so far. In a cattle farm located in Syracuse province, a 4-year-old Belgian blue bull born in Sicily displayed typical clinical signs of the sub-acute/chronic disease phase with thickening of the skin of the scrotum and testicular degeneration. Histological examination of scrotal biopsies revealed the presence of several tissue cysts of *B. besnoiti*. The serological analysis of the herd using a commercial ELISA revealed a high seroprevalence (45 out of 55; 82%) of antibodies against *B. besnoiti*. Few seropositive animals (5 out of 45; 11%) showed clinical signs, cysts in *vestibulum vaginae* (1 out of 31; 3.2%), and testicular degeneration (4 out of 14; 28.6%) assessed by ultrasonographic investigations. The paucity of clinical signs associated with the high seroprevalence in the farm led to hypothesize that bovine besnoitiosis is endemic in the area though further studies are needed. Local practitioners should be more aware of the disease to facilitate the early detection of cases, prevent the spread of infection, and avoid economic losses and animal health problem.

Keywords *Besnoitia besnoiti* · Clinical signs · ELISA · Histology · Cattle · Hypofertility

Introduction

Besnoitia besnoiti (Apicomplexa; Sarcocystidae), the causative agent of bovine besnoitiosis, is a tissue cyst-forming parasite belonging to the family Sarcocystidae (Mugridge et al. 2000),the life cycles of the organisms belonging to this family are complex and partially unknown.

The life cycle of *B. besnoiti* is not completely understood since the host range, including the definitive host, has still to be identified. Cattle act as intermediate hosts, whereas domestic felids were suspected as definitive hosts (Basso et al. 2011; Olias et al. 2011). In fact, in an experimental study, after feeding three domestic cats with tissue cyst of this parasite, two of them developed specific antibodies against the parasite, suggesting the susceptibility of cats to *B. besnoiti* infection (Basso et al. 2011). However, no animal species has been identified shedding oocysts of the parasite by natural or experimental infections so far (Basso et al. 2011). Under natural conditions, only cattle have been described as intermediate hosts (Jacquiet et al. 2010), while in laboratory conditions, rabbits have been infected by subcutaneous inoculation of bradyzoites, showing clinical signs (fever, photophobia, and edema) and developing tissue cysts (Basso et al. 2011; Liénard et al. 2015). Furthermore, experimentally infected cats, guinea pigs, gerbils, voles, and mice developed specific antibodies against *B. besnoiti* (Basso et al. 2011). However, the role of wild animals as possible

Section Editor: Berit Bangoura

✉ Emanuele Brianti
ebrianti@unime.it

[1] Department of Veterinary Sciences, University of Messina, Messina, Italy

[2] Department of Animal Pathology, Investigación en Sanidad Animal: Galicia (Grupo INVESAGA), Faculty of Veterinary Medicine, Universidade de Santiago de Compostela, Santiago, Spain

[3] Department of Veterinary Medicine, University of Bari, Valenzano, Bari, Italy

hosts of the parasite needs further exploration (Gazzonis et al. 2014).

The main transmission route of *B. besnoiti* infection appears to be by mechanical action of blood-sucking arthropods such as tabanid horse flies and stable flies (Hornok et al. 2015), although the iatrogenic route and the direct contact may represent other forms of transmission (Cortes et al. 2014). Infections through natural mating, as through transplacental and colostral routes are feasible (Álvarez-García et al. 2013) but are considered unlikely to occur (Hornok et al. 2015).

In bovine besnoitiosis, it is possible to distinguish two different phases of the disease: an acute stage (i.e., occurring 11–13 days after infection and lasting 6–10 days), followed by a chronic stage. During the acute stage of the disease, tachyzoites undergo proliferation in endothelial cells and fibroblasts (Pols 1960; Basson et al. 1970) that result in vasculitis and thrombosis. Infected animals display fever, nasal, and ocular discharge, photophobia, swelling of superficial lymph nodes, generalized skin edema, acute orchitis, and lameness. This latter is due to the pronounced thickening of the limbs and the consequent difficult and painful locomotion (Pols 1960; Basson et al. 1970; Jacquiet et al. 2010; Dubey et al. 2013; Cortes et al. 2014; Gollnick et al. 2015). In pregnant cattle, vasculitis and thrombosis can lead to abortion (Pols 1960; Cortes et al. 2014). The chronic form of besnoitiosis is characterized by the presence of thick-walled tissue cysts (i.e., up to 0.5 mm in diameter containing slowly proliferating bradyzoites) in the mucosae of the upper respiratory and the lower genital tract, in the scleral conjunctival, and in the dermis and tendons of the lower limbs which seems to be the preferred sites for cysts formation (Basso et al. 2013; Frey et al. 2013; Dubey et al. 2013; Gollnick et al. 2015).

