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Survival of contagious ovine digital dermatitis (CODD)-associated treponemes on disposable gloves after handling CODD-affected feet

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Both contagious ovine digital dermatitis (CODD) and bovine digital dermatitis (BDD) are causes of infectious lameness in sheep and cattle, respectively, and are strongly associated with the presence of specific treponemes, with three different cultivable phylogroups commonly isolated: *Treponema medium*, *Treponema phagedenis* and *Treponema pedis*. The aim of this study was to investigate the potential to transmit CODD-associated *Treponema* species via gloves used when handling visibly clinically affected animals. The feet of sheep with and without CODD were handled as part of routine examination with gloved hands. The gloves were then swabbed to detect the presence of treponemes immediately after handling. Detection methods included culture and isolation techniques together with DNA detection by PCR. In addition, the duration of survival in air was determined as well as the efficacy of common disinfectants to remove treponemes from gloves. In this study, we demonstrate that CODD-associated treponemes can survive on gloves used to handle the feet of CODD-affected sheep but may be removed effectively using common disinfectants. These data provide evidence of a potential route of transmission and identify a practical method to reduce this risk.

Introduction

Since its emergence in 1974, bovine digital dermatitis (BDD) has led to substantial levels of lost revenue for the cattle industry, and significant animal welfare problems (Cheli and Mortellaro 1974, Losinger 2006, Bruijnis and others 2012). A microbiologically related but clinically different and more severe manifestation of disease in sheep—contagious ovine digital dermatitis (CODD) was initially identified in the UK in 1997 (Harwood and others 1997), and further manifestations of other *Treponema* species-associated diseases with distinct clinical presentations, have recently been identified in goats and wild elk (Clegg and others 2015, Sullivan and others 2015b).

These different manifestations of digital dermatitis (DD) have all been shown to be associated with bacteria of the genus *Treponema* and comprehensive phylogenetic studies demonstrate that very similar or identical treponeme strains are shared across all the different host species (Clegg and others 2016a,b). As such, they have been classified into three common culturable phylogroups: *Treponema medium*, *Treponema phagedenis* and *Treponema pedis*.

Although treponemes are consistently associated with CODD lesions, other causes of infectious lameness are also common, with footrot (caused by *Dichelobacter nodosus*) in particular shown to be strongly associated with CODD (Angell and others 2015c). Furthermore, *Fusobacterium necrophorum,* another opportunistic bacterium, has been considered a precursor for subsequent infection with *D nodosus* in some footrot cases (Egerton and others 1969, Zhou and others 2009). It has also been more recently associated with clinical disease subsequent to *D nodosus* infection implying secondary invasion (Witcomb and others 2014).

BDD is distributed worldwide (Evans and others 2016) and CODD is now common in the UK and Republic of Ireland with approximately 50 per cent of farms affected (Sayers and others 2009, Angell and others 2014, Winter and others 2015) and has possibly been identified in Chile (Borkert and Galleguillos 2015) and Denmark (Rasmussen and others 2012). Recent studies have isolated the DD treponemes from knives used for hoof trimming infected cattle and sheep, with implications that this could be a potential route of transmission which could be easily managed (Sullivan and others 2014).

It is often considered that contact between animals is the most likely route of transmission. However, the isolation of

Veterinary Record (2017) doi: 10.1136/vr.104228

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Provenance: Not commissioned; externally peer reviewed

Accepted May 18, 2017

treponemes from hoof trimming knives has raised the issue that manual transmission by humans who work on and/or visit the farm could be risk factors for transmission between animals and between farms if strict biosecurity practices are not employed (Sullivan and others 2014).

When handling sheep and particularly when inspecting or treating infected feet, wearing gloves is a reasonable personal hygiene and biosecurity measure employed to protect the handler and to reduce the risk of transmission between animals. However, in our experience many farmers and vets choose not to wear gloves. The aim of this study was to investigate the potential to transmit CODD-associated *Treponema* species via gloves used when handling visibly clinically affected animals. The specific objectives were to investigate whether pathogenic *Treponema* species could be detected on the surface of gloves after handling sheep feet with clinical CODD and then to determine the length of time these anaerobic bacteria could survive on gloves following handling.

