Towards a greater understanding of contagious ovine digital dermatitis (CODD): an epidemiological approach

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by

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Therefore lift your drooping hands and strengthen your weak knees, and make straight paths for your feet, so that what is lame may not be put out of joint but rather be healed.

Hebrews 12 vs 12-13 (The Bible; English Standard Version)

Abstract

Towards a greater understanding of contagious ovine digital dermatitis (CODD): an epidemiological approach - *Joseph W. Angell*

Contagious ovine digital dermatitis (CODD) is a cause of severe lameness in sheep in the UK. The aim of this study was to gain a greater understanding of this disease and provide practical information for application on farms.

A cross-sectional postal survey of 511 farmers in Wales provided information on prevalence, geographical distribution and farmer reported risk factors. CODD was shown to be now endemic in the UK with 35% of farms affected, and an average on farm prevalence of 2.0% although some farms experienced a much greater prevalence. Larger farms were reportedly more at risk, as were those with bovine digital dermatitis (BDD) in their cattle. There has been a rapid increase in reports of CODD arriving on farms since 2000, and farmers considered concurrent footrot, buying in sheep, adult sheep, time of year and housing to be associated with CODD.

A four-point ordinal locomotion scoring tool was developed enabling farmers and veterinarians to score the severity of a locomotion abnormality in sheep. This had high levels of intra-observer repeatability: weighted kappa (κ_W) 0.81 for veterinarians and 0.83 for farmers.

A detailed clinical description and five-point lesion grading system were developed in order to aid diagnosis amongst veterinarians and farmers, and to aid communication. Radiographs also highlighted the extensive damage to soft tissues and bony structures that may occur in advanced clinical cases, and locomotion scoring demonstrated a variation in welfare impact by lesion grade.

Histopathology provided detailed evidence of the pathological processes occurring in clinical lesions. Early lesions were characterised by a lymphoplasmacytic infiltrate of the distal digital skin, with suppurative coronitis and intracorneal pustules. In more advanced lesions there was complete separation of the dorsal hoof wall with a necrotizing and fibrinosuppurative exudate and dermatitis. Later lesions were mostly resolved, but with milder suppurative changes remaining within the cornified layer and periosteal reaction of the dorsal aspect of the distal phalanx. Immunohistochemistry revealed large numbers of treponemal-like organisms particularly within early lesion grades specifically associated with the histopathological changes.

A longitudinal repeated cross-sectional field study of six farms with CODD over 16 months provided information on risk factors for CODD. Footrot was strongly associated with CODD at foot level OR: 7.7 (95%CI: 3.9-15.5), as were various pasture based factors. There was a temporal variation in the prevalence of CODD with increases observed in early Autumn and after housing.

The minimum inhibitory/minimum bactericidal concentrations (MIC/MBC) of twenty CODD associated *Treponema*. spp isolates to ten antimicrobials were determined using a microdilution method, with penicillins and macrolides demonstrating the lowest MIC/MBC values.

A cluster randomised controlled trial of 24 farms with CODD, using whole flock systemic metaphylactic tilmicosin, together with repeated treatment and isolation of clinical cases failed to eliminate CODD and footrot. The high failure rate (7/13 farms) was considered to be as a result of biosecurity breaches and one control farm managed to eliminate CODD without the whole flock approach.

List of abbreviations

Abbreviation	Expanded form
BDD	Bovine digital dermatitis
CODD	Contagious ovine digital dermatitis
DD	Digital dermatitis
FA	Foot abscess
FAWC	Farm Animal Welfare Council
FAWL	Farm Assured Welsh Livestock
FR	Footrot
GR	Granuloma
ID	Interdigital dermatitis
IH	Interdigital hyperplasia
IHC	Immunohistochemistry
IN	Injury
JI	Joint infection
κ	Kappa
κ_W	Weighted Kappa
OG	Overgrown
UK	United Kingdom of Great Britain and Northern Ireland
VS	Veterinary surgeon
WL	White line disease
WS	Warthin Starry

Chapter 1

Introduction and literature review

Lameness in sheep

'Disease is the price animals pay for domestication' (John Cox - Leahurst), and the domestication of sheep is thought to have begun nine-ten thousand years ago in Asia (Ryder, 1984; Henson, 2000). These primitive breeds, in particular the various mouflon species, inhabited an ecological niche within arid and mountainous regions such as Turkey and Iran, an environment markedly different from the temperate climate and comparatively lower altitudes of the UK. Since that time domestication has led to the development of specific breeds, particularly suited to different land types and production systems and there are a great number and variety of breeds established within the UK. As such, the process of domestication and exploitation of certain genetic traits over others may lead to adaptations that are not necessarily suited to the conditions of the UK, and to its semi-intensive lowland agricultural systems. Indeed, the broader subject matter of this thesis – lameness in sheep – could be considered an example of their genetic unsuitability to domestication in the UK.

The term 'lameness' can be defined as 'being incapable of normal locomotion' (Blood and Studdert, 1999). Whilst any adverse event to any of the structures of the limb or close to the limb (e.g. the udder) may cause signs of lameness, in sheep the vast majority of problems are associated with the foot (Winter, 2004a; Egerton, 2007), and it is on these issues that this review will concentrate.

Prevalence

Each year in June, the Department for the Environment Food and Rural Affairs (DEFRA) surveys the numbers of various farmed species in the UK. In 2011 the number of sheep in the UK was 31.6 million (DEFRA, 2011). This figure has remained similar for the past few years following a steady decline in the numbers of sheep over the previous 20 years. In 1994 a survey of 3250 sheep farms across the UK was conducted. Of these farms, 527 returned usable data and of this sample ovine lameness was present on 92% of farms. The average farmer reported prevalence of lameness on these farms was 8.3% (Grogono-Thomas and Johnston, 1997). A further survey of 3000 English sheep farmers was conducted in 2005. Of these 809 provided usable data and the farmer reported prevalence for all lameness on farms from this survey was 10.4% (Kaler and Green, 2008b). A follow-up from this survey on a different sample of farmers recently reported that the prevalence of lameness had now reduced to 4.9% (Winter et al., 2015).

In 2011 the Farm Animal Welfare Council (FAWC) published an opinion on lameness in sheep (FAWC, 2011). This document estimated that based on the reported prevalence of lameness, approximately 3 million sheep are lame in the UK at any one time and that possibly 6 to 9 million sheep become lame in the UK over the course of a year. As such lame sheep are commonplace, and the proportion affected until recently had remained static for some time.

Welfare considerations

Lameness is one of the most significant causes of poor welfare in sheep (Fitzpatrick et al., 2006; Goddard et al., 2006; Winter, 2008). Recently a study examined the opinions of an expert panel consisting of 33 individuals with extensive individual and collective experience in sheep farming in the UK, veterinary service provision and welfare research (Phythian et al., 2011). This study found that lameness was the sole indicator that was consistently identified as an on farm welfare issue for all four of the production stages of sheep - young lambs, growing lambs, ewes and rams.

In 1994, the FAWC produced a short check-list to aid in the assessment of animal welfare (FAWC, 1994). This check-list has become known as 'The Five Freedoms' and is used widely across the industry:

1. Freedom from hunger and thirst - by ready access to fresh water and a diet to maintain full health and vigour.
2. Freedom from discomfort - by providing an appropriate environment including shelter and a comfortable resting area.
3. Freedom from pain, injury or disease - by prevention or rapid diagnosis and treatment.
4. Freedom to express normal behaviour - by providing sufficient space, proper facilities and company of the animal's own kind.
5. Freedom from fear and distress - by ensuring conditions and treatment which avoid mental suffering.

When the Five Freedoms are applied to a lame sheep it does not experience freedom from pain (freedom three). Pain associated with the foot may also reduce the ability of the sheep to feed (freedom one) and this can be seen in the negative effects on bodyweight, lamb growth rates and wool growth (Stewart et al., 1984; Marshall et al., 1991). Pain can also lead to an individual being unable to express normal behaviour (freedom four) because the pain may, for example, limit the ability to move away easily when threatened by another individual. Consequently, much research has been devoted to attempting to provide an evidence base into the causes of lameness, into the welfare compromises and into preventative management and therapeutic strategies to effect long term and widespread improvements in lameness and consequently sheep welfare. In particular extensive work has been completed into the conditions interdigital dermatitis (ID) and footrot, as these are the most prevalent causes of lameness in sheep (Kaler and Green, 2008b).

Economic considerations

In 2005, Nieuwhof and Bishop estimated the cost of footrot in the UK to be £24 million annually. They looked at costs associated with labour, medicines, structures and lost production (Nieuwhof and Bishop, 2005). A similar estimation in New Zealand put the annual cost of treatment and production for the Merino sheep industry at NZ$ 11 million[1] (Hickford et al., 2005). The financial costs associated with other causes of lameness have not been quantified

[1] Approximately £4.75 million, at current (2015) exchange rates.

but would only serve to increase these estimates as to the true cost of lameness as a whole to the sheep industry.

Any disease that affects the ability of sheep to feed reduces its potential to produce. The effects of lameness on bodyweight, lamb growth rates, wool growth and milk production have been demonstrated (Stewart et al., 1984; Marshall et al., 1991; Gelasakis et al., 2010). For example, Marshall et al. (1991) demonstrated over a two year period that the mean body weight of sheep in a group with footrot infection was 7.3kg (11.6%) lower than the mean body weight of sheep in a group without footrot. They also demonstrated that the mean clean fleece weight of sheep from the infected group was 0.4kg (8%) lighter than that from sheep in the uninfected group. Stewart et al. (1984) showed a significant ($P<0.001$) mean loss of body weight for sheep infected with more virulent strains of *Dichelobacter nodosus* (the causative agent of footrot (Beveridge, 1941)) compared to less virulent strains, which was exacerbated by more severe foot lesions in some but not all sheep. Gelasakis et al. (2010) demonstrated that lameness at flock level, in particular that caused by white line foot abscesses and footrot, had a significant impact ($P<0.01$) on milk production, resulting in approximately 47kg less milk per ewe per lactation after adjusting for age, number of lactations and duration of lactation.

Locomotion scoring systems

In order to study lameness as an outcome, to measure the effect of an intervention and to use this behavioral expression of pain to investigate the effect of a lesion on a sheep it is necessary to have a reliable tool to measure it.

Locomotion scoring systems provide a way by which observations can be characterised, that is to say 'how lame' a sheep is. The standardisation and validation of any technique or tool then allows more than one observer to reliably make the same judgment enabling clearer decision making (Kraemer, 1992).

The clinical signs of a lame sheep vary from a mild nodding of the head, to the distinct favouring of a limb, through to an extreme case whereby the animal favours a recumbent position and can walk only with difficulty carrying the limb (Egerton, 2007; Kaler et al., 2009) (Figure 1). Currently there is no universally accepted 'gold standard' technique or method for determining how lame an individual sheep is or how lame a flock is.

Figure1: A severely lame sheep carrying the left hind leg.

Study	Locomotion scoring method	Number & type of observers	Reliability/repeatability data
Ley et al., (1989)	0-4 numerical rating scale	1 'independent observer'	No data provided
Welsh et al., (1993)	0-4 numerical rating scale and visual analogue scale	2 veterinarians experienced in assessing and teaching sheep lameness assessment	*Intra-observer agreement*: no significant difference between observation attempts *Inter-observer agreement*: no significant difference between observers
Kaler et al., (2009)	0-6 numerical rating scale	3 experienced researchers familiar with observing locomotion in sheep	*Inter-observer agreement*: Overall exact agreement between observers = 68% ICC 0.93 κ_w 0.93 *Intra-observer agreement*: Overall exact agreement within observers = 76% ICC 0.90 κ_w 0.91
Phythian et al., (2012)	Group observation using binary lame/not lame system	4 experienced (3 trained 1 untrained) 3 inexperienced (2 trained 1 untrained)	*Overall intra-observer agreement*: Cronbach's α = 0.99 *Overall inter-observer agreement*: computed mean of all combinations of Cronbach's α = 0.92

Table 1: Comparison of studies that detail sheep locomotion scoring systems.

Various attempts have been made to grade the severity of lameness in sheep (Table 1). Comparisons are difficult to make between these scoring systems due to the differing methods of analysis of reliability as well as the differences in quality of reporting, particularly of the practical and statistical methodologies used. Some of the studies make use of visual analogue methodology e.g. Welsh et al. (1993), and others numerical rating systems (Ley et al., 1989; Welsh et al., 1993; Kaler et al., 2009). Phythian et al. (2012) describes a binary lame/not lame system for observation of groups of sheep. This group method can be useful for determining the prevalence of lameness within a flock, and offers advantages in that sheep do not need to be stressed by gathering, and therefore it can be useful for assessing the overall impact of lameness and the implied welfare considerations in a flock. However, there are disadvantages in welfare assessment using this method in that it does not take into account the severity of lameness.

One method used by scientific researchers and cited in several studies (e.g. Wassink et al. (2010b)) in assessing the locomotion of individual sheep, is the seven-point scale described by Kaler et al. (2009). This method breaks down in detail the different effects on the body that pain in the foot may elicit. However, a seven-point scale may be less easily used by sheep farmers and general practice veterinary surgeons (VS) who may require a simpler more 'user-friendly' system. Anecdotally in my experience, many farmers and VS simply use untested and unvalidated arbitrary sound-mild-moderate-severe grading systems determined personally by each individual.

Any tool developed for use must be reliable. Reliability is the degree to which the results obtained by a measurement procedure can be replicated (Porta et al., 2008). Due to the absence of a gold standard, reliability data is important in ensuring observations are repeatable by the same and different observers (Kraemer, 1992). If a tool is to be used for repeated measurements over time, for example to determine the response to treatment then it must have a high degree of repeatability. Similarly, to be used by different observers (for example in welfare monitoring inspections) this will ensure that their observations are comparable. Three of the four studies demonstrate excellent repeatability, however, all the systems use small numbers of observers when assessing this (Table 1). They also all tend to use experienced research workers (except in the case of Phythian et al. (2012) where a variety of observers were used with a range of experience and training). Both these factors could reduce generalisability when considering use of the tool by less specialist individuals.

In summary, the lameness scoring systems previously described have limitations either in terms of their ease of use in the field, number of scoring categories or type of observers used. Consequently, there is no universally accepted technique or method for determining lameness in sheep at an individual or flock level.

Diseases of the ovine foot

There are numerous non-contagious causes of poor mobility in sheep e.g. soil balling (the accumulation of soil into hard impactions between the digits), interdigital hyperplasia, foot abscesses (usually secondary to white line lesions which generally do not cause lameness), pedal joint sepsis, granulomas, horn overgrowth, laminitis and post dipping lameness (caused by *Erysipelothrix rhusiopathiae*) (Winter, 2004a). Due to the fact that contagious agents cause the majority of lameness problems in sheep (Egerton, 2007), this review is going to focus specifically on contagious foot lesions that cause lameness, but in particular contagious ovine digital dermatitis (CODD). Footrot will also be considered in detail due to its close relationship with CODD.

Contagious ovine digital dermatitis

History and aetiology

Various historic descriptions of footrot have distinct suggestions of CODD e.g. that in Day et al. (1916) where the description reads '[contagious foot-rot] commences at the top of the foot, working downwards, and is most prevalent during the Summer and Autumn'. However, it was first officially reported in sheep in the UK in 1997 (Harwood et al., 1997). In this case the condition was believed to be a severe and virulent form of footrot, but rapidly researchers observed large numbers of viable spirochaete bacteria in many of the cases, and unusually failed to identify *D. nodosus* in many of the samples (Naylor et al., 1998). Following further investigation of similar cases, the different clinical appearance of these lesions compared with conventional cases with footrot, and the recurrent inability to isolate *D. nodosus* from the majority of cases the term contagious ovine digital dermatitis was coined (Davies et al., 1999; Lewis et al., 2001).

Using 16S rRNA gene sequencing and subsequent phylogenetic analysis, the isolated spirochaete from Naylor et al. (1998) was identified as closely related to *Treponema vincentii,* a spirochaete that has been implicated in human periodontitis and bovine digital dermatitis (BDD) (Collighan et al., 2000). Subsequently, a second spirochaete was isolated from a CODD lesion and identified as genetically different to that identified by Collighan et al. (2000), sharing more similarity to the human periodontal pathogen *Treponema denticola* and also being 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 ($P<0.001$) high anti-*Treponema* spp. antibody reactions compared to controls. Subsequently, Sayers et al. (2009) cultured treponemes from 7 out of 10 cases of CODD, with two single isolates and five cultures containing various combinations of *Treponema medium/T. vincentii*-like, *Treponema phagedenis*-like and *T. denticola/Treponema putidum*-like spirochaetes with each of these three phylogroups previously having been isolated from and identified as having a high association with BDD lesions (Evans et al., 2008; Evans et al., 2009c). Recently, the *T. denticola/T. putidium*-like BDD spirochete has been renamed *T. pedis* (Evans et al., 2009b).

Doubt as to treponemes being the sole agent of disease was introduced by Wassink et al. (2003b) and Moore et al. (2005b) as *D. nodosus* was found to be present in some CODD lesions. Moore et al. (2005b) demonstrated by 16s rRNA PCR the presence of *D. nodosus* in 37 (74%) CODD lesions, but only found it in 5 (38%) apparently healthy feet. Duncan et al. (2012) then found that sheep with footrot were subsequently more likely to develop CODD later on (OR: 3.83, 95%CI: 2.61-5.62, P<0.001). They also demonstrated that vaccination using a multivalent *D. nodosus* vaccine reduced the new infection rate for CODD from 26.4% to 18.2% (P=0.011) and an overall vaccine efficacy against CODD of 32%.

However, more recently Sullivan et al. (2015) demonstrated by PCR one or more of the BDD associated *Treponema* phylogroups in 100% of 58 CODD lesions investigated, and failed to amplify any *Treponema* spp. DNA from 56 healthy feet. They also demonstrated *D. nodosus* and *F. necrophorum* in 59% and 71% of CODD lesions and 39% and 9% of healthy foot tissues respectively.

As such, on balance BDD associated treponemes appear to be strongly associated with disease, however whether they are primary or secondary agents, or co-invaders is not clear.

Epidemiology

Estimates of the prevalence of CODD across the UK and on individual farms are scarce. A survey of 209 farmers in 2003 found that 5% (95%CI: 3-9%) of respondents had seen CODD in their flock and the earliest they had noticed CODD was 1996 (Wassink et al., 2003b). The median farmer reported flock prevalence in this survey was 25.6% (range 0.5-100%). A further recent postal survey found that 53% of flocks in England were affected by CODD (Kaler and Green, 2008b) and in this survey the on farm prevalence was 2.4%. In a more recent follow-up survey of 1260 farmers in England, 48.7% of respondents reported CODD on their farms with a mean on farm prevalence of 2.3% (Winter et al., 2015).

Contagious ovine digital dermatitis typically affects just one foot at any one time in affected animals, however it has been reported in some situations affecting up to all four feet at once (Duncan et al., 2011). It has also been shown to be significantly more prevalent in hind feet compared to front feet ($P<0.001$) (Duncan et al., 2011).

Due to the interest over the years in *Treponema* spp. associated with BDD, some authors have reported isolated anecdotal epidemiological links with cattle. For example Moore et al. (2005b) reported that one of the farms they studied had sent sheep on tack to a dairy farm over the winter months and the farmer then considered that CODD had been observed in his sheep from this point. Moore et al. (2005b) also reported that all 6 farms studied reported seeing most cases in the late summer months.

Clinical signs[2]

Several authors have broadly described the clinical features of CODD. Common themes include those features which seem pertinent in distinguishing it clinically from footrot, namely that lesions tend to commence at the coronary band (compared to the interdigital space) and then underrun the hoof horn capsule abaxially. In severe cases the whole horn may be avulsed leaving the sensitive lamellae exposed (Harwood et al., 1997; Naylor et al., 1998; Wassink et al., 2003b; Winter, 2008). Sheep tend to be extremely lame and this has reportedly led to various consequences such as poor body condition and increased recumbency (Harwood et al., 1997; Winter, 2008; Duncan et al., 2012).

Welfare

With reference to The Five Freedoms (see above), Duncan and Williams (2010) argue that sheep with CODD are not free from pain or disease - freedom 3 is compromised. The extent of the degree of lameness for CODD has not yet been formerly characterised, however, given the severe pathology seen in affected sheep it may be expected that sheep with CODD will be severely lame. This corroborates well with anecdotes from veterinarians and farmers experienced with dealing with this disease (Lewis, 1999). A lame sheep may have a reduced ability to compete for food or to be able to spend time grazing, thus compromising freedom 2 and being lame may also reduce the ability of an individual to express normal behaviour and consequently freedom 4 is compromised (Duncan and Williams, 2010).

[2] Detailed photographs of CODD lesions are supplied in Chapter 4.

A recent survey has suggested that in part due to a reported declining prevalence of footrot, the proportional impact of CODD has risen, with a greater proportion of lameness now being caused by this disease, although the proportion of farms affected appears to be relatively unchanged (Winter et al., 2015).

Economics

Given the prevalence of CODD and the severe compromise on animal welfare expected to be caused by this disease, it is possible that the economic costs associated with poor production, increased labour, the use of medicines and veterinary expertise may be similar to that of footrot. However, currently no formal estimates are published in the literature.

Treatment

Wassink et al. (2003b) reported that the diagnosis of CODD has been based on the failure to control lameness in sheep using the control methods recommended for footrot, indeed this was the reason for the initial investigation by Harwood et al. (1997). There are only a handful of studies examining therapeutic interventions in order to control CODD. Two studies use amoxicillin, on the basis of *in vitro* studies by Evans et al. (2009a). Duncan et al. (2011) used a randomised split flock intervention study in a group of 320 lambs, to compare foot bathing in a solution of 1% chlortetracycline alone (aureomycin soluble powder; Fort Dodge Animal Health), or together with a single injection of long-acting amoxicillin (Bimoxyl LA; Bimeda). The initial prevalence of disease on this farm was 22%. Amoxicillin was shown to increase the odds of recovery OR: 3.84

(95%CI: 1.05-14.0, P=0.008) and was also shown to almost halve the odds of a foot acquiring a new CODD lesion OR: 0.42 (95%CI: 0.15-1.14, P = 0.09).

Duncan et al. (2012) then repeated the randomised split flock intervention model in a group of 748 lambs, this time using targeted long acting amoxicillin injection alone, compared to targeted amoxicillin injection together with two doses of a multivalent footrot vaccine (Footvax, MSD). This study demonstrated a significant reduction in CODD in the vaccinated group compared with a significant increase in CODD in the unvaccinated group. Furthermore, new infection rates were significantly lower in the vaccinated group compared with the unvaccinated group OR: 0.68 (95%CI: 0.46-0.99, P<0.001). This study also showed cure rates for amoxicillin alone to be 71%.

In 2010 Judson (2010) demonstrated a significant improvement (P<0.01) in the prevention and treatment of sheep with CODD, footrot and ID. In this study lambs were treated using topical tylosin (Tylan powder; Elanco Animal Health, dissolved 1g/L water as a footbath) and a long acting oxytetracycline injection (Alamycin LA; Norbrook Ltd.). The unit of comparison was the pen lambs were housed in and none of the treated pens showed any disease, although there were significant numbers of cases of CODD, footrot and ID in the untreated pens.

Other isolated reports of successful treatments are anecdotal, (Moore et al., 2005b) describes one farmer who found tilmicosin injection (Micotil; Elanco Animal Health) to be effective, and similarly a case report by Watson (1999) indicated that a single injection of tilmicosin, together with topical oxytetracycline led to the clinical recovery of a severe ovine foot disease (clinically resembling CODD) in a group of approximately 100 lambs. In addition, very early on Davies et al. (1999) anecdotally reported excellent results to topically applied solutions of lincomycin and spectinomycin (Linco-Spectin; Zoetis). Given that both *D. nodosus* and treponemes are gram negative and macrolide antibiotics are known to be variably effective against such bacteria, these anecdotes should be investigated.

Footrot and interdigital dermatitis

History and aetiology

Footrot was well established in England during the 18th Century (Youatt, 1837; Mohler and Washburn, 1904) and it was noted to have been documented in continental Europe, Australia and the USA during the early 19th Century (Mohler and Washburn, 1904; Beveridge, 1941). From the earliest descriptions footrot was noted to be contagious and to be associated with damp and lush pasture (Youatt, 1837; Stewart, 1989).

Since very early in the investigations into the aetiopathogenesis of footrot a number of bacteria have been implicated, including *D. nodosus*, *F. necrophorum* and the spirochaete *Spirochaeta penortha*/*Treponema podovis* (Mohler and Washburn, 1904; Beveridge, 1941; Egerton et al., 1969; Bennett et al., 2009). Whilst there is still some debate, (e.g. Egerton (2014); Witcomb (2014)), extensive work has led to a general swathe of opinion and body of evidence that footrot is caused by the Gram-negative obligate, anaerobic bacterium *D. nodosus*

(Beveridge, 1941; Stewart, 1989; Grogono-Thomas and Johnston, 1997; Green and George, 2008; Bennett and Hickford, 2011). *Fusobacterium necrophorum* may damage the interdigital skin sufficiently to allow secondary colonization by *D. nodosus*, however it is not essential to disease and footrot may be observed in the absence of *F. necrophorum*. Interestingly various early workers on footrot noted spirochaetes in many cases, with some experimental work suggesting that the presence of *Spirochaeta penortha*/*Treponema pedovis* led to more severe footrot lesions than when absent (Beveridge, 1941; Egerton et al., 1969). Unfortunately this work has not been extended[3], but it is tantalizing that as descriptions by Day et al. (1916) imply it is possible that footrot and CODD have been confused for far longer than previously thought.

Currently there are identified 19 serotypes of *D. nodosus* which are classified into ten fimbrial serogroups, namely A-I and M, although only serogroups A-I have been identified in the UK (Ghimire et al., 1998; Moore et al., 2005a), and in a recent survey of 35 farms in the UK 60% of the flocks sampled had more than one serogroup identified, with serogroup H being most prevalent (Moore et al., 2005a). In this same survey, the virulence of the 706 isolated *D. nodosus* organisms was examined, and with the exception of five isolates all the rest were virulent using a gelatinase gel protease thermostability assay. There is some debate as to the ability of this assay to detect intermediate virulence strains and thus Moore et al. (2005a) suggested that the elastase test may have allowed

[3] A search for the 16S rRNA genetic record of these spirochaetes revealed zero results, implying that in all likelihood a genetic analysis was not carried out at the time and it is highly likely that these samples are no longer available rendering further retrospective investigation impossible.

greater interpretation in terms of determining intermediate virulence (Links and Morris, 1996).

Clinically, footrot is preceded by clinical signs of ID, and in general in the UK, the two diseases may be considered the same but at different stages of advancement (Wassink et al., 2010b; Witcomb et al., 2014). There are some cases of ID that are caused by *F. necrophorum*, however these typically do not develop into footrot unless *D. nodosus* invades concurrently and thus they tend to rapidly self-resolve (Beveridge, 1941; Egerton et al., 1969; Winter, 2008).

Footrot was and still remains one of the most economically significant diseases of sheep in the UK and in other parts of the world including Australia and New Zealand (Schwartzkoff et al., 1993; Nieuwhof and Bishop, 2005), and it is currently by far the most prevalent cause of lameness accounting for approximately 80-90% of all cases of lame sheep (Grogono-Thomas and Johnston, 1997; Kaler and Green, 2008b).