The infection by *B. besnoiti* leads to a debilitating disease with cutaneous and systemic manifestations (Álvarez-García et al. 2013), resulting in dramatically decreased body condition scores and infertility in bulls and abortion in pregnant cows (Cortes et al. 2014).

However, in endemic scenarios, most animals remain sub-clinically infected, and only 10% show clinical infection signs (Bigalke 1968; Fernandez-Garcia et al. 2010). The sub-clinically infected animals may serve as carriers (Álvarez-García et al. 2013; Frey et al. 2013) and may be responsible for spreading the infection due to animal trade and movement from endemic to *Besnoitia*-free areas. Besnoitiosis is a widespread disease in sub-Saharan Africa, Middle East, and Asia (Bigalke and Prozesky, 2004; Olias et al. 2011) and an emerging or re-emerging disease in Europe. Indeed, bovine besnoitiosis is now considered endemic in some areas of France, Spain, and Portugal (Álvarez-García et al. 2013). Cases of infection were also recorded in other European countries, including Germany, Switzerland, Hungary, Croatia, Belgium, and Ireland (Cortes et al. 2014; Hornok et al. 2015; Vanhoudt et al. 2015; Ryan et al. 2016). In the Northern and Central regions of Italy, autochthonous outbreaks of bovine besnoitiosis have been detected since 2009 (Manuali et al. 2011; Mutinelli et al. 2011; Gentile et al. 2012; Gazzonis et al. 2017; Villa et al. 2019), however, the parasite has not been reported in Sicily so far.

The present study describes an autochthonous outbreak of bovine besnoitiosis in a farm located in Southern Italy (Sicily) apparently not correlated with those previously reported in northern and central Italy. The investigation of the herd was preceded by the detection of a chronic clinical case of bovine besnoitiosis, confirmed by histopathology and serology.

Materials and methods

The first clinical case

In July 2019, the owner of a semi-extensive mixed (beef and dairy) cattle farm, located in Syracuse, of Southeastern Sicily, Italy (37,06,045°N; 14,9067°E), referred to the Department of Veterinary Sciences, University of Messina (Italy), due to the low fertility shown by the cows in his farm. The farm is located in an area with a high density of dairy cattle farms. The site has an altitude of about 670 m above sea level. The mean annual temperature is 17.3 °C (max 23.1 °C, min 11.6 °C); the mean annual humidity index is 69.9% with an average annual rainfall of 45.6 mm approximately. In the farm, the natural service of the reproductive program was ensured by a 4-year Belgian blue bull, born in Ragusa province (Sicily, Southern Italy). The owner detected a problem of low fertility in the breeding bull and to ensure the natural service of the reproductive program decided to introduce three new bulls in the farm. Although after the introduction of the three new bulls the fertility initially improved, it decreased again 1 month later.

In August 2019, the 4-year Belgian blue bull was subjected to clinical examination, including ultrasound of the genital tract. Upon physical examination, the scrotum skin appeared thickened, and biopsies were performed on the scrotum using a biopsy punch (diameter 6 mm) and also from the testicle using a semiautomatic biopsy needle (14 *gauge*). The biopsies were fixed in 10% formalin and processed for histological examination. In particular, the samples were dehydrated in an increasing alcohol series, diaphanized in xylene, and embedded in paraffin; 5-µm-thick sections were stained with hematoxylin–eosin (HE) and examined by a light microscope. On the same date also, the

other three breeding bulls were subjected to clinical examination and ultrasonographic testicular evaluation.

Herd examination

In November 2019, following the owner's consent, a herd survey was conducted in the farm.

