**Isolation of digital dermatitis treponemes from cattle hock skin lesions.**

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## Abstract

Bovine hock lesions present a serious welfare and production issue on dairy farms worldwide. Although many variables affect lesion prevalence, some hock lesions are seen on most cattle farms. Current theories suggest that trauma as an important in the formation of hock lesions, although infection may also play a role in increasing their severity and duration. This study, for the first time, describes an association of novel infectious agents with severe open hock lesions.

Digital dermatitis (DD) lesions in dairy cows are strongly associated with specific treponemal bacteria which are known to be opportunistic invaders of other skin lesions including digital dermatitis and pig lesions. This study used PCR assays and bacterial isolation to detect DD treponemes in hock lesions. Swabs and tissue samples of open (defined as those which are bleeding) and closed hock lesions (hair loss but no skin breakage) were taken from cattle on several farms during milking.

Digital dermatitis treponemes were detectable and isolated from open hock lesions only, with closed lesions showing no evidence of treponemal infection, either by PCR or bacterial culture. When analysed by 16S rRNA gene sequencing, the cultured treponeme DNA showed complete homology or were very similar to those found in foot lesions, providing further evidence that these bacteria can infect any open wounds on the cow. Additionally, skin swabs from near the open hock wound were also positive by PCR assay and isolation for the DD treponemes, suggesting that bacteria can act as a reservoir for infection, with bacteria being carried to other places on the body, by rain water, blood, or contact from other animals. Identification of the contribution of these infectious agents will allow for more

optimal treatments to be developed in the future in order to reduce the prevalence and healing times of both hock lesions and DD. It may allow for a potential antiseptic spray over the entire surface to reduce infection and promote healing.

## Introduction

Hock lesions are a major problem for farmers worldwide, affecting a large number of cattle, causing significant welfare issues. This can be through discomfort and pain to the animal, as well as leading to production issues and decreased longevity (Huxley et al, 2006; Bareille et al, 2003; Barberg et al, 2007). Prevalence varies across farms and countries, with various prevalence reports depending on the scoring systems used: 73% in British Columbia (Weary et al, 2000), 57% in France (Veissier et al, 2004), 50% in Austria and Germany (Brenninkmeyer et al, 2012), 47.3% in Denmark (Burow et al, 2012), 60.5% in Norway (Kielland et al, 2009), 81% in England (Potterton et al, 2011) and 42-81% in North America (Von Keyserlingk et al, 2012). There are many risk factors for hock lesions which include time of year, type of stalls, nature of bedding and stage of lactation (Potterton et al, 2010; Regula et al, 2004; Haskell et al, 2006; Rutherford et al, 2008; Brenninkmeyer et al, 2012). In addition, older, larger and higher milk yielding cows are more commonly affected by hock lesions (Rutherford et al, 2008). Interestingly, cattle with dirtier hocks are generally less affected by hock lesions, suggesting a protective effect from dirt (Potterton et al, 2011). Contradictory data exists about body condition score of cattle and risk of developing hock lesions (Kielland et al, 2009; Potterton et al, 2011; Espejo et al, 2006; Bicalho et al, 2009; Regula et al, 2004). There may also be a genetic predisposition, as Holsteins are generally more affected than other breeds, but this may reflect their predominance as a dairy cattle breed (Potterton et al, 2011; Burow et al, 2012; Keil et al, 2006). Decreased numbers of hock lesions are reported in organic and outdoor farming, whilst there is contradictory information with regards herd size contribution (Rutherford et al, 2008).

Positive correlations have been reported between hock lesion severity and lameness, and between hock lesions and other lesions or ill health in cattle, including carpal joint callosities, teat and udder injuries and somatic cell counts (Haskell et al, 2006; Fulwinder et al, 2007; Kielland et al, 2009; Regula et al, 2004). A study by Lim et al., (2013) suggested that hock lesions could lead to lameness, or alternatively lameness may lead to hock lesions due to increased recumbence time and lesions may also alter the gait of cattle (Potterton et al, 2011), potentially leading to increased lameness and impaired locomotion (Potterton et al, 2011). Furthermore, hock lesions can require treatment from veterinarians, and require structural modification to stalls on a farm, both of which can be economically expensive.

