

FINAL REPORT FOR HCC/EBLEX

An Investigation into Contagious Ovine Digital Dermatitis Lesion Treponeme Bacteria and their Antibiotic Sensitivities.

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Aims of Project

- 1) To investigate the variation in treponeme bacteria found in contagious ovine digital dermatitis lesions.
- 2) To investigate the variation in antibiotic susceptibilities of treponeme bacteria from contagious ovine digital dermatitis lesions.

Time Scale: September 2013- December 2014

Background

Contagious ovine digital dermatitis (CODD) is a cause of infectious lameness in sheep in the UK and has been shown to have a severe impact on the welfare of affected individuals. Recent surveys have shown that CODD may now affect between 35% and 53% of flocks in the UK, and whilst on farm prevalence is typically low, it may affect up to 50% of the flock at any one time.

Shortly after the first identification of CODD as a disease distinct from footrot (Harwood *et al.*, 1997), a spirochaete was isolated that was biochemically and enzymatically similar to strains isolated from bovine digital dermatitis (BDD) lesions (Naylor *et al.*, 1998). This spirochaete was then shown by PCR of the 16s rRNA gene to be closely related to

the treponemal phylotype *Treponema vincentii* (Collighan *et al.*, 2000). This treponeme has been associated with human periodontitis and BDD (Rijpkema *et al.*, 1997). Since then, a second spirochaete has been identified and shown to be genetically different to that identified by Collighan *et al.* (2000) and was also similar to a further unnamed spirochaete isolated in the USA associated with BDD (Demirkan *et al.*, 2001). The hypothesis that CODD and BDD have a similar spirochaetal aetiology was developed by Dhawi *et al.* (2005) who demonstrated that both cattle and sheep with BDD and CODD respectively, show significant (<0.001) high anti-treponeme antibody reactions compared to controls. Sayers *et al.* (2009) also cultured treponemes from 7 out of 10 cases of CODD, with various single isolates and combinations of isolates of *T. phagedenis*-like, *T. medium*/*T. vincentii*-like and *T. denticola*/*T. putidum*-like DD treponemes.

As such, information known about the microbiological flora of CODD lesions is limited, although there is a growing body of evidence linking BDD treponemes to CODD lesions, and as such they are currently considered to be one of if not the only probable primary aetiological agents (Duncan *et al.*, 2014).

The identification of CODD as a new disease as different from footrot, in part occurred due to the failure of response to conventional treatments for footrot. Consequently there has been a wide range of empirical treatments attempted in clinical cases (Davies *et al.*, 1999; Judson, 2010; Sawyer, 2010; Winter, 2004) although only one randomised controlled trial (RCT) has been conducted comparing parenteral amoxicillin with topical chlortetracycline (Duncan *et al.*, 2011). This study extrapolated data from DD treponemal isolates and one ovine isolate from Evans *et al.* (2009) in order to justify the antibiotic choice.

The successful treatment of BDD has remained problematic, with many farms now adopting a management and control strategy as opposed to affecting a cure (Laven and Logue, 2006). In order to inform the development of effective therapeutic strategies for clinical cases of CODD, the management of disease on affected farms, and responsible use of antibiotics, a greater understanding is required of the species of *Treponema* found

in affected feet, and of sensitivities of the treponemes found in CODD lesions to antibiotics currently available for use in sheep in the UK.

Thus the aims of this study were: 1) to culture and molecular type treponemes from feet affected by CODD. 2) Determine the *in vitro* susceptibility of the pure isolates to a panel of antibiotics used in the UK sheep industry.

Methods

Bacterial Isolation for Treponeme Culture and Molecular Typing

Sample collection

Thirty eight CODD lesions biopsies were collected, from 4 different farms in North Wales from March 2013 to July 2014 (Table 1). All farms had between 300-1000 breeding ewes and all were lowland farms except for a farm in Conwy which was located on hill farm land. The sheep breeds on these farms were mainly; Welsh Mountain, Scottish Blackface, Suffolk/Suffolk crosses, Lleyn/Lleyn crosses, Charolais crosses or easy care breed. Sheep identified with classic CODD lesions were examined and the lesions were biopsied using a 3mm punch biopsy under local anaesthesia (Demirkan *et al.*, 2001, Evans *et al.*, 2008). All CODD lesions biopsied were active lesions shown by tissue appearing haemorrhagic and granulomatous. Lesion samples were divided in two with half transferred into transport medium and placed on ice for subsequent *Treponema* culture. Transport medium consisted of oral treponeme enrichment broth (OTEB; Anaerobe Systems, Morgan Hill, CA, USA) and contained the antibiotics rifampicin (5 µg/ml) and enrofloxacin (5 µg/ml). The remaining tissues from lesions (for PCR analysis) were transported on ice and stored at -20 °C.

