

## Case Report

## First confirmed case of bovine besnoitiosis in Rwanda

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## ABSTRACT

An-eighteen months (one and half years) old heifer was presented with a progressive loss of weight leading to cachexia and was attended to by a team of doctors from the ambulatory clinic of the School of Animal Science and Veterinary Medicine, University of Rwanda. Clinical examination revealed scleroderma of both sides of the neck and pinpoint cysts in the sclera and conjunctiva as well as corneal opacity of the left eye. Histological examination of skin biopsy showed a mononuclear dermatitis with several cysts of 100–300 μm filled up with bradyzoites in the subcutis and dermis. These lesions are consistent with bovine besnoitiosis, a disease caused by *Besnoitia besnoiti* in cattle. To the best of our knowledge, this is the first confirmed case of bovine besnoitiosis in Rwanda.

## 1. Introduction

Besnoitiosis, is a mild to severe acute, subacute or chronic but usually non-fatal protozoan disease caused by one of the tissue-cyst-forming coccidian parasite known as *Besnoitia besnoiti* belonging to the family Sarcocystidae, subfamily Toxoplasmatinae (Fernandez-Garcia et al., 2010; Gutiérrez-Expósito et al., 2017a, 2017b; Langenmayer et al., 2015a; Langenmayer et al., 2015b). The parasite and the disease it causes were named by Franco and Borges in 1916, after Besnoit, a French parasitologist who together with Robin had described the parasite in 1912. Cadéac had previously in 1884 described it as sarcosporidiosis (Cortes et al., 2014). Thereafter, diseases caused by various *Besnoitia* spp. have been reported in several geographical locations among a wide range of hosts including domestic and wild animals and rodents (Cortes et al., 2014; Fernández-García et al., 2009; Gollnick et al., 2010; Hornok et al., 2014; Kumi-Diaka et al., 1981; Langenmayer et al., 2015b; Lesser et al., 2012; Manuali et al., 2011; Maqbool et al., 2012; Schares et al., 2016; Vanhoudt et al., 2015), goat (Mirzaie et al., 2007; Namazi et al., 2011), African antelope (Foley et al., 1990), wildebeest and several other wildlife species (Leighton and Gajadhar, 2001). The disease has also been produced experimentally in rabbits (Mbuthia et al., 1993). Following an incubation period varying from 2 weeks to 2 months, the clinical signs generally appear as fever (40 °C to 41.6 °C) that is associated with parasite multiplication and parasitemia (Álvarez-García et al., 2013; Gutiérrez-Expósito et al., 2017a, 2017b). This phase is followed by the invasion of the cardiovascular

and respiratory systems by the parasite leading to respiratory distress, tachycardia, anorexia and weight loss. The main lesions include oedema of the head, neck and anasarca in severe acute cases, vasculitis, hyperplasia, thrombosis, and necrosis of venules and arterioles (Frey et al., 2013; Gutiérrez-Expósito et al., 2017a, 2017b; Gutiérrez-Expósito et al., 2017a, 2017b).

After the acute phase, the cutano-mucous form follows. In this phase, observed signs and lesions include thickening and folding of the skin, alopecia and hyperkeratosis. The pathognomonic clinical signs are cysts in scleral conjunctiva and vaginal mucosa which generally appear 6 to 7 weeks post-infection (Álvarez-García et al., 2013; Dubey et al., 2013; Jacquet et al., 2010). The disease has been reported worldwide and is endemic in several Sub Saharan Africa (Chatikobo et al., 2013; Gutiérrez-Expósito et al., 2017a, 2017b; Malatji, 2015). However, to the best of our knowledge, there is no previous report of the disease in Rwanda either experimentally or under natural condition. This report is based on a case of a heifer with alopecia and emaciation that was attended to during our ambulatory clinic. It could be used as a baseline to study the epidemiology of the disease in Rwanda.

## 2. Case presentation

## 2.1. Case history

In December 2013, a farmer from Burera Village, Rugazi Cell, Katagamu Sector of Nyagatare District in the Eastern Province of

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Fig. 1. Sclero-dermatitis in a one and half year old heifer.