Hock lesions generally progress from hair loss, through to wounds and scabs, often terminating in severe swelling, typically involving the tarsus joint (Fulwinder et al, 2007).

Many species of bacteria can cause skin lesions. Recently, treponeme bacteria have been isolated from lesions on pigs (Svartstrom et al, 2013; Karlsson et al, 2013, 2014), and shown to be similar or identical at the 16s rRNA gene level to those isolated from cattle, and other species including goats, sheep and elk. (Dawson, 1998; Sullivan et al, 2014, Evans et al, 2009; Klitgaard et al., 2008; Nordhoff et al, 2008; Demirkan et al, 2001; Sayers et al, 2006; Sullivan et al, 2014; Clegg et al, 2014). Similar bacteria have also been isolated from 'non-healing' hoof lesions, including toe necrosis, white line disease and sole ulcers, and are thought to have an environmental causality (Evans et al, 2011).

Lesions from cattle, sheep goats and elk affected by digital dermatitis and from pigs affected by other skin lesions generally contain spirochetes from three previously isolated and characterised *Treponema* phylogroups: identified as "*Treponema medium* phylogroup, *T. phagedenis* phylogroup and *T. pedis* phylogroup digital dermatitis (DD) spirochetes (Evans et al, 2008; 2009b).

As a consequence of the isolation of DD treponemes from digital dermatitis in cattle, sheep, goats and elk and from skin lesions on pigs, it is possible that these promiscuous bacteria could either cause hock lesions or infect established lesions and exacerbate the problem. This study was designed to consider the hypothesis that digital dermatitis treponemes are contributing to the pathology of hock lesions in dairy cattle.

## Methods

### Hock lesion appearance

Lesions occurred on both the medial and lateral aspect of the hock, especially where there was skin overlying bone with minimal subcutaneous cushioning material.

Early changes were seen as hair loss only. This could then progress to a thickening of the skin with callous formation, especially on the lateral aspect; these were classed as closed lesions for this study. Eventually, there is a cracking of the thickened skin and exposure of sensitive tissue, often with a serous discharge or frank bleeding. These were classed as open lesions. Advanced cases may develop a pronounced swelling from an underlying bursitis, but such cases were rare.

