Genital tract involvements in a bull affected by bovine besnoitiosis

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SUMMARY

Bovine besnoitiosis is a parasitic disease of cattle caused by the cyst-forming coccidian *Besnoitia besnoiti*. The parasite targets mainly skin, mucosal membranes, scleral conjunctiva, endothelial cells of large vessels and testes. Severe necrotising inflammation of the latter (orchitis) may result in permanent infertility. The aim of these investigation was to describe the gross and histopathological testicular lesions observed in a 14-months old Limousine bull affected by besnoitiosis. Gross examination of the samples revealed characteristic diffuse scleroderma and hyperkeratosis, especially in scrotal skin. There was diffuse hyperemia of testes and sporadically visible presence of cysts at the level of the testicular coverings and the preputial mucosa. Microscopical observation revealed characteristic cysts of Besnoitia spp. distributed in subcutaneous tissue of scrotum. Many cysts were found also in penis, testicular coverings, testicular tubules, and epididymis. The observed cysts were variable in size from 250 to 300 μm in diameter, and showed the parasitophorous vacuole containing typical 7.0×2.0 μm basophilic banana-shaped Besnoitia spp. bradyzoites. A moderate inflammatory response, composed by macrophages, eosinophils and scattered lymphocytes, was seen near and around the cysts. The presence of multiple Besnoitia spp. cysts in different section of the reproductive tract of the bull is confirmed. Moreover, the presence of the cysts in the testicular tubules (parenchyma), epididymis, in the penis may explain the potential impaired fertility of the bulls.

KEY WORDS

Bovine besnoitiosis, Besnoitia cysts, testicular coverings.

INTRODUCTION

Besnoitia besnoiti (Protozoa, Apicomplexa) is a cyst-forming coccidian parasite. The biology of *B. besnoiti* and many aspects of the epidemiology are poorly understood. It has been suspected that *B. besnoiti*, like other cyst-forming coccidia, might have an indirect life cycle, with a carnivore as definitive host and cattle and wild bovids representing intermediate hosts.

However, the complete life cycle of *B. besnoiti* is still unknown and a definitive host has not been identified yet. The only experimentally confirmed modes of transmission among cattle are mechanically through blood-sucking insects and iatrogenically through hypodermic needles; also the transmission through direct contact seems to be possible[1]. However, there is epidemiological evidence in support of other modes of infection, i.e. close contact between animals. The presence of cysts in the genital mucosae may probably result in transmission during mating. In addition, recovered and sub clinically infected animals are thought to remain life-long carriers of *B. besnoiti* and are considered as important sources of infection for naïve cattle[2].

Bovine besnoitiosis has largely been reported in sub-Saharan Africa and Asia[3]. In Europe, the disease was initially reported in Portugal and France[4, 5]. Over the last 10 years, several epidemiological studies have confirmed an increase in the number of cases also in countries traditionally free of bovine besnoitiosis as Germany[6], Italy[7], Switzerland[8], Croatia[3], Hungary[2] and Belgium[9].

Bovine besnoitiosis has two distinct clinical stages: an acute stage which lasts approximately 1-2 weeks, followed by a chronic stage, which is lifelong. The chronic phase is characterized by scleroderma and alopecia, which are associated with formation of tissue cysts of up to 0.5 mm in diameter by slowly proliferating bradyzoites, which can persist for several years in different tissues of the host[3].

Bovine besnoitiosis is responsible for severe economic losses on affected farms due to mortality, weight loss, prolonged convalescence, decline in milk production and definitive or transient sterility in males[10]. Only a few studies have described lesions in testes and assessed seminal quality in chronically infected animals[11, 12, 13]. Infected bulls may present testicular atrophy with azoospermia. Indeed, numerous cysts have been observed in the testes, epididymis, and ampullae and in the walls of blood vessels in the pampiniform plexus that could interfere with normal spermatogenesis[11, 12, 14]. Therefore, the objective of this case report was to investigate and describe the gross and histopathological findings in the reproductive tract of a bull with chronic besnoitiosis.

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MATERIALS AND METHODS

The patient was a 14-months old Limousine bull, imported from France and referred for thickening of the skin and enlargement of the testis and finally diagnosed as affected by besnoitiosis. The diagnosis was confirmed by histological investigation of skin sampled via biopsy. The bull was slaughtered before the expected time due to retarded growth and only the genital tracts were presented to the Department of Veterinary Medical Science of the University of Bologna (Italy). No other biological matrices were delivered. Gross examination of the organs of genital tracts (skin of scrotum, entire testes, testicular coverings, epididymis, penis, and penis retractor muscle) was performed. Representative tissue sample were collected, fixed in 10% buffered formalin, embedded in paraffin wax, and processed for histological investigation.

