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INFECTIOUS DISEASE

Severe Foot Lesions in Dairy Goats Associated with Digital Dermatitis Treponemes

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Summary

Treponeme-associated foot disease has been described in cattle with digital dermatitis and sheep with contagious ovine digital dermatitis. In this study, severe foot lesions in dairy goats associated with digital dermatitis treponemes (i.e. *Treponema medium*, *Treponema phagedenis* and *Treponema pedis*) were characterized macroscopically, radiographically and histologically. The main macroscopic foot lesion was of extensive solar ulceration with or without exophytic papilliform hyperkeratosis. Radiographically, the distal phalanx and distal sesamoid bones were severely damaged and remodelled. Histologically, the lesion was categorized as a chronic lymphoplasmacytic, suppurative and ulcerative pododermatitis. Immunohistochemistry identified the spirochaetal microorganisms located extracellularly in the superficial horn. Study limitations mean that the treponeme bacteria could not be considered the sole or causal agents in the cases described.

Keywords: digital dermatitis; goat; treponeme

Introduction

Lameness in domesticated ruminants is widely recognized as an important animal welfare (Whay *et al.*, 1997; Angell *et al.*, 2015a) and economic problem (Reader *et al.*, 2011). In the UK, lameness in dairy goats has been identified as a common welfare problem (Anzuino *et al.*, 2010); however, the diseases causing lameness are less well described than in cattle (Weaver, 1972; Sibley, 2013) and sheep (Winter, 2011). Generally, goat lameness problems are described as similar to those found in sheep (Winter, 2011). However, interdigital dermatitis (Zhou *et al.*, 2009; Sullivan *et al.*, 2015a), foot rot (Piriz Duran et al., 1990), heel horn erosion (Christodoulopoulos, 2009), white line disease, foreign body penetrations (Mgasa and Arnbjerg, 1993) and overgrown feet (Anzuino *et al.*, 2010) have all been recorded in goats. Most recently, two research groups in the UK have reported a severe foot disease in dairy goat herds associated with *Treponema* spp. commonly detected in infectious foot lesions in farm animals (Groenevelt *et al.*, 2015; Sullivan *et al.*, 2015b).

These treponeme-associated foot diseases occur in several animal species. In each case, they have been associated with three cultivable digital dermatitis (DD) treponeme phylogroups: *Treponema medium*, *Treponema phagedenis* and *Treponema pedis*. These diseases include DD in dairy and beef cattle (Evans *et al.*, 2009), a disease of global distribution;

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contagious ovine digital dermatitis (CODD) in sheep in the UK (Dhawi *et al.*, 2005; Duncan *et al.*, 2014) and Ireland (Sayers *et al.*, 2009); foot lesions in wild elk (*Cervus elaphus*) in the USA (Clegg *et al.*, 2015) and equine canker (Sykora and Brandt, 2015). These treponeme-associated hoof lesions are markedly different between species in terms of their gross pathological appearance, even though detection and isolation of the same treponeme phylogroups occurs consistently. While interspecies transmission of *Treponema* spp. has not yet been demonstrated, it may be theoretically possible given the similarity of the infectious organisms.

In cattle, the three DD treponemes are considered to be the primary aetiological agents of DD (Evans *et al.*, 2009) and are hypothesized to be the primary infectious cause of CODD (Sullivan *et al.*, 2015a). However, in cattle, secondary infection of pre-existing claw horn defects with the DD treponeme bacteria also occurs, resulting in severe non-healing foot lesions (Evans *et al.*, 2011). In goats, it is unknown whether the treponeme-associated foot disease is a primary bacterial disease or represents secondary invasion of claw horn defects, as is seen in the non-healing cattle foot lesions.

The gross and histopathological aspects of goat treponeme-associated foot disease have not been formally described. Such data would help inform understanding of the disease process in this species, and consequently would inform disease control measures. The published clinical presentations of the disease are varied with no single distinct clinical pattern. Groenevelt *et al.* (2015) reported that the majority of cases had involvement of the sole to some extent. Sullivan *et al.* (2015b) observed some lesions, which included the coronary band and hoof wall, while other lesions had more solar horn involvement. The aim of the present study was to investigate further the disease process of treponeme-associated foot disease in goats by characterizing the clinical, radiographical and histopathological features of the disease in a UK dairy goat herd.

Materials and Methods

Farm Background

All observations and samples of diseased feet were collected from a single UK dairy goat farm with a recent history of treponeme-associated foot disease (Sullivan *et al.*, 2015b). The farm consisted of 856 milking goats of various breeds. The goats were housed all year round in four straw yards (approximately 250 goats per yard) and house and milking parlour hygiene was assessed subjectively as extremely good by two of the authors. The goats were milked twice daily and fed on a total mixed ration including silage and cereal. Foot care included twice weekly foot bathing on exit from the parlour in 10% zinc sulphate and regular preventive and therapeutic foot trimming (every 3 months). For those goats with foot lesions, topical treatment with Derm Paste™ (Hoofcare Supplies, Selby, UK) and systemic antibiotics were used as prescribed by the farmer's usual veterinary surgeon.

Ethical approval for this study was obtained from the University of Liverpool Veterinary School Ethics Committee.

Animal Sampling

Data from the study farm were collected on two separate occasions in 2014 and 2015 (6 months apart). All animal sampling was with owner's consent and therefore limited by the discretion of the farmer. At the first visit, seven lame goats identified for culling by the farmer were humanely destroyed. After death, the fore- and hindlimbs of these goats were removed by disarticulation at the carpal and the tarsal joints, respectively. The foot lesions were photographed and samples of tissue were collected and placed in 10% neutral buffered formalin within 30 min of death. Tissue samples taken from the coronary band region were frozen at −20°C for microbiological examinations (polymerase chain reaction [PCR] and culture). The formalin fixed feet underwent subsequent clinical, radiographical, histopathological and immunohistochemical investigations.

On the second visit, a whole herd lameness assessment was carried out and 10 lame goats were examined; their foot lesions described, photographed and swabbed for microbiological assessment.

Control material was obtained from goats from a fallen stock centre with no macroscopic foot lesions. One goat was used as a control for radiography while three control goats, provided 12 'healthy' control tissue samples for PCR.

Herd Lameness Score

The gait of all milking goats was observed (by HC-D) as the goats exited the milking parlour. Due to the number of goats and their speed on exiting the parlour, a simple binary scoring system of lame/not lame was used (Phythian *et al.*, 2013).

