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A molecular epidemiology of treponemes in beef cattle digital dermatitis lesions and comparative analyses with sheep contagious ovine digital dermatitis and dairy cattle digital dermatitis lesions

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ABSTRACT

Bovine digital dermatitis (BDD) is an infective foot disease commonly reported in dairy cattle where *Treponema* are considered as the primary causative infectious agents. There still remains little definitive information on the etiology of BDD in beef cattle suggesting further investigations are warranted. Beef BDD lesions ($n = 34$) and healthy beef foot tissues ($n = 38$) were analysed by PCR for three BDD-associated *Treponema* phylogroups and also for *Dichelobacter nodosus* and *Fusobacterium necrophorum*. Spirochete culture was attempted on all BDD lesion samples. One or more BDD-associated *Treponema* phylogroups were detected in 100% of beef BDD lesions. "*Treponema medium/Treponema vincentii*-like", "*Treponema phagedenis*-like" and *Treponema pedis* spirochetes were identified in 27/34 (79%), 31/34 (91%) and 24/34 (71%) of BDD lesions, respectively. No BDD-associated treponeme DNA was amplified from beef healthy foot tissues. *D. nodosus* and *F. necrophorum* were present in 24/34 (71%) and 15/34 (44%) of lesions and 10/38 (26%) and 12/38 (32%) of healthy foot tissues, respectively. Twenty spirochetes were isolated from beef BDD lesions; 19 were representatives of the three BDD-associated *Treponema* phylogroups. One spirochete isolate shared less than 97% 16S rRNA gene similarity to the three cultivable BDD-associated *Treponema* phylogroups and therefore may represent a novel taxa of *Treponema*. Upon comparison, sheep contagious ovine digital dermatitis (CODD), dairy cattle and beef cattle BDD lesions appear to have extremely similar bacteriological data and therefore provides evidence of a shared etiopathogenesis posing concerns for cross-species transmission.

1. Introduction

Bovine digital dermatitis (BDD) is an infectious ulcerative foot disease of the bovine digital skin (Cheli and Mortellaro, 1974) which causes severe lameness in dairy cattle worldwide. The primary causative agents of digital

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dermatitis (DD) in dairy cattle are considered to be spirochetal bacteria of the genus *Treponema* (Evans et al., 2011) and BDD is now recognised as being polytreponemal in etiology (Klitgaard et al., 2008; Nordhoff et al., 2008; Evans et al., 2009a). Three phylotypes have been isolated from dairy cattle lesions in the UK and the US (Stamm et al., 2002; Evans et al., 2008), described as '*Treponema medium/Treponema vincentii-like*', '*Treponema phagedenis-like*' and '*Treponema denticola/Treponema putidum-like*' DD spirochetes (Evans et al., 2008) with the latter now recognised as a new species, *Treponema pedis* (Evans et al., 2009b). The disease has been reported in dairy cattle in nearly all countries they are farmed.

The tendency of beef cattle to be different breeds, fed different diets and subjected to different housing regimes than dairy cattle, gives reason for investigations into beef cattle BDD. A recent report identified the same *Treponema* species in beef cattle BDD that are commonly found in dairy cattle lesions (Sullivan et al., 2013). Currently, there are many published dairy BDD lesion *Treponema* isolates which have had their 16S rRNA gene sequenced, however there is a need for more to be obtained from beef cattle BDD lesions. Furthermore, in the last 20 years, a form of DD has been reported in UK sheep, termed contagious ovine digital dermatitis (CODD), which is rapidly emerging as a severe infectious foot disease (Harwood et al., 1997; Davies et al., 1999; Sayers et al., 2009). Sequences of the 16S rRNA gene of treponemes isolated from sheep CODD lesions suggest that the bacteria are in most cases identical to those found in dairy cattle lesions (Sullivan et al., 2015a). The possible involvement of other organisms such as *Dichelobacter nodosus* and *Fusobacterium necrophorum* has been investigated in dairy cattle BDD lesions (Cruz et al., 2005; Klitgaard et al., 2008; Capion et al., 2012; Rasmussen et al., 2012; Knappe-Poindecker et al., 2013) and sheep CODD lesions (Sullivan et al., 2015a), however it is still undetermined whether these are secondary invaders.