RESULTS

Gross examination of the scrotal skin revealed the typical aspects of thickening and lichenification. Presence of *B. besnoiti* cysts were observed at the level of the testicular coverings and preputial mucosa (Figure 1). The right testicle showed and evident diffuse hyperemia, especially if compared with the contralateral one (Figure 2). Microscopical observations revealed the presence of a lot of *B. besnoiti* cysts, containing numerous banana-shaped bradyzoites and host cells nuclei in their wall, localized in subcutaneous tissue of scrotum (Figure 3A). Numerous *B. besnoiti* cysts were also located in the cross section of the penis, testicular coverings, testicular tubulin, and epididymis (Figure 3B, 3C). Plasmocytic and histiocytic inflammatory reaction, with sparse eosinophilic granulocytes infiltration, were seen around some broken cysts. A moderate inflammatory response, characterized by the pres-

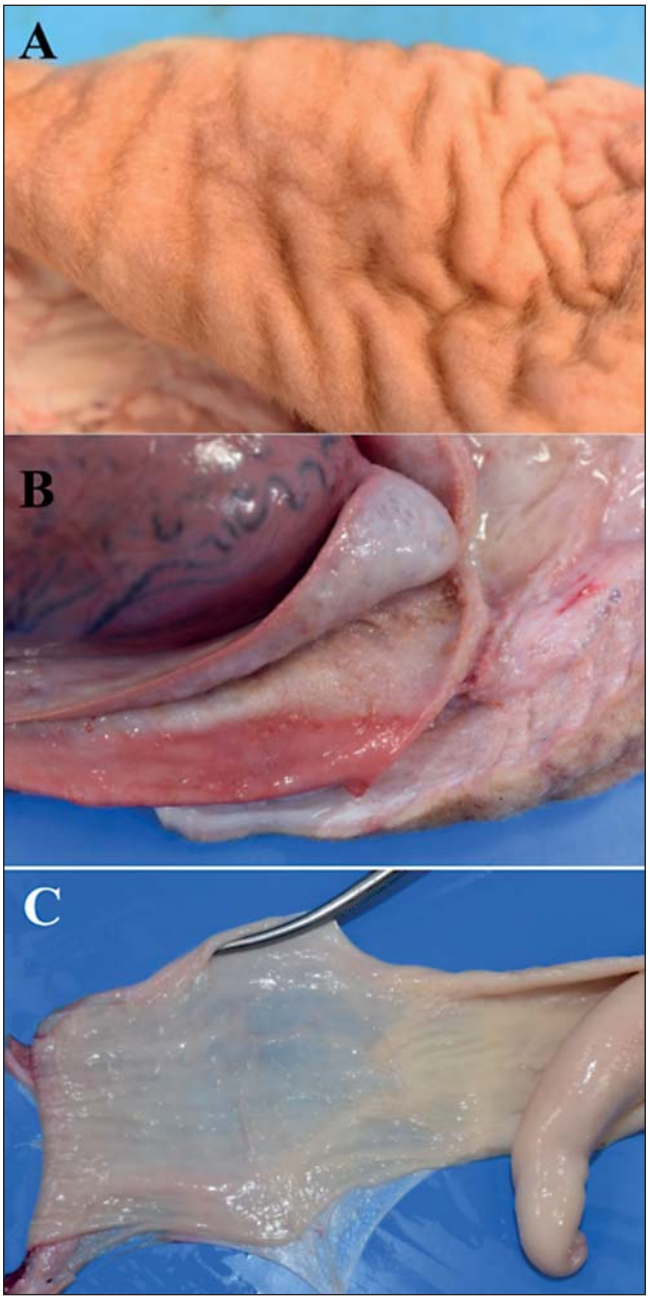

Figure 1 - Thickening and lichenification of the scrotal skin (**A**). Visible cysts at the level of the testicular coverings (**B**) and preputial mucosa (**C**).