Clinical Foot Lesion Descriptions

All foot lesions were photographed and the lesions categorized based on the foot lesion descriptors from the International Committee for Animal Recording

(ICAR) Claw Health Atlas (Egger-Danner *et al.*, 2015), clinical presentations of CODD (Angell *et al.*, 2015a) and clinical descriptors of ovine foot rot (Egerton and Roberts, 1971).

In addition, the following macroscopic pathological terms were applied. Gross enlargement was defined as the enlargement of the entire claw up to 2.5 times the control claws. Exophytic papilliform hyperkeratosis was new crumbly horn arranged in papillae, usually originating from the coronary band. In some cases of extensive solar ulceration, the presence or absence of deep crevasses due to hyperkeratosis of solar horn causing hyperkeratotic spurs was noted.

Clinical lesion descriptors were applied to all foot samples (using the photographs of the lesions) that were positive by PCR for any of the three DD treponemes (i.e. *T. medium*, *T. phagedenis* and *T. pedis*). These were 11 feet from the seven lame culled goats and 10 feet from 10 lame non-culled goats.

Isolation of Spirochaetes

Spirochaetes isolation was attempted on 11 tissue samples taken from the feet of the culled goats. These bacterial isolations were as described previously (Evans *et al.*, 2008).

Sample DNA Extraction

Plain cotton swabs were used to sample 10 foot lesions from the 10 non-culled goats and stored at −20°C for subsequent PCR analyses.

Tissue samples from the culled goats and the negative control goats were collected from lesions immediately *post mortem* and each sample was placed in sterile universal tubes on ice, prior to transportation to the laboratory and freezing at −20°C.

Tissue samples were collected from all four feet of three of the negative control goats from the fallen stock centre, providing 12 control samples for PCR.

DNA was extracted from the swabs and tissue samples from the affected and control feet using the QIAquick DNeasy® Blood and Tissue Kit (Qiagen, Manchester, UK) as per the manufacturer's instructions.

The bacterial genomic DNA was extracted using Chelex-100™ (Bio-Rad; Hercules, California, USA) as described previously (Chua *et al.*, 2005).

Treponeme PCR Assays

Swabs and tissues were subjected to nested PCR assays specific for the three DD-associated treponeme phylogroups (Evans *et al.*, 2009). For identification of bacterial isolates, PCR and gene sequencing of almost the entire 16S rRNA gene obtained from bacterial culture extracts were performed (Chua *et al.*, 2005; Evans *et al.*, 2008).

Each PCR assay included positive controls (bovine DD *Treponema* spp. genomic DNA from each of the three unique bovine DD *Treponema* phylogroups) and a negative control (water) (Evans *et al.*, 2009). All assays were carried out in triplicate.

All tissue samples were also subjected to the *Treponema* genus PCR assay, which detects all *Treponema* species, both pathogenic and commensal (Moore *et al.*, 2005; Evans *et al.*, 2009).

Sequencing and Sequence Analysis

Amplified PCR products were sequenced commercially (Macrogen, Amsterdam, The Netherlands) and gene sequences assembled using the Chromas Pro™ sequence analysis package (Technelysium Ltd., Brisbane, Queensland, Australia). Gene sequences were aligned using CLUSTALW as implemented in MEGA 5.0 (Tamura *et al.*, 2011). The nucleotide sequence alignment was subjected to Modeltest, as implemented in Topali (Milne *et al.*, 2009), which revealed that the best fit model was General Time Reversible (GTR). This model was used to produce nucleotide maximum likelihood phylogenetic trees (bootstrap values based on 10,000 iterations).

Radiography

Eleven feet from the seven culled goats ($n = 22$ digits) and two feet (four digits) from a control goat were radiographed. After 1 month of formalin fixation, the feet were sectioned into individual digits through the interdigital space. Lateromedial radiographs of each claw were obtained and processed as described by Angell *et al.* (2015a).

Radiographic descriptions were made on the points described in Table 1 with Fig. 1 demonstrating the anatomy of the distal phalanx.

Histology and Immunohistochemistry

Following radiography, the 11 feet from the seven culled goats and one from the control goat were subjected to histological and immunohistochemical examination. Each digit was sectioned sagittally as described by Angell *et al.* (2015b). The following samples were obtained from all digits: distal digital skin with coronary band; dorsal horn including laminae; and solar horn including laminae and the distal phalanx. These samples were processed routinely and embedded in paraffin wax. The horn and bone required softening or decalcification pretreatment (Angell *et al.*, 2015b) prior to embedding. Serial

Table 1
Definitions of radiographical descriptors for the foot lesions

	Mild	*Moderate*	*Severe/Marked*
Distal Phalanx (P3)			
Misshapen distal phalanx	The distal phalanx retains overall shape of P3 with <25% of the bone changed in shape	The distal phalanx is still recognizable as the distal phalanx but 25–50% of the bone has changed shape.	The distal phalanx is no longer recognizable and over 50% of the bone has changed shape
Proliferative new bone – extensor process	Irregular new bone extends up to the distal third of the middle phalanx and is <50% of the width of the extensor process	Irregular new bone extending up to the distal half of the middle phalanx and up to the width of the extensor process	Irregular new bone extending above the distal half of the middle phalanx and over the width of the extensor process
Proliferative new bone – solar cortical margin	Small fine new bone spicules covering <25% of the length of the cortical margin	New bone covering 25–50% of the length of the solar cortical margin	New bone covering >50% of the length of the cortical solar margin and any protruding bone spurs (even if <50%)
Bone lysis (evident as decreased radiodensity)	<25% of the distal phalanx has decreased radiodensity	25–50% of the distal phalanx has decreased radiodensity	>50% of the distal phalanx has decreased radiodensity
Dorsal protrusion of the dorsal cortical border of P3	Angle from solar cortical border to the mid-dorsal border is 50–75°, but this levels out or gently slopes to the dorsal notch	Angle from solar cortical border to the mid-dorsal border is 75–90°, but levels out or gently slopes to the dorsal notch	Angle from solar cortical border to the mid-dorsal border is >90° then has a >30° angle down toward the dorsal notch
Middle Phalanx (P2)			
Proliferation of new bone on the dorsal and palmar/plantar aspect (both aspects must have new bone)	New bone along <25% of the length of both margins	New bone along 25–50% of at least one margin (the other margin may have less)	New bone along >50% of one margin and any protruding bone spurs
Bone lysis	<10% radiolucency and in one area	10–25% of radiolucency; can be in multiple areas of the middle phalanx	>25% of radiolucency; can be in multiple areas of the middle phalanx
Proximal Phalanx (P1)			
Proliferative new bone along the dorsal or palmar/plantar aspect	New bone along <25% of the bone margin	New bone along 25–50% of the bone margin	New bone along >50% of the bone margin
Distal Sesamoid Bone			
Proliferation of new bone on the palmar/plantar aspect or palmaro/plantarodistal aspect or palmaro/plantaroproximal aspect or proximal aspect	New bone comprises <25% of the original size of the distal sesamoid bone	New bone comprises 25–75% of the original size of the distal sesamoid bone	New bone comprises >75% of the original size of the distal sesamoid bone

sections (4 μm) were stained by haematoxylin and eosin (HE) or subjected to immunohistochemistry (IHC).