Culture of spirochetes

Bacterial isolation, specifically for treponemes, was attempted on all CODD lesion samples (n=38). A (1-1.5mm) piece of tissue was transferred into an anaerobic cabinet (85% N₂, 10% H₂ and 5% CO₂, 36 °C). Each was inoculated into OTEB with 10% foetal calf serum (FCS) and the antibiotics rifampicin (5 µg/ml) and enrofloxacin (5 µg/ml). After 2-5 days, bacteria were then sub-cultured on fastidious anaerobe agar (FAA) plates

(LabM, Bury, UK) with 5% defibrinated sheep blood, 10% FCS and antibiotics as above. After 1-2 weeks, single colonies were inoculated into growth media and were checked for pure culture by phase contrast microscopy. DNA was extracted from treponeme cultures and the isolated organisms identified using a 16S rRNA gene PCR as previously described (Evans *et al.*, 2008). Bacterial culture was not attempted on sheep healthy foot tissue.

DNA extraction

For PCR analysis, tissues from the lesions and healthy tissues were thawed and DNA extracted using a DNeasy kit (Qiagen, United Kingdom), according to the manufacturer's instructions, and genomic DNA stored at -20°C.

Genus and phylogroup specific treponeme PCR assays

Samples were subjected to nested PCR assays specific for the three DD-associated treponeme groups, “*T. medium/T. vincentii*-like”, “*T. phagedenis*-like” and “*T. denticola/T. putidum*-like”, as described by Evans *et al.*, (2008, 2009b) with resulting PCR products encompassing 300 to 500bp of the 16S rRNA gene. All foot samples were also subjected to the *Treponema* genus PCR assay which detects all *Treponema* species both pathogenic and commensal (Moore *et al.*, 2005). To ensure validity in each assay, water was used as a negative control, and positive controls included genomic DNA from each of the three treponeme phylogroups. Primer sequences are shown in Table 1.

Bacterial isolation for Antibiotic Sensitivity Testing

Twenty spirochaete isolates were cultured from CODD lesions from 19 sheep collected from 6 farms in England and Wales. Isolates were used to investigate antimicrobial susceptibility (Table 2). All the isolates were obtained from sheep with CODD lesions. Included are five group 1 isolates (*Treponema medium/Treponema vincentii*-like), ten group 2 isolates (*Treponema phagedenis*-like) and five group 3 isolates (*Treponema denticola/Treponema putidum*-like).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing to determine the minimum inhibitory concentration (MIC) was performed by a broth microdilution method using sterile 96-well polystyrene flat-bottomed microplates (Appleton Woods, Birmingham, U.K.). In this study each microplate included positive controls (bacteria inoculated without antimicrobial agents), negative controls (no bacteria or antimicrobial), and serial twofold dilutions of each of ten antimicrobials, all in oral treponeme enrichment broth (OTEB; Anaerobe Systems, CA, USA) inclusive of the respective serum supplement. The respective OTEB serum supplements were 10% (v/v) rabbit serum (RS) for group 1 strains and 10% (v/v) foetal calf serum (FCS) for group 2 and 3 strains.

Microplates were incubated in an anaerobic cabinet for several hours prior to use. The group 1, 2 and 3 strain inoculums were taken from cultures grown for 6, 4 and 4 days respectively, with all cultures being grown at 36°C under anaerobic conditions in OTEB supplemented with respective sera. Based on Evans *et al.* (2009) the stock inoculum of group 1, 2 and 3 isolates contained 8.75×10^7 , 1.14×10^8 and 2.69×10^8 treponemal organisms/ml respectively with 50µl inoculation volumes used. Similarly to Evans *et al.* (2009), prior to inoculation with treponemes, each well had a volume of 150µl, thus resulting in a final volume after inoculation of 200µl. The microplates were incubated at 36°C under anaerobic conditions for the required time for MIC measurement.