The milking herd was housed in a free-stall barn with their calves, and only heifers were kept outdoors in confined pasture lots. Other animal species, including dogs and cats, were present in the farm, and, considering the farm's site, direct contact of the herd with wild animals (i.e., wild boar, fox, wild rabbit, and other micromammals) could be also possible. The farm consisted of 55 animals ranging from 5 to 144 months of age (Table 1). A complete clinical examination was carried out on all the cattle; physical examination of the external genitalia and in males the measurement of the scrotal circumference was performed; moreover, a visual exploration of the sclera was carried out. To perform serological investigation, from each animal, blood samples were collected from the middle coccygeal vein into 10 mL vacuum tubes. The blood samples were centrifuged at $1780 \times g$ for 10 min, and the sera were stored at − 20 °C until analysis.

A commercial indirect ELISA kit (ID Screen® Besnoitia Indirect 2.0, VET-Innovate ID Diagnostics, France) was used, according to the manufacturer's instructions, for the detection of antibodies against *B. besnoiti* in sera samples. All samples were analyzed in duplicate and optical density (OD) was read at 450 nm using a microwell plate reader (Sirio, SEAC, Florence, Italy).

The sample-to-positive percentage (S/P%) was calculated as follows:

S/P% = (net OD sample/net OD positive control) × 100.

The interpretation of the results was carried out by comparing the obtained percentage values with a cut-off indicated by the supplier. This last was identified as two threshold values: S/P of 25%, below which the results were considered negative, and an S/P of 30%, above which the samples were considered positive; the samples included in the S/P interval (25–30%) were considered doubtful.

In order to investigate the potential source of the infection, data on animal movement provided both by the owner and consulting the Italian National Data Bank for Livestock Registration (BDN, http://www.anagrafe.izs.it) for newly animals since 2006 were analyzed.

Table 1 Herd composition according to category, gender, and age of the animals in the farm

Category and age class	Male	Female
Calves < 12 months	6	4
Beef cattle 13–48 months	9	-
Adult female ≥ 11–144 months	-	32
Breeding bull 30–48 months	4	-

Results

The first clinical case

The 4-year Belgian blue bull (ID 11) showed clinical signs compatible with the sub-acute/chronic phase of besnoitiosis with thickening of the skin of the scrotum (Fig. 1a, b). Moreover, it showed an evident testicular degeneration upon ultrasonographic testicular evaluation (Fig. 1c). On histological examination, several spherical parasitic cysts were found in the scrotal subcutis with a density of over 30 cysts at a low magnification field. The diameter of cysts ranged from 250 to 450 µm, and they were lined by a thick (10 µm) capsule. Numerous bradyzoites of *B. besnoiti* were detected inside the cysts. A chronic inflammation associated with the cysts was found (Fig. 2). In the testicular samples, chronic interstitial orchitis associated with a severe testicular degeneration was observed with the total disappearance of the germ layers into the seminiferous tubules. The other three breeding bulls (IDs 14, 52, and 55) showed clinical signs compatible with acute besnoitiosis, mainly fever, orchitis, hydrocele, and scrotal edema at the first visit (Fig. 3).

As a result of the first visit, bovine besnoitiosis was identified as the cause of male infertility in the farm, and natural service was discouraged.

Herd examination

On the second visit to the farm (November 2019), only few animals showed clinical signs of besnoitiosis. In particular, the 4-year Belgian blue bull (ID 11) showed an accentuation of the chronic phase with thickening of the scrotal skin, cyst in the scleral conjunctiva, and testicular atrophy. Two breeding bulls (IDs 14 and 52) showed testicular atrophy, whereas the other bull used for breeding (ID 55) showed thickening of the skin of the scrotum, testicular atrophy, and crusty dermatitis on the left hind limb. One cow (i.e., ID 6) showed few cysts in the *vestibulum vaginae*.

Regarding serological analysis, 45 out of 55 animals (82%) tested positive for anti-*B. besnoiti* antibodies. In particular, 30 were adult females (66.7%), one 5-month-old female calf (2.2%), and 14 males (31.1%) between 10 and 48 months of age, including two offspring of the 4-year Belgian bull (IDs 2 and 4) of 10 months of age.