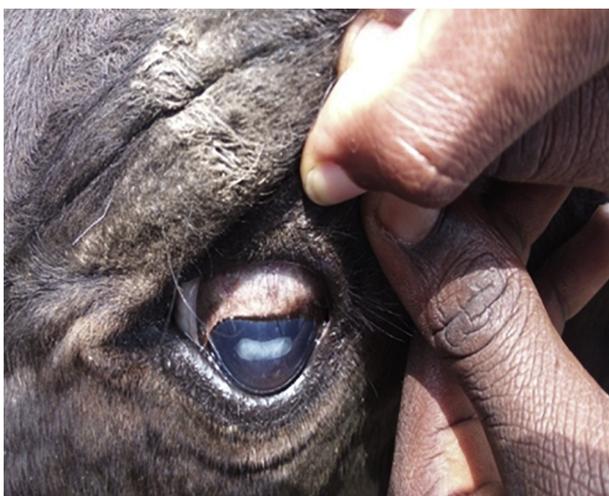


Fig. 2. Corneal opacity “pinpoint grayish white areas” due to besnoitiosis.

Rwanda transferred 20 weaned Friesian heifers to a hired farm that had been previously used for communal grazing. In December 2014, one of the Friesian heifers at 13 months of age developed skin lesions which consisted of generalised sclero-dermatitis (like those of this reported case). Despite the treatment given, it underwent progressive emaciation and died after 8 months (July 2015). The information gathered showed that the animal had been treated with an antibiotic (Oxytetracycline 20% LA 1 ml per 10 kg b.w. single injection) anti-inflammatory (a single dose of Phenylbutazone 20% injection at the dosage of 1 ml for 10 kg b.w.) and vitamin supplements. In December 2015, a second heifer of about the same age (the case of this report) was presented by the farmer. Clinical examination revealed macroscopic lesions of hard scaly corrugated skin affecting both sides of the neck (Fig. 1) and multiple pinpoint grayish white foci in the sclera and conjunctiva, as well as corneal opacity of the left eye (Fig. 2), were observed. The heifer was in a poor condition with a body condition score (BCS) of 2 on scale 5. A square centimetre (1 cm × 1 cm × 0.5 cm) skin biopsy from the affected neck was taken and fixed in 10% buffered formalin. After discussing with the farmer, the treatment options and assessing the risk of spreading the disease to the rest of the herd, the farmer decided to cull it. Although a systematic screening by serological tests was suggested, regrettably, it was not conducted due to lack of financial means from the farmer.

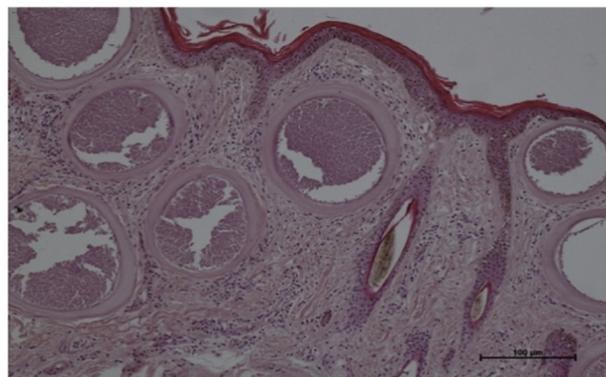


Fig. 3. Besnoitia cysts in the dermis.

## 2.2. Histopathology

The 10% formalin fixed biopsy samples were then processed by the routine histopathological technique and stained with Haematoxylin and Eosin for microscopical examination. Briefly, the formalin-fixed samples were trimmed, dehydrated through graded alcohol, cleared in xylene and embedded in molten paraffin wax. The sections were then cut by a tissue microtome to about 3 μm thick and stained with Haematoxylin-Eosin stain for microscopic examination. The microscopic examination of the stained sections revealed a mild mononuclear inflammatory reaction in the dermis and deep in the subcutis. Several besnoitia cysts (100–300 μm) filled with bradyzoites (Fig. 3) of *B. besnoiti* in the subcutis were observed. Some cysts were found deep in the subcutis of the skin (Fig. 4, arrows). These microscopic findings are consistent with the cutaneous form of bovine besnoitiosis.

