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INFECTIOUS DISEASE

Detection and Isolation of Digital Dermatitis Treponemes from Bovine Pressure Sores

S. R. Clegg*, H. E. Crosby-Durrani†, J. Bell*, R. Blundell†, R. W. Blowey‡, S. D. Carter* and N. J. Evans*

** Department of Infection Biology, Institute of Infection and Global Health, School of Veterinary Science, University of Liverpool, Liverpool Science Park IC2, 146 Brownlow Hill, Liverpool, † Department of Veterinary Pathology, School of Veterinary Science, Leahurst, Chester High Road, Neston and ‡ University of Liverpool and Wood Veterinary Group, Gloucester, Gloucestershire, UK*

Summary

Pressure sores cause severe pain and discomfort in hospitalized people and in farmed cattle and are often infected with unknown bacteria. Pressure sores occur on the upper legs of 6–10% of recumbent cattle and are generally considered to be caused by constant pressure, commonly on bony areas of the limbs. This study analyzed pressure sores taken from the upper limbs of 14 cattle using isolation in culture and nested polymerase chain reaction (PCR) to detect treponemes associated with digital dermatitis (DD). A 100% association of DD treponemes with the pressure sores was demonstrated, but treponemes were shown not to be part of the normal skin microbiota. Immunohistochemistry showed an association of DD treponemes with lesions and particularly with the hair follicles in lesions, identifying the bacteria deep within wounds, thereby suggesting that they could contribute to lesion pathogenesis. The bacteria isolated from the pressure sore lesions were similar or identical on analysis of the 16S rRNA gene to those found in DD foot lesions in cattle, suggesting the same bacteria can infect multiple lesions. Indeed, the results of this study suggest that these spirochaetal bacteria may be expanding in host range and in their ability to colonize different tissues and contribute to a range of disease manifestations in farm animals.

Keywords: bovine; pathology; pressure sore; *Treponema* spp.

Introduction

Pressure sores occur when there is unrelieved pressure on an area, such as when a person or animal is immobilized in a single position or constantly rubs against something in the environment, such as bedding. As such, pressure sores commonly occur in areas where bones are prominent, such as joints on the upper limb, which leads to a relative reduction in blood supply and subsequent skin erosion (Kosiak *et al.*, 1958). This can leave the skin open to bacterial infection, further complicating and slowing the healing process (Ceelen, 2003).

Treponemal bacteria are known to be closely associated with digital dermatitis (DD) lesions on the feet of cloven-hoofed animals including dairy and beef cattle worldwide (Dawson, 1998; Evans *et al.*, 2011; Sullivan *et al.*, 2014a), sheep from the UK (Dhawi *et al.*, 2005; Duncan *et al.*, 2014) and Ireland (Sayers *et al.*, 2009), UK goats (Sullivan *et al.*, 2014a) and also wild elk from the USA (Clegg *et al.*, 2014). Additionally, DD treponemes have been isolated from pigs with skin lesions on their shoulders, tail and ears (Karlsson *et al.*, 2013, 2014; Svartström *et al.*, 2013; Clegg *et al.*, 2016c), and detected in cases of horse canker in Austria (Sykora and Brandt, 2015).

These lesions generally contain spirochaetes from several DD *Treponema* phylogroups, which are

Correspondence to: S. R. Clegg (e-mail: S.R.Clegg@liv.ac.uk).

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considered to be motile, anaerobic microorganisms (Graves *et al.*, 1975; Baseman *et al.*, 1976). The treponemes in DD lesions have been isolated and characterized into three different phylogroups identified as the *Treponema medium* phylogroup, the *Treponema phagedenis* phylogroup and the *Treponema pedis* phylogroup (Evans *et al.*, 2008).

Previous studies have shown that DD treponemes are also associated with other lesions on the skin of cattle, including several non-healing foot lesions (Evans *et al.*, 2011), hock lesions (Clegg *et al.*, in press) and udder lesions including mammary dermatitis and ischaemic teat necrosis (Evans *et al.*, 2010; Clegg *et al.*, 2016b), which are generally slow to heal. Taken together, these studies suggest a potential for treponemes to infect skin wounds on areas other than the foot.

Pressure sores are also slow to heal, and due to their involvement in other skin lesions, the aim of the present study was to investigate the infective process of these lesions in cattle and particularly to determine whether the DD treponemes play a role in the pathogenicity of the lesions. If infection with treponemes is involved, then appropriate treatment regimens may be used to enable effective and rapid recovery from pressure sores and prevention measures can be considered. These lesions in cattle may also provide a naturally occurring model for research into human pressure sores.

Materials and Methods

Sample Collection

Tissue samples were collected from pressure sore lesions on the quadriceps mass of cattle. In addition, to allow for testing for common DD lesions, the two hind feet were also taken from animals presenting at a fallen stock yard serving the North West of England. This centre is used for the slaughter and removal of sick or already dead animals from farms, so all animals at the site were dead. To allow for minimal contamination *post mortem*, only animals that had been killed that day, by head shot, were tested.

Animals were categorized into four groups: those with quadriceps mass pressure sores, but no DD lesions on the feet ($n = 11$); those with pressure sores and DD lesions on the feet ($n = 3$); those with no pressure sores, but DD lesions on the feet ($n = 5$); and those with neither pressure sores nor DD lesions on the feet (controls; $n = 10$).

Only lesions between the coxofemoral and tarsal joints of the hindlimbs were classified as pressure sores and tested. Lesions were characterized as acute or chronic, where they had become highly necrotic, and both were tested. Other lesions may have been present on the animal, but were not tested. Animals with pressure sores generally appeared to have been recumbent for a long period of time and so in order to prevent contamination from external sources, deeper tissues were taken from the lesions. Pressure sore surfaces were also cleaned before and after sampling by washing with sterile, filtered phosphate buffered saline (PBS).

In addition, samples of three chronic pressure sore lesions were taken from three different animals and preserved in 10% neutral buffered formalin for use in histological and immunohistochemical studies.

When an animal had a pressure sore, a skin swab was taken from between the hock and the dew claw in order to ascertain whether DD treponemes were present. A swab was also taken from a similar area on an unaffected animal to ascertain whether the treponemes were part of the natural cutaneous microbiota of cattle.

As DD treponemes are isolated consistently from DD lesions of the interdigital space (IDS) or coronary band, these sites were also examined for the presence of DD lesions. Tissue samples were taken from the IDS and coronary band (the latter only if there was any evidence of abnormality suggestive of a lesion) from animals with and without pressure sores.

In order to determine whether the pressure sores of living cattle contained DD treponemes and their presence did not reflect post-mortem infection, dry swabs were taken from pressure sores on cattle from five living animals. Five swabs were also taken from the same area on healthy animals without evidence of pressure sores.

For culture, tissue samples were placed into oral treponeme enrichment broth (OTEB; Anaerobe Systems, Morgan Hill, California, USA) containing rifampicin (5 μg/ml) and enrofloxacin (5 μg/ml) and transferred to the laboratory where they were inoculated immediately. Swabs were stored at −20°C until subjected to DNA extraction.

Histology and Immunohistochemistry

Skin lesions were sampled from animals that had been shot on that day (within approximately 8 h of death) and preserved in 10% neutral buffered formalin. After fixation, the tissues were trimmed to 3−5 mm in thickness, processed routinely and embedded in paraffin wax. Sections (5 μm) were stained with haematoxylin and eosin (HE) and Warthin Starry stain to identify spirochaetal bacteria.

Immunohistochemistry (IHC) was used to investigate the presence of the three *Treponema* phylogroups,