According to animal movement information, all seropositive animals were born on the farm and never left it,

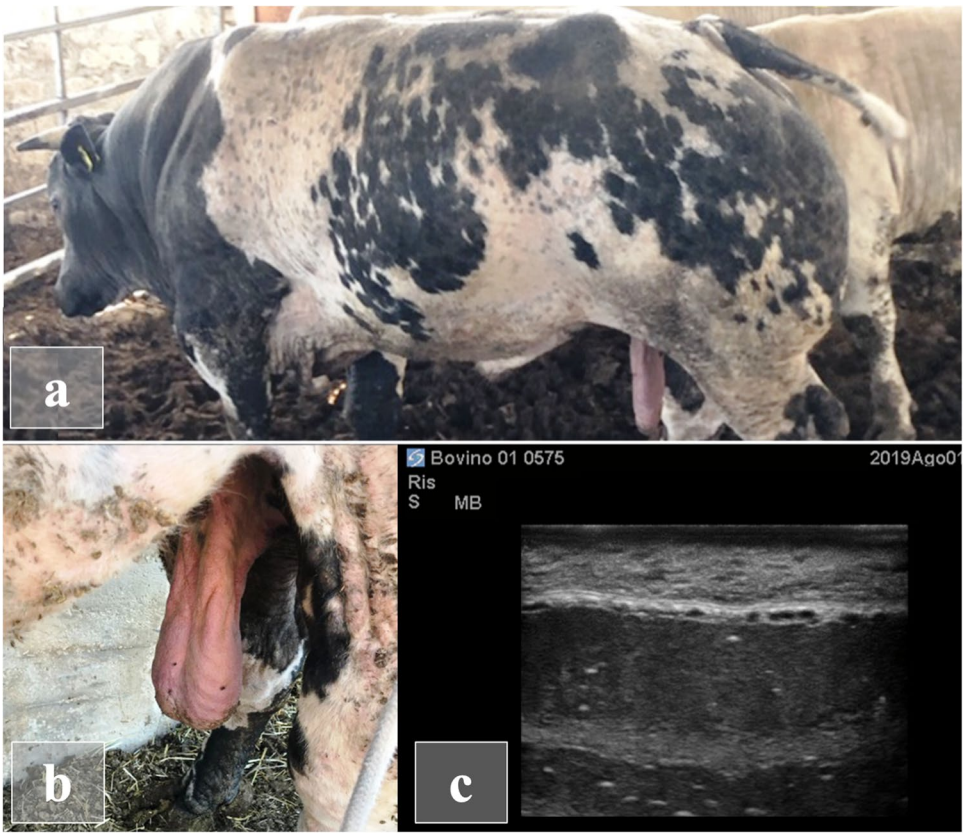

Fig. 1 **a** A 4-year Belgian blue bull (ID 11) at the first clinical inspection. Note thickening of the skin of the scrotum and severe testicular hypotrophy (**b**) confirmed upon ultrasound by the presence of hyperechoic parenchymal foci, enlarged mediastinum, and ectasia of the tunica albuginea network (**c**)

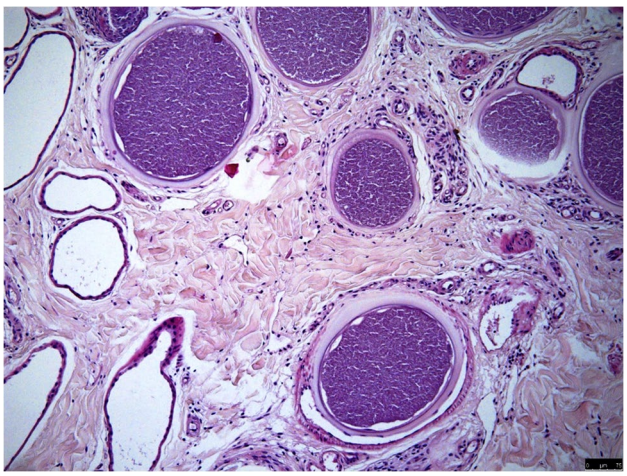

Fig. 2 Skin biopsy from the 4-year Belgian blue bull (ID 11) affected by besnoitiosis: cross-section of scrotal subcutis large circular cysts. Note the three layers of the wall: the outer hyaline one, the middle layer with host cell cytoplasm and nuclei, and the inner membrane with parasitophorous vacuole containing numerous bradyzoites. Cysts are surrounded by macrophages, eosinophils, and scattered lymphocytes. Bar, 100 μm

except for the three bulls (sample ID 11, 52, and 55). Particularly, the bull ID 11 was born in December 2015 on a farm located in Ragusa province (Sicily, Southern Italy), transferred to another farm of Syracuse province (Sicily) in May 2016, and finally, transferred to the study site in October 2017. The bull ID 52 was born in November 2017 on a farm in Syracuse province, transferred to another farm of the same province in April 2018, and finally, transferred to the study site in March 2019. The bull ID 55 was born in December 2017 on a farm of Syracuse province and transferred in the study site farm in March 2019. Another bull, ID 14, born on the farm in November 2017, was sold and transferred to another farm in the Campania region (Avellino, Central Italy) in October 2019 after the detection of reproductive problem.