Methods

Sample collection

Three related experiments were carried out. Experiment 1 was a case-control study comparing the presence of CODD-associated *Treponema* species on gloves following the handling of visibly clinically affected and clinically unaffected feet. Experiment 2 was a longitudinal study examining the duration of survival of the CODD-associated *Treponema* species in air. Experiment 3 used gloves from both experiments 1 and 2 to determine the viability of *Treponema* species following cleaning with various solutions.

This study was approved by the University of Liverpool Ethics committee (number VREC 417).

Experiment 1: case-control

Visibly clinical cases were identified (by JWA) as sheep with clinically active CODD based on having a CODD lesion graded 1–4 (Angell and others 2015a). Control sheep were those with clinically normal feet. All the feet of the sheep were handled as part of routine examination using sterile nitrile disposable gloves (Starguard; Starlab, Milton Keynes, UK), with a new pair worn for each sheep. After examination, two sterile cotton swabs (Copan, UK) were used to sample the right hand glove, including two fingers and the palm.

The sheep came from four commercial UK farms with CODD. Samples were obtained from 23 cases and 30 controls matched by farm and grazing group. Duplicate swabs were taken from gloves from both case and control sheep with each swab being drawn five times across each palm, thumb and fingers. In each case, one swab was to be used for *Treponema* species DNA detection by PCR and the other used for bacterial culture and isolation.

Fisher's exact test was used to investigate the presence of an association between the presence of CODD-associated *Treponema* species and the presence of *D nodosus* or *F necrophorum*, either individually or in combination, as detected by PCR.

Experiment 2: longitudinal survival

Twelve sheep with CODD were handled as described above, for routine foot examination. Five clean sterile gloves were used for each CODD-affected foot, each being used to examine the affected foot in the same way. The gloves were then removed and placed into a clean sterile plastic bag and transported to the laboratory. Here, gloves were removed from the plastic bags, placed on a sterile tray and kept untouched in the laboratory at room temperature and exposed to the air. Each day, two dry sterile cotton swabs were used to take a sample of material from one glove per case, as described for experiment 1 above. One swab was then inoculated into media for culturing of bacteria to ascertain viability, while the other was subjected to DNA extraction for diagnostic PCR analyses. This process was then repeated using a different glove, for five consecutive days, to determine the length of time bacteria could remain detectable and remain alive and therefore potentially viable and infectious.

Experiment 3: analysis of glove cleaning materials

As gloves may be used to handle multiple sheep, both with and without CODD lesions, it was of interest to ascertain whether the bacteria could be removed using common household and farm disinfectants.

Gloves used in the case-control experiment (experiment 1), and the gloves from days one and two from the longitudinal survival experiment (experiment 2) were used. After the first swabs were collected, the gloves were washed for five seconds while rubbing palms together using one of the following: (1) cold water, (2) warm water (at a temperature suitable to keep hand under without discomfort), (3) water and hand soap (Carex, PZ Cussons), (4) Virkon at a 1 per cent concentration (DuPont), (5) FAM 30 at a 1:90 dilution as recommended by the manufacturer (Evans Vanodine) and (6) 70 per cent ethanol. After washing, gloves were allowed to air dry before being sampled using sterile cotton swabs as described above, and then processed using diagnostic PCR assays and culture as described below.

To confirm that treponemes remained after the initial sampling as part of either experiment 1 or 2, one pair of gloves was swabbed without cleaning and used as a control.