The antibiotics tested were penicillin G, amoxicillin, oxytetracycline, tilmicosin, lincomycin, spectinomycin and tylosin (Sigma-Aldrich, Dorset, UK); and tildipirosin (Zuprevo; MSD Animal Health, Milton Keynes, UK); and tulathromycin (Draxxin; Zoetis UK Limited, London, UK); and gamithromycin (Merial LLC, Duluth, Georgia, USA).

Determination of MICs

In Evans *et al.* (2009) growth curves were determined for each BDD associated spirochaete group, in order to identify the late exponential/early stationary phase microdilution time point. The same time points from this study were then used for the current study, namely 3 days for group 3 isolates and 4 days for group 1 and 2 isolates.

The MIC for each antibiotic was taken as the lowest concentration of antibiotic that prevented growth in the wells. Cell growth was determined by comparison of the absorbance measurement immediately after inoculation with the absorbance measured at the late exponential/early stationary phase time point specific to the group that the tested isolate belongs. All of the absorbance measurements occurred at 540nm using a Multiskan microtitre plate reader (Thermo Scientific, Hampshire, UK). The MIC values were taken as the median of three repeat experiments, performed on different days, with freshly prepared inocula, medium and antibiotics.

Determination of Minimum Bactericidal Concentrations (MBC)

For each antibiotic, after MIC determination, 10µl of culture from the MIC well and the next three wells of increased antibiotic concentration were sub-cultured to new wells containing 190µl OTEB with the respective serum supplement, but with no antimicrobials included. Microplates were incubated for a further 5 days at 36°C under anaerobic conditions. Growth was assessed using phase contrast microscopy. The MBC was defined as the dilution that contained no treponeme cells in the sub-cultured media. The MBC values are reported as the median value of three experiments performed on different days.

Study validation

Control substances included penicillin, oxytetracycline, lincomycin and erythromycin, and used *T. phagedenis* biotype Reiter together with *T. phagedenis*-like BDD spirochete T320A as control microorganisms for testing. The control microorganisms were grown and susceptibility tested under the same conditions as the group 2 (*T. phagedenis*-like) treponeme group. To validate the method described here; the control data produced were then compared to previously described results from Abramson and Smibert (1971) and Evans *et al.* (2009) and compared statistically using linear regression.

Results

Genus and phylogroup specific treponeme PCR survey of CODD lesions

The results of the specific DD *Treponema* phylogroup PCR and *Treponema* genus-wide PCR assays in CODD lesions and are shown in Table 1.

All CODD lesions (n=38) were found to be positive for general *Treponema*. The phylotype specific PCR for “*T.medium/T. vincentii*-like”, “*T.phagedenis*-like” and “*T. denticola/T. putidum*-like” DD spirochetes, showed that they were individually present in 26/38 (68%), 36/38 (95%) and 26/38 (68%) of CODD lesions, respectively. All CODD lesions (100%) were positive for at least one or more of the DD-associated *Treponema* phylotypes, with 19/38 (50%) of CODD lesions positive for all three DD-associated *Treponema* phylotypes.

Antibiotic Sensitivity Testing

The individual *in vitro* susceptibilities of the 20 CODD associated treponemes to 10 antimicrobial agents are summarised in Tables 3 and 4 and Figures 1 and 2.

All groups were most susceptible to gamithromycin, and were least susceptible to lincomycin, spectinomycin and oxytetracycline.

The variation in sensitivity across the different treponeme groups was compared using the chi squared test. The variation across groups for each of the six of the seven macrolides was not significant (P=0.2), with gamithromycin approaching significance (P=0.08). The variation for penicillin was also not significant (P=0.2), however for amoxicillin and oxytetracycline group 2 bacteria were more sensitive compared to groups 1 and 3 (P=0.02 and P=0.004 respectively).

Study Validation

The MIC microdilution method described in this study was validated by comparing the results for four antibiotics (penicillin, oxytetracycline, lincomycin and erythromycin) to two control microorganisms *T.phagedenis* biotype Reiter and T320A. These MIC values

were compared to results previously obtained using a macrodilution method (Abramson and Smibert, 1971) and also results obtained using a similar microdilution method (Evans *et al.*, 2009). These all matched the previously described results except for oxytetracycline, which was a single serial dilution different to the results described by Abramson and Smibert (1971). Linear regression for these comparisons showed a strong correlation ($R=0.99$) indicating the efficacy and reproducibility of this method.

