Identification of Spirochetes associated with Contagious Ovine Digital Dermatitis

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Abstract

Spirochetes of the genus *Treponema* were cultured from 7 of 10 cases of digital dermatitis in sheep. Two cultures comprised *Treponema phagedenis*-like and *Treponema medium/Treponema vincentii*-like spirochetes respectively, whilst remaining cultures comprised mixed populations of *Treponema medium/Treponema vincentii*-like, *Treponema phagedenis*-like, and *Treponema denticola/Treponema putidum*-like organisms.

Text

Contagious Ovine Digital Dermatitis (CODD) is a disease of the ovine hoof which results in acute, severe lameness, Figure 1. In contrast to virulent foot rot, which is characterized clinically by lesions involving the heel and the interdigital area, CODD is characterised by ulcerative lesions of the coronary band which progress and result in disruption of the abaxial wall lining the hoof and loss of the horn case in untreated cases (1, 10, 12). The causative agent of CODD is unknown; however spirochetes have been associated with clinical cases of CODD (2, 3, 10). Whilst evidence of CODD in Ireland is sparse with only one documented case (3), anecdotal evidence from shepherds highlighting persistent ‘incurable’ footrot and ineffective vaccine strategies, suggest that CODD may be prevalent and being incorrectly diagnosed as virulent footrot.

Two geographically distinct lowland sheep farms with persistent flock lameness problems were identified. Charcoal anaerobic swabs were used to take samples from 10 crossbred sheep with acute lesions of the coronary band. Swab buds were buried deep in the lesion within the broken skin-horn junction and transported immediately to the
laboratory. Samples were dipped vigorously in 10ml fastidious anaerobic broth (Lab M Limited, UK) containing 20% foetal calf serum (Sigma, UK), rifampicin, enrofloxacin and marbofloxacin (Sigma, UK), 10 μg/ml each, and incubated at 37°C in anaerobic conditions.

Following 12 days incubation, dark-field microscopy confirmed spirochetes in seven of the 10 cultures from clinical cases of CODD. Contamination from other bacterial sources was confirmed negative in all cases by aerobic incubation on blood agar. Further morphological analysis was performed by scanning and transmission electron microscopy (EM), Figure 2 A, B and C. For scanning EM, an aliquot of spirochetes containing 2.5% glutaraldehyde in 0.1 M Sorensen Phosphate buffer (pH 7.3) was incubated at room temperature for one hour, centrifuged, and pellet post-fixed with 1% Osmium tetroxide in 0.1M Sorensen Phosphate buffer (pH 7.4) for one hour at room temperature and washed twice. The pellet was dispersed in water and applied to poly-l-lysine coated glass microscope slide (Menzel-Glaser, Germany), air dried and coated in gold (SEM coating unit Polaron E5100). Samples were examined using a JEOL JSM 5410LV (JEOL, UK) scanning electron microscope at 15kV. For transmission EM, an aliquot of spirochetes containing 2.5% glutaraldehyde in 0.1 M Sorensen Phosphate buffer (pH 7.3) was incubated at room temperature for one 1 hour, centrifuged, and post-fixed with 1% osmium tetroxide in 0.1M Sorensen Phosphate buffer (pH 7.3) for one hour at room temperature. Samples were embedded in Epon resin using standard methods and ultra-thin (80nm) were cut using a diamond knife and a Leica UC6 ultramicrotome, picked up on 200 mesh copper grids and contrasted with uranyl acetate (20min) and leaf citrate (10min). Sections were examined in a Tecnai 12 BioTwin
Transmission electron microscope (FEI Electron Optics, Netherlands) using an acceleration voltage of 120Kv and an objective aperture of 20 µm. Digital images at various magnifications were acquired with a MegaView 3 camera (Soft Imaging Systems, Germany). The basic defining features of treponemes were evident; helical shape, outer and inner membrane, and approximately five flagellar filaments located in the periplasmic space, Figure 2A, B & C (8).