Discussion

The present study represents the first report of besnoitiosis in Sicily with a high overall seroprevalence within one farm (82% of animals showed anti-*B. besnoiti* antibodies). This finding, coupled with the absence of clinical signs in the majority of seropositive animals, suggests that *B. besnoiti* infection in the farm was endemic rather than recently introduced. Bovine besnoitiosis is reported in the OIE Terrestrial code; additionally, in some countries such as Switzerland, it is a notifiable disease where an eradication plan has been set up (Basso 2018). The spread of the disease in Europe

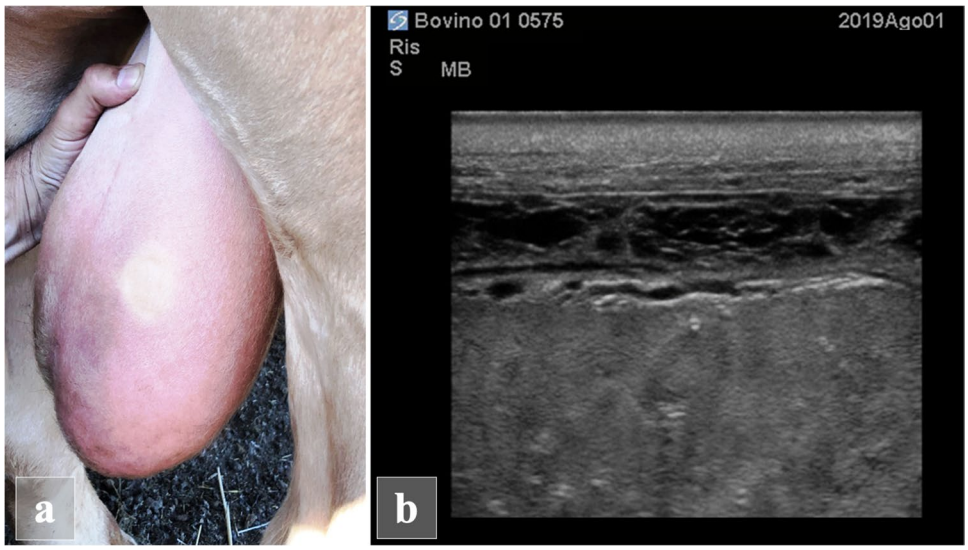

Fig. 3 Orchitis, hydrocele, and scrotal edema observed in a breeding bull (ID 14) during the clinical inspection on August 01, 2019 (**a**), and confirmed by ultrasonography (**b**)

prompted the European Food Safety Authority (EFSA) in 2010 to classify the illness as an emerging disease in the continent (EFSA, European Food Safety Authority, 2010). Until 2009, besnoitiosis was not considered endemic in Italy and the sole report of the disease in the country referred to animals imported from France (Agosti et al. 1994; Vacirca et al. 1994; Mutinelli et al. 2011). However, in the last decade, autochthonous outbreaks of bovine besnoitiosis were reported in herds of northern and central Italy leading to an increased awareness of the disease (Gollnick et al. 2010; Manuali et al. 2011; Gentile et al. 2012). More recently, besnoitiois has also been reported in southern regions of Italy where cattle seem to be the only domestic large ruminant sensitive to the infection (Ciuca et al. 2020).