Discussion

Genus and phylogroup specific treponeme PCR survey of CODD lesions

CODD leads to severe welfare issues in sheep, and understanding the aetiopathogenesis of the disease is key to developing the means of managing or preventing this debilitating disease. Since the first CODD report in 1997 (Harwood *et al.*, 1997) it has become apparent that there is an infective component and that the specific treponemes closely associated with DD in dairy (Stamm *et al.*, 2002; Evans *et al.*, 2008, 2009b; Klitgaard *et al.*, 2008) and beef (Sullivan *et al.*, 2013) cattle are clearly involved in CODD, and may be a primary initiating agent (Dhawi *et al.*, 2005; Angell *et al.*, 2014). However, the available data thus far has been limited and therefore not proven substantial evidence for a causative association. The current study is a comprehensive attempt to consider the link between BDD treponemes and CODD.

What is clear from the study is that the BDD treponemes (individually and collectively) are present in all CODD lesions examined and does provide further evidence for the treponemal associations with CODD. However interesting, it is accepted that this finding alone does not prove causality. Further investigations must include comparisons with healthy tissue, investigation of associations with other bacterial species and temporal associations with lesion development.

Thus, a key question is whether BDD treponemes are the primary or secondary infections leading to the development of CODD lesions. What is clear is that they are present in all CODD lesions in this study and thus must be hypothesized as the prime reason why sheep

develop these specific lesions. However, we must also consider that they may be secondary infections to other, possibly non-infective, lesions in sheep feet. It has become apparent that the BDD treponemes must be considered as promiscuous and opportunistic infective agents as it has been clearly demonstrated that they are able to invade other (non-infective) lesions in cattle feet such as white line disease and sole ulcers and clinically manifest as new serious infectious diseases which are very difficult to treat (Evans *et al.*, 2011).

Antibiotic Sensitivity Testing

The described microdilution method has been used previously against treponemes isolated from BDD lesions (Evans *et al.*, 2009) and the validation in this study allows comparisons between the two. In Evans *et al.*, 2009 the MIC for the ovine isolate tested was comparable to the MICs for the cattle isolates, and this study has added more data demonstrating comparable results for a further 20 isolates.

When including all the spirochetes studied, gamithromycin, tildipirosin, penicillin, tylosin and tilmicosin have the lowest MICs and MBCs and as such are likely to be the most effective antibiotics for the treatment of CODD.

In sheep, there have been very few robust *in vivo* studies examining effective treatment. In two studies (Duncan *et al.*, 2012; Duncan *et al.*, 2011) parenteral amoxicillin was found to have a clinical cure rate of approximately 80%. Anecdotally, parenteral tilmicosin used on a whole flock basis was also found to be effective (Sawyer, 2010) and parenteral oxytetracycline together with a tylosin footbath were shown to be an effective preventative method (Judson 2010). However, both these last two studies remain unpublished and lack control data. It is interesting to note that given that lincomycin and spectinomycin both demonstrated the highest MIC and MBC concentrations and that topical preparations of lincomycin and spectinomycin together have been advocated as a clinical treatment (Davies *et al.*, 1999). Furthermore, in the authors experience this treatment method is widely used in the industry.

This study is therefore timely for a number of reasons: 1) it is the first study to provide data in support of further *in vivo* studies, providing necessary *in vitro* information which may be needed when considering which antibiotics to use in clinical trials; 2) it provides evidence that current therapeutic strategies may include the use of antibiotics (e.g. lincomycin and spectinomycin) that are most likely to be ineffective and also most likely to drive resistance. Indeed, in Evans *et al.*, 2009 a group 2 treponeme (T167) was identified to be resistant to spectinomycin with an MIC 2048 times that of the other groups 2 isolates tested in that study.

To date no antibiotic product has a license to be used to treat CODD. The antibiotics studied here were selected to include antibiotics that already have a UK license for use in sheep (penicillin, amoxicillin, oxytetracycline and tilmicosin) together with those that in the authors' experience are already used (off label) in the sheep industry.

Therefore, when taking current UK medicines legislation into account, and given these data as a whole, penicillin and tilmicosin would appear to be the most likely candidates for future clinical trials as a consequence of their efficacy and availability as licensed products for use in sheep.