Epidemiology

Footrot is found in many countries worldwide (for example Nepal (Ghimire et al., 1999), Greece (Gelasakis et al., 2010) and India (Hussain et al., 2009)) but is of particular importance in countries with large sheep based economies such as New Zealand and Australia (Stewart, 1989; Kennan et al., 2011) as well as the UK (Green and George, 2008).

Current UK farmer reported prevalence estimates are reported to have decreased from around 8-10% of sheep (Grogono-Thomas and Johnston, 1997; Wassink et al., 2003b) to 3.1% (Winter et al., 2015) over the last decade, although there is significant variation between different farms.

With any infectious disease, there are some individuals that become infected and develop clinical signs, and some that don't. The pathogen *D. nodosus* requires a susceptible host and favorable environmental conditions in order to cause disease. In his benchmark publication Beveridge (1941) described the necessity for the interdigital skin to be damaged or scarified prior to inoculation with *D. nodosus* bacteria in order for clinical disease to be expressed. Subsequently, various risk factors have been identified as being associated with footrot:

1. *Environmental factors*

Studies in Australia find a greater association with *F. necrophorum* than studies in the UK (e.g. Egerton et al. (1969)), but this may be due to the differing climates of the two countries. Stewart (1989) reports that persistent rainfall (a common phenomenon in the UK) together with lush pasture and susceptible hosts are required for a footrot outbreak. Moisture may cause physical changes in the foot (e.g. tissue maceration) or may change the biology of the pathogens that cause footrot (Egerton et al., 1969; Bennett and Hickford, 2011). Lush pasture may lead to an increase in stocking densities and therefore an increased risk of transmission (Stewart, 1989). Temperatures above 10° Celsius are also required for effective transmission, and these two environmental factors aid predictions of footrot outbreaks (Stewart, 1989). There are very few longitudinal epidemiological studies on UK farms detailing the dynamic changes of footrot in association with climatic changes, however one recent study on three farms in the UK, suggested that footrot could be transmitted throughout a 12-month period. Footrot transmission was particularly influenced by wet and muddy conditions, but not influenced by housing or temperature (Ridler et al., 2009) [4]. Moisture and interdigital abrasions caused by coarse plant species on grazed pasture were also considered to increase the susceptibility of sheep to *D. nodosus* in Australia (Whittington, 1995). As such the damp and unpredictable climate of the UK is considered to be the main reason for difficulties experienced in eradication/elimination programmes in the UK (Abbott and Lewis, 2005; Green and George, 2008; Winter, 2009).

Fusobacterium necrophorum may be present in the environment, being shed in the faeces of sheep. However, *D. nodosus* may only thrive in the epidermal tissues of the hooves of ruminants infected with footrot, only surviving on pasture for approximately 1 week (Egerton, 2007). Transmission occurs when infective material is transferred from exposed lesions onto soil enabling another sheep to become contaminated. Tracks and feeding areas thus provide ideal areas for transmission (Stewart, 1989) (Figures 2 and 3), but transmission has also been observed in footrot free sheep that were yarded for 1 hour in a yard

[4] Unfortunately this study has only been published as an abstract in the conference proceedings of the 7th International Sheep Veterinary Congress and thus is difficult to interrogate in more detail.

that had the same day previously contained another group with sheep with <1% prevalence of footrot lesions (Whittington, 1995).

Benign strains of *D. nodosus* can also be carried by cattle and strains of *D. nodosus* from footrot may be cultured from goats, and thus these species can also form reservoirs of infection on farms that have them (Stewart, 1989; Ghimire et al., 1999).

Figure 2: A mineral lick placed in a field to provide mineral supplementation to sheep at pasture. The poaching around it illustrates the concentration of animals at such sites in fields and highlights the risk for transmission at these areas.

Figure 3: A gateway demonstrating poaching and highlighting the risks for transmission at areas of high traffic.

2. Host effects

All infectious agents need a susceptible host to parasitise, and in terms of the susceptibility of sheep to footrot there is variation in the susceptibility of some breeds to others. For example in Australia, Romney Marsh sheep were shown to demonstrate greater resistance to Merinos despite similar immunological responses (Emery et al., 1984). This difference may be due to genetic differences possibly associated with changes in phenotype conferring resistance e.g. small hard hooves (Green and George, 2008). The heritability of resistance to footrot has been poorly studied, but estimates for eight clinical indicators of resistance to footrot were examined in 1562 Merino sheep by Raadsma et al. (1994). In that study heritability estimates of liability to footrot ranged between 0.09 and 0.41 with variation occurring due to differences in the time between the challenge with *D. nodosus* and the diagnostic inspection.

When considering the foot *per se*, Kaler et al. (2010a) demonstrated that ewes with poor foot conformation were more likely to become lame OR: 1.83 (95%CI: 1.24-2.67) and to be older than 4 years OR 1.50 (95%CI: 1.09-0.75). They also demonstrated that the probability of a ewe becoming lame increased when at least one of her offspring was lame OR: 2.03 (95%CI: 1.42-2.92). Furthermore, they showed that lambs were more likely to become lame if they were male OR: 1.42 (95%CI: 1.01-2.01), a single (rather than a twin/triplet) OR: 1.86 (95%CI: 1.34-2.59) or had a lame dam or sibling OR: 3.10 (95%CI: 1.81-5.32).

One of the biggest causes of the introduction of footrot, or new strains of *D. nodosus* into flocks is the introduction of sheep from affected flocks (Stewart, 1989; Egerton, 2007), this is corroborated by Wassink et al. (2003b) who reported that the isolation of bought in sheep was associated with a lower prevalence of footrot. *Dichelobacter nodosus* may also be transmitted via soil contact (Beveridge, 1941; Whittington, 1995) and this has been used to suggest that higher stocking rates may lead to increased transmission rates, however this was not borne out by Wassink et al. (2003b).

Clinical signs

Interdigital dermatitis presents as inflammation of the interdigital skin (Figure 4), and as such the appearance is a blanching or reddening of the skin and often a yellow/white, grey exudate on the surface of the lesion (Winter, 2004a; Green and George, 2008). In simple cases of ID there is no separation of the horn and in many cases it causes surprisingly severe lameness (Winter, 2004a).

Figure 4: An interdigital dermatitis lesion with inflammation of the interdigital skin.

Footrot (Figure 5) begins clinically as ID, and in early stages the clinical presentation of ID caused by *F. necrophorum* and that caused by *D. nodosus* are indistinguishable (Winter, 2004a). If there is colonisation with *D. nodosus* then under-running of the hoof horn, usually starting adjacent to the lesion towards the heel will occur. Depending then upon the virulence of the strain of *D. nodosus* present, the under-running of the hoof horn may produce a spectrum of clinical signs, from slight under-running, damage to the sole through to total evulsion of the hoof capsule (Stewart, 1989; Winter, 2004a, 2008). Together with these clinical signs there is usually a distinctive pungent smell, a grey discharge and variable degrees of lameness (Egerton, 2007; Green and George, 2008; Winter, 2008). Sheep with severe footrot may graze on their knees and may be recumbent for extended periods (Stewart, 1989; Egerton, 2007).

Figure 5: A footrot lesion with typical damage to the interdigital space and the majority of the sole.

Welfare

'The prevalence of many endemic diseases in farm animals is too high' (FAWC, 2012). This recent report also highlights specifically that the prevalence of lameness in sheep has not reduced for a number of years. The clinical signs associated with footrot are associated with varying degrees of adverse welfare in sheep, and obviously the more severe cases of footrot experience more indicators of poor welfare, such as restricted feeding, reduction in bodyweight and poor wool quality (Stewart et al., 1984; Marshall et al., 1991; Egerton, 2007). In terms of The Five Freedoms (see above) welfare may be compromised in much the same way as for CODD.

Treatment and control

Despite extensive research over 200 years and comprehensive knowledge of the disease, footrot remains a problem worldwide (Bennett and Hickford, 2011). Control has remained difficult on farms in the UK although a recent report suggests that the on farm prevalence of footrot in the UK has reduced significantly over the last few years (Winter et al., 2015). Strategies for control have included routine preventative and therapeutic foot trimming together with foot bathing in various chemicals, vaccination and the use of systemic and topical antibiotics (Abbott and Lewis, 2005; Winter, 2009).

In some countries, in particular some areas of Australia and Nepal, the eradication/elimination of footrot from farms has been possible (Egerton et al., 2002; Dhungyel et al., 2008). Success has in part been due to the use of specific monovalent/bivalent vaccines prepared from isolates cultured from cases on affected farms.

Recently in the UK, the industry has put great efforts into trying to educate farmers as to appropriate foot care and a ‘Five Point Plan’ has been advocated (Figure 6) (Balsom, 2012; Clements and Stoye, 2014). This has been developed by the Food Animal Initiative (FAI Farms Ltd.) and has been reported to be effective on a number of farms to reduce the incidence of footrot to minimal levels if used rigorously (FAI-Farms, 2011), although no formal validation has been carried out for the plan as a whole. It is deemed that in the UK the weather (together with a lack of coordinated effort by the industry) plays a significant part in the failure to achieve eradication due to the lack of predictably dry periods when transmission might be deemed to be low risk. The five points of the FAI-Farms’ plan are:

1. Quarantine incoming animals
2. Culling persistently affected animals
3. Treat clinical cases early
4. Avoid spread at handling and in fields
5. Vaccination

Despite a lack of validation or testing across a broad range of enterprises it serves as a practical approach in controlling ID and footrot. The only study using it to show a positive effect is that on FAI Farms’ own farm where a sustained reduction in lameness prevalence and associated number of ewe treatments was demonstrated (Figure7).

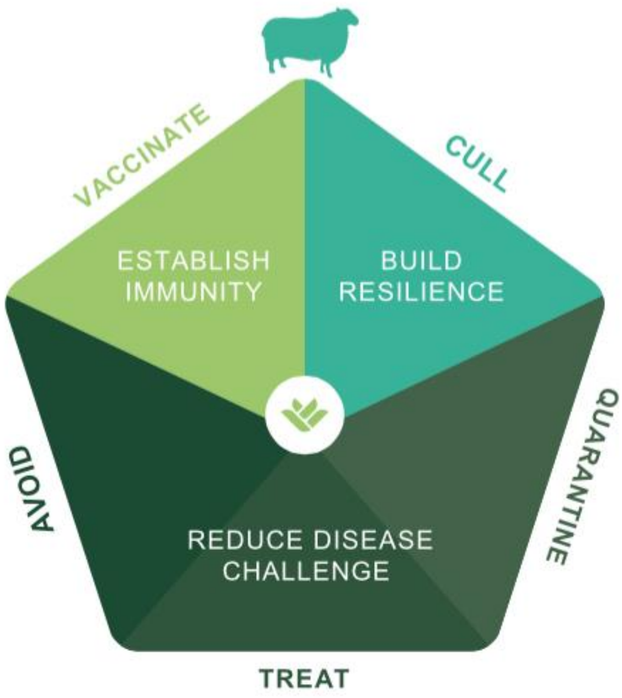

Figure 6: The Five Point Plan for Lameness in Sheep; reproduced with kind permission from Ruth Clements, FAI Farms Ltd, Wytham, Oxfordshire, UK.

A brief review of the published evidence for each part of the Five Point Plan was considered in order to understand the underlying rationale:

Quarantining incoming animals

For any contagious disease the quarantine or isolation of new individuals before introducing them into a group is a logical step to reduce the risk of bringing in any new pathogens. It also allows appropriate biosecurity measures such as vaccination or inspection of animals to be carried out prior to mixing new entrants with the rest of the flock (Winter, 2004b). Unfortunately there are no studies specifically assessing this intervention in a controlled way for sheep in general or for footrot in particular, although the quarantining of sheep has been used as a component factor in footrot eradication programmes in Australia and these programmes have been successful (Mills et al., 2012). Quarantining has also been associated with a reduced prevalence of footrot in two surveys in the UK (Wassink et al., 2003b; Winter et al., 2015).

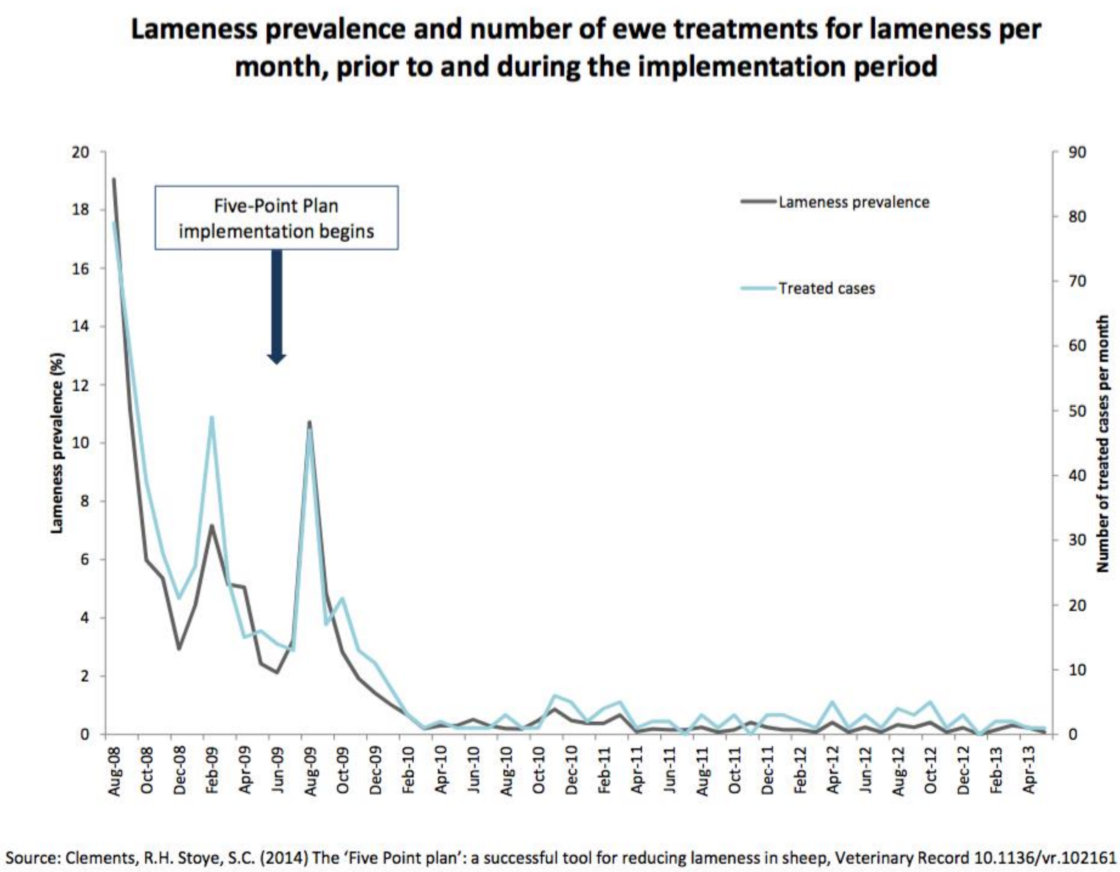

Figure 7: Lameness prevalence and number of ewe treatments for lameness per month, prior to and during the implementation period of the FAI Farms' Five Point Plan; reproduced with kind permission from Ruth Clements of FAI Farms Ltd. Wytham, Oxfordshire, UK.

Voluntary biosecurity principles are understood and implemented much better in the pig and poultry industries; a literature search will identify numerous articles assessing many aspects of this subject and personal experience of visiting such farms serves only to confirm this. In a recent survey of cattle farms in the North-West of England biosecurity policies were extremely varied and disorganised and none of the 56 cattle farms surveyed had rigorous and consistent policies or practices despite biosecurity being a major part of all farm assurance scheme requirements (Brennan and Christley, 2012).

Culling persistently affected animals

Culling persistently affected animals has been advocated as part of footrot control strategies in the UK (Abbott and Lewis, 2005; Winter, 2009) and destocking and culling form a major part of successful footrot eradication programmes in Australia (Mills et al., 2012). This recent study has demonstrated these two methods to be effective and to shorten the time to eradication over and above the use of antibiotics and foot bathing (Mills et al., 2012). However, conversely a recent survey of sheep farmers in England found no association with culling and a reduced prevalence of lameness, after controlling for confounders, although this may be reflective of these farmers adopting other control strategies and thus not needing to cull (Winter et al., 2015).

Treat clinical cases early

Dichelobacter nodosus has been shown in numerous *in vitro* and *in vivo* studies to be effectively killed by numerous antibiotics, in particular oxytetracycline (Grogono-Thomas et al., 1994). In considering treatment further, the prompt treatment of clinical cases has been investigated through a number of studies, and two recent randomized controlled trials have demonstrated convincingly the benefits of prompt individual treatment with parenteral and topical antimicrobials over and above delayed treatment without systemic antimicrobials (Kaler et al., 2010a; Wassink et al., 2010b). Both these studies have also reported the negative effects of foot trimming as a therapeutic measure, demonstrating delayed healing of lesions and a return to soundness. These effects are also supported by observational data by Winter et al. (2015).

The data collected in the study by Wassink et al. (2010b) was also analysed to investigate the effects of treatment on foot conformation, and these analyses demonstrated that prompt treatment with parenteral and topical antimicrobials, without foot trimming, also led to the prevention and/or recovery of poor foot conformation (Kaler et al., 2010b). This resolution and/or maintenance of good foot conformation also led to a reduced risk of developing a new case of footrot in the future.

Foot bathing was once a commonly advocated therapeutic and preventative measure, but recently has been reported to only be effective where the facilities are of an excellent standard (Wassink et al., 2003b). Furthermore, it has recently been suggested that foot bathing may be associated with farmer behaviors that lead to the delayed treatment of lame sheep and as such a reliance on this as a control method may encourage the development of more chronic lesions and increased horizontal spread within a flock (Winter et al., 2015).

Avoid spread at handling and in fields

Avoiding spread at handling and in fields again has not been investigated robustly from an intervention perspective, however it would appear to be based on standard contagious disease principles. For example Wassink et al. (2003a) reported that those farmers who 'always' separated diseased sheep from the main flock reported significantly lower levels of footrot than those where infected sheep were 'sometimes' or 'never' separated (3.3 v 8.6 and 10.9 cases per 100 ewes per day respectively). Practically it has been suggested to use hydrated lime as a disinfectant around high traffic areas such as gateways and tracks and zinc sulphate impregnated mats in front of feeding areas in housed animals, as well as adopting cleaning and disinfection practices in handling pens (Ruth Clements; personal communication; (Winter, 2004b)).

Vaccination

Vaccination against *D. nodosus* has been investigated in a number of studies, and vaccination *per se* is a logical step when considering how to prevent any contagious disease in individuals and flocks. Recent evidence suggests that most farms may have a combination of serogroups present at one time and these are likely to vary from farm to farm, and indeed sheep to sheep (Moore et al., 2005a). Currently in the UK there is licensed for use only one multivalent vaccine consisting of formalin-killed cells of all nine serogroups suspended in an oily adjuvant (Footvax; MSD). A recent study using this vaccine suggested that in an outbreak situation the protection afforded was only 62% (Duncan et al., 2012), which compares to 55% and 73% in two previous studies in Australia (Kennedy et al., 1985; Liardet et al., 1989). Part of this lack of efficacy might be due to antigenic competition for major histocompatibility complex binding sites, made worse through the use of whole cells rather than immunogenic specific pilli antigens by the presentation of unnecessary extraneous antigens (Schwartzkoff et al., 1993). Monovalent or bivalent vaccines, ideally using pilli antigens as opposed to whole cells are likely to afford higher levels of protection (>90%) but are not commercially available in the UK (Schwartzkoff et al., 1993; Egerton et al., 2002; Dhungyel et al., 2008). As such, vaccination on its own is unlikely to deliver the protection necessary to be used as the sole management control method for footrot in the UK, however as part of a combined approach it may be useful on some farms (Winter et al., 2015).

The Five Point Plan is just one combination strategy available to farmers and their advisors in the UK, and whilst based partly on sound, specific experimental evidence it is also partly based just on logical extrapolation of basic contagious disease principles. As such, control *may* be achieved through the use of this approach, but may also be achieved through other approaches or indeed through employing just one or two elements of the plan. As to which of the five elements are critical to control is not clear, nor if the benefits are only achieved through the accumulation of gains made in each area. As such, although the UK sheep industry has responded largely in support of this approach, a more in-depth understanding of the experimental evidence (or lack of it) may ensure a more intelligent and tailored delivery.

Conclusions

There is an extensive and deep knowledge base for footrot, which has led to the development of several effective strategies for control and treatment. It appears that whilst it is improbable to expect farmers to be able to eradicate footrot in the UK, there is little reason not to be able to manage disease to a consistently low level.

For CODD however, much less is known. Indeed the aetiology has yet to be established conclusively, although there is a body of evidence to suggest that the BDD associated *Treponema* spp. are probably involved. The disease has yet to be formerly described in detail in terms of its clinical appearance, gross and histopathology and its impact on welfare. Prevalence estimates are scarce, as are risk factors for sheep and farms, and there are very few robust studies investigating the treatment of clinical cases and control/eradication/elimination of disease from affected farms.

Objectives

Towards a greater understanding of contagious ovine digital dermatitis (CODD) - an epidemiological approach

Through considering the current knowledge base for CODD, a number of pertinent questions become apparent, which require answers for appropriate action to be taken on affected farms, and for appropriate advice to be given. These include:

- What is CODD?
- What causes CODD?
- What is the extent of the problem?
- What can be done to make it less likely for sheep/flocks to get CODD?
- Is it possible to effectively treat clinical cases?
- Is it possible to control CODD on farms to a very low level?
- Is it possible to eradicate CODD from farms?

In this thesis I will attempt to address these questions in part, and begin to provide some evidence upon which to base the answers. The answers are likely to be incomplete and are likely to require changes as new research is done. The following objectives were formulated in order to provide a framework around which to design studies in an attempt to provide some answers to the questions of interest:

Objective 1:

To determine the current between and within farm prevalence of CODD and to determine reported epidemiological factors associated with disease.

Objective 2:

To develop a practical tool for use by general practitioners and farmers in the assessment of individual sheep lameness.

Objective 3:

To describe in detail the clinical, gross and histopathological, and radiographical features of CODD.

Objective 4:

To determine key sheep and farm level epidemiological risk factors for CODD that could be used to help inform treatment programmes and manage disease on affected farms.

Objective 4:

To determine the *in vitro* sensitivity of cultured treponemes associated with CODD to pharmacological agents.

Objective 5:

To investigate a therapeutic strategy for eliminating CODD from affected farms.

These objectives are addressed in the remainder of this thesis.

Chapter 2

Farmer reported prevalence and factors associated with Contagious Ovine Digital Dermatitis in Wales: a questionnaire of 511 sheep farmers

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Abstract

In 2012, 2000 questionnaires were sent to a random sample of Welsh sheep farmers. The questionnaire investigated farmers' knowledge and views on Contagious Ovine Digital Dermatitis (CODD) - an emerging disease of sheep responsible for causing severe lameness, welfare and production problems. The overall response rate was 28.3% (95%CI: 26.3-30.3%) with a usable response rate of 25.6% (95%CI: 23.7-27.5%). The between farm prevalence of CODD was 35.0% (95%CI: 31.0-39.3%) and the median farmer estimated prevalence of CODD was 2.0% (IQR 1.0-5.0% [range 0.0-50.0]). The disease now appears endemic and widespread in Wales. Furthermore, there has been a rapid increase in reports of CODD arriving on farms since the year 2000. Risk factors for CODD identified in this study include the presence of Bovine Digital Dermatitis (BDD) in cattle on the farm and larger flocks. Farmers also consider concurrent footrot/interdigital dermatitis, buying in sheep, adult sheep, time of year and housing to be associated with CODD. Further experimental research is necessary to establish whether these observations are true associations.

Introduction

Lameness is one of the most significant causes of poor welfare in sheep (Fitzpatrick et al., 2006; Goddard et al., 2006; Winter, 2008). According to a recent sheep welfare expert panel, lameness was the only indicator that was consistently identified as an on farm welfare issue for all four of the production stages of sheep - young lambs, growing lambs, ewes and rams (Phythian et al., 2011). A recent Farm Animal Welfare Council (FAWC) report (FAWC, 2011) estimated that at least three million sheep are lame in the UK at any one time and that six to nine million sheep become lame in the UK over the course of a year.

In the majority of cases, lameness in sheep is caused by contagious agents and there are three main expressions of disease. Footrot and Interdigital Dermatitis (ID) cause over 90% of lameness in sheep in the United Kingdom (Kaler and Green, 2008a). They have been extensively researched in the UK, Australia and New Zealand (and to a lesser extent elsewhere) and effective control strategies have been established (Kaler and Green, 2008a; Bennett and Hickford, 2011; Raadsma and Egerton, 2013).

In 2005 Nieuwhof and Bishop estimated the cost of footrot (FR) in the UK to be £24 million annually, including costs associated with labour, medicines, structures and lost production (Nieuwhof and Bishop, 2005). A similar estimate for New Zealand put the annual cost of treatment for FR together with associated production losses for the Merino sheep industry at NZ$ 11 million (Hickford et al., 2005).

Contagious ovine digital dermatitis (CODD) is an emerging disease and currently is less well understood. It was first reported in sheep in the UK in 1997 (Harwood et al., 1997) and is reported to occur across the UK and Ireland (Kaler and Green, 2008a; Scott, 2010). The FAWC lameness report (2011) highlighted the as yet scant evidence into the prevalence, incidence and aetiology of CODD although it notes that CODD causes severe lameness and consequently is a significant welfare concern (FAWC, 2011). The most recent surveys indicate a farm level prevalence of between 13% and 53% (Wassink et al., 2003a; Kaler and Green, 2008a). Recent work has suggested a role for treponemes in the aetiology of CODD similar to bovine digital dermatitis (BDD) (Dhawi et al., 2005; Sayers et al., 2009). However, there is some question over the possible interaction of other organisms such as *Dichelobacter nodosus* and *Fusobacterium necrophorum* (Moore et al., 2005b).

Several authors have described the clinical features of CODD, and currently they are still the mainstay of disease diagnosis (Winter, 2008). Common themes include those features which seem pertinent in distinguishing it clinically from FR, namely that lesions tend to commence at the coronary band (compared to the interdigital space) and then quickly under run the hoof horn capsule abaxially. In severe cases the whole horn may be shed leaving the sensitive lamellae exposed (Harwood et al., 1997; Naylor et al., 1998; Wassink et al., 2003b; Winter, 2008). Sheep tend to be extremely lame with severe consequences such as loss of body condition and increased recumbency (Harwood et al., 1997; Winter, 2008; Duncan et al., 2012). With little recent epidemiological data available, it is not easy to determine the true spread of CODD since its first report, or current prevalence in the UK.

Postal questionnaires have previously been used to assess farmer's views on lameness in sheep in the UK (Grogono-Thomas and Johnston, 1997; Kaler and Green, 2008a; Wassink et al., 2010a). These have described the prevalence and risk factors for FR, and the between and within farm prevalence of CODD. Other epidemiological aspects of CODD such as factors associated with disease have not yet been examined.

The aim of this study was to determine the farmer reported prevalence and factors associated with CODD using a postal questionnaire.