Cultured spirochete species were also typed at the molecular level. Briefly, genomic DNA was extracted from 250 µl of cultured broth using DNeasy Blood & Tissue Kit (Qiagen, UK) and stored at -20°C until use. The 16S rDNA and 16S-23S rDNA Intergenic Spacer Region 2 region were amplified, sequenced and analysed for species identification (11). Typing of mixed cultures implemented a PCR method used to identify the association of Treponema medium/Treponema vincentii-like, Treponema phagedenis-like, and Treponema denticola/Treponema putidum-like digital dermatitis (DD) treponemes with bovine DD lesions, which uses species-specific primers located within the 16S rRNA gene (6, 7). All 7 cultures of spirochetes were typed within the genus Treponema, Table 1. Two of seven samples were pure cultures of T. phagedenis-like (100% homology to accession number EF057411) and T. medium/T. vincentii-like (99.4% homology to accession number EF061252) DD treponemes (data not shown). Mixed cultures included various permutations of the T. medium/T. vincentii-like, T. phagedenis-like, and T. denticola/T. putidum-like DD treponemes, as shown in Table 1.

In this study, Treponemes were identified in 70% of CODD lesions, bearing similarity to results of a study by Moore et al. (9). Negative culture results should be interpreted carefully in light of the fastidious nature of culturing treponemes and the...
variability in swab sampling techniques, leading to potential false negative results. *T.* phagedenis-like and *T. medium/T. vincentii*-like species have also been previously associated with digital dermatitis in cattle (4, 6, 11). This suggests that these *Treponemas* are associated with digital dermatitis in both cattle and sheep, highlighting the potential for inter-species transmission, as noted in previous studies (2, 5). This transmission potential raises specific disease control and bio-security issues for multi-enterprise farming and may account for the inability for some enterprises to achieve lameness-free status in cases where alternate housing units and grazing of paddocks were used for sheep and cattle as a means of disease control. The two farms investigated in this study were combined cattle and sheep enterprises.

This study has identified spirochetes in clinical cases of digital dermatitis in sheep and has characterised these spirochetes as the genus *Treponema*. A definitive population of culturable spirochetes from digital dermatitis lesions in cattle and sheep is emerging and an immune response to spirochete infection in sheep has been observed (5). In order to develop potential immuno-prophylactic control measures for digital dermatitis, the nature, extent and specificity of this immune response will need to be determined in both cattle and sheep.

Partial 16S rRNA gene sequences for isolates designated Mayo A and Wicklow 8 (Table 1) have been deposited in Genbank; accession numbers FM210038 and FM210039 respectively.
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Figure Legends

Figure 1: An acute lesion of contagious ovine digital dermatitis on the coronary band. If untreated, the infection will lead to loss of the hoof case.

Figure 2: Electron Microscopy: A; SEM of a spirochete cultured from the CODD lesion shown in Figure 1. The spirochete was identified as Treponema phagedenis-like based on 16S rDNA phylogenetic analysis. B; TEM of a cultured spirochete, Treponema phagedenis-like 1: Outer membrane; 2: Inner membrane; 3: Periplasmic flagella. C: Cross section of a cultured Treponema phagedenis (TEM). Arrows indicate evidence of periplasmic flagella.
<table>
<thead>
<tr>
<th>Sample No.</th>
<th>PCR*</th>
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<tr>
<td>Mayo A</td>
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<td>Wicklow 2</td>
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<td>Wicklow 6</td>
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<tr>
<td>Wicklow 8</td>
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*Group 1: *Treponema medium/*Treponema vincentii-like*, Group 2: *Treponema phagedenis-like*, Group 3: *Treponema Denticola/*Treponema putidum-like*

**Table 1**: Genetic analysis of cultured spirochetes. ‘Mayo A’ was 100% homologous to *T. phagedenis-like* (comparative to accession No. EF057411) and ‘Wicklow 8’ was 99.4% homologous to *T. medium/Treponema vincentii-like* (comparative to accession No. EF061252). The remaining samples were mixed cultures.
References