IHC was employed to investigate the presence of *T. medium*, *T. phagedenis* and *T. pedis* phylogroups, using a rabbit polyclonal antibody (Evans *et al.*, 2009). Sections were dewaxed and subjected to antigen retrieval using pH 6.1 EnVision™ FLEX target retrieval solution (Dako, Agilent Technologies, Carpinteria, California, USA) at 95°C for 25 min. The slides were run through a DAKO Autostainer Link 48 (Dako) with the following steps. Slides underwent a series of washes with EnVision™ FLEX wash buffer (Dako) prior to addition of EnVision™ FLEX peroxidase block (Dako) to block endogenous peroxidase and then washed again. The primary antibody (1 in 4,000 dilution) was diluted with EnVision™ FLEX antibody diluent (Dako) prior to application to each slide and incubated for 20 min. A wash was performed and the slides incubated with labelled polymer, EnVision™ FLEX/HRP (Dako) for 20 min before a series of washes. EnVision™ FLEX DAB + chromogen (Dako) was used to detect the bound antigen. The slides were counterstained with haematoxylin. In addition to this procedure, a sample that displayed positive immunolabelling was processed without the primary antibody to ensure that the secondary antibody did not cause background labelling. No labelling was observed in this sample.

Following optimization, a grading system of no labelling, mild granular (interpreted as background) or intense labelling was used to give an indication of

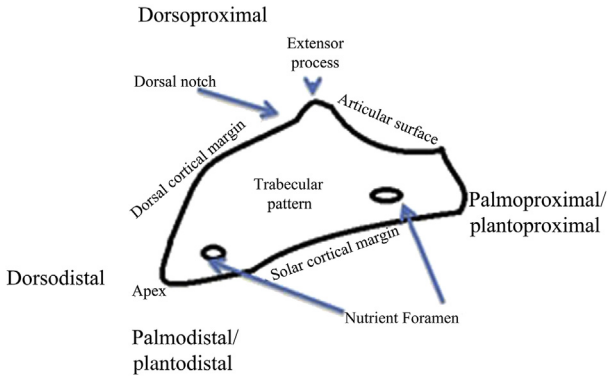

Fig. 1. Labelled parts of distal phalanx used for radiographical interpretation.

the varying intensity of treponeme antigen presence and whether treponeme morphology was observed.

Results

Lameness Score

At the second visit, lameness scoring of milking goats was performed as they exited the milking parlor. Using a binary scoring system of lame/not lame, 209 of the 856 goats (24.4%; 95% confidence interval [CI]: 21.6–27.4%) were lame.

Clinical Foot Lesion Descriptions

Foot lesions were described for the seven culled goats and the 10 lame non-culled goats. Of the 17 lame animals observed, 12 had lesions in one foot, three had lesions in two feet, two had lesions in three feet and none had lesions in all feet. Some of the foot lesion types are shown in Figs. 2 and 3. Table 2 shows the frequency of the different foot lesions. The predominant feature was ulceration of the solar aspect of the foot, with 23 of the 24 (95.8%; 95% CI: 78.9–99.9%) foot lesions being a form of ulcer. Gross enlargement and severe hyperkeratosis of the distal haired skin and coronary band were only observed in conjunction with ulcers, which all contained extensive granulation tissue. In all culled goats, the extensive solar ulceration presented with hyperkeratotic horn spurs surrounding the ulcer site (Figs. 3a,b).

Treponeme Polymerase Chain Reaction

All 21 diseased feet (100%) and nine of the 12 (75%) control feet were positive for the general treponeme species PCR. Ten of the 21 (48%) diseased feet were positive for the *T. medium* phylogroup and seven of the 21 (33%) diseased feet were positive for the *T. phagedenis* phylogroup. Eighteen of the 21 (86%) diseased feet were positive for the *T. pedis* phylogroup.

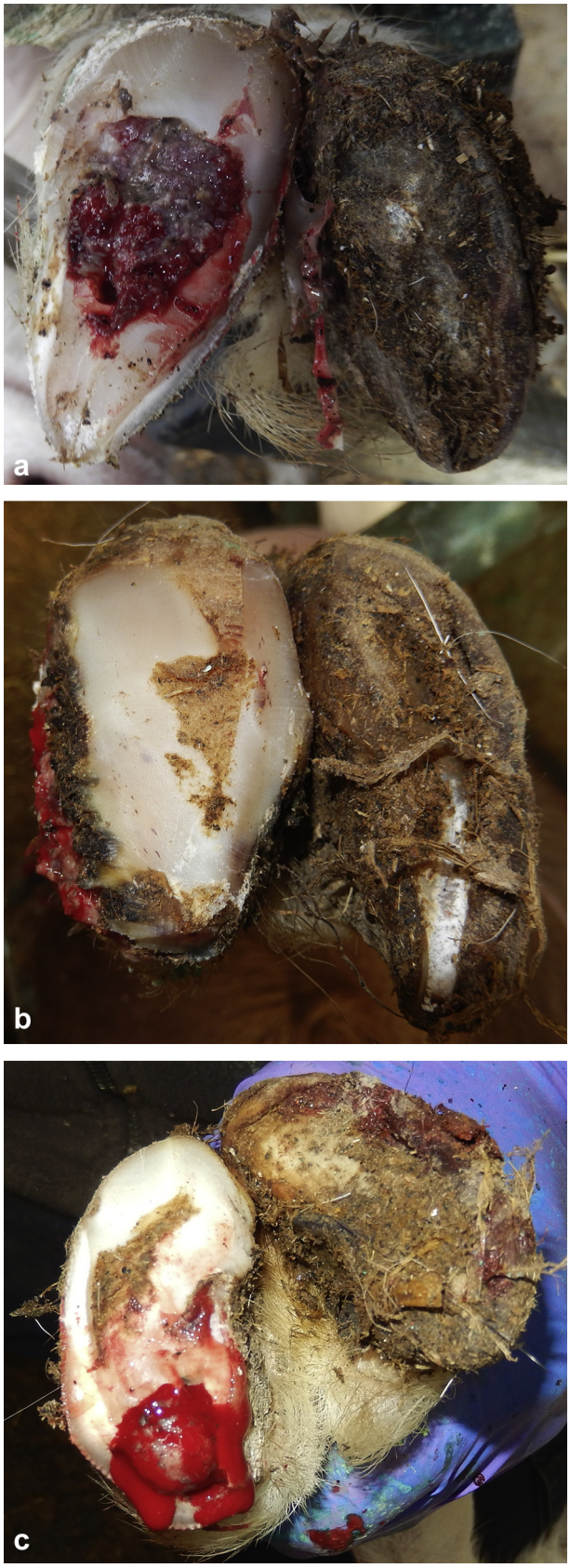

Fig. 2. Macroscopic foot lesions observed in the milking goat herd. (a) Sole ulcer. (b) Wall ulcer. (c) Toe ulcer.