Laboratory methods

Bacterial isolation

Spirochaete isolation attempts were carried out on all swabs taken from gloves used to handle the 23 cases with CODD and 30 control sheep showing no signs of foot disease. Each swab head was removed from its wooden handle using a sterile scalpel blade, and placed into oral treponeme enrichment broth (OTEB, Anaerobe Systems, Morgan Hill, California). To maximise isolation attempts, samples were inoculated into OTEB containing fetal calf serum (Gibco, Paisley, UK), to maximise growth of *T phagedenis* and *T pedis* phylogroup treponemes and rabbit serum (GE Healthcare Life Sciences, Buckinghamshire, UK) to maximise growth of *T medium* phylogroup treponemes (Evans and others 2008). Cultures were examined weekly for up to six weeks to confirm positive or negative results.

To attempt to obtain a pure culture of treponemes and remove other contaminating bacteria, bacterial cultures were reinoculated into fresh OTEB including rifampicin (5 µg/ml) and enrofloxacin (5 µg/ml). All isolation attempts were carried out in an anaerobic cabinet (85 per cent N_2, 10 per cent H_2 and 5 per cent CO_2, 36°C). Cultures were screened by phase contrast microscopy and analysed by specific nested PCR assays to identify whether specific DD treponeme phylogroups were present.

DNA extraction

For collection of bacterial genomic DNA from cultures, 2 ml of the culture was centrifuged (5000*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 and others 2005) and stored at −20°C.

For extraction of bacterial DNA from swabs, the QIAquick DNeasy blood and tissue kit (Qiagen, Manchester) was used following the manufacturer's instructions.

PCR assays

Cultures of swabs taken from both sheep affected and unaffected by CODD were subjected to nested PCR assays specific for the three CODD-associated treponeme phylogroups, *T medium, T phagedenis and T pedis* as described previously (Evans and others 2008) with resulting PCR products encompassing 300–500 bp of the 16S rRNA gene. More recent studies (Sullivan and others 2015a) have also demonstrated that these same phylogroups are highly associated with CODD lesions, as well as BDD lesions.

To validate PCR assays, each experiment included positive controls (previously isolated DD treponeme genomic DNA from each of the three unique DD treponeme phylogroups) and a negative control (water) as described previously (Evans and others 2008) and all assays were carried out in triplicate.

All swabs were also subjected to the *Treponema* genus PCR assay (Moore and others 2005), which detects all *Treponema* species, both pathogenic and commensal, using the same positive and negative controls. PCR assays were also carried out to ascertain if *F necrophorum* and *D nodosus* were also present on the gloves (Sullivan and others 2015a). To assess bacterial removal postcleaning, a PCR assay designed to amplify the entire 16S rRNA gene was also performed (Evans and others 2008).

Results

Experiment 1: case-control study

In total, 53 swabs were taken from gloves used to handle sheep feet, 23 cases affected by CODD and 30 from control animals as described.

No CODD-associated treponemes were detected (either by isolation or by PCR) from any of the gloves used to handle the control sheep. However, treponeme growth did occur from three of the swabs taken from gloves used to handle sheep visibly unaffected by CODD, but these were shown by PCR not to be any of the three pathogenic phylogroups, but were more likely intestinal/faecal commensal treponemes. By contrast, CODD-associated treponemes were detected by culture from 21 (91 per cent (95 per cent CI 69 to 98 per cent)) of the samples taken from gloves used to handle the cases and from 100 per cent by PCR (Table 1). Of these, *T medium* was isolated from 11 (48 per cent (95 per cent CI 27 to 69 per cent)), *T phagedenis* was isolated from 18 (78 per cent (95 per cent CI 55 to 91 per cent)) and *T pedis* was isolated from 12 (52 per cent (95 per cent CI 31 to 73 per cent)).