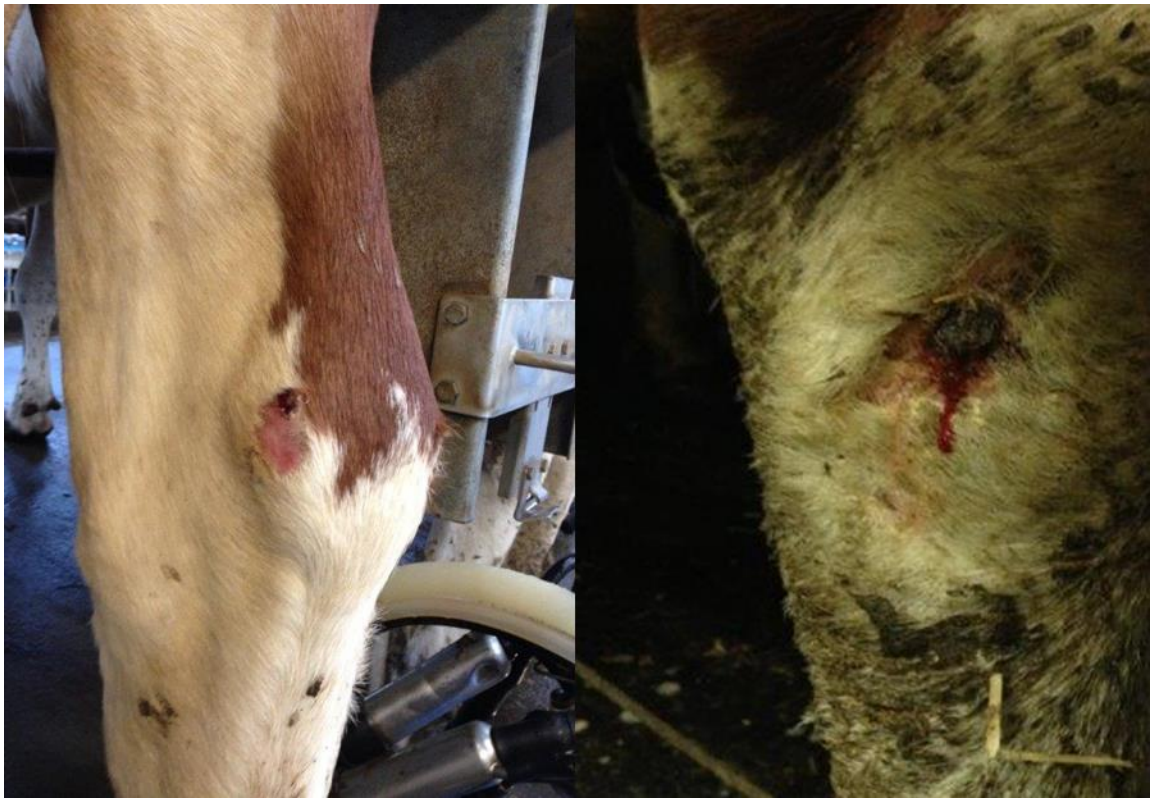

**Figure 1**. A closed hock lesion (left) and an open hock lesion (right). The open hock wound is oozing blood, suggesting the skin has been broken, with signs of scabbing around the edges. A closed lesion was characterised as having hair loss but no current skin break.

Farm data

Swabs and tissue samples were taken from hock lesions from two farms, based in the south west of England. These farms housed approximately 200 and 420 milking cows respectively. The cows were housed in concrete cubicles covered with a mattress, on top of which they had either straw (Farm B) or sawdust (Farm R). Both farms had DD lesions apparent on some cattle feet. Samples were taken during routine milking.

Sample collection

1. Twenty one swabs were taken from open and closed hock wounds on the lateral (*n* = *15*) and medial (*n*= *6*) sides of the hock.
2. Swabs (n = 15) were taken from the skin, approximately 2 cm from the hock lesion (11 by open lesions, 4 by closed lesions), to ascertain if the bacteria were spreading out from the lesion over the skin.

3. An additional 15 swabs were taken from normal hocks from animals on farms which had stock affected by hock lesions, to confirm that they were not present on normal skin on affected farms.
4. Tissue samples were obtained from seven open hock lesions, three of which were on the lateral hock and four of which were from the medial hock. Loose skin and crust were removed by hand. None of the sampled cows had obvious signs of DD when observed in the milking parlour by an experienced veterinarian.
5. Tissue samples from closed lesions (n = 7) were taken from cows at a local fallen stock centre, from animals which had died due to other, unknown reasons.
6. In order to confirm that any bacteriological findings were not a common feature of the normal skin, 15 normal control tissue samples were taken from animals without any hock lesions (7 from the lateral hock, 8 from the medial side) (Table 1). These samples were deep tissue samples taken from cows from other farms which presented at a local fallen stock centre when an animal had died due to other, unknown reasons. These control animals were also confirmed to be DD-free.

Tissue samples were placed in oral treponeme enrichment broth (OTEB: Anaerobe Systems, Morgan Hill, CA, USA) containing rifampicin (5 µg/ml) and enrofloxacin (5 µg/ml)) and transferred to the laboratory where they were used to inoculate fresh media immediately for culture and isolation of DD treponemes.

