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Does bovine besnoitiosis affect the sexual function of chronically infected bulls?

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ABSTRACT

Bovine besnoitiosis is a reemerging disease in Europe. The clinically *Besnoitia besnoiti* infection in bulls is characterized by fever, nasal discharge, and orchitis in the acute phase and by scleroderma in the chronic phase. However, in many bulls, *B besnoiti* infection remains at a subclinical stage. Bull infertility is an economically relevant consequence of besnoitiosis infection. It is not clear, however, if semen quality returns to normal levels when infected animals have clinically recovered. The aim of this study was to examine the relationship between chronic besnoitiosis and bull sexual function in a region of eastern France, where the disease is reemerging, by comparing semen quality and genital lesions in 11 uninfected, 17 subclinically infected, and 12 clinically infected bulls. The presence of anti-*B besnoiti* antibodies was detected by Western blot test. Semen was collected by electroejaculation. Bulls clinically infected with *B besnoiti* showed significantly more genital tract alterations than uninfected or subclinically infected bulls. No relationship was evidenced between besnoitiosis infectious status and semen quality, whereas a significant relationship was noted between genital lesions and semen score. This means that in the absence of moderate to severe genital lesions, chronic bovine besnoitiosis is unlikely to alter semen quality. However, as the presence of infected animals could lead to spread of the disease, culling or separation of clinically infected bulls from the remaining healthy animals is strongly recommended.

1. Introduction

Bovine besnoitiosis is a parasitic disease caused by the apicomplexan protozoan *Besnoitia besnoiti*, traditionally endemic in Sub-Saharan Africa and Asia. In Europe, cattle besnoitiosis has been reported in Portugal [2], Spain [3], and Italy [4], and recently in Germany [5], Switzerland [6], Hungary [7], and Greece [8]. In France, the disease was first

Ethical considerations: The electroejaculation technique is occasionally used in Europe to evaluate sexual function in bulls. A previous study has provided evidence that this semen collection method does not result in pain to the bull [1]. Blood and skin samples are commonly used in the diagnosis of parasitic disease. All these samplings were done by veterinarians.

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described in the Pyrenean area and has been rapidly spreading in central, southeastern, and western regions since the 1990s [9]. The European Food Safety Authority therefore considered bovine besnoitiosis as a reemerging disease in Europe in 2010 [10]. The clinical form of *B besnoiti* infection exhibits three consecutive stages: an acute stage with fever, a subacute phase with edemas, followed by a chronic stage with scleroderma, resulting from the formation of numerous tissue cysts in the derma, mucous membranes, and scleral conjunctiva. However, only a few animals in a *B besnoiti*-infected herd will develop clinical signs [11,12], whereas most of them will be seropositive but remain subclinically infected. Moreover, some chronically infected animals can recover, at least partially, but remain infected.

In bulls, *B besnoiti* may cause orchitis in the acute stage of the disease, and numerous cysts have been observed in the testes, epididymis, ampullae, and in the walls of blood vessels in the pampiniform plexus in chronically infected bulls [2,13,14]. These numerous besnoitiosis cysts can result in fibrotic lesions of the testis and a thickened scrotum, thus interfering with normal spermatogenesis. Bulls can be rendered permanently infertile by unilateral or bilateral testicular degeneration.

Bull infertility is one of the most relevant economic consequences of besnoitiosis infection, particularly in extensive natural-service herds. However, it is not clear if semen quality returns to normal levels when infected animals are still subclinically infected or exhibit clinical recovery. The aim of this study was to examine the relationship between chronic besnoitiosis and sexual function in naturally infected bulls from a region of eastern France where the disease is reemerging, by comparing semen quality and genital lesions in uninfected, subclinically infected, and clinically infected bulls.

2. Material and methods

2.1. Animals

All animal procedures were carried out in accordance with the national regulations regarding ethics and best practices in veterinary care. Forty sexually mature bulls from 31 extensively farmed beef cattle herds using natural service were included in this study in September 2013. They came from the “Alpes-de-Haute-Provence” department in southeastern France, mountainous area where bovine besnoitiosis has been reemerging since the first cases of the disease were reported 10 years ago. The herd prevalence in this region currently varies from 50% to 74%, and the animal prevalence within herds ranges from 18% to 70% [15].

The breeds of these bulls were Charolais (n = 21), Limousin (n = 16), Blonde d’Aquitaine (n = 1), Abondance (n = 1), and Angus (n = 1), and the bulls were between 1.5 and 8.5 year old (3.8 ± 1.9 years). They were selected from herds with prior serological assessment of besnoitiosis and where bull infertility was suspected because of abnormally long calving intervals. The clinical cases of besnoitiosis in cows and bulls are recorded in Table 1. All bulls were in good body condition (mean ± standard deviation [SD]: 3.4 ± 0.42, range: 2.5–4) on the basis of a scale of 1 to 5.

Each bull was carefully checked for clinical signs indicative of bovine besnoitiosis, i.e., cysts on the sclera conjunctiva and thickening of the scrotal and leg skin.

2.2. Breeding soundness assessment

The clinical examinations and sampling were carried out in the equine breeding center of Seyne Les Alpes (Alpes-de-Haute-Provence). Each bull was placed in an individual restraining cage and subjected to clinical genital examinations, which included visual inspection of the scrotum and penis (if exteriorized); measurement of scrotal circumference; palpation of the scrotum, testis, epididymis, prostate, and vesicular glands; and ultrasonography of the testis and epididymis. Testis calcification was assessed from a transmediastinal section (Fig. 1) using the method described by Barth et al. [16]. A clinical score was established for each bull on the basis of severity of the genital lesions: normal included normal genital tract or mild testis fibrosis or mild, localized thickening of the scrotum; moderate lesions: moderate testis fibrosis or scrotal thickening, unilateral epididymal or testis lesion; and severe lesions: severe and/or bilateral testis fibrosis, severe scrotal thickening or abcess, bilateral epididymal cysts (Table 1).

2.3. Samples collection

Blood samples were obtained by caudal vein puncture, and the sera were separated by natural precipitation in the sampling tube (Venosafe Plastic Tube: Serum gel–VF-054SAS02). All sera were stored at −20 °C until used.

Semen was collected by electroejaculation (Electrojac5; Ideal Instruments, MI, USA). A previous study has provided evidence that this semen collection method does not result in pain to the bull [1]. In addition, to preserve the welfare of the bull, stimulation was discontinued if signs of stress or physical discomfort were detected. When the ejaculates turned cloudy, after the emission of clear seminal plasma, the semen was collected separately into prewarmed tubes through a cone. In four bulls, the stimulation was unsuccessful in eliciting ejaculation. Each ejaculate was kept in a water bath at 37 °C until semen examination.

2.4. Semen examination

The following semen characteristics were analyzed: volume (mL), gross and progressive motility, and abnormalities after eosin-nigrosin staining (Sperm VitalStain; Nidacon, Sweden) and embedding in resin (Histolaque LMR; LaboModerne, Paris). Sperm concentration was measured by spectrophotometry (Accuread, IMV technologies, L’Aigle, France). A seminal score on the basis of number of spermatozoa in the ejaculate, the individual motility, and the percentage of abnormal spermatozoa was determined for each bull [17,18] (Table 2).

2.5. Serological analysis

Specific antibodies against bovine besnoitiosis were detected in the bulls by analyzing blood sera by