Materials and Methods

Study Population

Welsh sheep farms were chosen as the study population as CODD in this population has not previously been studied in detail, Wales has a substantial sheep population and the industry forms a significant part of the Welsh economy - in June 2011 there were 14,184 registered agricultural holdings with sheep (Welsh Assembly Government, 2012), although many farmers may have more than one holding. Simple randomisation, using random numbers, was used to select 2000 farmers registered with the Farm Assured Welsh Livestock (FAWL) database. The FAWL farm assurance scheme is a voluntary scheme although due to the financial benefits of belonging, the vast majority of sheep farmers are members and currently FAWL reports it has over 7,200 members (FAWL, 2013).

Sample Size Calculation

Given the response rates of previous published lameness surveys, together with the response rates from other farm questionnaires by The University of Liverpool, a response rate of approximately 50% was predicted. Sample size calculations were performed using Stata IC 12 (Statacorp; Texas). To detect a 10% difference in prevalence with 90% power and alpha at 5%, a sample size of 1076 would be required. The questionnaire was sent by post to the 2000 selected farmers in spring 2012. This equates to approximately 27% (95%CI: 26-28%) of the sampling frame.

Description of the Questionnaire

A pilot questionnaire was tested on a sample of 20 farmers. Adjustments to the questionnaire were made on the feedback responses to the pilot questionnaire.

The final 15 page questionnaire written in English, together with a cover letter written in English and in Welsh stressing the anonymity and confidentiality of the data and a reply paid envelope, was sent to the selected farms[5]. Using a mixture of open and closed question techniques, general and detailed questions were asked with regards to contagious foot conditions, farm and management factors, together with questions looking at the views and opinions of farmers on sheep lameness issues. Questions included factual based responses e.g. ‘Do you lamb your sheep inside?’ and opinion based responses e.g. ‘Do you think CODD on your farm is worst when sheep are housed?’. When considering their opinion,

[5] See Appendix A

farmers were asked to consider when most problems occurred, or when CODD was at its worst.

Farmers were asked to base their classification of foot diseases on a pictorial guide (see supplementary materials[6]) which demonstrated the key diagnostic features of CODD, FR and ID. The pictorial guide was developed in the pilot study by sheep veterinarians and sheep farmers in order to ensure valid diagnosis. The farmers had the option to request all the documents, in particular the questionnaire in Welsh. All returned questionnaires were entered into a cash prize draw.

One month after the initial survey a second set of documents was sent out to all non-responding farmers with an adjusted covering letter encouraging farmers to respond. Again farmers had the option to request the questionnaire in Welsh. Due to financial constraints only one reminder could be sent.

Data Analysis

The questionnaires were returned to The University of Liverpool and checked manually for inconsistencies. The data were then entered into a Microsoft Access Database (Microsoft; USA) followed by further range and consistency checks.

[6] See Appendix A.

All analyses were conducted using Stata IC 12 (Statacorp; Texas). Descriptive statistics were estimated, together with regression analyses where appropriate. Probability values of < 0.05 were taken as significant.

The primary outcome variable was the presence of CODD. Univariable logistic regression was employed to investigate associations between the reported presence of CODD and the following putative exposures: geographical area (county), farm size (ha), flock size, farm land type, time of year, housing, group (e.g. ewes, lambs etc.), isolation of purchased animals, foot trimming, the presence of cattle, BDD in cattle on the farm and the presence of concurrent disease - both foot and systemic disease. Foot trimming variables included: the presence or absence of foot trimming, trimming when lame, trimming when overgrown, routine foot trimming once per year and routine foot trimming more than once per year. From these foot trimming variables, the following composite variables were created: reactive foot trimming - this comprised affirmative responses to trimming when lame or trimming when overgrown, and preventive foot trimming - this included affirmative responses to routine foot trimming once per year and routine foot trimming more than once per year.

A multivariable model was fitted using a backward elimination strategy whereby a full model was built and then each variable removed in turn, a likelihood ratio test performed and the resultant P value noted. The variable with the highest P value was then omitted and the process repeated. This process was repeated until only variables with $P<0.05$ remained in the model. The omitted variables were then added back in turn, starting with the lowest P value, a likelihood ratio test performed after each addition, and the variable retained if $P<0.05$. This process was continued until no further variables could be added, to produce the final model. Interactions between variables in the final model were considered for inclusion if considered plausible and retained if they improved model fit as judged by the likelihood ratio test. The final model fit was assessed using the Hosmer-Lemeshow goodness of fit test.

The following explanatory variables were entered in the initial model: farm size (ha), flock size (total number of breeding ewes), lambing inside, the composite variables preventive foot trimming (routine foot trimming once per year or routine trimming more than once per tear) and reactive foot trimming (trimming when lame or when overgrown), the isolation of purchased animals and BDD in cattle.

Results

Response Rate

Of the 2000 questionnaires sent out, 565 were returned giving an overall response of 28.3% (95%CI: 26.3-30.3%). Of these 54 were not usable, the reasons for this are outlined in Table 1. This left 511 - a usable response of 25.6% (95%CI: 23.7-27.5%); not all respondents answered all questions. Excluding Gloucestershire, Herefordshire and Wrexham (only 1 - 3 farms were sampled from these counties) logistic regression demonstrated that the proportion of returns was not significantly different between counties.

Reason for removal	Number of questionnaires
Questionnaire returned unanswered	24
No sheep on the farm	24
Addressee gone away	2
Too busy	2
Farmer died	1
Farmer ill	1
Total	54

Table 1: Breakdown of reasons for removal of questionnaire from dataset of the survey of sheep farmers in Wales in 2012.

Farm Attributes

Median farm size was 101.2 hectares (IQR 52.6-157.8 hectares [range 5.3-1841.4 hectares]). The median number of sheep per holding was 704 (IQR 266-1250). This was 96 sheep more than that reported by the Welsh Government for the same year (Average flock size 608 June 2011) (Welsh Assembly Government, 2012) and 202 sheep more than the mean flock size (n = 390) reported by The Farm Business Survey in Wales (Mean flock size 502 (2011/2012)) (Farm Business Survey, 2011/2012).

Of the 511 respondents, 425 (83.2% (95%CI: 79.6-86.3%)) indicated the breeds of sheep kept. There were 38 different breeds represented but the top five ones were Welsh Mountain, Texel/Texel Cross, Suffolk/Suffolk Cross, Charollais/Charollais Cross and Mule (Table 2).

Breed of sheep	Number (% [95%CI]) of farmers reporting sheep of particular breeds		
	Ewes (n=425)	Lambs (n=368)	Rams (n=346)
Welsh Mountain	142 (33.4 [28.9-38.1])	106 (28.8 [24.2-33.7])	103 (29.8 [25.0-34.9])
Texel/Texel Cross	79 (18.6 [15.0-22.6])	96 (26.1 [21.7-30.9])	100 (28.9 [24.2-34.0])
Suffolk/Suffolk Cross	53 (12.5 [9.5-16.0])	49 (13.3 [10.0-17.2])	50 (14.5 [10.9-18.6])
Charollais/Charollais Cross	12 (2.8 [1.5-4.9])	36 (9.8 [6.9-13.3])	39 (11.3 [8.1-15.1])
Mule	49 (11.5 [8.7-15.0])	12 (3.3 [1.7-5.6])	1 (0.3 [0.01-1.6])

Table 2: Top five breeds of sheep kept by Welsh sheep farmers, from the survey of 511 sheep farmers in wales in 2012.

Prevalence

Ninety-five percent (95%CI: 93-97%) of farmers reported that they saw lame sheep on their farm. However, 5% (95%CI: 3-7%) reported that they did not, but then all of these farmers went on to confirm that they saw contagious foot diseases on their farm. When asked how much of a problem lameness was on their farms, 36.2% (95%CI: 32.0-40.6%) of respondents reported that lameness was either a moderate or a severe problem and 59.8% (95%CI: 55.5-64.1%) reported it to be a minor problem. Only 4.0% (95%CI: 2.6-6.1%) of respondents said that they did not consider lameness to be a problem at all.

Contagious ovine digital dermatitis was reported on 35.0% (95%CI: 31.0-39.3%) of farms. The median farmer estimated on farm prevalence of CODD on farms was 2.0% (IQR 1.0-5.0% [range 0.0-50.0%]).

The earliest reported observation of CODD on a farm was in 1960 (Figure 1); however, 88.4 % (95%CI: 82.2-92.6%) reported seeing it on their farm after 1997. Visual inspection of Figure 1 reveals a rapid increase in the firstobservance of CODD on farms since the year 2000. Of the 182 respondents who offered an opinion of how CODD arrived on their farm, 47.3% (95%CI: 40.0-54.6%) reported that they did not know, with 43.5% (95%CI: 36.3-50.8%) reporting that they thought CODD had arrived following the purchase of infected sheep.

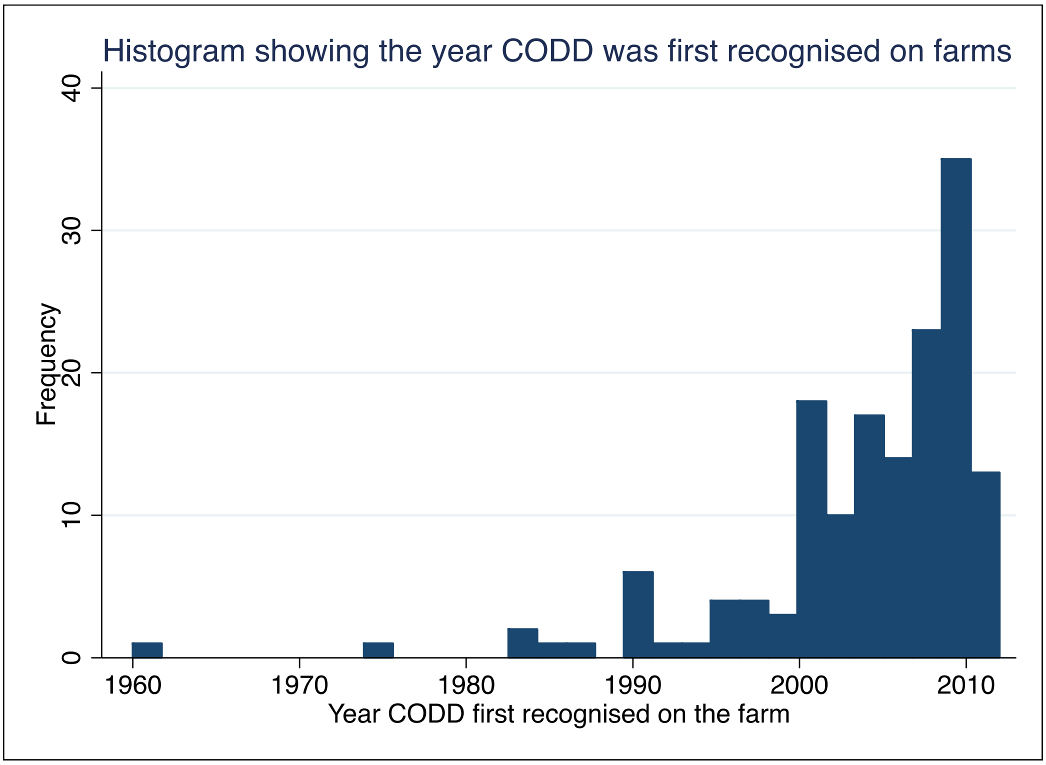

Figure 1: Histogram showing farmers opinions of when they believe CODD first arrived on their farm, from the survey of 511 sheep farmers in Wales in 2012.

Farm and management factors and their relationship with CODD

Univariable Analysis

There was no apparent county level variation in farmer reports of CODD across Wales. There was no association with land type (hill/mountain, upland or lowland). Larger farms (ha) were more likely to report CODD than smaller ones as were farms with larger flocks (Table 3). There was no association between farmer reported CODD and the following management practices: lambing inside, isolating purchased animals, gathering sheep and winter feeding practices such as the use of ring feeders and other field furniture that could lead to increased poaching.

Foot trimming of lame sheep was weakly associated with CODD. Trimming when overgrown was more strongly associated with CODD, although the effect was similar. Annual routine foot trimming was protective for CODD, but there was no association between CODD and routine trimming more than once per year. The composite variable preventive foot trimming was negatively associated with CODD and the composite variable reactive foot trimming was not associated with CODD.

Whilst the presence of cattle on the farm was not associated with CODD, the reporting of BDD in cattle on the farm was positively associated with CODD. Farmers were asked whether when they had CODD in their sheep if they observed it in isolation or whether it occurred concurrently with other diseases, either foot diseases or other diseases. Of the 179 farmers who reported seeing CODD on their farm, 164 (91.6% (95%CI: 86.6-95.2%)) answered this question. Of these, 35.0% (95%CI: 27.5-42.6%) reported that they saw CODD in isolation with 65.0% (95%CI: 57.4-72.5%) reporting that they saw it in association with other diseases. However, only 43 farmers gave details as to the other disease(s) they saw concurrently. Table 5 shows that the majority of those who did specify the concurrent disease see CODD associated with either FR or ID.

Variable (n=number of responses to question)	Number of farms with CODD	% of farms with CODD	OR	95% CI	P value
Farm size (ha)(n=499)					
5.0-46.9 (n=101)(Baseline)	20	19.8			
47.0-81.9 (n=111)	40	36.0	2.3	1.2-4.3	0.01
82.0-121.9 (n=110)	38	34.6	2.1	1.1-4.0	0.018
122.0-182.9 (n=82)	31	37.8	2.5	1.3-4.8	0.008
183.0-1842.0 (n=95)	46	48.4	3.8	2.0-7.2	<0.001
Flock size (number of ewes)(n=503)					
5-120 (n=102)(Baseline)	20	11.6			
121-285 (n=100)	28	15.9	1.6	0.8-3.1	0.163
286-480 (n=102)	35	19.9	2.1	1.1-4.1	0.019
481-750 (n=103)	42	23.8	2.8	1.5-5.3	0.001
751-3150 (n=96)	51	29.0	4.6	2.5-8.7	<0.001
Foot trimming (n=511)[7]					
Some foot trimming (n=506)(Baseline)	178	35.2			
Never foot trim (n=5)	1	20.0	0.5	0.1-4.2	0.49
Do not trim when lame (n=116)(Baseline)	32	27.6			
Trim when lame (n=395)	147	37.2	1.6	1.0-2.5	0.057
Do not trim when overgrown (n=190)(Baseline)	52	27.4			
Trim when overgrown	127	39.6	1.7	1.2-2.6	0.005
Do not trim once a year (n=413)(Baseline)	154	37.3			
Routine trim once a year (n=98)	25	25.5	0.6	0.4-0.9	0.029
Do not routine trim >once a year (n=436)(Baseline)	157	36.0			
Routine trim >once a year (n=75)	22	29.3	0.7	0.4-1.3	0.264
Composite foot trimming variables (n=511)					
No reactive foot trimming (n=68)(Baseline)	17	25.0			
Reactive foot trimming (n=443)	162	36.6	1.7	1.0-3.1	0.065
No preventive foot trimming (n=344)(Baseline)	134	39.0			
Preventive foot trimming (n=167)	45	26.9	0.6	0.4-0.9	0.008
Lambing inside/outside (n=500)					
Outside (n=111)(Baseline)	39	35.1			
Inside (n=389)	136	35.0	1.0	0.6-1.5	0.973
Isolated purchased stock (n=511)					
Isolate (n=312)(Baseline)	115	36.9			
Do not isolate (n=199)	64	32.1	0.8	0.6-1.2	0.278
BDD in cattle on the farm (n=357)					
No BDD (n=260)(Baseline)	71	27.3			
BDD (n=97)	55	56.7	3.5	2.1-5.7	<0.001

Table 3: Univariable analysis of farm and management factors and their relationship with CODD, from the survey of 511 sheep farmers in Wales in 2012.

[7] Farmers could include more than one response to the question on foot trimming, e.g. they might record they foot trim when lame, when overgrown and routinely once per year. Reactive foot trimming includes responses for trimming when lame and when overgrown and preventive foot trimming includes responses for routine trimming once per year and more than once per year.

Multivariable Analysis

The final multivariable model included the total number of breeding ewes and BDD in cattle on the farm as the remaining explanatory variables (Table 4). No significant interaction was found between the variables in the final model. The Hosmer-Lemeshow test indicated evidence of a good fit (P=0.938, 7 degrees of freedom).

Variable	OR	95% CI	P value
Flock size (number of ewes)	1.4	1.2-1.7	<0.001
BDD in cattle	3.1	1.9-5.1	<0.001
Baseline odds (102 ewes; no BDD)	0.1[8]	0.1-0.2	<0.001
Hosmer-Lemeshow goodness of fit test (7 d.f.)			0.938

Table 4: Final multivariable model, from the survey of 511 sheep farmers in Wales in 2012.

Disease	Number of farmers reporting	% farmers reporting	95% CI
Footrot	16	9.8	5.2-14.3
ID	19	11.6	6.6-16.5
Granuloma	1	0.6	0.0-1.8
White line disease	1	0.6	0.0-1.8
Pneumonia	2	1.2	0.0-2.8
Worms	2	1.2	0.0-2.8
Ill thrift	2	1.2	0.0-2.8

Table 5: Diseases farmers report to be concurrent with CODD, from the survey of 511 sheep farmers in Wales in 2012.

[8] Baseline odds

Farmer perception of risk factors associated with CODD

These data are displayed in Table 6. Farmers perceive their sheep to be similarly affected with CODD for most of the year, with a slight improvement in the summer. Farmers consider housing to make CODD worse, and they think that CODD affects their breeding ewes significantly more than other groups.

Variable	Number reporting variable as risk factor	% reporting variable as a risk factor	95% CI
Time of year (n=179)			
Winter (December January February)	87	48.6	41.2-56.0
Spring (March April May)	67	37.4	30.3-44.6
Summer (June July August)	48	26.8	20.3-33.4
Autumn (September October November)	79	44.1	36.8-51.5
Do not know	35	19.6	13.7-25.4
Housing (n=234)			
Yes	91	38.9	32.6-45.2
No	64	27.4	21.6-33.1
Do not know	79	33.8	27.7-39.9
Group (n=179)			
Lambs (new born to weaning)	3	1.7	0.0-3.6
Growing lambs (weaning to 1 year old)	46	25.7	19.2-32.2
Ewe replacements (ewe lambs up to 2 years of age)	36	20.1	14.2-26.0
Breeding ewes (2 years and older)	117	65.4	58.3-72.4
Breeding rams	54	30.2	23.4-37.0

Note: Farmers could include more than one response to questions on time of year and group, e.g. they may report more CODD in Winter and Spring, or in breeding ewes and breeding rams.

Table 6: Direct responses from farmers to questions on their views on prospective risk factors for CODD, from the survey of 511 sheep farmers in Wales in 2012.

Discussion

These current data serve to increase our understanding of CODD, specifically identifying potential key risk factors for CODD. This study represents the largest study to date to specifically address farmers' collective knowledge and views on CODD.

The overall response rate of 28.3% (95%CI: 26.3-30.3%) was low in comparison to recent sheep lameness surveys (Kaler and Green, 2008a; Wassink et al., 2010a). A second reminder may have increased the response rate, but due to financial constraints this was not possible. It is not clear whether the response was made worse due to Wales being used as the sampling area rather than using other regions or the UK as a whole. For example Grogono-Thomas and Johnston (1997) surveyed 3250 sheep holdings in 1994 and showed a significantly poorer response from Welsh sheep farmers (10%) compared with all other areas in the UK (20%).

Due to the relatively recent arrival of CODD, diagnosis may still be an issue, indeed three farmers commented that until completing the questionnaire they had not realised that they had CODD in their sheep. To aid in diagnosis a pictorial guide was included in the questionnaire. A similar method was used by Kaler and Green (2008a) in their questionnaire. Farmer based diagnosis should always be interpreted cautiously. The survey by Kaler and Green (2008a) demonstrated the confusion of many farmers when attempting to diagnose different foot conditions. The pictorial guide in this current study has not been validated as a diagnostic tool, however it was developed in conjunction with sheep vets and piloted with 20 farmers. This helped determine that it would be effective as an aid to diagnosis.

This study reports a lower between farm prevalence of CODD across Wales (35% (95%CI: (95%CI: 31.0-39.3%)) compared to that across England (53%) (Kaler and Green, 2008a). However, the within farm prevalence is very similar. Based on our geographical data the disease has a similar prevalence across all counties of Wales. Therefore, CODD is now common and endemic in Wales. Given that the most common reason for the introduction of diseases suggested by farmers in this survey was the purchase of new stock, biosecurity practices are likely to be critical in preventing disease in the 65% (95%CI: 60.7-69.0%) of flocks which do not yet report CODD.

The current survey showed a response bias towards farmers with larger flocks which may influence the results - for example with farms with larger flocks responding the data may be less applicable to those with smaller flocks. However, as the trend for bigger farms develops the results are likely to become increasingly representative.

Farmers with larger flocks are possibly those whose farming enterprise would be impacted more by disease, and also this study shows, are more likely to report CODD. Larger farms are likely to have more animal movements (Green et al., 2007a) (e.g. larger flocks will need to purchase a greater number of rams and/or ewe replacements) which farmers report is the main route, in their opinion through which disease may be introduced.

There was some reported seasonal variation of CODD with a slight improvement in summer. In Australia there is a strong seasonal variation in FR incidence in relation to precipitation - increased rainfall leading to increased disease (Abbott and Lewis, 2005). This association is not demonstrated as clearly in the UK (Wassink et al., 2003a; Abbott and Lewis, 2005) due to more variable rainfall patterns (Green and George, 2008).

Despite a poor response rate to the pertinent questions regarding concurrent diseases, a number of farmers (n=35) did report an association between CODD and FR/ID. Duncan et al. (2012) also demonstrated that FR was a risk factor for CODD and a recent bacteriological study found an association between *D. nodosus* and CODD lesions (Moore et al., 2005b).

This study did not demonstrate any association with cattle kept on the same farms, however farmers reporting BDD in their cattle were more likely to report CODD in their sheep. This lends weight to the theory that there is a common microbiological aetiology and that the disease in sheep is likely to have come originally from cattle. Therefore it would be prudent, to reduce the risk of introducing CODD to sheep, not to co-graze cattle and sheep.

It is interesting to note that the early reports of the occurrence of CODD in this study coincide with the early recognition of BDD in Italy in 1974 (Cheli and Mortellaro, 1974), and in the UK in 1988 (Blowey and Sharp, 1988). Indeed, there is an early description of FR (1916) which bears distinct similarities with current descriptions of the clinical appearance of CODD, namely that lesions commence at the top of the foot and work downwards (Day et al., 1916).

Foot trimming was shown to be weakly associated with CODD and annual foot trimming was shown to be protective. For FR, foot trimming has also been shown to be associated with an increased prevalence and has also been shown to delay recovery (Kaler et al., 2010a; Wassink et al., 2010a). This similar pattern may suggest that the underlying mechanisms are similar.

In an attempt to investigate potential risks for the transmission of disease, we investigated the relationship between the presence of CODD on a farm and winter feeding practices, housing and the use of field furniture (e.g. ring feeders). Given that such factors are believed to be important in the transmission of *D. nodosus*, we found no evidence of associations for them and CODD on the farm. However, we recognise that more detailed on farm experimental studies would be a more appropriate method to investigate foot level disease transmission.

Conclusion

Contagious ovine digital dermatitis now appears to have become endemic and widespread in this study area. Risk factors associated with disease are the presence of cattle with BDD on the farm and the size of the flock. Farmers also consider adult sheep to be most at risk and there to be some seasonal variation. Other risk factors may include concurrent FR or ID, and housing. In order to devise effective treatment and control strategies for farms with multiple infections and in particular CODD, research should be directed to on farm epidemiological studies investigating specific potential risk factors for disease together with the aetiopathologic agents.

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Chapter 3

A practical tool for locomotion scoring in sheep: reliability when used by veterinary surgeons and sheep farmers

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Abstract

A four-point locomotion scoring tool for sheep was developed and tested on 10 general practice veterinary surgeons (VS) and 10 sheep farmers. Thirty-four video clips of sheep displaying different locomotion scores were recorded and randomly assorted. Following a set period of training using four other video clips typical of the four locomotion scores, participants then scored the 34 test clips. The participants repeated the training and the exercise one month later. There were high levels of intra-observer repeatability: weighted kappa (κ_W) 0.81 for VS and 0.83 for farmers. There was no difference in intra-observer repeatability between vets and farmers (Wilcoxon signed rank P = 0.8). When considering the overall distribution of scores within the video-package, there were high levels of inter-observer repeatability: mean κ_W 0.73 for VS and 0.72 for farmers. However, the repeatability for the individual locomotion scores was only fair to moderate. It is therefore recommended that when observations are repeated on different occasions they are made by the same observer.

Introduction

Lameness in sheep is a priority welfare concern for the UK sheep industry (Phythian et al., 2011). Current understanding and consensus of opinion have led to recommendations to farmers to identify and treat lame sheep early (FAWC, 2011).

Locomotion scoring identifies lame individuals and can be used to determine flock prevalence. As detailed in Chapter 1, various attempts have been made to grade the severity of lameness in sheep (Table 1 in Chapter 1). Previous scoring tools e.g. Ley et al. (1989); Kaler et al. (2009); Phythian et al. (2012) and Welsh et al. (1993) all have limitations, being either overly detailed or simplistic. Furthermore, they all use small numbers of experienced researchers, which could reduce generalisability[9].

For example, Phythian et al. (2012) describes a binary lame/not lame system for observation of groups of sheep. This group method can be useful for determining the prevalence of lameness within a flock, and offers advantages in that sheep do not need to be stressed by gathering, and therefore it can be useful for assessing the overall impact of lameness and the implied welfare considerations in a flock. However, there are disadvantages in welfare assessment using this method in that it does not take into account the severity of lameness.

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[9] For a more detailed comparison of these studies see Table 1 (p7) in the Introduction.

In comparison, the seven-point scale described by Kaler et al. (2009) breaks down in detail the different effects on the body that pain in the foot may elicit thus accounting for differences in severity. However, a seven-point scale may be less easily used by sheep farmers and general practice veterinary surgeons (VS) who may require a simpler more ‘user-friendly’ system. Together with this, the large number of subdivisions does not seem to offer any advantage over a sound-mild-moderate-severe type system, which anecdotally would be used widely within the industry. In addition, it has only been tested for reliability using experienced sheep researchers, which could reduce generalisability when considering use of the tool by less specialist individuals.

Therefore, there is a need for a locomotion scoring tool that is simple to use, has the minimum number of subdivisions necessary to determine severity and is tested on the target population.

The aim of this study was to develop a locomotion scoring tool for use by farmers, and veterinary surgeons (VS) to assess lameness severity in individual sheep, and severity and prevalence in flocks.

Materials and Methods

Locomotion Scoring

A four-point system was developed by combining the Kaler system (Kaler et al., 2009) with the DairyCo Mobility Scoring System (DairyCo, 2009):

0: (SOUND) Bears weight evenly on all four feet and walks with an even rhythm.

1: (MILDLY LAME) Steps are uneven but it is not clear which limb or limbs are affected.

2: (MODERATELY LAME) Steps are uneven and the stride may be shortened; the affected limb or limbs are identifiable.

3: (SEVERELY LAME) Mobility is severely compromised such that the sheep frequently stops walking or lies down due to obvious discomfort. The affected limb or limbs are clearly identifiable and may be held off the ground whilst walking or standing.

Ethical approval was provided by the University of Liverpool (VREC13).