In conclusion, this study provides the first detailed examination of the antimicrobial susceptibilities of antibiotics to all three groups of treponemes cultured from CODD lesions, using the microdilution method. As such they provide important information on the current antibiotics used to treat this disease and these data should help inform researchers which products to include when planning further CODD clinical treatment trials.

Further work

1) Investigations into the Variation in Treponeme Bacteria Identified in Contagious Ovine Digital Dermatitis Lesions.

By providing additional CODD biopsy material, the work funded here has substantially enhanced the on-going programme of Leigh Sullivan's EBLEX/HCC funded PhD. In that project she has examined many more CODD biopsy samples for treponeme bacteria and looked for the presence of other bacteria such as *Dichelobacter nodosus* and *Fusobacterium necrophorum*. In addition, she has bacteriologically investigated feet from healthy sheep, as well as undertaking a phylogenetic analysis of treponema isolates.

Beyond this project we consider the next step in establishing the microbial causality of CODD is to investigate the temporal associations of the various bacteria associated with the disease during an experimentally induced disease outbreak (subject of BBSRC EBLEX HCC Industrial Partnership Award Application.)

2) Investigation of the Antibiotic Sensitivities of Treponeme Bacteria from Contagious Ovine Digital Dermatitis Lesions.

The work described here has already moved on to the next logical research step which is examining the efficacy of antibiotic treatment of sheep affected by CODD. The research group are currently undertaking a large scale treatment trial on 30 farms investigating the efficacy of whole flock antimicrobial treatment with tilmicosin on the prevalence of CODD. As previously discussed whole flock treatments with tilmicosin have been undertaken by vets and farmers in an attempt to eradicate CODD and footrot from their flocks. However, currently there is no evidence base to support this strategy. The work funded by EBLEX and HCC has shown that in the laboratory at least, tilmicosin is a sensible choice for treatment of CODD associated treponeme infections. The results of the field trial will be complete by December 2015.

Dissemination to Industry

As discussed, the outputs of this project are 1) improved understanding of the aetiology of CODD and 2) improved understanding of the appropriate antibiotic choices for treatment. These results have gone on to inform the work of two larger projects; Leigh Sullivan PhD (funded by EBLEX/HCC) and Joseph Angell PhD (funded by BVAAWF). Both these projects will complete in 2015 and it is planned to work closely with HCC/EBLEX in a knowledge exchange programme to farmers and vets to disseminate the outputs of all current research work at University of Liverpool on CODD.

Table 1: Isolation and PCR strain type detection of treponemes from 38 CODD lesions.

Details (Farm location, sheep number)	Treponeme isolated	Specific PCR for group ^a :		
		1	2	3
Anglesey, 1	G2SL1	+	+	+
Anglesey, 97	IF	-	+	-
Anglesey, 73	IF	-	+	-
Anglesey, 30	G2SL5	+	+	+
Anglesey, 63	G12F2	+	+	+
Anglesey, 229	G12F2, G23F1	+	+	+
Anglesey, 36 back left	IF	+	+	+
Anglesey, 36 back right	IF	+	+	+
Anglesey, 2	IF	+	+	+
Denbighshire, 3	G16F2, G26F1	+	+	-
Conwy farm 1, 218	IF	-	+	+
Conwy farm 1, 10	G2F2C10, G2ST24	-	+	-
Conwy farm 1, 49	G2F3C12, G2F3	+	+	+
Conwy farm 1, 4	G2F4C4	+	+	+
Conwy farm 1, 53	IF	+	+	+
Conwy farm 1, 12	G2F6C6	+	+	+
Conwy farm 1, 33	G1F7C5	+	+	+
Conwy farm 1, 8	IF	+	+	+
Conwy farm 1, 86	G1F9C27, G2F9	+	+	+
Conwy farm 1, 85	G2F10C10	+	+	+
Conwy farm 1, 62	G2F11C11	+	+	+
Conwy farm 1, 96	IF	+	+	+
Conwy farm 2, 5	G2138C	+	+	+
Conwy farm 2, 6	G2148C	-	+	+
Conwy farm 2, 900	G2158C	-	+	+
Conwy farm 2, 930	IF	+	+	+
Anglesey, 38	IF	-	+	-
Anglesey, 653	IF	+	-	-
Anglesey, 58	IF	+	+	-
Anglesey, 40	IF	-	+	-
Anglesey, 74	IF	+	+	+
Anglesey, 60	G21C11	-	+	-
Anglesey, 59	G22C4	+	+	+
Anglesey, 41	IF	-	+	-
Anglesey, 39	IF	+	+	-
Anglesey, 651	IF	+	-	+
Anglesey, 652	IF	-	+	-
Anglesey, 33	IF	-	+	+

^a1, 2, 3 correspond to *T. medium*-like, *T. phagedenis*-like and *T. pedis* DD spirochete PCR assays respectively.