TABLE 1: Phase contrast microscopic and PCR analysis of swabs taken from gloves used to handle sheep feet affected by or unaffected by CODD in the case-control experiment

				Bacterial culture PCR results				Swab PCR results					
Sample ID	Animal status (CODD+/–)	Farm ID number	Microscopy	Treponeme genus	DD1	DD2	DD3	Treponeme genus	DD1	DD2	DD3	*Dichelobacter nodosus*	*Fusobacterium necrophorum*
a	+	1	+	+	+	+	+	+	+	+	–	+	+
b	+	1	+	+	–	+	+	+	–	+	+	+	+
c	+	2	+	+	–	+	–	+	+	+	+	+	+
d	–	2	–	–	–	–	–	+	–	–	–	+	+
e	+	2	–	–	–	–	–	+	+	+	+	+	–
f	–	2	–	–	–	–	–	+	–	–	–	–	–
g	+	2	+	+	+	+	+	+	+	+	+	–	–
h	+	2	–	–	–	–	–	+	+	–	+	–	–
i	–	2	–	–	–	–	–	+	–	–	–	+	–
j	+	2	+	+	+	+	–	+	+	+	+	–	+
k	–	2	–	–	–	–	–	–	–	–	–	+	+
1	–	3	–	–	–	–	–	+	–	–	–	–	+
2	–	3	–	–	–	–	–	+	–	–	–	+	+
3	–	3	–	–	–	–	–	+	–	–	–	+	+
4	–	3	–	–	–	–	–	+	–	–	–	–	+
5	–	3	–	–	–	–	–	–	–	–	–	–	–
7	–	3	–	–	–	–	–	–	–	–	–	–	–
8	–	3	–	–	–	–	–	+	–	–	–	–	–
9	–	3	–	–	–	–	–	–	–	–	–	+	+
10	–	3	–	+	–	–	–	–	–	–	–	+	+
11	–	3	–	–	–	–	–	–	–	–	–	+	+
12	–	3	–	–	–	–	–	+	–	–	–	–	+
13	–	3	–	–	–	–	–	–	–	–	–	–	+
14	–	3	–	–	–	–	–	+	–	–	–	–	+
16	–	3	–	–	–	–	–	–	–	–	–	–	–
17	–	3	–	–	–	–	–	–	–	–	–	–	–
18	–	3	–	–	–	–	–	–	–	–	–	+	–
19	–	3	–	–	–	–	–	–	–	–	–	+	+
20	–	3	–	–	–	–	–	–	–	–	–	+	–
21	–	3	–	+	–	–	–	+	–	–	–	+	–
22	–	3	–	+	–	–	–	+	–	–	–	+	–
24	–	3	–	–	–	–	–	–	–	–	–	–	+
25	–	3	–	–	–	–	–	+	–	–	–	–	+
26	–	3	–	–	–	–	–	+	–	–	–	+	+
27	–	3	–	–	–	–	–	–	–	–	–	+	+
28	–	3	–	–	–	–	–	–	–	–	–	+	+
29	–	3	–	–	–	–	–	+	–	–	–	+	–
31	+	3	+	+	+	+	+	+	+	+	+	+	–
32	+	3	+	+	–	+	–	+	+	+	–	+	–
33	+	3	+	+	+	+	–	+	+	+	–	–	+
34	+	3	–	+	–	+	–	+	–	+	+	–	+
35	+	3	+	+	–	+	+	+	+	+	+	–	+
36	+	3	–	+	–	–	+	+	+	–	+	–	+
37	+	3	+	+	+	+	+	+	+	+	+	–	+
38	+	3	–	+	+	+	–	+	+	+	–	+	+
39	+	3	–	+	+	+	+	+	+	+	+	+	+
40	+	3	+	+	+	–	+	+	+	–	+	+	+
10B	+	2	+	+	–	+	+	+	+	+	+	+	–
20B	+	4	+	+	+	+	–	+	+	+	–	+	+
9B	+	4	+	+	–	+	–	+	–	+	–	–	+
21B	+	2	+	+	–	+	–	+	–	+	–	–	+
29B	+	4	+	+	+	+	+	+	+	+	+	–	–
30B	+	2	+	+	–	–	+	+	+	–	+	–	–

DD1 refers to the *Treponema medium* phylogroup, DD2 refers to the *Treponema phagedenis* phylogroup and DD3 refers to the *Treponema pedis* phylogroup
CODD, contagious ovine digital dermatitis

Growth of treponemes occurred in inoculates from 24 of the samples taken, confirming that the bacteria were viable. However, there were numerous other unidentified contaminating bacteria within the culture, which made production of a pure treponeme culture difficult. Unfortunately, no pure cultures could be isolated for sequencing of 16S rRNA genes.