Isolation of spirochetes

Spirochete isolation attempts were made on all tissue samples taken from affected hock lesions, including seven affected open hock lesions, seven closed hock lesions and fifteen unaffected hock skin samples. These bacterial isolations were as described previously using oral treponeme enrichment broth (OTEB) (Evans et al, 2008) including rifampicin (5 µg/ml) and enrofloxacin (5 µg/ml). To maximise isolation attempts, samples were inoculated into OTEB containing foetal calf serum (FCS) (Gibco, Paisley, UK), to maximise growth of *T. phagedenis* and *T. pedis* phylogroup treponemes, and rabbit serum (RS (GE Healthcare Life Sciences, Buckinghamshire, UK) to maximise growth of *T. medium* phylogroup treponemes. All isolation attempts were carried out in an anaerobic cabinet (85% $N_2$, 10% $H_2$ and 5% $CO_2$, 36ºC). Cultures were screened by phase contrast microscopy and analysed by species-specific nested PCR assay to identify specific treponeme phylogroups present as described below. Cultures were deemed negative after 6 weeks.

Passage was continued using fastidious anaerobe agar (FAA: Lab M; Manchester, UK) plates, supplemented with 5% defibrinated sheep blood and antibiotics as above. Single colonies from the plates were inoculated into OTEB tubes as described above to allow pure bacterial cultures to be obtained.

In order to ascertain if swabs taken from the skin near to open hock lesions contained viable DD treponeme bacteria, small samples of swabs were pooled and inoculated in small amounts of OTEB media. This culture was then analysed using phase contrast microscopy and PCR.

DNA extraction

For isolation of bacterial genomic DNA from OTEB cultures, 2 ml of each culture was centrifuged (5000 X *g*, 10 min, 4°C) in a bench-top centrifuge. DNA was then extracted from the cell pellet using Chelex-100, as previously described (Chua et al, 2005) and stored at -20°C. For extraction of DNA from tissues and swabs, a QIAquick DNeasy blood and tissue kit (QIAGEN, Manchester, UK) was used following manufacturer's instructions.

PCR assays

Tissues taken from open and closed hock lesions on affected animals and from unaffected hocks, and culture samples were subjected to nested PCR assays specific for the three DD-associated treponeme phylogroups, *T. medium*, *T. phagedenis and T. pedis* described previously (Evans et al, 2008, 2009) with resulting PCR products encompassing 300 to 500bp of the 16S rRNA gene. For identification of bacterial isolates, PCR and gene sequencing of almost the entire 16S rRNA gene was carried out (Evans et al, 2008).

In order to validate PCR assays, each experiment included positive controls (bovine DD treponeme genomic DNA from each of the three unique bovine DD treponeme phylogroups) and a negative control (water) as described previously (Evans et al, 2009). All assays were carried out in triplicate.

All swab and tissue samples were also subjected to the *Treponema* genus PCR assay which detects all *Treponema* species, both pathogenic and commensal (Moore *et al.,* 2005), using the same positive and negative controls.

Sequencing and sequence analysis

Amplified PCR products were sequenced commercially (Macrogen, Amsterdam) and gene sequences assembled using Chromas Pro sequence analysis package (Technelysium Pty ltd). 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 TrN93 (Tamura et al, 1993). This was used to produce nucleotide maximum likelihood phylogenetic trees (bootstrap values based on 10000 iterations).

## Results

### Spirochaete isolations from tissues

None of the tissue samples taken from unaffected animals showed any signs of treponeme presence or growth in culture, when analysed by phase contrast microscopy and the tissue and bacterial cultures were also negative when tested by the nested PCR assay for all three DD treponeme phylogroups.

Of the seven tissues taken from open hock lesions, all of the resulting cultures were positive for treponeme growth upon analysis by phase microscopy, and this was confirmed by *Treponema* genus PCR assay and nested PCR assay for all three DD treponeme phylogroups (Table 1).

Of the seven closed hock lesions, one of the cultures was weakly positive for bacteria which appeared to be spirochaete-like using phase contrast microscopy, but was negative when tested by nested PCR. This culture was not tested further. All other closed lesions were negative by microscopy and *Treponema* nested PCR assay.

In four of the tissues used for isolation studies, contamination with other bacteria was low, so isolation of a single treponeme phylogroup was possible. The other three were contaminated with unidentified bacteria and isolation of a unique treponeme was not possible.