Abbreviations: IF, isolation failed.

Table 2. Treponemes tested for susceptibility to antimicrobial agents

Strain no.	Strain	UK Region	Nearest Relative ^a	Group ^b
1	F7	Conwy	<i>Treponema medium/Treponema vincentii</i>	1
2	F9	Conwy	<i>Treponema medium/Treponema vincentii</i>	1
3	OV11R	Gloucester	<i>Treponema medium/Treponema vincentii</i>	1
4	S2R	Wirral	<i>Treponema medium/Treponema vincentii</i>	1
5	ST27	Conwy	<i>Treponema medium/Treponema vincentii</i>	1
6	3F2	Anglesey	<i>Treponema phagedenis</i>	2
7	C2F	Gloucestershire	<i>Treponema phagedenis</i>	2
8	S4F	Cheshire	<i>Treponema phagedenis</i>	2
9	F3	Conwy	<i>Treponema phagedenis</i>	2
10	3F3	Denbighshire	<i>Treponema phagedenis</i>	2
11	SL1	Anglesey	<i>Treponema phagedenis</i>	2
12	3F1	Anglesey	<i>Treponema phagedenis</i>	2
13	S3R2	Cheshire	<i>Treponema phagedenis</i>	2
14	ST24	Conwy	<i>Treponema phagedenis</i>	2
15	C2R	Gloucestershire	<i>Treponema phagedenis</i>	2
16	Ovine	Gloucester	<i>Treponema denticola/Treponema putidum</i>	3
17	G3ST1	Shrewsbury	<i>Treponema denticola/Treponema putidum</i>	3
18	G3545	Shrewsbury	<i>Treponema denticola/Treponema putidum</i>	3
19	G3T1	Shrewsbury	<i>Treponema denticola/Treponema putidum</i>	3
20	G3T7	Shrewsbury	<i>Treponema denticola/Treponema putidum</i>	3

^aAs determined by 16S rRNA gene phylogenetic analysis.

^b 1, 2, 3 correspond to *T. medium*-like, *T. phagedenis*-like and *T. pedis* DD spirochetes.

Table 3. Contagious ovine digital dermatitis associated treponeme minimum inhibitory concentrations (MIC) to ten antimicrobial agents

Strain no. ^a	Median MIC (mg/L)									
	Penicillin	Amoxicillin	Oxytetracycline	Tilmicosin	Lincomycin	Spectinomycin	Tildipirosin	Tulathromycin	Gamithromycin	Tylosin
1	0.0750	0.5625	3	0.0703	24	12	0.0234	0.2930	0.0469	0.0469
2	0.0375	0.2813	3	0.0234	48	24	0.0234	0.2930	0.0234	0.0234
3	0.0375	0.5625	1.5	0.0117	48	12	0.0469	1.1719	0.0469	0.0234
4	0.0750	0.2813	1.5	0.0234	24	12	0.0469	0.2930	0.0234	0.0469
5	0.0750	0.5625	3	0.0117	24	24	0.0469	1.1719	0.0469	0.0469
6	0.0375	0.1406	0.75	0.0234	24	12	0.0469	0.5859	0.0469	0.0469
7	0.0188	0.1406	0.75	0.0469	12	12	0.0938	0.5859	0.0117	0.0469
8	0.0750	0.2813	0.375	0.0094	12	12	0.0469	0.2930	0.0117	0.0469
9	0.0375	0.1406	0.75	0.1875	24	12	0.0469	0.5859	0.0234	0.1875
10	0.0188	0.1406	0.75	0.0234	6	12	0.0117	0.5859	0.0469	0.0469
11	0.0188	0.2813	0.75	0.0234	3	6	0.0938	0.1465	0.0117	0.0469
12	0.0750	0.1181	0.375	0.0059	6	3	0.0469	0.2930	0.0029	0.0059
13	0.0375	0.1181	0.375	0.375	12	12	0.0234	0.5859	0.0938	0.1875
14	0.0750	0.1181	0.375	0.0938	48	12	0.0234	0.5859	0.0938	0.0234
15	0.0188	0.1406	1.5	0.1875	96	24	0.0469	0.2930	0.0234	0.0938
16	0.0750	0.2813	1.5	0.0234	24	24	0.0234	0.5859	0.0234	0.0938
17	0.0375	0.5625	6	0.0234	48	24	0.0234	0.5859	0.0469	0.0469
18	0.0750	0.5625	3	0.0234	48	12	0.0469	0.5859	0.0234	0.0469
19	0.0750	0.2813	1.5	0.0117	48	24	0.0469	0.5859	0.0234	0.0938
20	0.0750	0.5625	6	0.0117	96	24	0.0234	1.1719	0.0234	0.0938
MIC ₉₀ ^b	0.0750	0.5625	3	0.1875	48	24	0.0469	1.1719	0.0469	0.0938