D nodosus was detected by PCR from 11 gloves (48 per cent (95 per cent CI 27 to 69 per cent)) used to handle CODD-affected sheep, and from 17 gloves (57 per cent (95 per cent CI 38 to 74 per cent)) used to handle the control sheep. *F necrophorum* was detected by PCR from 15 gloves (65 per cent 95 per cent CI 43 to 83 per cent)) used to handle CODD-affected sheep, and from 18 gloves (60 per cent (95 per cent CI 41 to 77 per cent)) used to handle the control sheep.

For those gloves used to handle CODD-affected feet (n=23), there was no association between the presence of CODD-associated *Treponema* species as detected by bacterial culture or PCR and either *D nodosus* or *F necrophorum*, individually or in combination as detected by PCR (P>0.1).

Experiment 2: longitudinal survival

Swabs were taken on five sequential days from one of five gloves used to handle the same foot with CODD, and subjected to bacterial culture and PCR (Table 2). For *T medium,* bacteria were cultured in OTEB and detected using PCR assays from gloves from eight sheep (67 per cent (95 per cent CI 33 to 89 per cent)) and were viable for up to one day. For *T phagedenis*, bacteria were cultured from gloves from nine sheep (75 per cent (95 per cent CI 43 to 95 per cent)) and detected by PCR from 10 (83 per cent (95 per cent CI 46 to 97 per cent)). For *T pedis,* bacteria were cultured and detected by PCR from eight sheep (67 per cent (95 per cent CI 33 to 89 per cent)). For both *T phagedenis* and *T pedis*, the treponemes were viable for up to three days (median 2 days (range 0–3 days)). For all the sheep where treponemes were detected from the gloves by PCR, the treponemes remained detectable for all five days (Table 2).

TABLE 2: Detection of isolates by phase contrast microscopy (isolation) and PCR analysis of swabs taken from gloves used to handle CODD-affected feet and sampled daily in the longitudinal survival study

		Day treponemes were detected				
Animal ID	Detection method	1	2	3	4	5
40	Isolation	1, 2, 3	2, 3	–	–	–
	PCR	1, 2, 3	1, 2, 3	1, 2, 3	1, 2, 3	1, 2, 3
37	Isolation	1, 2, 3	2, 3	2, 3	–	–
	PCR	1, 2, 3	1, 2, 3	1, 2, 3	1, 2, 3	1, 2, 3
39	Isolation	2, 3	2, 3	–	–	–
	PCR	2, 3	2, 3	2, 3	2, 3	2, 3
46	Isolation	1, 2	2	–	–	–
	PCR	1, 2	1, 2	1, 2	1, 2	1, 2
48	Isolation	1, 3	3	–	–	–
	PCR	1, 3	1, 3	1, 3	1, 3	1, 3
35	Isolation	2	2	–	–	–
	PCR	2	2	2	2	2
7	Isolation	1, 3	3	–	–	–
	PCR	1, 3	1, 3	1, 3	1, 3	1, 3
4	Isolation	1, 3	3	–	–	–
	PCR	1, 2, 3	1, 2, 3	1, 2, 3	1, 2, 3	1, 2, 3
12	Isolation	2	2	–	–	–
	PCR	2	2	2	2	2
14	Isolation	2, 3	2, 3	–	–	–
	PCR	2, 3	2, 3	2, 3	2, 3	2, 3
44	Isolation	1, 2	2	–	–	–
	PCR	1, 2	1, 2	1, 2	1, 2	1, 2
42	Isolation	1, 2, 3	2, 3	2, 3	–	–
	PCR	1, 2, 3	1, 2, 3	1, 2, 3	1, 2, 3	1, 2, 3