For the four tissue samples from which a pure treponeme isolate was obtained the bacteria were further analysed by 16S rRNA gene sequencing.

| Sample name | Lesion area | Isolation | All trep | DD1 | DD2 | DD3 |
|---|---|---|---|---|---|---|
| B1 | Lateral | + | + | + | + | - |
| B6 | Medial | + | + | - | +(HL1) | + |
| B7 | Medial | + | + | - | + (HL2) | - |
| B10 | Medial | + | + | + | + | + |
| R6 | Lateral | + | + | - | + (HL3) | + |
| R7 | Medial | + | + | + (HL4) | + | - |
| R11 | Lateral | + | + | + | + (HL5) | - |

**Table 1**. Digital dermatitis treponeme detection in seven open hock lesion samples as confirmed by phase contrast microscopy and nested PCR. Additionally, seven closed hock lesions were tested, and 15 unaffected hock samples, but as these cultures were all negative for treponemes by phase contrast microscopy and PCR and are not included in the above table for clarity. The names shown in parentheses show the isolate ID, as used in the phylogenetic tree (Fig 2).

Key: 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.*

**PCR detection in hock lesion swabs**

The 21 swabs from hock lesions were subjected to DNA extraction and analysed by PCR for the presence of DD-associated treponemes. Of these, 15 of the swabs were taken from open hock lesions, and all were positive for DD treponemes. Seven of these swabs were taken from the same cows as the tissue sections were obtained, and the results matched in all cases (B1, B6, B7, B10, R6, R7 and R11).

When tested by nested PCR, one or more DD treponemes were detected in swabs taken from open wounds. These bacteria were motile, suggesting that they were viable and not from the original swabs. By comparison, all tissue samples and swabs taken from closed hock lesions, or from unaffected hocks were negative for DD treponemes (Table 2).

| Sample name | Lesion area | Open lesion | Isolation | Treponeme whole genus | DD1 | DD2 | DD3 |
|---|---|---|---|---|---|---|---|
| B1 | Lateral | Yes | + | + | + | + | - |
| B3 | Medial | Yes | N/A | + | + | + | - |
| B5 | Medial | Yes | N/A | + | + | + | - |
| B6 | Medial | Yes | + | + | - | + | + |
| B7 | Medial | Yes | + | + | - | + | - |
| B8 | Medial | Yes | N/A | + | - | + | - |
| B9 | Lateral | Yes | N/A | + | - | + | + |
| B10 | Medial | Yes | + | + | + | + | + |
| R5 | Lateral | Yes | N/A | + | - | + | - |
| R6 | Lateral | Yes | + | + | - | + | + |
| R7 | Medial | Yes | + | + | + | + | - |
| R8 | Lateral | Yes | N/A | + | + | + | + |
| R9 | Lateral | Yes | N/A | + | - | + | + |
| R10 | Medial | Yes | N/A | + | - | + | + |
| R11 | Lateral | Yes | + | + | + | + | - |
| B2 | Lateral | No | N/A | + | - | - | - |
| B4 | Lateral | No | N/A | + | - | - | - |
| R1 | Lateral | No | N/A | + | - | - | - |
| R2 | Lateral | No | N/A | + | - | - | - |
| R3 | Lateral | No | N/A | + | - | - | - |
| R4 | Lateral | No | N/A | + | - | - | - |

**Table 2**. PCR detection of DD treponemes in swabs taken from hock lesions. An additional 15 swabs not shown in the table were taken from animals unaffected by hock lesions, and were all negative by PCR.

Key: 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.*

Of the 15 hock lesion samples which were positive for DD treponemes, at least one of the three DD-associated treponeme phylogroups was detected by PCR. In total, *T. medium* phylogroup treponemes (DD1) were detected in 7 of 15 positive samples (47%)*, T. phagedenis* phylogroup treponemes (DD2) were detected in 15/15 (100%) and *T. pedis* phylogroup treponemes (DD3) were present in 7/15 (47%).

For the 15 positive hock lesions, two (13%) of the lesions contained three DD-treponeme phylogroups, ten (67%) contained two, and three (20%) contained just one.