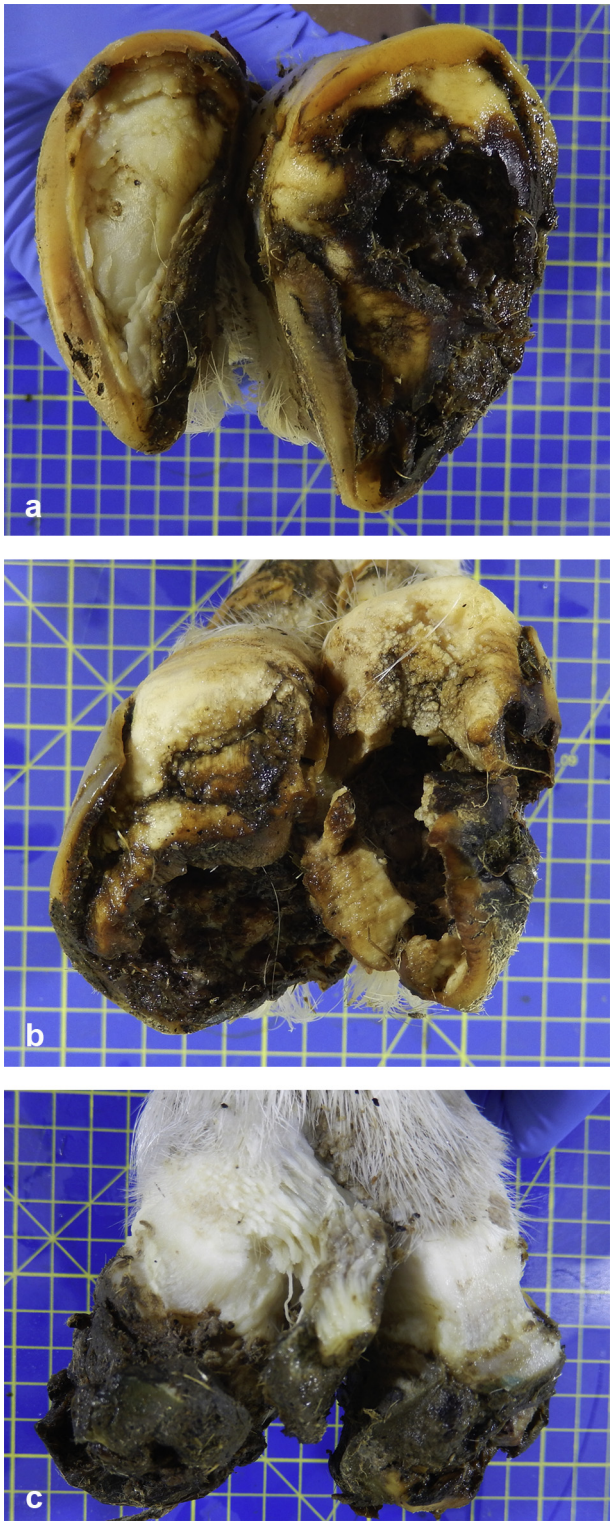

Fig. 3. Macroscopic lesions observed in the culled goats. (a) Solar view of unilateral solar necrosis without hyperkeratosis. (b) Solar view of bilateral solar necrosis without hyperkeratosis. (c) Dorsal view of bilateral solar necrosis with hyperkeratosis.

Table 2
Frequency of different lesions observed

Type of lesion	*Number of goats (n = 17)*	*Number of feet (n = 68)*	*Number of foot lesions (n = 24)*
Healthy/slightly overgrown horn	17 (100%)	44 (64.7%)	N/A
Toe ulcer	3 (17.6%)	3 (4.4%)	3 (12.5%)
Sole ulcer	3 (17.6%)	3 (4.4%)	3 (12.5%)
Wall ulcer	1 (5.9%)	1 (1.5%)	1 (4.2%)
Extensive sole ulcer (including wall and or toe region)	10 (58.8%)	16 (23.5%)	16 (66.7%)
Total with ulcers	17 (100.0%)	23 (33.8%)	23 (95.8%)
Hyperkeratosis	12 (70.6%)	15 (22.1%)	15 (62.5%)
Gross enlargement	12 (70.6%)	18 (26.5%)	18 (75.0%)
Chalky horn	1 (5.9%)	1 (1.5%)	1 (4.2%)

All diseased feet (100%) were positive for at least one of the three DD-associated treponemes. The control samples without foot lesions were all negative (Table 3).

Spirochaete Isolation and Treponeme Polymerase Chain Reaction from Tissue Samples

No *Treponema* spp. were cultured from the tissue samples from the control animals.

All goat foot lesion samples were positive for spirochaete growth on analysis by phase contrast microscopy, and the presence of the DD treponeme phylogroups was confirmed by nested PCR assays for all three DD treponeme phylogroups.

For each of the 11 lesions tested by the treponeme PCR assays, at least one of the three DD-associated treponeme phylotypes was detected. On culture, at least one of *T. medium*, *T. phagedenis* or *T. pedis* phylogroup treponemes were present in each foot lesion sampled. One of the 11 (9%) lesions cultured contained all three DD-treponeme phylogroups, five of the 11 (45.5%) contained two, and five of the 11 (45.5%) just one phylogroup.

16S rRNA Gene Sequence Analysis

Six pure treponeme culture isolates, obtained from lesions from the goats' feet, were subjected to 16S rRNA gene amplification by PCR and sequencing. To determine the relationship of the goat treponeme isolates to those found in other animals (i.e. sheep, elk, man and cattle), the 16S rRNA gene sequences were compared with other previously sequenced isolates by phylogenetic analysis.

All six isolated treponemes showed high 16S rRNA gene sequence similarity to previously isolated treponemes from a variety of hosts. The isolates 6 and 3

Table 3
PCR results for different *Treponema* spp.