Thirty-eight video clips of sheep walking and standing were made – representing all four scores. To ensure a range of severities was represented, these were scored by three experienced sheep VS to collectively determine the 'true' score. Four of the clips were used to train the participants. The other 34 were shown in random order to the participants. If there was more than one sheep visible, a red circle was drawn around the relevant individual.

Study Population

The tool was tested on a convenience, non-random sample of 10 general practice VS and 10 sheep farmers.

Training

Each participant was trained using clips typical of each score. Participants then watched the test clips taking as long as needed and were allowed to watch each clip as many times as necessary. No help was given during the test period. The training and assessment were repeated one month later.

Data Analysis

Intra-observer agreement

a) Bias between attempts

For each clip, the score from an individual's second attempt was subtracted from their initial score. Differences were investigated using one-sample t-tests.

b) Exact agreement

Percent agreement for an observer was determined from the number of observations that matched exactly:

$$\frac{(\text{number of matching observations})}{34} \times 100$$

The mean percent agreement was calculated for VS and farmers, and compared using the Chi-squared test. Similar data were compiled for the one and two point differences.

c) Pairwise Kappa

Weighted Kappa (κ_W) was calculated between each pair of observations by each observer using quadratic weights and interpreted using Landis and Koch (1977), Table 2.

Inter-observer agreement

a) Kappa between observers

For each observer, a κ_W was created with each member of their group. The mean of these nine values was that individual's inter-observer agreement.

b) Kappa for locomotion scores

To examine the repeatability of recording different severities of locomotion score, unweighted κ was obtained for all clips that had received the given score.

c) Median scores

Median scores for each clip were calculated for both VS and farmers. Differences were assessed using the Wilcoxon signed-rank test.

Statistical significance was set at <0.05. All analyses used STATA 13 (StataCorp, Texas).

Results

All participants found the tool easy to use. They found it hardest to distinguish between scores 1 and 0.

The mean proportion of scores attributed from the first set of observations was: score 0: 8.7 (26% (95%CI: 6.5-44.7%)), score 1: 9.9 (29% (9.1-48.9%)), score 2: 9.8 (29% (95%CI: 8.9-48.5%)) and score 3: 5.7 (17% (0.4-33.1%)).

Intra-observer agreement

a) Bias between attempts

Bias was present within and between observers and was significant for three VS and five farmers (Table 1). The largest differences in scores were -0.21 and 0.25 respectively.

b) Exact agreement

The mean overall exact agreement within individual observers was 65.0% (SD 8.7) for VS and 68.3% (SD 12.2) for farmers (P = 0.5).

c) Pairwise kappa

The mean κ_W at intra-observer level was 0.81 for VS and 0.83 for farmers (P = 0.8).

Individual Observers	Locomotion Score Mean (SD)	Mean observer difference in locomotion scores between observations	t-test of mean observer difference compared to zero (P value)	Intra-observer agreement (%)			Intra-observer κ_W for each individual observer comparing first and second observations	Inter-observer agreement: Mean κ_w for each observer versus all 9 other observers in group
				Exact agreement	+/- 1 point difference	+/- 2 point difference		
Veterinary Surgeon Observers								
Vet 1	1.31 (1.16)	-0.12	0.013	61.8	100	100	0.86	0.78
Vet 2	1.37 (1.09)	-0.06	0.344	79.4	100	100	0.91	0.75
Vet 3	1.56 (1.15)	0.13	0.076	70.6	100	100	0.89	0.75
Vet 4	1.46 (0.97)	0.03	0.647	55.9	94.1	100	0.67	0.72
Vet 5	1.22 (1.13)	-0.21	0.003	79.4	100	100	0.92	0.76
Vet 6	1.38 (1.07)	0.19	0.001	61.8	97.1	100	0.74	0.74
Vet 7	1.41 (1.03)	-0.02	0.762	61.8	97.1	100	0.77	0.76
Vet 8	1.41 (1.03)	-0.02	0.825	55.9	97.1	100	0.75	0.64
Vet 9	1.54 (0.97)	0.11	0.129	58.8	97.1	100	0.73	0.68
Vet 10	1.41 (1.03)	-0.02	0.782	64.7	97.1	100	0.83	0.70
Overall Mean (SD)		**-0.01 (0.05)**		**65.0 (8.7)**	**98.0 (2.0)**		**0.81 (0.1)**	**0.73 (0.04)**
Farmer Observers								
Farmer 1	1.04 (0.95)	-0.27	<0.001	79.4	100	100	0.89	0.71
Farmer 2	1.26 (1.07)	-0.05	0.530	52.9	94.1	100	0.72	0.67
Farmer 3	1.63 (1.16)	0.32	<0.001	64.7	97.1	100	0.83	0.70
Farmer 4	1.31 (1.22)	0.00	0.952	70.6	97.1	100	0.87	0.77
Farmer 5	1.56 (0.92)	0.25	<0.001	76.5	100	100	0.86	0.69
Farmer 6	1.43 (1.01)	0.11	0.026	61.8	100	100	0.81	0.73
Farmer 7	1.26 (1.05)	-0.05	0.306	67.7	97.1	100	0.81	0.77
Farmer 8	1.07 (1.11)	-0.24	<0.001	91.2	100	100	0.96	0.78
Farmer 9	1.22 (1.01)	-0.09	0.198	67.7	94.1	100	0.75	0.70
Farmer 10	1.34 (1.05)	0.35	0.678	50.0	100	100	0.77	0.73
Overall Mean (SD)		**0.00 (0.06)**		**68.3 (12.2)**	**98.0 (2.4)**		**0.83 (0.1)**	**0.72 (0.04)**
Comparison of difference in means between VS and Farmer observers (P value)				**0.5**	**1.0**		**0.8**	**0.8**

Table 1: Intra- and Inter-observer agreement for veterinary surgeon and farmer observers.

Inter-observer agreement

a) Kappa between observers

The mean κ_w at inter-observer level was 0.73 (SD 0.04) for VS and 0.72 (SD 0.04) for farmers (P = 0.8) (Table 1).

b) Kappa for locomotion scores

Overall, for score 3 there is substantial agreement between observers. For other scores, there is moderate or fair agreement (Table 2).

c) Median scores

The median score assigned to each video clip by VS was not significantly different to that assigned by farmers (P = 0.18) (Table 2).

Locomotion score	**Overall κ VS and Farmers§**	**Overall κ VS§**	**Overall κ Farmers§**
0	0.37 Fair	0.30 Fair	0.43 Moderate
1	0.22 Fair	0.27 Fair	0.18 Slight
2	0.43 Moderate	0.47 Moderate	0.41 Moderate
3	0.62 Substantial	0.67 Substantial	0.58 Moderate
Combined	**0.40 Fair**	**0.42 Moderate**	**0.39 Fair**

Table 2: Interobserver agreement for individual locomotion scores

§ Interpretations are taken from Landis and Koch (1977): 0 = poor; 0.01 to 0.20 = slight; 0.21 to 0.40 = fair; 0.41 to 0.60 = moderate; 0.61 to 0.80 = substantial; 0.81 to 1.00 = almost perfect.

VS, veterinary surgeon

Discussion

There were score differences between observation attempts, however we consider this bias, whilst present, is too small to invalidate the scoring system. The variation in locomotion scores (Table 2) indicates bias between observers and may have led to smaller κ values than if the scores had equal prevalence within the video package (Byrt et al., 1993). However, given that the lowest prevalence score (score 3) had the highest levels of repeatability between observers, a more equal prevalence would likely have had little impact on the κ values.

For this locomotion scoring tool both intra- and inter-observer repeatability were substantial indicating that this tool could be used reliably in monitoring lameness in individuals over time and enable different observers to reliably measure lameness across farms. However, the inter-observer repeatability of locomotion scores was slight to moderate, except for score 3. Therefore, whilst different observers scored similar proportions of sheep with each locomotion score, the ability to score the same individual with the same score was unsatisfactory.

Therefore, at a whole flock level, where different observers score individuals to compile an aggregate overall lameness assessment, the repeatability of the flock distribution of lameness scores is reliable. However, for a specific individual animal, different observers are less likely to attribute the same locomotion score. Consequently, these data would suggest that given the high levels of intra-observer repeatability a single observer should make the repeat assessments.

An example of where this might be necessary could be in the repeated assessment of an individual sheep in response to treatment.

This locomotion scoring tool addresses some of the weaknesses found in previous examples. For instance it can be used to determine the severity of lameness, compared to a binary scoring system such as that used by (Duncan et al., 2012); Phythian et al. (2012), but yet is not overly detailed and has been tested on both general practice veterinary surgeons and sheep farmers so may be more generalizable than that developed by Kaler et al. (2009). However, the results indicate that this study population found it difficult to determine when an individual should be scored as mildly lame (score 1). This is comparable with the results of Kaler et al. (2009) who report that 'the discrepancy between observers' scores was mainly for scores 0 and 1' – score 1 being 'uneven posture and/or short stride on one leg compared with others'. In other studies farmers have reported that they can easily recognize lame sheep when a head nod becomes present (King and Green, 2011). However, these workers describe a head nod being present when lameness has already progressed so far as to be quite pronounced and thus we considered a head nod to not be an indicator of early or mild lameness. These studies along with the current report serve to illustrate the consistent difficulty in detecting the early and crucial changes of the onset of lameness.

Training and practice have been shown to improve the repeatability of many observational tests (Vasseur et al., 2013). The study by King and Green (2011) and that by Phythian et al. (2012), also demonstrate the need for specific training, experience and practice in scoring in order to help observers detect subtle changes in behaviour indicating lameness. Two shrewd comments by observers in this study appear helpful. The first is that "mildly lame sheep appear a bit uncomfortable and may be 'a bit tender' on their feet". The second is that "if you are in a hurry you don't see any lame sheep" – that is to detect early and subtle signs of alterations in locomotion an observer needs time, patience and practice to make an accurate observation. It is likely that more extensive training, experience and practice by the observers in this study would improve the repeatability.

The tool appears to be readily applicable to the main sheep industry users. The large number and type of observers used in this study strengthen it, and show that mixed practice VS and sheep farmers can use this tool successfully. Furthermore, there was no significant variation between the VS group or farmer group. Some of the farmers who were trained to use the scoring system in this study reported that they have become more aware of their mildly lame sheep and several of the VS in the study now use the system routinely in their work.

In conclusion, this study provides a tool that may be easily used successfully by VS and sheep farmers for the locomotion scoring of sheep. The high levels of intra- observer agreement ensure that with only limited training it may be used reliably to evaluate and monitor the locomotion of sheep. However, there is less inter-observer agreement with allocating the same sheep with the same locomotion score by different observers, and whilst the distribution of scores within a group have higher levels of repeatability we would suggest that for repeated observations of the same flocks, or in particular, observations of individual animals should be made by the same observer.

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Chapter 4

Clinical and radiographic features of contagious ovine digital dermatitis, and a novel lesion grading system

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Abstract

Contagious ovine digital dermatitis (CODD) is an infectious foot disease of sheep causing severe lameness. Diagnosis is currently made using broad anecdotal descriptions. The aim of this study was to systematically and formally describe the clinical presentation of the disease in terms of 1) a lesion grading system; 2) associated radiographic changes and 3) severity of associated lameness.

A five-point lesion grading system was developed and applied to 908 sheep affected by CODD from six farms. Sheep with lesions typical of each grade were euthanased and their feet radiographed. Radiographic abnormalities including soft tissue and bony changes were evident in feet with lesions graded 2-5.

In order to quantify the welfare impact of CODD, all the 908 sheep were locomotion scored. Five hundred and eighty-five (64.5% [95%CI: 61.4-67.6%]) were lame. The locomotion score for affected sheep increased with worsening pathological changes. Once healing had begun the locomotion score decreased.

In conclusion the 5-point grading system may be used to clinically describe stages of CODD lesions. The radiographic changes revealed examples of deeper pathological changes and there was a strong association between the lesion grading system and locomotion score in affected sheep.

Introduction

Contagious ovine digital dermatitis (CODD) is a relatively new cause of lameness in sheep in the UK, (Harwood et al., 1997). Recent surveys of sheep farmers have revealed that CODD has now become common, with farmer reported prevalence of 35% (95%CI: 31.0-39.3%) (Angell et al., 2014) and 53% (95%CI: 50-57%) (Kaler and Green, 2008b) identified, and whilst on most affected farms the on farm prevalence of affected sheep is 2.0% (IQR 1.0-5.0% [range 0.0-50.0%]), it can be as much as 50% (Angell et al., 2014).

The study of any disease requires a clear case definition to enable diagnosis and monitoring for clinical and research purposes. Currently, CODD is poorly defined in terms of its clinical presentation and the severity of associated lameness. At present, the clinical description of the disease (which is used as the basis of veterinary diagnosis) is limited to anecdotal, broad descriptions of typical foot lesions (Winter, 2004a; Sargison, 2008). Visual inspection of foot lesions is an accepted method of diagnosis for other foot conditions in sheep (Egerton, 1989; Foddai et al., 2012), and clinical descriptions of the lesions are aided by lesion scoring systems in order to describe specific features in more detail. Currently, there is no standard clinical lesion scoring system for CODD.

Treponemes have been demonstrated in CODD lesions and have been associated with disease (Dhawi et al., 2005). In other treponemal associated diseases tissue damage may be extensive and in some cases has had bony involvement, for example in cases of periodontal disease in humans and toe necrosis in cattle, (Kofler, 1999; Loesche and Grossman, 2001; Evans et al., 2011; Blowey et al., 2013). Scott (2010) reported a case series of CODD-like lesions in a group of ten Cheviot ewes, and using radiography demonstrated osteopenia and resorption/re-modelling of the third phalanx of affected digits in some of these sheep.

Sheep with CODD have been observed to be severely lame (Davies et al., 1999; Phythian et al., 2013), however systematic locomotion scoring, and therefore the implied welfare impact on affected sheep at different stages of disease, has yet to be described.

Therefore, the aim of this paper is to describe the clinical features of CODD in terms of 1) a lesion grading system 2) associated radiographic changes and 3) the severity of associated lameness.

Materials and Methods

The study protocol was approved by The University of Liverpool ethics committee (VREC 13) on 24th August 2011.

Development of lesion grading system

Current published clinical descriptions were initially used to establish a case definition of CODD as distinct from other foot conditions, in particular footrot.

CODD was described as an ulcerative or chronic inflammatory lesion at the coronary band, which may extend dorsally and abaxially under the hoof wall and in many cases leads to avulsion of the hoof capsule. The affected digit may be swollen and shortened (Winter, 2004a; Scott, 2010).

Footrot was described as an erythematous and/or an erosive or ulcerative lesion affecting the inter-digital space leading to hoof horn separation axially which may lead to necrosis of the sole and in very severe cases lead to avulsion of the hoof capsule (Winter, 2004a; Sargison, 2008).

The definition for CODD was then further developed using previous clinical experience of the authors, together with examination of a photographic archive of CODD lesions to produce the following five-point lesion grading system:

Grade 1	Erosion/ulceration with or without alopecia specifically at the level of the dorsal coronary band.
Grade 2	Erosion/ulceration of the skin at the coronary band with partial (<50%) under-running of the hoof horn dorsally, abaxially and tending towards circumferential under-running.
Grade 3	Erosion/ulceration of the skin at the coronary band with 50% - 100% under-running of the hoof horn with possible hoof horn avulsion.
Grade 4	Healing foot with the horn beginning to regrow but an active lesion still present.
Grade 5	Healed foot, often with deformation of the regrown horn.

Farm	Land Type	Total size (Hectares)	Total number of breeding ewes	Breeds
1	Hill	186.2	740	Welsh Mountain (99.4% (95%CI: 96.0-99.9%)) Other breeds (0.6% (95%CI: 0.1-4.0%))
2	Lowland	360.2	500	Scottish Blackface (21.4% (95%CI: 19.1-23.8%)) Mule (43.7% (95%CI: 40.8-46.5%)) Suffolk/Suffolk Cross (20.7% (95%CI: 18.4-23.2%)) Other breeds/ crossbreeds (14.2% (95%CI: 12.3-16.4%))
3	Lowland/Upland	111.3	870	Lleyn/Lleyn Cross (31.3% (95%CI: 28.8-33.9%)) Easy Care/Easy Care Cross (63.2% (95%CI: 60.5-65.8%)) Other Lowland Crossbreeds (5.5% (95%CI: 4.3-6.9%))
4	Lowland	72.8	300	Mule (70.5% (95%CI: 67.8-73.0%)) Charollais Cross (20.5% (95%CI: 18.3-22.9%)) Other Lowland Crossbreeds (9.0% (95%CI: 7.4-10.7%))
5	Lowland/Upland	68.8	460	Suffolk Cross (43.4% (95%CI: 39.0-47.8%)) Mule (34.8% (95%CI: 30.6-39.2%)) Texel Cross (18.7% (95%CI: 15.5-22.4%)) Other Lowland Crossbreeds (3.1% (95%CI: 1.7-5.0))
6	Upland	97.1	400	Lleyn/Lleyn Cross (85.1% (95%CI: 82.3-87.6%)) Mule (10.7% (95%CI: 8.6-13.1%)) Other Lowland Crossbreeds (4.2% (95%CI: 2.9-5.9%))

Table 1: Farm attributes of the six study farms in North Wales.

Subsequently, this lesion grading system was applied in the examination of sheep on six farms confirmed as affected by CODD (Table 1). Over a one-year period each flock was repeatedly inspected between 3 and 6 times. At each visit all the sheep in the flocks were inspected closely in the handling pens. All lame sheep were then restrained in dorsal recumbency for detailed examination of their feet. Together with this, depending on feasibility and practical constraints, up to half of the remaining non-lame sheep were also examined in detail. This strategy identified 908 sheep (from 5,498 examined) with foot lesions consistent with CODD. The proposed lesion grading system was then used to categorise (by JWA) the 908 sheep affected by CODD in this study and further subtle details and variations within each grade were gathered as a result and used to add detail to the final five-point lesion grading system.

Radiographic descriptions

Sheep with lesions typical of the five grades were identified and, with the farmers consent, were euthanased on the farms using pentobarbital sodium (Pentoject; Animal Care). At post mortem, their feet were immediately removed by disarticulation of the radiocarpal or tibiotarsal joints and after gentle washing with a stream of cold water to remove gross contamination were placed immediately in 10% formalin (GCC Diagnostics). This was necessary as the feet were intended to undergo further gross and histopathological examination.

Locomotion scoring sheep affected by CODD

The CODD lesion grade of each of the 908 sheep diagnosed with CODD was recorded. In addition, the gait of all the sheep was observed (prior to clinical examination) whilst standing and walking to determine their locomotion score using a four-point ordinal locomotion scoring system (Angell et al., 2015c):

Score 0 (SOUND) Bears weight evenly on all four feet and walks with an even rhythm.

Score 1 (MILDLY LAME) Steps are uneven but it is not clear which limb or limbs are affected.

Score 2 (MODERATELY LAME) Steps are uneven and the stride may be shortened; the affected limb or limbs are identifiable.

Score 3 (SEVERELY LAME) Mobility is severely compromised such that the sheep frequently stops walking or lies down due to obvious discomfort. The affected limb or limbs are clearly identifiable and may be held off the ground whilst walking or standing.

This scoring system was shown to have high levels of intra-observer repeatability when tested by 10 veterinary surgeons (κW 0.81) (Angell et al., 2015c).

Data were entered into a Microsoft Access database (Microsoft, USA). Graphs and descriptive statistics were performed using STATA IC 13 (Statacorp, Texas). Median data are presented for the locomotion scoring due to the ordinal nature of the locomotion-scoring tool.

Results

Descriptive statistics

Of the 908 sheep with CODD, 866 (95.4% [95%CI: 94.0-96.7]) had CODD in one foot, 42 (4.6% [95%CI: 3.3-6.0]) had CODD present in more than one foot. The clinical grading system was used for all 908 sheep and lesions were readily classified using the system. The number of sheep with each grade was not uniform with grades 2 and 5 most commonly represented and grade 1 least represented (6.9% [95%CI: 5.3-8.6]) (Table 2).

CODD grade	Number of sheep with CODD locomotion scored (% [95% CI])	Median locomotion score (IQR[range])
1	63 (6.9% [5.3-8.6])	1 (1-2[0-3])
2	237 (26.1% [23.2-29.0])	2 (1-3[0-3])
3	149 (16.4% [14.0-18.8])	3 (2-3[0-3])
4	152 (16.7% [14.3-19.2])	1 (0-2[0-3])
5	265 (29.2% [26.2-32.1])	0 (0-0[0-3])
CODD in more than one foot	42 (4.6% [3.2-6.0])	2 (1-2[0-3])
Total	908	

Table 2: The number and proportion of sheep with different grades of CODD, together with the median locomotion score of sheep with that grade, from a sample of 908 sheep with CODD on 6 farms in North Wales.

Lesion grading system

Summary observations and photographs of CODD lesions from using the descriptors in this study include the following:

Grade1 (Figures 1 and 2)

These lesions were focally extensive, erosive or ulcerative affecting the digital skin and coronary band. They could be raised (proliferative) or depressed (erosive/ulcerative) and could be reddened or yellow-white (purulent) in colour. At this stage there was no under-running of the hoof capsule.

Grade 2 (Figures 3 and 4)

The separation occured between the hoof capsule and the lamellae. The under-running varied from the separation of a narrow section of the dorsal hoof wall, to up to 50% of the dorsal and abaxial hoof wall. These digits appeared swollen and grossly the surface of the lamellae appeared rounded and in many cases non-existent. The surface could appear dark red (haemorrhagic) and there was often purulent material adherent to the surface, together with a foul smell.

Grade 3 (Figures 5 and 6)

As for grade 2 lesions, however, the under-running of the hoof horn varied from >50% of the hoof wall, to complete avulsion of the hoof capsule.

Grade 4 (Figures 7 and 8)

Up to 100% of the hoof capsule was absent. Affected digits appeared swollen and the subcorneal tissue exposed by the loss of the hoof wall appeared roughened, dark red (haemorrhagic/necrotic) and friable and could be easily traumatised leading to further bleeding. Often there was obvious shortening of the digit.

Grade 5 (Figures 9 and 10)

Horn regrowth was apparent. The surface was usually smooth but distorted by circumferential ridges or creases. The affected digit was usually still wider and shortened in comparison to the unaffected digit.

Radiographic changes (Figures 11-15)

Two digits of each grade, from two different feet in each case were radiographed. Lateromedial views are presented as examples only[10]. Key features to note are the extent and appearance of the hoof structures in relation to the bony structures underneath. Demarcation of the laminae is not possible as the hoof horn and laminae are of similar radio density.

[10] Dorso-medial palmaro-lateral oblique views are presented in Appendix .

Figure 1: Grade 1 contagious ovine digital dermatitis lesion, erosion/ ulceration with or without alopecia loss specifically at the level of the dorsal coronary band. This example demonstrates a 1 cm erosive/ ulcerative slightly raised purulent lesion with minimal alopecia.

Figure 2: Grade 1 contagious ovine digital dermatitis lesion, erosion/ ulceration with or without alopecia specifically at the level of the dorsal coronary band. This example demonstrates a 1.5 cm erosive/ ulcerative lesion; the lesion is slightly depressed and the edges are thickened and white.

Figure 3: Grade 2 contagious ovine digital dermatitis lesion, erosion/ ulceration of the skin at the coronary band with partial (<50%) underrunning of the hoof horn dorsally and abaxially. This example demonstrates the partial underrunning of the hoof horn visible when flexed downwards - it is still attached plantarly. The lesion is purulent and the laminae are visibly blunted and rounded.

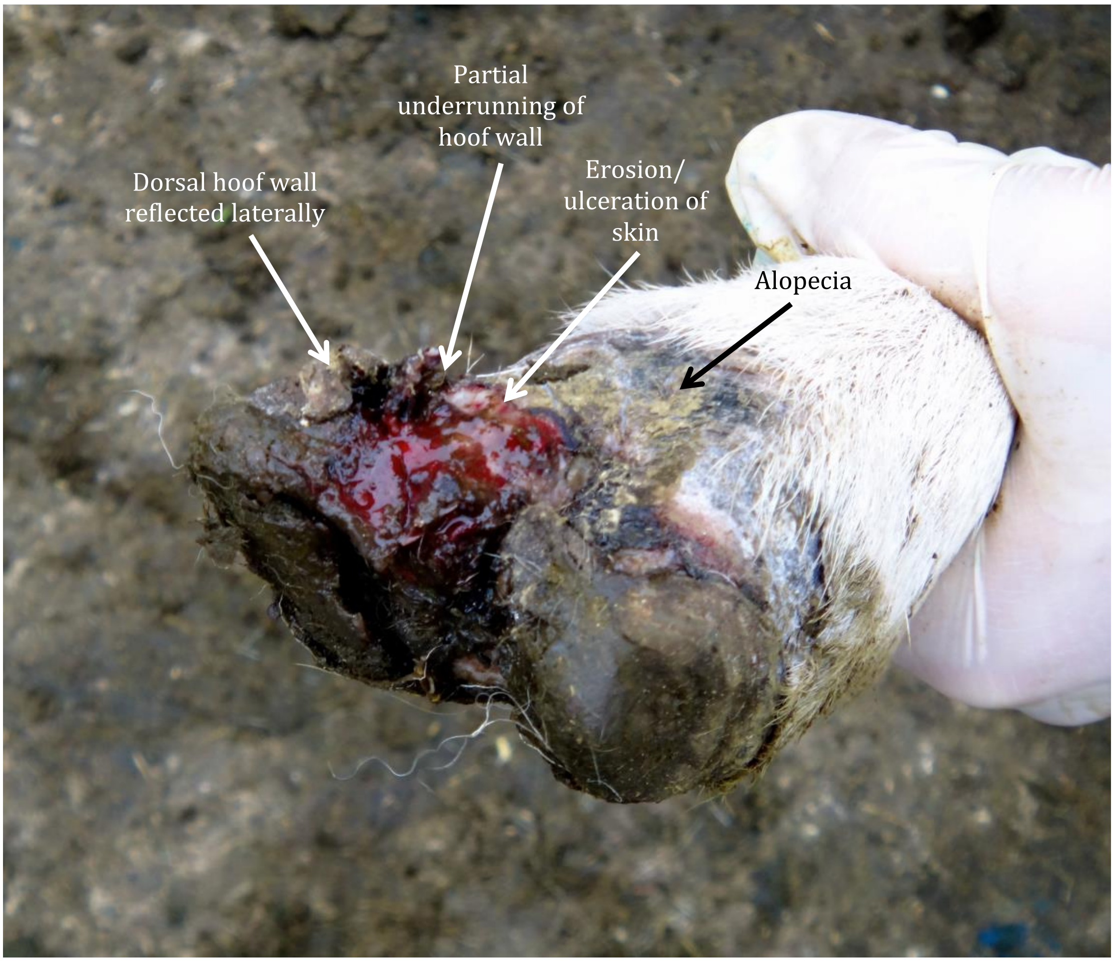

Figure 4: Grade 2 contagious ovine digital dermatitis (CODD) lesion, erosion/ulceration of the skin at the coronary band with partial (<50%) underrunning of the hoof horn dorsally and abaxially. There is alopecia proximal to the lesion and the dorsal hoof wall is missing being reflected laterally.

Figure 5: Grade 3 contagious ovine digital dermatitis lesion, erosion/ ulceration of the skin at the coronary band with >50–100% underrunning of the hoof horn with possible hoof horn evulsion. In this example the whole hoof horn has been avulsed, the digit is visibly swollen, the laminae appear grossly non-existent and the surface is purulent, bleeding easily if disturbed.