In the studied herd, a high seroprevalence was observed with an 82% ELISA positivity to anti-*B. besnoiti* which is significantly higher than the one reported in similar studies conducted in Northern (36.5%) (Gazzonis et al. 2017), Central (9.7%) (Gentile et al. 2012), and Southern Italy (44.1%) (Rinaldi et al. 2013). Interestingly, all the seropositive animals were born in the farm or in other Sicilian farms, and no history of travel to endemic regions was reported. It is likely, therefore, that the animals acquired from other farms had been already in contact with other *B. besnoiti*-positive cattle before they arrived at the farm, or they may have been exposed through mechanical transmission by blood-sucking arthropods (i.e., tabanid horse flies, stable flies) that represent the main infection transmission route (Olias et al. 2011; Hornok et al. 2015). Indeed, the lack of insect control strategies could have allowed the spreading of the disease in the study farm. Therefore, the animal trade within the region might be considered the way of disease introduction in the farm. The lack of evidence of direct links of the studied farm with endemic areas, the high seroprevalence, and the chronic presentation of the disease suggest that the studied farm is not the only positive farm in Sicily. The bulls acquired to supply the natural reproductive service became serologically positive and showed the clinical signs of the disease soon after the contact with the females, which, in turn, were probably infected by the 4-year-old Belgian blue bull, indicating that mating or mechanical transmission through blood-sucking insects may have led to the spread of the disease inside the herd (Majzoub et al. 2010). The former route of transmission is expected to occur since parasitic cysts lie in superficial mucosal tissues such as the mucous membrane of the vagina (Nobel et al. 1977), *vestibulum vaginae* (Majzoub et al. 2010; Rostaher et al. 2010), and in the mucosal membranes of the penis. Hence, it seems likely that superficially based cysts in the genital tract may rupture due to the mechanical insult of mating and bradyzoites could be released and enter the bloodstream of non-infected animals through micro-lesions. Therefore, as previously demonstrated (Gazzonis et al. 2017), age and productive category represent risk factors for the parasite infection, with older cattle having a higher probability of exposure and infection.

All cattle examined at the second visit that were born on the farm were normothermic and did not show clinical signs ascribable to the acute phase of bovine besnoitiosis. In addition, only recently acquired animals and a young calf were seropositive and showed clinical signs. Conversely, the majority of seropositive cattle were sub-clinically infected, representing a high risk for parasite transmission, being a source of infection for other animals of the same farm, as well as for animals housed in the neighboring farms.

The low number of animals with clinical signs suggests that *B. besnoiti* infection might have been undetected in the farm for months or even years. In areas where the infection is endemic, the proportion of infected cattle developing the clinical disease is low (Liénard et al. 2011; Álvarez-García et al. 2014; Villa et al. 2019),therefore, it could be

hypothesized that bovine besnoitiosis is endemic in that area of Sicily and further studies are needed to determine the real epidemiological scenario.

Data availability Raw data and derived data supporting the findings of this study are available from the corresponding author EB on request.

Declarations

Ethics approval All experimental procedures were carried out in accordance with the European legislation regarding the protection of animals used for scientific purposes (European Directive 2010/63), as recognized and adopted by the Italian law (D.Lgs. 26/2014).

Conflict of interest The authors declare no competing interests.

References

Agosti M, Belloli A, Morini M, Vacirca G (1994) Segnalazione di un focolaio di Besnoitiosi in bovini da carne importati. Praxis 15:5–6

Álvarez-García G, Frey CF, Mora LM, Schares G (2013) A century of bovine besnoitiosis: an unknown disease re-emerging in Europe. Trends Parasitol 29:407–415. https://doi.org/10.1016/j.pt.2013.06.002

Álvarez-García G, García-Lunar P, Gutiérrez-Expósito D, Shkap V, Ortega-Mora LM (2014) Dynamics of *Besnoitia besnoiti* infection in cattle. Parasitol 141:1419–1435. https://doi.org/10.1017/S0031182014000729

Basso W. (2018) Chapter "Besnoitiosis" in Coetzer J.A.W., Thomson G.R., Maclachlan N.J. and Penrith M.L. (Eds.) Anipedia, infectious diseases of livestock. Part I

Basso W, Lesser M, Grimm F, Hilbe M, Sydler T, Trösch L, Ochs H, Deplazes BU, P, (2013) Bovine besnoitiosis in Switzerland: imported cases and local transmission. Vet Par 198:265–273. https://doi.org/10.1016/j.vetpar.2013.09.013

Basso W, Schares G, Gollnick NS, Rutten M, Deplazes P (2011) Exploring the life cycle of *Besnoitia besnoiti*: experimental infection of putative definitive and intermediate host species. Vet Par 178:223–234. https://doi.org/10.1016/j.vetpar.2011.01.027

Basson PA, McCully RM, Bigalke RD (1970) Observations on the pathogenesis of bovine antelope strains of *Besnoitia besnoiti* (Marotel, 1912) infection in cattle and rabbits. Onderstepoort J Vet Res 37:105–126

Bigalke RD (1968) New concepts on the epidemiological features of bovine besnoitiosis as determined by laboratory and field investigations. Onderstepoort J Vet Res 35:3–137