Sample (goat ID)	Treponema *genus PCR*	Treponema *groups*		
		Treponema medium	Treponema phagedenis	Treponema pedis
Control goat feet				
1	−	−	−	−
2	+	−	−	−
3	+	−	−	−
4	+	−	−	−
Non-culled goat feet				
1	+	−	−	+
2	+	−	−	+
3	+	−	+	+
4	+	+	−	+
5	+	−	−	+
6	+	−	−	+
7	+	−	−	+
8	+	−	−	+
9	+	−	−	+
10	+	+	+	+
Culled goat feet				
1A	+	−	+ (1A)	−
1B	+	+	−	+
2A	+ (2A)	+	+	+
2B	+	+	−	+
3	+	+	−	+ (3)
4A	+	+	+	−
4B	+	+	−	+
5A	+	−	+ (5A)	+
5B	+	+	−	−
6	+	+	−	+ (6)
7	+	−	+ (7)	+

+, positive; −, negative. The names shown in parentheses are the isolate name, as per the phylogenetic tree (Fig. 4).

belonged to the *T. pedis* phylogroup and had an identical 16S rRNA gene to treponemes previously isolated from cases of DD in sheep and cattle (Fig. 4). They were 20/1,548 (0.01%) nucleotides different to previously isolated treponemes from goats. Isolates 1A, 5A and 7 belonged to the *T. phagedenis* phylogroup and were identical on 16S rRNA gene sequence analysis to previous isolates taken from goats, cattle, sheep and people. Isolate 2A belonged to the *T. medium* phylogroup, with a 16S rRNA gene sequence identical to previously isolated sheep and bovine DD treponemes.

Overall, there was relatively little diversity seen within the 16S rRNA gene between the goat isolates when compared with isolates of the same treponeme phylogroups from a range of host species (Fig. 4).

Radiography

The distal phalanx in the negative controls varied slightly in appearance on radiographs; however, there were general similarities to the radiographical appearance of the normal ovine digit (Duncan *et al.*, 2013). The control digits (Fig. 5a) did not display any radiographical abnormalities.

The feet with severe solar necrosis and no hyperkeratosis exhibited mild to moderate radiographical abnormalities of the distal phalanx, with mild new bone formation along the solar aspect and the occasional presence of mild to moderate bone lysis (Fig. 5b). The digits with hyperkeratosis and solar necrosis had the most severe distal phalanx radiographical abnormalities, with the consistent finding of marked new bone formation on the extensor process (Fig. 5c). The other radiographical abnormalities of the distal phalanx were of variable severity, with bone lysis not a feature of cases with hyperkeratosis. A consistent finding between the feet with and without hyperkeratosis was the moderate to marked formation of new bone around the various palmar/plantar aspects of the distal sesamoid bone. Moderate to marked new bone formation on the cortical margins of the middle phalanx was sometimes present in cases with and without the hyperkeratosis.

Histopathology

Sixteen of the 22 digits and one control digit were considered suitable for further histopathological examination. At least one digit was examined from each of the 11 affected feet. A summary of the histopathology and IHC results is given in Table 4.

Coronary Band. The main lesions of the coronary band region in the abnormal feet were moderate to severe, irregular, and mostly orthokeratotic hyperkeratosis of the stratum corneum ($n = 15$) and multifocal to coalescing mild to severe perivascular, perifollicular and peri-adnexal lymphoplasmacytic infiltrates ($n = 14$) with neutrophils ($n = 3$) in association with epidermal erosion/ulceration. Moderate to severely hyperplastic, often irregular, epidermis ($n = 9$) with many scattered keratohyalin granules ($n = 10$) in the superficial stratum granulosum, with or without a serocellular crust, was also commonly observed.

Dorsal Horn. The histopathological changes observed were broadly categorized into two groups: a chronic–active process and a chronic process. Ten digits were categorized as chronic–active and six digits as chronic.

In the chronic–active lesions, the main histopathological changes were severe, irregular, mostly orthokeratotic hyperkeratosis ($n = 8$); erosion and or ulceration ($n = 8$) with serocellular crusting ($n = 7$) consisting of myriad mixed bacteria, degenerate neutrophils, fibrin and scattered lymphocytes; and