^a Isolates 1-5 are group 1 CODD treponemes with antibiotic test ranges ($\mu\text{g/L}$) of: penicillin ; amoxicillin ; oxytetracycline ; tilmicosin ; lincomycin ; spectinomycin ; tildipirosin ; tulathromycin ; gamithromycin and tylosin .

Isolates 6-15 are group 2 BDD treponemes with antibiotic test ranges ($\mu\text{g/L}$) of: penicillin 0.75-0.0059; amoxicillin 2.25-0.0176; oxytetracycline 12-0.0938; tilmicosin 0.375-0.0029; lincomycin 192-1.5; spectinomycin 48-0.375; tildipirosin 0.75-0.0059; tulathromycin 9.375-0.0732; gamithromycin 0.188-0.0015; and tylosin 0.375-0.0029..

Isolates 16-20 are group 3 CODD treponemes with test ranges as group 2

^b Cumulative susceptibility results across all treponemes tested are expressed as MIC_{90} , the concentration at which 90% of CODD associated treponemes are inhibited.

Table 4. Contagious ovine digital dermatitis associated treponeme minimal bactericidal concentrations (MBC) to ten antimicrobial agents

Strain no. ^a	Median MBC (mg/L)									
	Penicillin	Amoxicillin	Oxytetracycline	Tilmicosin	Lincomycin	Spectinomycin	Tildipirosin	Tulathromycin	Gamithromycin	Tylosin
1	0.0750	0.5625	6	0.0938	48	12	0.0469	0.5859	0.0469	0.0938
2	0.0750	0.5625	3	0.0469	96	24	0.0469	0.2930	0.0469	0.0938
3	0.0750	1.1250	3	0.0234	48	24	0.0469	1.1719	0.0469	0.0938
4	0.0750	0.5625	3	0.0469	48	24	0.0469	0.5859	0.0234	0.0938
5	0.0750	0.5625	6	0.0234	24	24	0.0469	1.1719	0.0469	0.0938
6	0.0375	0.5625	6	0.0469	48	12	0.0938	0.5859	0.0469	0.3750
7	0.0375	0.5625	3	0.1875	24	24	0.0938	0.5859	0.0234	0.0938
8	0.0750	0.5625	3	0.1875	24	12	0.0469	1.1719	0.0234	0.3750
9	0.0750	0.5625	6	0.1875	96	24	0.0938	0.1172	0.0469	0.1875
10	0.0375	0.2813	6	0.0234	96	12	0.0234	0.5859	0.0469	0.0469
11	0.0189	0.2813	3	0.0234	48	6	0.3750	0.5859	0.0117	0.0469
12	0.0750	0.2813	1.5	0.0117	24	6	0.0469	0.5859	0.0117	0.0117
13	0.375	0.1181	0.75	0.1875	24	12	0.0234	1.1719	0.0938	0.1875
14	0.0750	0.5625	0.375	0.0938	48	12	0.0234	0.5859	0.0938	0.0938
15	0.0188	0.2813	0.75	0.1875	24	12	0.0469	0.5859	0.0234	0.0938
16	0.0750	0.5625	3	0.0234	48	24	0.0469	0.5859	0.0469	0.0938
17	0.0750	0.5625	6	0.0234	96	24	0.0469	0.5859	0.0469	0.0938
18	0.0750	0.5625	6	0.0469	48	12	0.0469	1.1719	0.0234	0.0938
19	0.0750	0.5625	6	0.0469	48	24	0.0469	1.1719	0.0469	0.0938
20	0.0750	0.5625	6	0.0234	96	24	0.0234	1.1719	0.0469	0.0938
MBC ₉₀ ^b	0.0750	0.5625	6	0.1875	96	24	0.0938	1.1719	0.0469	0.1875