Figure 6: Grade 3 contagious ovine digital dermatitis lesion, erosion/ulceration of the skin at the coronary band with >50–100% underrunning of the hoof horn with possible hoof horn avulsion. In this example the whole hoof horn has been avulsed, the laminae appear grossly non-existent and the surface is roughened with adherent purulent material.

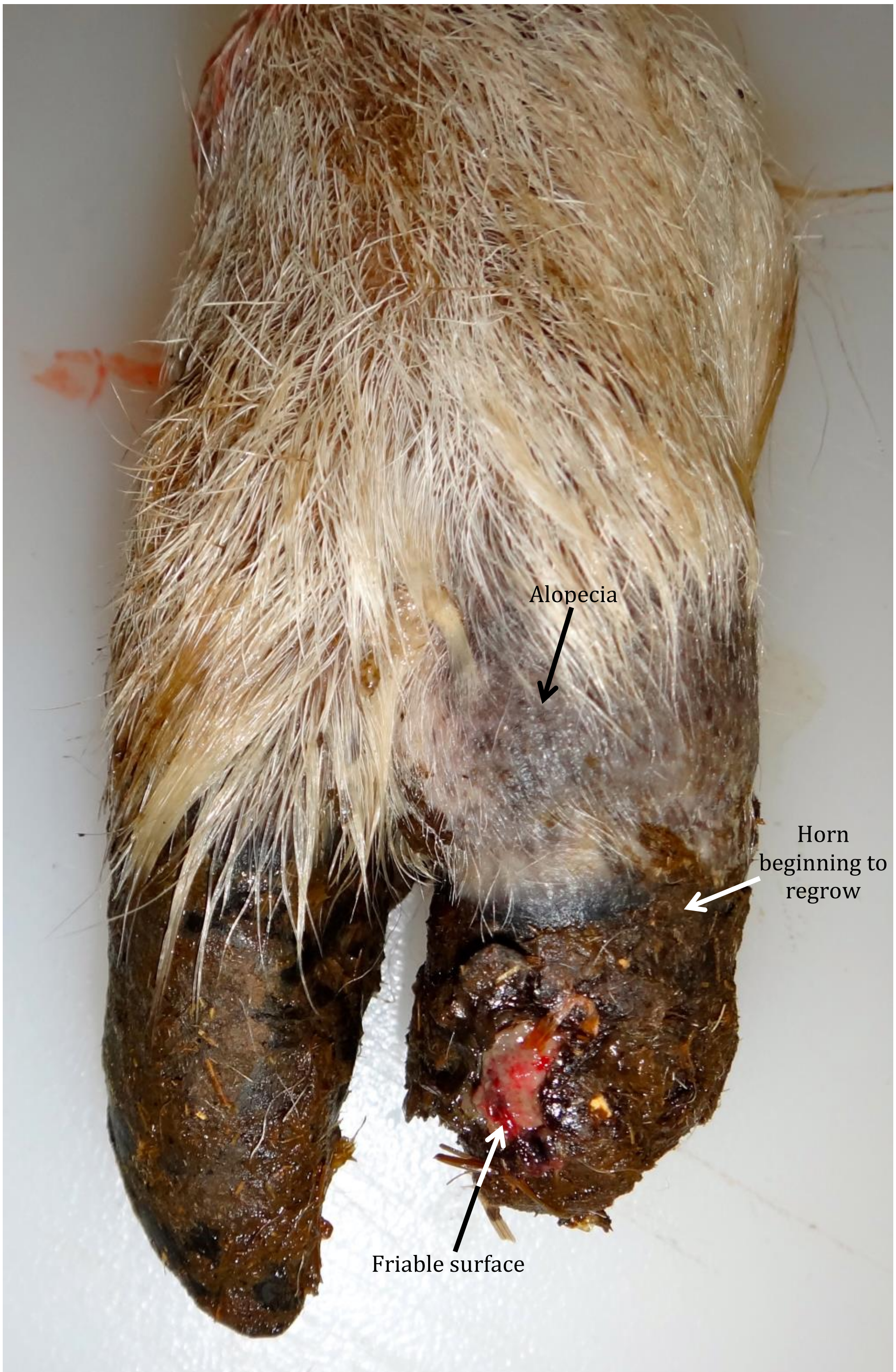

Figure 7: Grade 4 contagious ovine digital dermatitis lesion, healing foot with the horn beginning to regrow but an active lesion still present. This example demonstrates extensive alopecia proximal to the coronary band; the digit is visibly swollen and shortened. The hoof horn is beginning to regrow proximally but the uncovered surface is still very friable and bleeds easily if disturbed.

Figure 8: Grade 4 contagious ovine digital dermatitis lesion, healing foot with the horn beginning to regrow but an active lesion still present. The hoof horn has regrown covering about half of the digit. The digit remains shortened and the uncovered tissues still bleed if disturbed.

Figure 9: Grade 5 contagious ovine digital dermatitis lesion, healed foot, often with deformation of the regrown horn. The affected digit is shortened and wider than the unaffected and whilst the horn has regrown it is deformed by circumferential creasing.

Figure 10: Grade 5 contagious ovine digital dermatitis lesion, healed foot, often with deformation of the regrown horn. The digit is visibly swollen and shortened; the hoof horn has regrown but is grossly distorted.

Figure 11: Lateromedial radiograph of a digit with a grade 1 contagious ovine digital dermatitis lesion. No radiographic abnormalities are evident.

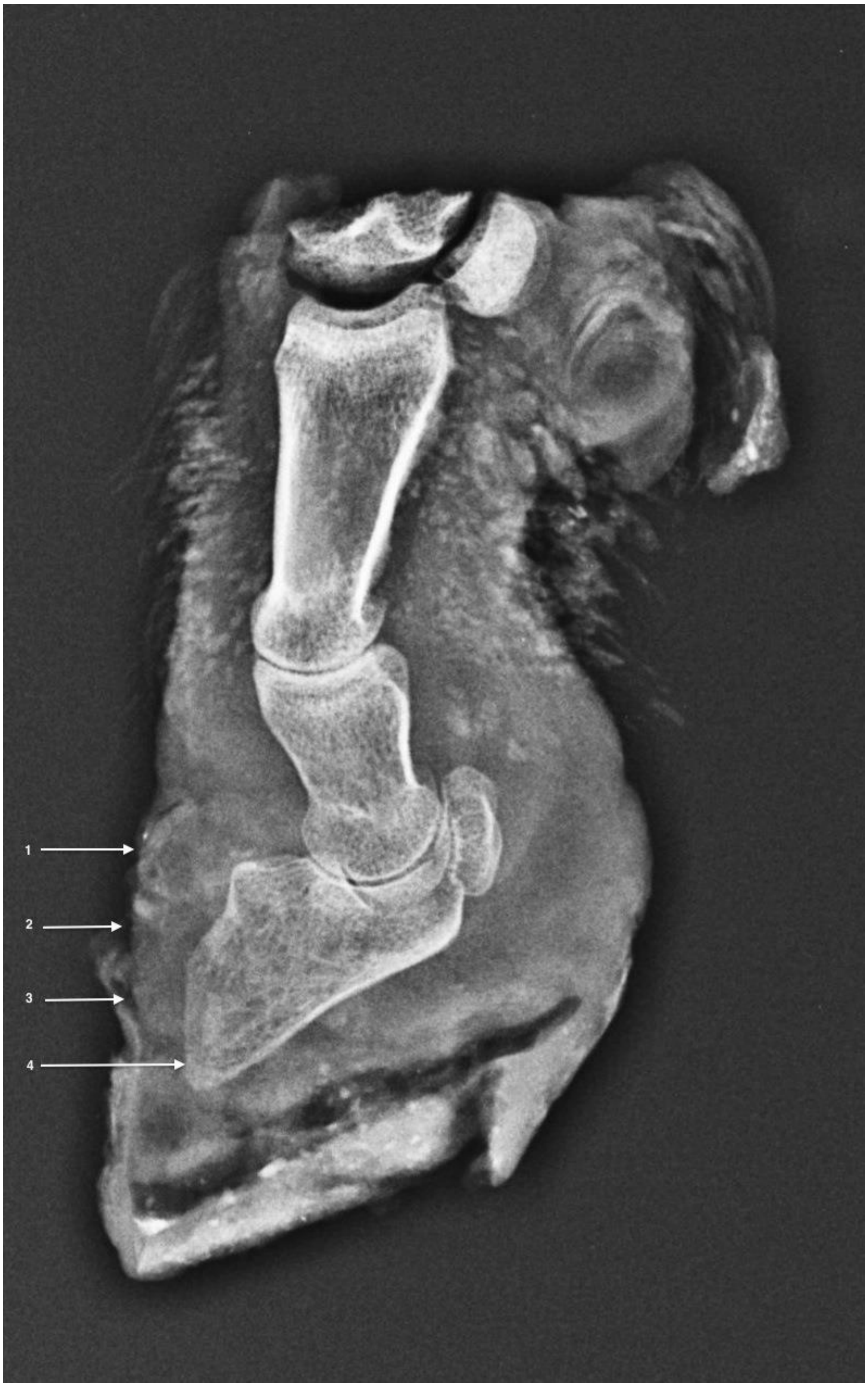

Figure 12: Lateromedial radiograph of a digit with a grade 2 contagious ovine digital dermatitis lesion. The lesion at the coronary band is evident as a decrease in radiodensity in the soft tissue structures at this point (1). There are hair-like radiolucencies evident within the soft tissues and the surface of the soft tissue appears roughened (2). The separation of the hoof wall from the underlying phalanx is clearly evident as a radiolucent line (3). The surface of the lamellae appears disrupted. There is some mild osteophytic change to the dorsal aspect of the distal phalanx (4).

Figure 13: Lateromedial radiograph of a digit with a grade 3 contagious ovine digital dermatitis lesion. The complete underrunning of the hoof horn is evident in both views. A radiolucent tract is visible from the coronary band to the dorsal aspect of P3 (1). There is mild osteophytic reaction to the dorsal aspect of P3 (2). The hoof horn appears avulsed (3).

Figure 14: Lateromedial radiograph of a grade 4 contagious ovine digital dermatitis lesion. The soft tissue surrounding the distal aspect of P3 appears to have reduced radiodensity (1). The dorsal and distal aspects of P3 demonstrate extensive demineralisation (2) to the extent that there appears to be a section missing from the dorsal and distal aspect of P3 (3).

Figure 15: Lateromedial radiograph of a grade 5 contagious ovine digital dermatitis lesion. The soft tissue structures surrounding the distal aspect of the foot generally appear of more uniform soft tissue density compared with the grade 4 foot (1), although the distance between the outer margin and P3 appears reduced when compared with the grade 1 foot (2). Osteophytic change is evident on the dorsal aspect of P3 (3).

The locomotion scores of sheep affected by CODD

The locomotion score for affected sheep increased with the grade of CODD, corresponding with a greater degree of underrunning of the hoof horn (Table 2 and Figure 16). Once the hoof capsule had been shed and healing had begun (grade 4) the locomotion score for affected sheep decreased, although sheep with a healed lesion (grade 5) were sometimes still lame. Sheep with more than one foot affected demonstrated a median locomotion score of 2 (IQR 1-2 [range 0-3]). Sheep with all grades of CODD could demonstrate sound gait or severe lameness, and overall, of the 908 sheep with CODD, 585 (64.5% [95%CI: 61.4-67.6]) were classified as being lame.

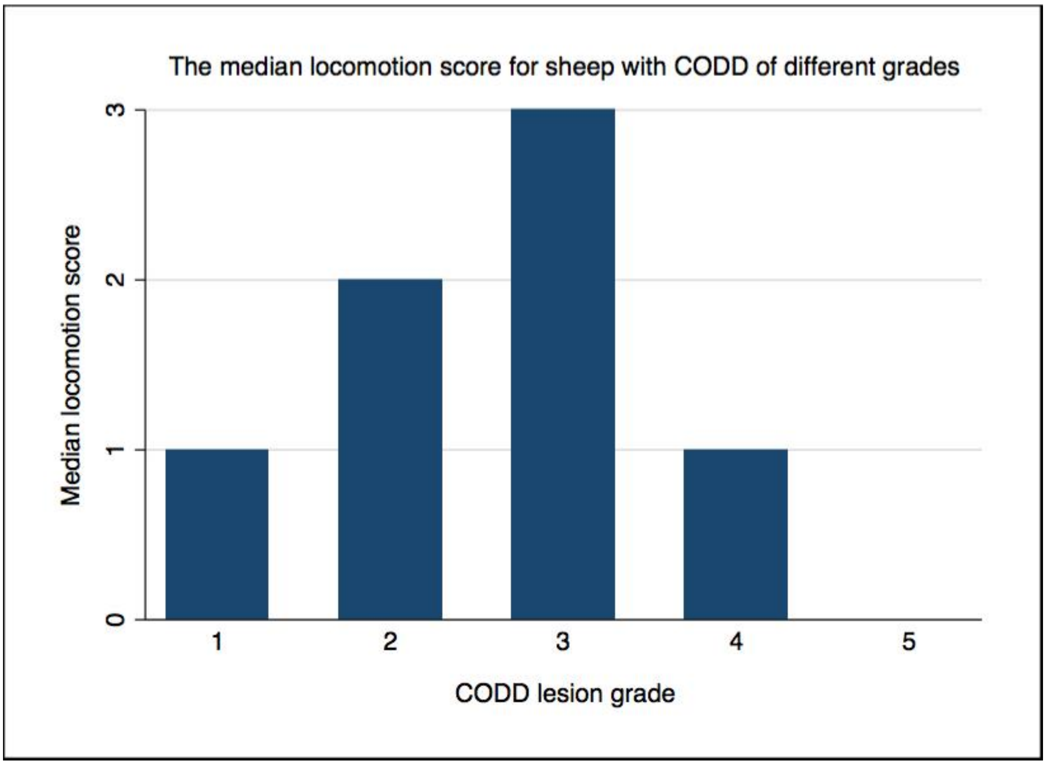

Figure 16: Bar chart of contagious ovine digital dermatitis (CODD) grade by median locomotion score, of 908 sheep with CODD on six farms in North Wales.

Discussion

This paper provides a thorough, systematic and detailed description of the clinical and radiographic presentations of CODD. It documents the range of disease presentation observed on six farms over one year and a grading system is proposed by which veterinary practitioners and researchers may describe the clinical signs more consistently. It also provides some radiographic evidence of the changes in feet with CODD at each grade, and using locomotion scoring quantifies the welfare impact to sheep that this disease causes.

Clinical description and grading system

Early in the identification of CODD there was some confusion as to the demarcation of CODD from other differentials (Davies et al., 1999; Lewis, 1999) in particular footrot. Footrot starts as inflammation of the interdigital skin and may progress axially to under-running of the hoof horn medially and in extreme cases lead to shedding of the hoof horn capsule (Winter, 2004a, 2008). It is also possible to observe mixed infections with lesions showing characteristics of both CODD and footrot in some feet (Duncan et al., 2012), making diagnosis difficult. Anecdotally there has also been some confusion with cases of Strawberry Footrot, foot abscesses and joint infections. Due to this, careful diagnostic evaluation of foot lesions is required, and in diagnosing CODD in a flock the authors prefer to examine a number of affected sheep with a range of clinical presentations. As such the detailed description of lesions here and the grading system should aid in the diagnosis of CODD and in differentiating it from other lesions.

In this study, the distribution of lesions was not even - there being more grade 2 and 5 lesions represented and fewer grade 1 lesions. This may be due to the sampling strategy of preferentially selecting lame sheep for examination, or as is suspected, if the lesion grades form a continuum this may reflect that some stages of the disease progress more rapidly than others. Repeated observation of individuals with CODD over time may help to understand this.

For some time BDD and CODD have been hypothesized to be the same disease but in different species (Demirkan et al., 2001; Dhawi et al., 2005; Duncan et al., 2014). However, the typical clinical picture as evidenced here in sheep is strikingly different from that for cattle. As such, any detailed comparisons between lesion scoring systems for cattle e.g. Dopfer et al. (1997) are difficult.

Radiographic changes

Given the small sample size, the radiographic changes presented here are examples only and may not be representative of all feet at each grade. In these examples, in early CODD lesions (grade 1) very little radiographic changes were evident. As the hoof horn was under-run, the damage to the foot became not only more extensive but progressed deeper into the tissues as well, leading to inflammatory bony changes on P3 and in some cases leading to extensive damage to P3.

These radiographs highlight the possible extent of the disease if it is unchecked, and may provide some explanation for why sheep can remain lame once healing has occurred. As treatment strategies are developed, these should focus on early identification of CODD and early intervention - so that the progression of the disease may be halted before it leads to extensive and potentially irreversible damage. It would also suggest that systemic rather than topical treatments may be more appropriate, particularly for grade 2 -4 lesions due to the depth of the inflammatory changes and the possible penetration of infection to the deeper tissues.

Further work would examine the pathological and histopathological changes of the feet at these various grades to understand the mechanisms and pathophysiology of the disease better.

Lameness

The changes in locomotion in affected sheep suggest pain is likely to be associated with CODD lesions, indeed there is an association between the locomotion score and the grade of disease. All sheep were locomotion scored during handling in pens in order to examine the sheep, and prior to foot examination to reduce bias. It is well known that on the basis of their status as a prey species, sheep can reduce the behaviours associated with pain when handled (Fitzpatrick et al., 2006; Phythian et al., 2013) and so in a field situation sheep may appear more lame than when gathered and handled. Unfortunately, due to practical constraints it was not possible to locomotion score individual sheep undisturbed in a field and then subsequently grade the lesion.

Grade 1 CODD lesions tended to show only mild lameness and sheep with these lesions may be difficult to observe when standing or walking, particularly in a group situation or when handling sheep in pens (median locomotion score 1).

In Angell et al. (2014) 47.3% (95%CI: 40.0-54.6%) of farmers reported that they did not know how CODD had arrived on their farm. However, a further 43.5% (95%CI: 36.3-50.8%) reported that they thought CODD was introduced into their flocks following the purchase of infected sheep. It is therefore possible that sheep with a grade 1 lesion may not be noticed until infection has already spread to the rest of the flock. This has important implications for flock biosecurity. Close inspection of the feet of all purchased animals, together with a period of isolation away from the rest of the flock would be recommended in order to identify any mild lesions that are not causing any locomotor changes. This would seem particularly important for naïve flocks due to the contagious nature of the disease (Harwood et al., 1997; Davies et al., 1999), the high prevalence seen on some farms (Angell et al., 2014) and the current lack of effective treatment strategies.

Grade 1 lesions appeared to progress to grade 3 over time, and sheep with these lesions appear severely lame (median locomotion score 3). It is the authors' view that this is likely to be due to the movement of the horn causing pinching of the underlying soft tissue, and the associated laminitis.

As would be expected, a greater degree of under-running led to increased locomotion scores with CODD grade 3 lesions showing increased scores compared to grade 2 lesions. Shedding of the hoof horn led to a reduction in locomotion score which may indicate that this helped alleviate some of the pain. Interestingly all six farmers observed that sheep tended to recover faster if the underrun hoof horn was gently and carefully removed by trimming, taking care to avoid damage to the underlying soft tissue structures. This is consistent with other anecdotal observations of the disease (Harwood et al., 1997).

During recovery the hoof horn regrew, although there was usually some deformation of the new horn growth and the affected digit was appreciably shortened. This suggests that the damage to the coronary band was not total thus allowing the horn to regrow. It is notable that some of the sheep with healed lesions remained lame, which may be due to damage to the deeper structures including the distal phalanx. Interestingly all the farmers reported that some individuals were lame for many weeks after regrowth and this may suggest irreversible damage to underlying structures or damage to nerve endings resulting in hyperaesthesia and allodynia (Lumb and Jones, 1996), and is consistent with observations made by Scott (2010).

Conclusion

Contagious ovine digital dermatitis is a severe and debilitating disease of sheep that has a range of clinical signs. Lesions commence at the coronary band leading to underrunning of the hoof horn capsule dorsally and abaxially resulting in sloughing of the hoof horn capsule. Horn regrowth and recovery is possible although sheep may remain lame. The disease can cause severe damage to the soft tissues of the ovine foot and can lead to bony changes in the distal phalanx. Effective treatment and prevention strategies are urgently warranted in order to improve the welfare of sheep and the profitability of affected sheep farms.

Acknowledgements

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Chapter 5

Histopathological Characterization of the Lesions of Contagious Ovine Digital Dermatitis and Immunolabelling of *Treponema*-like Organisms

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Abstract

Contagious ovine digital dermatitis (CODD) is a cause of severe lameness in sheep and the three *Treponema* phylogroups *Treponema medium/Treponema vincentii*-like, *Treponema phagedenis*-like and *Treponema pedis* have been associated with clinical disease. The aims of this study were: (1) to describe the histopathological changes associated with each previously established grade of clinical lesion, and (2) to investigate immunohistochemically the association of the *Treponema*-like organisms with the observed histopathological changes. Early lesions were characterized by lymphoplasmacytic infiltration of the distal digital skin, with suppurative coronitis and intracorneal pustules. In more advanced stages of the disease there was complete separation of the dorsal wall of the hoof with a necrotizing and fibrinosuppurative exudate and dermatitis. The later lesions were mostly resolved, but with milder suppurative changes remaining within the cornified layer and periosteal reaction of the dorsal aspect of the distal phalanx. Large numbers of *Treponema*-like organisms were identified within early grade lesions (as well as later, more advanced grade lesions) and were specifically associated with the observed histopathological changes. The results of this study provide some evidence in support of the hypothesis that the three CODD-associated *Treponema* phylogroups are involved in the aetiopathogenesis of this disease.

Introduction

In 1997, a newly emerged disease of the ovine digit was observed in the UK, the lesions of which led to severe clinical signs in affected animals (Harwood et al., 1997). The distinction between this disease and ovine foot rot was made by the differences in clinical appearance, the failure to respond to conventional footrot therapies, and the isolation of *Treponema* spp. from the lesions; with and without concurrent detection of the causative agent of foot rot, *Dichelobacter nodosus* (Naylor et al., 1998; Davies et al., 1999; Lewis et al., 2001). The disease was named contagious ovine digital dermatitis (CODD) (Davies et al., 1999) and further work has since identified three *Treponema* phylogroups to be associated with clinical disease, namely *Treponema medium*/*Treponema vincentii*-like, *Treponema phagedenis*-like and *Treponema pedis* (Sullivan et al., 2015). These same three *Treponema* phylogroups are reported to be the cause of bovine digital dermatitis (BDD) (Evans et al., 2008), although not all BDD lesions may necessarily contain all three phylogroups (Evans et al., 2009c).

Recently, the clinical presentation of CODD was described in detail for the first time, and a five-point lesion grading system was proposed (Angell et al., 2015a). In that study, the range of lesions was organized into a clinical progression of disease such that lesions progressed from grade 1 to 5. Briefly, the disease begins at the coronary band and extends distally, resulting in progressive, and eventually total, separation of the dorsal hoof wall. The lesions may resolve and new horn eventually replaces the horn lost in the fulminant stage of the disease, with some deformity of the regrown hoof. To date, there has been no histopathological characterization of these lesions or immunohistochemical investigation of the presence of *Treponema* spp. associated with the pathological changes.

In cattle, immunohistochemistry (IHC) has been used to identify *Treponema*-like organisms in BDD lesions, and these are associated with hair follicles and sebaceous glands (Evans et al., 2009c). In the present study, IHC was used to investigate the presence/absence of CODD-associated *Treponema* spp. It was considered that *Treponema* spp. would more likely be identified in earlier grades of lesion, particularly those of grade 1. The organisms may persist throughout all of the stages of the clinical disease, but might not be identifiable due to competition by secondary invaders, tissue necrosis or damage/removal by the immune system.

The aims of this study were: (1) to describe the histopathological changes observed for each grade of clinical lesion and to investigate whether these grades reflected a progression of disease, and (2) to investigate whether the spirochaetes of the three phylogroups *T. medium/T. vincentii*-like, *T. phagedenis*-like and *T. pedis* were associated with clinical disease.

Materials and Methods

The study protocol was approved by The University of Liverpool ethics committee (VREC 13) on 24th August 2011.

Sampling

In a previous study, a novel five-point lesion grading system was proposed to describe clinical cases of CODD (Angell et al., 2015a). In that study, clinical cases deemed typical of each lesion grade were obtained and the digits were sampled *post mortem* for further radiographic analysis. These digits were removed immediately *post mortem* and placed in 10% neutral buffered formalin. These same digits were used in the current study. In addition, two sheep were purchased from a farm with no clinical history or evidence of CODD and digits from these were used as negative controls, together with interdigital skin from the footpad of a dog with exudative interdigital pyoderma.

Fifteen digits were used, including the two normal digits from the two negative control sheep, a clinically normal digit from a sheep that had CODD in other digits in other feet, five digits with lesions of clinical grade 1, two digits with grade 2 lesions, two digits with grade 3 lesions, one digit with a grade 4 lesion and two digits with grade 5 lesions.

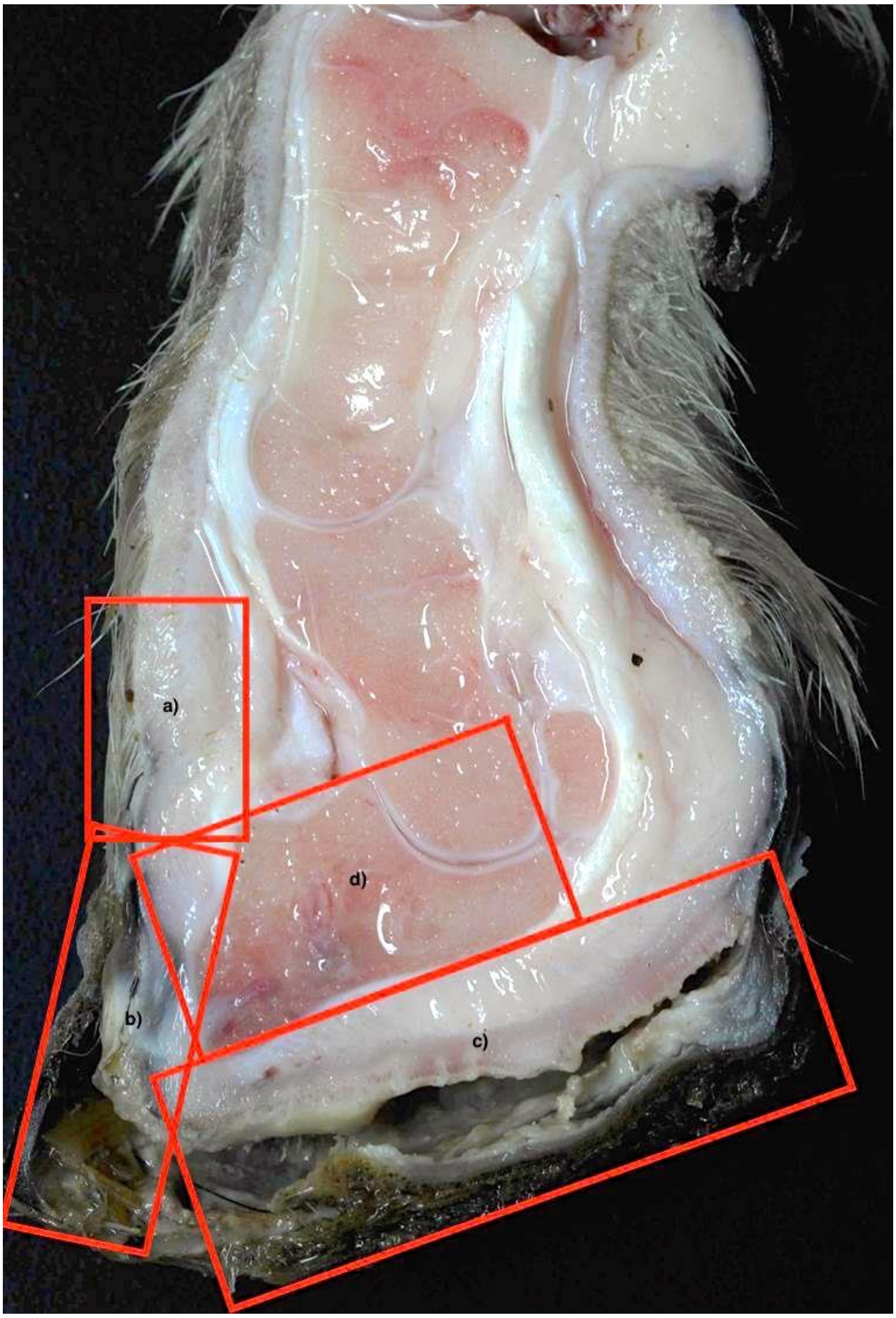


Figure 1: Sagittal section from an ovine foot with CODD, illustrated to indicate the sites from which tissue samples were obtained including: a) distal digital skin including coronary band; b) dorsal horn with laminae; c) solar horn with laminae and d) the distal phalanx.