## 16S rRNA gene analysis

Five pure treponeme culture isolates were obtained from hock lesion tissue samples and the resulting 16S rRNA gene amplification PCR products were sequenced. To determine the relationship of the hock lesion treponeme isolates to those commonly found in DD lesions in cattle, the 16S rRNA gene sequences were compared to other previously sequenced isolates using phylogenetic analysis (Fig 2). All five isolated treponemes showed high similarity to previously isolated bacteria from DD lesions in cattle. The isolate named hock lesion 4 (Fig 2) showed a high level of similarity to human and bovine *T. medium*, and the other four hock lesions showed high similarity to *T. phagedenis* isolates from human and bovine hosts.

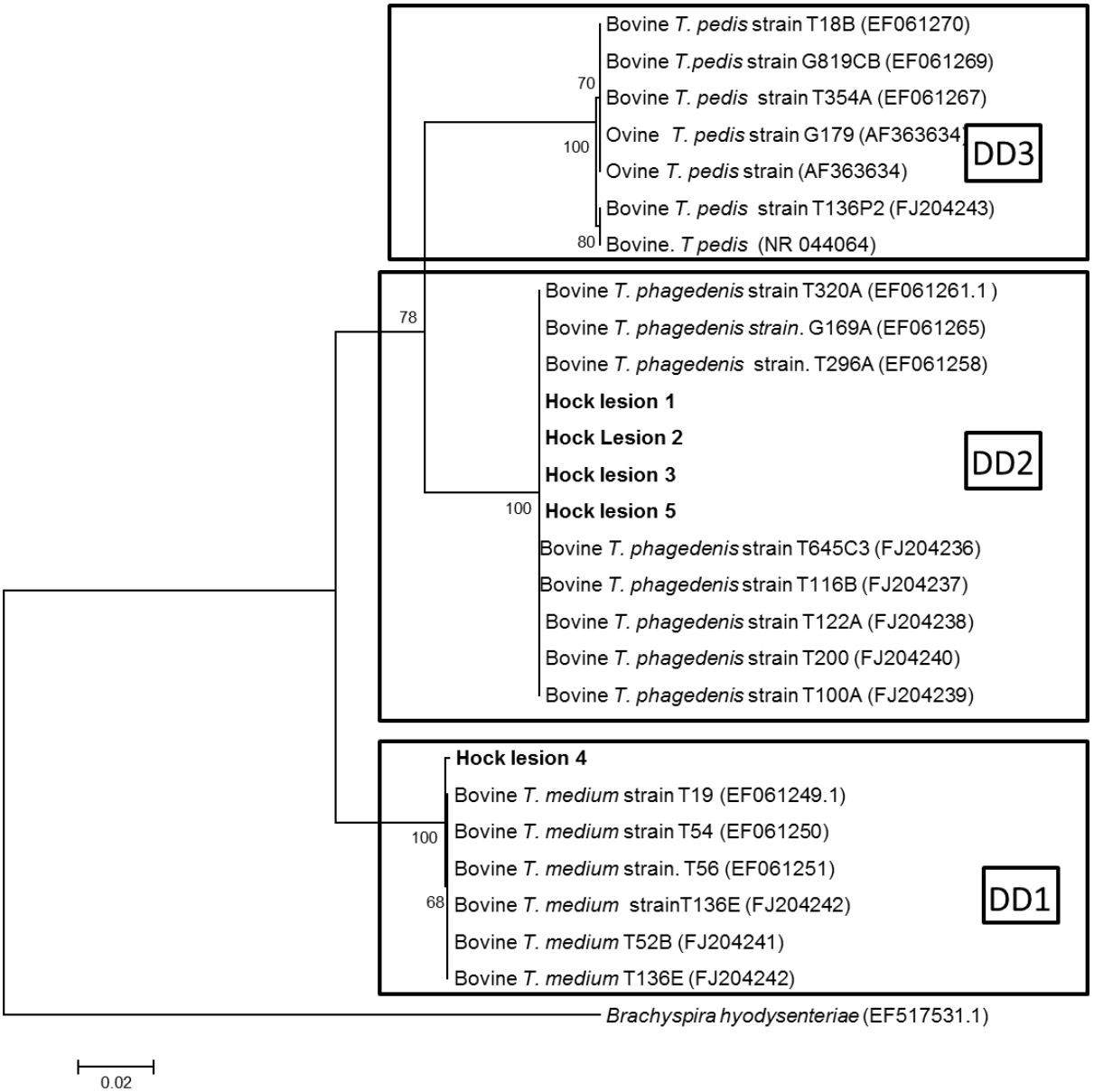

**Figure 2. 16S rRNA gene sequence phylogeny of treponemes cultured from open hock lesions on cattle.** Comparison of 16S rRNA gene sequences from pure treponeme isolated from open hock lesions in this study to those isolated from typical digital dermatitis lesions in cattle feet (for clarity, bootstrap values below 65 were removed). Sequences from Genbank of cattle treponemes, and other related treponemes are also shown, with the accession number in parentheses.

The sequences from isolates in this study are labelled with hock lesion number (as shown in table 1).

Key: Key: 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.*

## Analysis of swabs of normal skin adjacent to hock lesions

Fifteen swabs were taken from skin approximately 2 cm away from the hock lesions (11 by open lesions, 4 by closed lesions) to ascertain if treponeme bacteria were confined to a wound, or if they spread out over the surface near the lesion, if present. All swabs were positive using the treponeme genus PCR, as might be expected due to contamination from the farm environment. Only those swabs taken from close to open hock lesions were PCR-positive for DD treponemes, with all the swabs from skin near to closed lesions being negative.

Of those swabs taken from close to the 15 open hock lesions, 11 were positive for DD treponemes. The T. phagedenis phylogroup treponemes were present in all of the skin swabs tested, with T. medium phylogroup treponemes being found in four skin swabs (compared to five in lesions) and T. pedis phylogroup treponemes being found in five skin swabs (compared to seven in lesions).