^a 1-5, group 1; 6-15, group 2; 16-20, group 3. ^b Cumulative susceptibility results across all treponemes tested are expressed as MBC₉₀, the concentration at which 90% of CODD associated treponemes are killed

Figure 1. Contagious ovine digital dermatitis associated treponeme minimum inhibitory concentrations (MIC) to ten antimicrobial agents

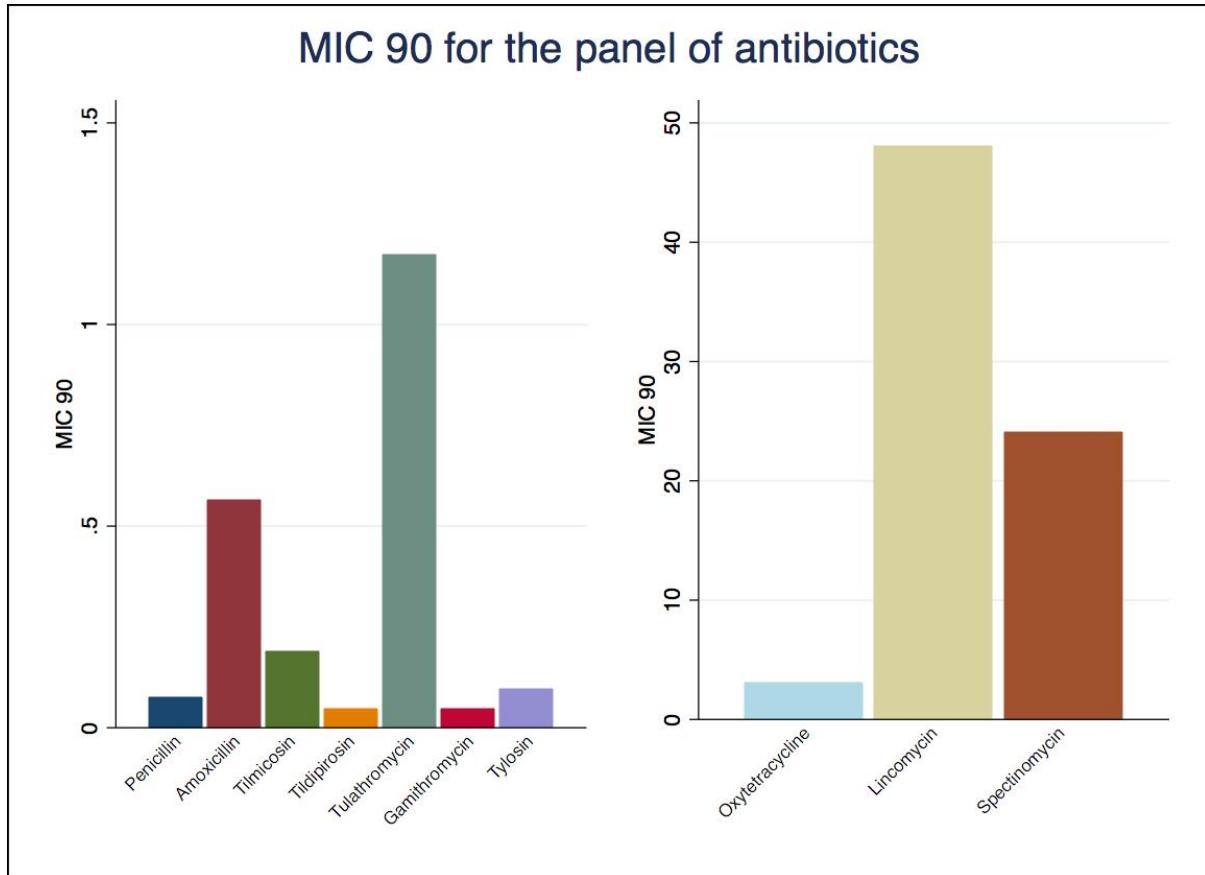


Figure 2. Contagious ovine digital dermatitis associated treponeme minimum bacteriocidal concentrations (MBC) to ten antimicrobial agents.