Histopathology

Each digit was sectioned sagittally to obtain a longitudinal slice of approximately 3–5 mm incorporating hoof horn, phalangeal bones and soft tissues up to and including the proximal phalanx. In all cases the following tissue samples were obtained (Figure 1): (1) distal digital skin including the coronary band, (2) dorsal horn with laminae, (3) solar horn with laminae, and (4) the distal phalanx. These tissues were embedded in paraffin wax. Blocks containing horn underwent a softening pretreatment with hair removal lotion (Nair Hair Removal Lotion; Church & Dwight, Folkestone, UK) and blocks containing bone were decalcified using a decalcification product (Calci-Clear; National Diagnostics, Hessle, UK), respectively. These were then sectioned into 5 μm slices and stained with haematoxylin and eosin (HE). A selection of tissues from different digits with necrosuppurative lesions with numerous bacterial colonies were also stained by the Warthin–Starry method.

Immunohistochemistry

IHC was employed to investigate the presence of the three CODD associated *Treponema* spp. in tissue samples using a polyclonal antibody raised in a rabbit (Evans et al., 2009c). Sections were dewaxed using a series of xylene and ethanol baths followed by pretreatment with 6ml of 30% H_2O_2 with 360ml of 100% methanol to block endogenous peroxidase activity. Antigen retrieval was performed using bacterial proteinase type XXIV (Sigma; Poole, Dorset, UK) for 5 min before blocking with normal goat serum for 10 min. The primary antibody (1 in 4,000 dilution) was incubated overnight and then slides were washed and incubated with secondary goat anti-rabbit antibody (1 in 100 dilution) for 30 min before being washed again. An avidin–biotin complex method was then used to detect binding. The slides were then counterstained with haematoxylin. Following optimization and examination under low power to survey tissue areas, a grading system of no labelling (0), mild (1), moderate (2) or strong (3) labelling was used to give an indication of the varying intensity of the *Treponema* spp. antigen detection. None of the control tissues exhibited any positive immunolabelling and the bacteria in the canine skin sample were not labelled.

Results

Details of each lesion are summarized in Table 1; key features from each clinical lesion grade are summarized as follows.

Clinically normal digits and negative controls

There was mild eosinophilic and lymphoplasmacytic dermatitis of the distal digital skin. No significant change was observed in the hoof. There were no organisms detected by Warthin–Starry stain and there was no specific immunohistochemical labelling. Representative images are shown in Figure 2.

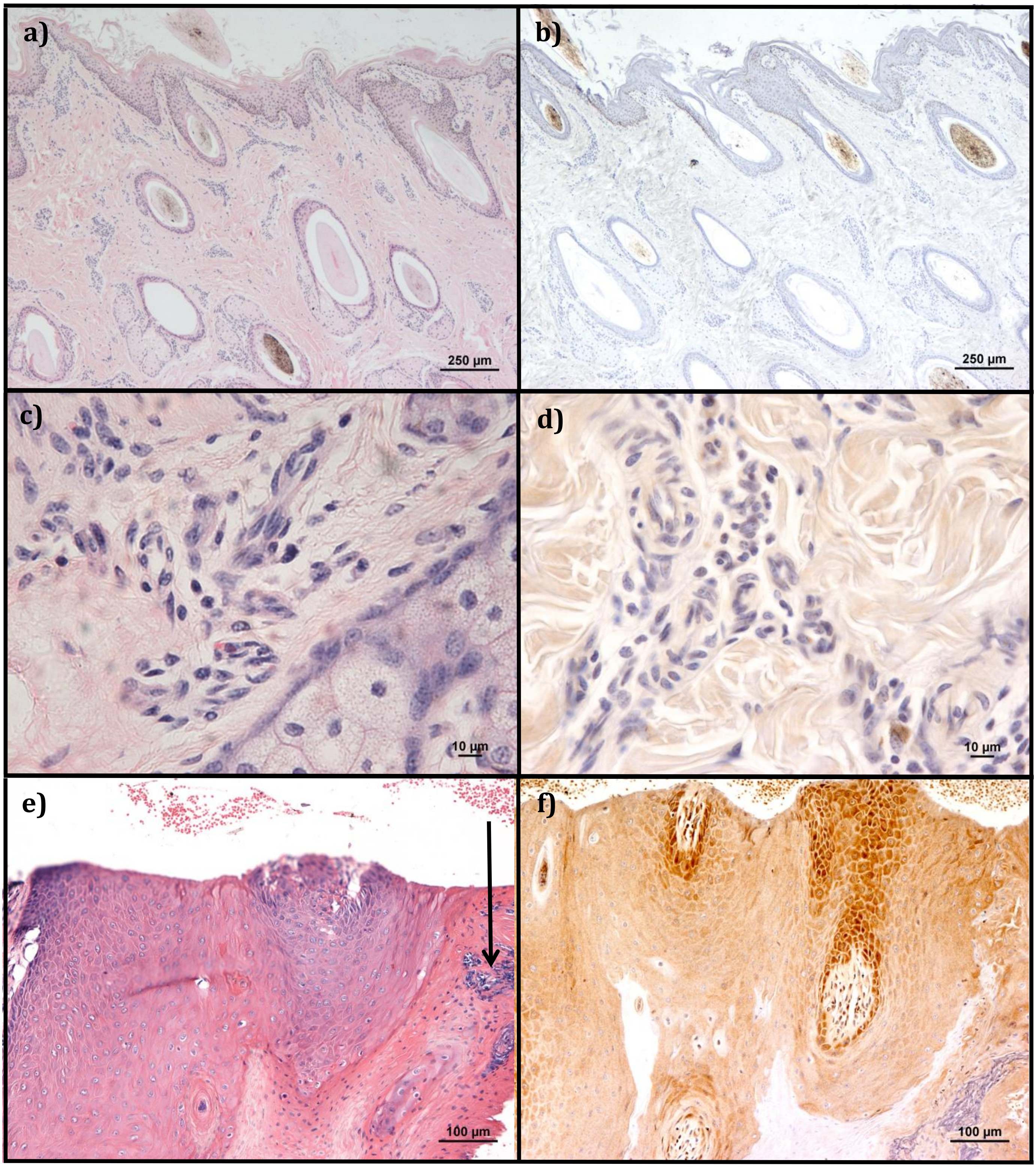


Figure 2: Skin from a clinically unaffected sheep from a farm without CODD (a – d) and tissue from a canine interdigital skin with an exudative pyoderma (e and f): H & E and *Treponema* spp. IHC

a) H & E, mild perivascular and periadnexal dermatitis; b) IHC, demonstrating very mild background immunolabelling; c) H & E, perivascular lymphoplasmacytic dermatitis; d) IHC, very mild background labeling e) H & E, epidermis of a canine interdigital skin with bacterial colonies to the right of the image (arrow); f) IHC, intracytoplasmic granular background labeling of epidermal keratinocytes together with unlabeled bacteria.

Clinical Grade 1

The distal digital haired skin and coronary band exhibited mild to severe, multifocal, periadnexal to perifollicular lymphoplasmacytic infiltration within the superficial dermis, in some cases extending into the deep dermis. In some digits there was moderate epidermal hyperplasia and orthokeratotic and/or parakeratotic hyperkeratosis. Bacterial colonies of variable morphology, with haemorrhage and an infiltrate of neutrophils, were sometimes present at the coronary band. At the coronary band and distally in the superficial dorsal hoof horn there was strong extracellular immunolabelling. Often there was strong and specific immunolabelling of organisms with clear *Treponema* spp. morphology, admixed with the neutrophilic infiltrate, often in the hyperkeratotic regions, at the sites of hoof wall detachment and between the cells of the stratum corneum and stratum spinosum (Figure. 3).

Within the subcorneal epidermal laminae there was mild to severe, multifocal, dermal perivascular lymphoplasmacytic infiltration, sometimes with pustules associated with degenerate keratinocytes. In some digits the proximal dorsal hoof horn (stratum corneum) had become detached from the remainder of the epidermis. Where the horn had separated, the eroded epidermis was lined by a superficial layer of proteinaceous debris, degenerate neutrophils, necrotic keratinocytes and bacterial colonies of variable morphology. The neutrophils extended into the remaining epidermis and were present in the dermal and epidermal laminae.

In the regions of epidermal erosion and suppurative inflammation, superficially there were large, multifocal areas of strong immunolabelling with large numbers of organisms exhibiting clear *Treponema* spp. morphology. These were located extracellularly between the (sometimes sloughed) keratinocytes of the stratum corneum and stratum spinosum. Occasionally, there were small areas of strong extracellular labelling with several organisms exhibiting *Treponema* spp. morphology in the deeper epidermis. Representative images are shown in Figure 3.

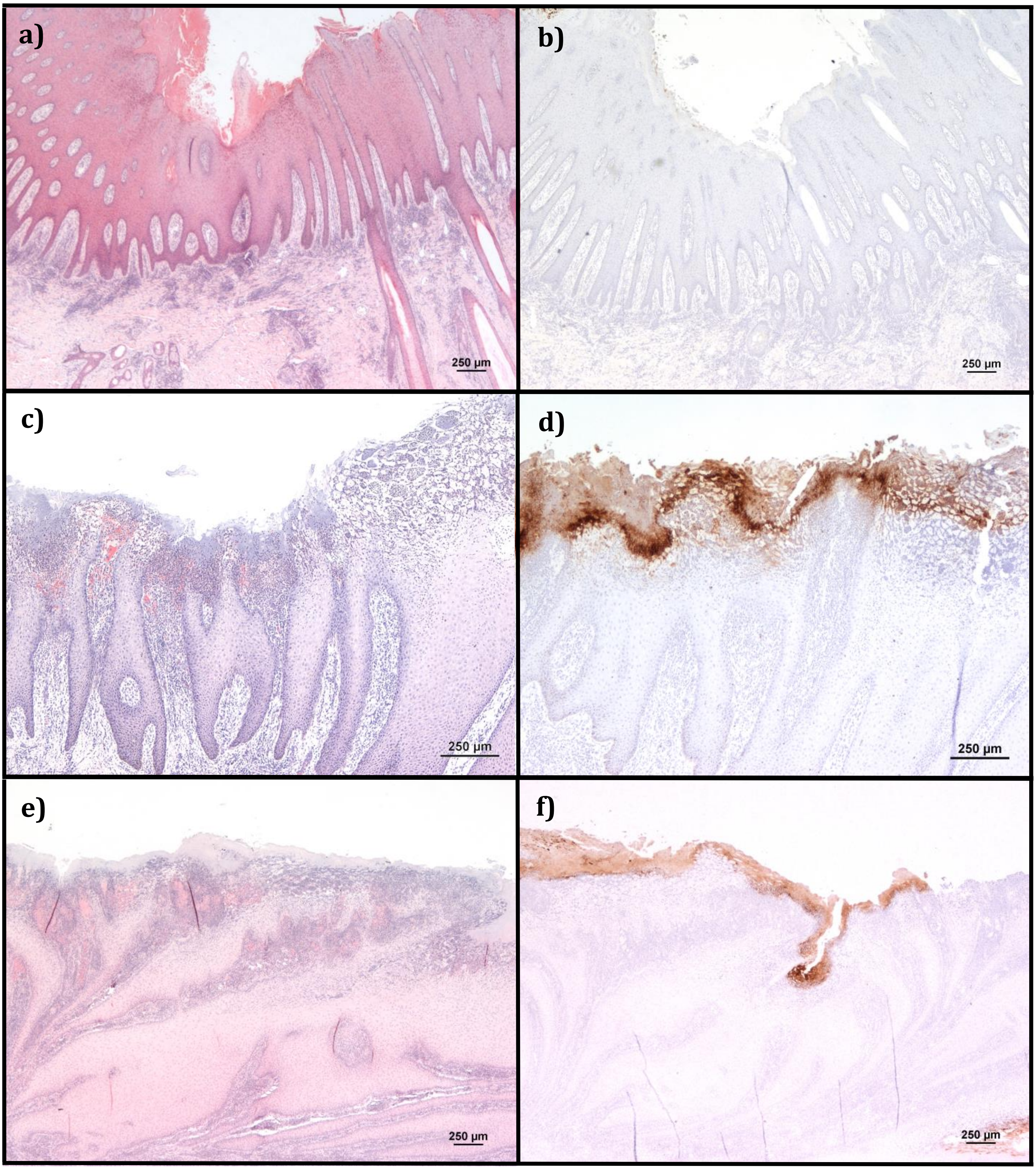


Figure 3: Tissues from a clinical grade 1 CODD lesion: H & E and *Treponema* spp. IHC.

a) H & E, skin/coronary band with moderate, multifocal, periadnexal/perifollicular lymphoplasmacytic dermatitis with hyperplasia and orthokeratotic hyperkeratosis (left of the image) b) IHC, very mild background immunolabelling in the superficial stratum corneum; c) H & E, diffuse severe erosion of the dorsal horn with suppurative inflammation, haemorrhage and oedema; d) IHC, mild extracellular labelling of the exudate with strong labelling of the superficial eroded dorsal horn; e) H & E, superficial inflammatory exudate and intracorneal pustules in the dorsal horn adjacent to the coronary band; f) IHC, extracellular labelling of eroded material of skin/dorsal horn at coronary band region, together with immunopositive labelling within a fissure extending deeper into the epidermis, the IHL becoming more intense at the deepest point.

Clinical Grade 2

Within the distal digital haired skin there was moderate to severe epidermal hyperplasia with rete ridge formation and orthokeratotic hyperkeratosis. There was moderate, multifocal to diffuse, lymphoplasmacytic infiltration of the superficial and mid dermis, becoming progressively more severe towards the coronary band. No immunolabelling was observed in the distal digital skin.

There was complete loss of the superficial cornified layer (horn) of the proximal dorsal hoof wall, with replacement by a band of degenerate neutrophils, fibrin, necrotic debris, haemorrhage and numerous colonies of coccobacilli. The epidermal laminae were markedly infiltrated by the suppurative inflammatory process, which extended into the dermal laminae becoming mixed with a moderate to severe lymphoplasmacytic infiltrate. In the superficial stratum corneum there were numerous groups of bacteria with coccoid, rod and spirochaetal morphology.

There were large multifocal to coalescing areas of strong extracellular immunolabelling in the eroded dorsal horn, which extended to the dorsal laminae and was admixed with large numbers of positively labelled organisms with clear *Treponema* spp. morphology. This was mostly observed superficially in areas of suppurative inflammation, but strong extracellular immunolabelling was also found deep within the horn.

In the caudal solar horn and laminae there was background mild lymphoplasmacytic infiltration throughout the superficial dermis. In the more distal sections, the horn was separated from the remainder of the epidermis. Superficially, there was marked infiltration of neutrophils with fibrin, necrotic debris and haemorrhage. The dermal laminae were similarly infiltrated by neutrophils, mixed with lymphocytes and plasma cells. Subjacent to the epidermis was a lymphoplasmacytic band with fewer neutrophils, throughout the superficial dermis and almost obscuring the dermo-epidermal junction. There was a distinct boundary (distal to caudal) between the severely affected tissue and relatively mildly affected tissue, which exhibited an intact cornified epidermis and mild lymphoplasmacytic and very mild suppurative dermatitis of the dermal laminae. No immunolabelling was observed in the caudal and solar horn/laminae.

In the distal phalanx, in one digit, the cambium layer of the periosteum subjacent to the dermal inflammation consisted of a continuous row of plump (activated) osteoblast-like cells. There were no organisms detected in the distal phalanx by Warthin–Starry staining and no immunolabelling was observed. Representative images are shown in Figures 4 and 5.

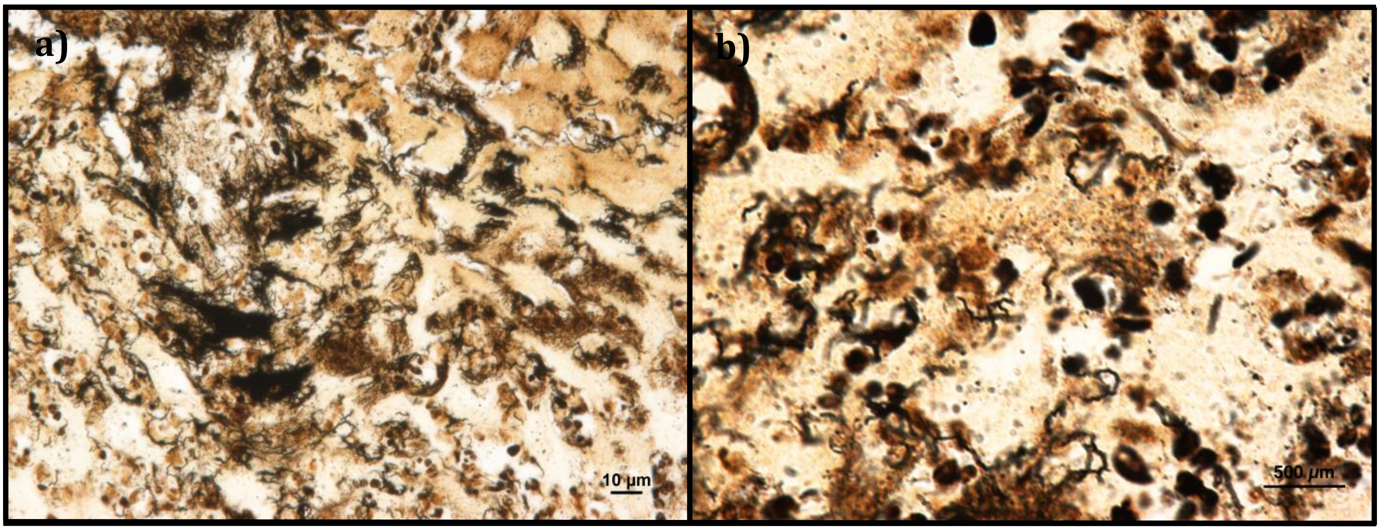


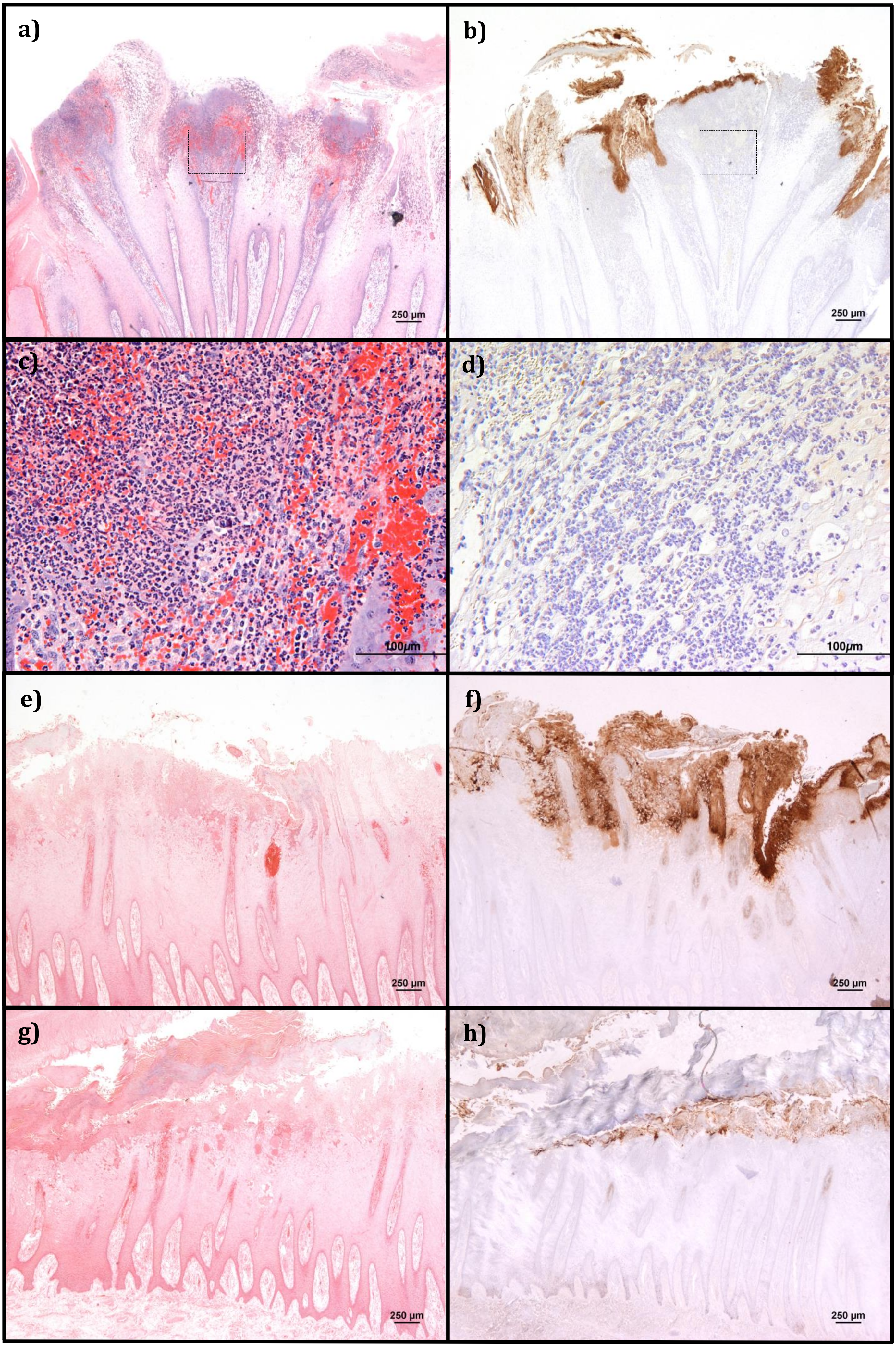
Figure 5: Tissues from a grade 2 and a grade 3 lesion stained with Warthin Starry:

a) WS, tissue from the dorsal horn from a grade 2 lesion demonstrating myriad bacteria, with many of spirochaetal morphology; b) WS, tissue from the dorsal horn from a grade 3 lesion clearly demonstrating bacteria with clear spirochaetal morphology.

There were large multifocal to coalescing, areas of strong extracellular immunopositive labelling in the eroded dorsal horn, which extended to the dorsal laminae admixed with large numbers of positively immunolabelled organisms exhibiting clear treponema morphology. This was mostly observed superficially in areas of suppurative inflammation but strong extracellular immunopositive labelling was also found embedded deep within the horn.

In the caudal solar horn and laminae there was a (background) mild lymphoplasmacytic infiltrate throughout the superficial dermis. In the more distal sections, the horn was separated from the remainder of the epidermis. Superficially, there was a severe infiltrate of neutrophils, fibrin, necrotic debris and haemorrhage. The dermal laminae were similarly infiltrated by neutrophils, mixed with lymphocytes and plasma cells. Subjacent to the epidermis was a lymphoplasmacytic band with smaller numbers of neutrophils, throughout the superficial dermis almost obscuring the dermo-epidermal junction. There was a distinct boundary (distal to caudal) between the severely affected tissue and relatively mildly affected tissue, which exhibited an intact cornified epidermis and a mild lymphoplasmacytic and very mild suppurative dermatitis of the dermal laminae. No immunopositive labelling was observed in the caudal and solar horn/laminae.

In the distal phalanx, in one digit, the cambium layer of the periosteum subjacent to the dermal inflammation consisted of a continuous row of plump (activated) osteoblast-like cells. There were no organisms detected in the distal phalanx using Warthin Starry staining and no immunopositive labelling was observed.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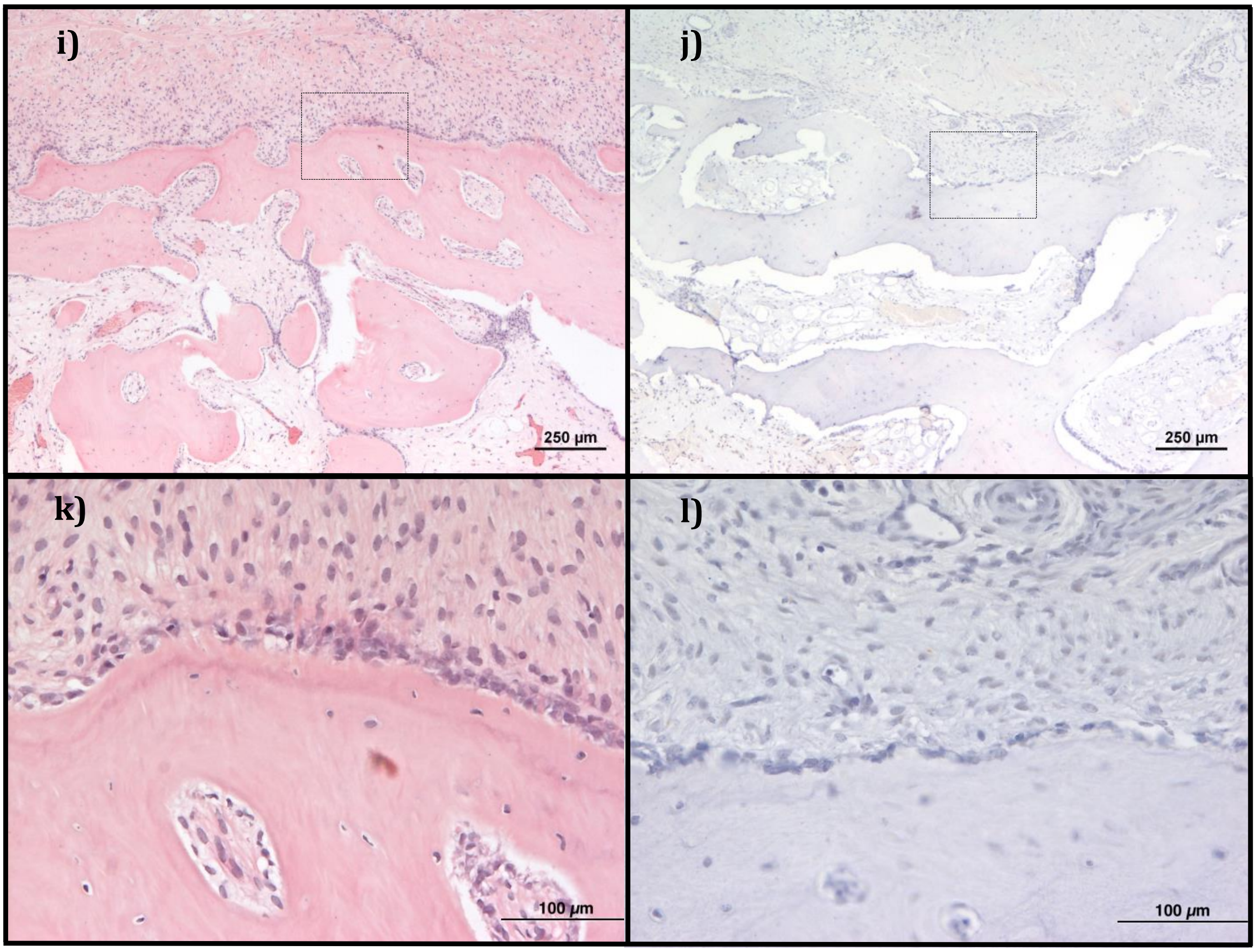


Figure 4**: Tissues from a clinical grade 2 CODD lesion: H & E and *Treponema* spp. IHC**

a) H & E, erosion of the dorsal horn with multifocal to coalescing suppurative infiltrates and haemorrhage; b) IHC, extracellular labelling in the exudate and the eroded dorsal horn, the immunolabelling is of greater intensity deeper into the fissures formed in the lesions extending deeper into the epidermis; c) H & E, increased magnification of boxed area of a); d) IHC, increased magnification of boxed area of b), there is only very mild background immunolabelling; e) H & E, erosion of the dorsal horn together with an exudate; f) IHC, extracellular labelling in the exudate of the eroded dorsal horn, together with immunopositive labelling within a fissure extending deeper into the epidermis – the immunopositive labelling is more intense at the deepest part of the fissure; g) H & E, partial separation of the superficial dorsal horn together with a superficial exudate; h) IHC, increased detail of the partial horn separation with immunopositive labelling between the separated layers where the dorsal hoof wall was detaching from the remainder of the epidermis; i) H & E, periosteal reaction of the distal phalanx; j) IHC, very mild background staining; k) H & E increased magnification of boxed area of i) demonstrating the activated osteocytes; l) IHC, no evidence of any immunolabelling.