1—*Treponema medium* phylogroup, 2—*Treponema phagedenis* phylogroup and 3—*Treponema pedis* phylogroup
CODD, contagious ovine digital dermatitis

TABLE 3: The detection of CODD-associated treponemes following treatment with different chemicals

Treatment	N	Positive culture, N (%)	Treponeme PCR positive, N (%)	16S rRNA PCR positive, N (%)
Cold water	10	10 (100)	10 (100)	10 (100)
Hot water	10	10 (100)	10 (100)	10 (100)
Hand soap	10	0 (0)	1 (10)	1 (10)
Ethanol	10	0 (0)	0 (0)	0 (0)
FAM 30	10	0 (0)	0 (0)	0 (0)
Virkon	10	0 (0)	1 (10)	1 (10)

Ten swabs were taken from gloves which were known to be positive for treponeme growth following treatment with the chemical. Treponeme growth in culture was determined by specific phylogroup PCR, and the swabs were also exposed to phylogroup-specific PCRs
CODD, contagious ovine digital dermatitis

Although *T medium* phylogroup treponemes were detected on 8 of the 12 gloves at the start of the study, they were only detected in culture samples on day one, and not on day two or three. Conversely, *T phagedenis* and *T pedis* phylogroup treponemes were detected on day one, and grew until day two or three, depending on the sheep tested.

Experiment 3: glove cleaning

CODD-associated treponemes were detected by culture and diagnostic PCR from all 10 gloves following cleaning by washing with cold and warm water. However, none was detected by culture following cleaning with any of the other four methods, although CODD-associated treponemes were detectable by PCR on one of the gloves cleaned with hand soap and one with Virkon (Table 3).

The eubacterial 16S rRNA gene PCR assay further identified that ethanol and FAM 30 are suitable disinfectants to remove all bacteria, including treponemes and other potential lameness-associated microbes (such as *D nodosus* and *F necrophorum*). Additionally, as well as removing the viable bacteria, all results with these cleaning agents were PCR negative, suggesting that the disinfectant destroyed all the bacteria present.

Discussion

This study shows for the first time that CODD-associated treponemes are present and can survive on the gloves of personnel working with CODD-affected sheep feet, providing novel information regarding CODD treponeme fomites adding to the previous report of their presence on hoof trimming knives (Sullivan and others 2014). The high percentage (91 per cent (95 per cent CI 69 to 98 per cent)) of gloves that were contaminated with DD treponemes after handling CODD-affected feet reiterates the importance of control measures between affected and unaffected animals. In addition, the detection of *D nodosus* and *F necrophorum* on the gloves by PCR (although viability was not ascertained) also raises the possibility that these pathogens may also be transmitted through contact with contaminated gloves.

In previous studies, a strong positive association between the clinical diseases CODD and footrot has been shown (Angell and others 2015c, Duncan and others 2012). In this study, there was no association found between the presence of the CODD-associated *Treponema* species (on gloves) and the causative agent of footrot—*D nodosus*, or the pathogen *F necrophorum*. These bacteria were also detected less frequently when compared with the CODD-associated *Treponema* species, which is in agreement with Sullivan and others (2015a), and strengthens the argument for these *Treponema* species to be considered as a necessary cause of CODD.

Working with the assumption that the associated *Treponema* species are a necessary cause of disease (Duncan and others 2014, Angell and others 2015b, Sullivan and others 2015a), after handling sheep with CODD, there is the potential for these bacteria to be passed from one sheep to another and this may be a

potential route of transmission. A preventive strategy could therefore involve simply changing or at least cleaning gloves following the handling of diseased sheep.