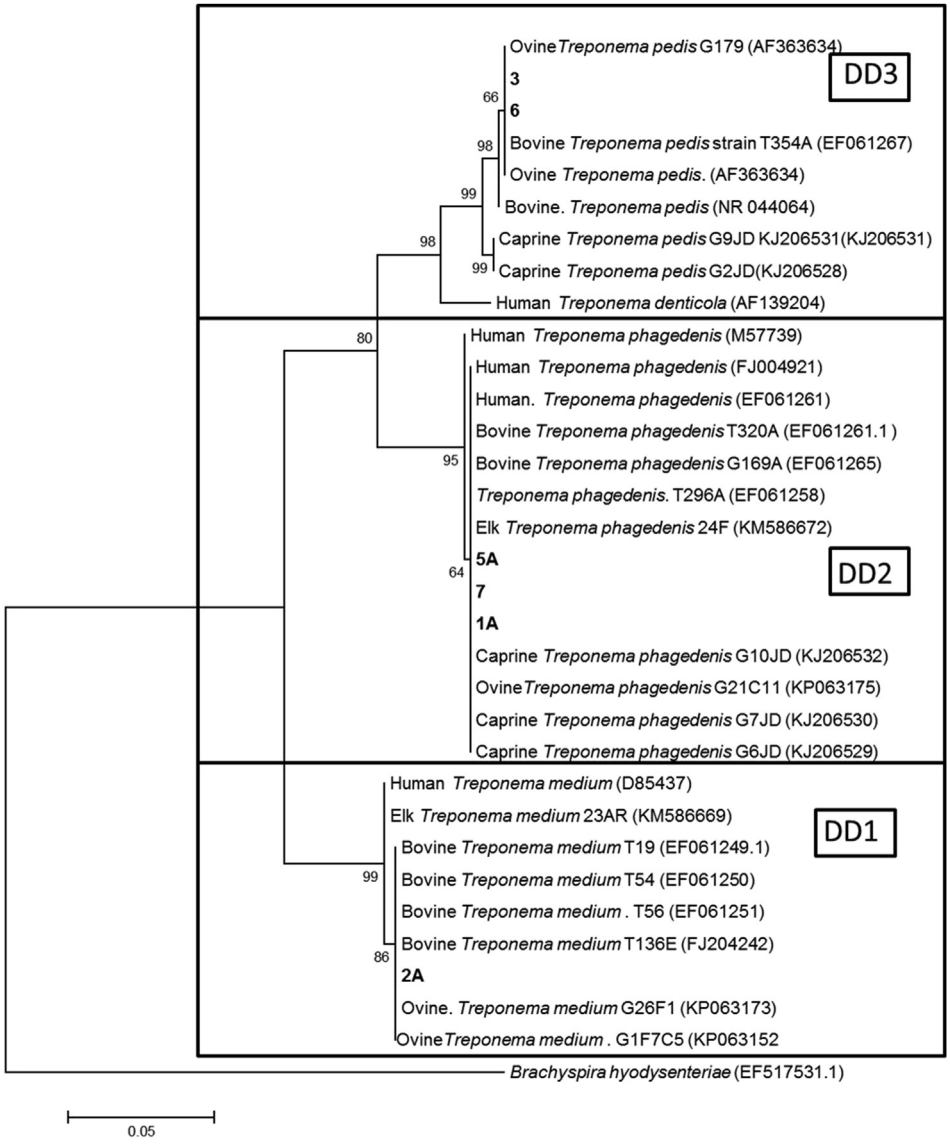

Fig. 4. 16S rRNA gene sequence phylogeny of treponemes cultured from goat foot lesions. Comparison of treponeme sequences isolated from goat foot lesions in this study with those isolated from cattle, man, goats, elk and sheep in previous studies (for clarity, bootstrap values below 65 were removed). Sequences from Genbank of human treponemes and other related treponemes are also shown, with the accession number in parentheses. The sequences from isolates in this study are labelled with goat number (as shown in Table 3). DD1, DD2 and DD3 refer to the DD treponeme phylogroups, where DD1 is *T. medium* phylogroup, DD2 is *T. phagedenis* phylogroup and DD3 is *T. pedis phylogroup*.

dystrophic calcification ($n = 8$). Often there were mild to severe, multifocal to coalescing perivascular lymphoplasmacytic infiltrates in the dermis and dorsal laminae ($n = 11$). Within the stratum spinosum, intracellular and intercellular oedema of keratinocytes was observed regularly. Sometimes, multifocal areas of ulceration were filled by large quantities of granulation tissue with or without embedded plant material and/or myriad mixed bacteria and degenerate neutrophils. Irregular, fused and blunted laminae were observed in 10 cases. The chronic cases were defined as lacking suppurative inflammation, oedema, ulceration or granulation tissue, but with marked epidermal hyperplasia and exuberant hyperkeratosis.

Solar Horn. Lesions within the solar horn (Figs. 6a,b) were dominated by severe irregular thickening of the stratum corneum ($n = 12$) with myriad mixed bacteria on the surface of the lesion and a moderate to severe dermal/dorsal laminae infiltrate of plasma cells and lymphocytes.

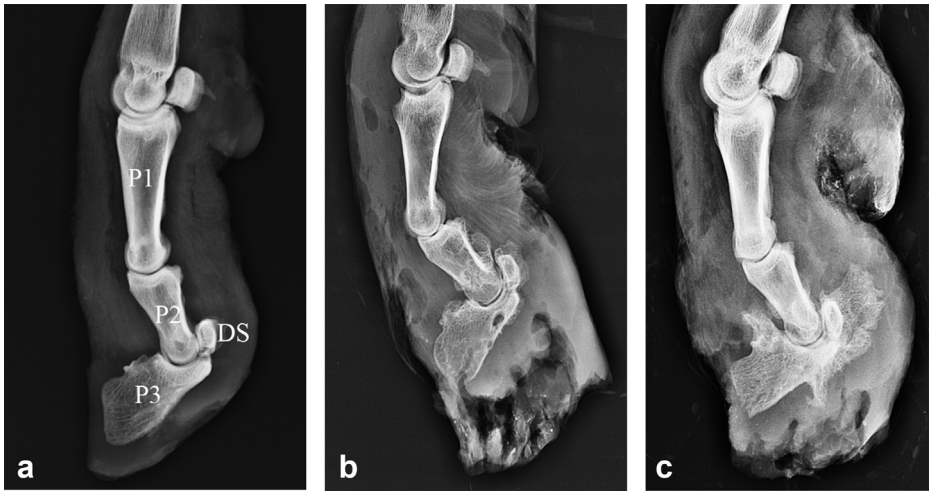

Fig. 5. Radiographs of individual digits. (a) Control. P1, proximal phalanx; P2, middle phalanx; P3, distal phalanx; and DS, distal sesamoid bone. (b) Foot with solar necrosis without hyperkeratosis. (c) Foot with solar necrosis and hyperkeratosis.

Granulation tissue was a predominant feature of the solar horn lesion and was always associated with large numbers of degenerate neutrophils, myriad mixed bacteria and multifocal areas of ulceration and erosion ($n = 8$). Often within the epidermis and dermis (in ulcerated regions) there was dystrophic calcification ($n = 6$). Irregular, fused and blunted laminae were observed in eight cases.

Distal Phalanx (P3). Every distal phalanx, except for the control, had an irregular surface with moderate to large numbers of mineralized bony projections and separate islands of mineralized new bone. All P3 bones also had multifocal areas adjacent to the periosteum of plump (activated) osteoblasts embedded within osteoid, scalloped edges ('reversal lines' indicative of bone remodelling) and moderate to severely

Table 4
Summary of the histopathology and immunohistochemical detection of treponemes

	Histological features of severely affected digits	*Immunohistochemistry*
Coronary band	Moderate to severe epidermal hyperplasia Moderate to severe orthokeratotic hyperplasia, often irregular Mild to moderate perivascular and peri-adnexal lymphoplasmacytic dermatitis +/− serocellular crust	Three out of 10 intense labelling, two with clear treponeme morphology
Dorsal horn		
Chronic–active	Multifocal epidermal erosion/ulceration with multifocal hyperkeratosis and a serocellular crust Severe suppurative dermal/epidermal infiltrate Epidermal intra- and intercellular oedema	Four out of 10 showed intense labelling, two with clear treponeme morphology (all cases with labelling were from the chronic–active dorsal horn)
Chronic	Granulation tissue Irregular and fused laminae Moderate to severe interface lymphoplasmacytic dermatitis Diffuse severe hyperkeratosis Mild to moderate perivascular lymphoplasmacytic dermatitis	
Solar horn	Severe multifocal alternating hyperkeratosis with eroded/ulcerated epidermis and multifocal replacement with granulation tissue and a serocellular crust Severe suppurative dermal/epidermal infiltrate Irregular and fused laminae Severe interface lymphoplasmacytic dermatitis	Six out of 11 showed intense labelling, all with clear treponeme morphology and labelling extending into the fissure of the horn
Distal phalanx	Multifocal periosteal projections Presence of cement lines, osteoclasts and plump osteoblasts Often with chondroid metaplasia.	No labelling