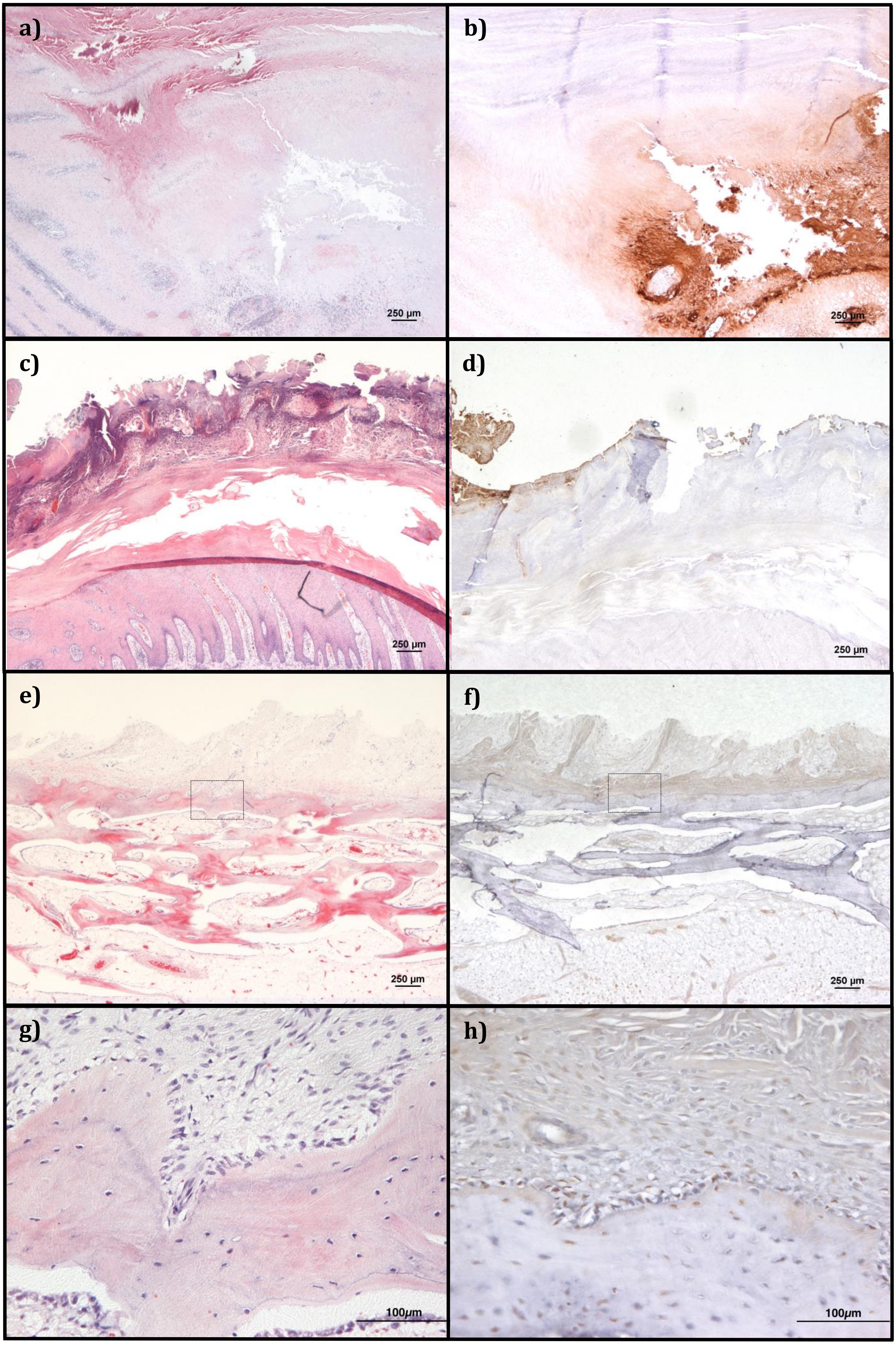
Clinical Grade 3

The histological appearance was similar to that of the grade 2 lesions, although there was moderate, homogeneous non-specific immunolabelling at the level of the coronary band.

The stratum corneum of the dorsal hoof horn was markedly and irregularly thickened where present, but in some areas was eroded or partially detached exposing the subcorneal layers of the epidermis, which was, in some areas, expanded by intracorneal pustules. The eroded tissues and subjacent dorsal laminae exhibited histological changes similar to those of the grade 2 samples, also demonstrating strong extracellular immunolabelling, but there were fewer organisms exhibiting clear *Treponema* spp. morphology.

The solar horn was thickened by moderate diffuse orthokeratotic hyperkeratosis. Within the solar laminae and subjacent dermis there was moderate, multifocal to coalescing lymphoplasmacytic infiltration. There was no evidence of suppuration within the solar horn or associated laminae or dermis.

The periosteum on the dorsal aspect of the distal phalanx was activated with mild to moderate new bone growth dorsally. Within the adjacent dermis there were mild lymphoplasmacytic infiltrates, together with variably sized collections of activated fibroblasts. No immunolabelling was present. Representative images are shown in Figure 6.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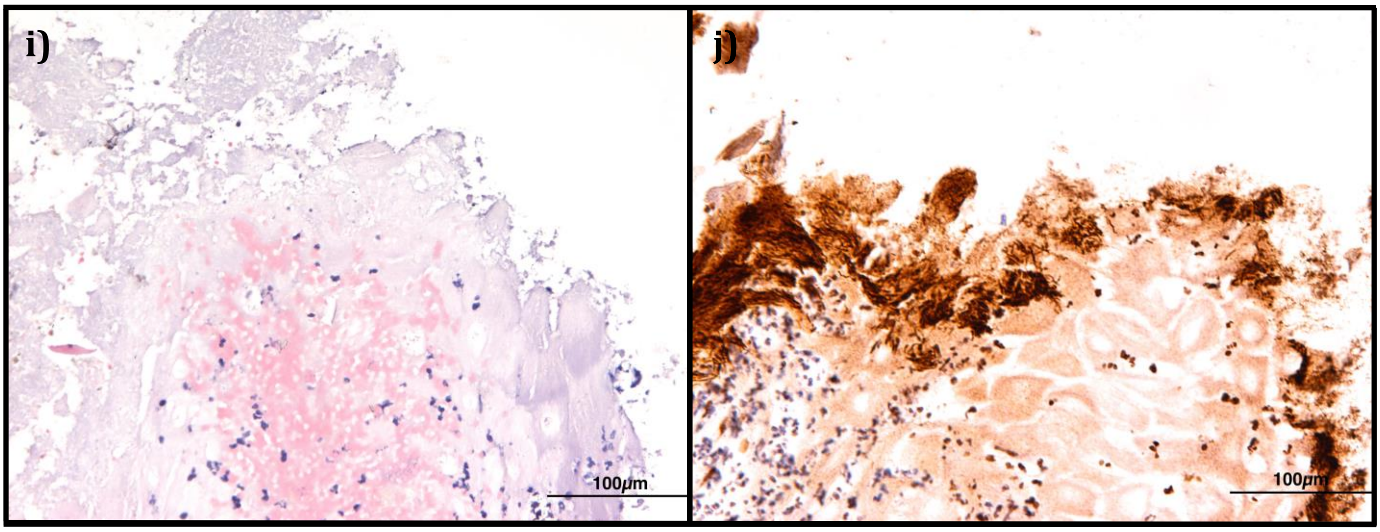


Figure 6: Tissues from a clinical grade 3 CODD lesion: H & E and *Treponema* spp. IHC

a) H & E, an intracorneal pustule in the dorsal horn; b) IHC, immunopositive labelling of an intra-corneal pustule within the dorsal horn; c) H & E, erosion of stratum corneum and separation of the dorsal horn containing intracorneal pustules; d) IHC, very mild immunopositive labelling of the eroded horn; e) periosteal reaction of P3, together with evidence of new bone growth; f) IHC, mild background immunolabelling of the distal phalanx and surrounding collagen; g) H & E, increased magnification of boxed area of e) showing periosteal reaction of the distal phalanx; h) IHC, increased magnification of boxed area of f) showing mild background immunolabelling; i) H & E, .superficial proteinaceous exudate admixed with neutrophils and bacteria; j) IHC, extracellular, immunopositive labelling of superficial exudate, with individuals and groups of immunopositive organisms of *Treponema* spp. morphology.

Clinical Grade 4

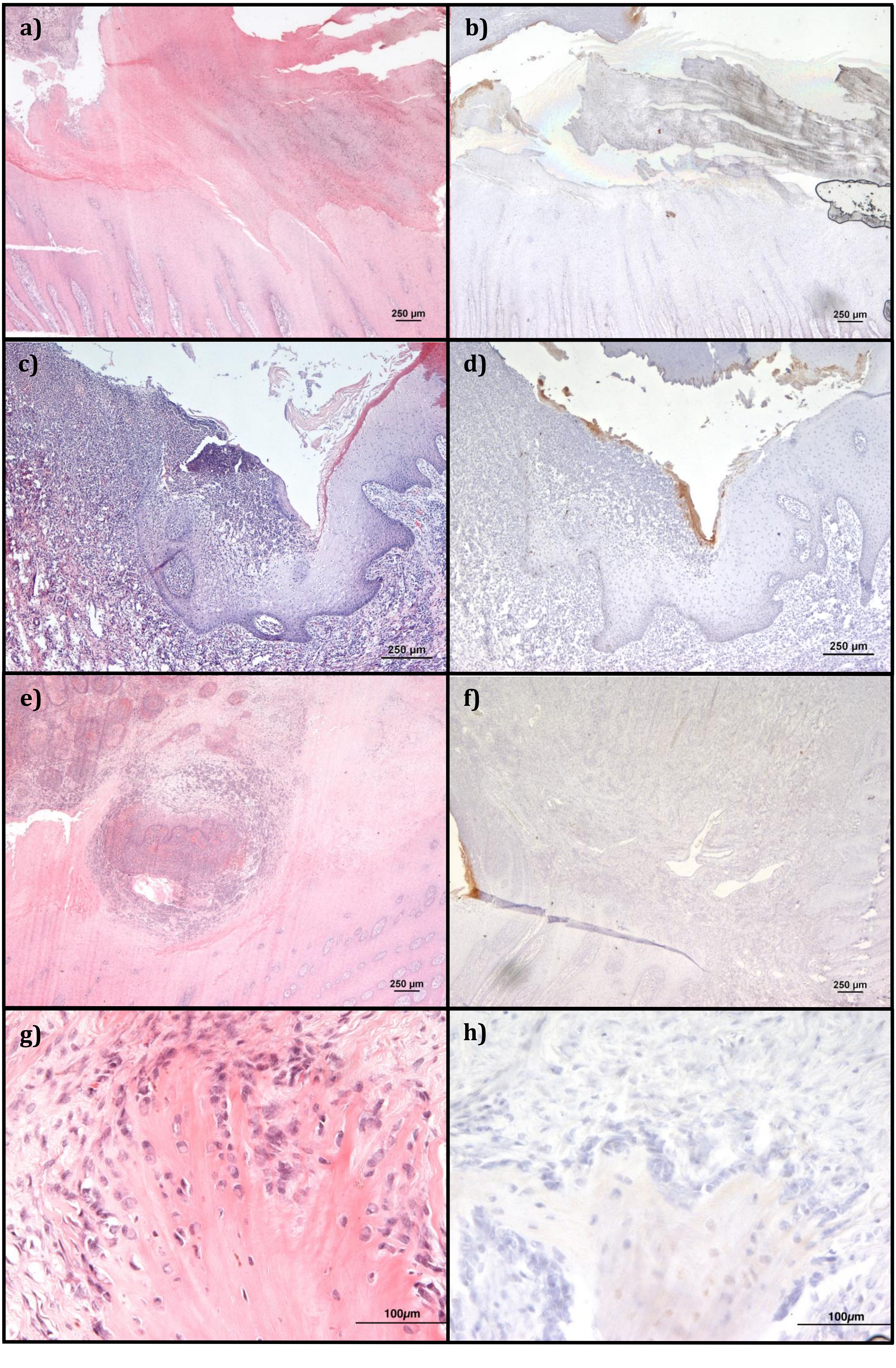
There was moderate epidermal hyperplasia and orthokeratotic hyperkeratosis of the distal digital haired skin. There was mild multifocal lymphoplasmacytic infiltration of the dermis, but the epidermis of the coronary band appeared unaltered. In the skin and coronary band there were numerous groups of bacteria with mostly coccoid and rod morphology superficially, but no immunolabelling was observed.

The superficial cornified layer of the dorsal hoof horn exhibited extensive areas of marked infiltration by neutrophils admixed with fibrin and necrotic debris, together with proliferation of well vascularized fibrous tissue (granulation tissue) with plump fibroblast and endothelial cell nuclei. Superficially within the suppurative and necrotic areas there were numerous groups of bacteria with mostly coccoid, rod and spirochaetal morphology, and there was strong extracellular immunolabelling and labelling of small numbers of organisms exhibiting clear treponemal morphology. These areas were not as extensive and there appeared to be fewer organisms than in grade 1 and 2 lesions, and there was no involvement of the solar horn and laminae. The dorsal epidermal laminae were hyperplastic and the dermal laminae and sub-epidermal dermis exhibited background mild multifocal lymphoplasmacytic infiltration.

Within the connective tissue between the dorsal hoof wall and the distal phalanx, there was moderate, multifocal lymphoplasmacytic infiltration within moderately well vascularized, dense, fibrous connective tissue with plump fibroblast nuclei (mature granulation tissue). The dorsal surface of the distal phalanx was highly irregular with multifocal projections extending from the bone into the overlying connective tissue. The projections were well mineralized closer to the bone, but more distally were predominantly fibrous, and distally they were poorly demarcated from the surrounding connective tissue and had no periosteal lining. There were islands of osseous tissue separate from the distal phalanx within the fibrous connective tissue. The distal tip of the distal phalanx was partially lost in one of the digits, and replaced by fibrous connective tissue. Where present, the periosteal cambium exhibited hyperplastic osteoblast-like cells. No immunolabelling was present in the distal phalanx. Representative images are shown in Figure 7.

Figure 7 (overleaf): Tissues from a clinical grade 4 CODD lesion: H & E and *Treponema* spp. IHC

a) H & E, hyperplastic dermal laminae and granulation tissue reaction of the dorsal horn; b) IHC, very scant, superficial immunopositive background labelling of the granulation tissue reaction; c) H & E, marked suppurative inflammatory infiltration surrounding a fissure deep within the dorsal horn; d) IHC, very scant, superficial immunopositive labelling deep in the fissure in the dorsal horn; e) H & E, intracorneal pustule in the dorsal horn; f) IHC, scant background immunopositive labelling of the tissue, g) H & E, distal phalanx showing osteoblast like cells and periosteal reaction, h) IHC, very scant background immunopositive labelling of the periosteal reaction.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Clinical Grade 5

In the distal digital skin/coronary band there was a mild diffuse hyperplasia, with mild diffuse orthokeratotic hyperkeratosis together with mild to moderate multifocal lymphoplasmacytic infiltration of the superficial and mid dermis. In the necrotic debris sloughed from the distal skin there were small bacterial colonies mostly with indistinguishable morphology. No immunolabelling was observed in the distal digital skin/coronary band or the sloughed necrotic debris.

The cornified layer of the dorsal horn was largely intact, but exhibited numerous areas of nucleation of corneal keratinocytes. There were multifocal intracorneal aggregates of degenerate neutrophils and necrotic cells admixed with haemorrhage and multifocal small bacterial colonies mostly with coccoid, rod or indistinguishable morphology. The epidermal laminae were shortened, thickened and often fused into wide bands. There was mild to moderate multifocal lymphoplasmacytic infiltration, together with active fibroblasts. No immunolabelling was observed in the dorsal horn.

The dorsal margin of the distal phalanx was mild to moderately uneven with numerous small spicules. The cambium layer of the periosteum contained numerous plump osteoblast-like cells. No immunolabelling was observed. Representative images are shown in Figure 8.

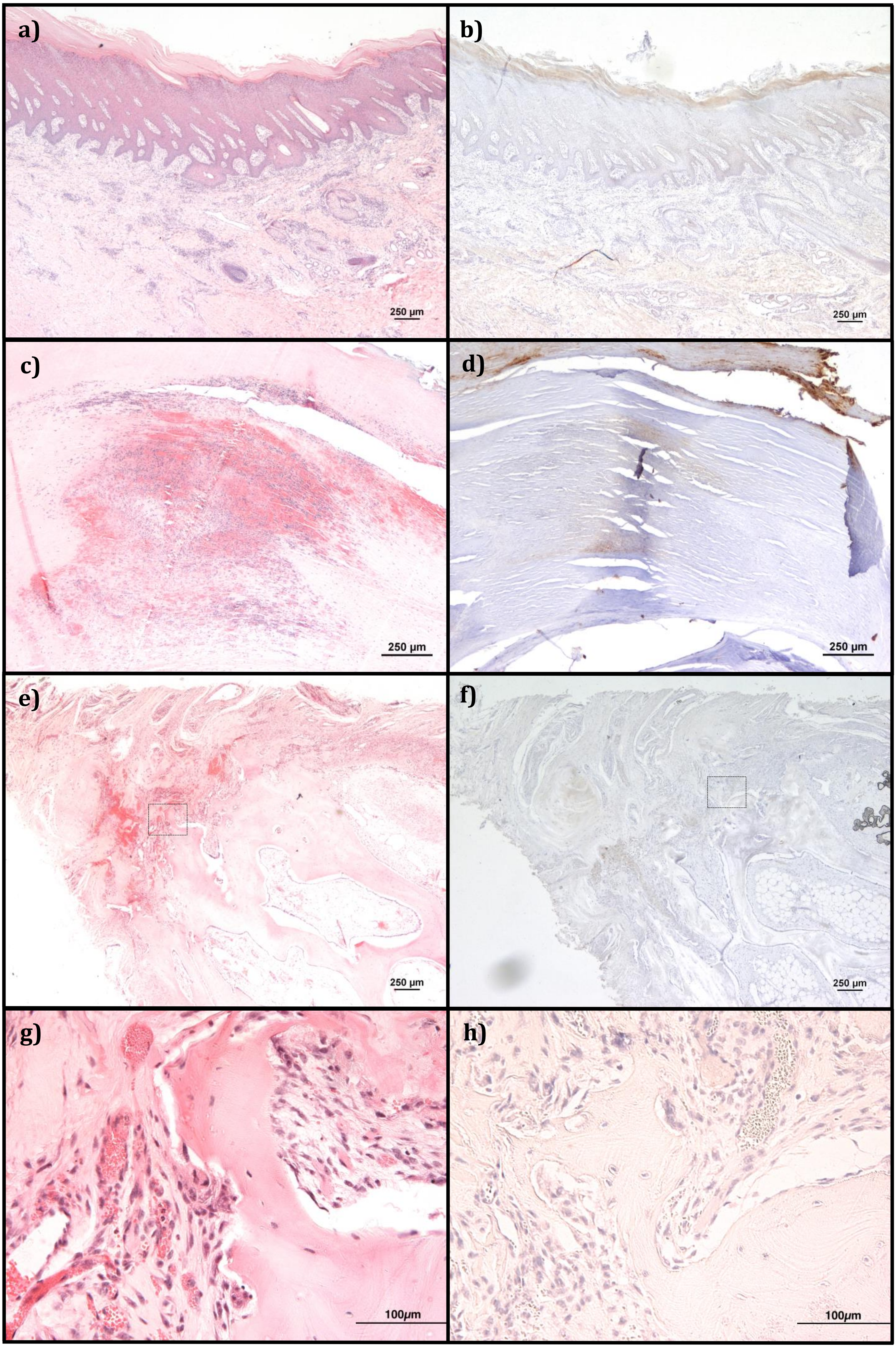
a)
250 µm
b)
250 µm
c)
250 µm
d)
250 µm
e)
250 µm
f)
250 µm
g)
100µm
h)
100µm

Figure 8: Tissues from a clinical grade 5 CODD lesion: H & E and *Treponema* spp. IHC

a) H & E, moderate multifocal chronic lymphoplasmacytic dermatitis of the distal haired skin/coronary band region; b) IHC, mild background immunolabelling of the superficial stratum corneum; c) H & E, intracorneal pustule in the dorsal horn; d) IHC, scant background immunolabelling of the pustule; e) H & E, periosteal reaction of the distal tip of the distal phalanx f) IHC, mild background immunolabelling of the distal tip of the distal phalanx; g) H & E, increased magnification of boxed area of e) showing osteoblasts and osteoclast periosteal reaction; h) IHC, increased magnification of boxed area of f) showing mild background immunolabelling of the distal phalanx.

Overall summary description

There was a background diffuse, mild, perivascular, dermal inflammatory infiltrate of eosinophils, lymphocytes and plasma cells in the majority of sections of distal digital haired skin, which was also present in control animals. In the grade 1 lesions, this was more severe and was mildly suppurative and there were intracorneal pustules within the stratum corneum of the coronary band and proximal hoof wall. In grade 2 lesions, severe distal digital lymphoplasmacytic dermatitis was associated with rete ridge formation and epidermal hyperplasia and hyperkeratosis. The superficial cornified layer of the proximal dorsal horn (i.e. hoof wall) was partially or totally absent and replaced by inflammatory cells, bacteria, protein and associated necrotic debris. In grade 3 lesions, the pathological changes were similar to those of grade 2, but were more severe and extensive (reflecting the clinical grading determined by the extent of horn loss). In grade 4 lesions, the lesions of the distal digital skin and coronary band were chronic and less severe, although the superficial cornified layer of the dorsal hoof wall was still multifocally infiltrated by neutrophils and necrotic debris and there was granulation tissue formation. Similarly in the connective tissue between the hoof wall and the distal phalanx there was lymphoplasmacytic infiltration within the granulation tissue, and on the surface of the distal phalanx there were small mineralized projections. In grade 5 lesions, the skin remained hyperplastic, the laminae were shortened, thickened and fused into wide bands, the dorsal hoof wall was intact although with multifocal small intracorneal pustules still present, and on the surface of the distal phalanx there were mineralized projections of new bone growth. These findings are summarized in Table 1.

Summary of immunohistochemical observations

Immunolabelling was observed, predominantly extracellularly, in lesions graded 1–4. The labelling was strongest in the superficial tissues and associated with the fibrinosuppurative exudates present in areas of separated horn with numerous bacterial colonies, including intracorneal pustules. *Treponema* spp. organisms were labelled and observed, in particular, at the leading edge of the separation of the horn from the underlying epidermis, and in grades 1 and 2 lesions, organisms also tracked between keratinocytes and were occasionally deep within the epidermis. In many sections, immunolabelled bacteria exhibiting distinct, spirochaetal morphology were observed within areas of more strongly immunopositive homogeneous material. The numbers of organisms with *Treponema* spp. morphology was greater in earlier lesions (i.e. grades 1 and 2), while numbers declined in later lesions (i.e. grades 3 and 4) and positive immunolabelling was completely absent in grade 5 lesions. Much of the immunolabelling appeared homogeneous and was concentrated in the areas of necrosis and exudation. Spirochaetal organisms were observed within this material, but in many areas the labelling appeared amorphous in addition to being strongly and specifically positive. This reaction was markedly stronger than the very faint background labelling observed in clinically normal tissues.

Clinical grade	Anti-Treponema immunolabeling	Distal digital skin	Coronary band	Dorsal hoof wall	Solar horn	P3
0		Mild, multifocal perivascular lymphoplasmacytic dermatitis.	NAD*	NAD	NAD	NAD
	TM†	-	-	-	NE	-
	HEL‡	-	-	-		-
1		Mild to severe multifocal, lymphoplasmacytic dermatitis. Moderate to marked epidermal hyperplasia.	Moderate to severe lymphoplasmacytic and mild to marked suppurative coronitis; intracorneal pustules; myriad colonies of coccobacillary and spirochaetal bacteria.	Mild focal suppurative dermatitis to severe suppurative infiltration of the horn and subcorneal epidermis. Spirochaetes within dorsal horn.	Mild lymphoplasmacytic dermatitis.	NAD
	TM	-	+++	+++	NE§	-
	HEL	-	+++	+++		-
2		Moderate multifocal, lymphoplasmacytic dermatitis. Marked epidermal hyperplasia.	Complete loss of the superficial cornified layer, fibrinopurulent exudate, necrotic debris. Lymphoplasmacytic dermatitis.	Complete separation of horn, replaced by necrotic debris, marked suppurative infiltrate of epidermal and dermal laminae. Colonies of coccobacillary and spirochaetal bacteria. Lymphoplasmacytic dermatitis.	Mild lymphoplasmacytic dermatitis. One foot - loss of horn and suppurative dermatitis.	Activation of the cambium layer.
	TM	-	-	+++	NE	-
	HEL	-	-	+++		-
3		Mild, multifocal perivascular lymphoplasmacytic dermatitis. Marked epidermal hyperplasia.	Mild, multifocal perivascular lymphoplasmacytic coronitis; intracorneal pustules.	Complete separation of horn, replaced by necrotic debris, marked suppurative infiltrate of epidermal and dermal laminae. Colonies of coccobacillary and spirochaetal bacteria. Severe lymphoplasmacytic dermatitis.	NE	Activation of the cambium layer with mild to moderate new bone growth dorsally.
	TM	-	-	+	NE	-
	HEL	-	-	+++		-
4		Mild, multifocal perivascular lymphoplasmacytic dermatitis. Marked epidermal hyperplasia.	NAD	Severe necrotising fibrinosuppurative infiltrate of superficial cornified layer. Hyperplastic epidermal laminae. Colonies of coccobacillary bacteria. Granulation tissue formation.	Mild lymphoplasmacytic dermatitis.	Multifocal periosteal projections from the dorsal surface.
	TM	-	-	+	NE	-
	HEL	-	-	+++		-
5		Mild, multifocal perivascular lymphoplasmacytic dermatitis. Mild epidermal hyperplasia.	NAD	Largely intact, multifocal small intracorneal pustules.	NAD	Dorsal surface of P3 mild to moderately uneven with numerous small bony spicules.
	TM	-	-	-	NE	-
	HEL	-	-	-		-

Table 1: Summary of typical lesions exhibited in each of the 5 clinical grades of CODD at the sites sampled from the sheep feet.

