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Bovine besnoitiosis in Switzerland: Imported cases and local transmission

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ABSTRACT

Bovine besnoitiosis is an economically important disease of cattle, caused by *Besnoitia besnoiti* (Protozoa, Apicomplexa). A considerable spreading of this parasitic infection has been observed in Europe in the last ten years, mainly related to animal trade. In order to investigate the possibility of *B. besnoiti* being unnoticed introduced and getting established in Switzerland through the import of breeding cattle from France, a total of 767 animals (650 cattle imported from France and 117 cattle that had contact with *B. besnoiti* positive cattle in Swiss farms) were screened for antibodies against *B. besnoiti* by both a commercial ELISA and by the indirect fluorescent antibody test (IFAT). A total of 101 (13.17%) samples showed a positive reaction in ELISA (cut-off: percent of positivity [PP] ≥15) and 16 (2.09%) samples had IFAT titers ≥ 1:100. Eight of those samples reacted positive in Western blot (WB), corresponding to five imported Limousin cattle (two cows and one bull from France and two cows from Germany) and to three cattle born in Switzerland (one Limousin heifer born from one of the positive German cows, and two adult Braunvieh cows, that had been in contact with one of the French cows at a Swiss farm). Seven of those animals were subclinically infected and one animal showed only very mild signs. They were subsequently slaughtered, and the serological diagnosis could be confirmed by real-time PCR and/or histopathology in seven animals. The most frequent parasite localizations were the tendons and surrounding connective tissue of the distal limbs and the skin of the head region. Furthermore, *B. besnoiti* could be successfully isolated *in vitro* from one French, one German and one Swiss cattle (isolates Bb-IPZ-1-CH, Bb-IPZ-2-CH and Bb-IPZ-3-CH). In the current situation in Switzerland, prophylactic and control measures should include a serological examination of cattle to be imported from endemic areas and the culling of all confirmed positive animals from the herd. The evidence of *B. besnoiti* infection in both imported and locally born cattle shows that the conditions for the establishment and dissemination of this parasite in Switzerland seem to be adequate.

1. Introduction

Bovine besnoitiosis is caused by the cyst-forming coccidian parasite *Besnoitia besnoiti* (Protozoa, Apicomplexa), closely related to *Toxoplasma gondii*, *Neospora caninum* and *Sarcocystis* spp. This parasite may affect cattle of all

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breeds and ages and can lead to substantial economic losses through reduction of milk and meat production, infertility of bulls, skin damage, premature slaughter and death. Clinical signs may occur during the acute stage (*i.e.* fever, anorexia, nasal and ocular discharge, tachycardia, tachypnoea, salivation, subcutaneous edemas and orchitis) or during the chronic stage of infection (*i.e.* thickening, hardening and folding of the skin, hyperkeratosis, alopecia, presence of macroscopically visible thick-walled tissue cysts in the scleral conjunctiva and genital mucosa) (Bigalke, 1968; Deplazes et al., 2013). Nevertheless, it is estimated that less than 1/3 of the infected animals in a herd develop clinical signs (Fernandez-Garcia et al., 2010; Jacquiet et al., 2010). On the other hand, subclinically infected animals are thought to be epidemiologically important, as they might be unnoticed introduced into naïve herds through animal trade, constituting a source of infection. However, studies dedicated to evaluate the real significance of these animals as infectious sources are scarce (Frey et al., 2013), and many aspects of the epidemiology of bovine besnoitiosis are still poorly understood. It has been suspected that *B. besnoiti*, like other cyst-forming coccidia, has an indirect life cycle with bovids as intermediate hosts and a carnivore as definitive host. Early studies in the former Soviet Union (Peteshev et al., 1974) implicated domestic (*Felis catus*) and wild cats (*Felis libyca*) as definitive hosts of *B. besnoiti*, but this could not be confirmed in later studies. Further attempts to identify a definitive host of *B. besnoiti* by feeding infected tissues to domestic dogs and cats and to wild carnivores, including several species of mammals, birds and reptiles, failed (Basso et al., 2011; Diesing et al., 1988; Rommel, 1975). So far, the only experimentally confirmed modes of transmission among cattle are mechanically through blood-sucking insects (Tabanids, *Stomoxys calcitrans*) and iatrogenically through hypodermic needles (Bigalke, 1967, 1968).