Ciuca L, Borriello G, Bosco A, D'Andrea L, Cringoli G, Ciaramella P, Maurelli MP, Di Loria A, Rinaldi L, Guccione J. (2020) Seroprevalence and clinical outcomes of *Neospora caninum, Toxoplasma gondii* and *Besnoitia besnoiti* infections in water buffaloes (*Bubalus bubalis*). Animals (Basel). 10 (3): 532

Cortes H, Leitao A, Gottstein B, Hemphill A (2014) A review on bovine besnoitiosis: a disease with economic impact in herd health management, caused by *Besnoitia besnoiti*. Parasitology 141:1406–1417. https://doi.org/10.1017/S0031182014000262

Dubey JP, van Wilpe E, Blignaut DJC, Schares G, Williams JH (2013) Development of early tissue cysts and associated pathology of *Besnoitia besnoiti* in a naturally infected bull (*Bos taurus*) from South Africa. J Parasitol 99:459–466. https://doi.org/10.1645/12-128.1

EFSA European Food Safety Authority, 2010. Bovine besnoitiosis: an emerging disease in Europe. Scientific statement on Bovine Besnoitiosis, Question No EFSA-Q-2009–00879, adopted 28.01.2010, *EFSA Journal* **8**:1499

Fernandez-Garcia A, Alvarez-Garcia G, Risco-Castillo V, Aguado-Martinez A, Marcen JM, Rojo-Montejo S, Castillo JA, Ortega-Mora LM (2010) Development and use of an indirect ELISA in an outbreak of bovine besnoitiosis in Spain. Vet Rec 166:818–822. https://doi.org/10.1136/vr.b4874

Frey CF, Gutierrez-Exposito D, Ortega-Mora LM, Bena-vides J, Marcen JM, Castillo JA, Casasus I, Sanz A, Garcia-Lunar P, Esteban-Gil A, Alvarez-Garcia G (2013) Chronic bovine besnoitiosis: intra-organ parasite distribution, parasite loads and parasite-associated lesions in subclinical cases. Vet Par 197:95–103. https://doi.org/10.1016/j.vetpar.2013.04.023

Gazzonis AL, Alvarez Garcia G, Zanzani SA, Garippa G, Rossi L, Maggiora M, Dini V, Invernizzi A, Luini M, Tranquillo VM, Ortega Mora L, Manfredi MT (2014) *Besnoitia besnoiti* among cattle in insular and northwestern Italy: endemic infection or isolated outbreaks? Parasit Vectors 7:585. https://doi.org/10.1186/s13071-014-0585-4

Gazzonis AL, Alvarez-Garcia G, Maggioni A, Zanzani SA, Olivieri E, Compiani R, Sironi G, Ortega-Mora LM, Manfredi MT (2017) Serological dynamics and risk factors of *Besnoitia besnoiti* infection in breeding bulls from an endemically infected purebred beef herd. Parasitol Res 116:1383–1393. https://doi.org/10.1007/s00436-017-5418-x

Gentile A, Militerno G, Schares G, Nanni A, Testoni S, Bassi P, Gollnick NS (2012) Evidence for bovine besnoitiosis being endemic in Italy: first in vitro isolation of *Besnoitia besnoiti* from cattle born in Italy. Vet Par 184:108–115. https://doi.org/10.1016/j.vetpar.2011.09.014

Gollnick NS, Gentile A, Schares G (2010) Diagnosis of bovine besnoitiosis in a bull in Italy. Letter Vet Rec 166:599. https://doi.org/10.1136/vr.c2314

Gollnick NS, Scharr JC, Schares G, Langenmayer MC (2015) Natural *Besnoitia besnoiti* infections in cattle: chronology of disease progression. BMC Vet Res 11:35. https://doi.org/10.1186/s12917-015-0344-6

Hornok S, Fedák A, Baska F, Basso W, Dencső L, Tóth G, Szeredi L, Abonyi T, Dénes B (2015) Vector-borne transmission of *Besnoitia besnoiti* by blood-sucking and secretophagous flies: epidemiological and clinicopathological implications. Parasit Vectors 8:450. https://doi.org/10.1186/s13071-015-1058-0

Jacquiet P, Lienard E, Franc M (2010) Bovine besnoitiosis: epidemiological and clinical aspects. Vet Par 174:30–36. https://doi.org/10.1016/j.vetpar.2010.08.013