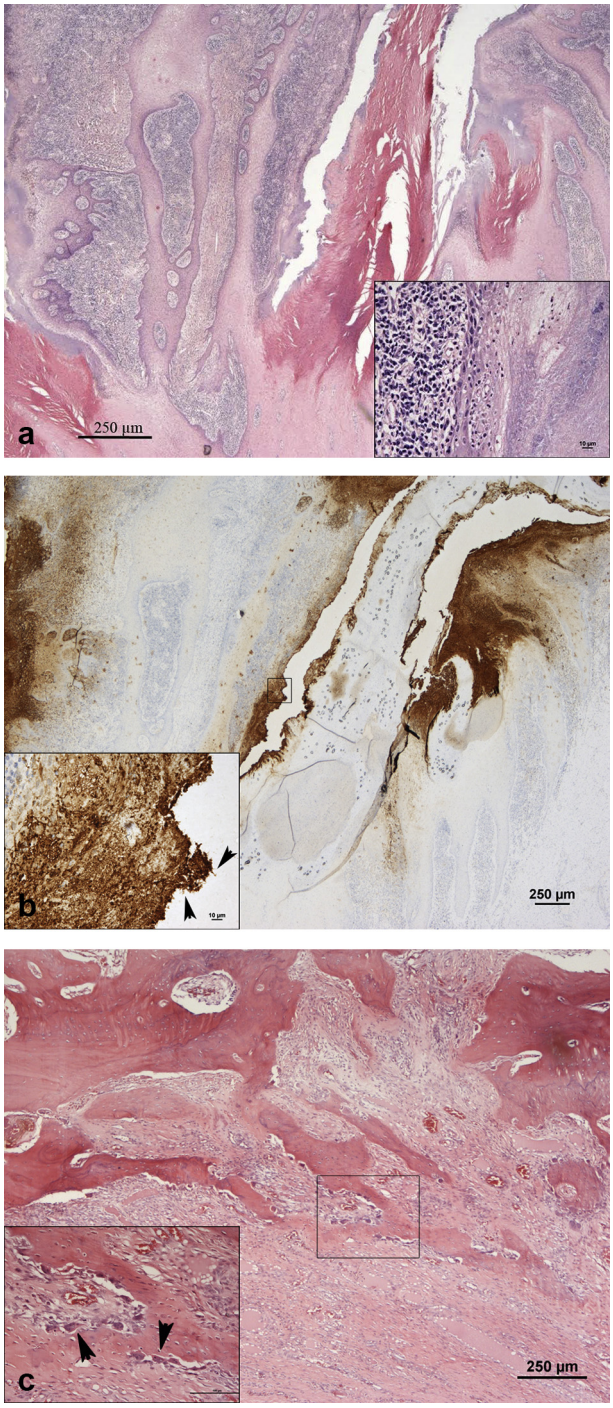

Fig. 6. Histopathology and immunohistochemical detection of *Treponema* spp. antigens. (a) Solar horn with hyperkeratosis and ulceration. HE. Bar, 250 µm. Inset shows higher magnification detail. (b) IHC of the same field shown in (a). Inset shows higher magnification detail. (c) Distal phalanx with increased osteoclast activity. HE. Inset shows detail of osteoclasts (arrowheads).

increased numbers of osteoclasts (Fig. 6c). Multifocally, there were small to large areas of woven bone with non-mineralized areas. In the surrounding connective tissue, there were mild to moderate lymphoplasmacytic infiltrates with increased numbers of plump (activated) fibroblasts, with occasional multifocal chondroid metaplasia ($n = 4$). The articular surface in some sections was irregular.

Immunohistochemistry

The sections from feet with no macroscopic, radiographical or histological lesions did not demonstrate positive labelling for *Treponema* spp. or there was a small amount of multifocal granular labelling, which was interpreted as background. In affected tissues, there was intense labelling of the solar horn (Table 4, Fig. 6b).

Discussion

Lameness is a substantial welfare issue in small ruminants. This study describes the clinical, microbiological, pathological and radiographical features of a severe, newly emerging treponeme-associated foot disease in dairy goats. There are a number of limitations of the study, which should be considered in the interpretation of the results. Firstly, the control healthy foot samples (which were negative for treponeme bacteria) were collected from goats from a fallen stock centre rather than animals from the same farm, which may reduce the comparability. Secondly, the study did not consider detection of microbiological agents other than treponeme bacteria. Therefore, it cannot be concluded that the treponeme bacteria are causal organisms or are the only organisms associated with the disease process. Finally, this study is based on a single goat farm and only the most severe cases could be characterized fully, due to the limited number of samples available. Despite these limitations, the descriptions of the clinical presentations of the milder lesions, together with the microbiological assessment and the further characterization of the severe cases, provide a useful insight into the disease process of this novel foot disease in goats.

The 24% prevalence of lameness on this single farm at one visit lies within the range of 12–67% reported by Groenevelt *et al.* (2015) and is similar to the figure of 19.2% reported by Anzuino *et al.* (2010), demonstrating that this goat herd had typical lameness prevalence. The cause of lameness was not determined in all the lame animals in this study. The lameness percentage of 65% reported by Sullivan *et al.* (2015b) was from the same farm as this study, but 12 months earlier; therefore, the prevalence of lameness in this

herd had decreased substantially. It was considered by the farmer to have been achieved via culling of lame goats in parallel with changes to hygiene practices and treatment protocols.

Clinically, the predominant foot lesion in both the severe and milder cases was solar ulceration with granulation tissue, with or without hyperkeratosis and gross enlargement of the foot. Hyperkeratosis was present in approximately half of foot lesions and was present in all the more severely affected cull goats. All foot lesions examined in the study were confirmed by PCR as being positive for one of the three DD-associated treponeme bacteria.

The first reports of treponeme-associated foot disease in dairy goats (Sullivan *et al.*, 2015b) described the clinical presentation of the foot lesions as 'footrot-like' lesions with extensive solar horn ulceration or 'CODD-like' lesions with extensive underrunning and separation of the hoof wall. The lesions examined in that study were chronic and severe with no early stage lesions observed. However, extensive ulceration of the solar and wall horn with granulation tissue was a common feature between this and previous studies. With limited material available in the current study, a direct comparison of the macroscopical pathology of CODD in sheep and treponeme-associated foot disease in goats was not possible. In the previous study, the lesions were only assessed for presence of the three DD treponemes, not for *Dichelobacter nododus*, the causal agent of footrot in sheep (Egerton, 2014) nor for *Fusobacterium necrophorum* (also associated with footrot and interdigital dermatitis in sheep). To determine whether the treponeme-associated foot disease is a manifestation of footrot in goats, microbiological assessment for the presence of *Dichelobacter nodosus* would be required. Interestingly, in the study by Groenevelt *et al.* (2015), very low levels of *D. nodosus* were detected in their 'atypical foot lesions', suggesting that they were not footrot. In the current study, the presence of extensive solar lesions without coronary band involvement also suggests that the goat disease, at least in terms of its gross pathology, is different to CODD in sheep. In one of the other studies of treponeme-associated foot disease in dairy goats (Groenevelt *et al.*, 2015) the disease picture is described as foot lesions that start from the white line or sole and involve varying degrees of solar horn loss.