In order to ascertain if the bacteria were viable, small samples of individual swabs were pooled, and inoculated into OTEB to determine if any bacteria could be isolated. Isolation of treponemes from cotton swabs is notoriously difficult and, unsurprisingly, a pure culture isolate was not achieved. However, when the pooled culture was analysed by phase contrast microscopy, small numbers of treponemes were clearly visible. Subsequently, when analysed by treponeme phylogroup-specific PCR, all three phylogroups were identified, suggesting that the bacteria were surviving on the skin outside the lesion.

## Discussion

Our data identify an association in cattle hock lesions with the treponemal bacteria previously identified in digital dermatitis lesions in dairy cattle, beef cattle, sheep, goats and wild elk (Dawson, 1998; Sullivan et al, 2014; Evans et al, 2009; Klitgaard et al., 2008; Nordhoff et al, 2008; Demirkan et al, 2001; Sayers et al, 2006; Sullivan et al, 2014; Clegg et al, 2014; Svartstrom et al, 2013; Karlsson et al, 2013, 2014). Importantly, these bacteria have also been shown to be very prevalent in other foot and hoof lesions, including non-healing forms of toe necrosis, white line disease and sole ulcers (Evans *et al*, 2011).

Whilst there is now significant evidence of DD-associated treponemes being present in multiple lesions in a number of domesticated (and wildlife) species, there is no firm evidence that they are primary aetiopathogenic agents causing hock lesions. However, as these skin lesions may have a range of initial triggers (such as trauma), it is very likely that they are opportunistic secondary invaders and contribute to skin pathology in a significant manner. Their importance as a potential cause of skin lesions cannot be ignored as treponemal infections are notoriously difficult to treat with current available antimicrobial agents, particularly in milk producing animals where the prime, penicillin based products cannot be used without significant milk withhold. Thus, understanding routes and mechanisms of infection/transmission are key to our need to control these lesions and prevent spread of infection to or from other parts of the body. Consequently, our data showing that the DD treponemes may be detected on the skin surface near active lesions, would strongly suggest that these highly motile organisms are able to migrate over the animal, possibly in blood, or water, or through contact with other animals. This could easily be a way in which they move from one site on an animal to a new site that has suitable conditions for growth, colonisation and further lesional development, or by direct contact, from animal to animal. This swarming motility has been reported in other flagellated bacteria previously (Kearns, 2010).