The current study aimed to further our understanding of the etiology of beef cattle BDD by surveying a large number of lesions and healthy foot tissue for detection and isolation of DD-associated treponemes and other lameness associated bacteria. Additionally, comparisons were made to compare the bacteriology of DD lesions in beef cattle, dairy cattle and sheep to enable effective prevention and intervention measures for affected animals in the future.

2. Materials and methods

2.1. Sample collection

Beef cattle BDD lesions were sampled from four different farms between December 2012 and July 2014. These farms were located in Gloucestershire (Gloucestershire farm 1 and farm 2), and North Wales (North Wales farm 1 and farm 2). From these farms a total of 26 BDD lesion samples were obtained, of these 21 were surgical biopsies and five were swabs of lesions. Four of the beef BDD samples obtained from Gloucestershire used in this study had previously been investigated by authors (Sullivan et al., 2013) and were included for further investigation. Additionally, eight surgical biopsies of beef cattle BDD lesions and 38 healthy beef cattle foot skin biopsies were collected from a fallen stock centre (March 2014–June 2014). The healthy foot tissue samples were obtained from beef cattle that did not have any evidence of BDD or any other foot lesions. These cattle were from a fallen stock centre which received animals from farms within Lancashire, Cheshire and South Cumbria. This gave a total of 34 beef cattle BDD lesion samples (surgical biopsies and swab samples) and 38 healthy foot tissue samples.

Briefly, Gloucestershire farm 1 was a beef-rearing unit, with around 120 beef cattle and farm 2 was a finishing unit, finishing around 3000 animals each year. North Wales farm 1 was a beef-finishing unit, finishing around 800–1000 beef animals per year and farm 2 was a beef suckler herd of about 60 cattle.

On all farms from which BDD surgical biopsies were obtained, farmers had isolated lame animals suspected of suffering from BDD. These lame animals were then all inspected for BDD lesions. A cow was defined as having BDD if one or more feet had a clear lesion consistent with the clinical signs of BDD (Cheli and Mortellaro, 1974; Blowey and Sharp, 1988). Typical lesions presented as 20–60 mm diameter (across the largest dimension) circular areas of gray/brown moist exudate, primarily in the region of the caudal interdigital cleft, at the junction of the skin with the soft perioplic horn of the heel, with an underlying raw proliferative area which appeared erosive and granular with a stippled appearance. Some lesions extended into the interdigital cleft, occasionally on the surface of interdigital skin, or extended dorsally to the accessory digits. All beef cattle BDD lesions from which samples were obtained were classified as the classical ulcerative stage defined as 'M2' grade lesions (Döpfer et al., 1997).

The beef cattle identified with these classic BDD lesions were examined and the lesions were biopsied using a 3 mm punch biopsy under local anaesthesia (Evans et al., 2008; Demirkan et al., 2001). Tissue biopsy samples were divided in two with half transferred into transport medium and placed on ice for subsequent *Treponema* culture. Transport medium consisted of oral treponeme enrichment broth (OTEB; Anaerobe Systems, Morgan Hill, CA, USA) and contained the antibiotics rifampicin (5 μg/ml) and enrofloxacin (5 μg/ml). The remaining tissues from lesions, for PCR analysis, were transported on ice and stored at −20 °C.

On some farms, BDD lesions were sampled by swabbing. Local anaesthesia was not used and rather than a punch biopsy, a plain sterile cotton swab was used to sample the lesions. This was done by running the swab over the active lesion where it appeared haemorrhagic, granulomatous and/or necrotic. Swab samples were then processed according to biopsy samples. All samples obtained from the fallen stock centre were collected using the same methods as per BDD lesion sample collection from farms, excluding the use of anaesthesia.

All samples from beef cattle BDD lesions (biopsies and swab samples) were used for DNA extraction and *Treponema* culture, whereas healthy foot tissue samples were only used for DNA extraction and subsequent PCR