The current study has allowed further characterization of treponeme-associated foot disease. Solar ulceration, sometimes accompanied by toe and wall ulceration, appears to be a consistent feature of treponeme-associated foot disease in all three studies (Groenevelt *et al.*, 2015; Sullivan *et al.*, 2015b). To our knowledge, solar ulcers, as described commonly in early lactation dairy cattle, have not been reported in dairy goats, but white line lesions have been recorded (Hill *et al.*, 1997). Caprine treponeme-associated foot disease may be a primary bacterial infection, as is considered with DD in cattle (Wilson-Welder *et al.*, 2015), or it may reflect secondary treponeme invasion of pre-existing foot lesions, as seen in non-healing lesions such as toe necrosis, non-healing white line disease and non-healing solar ulcers reported in cattle feet (Evans *et al.*, 2011). Although DD treponemes were identified in the foot lesions present in this study, this does not indicate that they are the only organism involved in the foot lesions and they may be only casual agents.

Molecular detection (PCR) was used to confirm that all foot lesions included in the study were DD treponeme associated as previously reported (Sullivan *et al.*, 2015b). The PCR results showed at least one of the DD-associated treponeme phlyogroups to be present in foot lesions and absent in the control feet. Two animals had all three DD-associated treponemes present. However, a limitation of the control sampling of apparently healthy feet is that the microbiological samples were not collected from the same farm as the diseased goats, but were collected from goats at a fallen stock centre. Therefore we cannot conclusively state that DD treponemes would not be found in healthy feet from goats on the farm containing the diseased animals. The findings of the same bacteria by PCR in novel goat foot lesions correlates with the findings of Sullivan *et al.* (2015b) and Groenevelt *et al.* (2015). All 11 foot tissue samples from the culled goats were culture positive, indicating that the treponemes were not only present in the lesions, but were also alive, with isolation of a pure culture possible in six cases. The 16S rRNA gene sequences showed clear homology, or a high level of similarity, to other treponeme sequences obtained from cattle, sheep and elk, suggesting that the same bacteria may infect different animal hosts, raising the risk of transmission between hosts (Duncan *et al.*, 2014; Clegg *et al.*, 2015; Sullivan *et al.*, 2015b). The *T. pedis* isolates obtained from these goats are different to those previously isolated in goats, which suggests a different origin.

Only goats with advanced lesions were available for radiographical and further pathological characterization, therefore the results cannot be considered as representative for all stages of disease. All feet that were radiographed showed significant abnormalities of the distal phalanx and the distal sesamoid bone. The macroscopic lesion of hyperkeratosis appeared to be consistent with the chronic lesions, since digits

manifesting hyperkeratosis all had extensive radiographical abnormalities of the distal phalanx, distal sesamoid bone and the middle phalanx. Changes to the distal phalanx have been documented in sheep with CODD (Angell *et al.*, 2015a) and in cattle with *Treponema*-associated toe necrosis (Kofler, 1999; Evans *et al.*, 2011; Blowey *et al.*, 2013). Bone changes have been documented in rabbits with syphilis due to *Treponema pallidum* (Brown *et al.*, 1921) and in people with periodontal disease (Loesche and Grossman, 2001). Therefore, the pathogenesis of the development of the bone changes and the association of *Treponema* spp. in the hoof horn needs to be investigated further, as bone remodelling appears to be a consistent finding between species of animals.

Histopathology revealed the nature of the hoof lesions to be chronic lymphoplasmacytic, suppurative and ulcerative pododermatitis, with many areas of the digit affected. The consistent predominating lesion was in the solar horn and was a mixture of hyperkeratosis with erosion/ulceration and multifocal replacement by granulation tissue and a serocellular crust. A suppurative dermal/epidermal infiltrate was also a common finding in the solar horn. Often, the dorsal horn exhibited similar lesions to the solar horn, but there were cases that were only chronic with severe diffuse hyperkeratosis and a mild to moderate perivascular dermatitis was predominant. The main observation concerning the coronary band and distal digital haired skin was of orthokeratotic hyperkeratosis.

The intensity of immunolabelling was not consistent across the specimens. Thirteen sections had intense labelling, confirming the presence of *Treponema* spp. bacteria, with the spirochaetal morphology clearly observable at the tissue periphery in 10 cases. The intense labelling was always superficial, with the *Treponema* spp. bacteria located between keratinocytes, as seen in CODD (Angell *et al.*, 2015b). IHC showed that the bacteria were only present occasionally in the dermis when epidermal erosion or ulceration was present. This consistent localization suggests that these bacteria are surface dwelling and trophic to the superficial keratinocytes in the abnormal horn. *Treponema* spp. were not observed in the haired skin, which is different to cattle with DD (Evans *et al.*, 2009). It is possible that sloughing of the superficial epidermis and the associated treponemes may indicate a potential mode of transmission that has not been demonstrated to date.

In summary, this is the third report of treponeme-associated foot disease in dairy goats in the UK. The predominant clinical presentation was solar ulceration of varying extent, with or without foot enlargement and hyperkeratosis. The radiographical study demonstrated the severity of the bone pathology associated with disease, which likely contributes to the severe and chronic pain/lameness in the goats. The histopathology of the disease allows it to be classified as a chronic lymphoplasmacytic, suppurative and ulcerative pododermatitis. Immunohistochemical and bacteriological results confirm the presence of the three DD-associated treponemes found in cattle, sheep and elk foot lesions in the affected goat tissues. The current study is not conclusive about the disease process, but the clinical descriptions of the secondary non-healing disease in cattle bears a strong similarity to the disease in goats, an observation that was also made by Groenevelt *et al.* (2015). As to the nature of any underlying primary lesion, the authors can only speculate. It is unknown whether the disease is a primary treponemal disease or the result of secondary invasion of pre-existing foot lesions. Substantial comprehensive longitudinal studies of the disease process, including clinical, microbiological and pathological aspects, are required urgently.

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Conflict of Interest Statement

The authors declare they have no conflicts of interest with respect to publication of this manuscript.

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