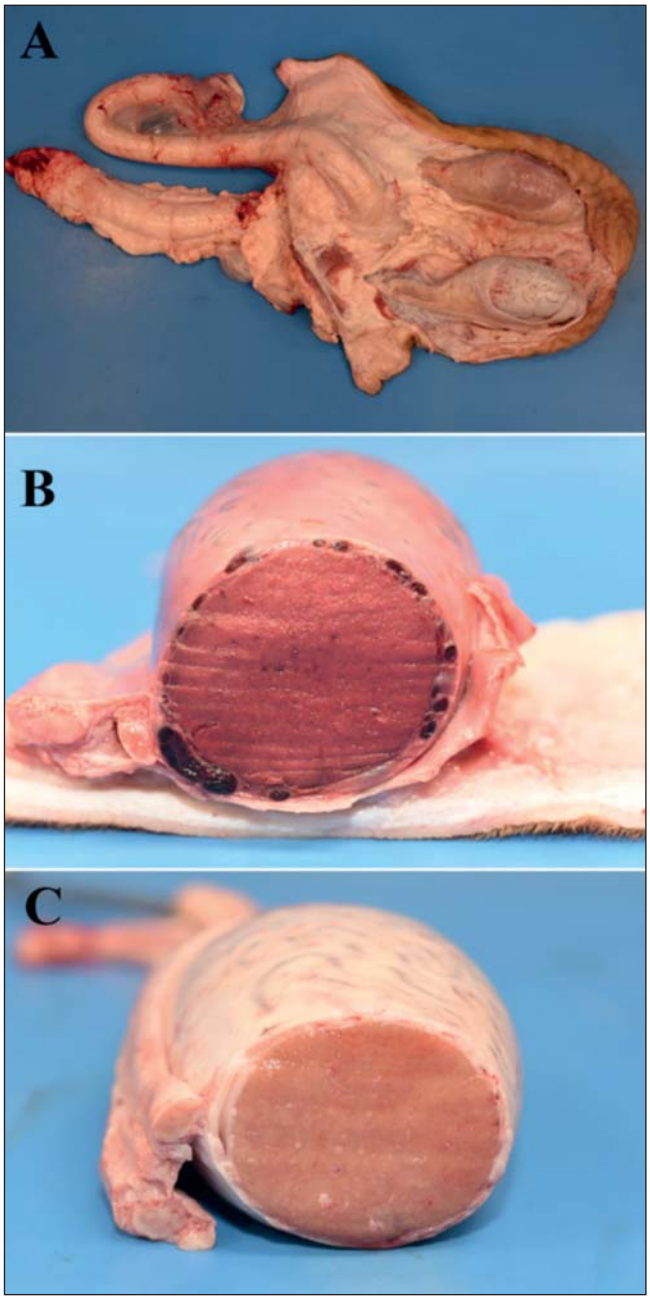

Figure 2 - Evident hyperemia of the right testicle (**A**, **B**), especially if compared with the left one (**C**).