Treponemes are considered to be anaerobic, and as such all studies which have cultured them have used anaerobic chambers to do so (Sabo and others 1988). The survival of treponemes on gloves for two to three days in air in this study suggests that these bacteria may not be strict anaerobes but may be able to tolerate oxygen for short periods. Careful disposal of gloves would be necessary to prevent inadvertent infection through contact with the gloves, and by extrapolation, it is possible that being able to survive for short periods in aerobic conditions may increase the chances of survival of these bacteria in the farm environment. This in turn may potentially increase the risk of infection from contaminated areas as there will be significantly more areas where aerobic bacteria can survive compared with anaerobic bacteria. It also raises the possibility of a risk of transmission between farms by contaminated material being transferred on personnel and equipment and reiterates the need for considered and rigorous biosecurity policies and controls for farmers and professional visitors. Furthermore, in this study only one type of glove material was investigated and other materials may increase or decrease the risk of transmission.

In this study, there was variation in the duration of survival between the different phylogroups, with those of the *T medium* phylogroup only surviving for one day on the gloves. This could be due to a number of reasons but may suggest that they are more sensitive to oxygen than the other two phylogroups. Alternatively, it may reflect that this phylogroup remains the most difficult to isolate using the protocol described.

No CODD-associated treponemes were detected on gloves after handling the feet of sheep without CODD lesions, which is in agreement with previous data where these bacteria were only found on clinically affected feet (Sullivan and others 2015a). However, it remains important to maintain high levels of biosecurity including changing gloves between animals as *D nodosus* and *F necrophorum*—causative agents of footrot and scald (Egerton and others 1969, Zhou and others 2009), can also be found on gloves after handling clinically unaffected feet, which is also in agreement with previous data (Sullivan and others 2015a). The viability of these bacteria was not ascertained in this study, so the potential for transmission via gloves of these bacteria remains unknown.

Although the same *Treponema* species appear to be able to cause the different DD-like diseases in different host species (Clegg and others 2016b), the clinical pathology of these diseases is strikingly different between species, and this may affect the contamination of gloves posthandling. The disease manifestation in cattle may limit the contact of gloves with the lesion, and the treponemes are generally found deeper in the lesions (Evans and others 2009), compared with sheep and goats where the treponeme bacteria are generally found on the surface which may increase the risk of glove contamination (Angell and others 2015b, Crosby-Durrani and others 2016).

It is recognised that many farmers and foot trimmers may find it impractical to change gloves between handling every foot (although this would be ideal), therefore cleaning gloves following the handling of affected feet was tested using common chemicals found on farm. Use of common antibacterial hand soap (Carex), ethanol, FAM 30 and Virkon all removed live bacteria. In most cases, all traces of bacterial DNA were removed using FAM 30 and 70 per cent ethanol, and in 9/10 cases, hand soap and Virkon removed all CODD-associated treponeme DNA from the gloves. Therefore, a practical approach could employ the use of a bucket of disinfectant close to the work area to be used to wash the gloved hands between each animal. Constant use of chemicals on the gloves can cause them to degrade so they would still need regular replacement, although household 'rubber gloves' which are thicker may withstand these chemicals better. These disinfectant and cleaning chemicals may be useful for reducing the risk of transferring infective material on other equipment, for example, other personal protective equipment, tools and equipment.

Conclusions

Handling sheep may lead to the transmission of disease between CODD-affected and CODD-unaffected animals. *Treponema* species associated with CODD lesions can survive on gloves used to handle affected feet for up to three days in the air and may represent a biosecurity risk, although they are readily removed and killed with several commonly available cleaning/disinfection chemicals. Appropriate and affordable controls may include changing gloves between sheep or washing gloved hands between sheep in an appropriate disinfectant.

Acknowledgements

The authors would like to thank the farmers for their participation in this study.

Funding This work was funded by a Biotechnology and Biological Sciences Research Council (BBSRC) Industrial Partnership Award (IPA) Research Grant (BB/N002121/1) partnering with Hybu Cig Cymru HCC/Meat Promotion Wales and AHDB Beef and Lamb (a division of the Agriculture and Horticulture Development Board, UK).

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Veterinary Record published online June 22, 2017