## 3. Discussion

Bovine besnoitiosis is generally a chronic and debilitating disease that causes severe economic losses to farmers in endemic areas (Frey et al., 2013). In non-endemic areas, the acute form of the disease in cattle has also been reported during outbreaks (Gutiérrez-Expósito et al., 2017a, 2017b). However, in some cases, the disease may run a subclinical course and go unnoticed (Frey et al., 2013; Jacquet et al., 2010). This is the first confirmed case of clinical besnoitiosis in cattle in Rwanda. Presently, it is hard to determine the true origin of the infection and this requires an epidemiological investigation that would shed some light on the primary source of infection. Considering that the disease is endemic in some neighbouring countries (Uganda, Tanzania, Democratic Republic of Congo) and South Africa (Álvarez-García et al., 2013; EFSA, 2010) where most of the cattle are imported from, we suspect that the parasite was most likely introduced into Rwanda

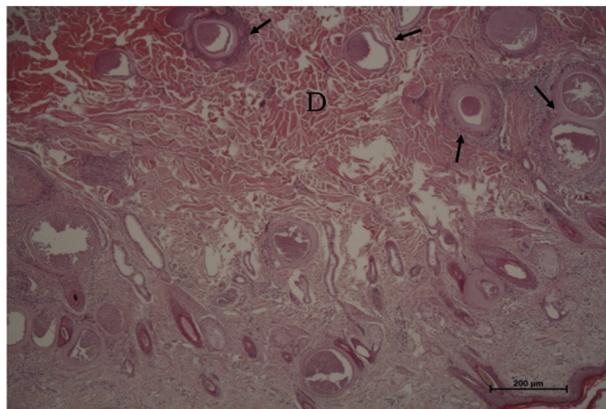


Fig. 4. Besnoitia cysts (arrows) deep in the subcutis (D).

through importation of infected animals under One Cow Per Poor Family programme also known as “*Girinka* programme”. This view is supported by the report of Bwangamoi et al. (2002) in which they reported cases of besnoitiosis in Uganda in imported goats from Kenya. However, given that the farm is not far away from the Akagera National Park, the possibility for the initial case coming from other wild animal species such as wild ruminants like Impala cannot be ruled out. Alternatively, this being possibly the second case on the same farm, iatrogenic transmission from the initial case through the use of a single needle on different animals should be considered. Whatever the case, the source of this first case of besnoitiosis in Rwanda remains a mystery that needs to be solved through a thorough epidemiological investigation.

There is a need to contain the disease in the country earlier enough, considering its economic impact through abortions of infected pregnant cows and infertility of infected bulls (Cortes et al., 2014). This confirmation of a case of besnoitiosis in the main cattle corridor of Rwanda's Eastern province, therefore, is a threat to the economy of the country that seeks to promote “Made in Rwanda” through the transformation of green leather into finished products. Currently, Rwanda earns over USD 6,5 million annually from green hides and skins and it is estimated that if the hides and skins are transformed into finished products, they can generate about US\$117 million to the country. An estimate of hundreds of jobs would also be created among the local population. The severe damage to the skin and presence of *Besnoitia* cysts of the size of sugar granules in the subcutis and intermuscular fascia may sometimes lead to carcass condemnation during meat inspection but also the loss of the skins and hides since they cannot be processed. This would be a big setback to the growing leather industry in particular, food security and meat trade in general.

It is important to highlight that farmers and veterinarians are not aware of bovine besnoitiosis risks in newly affected countries such as Rwanda. We therefore recommend that the Rwandan government raises awareness about this disease regarding clinical signs and the transmission mode. It is recommended that an epidemiological investigation covering the whole country should be conducted to establish the extent of the infection in the country and to understand the origin of the disease in Rwanda.

### Conflicts of interest

The authors declare that they have no financial or personal relationships which may have inappropriately influenced them in writing this article.

### Authors' contributions

G.H. contributed to the write-up, editing and coordinated the publication of the manuscript. C.N. did clinical examination and contributed to the initial write up of the manuscript. B.A. and B.T. contributed to the clinical examination. L.O. did the histopathological examination and manuscript editing. All authors approved the final version of the manuscript.

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### Ethical statement

Ethical approval for this study was obtained from the research committee of the School of Animal Sciences and Veterinary Medicine,

College of Agriculture, Animal Sciences and Veterinary Medicine of the University of Rwanda. We obtained a written consent from the owner of the heifer to publish this case. Animal care and used protocol adhered to World Organisation for Animal Health (OIE) Terrestrial Animal Health Code 2012 (use of animals in research and education).

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