In addition to contributing to lesional pathology, the treponemes in the skin lesions may act as a reservoir of the bacteria for infection of other skin wounds, and even foot lesions. Therefore, clearance of treponemes from one wound may lead to re-infection with treponemes from another wound on the same animal, e.g. bacteria could be cleared from the foot with topical treatment, but then become re-infected by bacteria migrating over the skin from hock wounds. Alternatively, infection may be from other sources, such as farm machinery, direct contact with a classical foot DD lesion of another animal, or potentially from faecal contamination, where DD treponemes have recently been detected in small quantities using metagenomic studies (Klitgaard et al., 2014).

The lack of treponeme isolation from closed hock lesions, combined with the high prevalence of them in the open lesions further suggests that the treponemes are opportunistic invaders of wounds such as hock lesions, and potentially foot lesions.

Additionally, treponemes have been shown to invade pig skin lesions, possibly opportunistically after trauma or if combat causes an initial skin opening (Karlsson et al, 2013; 2014; Svartström et al, 2014).

*Treponema denticola*, a closely related human pathogen has been shown to have a chymotrypsin enzyme encoded within its genome. Preliminary analysis of genomes of the three different phylotypes of DD treponemes (Clegg *et al*, unpublished) suggest that they could have the same enzyme encoded within their genomes. This enzyme degrades connective tissues and immunoglobulins, so may explain the slow healing of wounds infected by treponemes (Uitto et al, 1988). Additionally, as the enzyme degrades connective tissues, it may be that hock infection with the bacteria leads to the skin thinning, making it more prone to splitting with trauma, or potentially splitting due to infection. Additionally, treponeme shave been shown to have a high affinity for fibrinogen, an important protein in blood clotting (Edwards et al, 2003). Infection of wounds with treponemes may thus prevent adequate clotting, further contributing to the pathology and severity of the lesions.

Closed herds tend to have less hock lesions than open herds, further suggesting that the presence of these lesions are linked to an infectious agent being brought into the herd (Rutherford et al, 2008). This is similar to the situation with digital dermatitis (Evans et al 2009). In terms of clinical effect, it is the more severe hock lesions which are often open and swollen which can lead to lameness, or altered gait of the cow, and thus the greatest economic loss (Brennickmeyer et al, 2012). As these are the lesions closely associated with treponeme infection, this provides strong supporting evidence of a need to break infection transmission cycles to prevent these lesions becoming progressively more severe. Furthermore, it has been suggested that bacterial infection of the hock can lead to a systemic spread of infection via the blood or lymphatic systems (Kester et al, 2014).

The 100% prevalence of *T. phagedenis* phylogroup treponemes is similar to the prevalence found in digital dermatitis lesions on dairy cattle (Evans et al, 2009), but different to the prevalence seen in lesions on elk, sheep, and digital dermatitis lesions on cattle where this phylotype is generally found in lower levels (Sullivan  et al, 2015; Clegg et al, 2014). This may suggest that *T. phagedenis* phylogroup treponemes are the pathogenic organism in hock lesions, or that this particular treponeme phylogroup is responsible for holding open wounds infected by treponemes so that a recruiting process occurs, allowing invasion of other treponeme groups, and/ or other bacteria, making the wound more severe, and leading to the swelling commonly seen with it.

Additionally, for treatment, it may be that a full body spray treatment is better, rather than a topical spray commonly used to treat digital dermatitis.

It is apparent that since the first description of BDD in 1974 (Cheli et al, 1974) and the subsequent spread to foot and skin lesions of multiple host species, the DD-associated treponemes have expanded not only their host range, but also their tissue specificity. As such, these organisms are providing an increasing challenge in farming and to veterinary science.

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