Liénard E, Pop L, Prevot F, Grisez C, Mallet V, Raymond-Letron I, Bouhsira É, Franc M, Jacquiet P (2015) Experimental infections of rabbits with proliferative and latent stages of *Besnoitia besnoiti*. Parasitol Res 114:3815–3826. https://doi.org/10.1007/s00436-015-4612-y

Liénard E, Salem A, Grisez C, Prévot F, Bergeaud JP, Franc M, Gottstein B, Alzieu JP, Lagalisse Y, Jacquiet P (2011) A longitudinal study of *Besnoitia besnoiti* infections and seasonal abundance of *Stomoxys calcitrans* in a dairy cattle farm of southwest France. Vet Par 177:20–27. https://doi.org/10.1016/j.vetpar.2010.11.030

Majzoub M, Breuer W, Gollnick N, Rostaher A, Schares G, Hermanns W (2010) ein ausbruch von besnoitiose bei rindern in Deutschland. Wien Tierärztl Mschr 97:9–15

Manuali E, Lepri E, Salamida S, D'Avino N, Mangili P, Vitellozzi G, Grelloni V, Filippini G (2011) An outbreak of bovine besnoitiosis in beef cattle born in Central Italy. Transboud Emerg Dis 58:464–467. https://doi.org/10.1111/j.1865-1682.2011.01220.x

Mugridge NB, Morrison DA, Jakel T, Heckeroth AR, Tenter AM, Johnson AM (2000) Effects of sequence alignment and structural domains of ribosomal DNA on phylogeny reconstruction for the protozoan family Sarcocystidae. Mol Biol Evol 17:1842–1853. https://doi.org/10.1093/oxfordjournals.molbev.a026285

Mutinelli F, Schiavon E, Ceglie L, Fasolato M, Natale A, Rampin F, Carminato A (2011) Bovine besnoitiosis in imported cattle in Italy. Vet Par 178:198. https://doi.org/10.1016/j.vetpar.2010.12.019

Nobel TA, Neumann M, Klopfer U, Perl S (1977) Kystes de *Besnoitia besnoiti* dans les organes génitaux de la vache. Bullet Acad Vét Fr 50:569–574

Olias P, Schade B, Mehlhorn H (2011) Molecular pathology, taxonomy and epidemiology of *Besnoitia* species (Protozoa: Sarcocystidae). Infect Genet Evol 11:1564–1576. https://doi.org/10.1016/j.meegid.2011.08.006

Pols J (1960) Studies on bovine besnoitiosis with special reference to the aetiology. Onderstepoort J Vet Res 28:265–356

Rinaldi L, Maurelli MP, Musella V, Bosco A, Cortes H, Cringoli G (2013) First cross-sectional serological survey on *Besnoitia besnoiti* in cattle in Italy. Parasit Res 112:1805–1807. https://doi.org/10.1007/s00436-012-3241-y

Rostaher A, Mueller RS, Majzoub M, Schares G, Gollnick NS (2010) Bovine Besnoitiosis in Germany. Vete Dermatol 21:329–334. https://doi.org/10.1111/j.1365-3164.2009.00813.x

Ryan EG, Lee A, Carty C, O'Shaughnessy J, Kelly P, Cassidy JP, Sheehan M, Johnson A, de Waal T (2016) Bovine besnoitiosis (*Besnoitia besnoiti*) in an Irish dairy herd. Vet Rec 178:608. https://doi.org/10.1136/vr.103683

Vacirca G, Agosti M, Belloli A, Greppi G, Morini M (1994) Indagine siero immunologica in un focolaio di besnoitiosi in bovini da carne importati. In: Proceedings of the XVIII. World Buiatrics Congress, Bologna, Italy, August 29–September 2, 1994, 389–392.

Vanhoudt AP, B, De Schutter P, Bosseler T, Sarre C, Vercruysse J, Deprez P, (2015) First confirmed case of bovine besnoitiosis in an imported bull in Belgium. Vlaams Diergeneeskund Tijdschr 84:205–211

Villa L, Gazzonis AL, Zanzani SA, Perlotti C, Sironi G, Manfredi MT (2019) Bovine besnoitiosis in an endemically infected dairy cattle herd in Italy: serological and clinical observations, risk factors, and effects on reproductive and productive performances. Parasit Res 118:3459–3468. https://doi.org/10.1007/s00436-019-06501-9