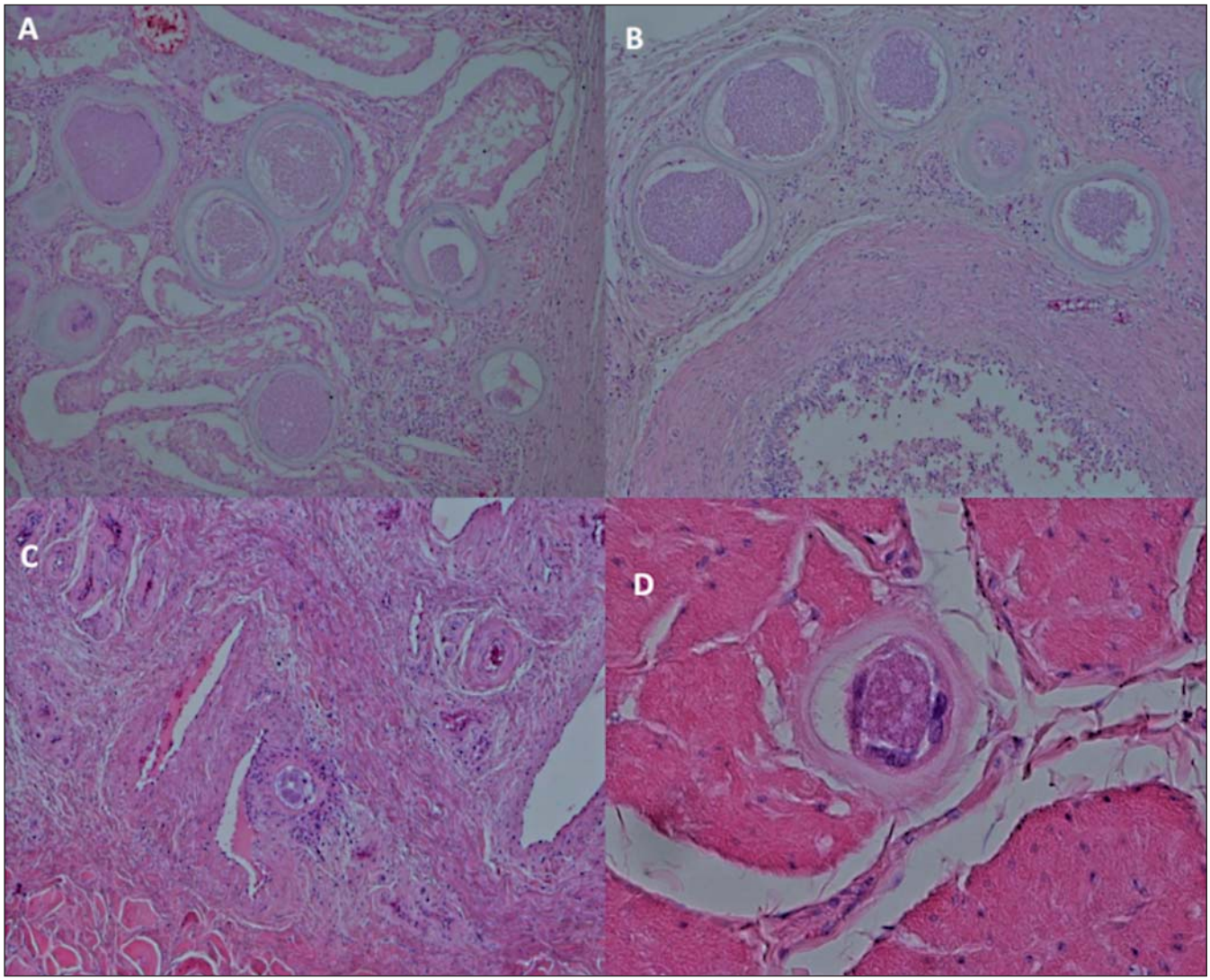

Figure 3 - *B. besnoiti* cysts in the skin and subcutaneous tissue of the scrotum, in the testicular coverings as well as in the testicular tubules (**A**) (10x), and epididymis (**B**) (10x). Plasmocytic and histiocytic inflammatory reaction, with sparse eosinophilic granulocytes infiltration were observed around some broken cysts **(C)** (10x). Numerous *B. besnoiti* cysts were evidenced also in the cross section of the penis (**C**) (10x) and the penis retractor muscle (**D**) (20x).

ence of macrophages, eosinophils and scattered lymphocytes, was seen near and around the cysts (Figure 3C). The *B. besnoiti* cysts were variable in size from 250 to 300 μm in diameter, showed a wall of 10-20 μm in thickness with three layers: the outer hyaline one, the middle layer with host cells cytoplasm and nuclei, and the inner membrane with the parasitophorous vacuole containing typical 7.0×2.0 μm basophilic banana-shaped Besnoitia spp. Bradyzoites (Figure 3).

DISCUSSION

Similarly to other reports[4, 11, 15, 16], the lesions observed in the current case showed a strong affinity of the *B. besnoiti* cysts for all the tissues of the male genital system: scrotal skin, preputial mucosa, penis, testicular coverings, testicular tubulin, epididymis and, finally, testis. However, it should be emphasized that previous reports evidenced the possibility of some tissue to be preserved by the cysts invasion, such as the testicular parenchyma[1] or the penis[16].

The observed *B. besnoiti* cysts were variable in size from 250 to 300 μm in diameter and showed a wall of 10-20 μm in thickness with three layers. The literature shows both reports with overlapping cysts' dimensions[16], and reports with smaller cysts' diameters, the latter describing cyst diameter of 97.2, 90.68 and 20.0 μm in the pampiniform plexus, scrotal skin and testicular parenchyma, respectively.

The cysts were surrounded by macrophages, eosinophils and scattered lymphocytes, evidencing an inflammatory and degenerative response that might be considered at the base of the risks of temporary or permanent infertility[12].

It has been hypothesized that the presence of numerous *B. besnoiti* cysts in the interstitial spaces and under the epithelial cells in the seminiferous tubules might displace the normal cells and cause an increase of direct pressure on the germinal epithelial cells, interfering with normal spermatogenesis. This would be finally provoked by the pressure atrophy of epididymal and/or testicular tissues[11]. This would be finally provoked by the pressure atrophy of epididymal and/or testicular tissues[11]. Additionally, the testicular degeneration might be also the consequence of (i) thermoregulation failure induced by vascular lesions in pampiniform plexus and scrotal skin lesions; (ii) severe vascular wall injury induced by the inflammatory response in the testis; and (iii) blood-testis barrier damage and alteration of spermatogenesis by immunoresponse[15].

CONCLUSIONS

This case of a chronically affected bull confirmed the severe repercussions of a besnoitiosis infection on the genital tract, evidencing the characteristic inflammatory and degenerative lesions that could ends up with an alteration in the testicle function. Although no bull fertility studies were conducted in our study, the injury to the genital tract appeared severe enough to suggest infertility as a possible consequence. In conclusion, besides the potential economic losses due to the involvement of the tegumentary tissue and the metabolic repercussions, impairment of reproductive performances should also be considered an important concern in case of besnoitiosis.

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Conflict of Interest

The authors declare that there were no conflicts of interest.

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