In Europe, since its first description about 100 years ago (Besnoit and Robin, 1912), *B. besnoiti* seemed to be restricted to endemic areas in the South of France, Portugal and Spain. However, in the last 10 years, a considerable spread of the disease within these countries was observed (Alzieu et al., 2007; Cortes et al., 2006b; Fernandez-Garcia et al., 2009, 2010; Liénard et al., 2011), and recently, cases of bovine besnoitiosis were also reported from countries considered free of the parasite, like Germany (Schares et al., 2009) and Italy (Gentile et al., 2012; Gollnick et al., 2010; Manuali et al., 2011). In 2010, the European Food Safety Authority (EFSA) classified the bovine besnoitiosis in Europe as an emerging disease (http://www.efsa.europa.eu/en/scdocs/scdoc/1499.htm). It is suspected that animal trade is one of the main factors involved in the spread of this disease (Olias et al., 2011). Recently, bovine besnoitiosis was for the first time diagnosed in a Swiss farm during a preliminary monitoring of imported breeding cattle, highlighting the importance of animal import for the dissemination of this parasitic infection (Lesser et al., 2012). After this initial case, the monitoring of cattle imported from France, where bovine besnoitiosis occurs endemically, was extended. In addition, also local cattle that had been in contact with confirmed positive animals were tested in order to determine whether *B. besnoiti* can be transmitted from imported to local cattle and disseminate under Swiss farming conditions. Furthermore, different methods available for the diagnosis of bovine besnoitiosis were performed to detect subclinical infections and were evaluated for their use in epidemiological studies.

2. Materials and methods

2.1. Sampling

A total of 716 breeding cattle imported between 2005 and 2012 from France into Switzerland were identified with the help from the Swiss Federal Veterinary Office (BVET) and the Cantonal Veterinary Services. The farmers were informed about the study, and blood samples from 650 (90.8%) of those animals could be collected by the local veterinarians. The sampled animals derived from 168 farms, localized in 16 Swiss Cantons. Animals that had been introduced into Switzerland for short periods only (*i.e.* for summer grazing) or for direct slaughter were not included in the sampling. In farms with serologically positive imported animals (confirmed by Western blot, see below), the whole herd was serologically tested in order to detect local transmission. Also animals sold to other farms, which had been in contact with positive animals, were localized and tested. Geoprocessing was performed with the Quantum GIS software, version 1.8.0 (QGIS Development Team, 2013) (Fig. 1).

Confirmed serologically positive animals were clinically examined and slaughtered. Tissue samples (liver, heart, spleen, kidney, lung, trachea, brain, tendons of hind limb flexor muscles, fasciae of hind limb muscles, dorsal eyelid, skin of ventral and head region, mucosa of *vestibulum vaginae*, uterus, udder, teat, testicle and scrotum) were collected using individual disposable materials to avoid cross contaminations among the samples. Aliquots were stored at −20 °C for real time-PCR analysis. For histopathology, the samples were fixed in 10% buffered formalin. For parasite isolation *in vitro*, samples of tendons and fasciae from hind limbs were conserved at 4 °C and processed within 24 h.

2.2. Serological analysis

All serum samples were double-screened for antibodies against *B. besnoiti* by both a commercial ELISA and by an indirect fluorescent antibody test (IFAT). Samples reacting positive in the ELISA and/or with IFAT titers $\geq$ 1:100 were subsequently tested by Western blot to confirm the screening results. Positive control sera were obtained from naturally infected cattle in which the infection was confirmed by histopathological examination and by PCR (Schares et al., 2009).

2.3. ELISA

A commercial ELISA (PrioCHECK® Besnoitia Ab 2.0, Prionics AG, Zurich, Switzerland, Lot No. U120601; in the following referred to as PrioCHECK Besnoitia ELISA) designed to detect antibodies against *B. besnoiti* in cattle was performed according to the manufacturer's