Table 2: Summary of typical lesions exhibited in each of the 5 clinical grades of CODD at the sites sampled from the sheep feet.

The anti-*Treponema*-spp. immunolabelling patterns exhibited in each of the 5 clinical grades of CODD at the sites sampled through the sheep feet are indicated: (-) no labelling observed; (+) mild labelling; (++) moderate labelling; (+++) strong labelling. Immunolabelling highlighting organisms with distinct spirochaetal morphology were present in the largest numbers at the coronary band and dorsal hoof horn in grade 1 and the dorsal hoof horn at grade 2; smaller numbers were observed in the dorsal hoof wall at grades 3 and 4. Strong homogenous extracellular immunolabelling was present in the dorsal hoof horn from grades 1 to 4. Positive immunolabelling was absent from grade 5 and negative controls (grade 0).

*NAD: No abnormalities detected; †TM: *Treponema* spp. morphology clearly observed; ‡HEL: Homogenous extracellular immunolabelling; §NE: Not examined.

Discussion

The sampling strategy used in this study ensured that fresh specimens of clinical cases were obtained and placed in fixative within minutes of death. However, due to on-farm practical constraints and in order to focus resources, this meant that the number of lesions in each grade was small and was not uniform. However, this bias is not considered to invalidate the histopathological description due to the consistent changes noted across the different lesion grades, nor invalidate the immunolabelling due to the consistent and predictive pattern of reaction. Attention was focused on the early lesions (i.e. grade 1) as clinically these lesions appear to progress to grades 2–5 (Angell et al., 2015a) and it was considered that information from these early lesions would add more in understanding the aetiopathogenesis of the disease. Furthermore, in considering the associations between *Treponema* spp. and the pathological changes present, and their putative role as causal agents, it was considered important to identify whether the bacteria could be detected in early lesions and thus help to determine their role in the aetiopathogenesis of CODD.

In the tissues obtained from the clinically normal negative control animals, there was a mild, perivascular, dermal, mainly lymphoplasmacytic infiltrate. To our knowledge, this 'background infiltrate' has not been reported in sheep before and we are not aware of any similar reports in other species. Indeed, there has been limited published histological investigation of the ovine digit and as such this finding should be interpreted cautiously. It is not necessarily surprising that this part of the skin, being exposed to wet conditions and persistent low-level trauma, should have a low-grade chronic inflammatory reaction present in clinically normal animals. However, in viewing and interpreting the pathological changes described in this paper, this background infiltrate should be borne in mind.

This is the first time to the knowledge of the authors that CODD lesions have been documented histologically and the first time immunohistochemical techniques have been used to determine the presence and location of *Treponema* spp. in ovine feet.

In an earlier paper (Angell et al., 2015a), it was suggested that the clinical lesion grades may form a progression of disease such that CODD may initially present as a grade 1 lesion and then progress through the other grades. The histological observations made in this current study appear to support this theory, in that acute changes were first observed at the coronary band, followed by very severe necrotizing and fibrinosuppurative lesions with separation of the horn in tissues from grade 2 and 3. Tissues from grade 4 lesions showed signs of chronicity (e.g. granulation tissue), but with suppurative lesions still present, and grade 5 lesions showed evidence of healing, albeit with mild intracorneal suppurative changes still present in some cases. The histological changes in the low-grade lesions clinically appear to commence at the coronary band and then the lesion appears to progress distally down the dorsal horn while the initial site (coronary band) heals. As such, the clinical and histopathological descriptions from both this current study and the previous clinical description appear well correlated and this may support the use and reliability of the CODD lesion grading system.

In cattle, BDD-associated *Treponema* spp. have been observed in and around hair follicles and sebaceous glands, and these structures have been suggested as a potential route of entry/exit of the bacteria into the tissues (Evans et al., 2009c). In the current study, the distal digital skin above the coronary band exhibited progressive lymphoplasmacytic dermatitis with epidermal hyperplasia, but in no cases was the very destructive suppurative lesion present in this location. Furthermore, no *Treponema*-like organisms were observed in hair follicles or sebaceous glands. This may suggest that in sheep the pathological processes target the keratin of the hoof horn more specifically.

In this study it was considered that CODD-associated *Treponema* spp. would be observed in clinically active lesions, but possibly be absent in healed (grade 5) lesions, which appears to be the case in these samples. The immunolabelling showed microorganisms with morphology consistent with *Treponema* spp. predominantly present extracellularly between the keratinocytes. Semiquantitatively, there appeared to be stronger immunolabelling in the earlier lesions (i.e. grades 1 and 2) compared with lesions of grades 3 and 4, and certainly greater numbers of organisms with *Treponema*-like morphology were readily identified in the earlier lesions. When present, these organisms appeared to penetrate for a short distance between the keratinocytes. These findings demonstrate that *Treponema*-like organisms are associated with active lesions, and it may be that they are involved in the initiation and/or propagation of the disease. This was further supported by the absence of *Treponema*-like organisms in healed (grade 5) lesions.

It was beyond the scope of this study to determine the causal nature of the *Treponema*-like organisms identified; however, the distribution within the tissues strongly suggests that they are causally involved to some extent. For example, in Fig. 4 the immunolabelling is stronger at the leading edge of the fissuring in (b) and (f), and in image (e) the immunolabelling is strongly associated with the pathological separation of the superficial dorsal horn from the remaining epidermis. Furthermore, intracorneal pustules were observed in many sections and the examples presented here demonstrate a possible association of *Treponema* spp. immunolabelling with these intracorneal pustules.

In the sections with strong, specific immunolabelling, there were extensive areas of amorphous positively-labelled material, centred on the exudates in the areas of sloughed horn. The *Treponema* spp. organisms were often embedded within this exudate, but the exact nature of the homogeneous labelling is unclear. It could either represent excreted *Treponema* spp. antigen, either free or bound by local tissues, or antigen released as a result of organism degeneration. It is possible that *Treponema* spp. organisms are lysed as a result of enzymes secreted during necrosis and/or inflammation or as a result of the strong host immune response (Dhawi *et al.*, 2005). This detection of *Treponema* spp. antigen in tissues, in the relative absence of intact *Treponema* spp. in late-stage CODD, again supports the hypothesis of a potential key role for the organisms in initiating the lesions of CODD lesions. The exudate in these lesions appeared to label strongly and specifically with the anti-*Treponema* spp. antibody, and negative control tissues (i.e. unaffected sheep feet and exudative pyoderma from a dog) were negative with only very faint background labelling, which in the case of the dog was also predominantly intracellular.

A further possibility is that in more chronic lesions with avulsion of the hoof horn capsule, the *Treponema*-like organisms are fewer in number due to them being shed with the keratin. Further testing is required in order to investigate these observations further.

In conclusion, CODD remains a challenge to the sheep industry. This study documents for the first time the histopathological changes associated with clinical lesions of each grade and provides clear evidence of how the lesions develop as the disease progresses. Furthermore, the presence of *Treponema*-like organisms associated with active lesions adds weight to the hypothesis that these bacteria are involved in the aetiopathogenesis of the disease.

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Chapter 6

Sheep and farm level factors associated with contagious ovine digital dermatitis: a longitudinal repeated cross-sectional study of sheep on six farms

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Abstract

Contagious ovine digital dermatitis (CODD) is a cause of severe lameness in sheep in the UK currently affecting approximately 50% of farms. Six farms were studied in North Wales to investigate 1) the prevalence dynamics of CODD, 2) the association between sheep with CODD and potential risk factors and 3) the impact of CODD on lameness in sheep. The farms were visited at approximately two-month intervals between June 2012 and October 2013 and 6,515 sheep were examined.

The mean sheep level prevalence of CODD varied between farms (2.5-11.9%). Within farms, prevalence may increase in the late summer/early autumn and after housing. Environmental risk factors included larger flocks, lowland pasture, lush pasture and poached pasture. Co-infection of a foot with footrot was strongly associated with CODD in that foot (OR: 7.7 95%CI: 3.9-15.5 P<0.001) but negatively associated with co-infection of a foot with interdigital dermatitis (OR: 0.04 95%CI: 0.02-0.1 P<0.001). Reinfection with CODD was observed in 78 individual sheep but there was no re-infection at foot level.

Lameness on all farms reduced during the study and seasonal changes in lameness followed similar patterns to those for CODD. Infection with CODD leads to a greater increase in locomotion score compared to footrot or interdigital dermatitis and CODD lesion grade was strongly associated with being lame. Sheep with CODD in more than one foot were more likely to be lame (OR: 25.0 95%CI: 12.5-49.9 P<0.001) than those with just one foot affected (OR: 10.0 95%CI: 8.6-11.6 P<0.001).

The biggest risk factor for CODD is co-infection with footrot and therefore control of footrot should help reduce the risk of CODD on affected farms. Furthermore environmental risk factors for CODD are similar to those for footrot adding weight for control strategies that target both diseases in tandem. The routine repeated gathering of sheep for the purposes of treating all lame sheep might be an effective control strategy for lameness on some sheep farms. Effective systemic immunity to CODD in sheep appears to be lacking, as 78 sheep were observed to be re-infected with CODD during the survey. However, there is epidemiological evidence that there may be some local immunity within the foot warranting further investigation.

Introduction

Contagious ovine digital dermatitis (CODD) was first identified in the UK in 1997 (Harwood et al., 1997) and since then the proportion of UK farms affected has increased to approximately 50% (Duncan et al., 2014). The disease affects the digits of sheep and causes lameness due to the severe damage caused in affected feet (Winter, 2008; Phythian et al., 2013). A diagnosis is made on the basis of clinical signs (Angell et al., 2015a) and effective treatment remains problematic, partly due to the fact that as yet the aetiology is unclear (Duncan et al., 2014). Whilst the aetiopathogenesis of CODD is currently unclear, pathogens implicated in the aetiology of CODD are *Treponema* spp. including those associated with Bovine Digital Dermatitis (BDD) - *Treponema medium/Treponema vincentii*-like, *Treponema phagedenis*-like and *Treponema pedis* (Sullivan et al., 2015). Other pathogens implicated in CODD include *Dichelobacter nodosus* and *Fusobacterium necrophorum* (Naylor et al., 1998; Moore et al., 2005a).

A recent review highlighted the large gaps in our knowledge of this disease (Duncan et al., 2014) but we have identified a number of putative risk factors for CODD at farm level, from a questionnaire survey of 511 farms in Wales, UK (Angell et al., 2014). These included concurrent digital dermatitis in cattle on the farm, increasing flock size, concurrent footrot (FR), buying in sheep, adult sheep, time of year and housing sheep. To date though there have been no on farm epidemiological studies investigating risk factors for CODD on naturally affected farms. Furthermore, there has been recent interest in this disease from a welfare perspective due to the severity of lesions and the significant impact this is likely to have both in individual sheep and on the sheep industry as a whole (FAWC, 2011).

The aim of this study was to examine the epidemiology of CODD on six farms in North Wales. The three main objectives were: 1) to describe any temporal variation in CODD prevalence; 2) to investigate the association between sheep with CODD and potential risk factors; 3) to investigate the impact of CODD on lameness in sheep.

Materials and Methods

The study protocol was approved by The University of Liverpool ethics committee (VREC 13) on 24th August 2011.

Study design and study population

The study is a prospective, repeated cross-sectional field survey of six sheep farms in North Wales, selected on known presence of CODD on the farm and farmer willingness to collaborate. Farms were visited approximately bi-monthly over a 12 month period (June 2012 – October 2013), although the visiting schedule was impacted by several factors including bad weather and breeding events. Farms with a range of production systems and breeds were selected, including hill, upland and lowland.

Sampling

At each visit all sheep on the farm were gathered from fields to handling pens and then visually inspected in groups of approximately ten sheep. All sheep on the farm were lameness scored in the pen using a four-point ordinal locomotion scoring system (Angell et al., 2015c). All lame sheep (score 1-3) were selected for further detailed examination, together with an equal or greater number of non-lame (score 0) control sheep, randomly selected from the same pen.

This sampling strategy was adopted as CODD is typically reported to be of low prevalence on affected farms (2.0% (IQR 1.0-5.0%)) (Angell et al., 2014).

Furthermore not all sheep with CODD are lame (Phythian et al., 2013) thus non-lame sheep were also examined. Each selected sheep was examined in detail and covariate data recorded and entered into a Microsoft Access Database (Microsoft; USA).

Due to the dynamic nature of sheep flocks, it was impossible to re-sample the same animals at each visit. As such, a repeated cross-sectional sampling at farm level strategy, as described, was adopted. Therefore at each visit, a combination of previously sampled and previously un-sampled sheep were sampled (Table 1). All the data collection, observations and examinations were made by the same person (JA) in order to reduce the risk of differential misclassification by different observers.

Number of times a sheep was sampled	Total number (n=6,515)	N (% [95%CI]) Lame (n=1,447)		N (% [95%CI]) Non-lame (n=4,812)		N (% [95%CI]) with missing lameness data (n=256)		N (% [95%CI]) with active CODD (n=733)	
1	2497	472	(18.9 [17.4-20.5])	1946	(77.9 [76.3-79.5])	79	(3.2 [2.5-3.9])	236	(9.5 [8.3-10.7])
2	2054	452	(22.0 [20.2-23.9])	1508	(73.4 [71.5-75.3])	94	(4.6 [3.7-5.6])	228	(11.1 [9.8-12.5])
3	1197	318	(26.6 [24.1-29.2])	812	(67.8 [65.1-70.5])	67	(5.6 [4.4-7.1])	186	(15.5 [13.5-17.7])
4	416	118	(28.4 [24.1-33.0])	283	(68.0 [63.3-72.5])	15	(3.6 [2.0-5.9])	53	(12.7 [9.7-16.3])
5	235	54	(23.0 [17.8-28.9])	180	(76.6 [70.7-81.9])	1	(0.4 [0.01-2.3])	17	(7.2 [4.3-11.3])
6	116	33	(28.5 [20.5-37.6])	83	(71.6 [62.4-79.5])	0	(0.0 [-])	13	(11.2 [6.1-18.4])

Table 1: Number of times individual sheep were sampled, stratified by whether they were lame or not lame and whether they had active CODD.

Farm and group level factors

Farm stocking density (Livestock units/hectare (Ha)) (DEFRA, 2010) was estimated using data supplied by the farmer (number and type of animals: grazeable land area). For beef cattle an average LU of 0.65 was used to take account of the different, varying and unquantifiable ages of beef cattle on the farm. At each visit, the total number of sheep in each field was counted and field stocking density (sheep/Ha) for that visit calculated.

The pasture moisture was assessed as: 1) 'Dry' – no moisture was observed on footwear following walking through the pasture, 2) 'Damp and well drained' – the ground was firm but the grass damp to touch and moisture evident on footwear, 3) 'Wet' - the ground would bear weight but was squelchy, 4) 'Boggy' – the ground was saturated and in places footwear would sink someway into the ground (Figure 1).

Pasture quality was determined as: 1) 'Lush' – 80% rye grasses, mostly leaf rather than stalk present, 2) 'Average' – approximately 50% rye grasses, some stalk and some leaf and 3) 'Rough' – moorland, marshland, virtually no rye grasses, and rushes, heather or bracken present (Figure 2).

The mean compressed sward height in each field was measured using a plate meter (Filips Manual Folding Plate Meter; Jenquip, Feilding, New Zealand, (Jenquip, 2004))with the observer walking each field in a zig-zag pattern taking recordings every ten paces to obtain an accurate mean. Between 18 and 445 readings were taken per field (depending on size) to obtain the mean compressed sward height.

The sward cover in each field may vary and the amount of cover in each field was determined by eye as: 1) ‘Good Coverage’ – all the field was visibly covered in grass, 2) ‘Patches’ – incomplete sward cover; some areas of the field were just soil (>5% to <50%), 3) ‘Heavily Poached’ – >50% of the sward was absent (Figure 3).

Figure 1: Scoring system for recording the amount of moisture on the pasture.

Figure 2: Scoring system for recording the quality of the pasture.

Figure 3: Scoring system for recording the cover of the pasture.

Figure 4: Scoring system for recording the cleanliness of the tail and perineal wool used in the cohort study on six sheep farms in North Wales.

Sheep factors

For each sheep examined in detail, the following data were recorded: ear tag number; locomotion score (Angell et al., 2015c); body condition score (Russel, 1984); age (estimated in whole years from the number of incisor teeth present (Spence and Aitchison, 1986)) and breed. The cleanliness of the tail and perineal wool was recorded using an ordinal scoring system: 0) 'Clean', 1) 'Mild Staining' – small amounts of faecal material adhering to the wool, 2) 'Dirty' – obvious staining to wool and perineum, with/without small 'dags' (hardened accumulations of faecal debris adhering to the wool), 3) 'Large Dags' – heavily stained wool and perineum; large dags obviously present (Figure 4).

For each examined sheep, each foot was examined and the following foot lesions were recorded if present: CODD, FR, interdigital dermatitis (ID), white-line/shelly hoof (WL), foot abscess (FA), granuloma (GR), interdigital hyperplasia (IH), joint infection (JI), injury (IN) and overgrown horn (OG). For the contagious foot conditions – FR, ID and CODD – if more than one lesion appeared to be present in the same foot e.g. footrot and CODD together, the combination was recorded. All CODD lesions were graded (1-5), and active CODD was defined as a lesion graded 1-4, grade 5 being a healed lesion (Angell et al., 2015a). All sheep were restrained and examined in dorsal recumbency using a Turn Over Crate (Harrington, Yorkshire, UK).

To ensure the welfare of the sheep, all sheep with a foot lesion were treated as per the usual protocol for that farm agreed between the farmer and their own veterinary surgeon. Treatments included long acting oxtetracycline injection (Alamycin LA; Norbrook), or long acting amoxicillin injection (Betamox LA; Norbrook), oxytetracycline spray (Alamycin spray; Norbrook) and foot trimming.

Data Analysis

The data were cleaned and checked for inconsistencies. All analyses were conducted using STATA IC 13 (Statacorp, TX). Probability values of <0.05 were taken as significant. Three analysis strategies were employed: 1) the prevalence of lameness and lesions were estimated; 2) modelling associations between farm, environmental, sheep and foot factors and the primary outcome, namely the presence of active CODD at foot level; 3) examining associations between farm, environmental and sheep factors with the primary outcome, namely a sheep being recorded as lame (locomotion score >0).

1) <u>Prevalence</u>

Lameness and lesion prevalence were calculated as the number of sheep affected, as a proportion of the flock. Due to the sampling strategy, the true flock prevalence of lameness or specific foot lesions was estimated from the sampled data using the formula:

$$P = \frac{(N_{case} + N_{est})}{N_{tot}}$$

P estimated prevalence

N_{case} number of recorded cases in examined sheep – lame and not lame.

N_{est} estimated number of cases in un-sampled flock – not lame.

N_{tot} total number of sheep in the flock.

The estimated number of cases in the un-sampled flock (N_{est}) was calculated from the sample data using the formula:

$$N_{est} = \left[\frac{NL_{case}}{NL_{tot}} \right] X \ N_{uns}$$

NL_{case} the number of sheep with a specified foot disease that were not lame at examination

NL_{tot} the total number of examined sheep that were not lame

N_{uns} N_{tot} minus the total number of sheep sampled

The estimated prevalence for lameness, and for each lesion was calculated for each visit and for each farm and then from these individual estimates, an overall mean estimated prevalence was calculated for all six farms over all 32 visits.

Distribution of lesions

The distribution of specific lesions by age was assessed using the Chi-squared test. The proportional distribution of lesions between front and back feet was investigated using the Z test.

2) <u>CODD as an outcome</u>

Descriptive statistics for CODD

The proportional distribution of CODD lesion grades in affected feet was investigated using the Z test.

Modeling

Univariable and multivariable logistic regression were employed to investigate associations between the primary outcome variable - presence of active CODD at foot level - and potential farm, environmental, sheep and foot explanatory variables (Table 2). A foot was considered the primary unit rather than a sheep as CODD often affects only one or two feet in a sheep (Duncan et al., 2011; Angell et al., 2015a).

Due to the non-random sampling strategy, probability weights were used in all regression models, defined as the probability of a non-lame sheep being sampled (PWT). For lame sheep PWT = 1 as all lame sheep were sampled. For non-lame sheep PWT was calculated as:

$$PWT = \left[\frac{N_{nl}}{FS_{visit} - N_{lame}} \right]^{-1}$$

N_{nl} the number of non-lame sheep examined at the visit

FS_{visit} total flock size at the visit

N_{lame} the total number of lame sheep at the visit

A multivariable logistic regression model with the binary outcome variable being the presence of active CODD at foot level was fitted using a backward elimination strategy whereby a full model was built and then each variable removed in turn. Model fit was assessed using the Bayesian Information Criterion (BIC) (Long, 1996). For a model M_k with deviance $D(M_k)$ the BIC is estimated as: $BIC_k = D(M_k) - df_k * \ln N$, where df_k is the degrees of freedom associated with the deviance and N

is the sample size. The more negative the BIC_k the better the model fit, with an absolute difference in BIC between two models of >6 offering strong support for the model with the smallest BIC. Variables were removed if this led to improved model fit. The omitted variables were then added back in turn and the variable retained if it improved model fit, until no more variables could be added. Interactions in the final model were considered for inclusion if considered plausible and retained if they improved model fit.

Variables were generated to code for the presence of lesions in the other three feet belonging to the sheep e.g. a binary variable for 'other feet in a sheep with FR' coded as 1 if any of the three other feet in the sheep had FR together with the foot in question, or coded as 0 if the other three feet did not have FR (Table 2).

Clustering of feet within sheep was accounted for by fitting the model using robust standard errors – random effects could not be used due to the use of probability weights. This final model was then used to assess the associations between the outcome and the included covariates adjusted for each other.

Variable	Description and coding of variable
Farm and environment	
Flock size / 50	Number of sheep in the flock divided by 50.
Land type	1=Hill 2=Upland/lowland 3=Lowland
Farm stocking density	The number of LU per hectare for the farm
Field stocking density	The number of sheep per hectare in each field at sampling
Pasture moisture	1=Dry 2=Damp and well drained 3=Wet 4=Boggy
Pasture quality	1=Lush 2=Average 3=Rough
Sward height (cm)	The mean compressed sward height in each field at sampling
Sward cover	1=Good coverage 2=Patches 3=Heavily poached
Sheep variables	
Age	1=Lamb 2=Yearling 3=Adult
Body condition score	1=Very thin 2=Lean 3=Average 4=Fat 5=Obese
Perineal cleanliness	0=Clean 1=Mild staining 2=Dirty 3=Large dags 0=Clean 1=Some dirt present
Foot lesions	
Other digit with active CODD	0=No other digit in same sheep with active CODD 1=1 or more digits in same sheep with active CODD
Other digit with healed CODD	0=No other digit in same sheep with healed CODD 1=1 or more digits in same sheep with healed CODD
Concurrent FR	0=No concurrent FR present in foot 1=Concurrent FR present in foot
Other digit with FR	0=No other digit in same sheep with FR 1=1 or more digits in same sheep with FR
Concurrent ID	0=No concurrent ID present in foot 1=Concurrent ID present in foot
Other digit with ID	0=No other digit in same sheep with ID 1=1 or more digits in same sheep with ID
Other digit with WL	0=No other digit in same sheep with WL 1=1 or more digits in same sheep with WL
Other digit with FA	0=No other digit in same sheep with FA 1=1 or more digits in same sheep with FA
Other digit with GR	0=No other digit in same sheep with GR 1=1 or more digits in same sheep with GR
Other digit with IH	0=No other digit in same sheep with IH 1=1 or more digits in same sheep with IH
Other digit with IN	0=No other digit in same sheep with IN 1=1 or more digits in same sheep with IN
Other digit with OG	0=No other digit in same sheep with OG 1=1 or more digits in same sheep with OG
Other digit with any other foot disease	0=No other digit in same sheep with any other foot disease 1=1 or more digits in same sheep with any other foot disease

Table 2: Description of variables collected at sampling visits for initial inclusion in statistical analyses.

Time covariates

To allow modeling of seasonal changes if present, four time covariates (X_1 X_2 X_3 X_4) were generated as follows: $X_1 = \cos(2\pi t/365.25)$, $X = \text{sine}(2\pi t/365.25)$, $X_3 = \cos(4\pi t/365.25)$, $X_4 = \text{sine}(4\pi t/365.25)$ where t = day with day 1 being the first sample date. These were forced into all the models as a composite (harmonic regression (Stolwijk et al., 1999)) together with the interaction with year. All four covariates were included to allow the modeling of annual and six-monthly variation within the study period.

Seasonal changes in the prevalence of CODD were described graphically by obtaining the logit prediction of a foot having active CODD estimated from the regression models, using the time covariates with year included as an interaction term as the sole explanatory variables, and then calculating the inverse logit i.e. the prevalence. Confidence intervals were not presented due to there being only one farm visited on each day.

3) Lameness as an outcome

Due to the sampling strategy (described above) the data could be examined as a case control study with lame sheep as cases, and non-lame sheep as randomly selected controls.

Statistical analysis

Descriptive statistics were estimated at sheep level, together with univariable logistic regression analyses where appropriate. The Kruskal-Wallis test was used to compare the median locomotion score of sheep with different combinations of different contagious foot lesions.

With regards to the presence of CODD, for all those sheep with just one foot affected (which was the majority with that lesion) the probability of lameness at sheep level was modelled by CODD grade, and marginal means were used to represent this graphically. Multivariable analyses were not attempted with lameness as an outcome, as following the construction of a theoretical causal diagram (Figure 5) it is clear that lameness – a behaviour associated with the perception of pain when moving - is most commonly due to the presence of a foot lesion. Other factors operate at a level above - at sheep level, and in order to model lameness at foot level it would be necessary to know which leg specifically the sheep was lame on. For some animals (e.g. those observed to be locomotion score 1, this was not possible and as such the data were therefore not available for this type of analysis.

Time

Seasonal changes for the probability of a sheep being lame were described by plotting the logit prediction of a sheep being lame from a regression model, using the time covariates with year included as an interaction term.

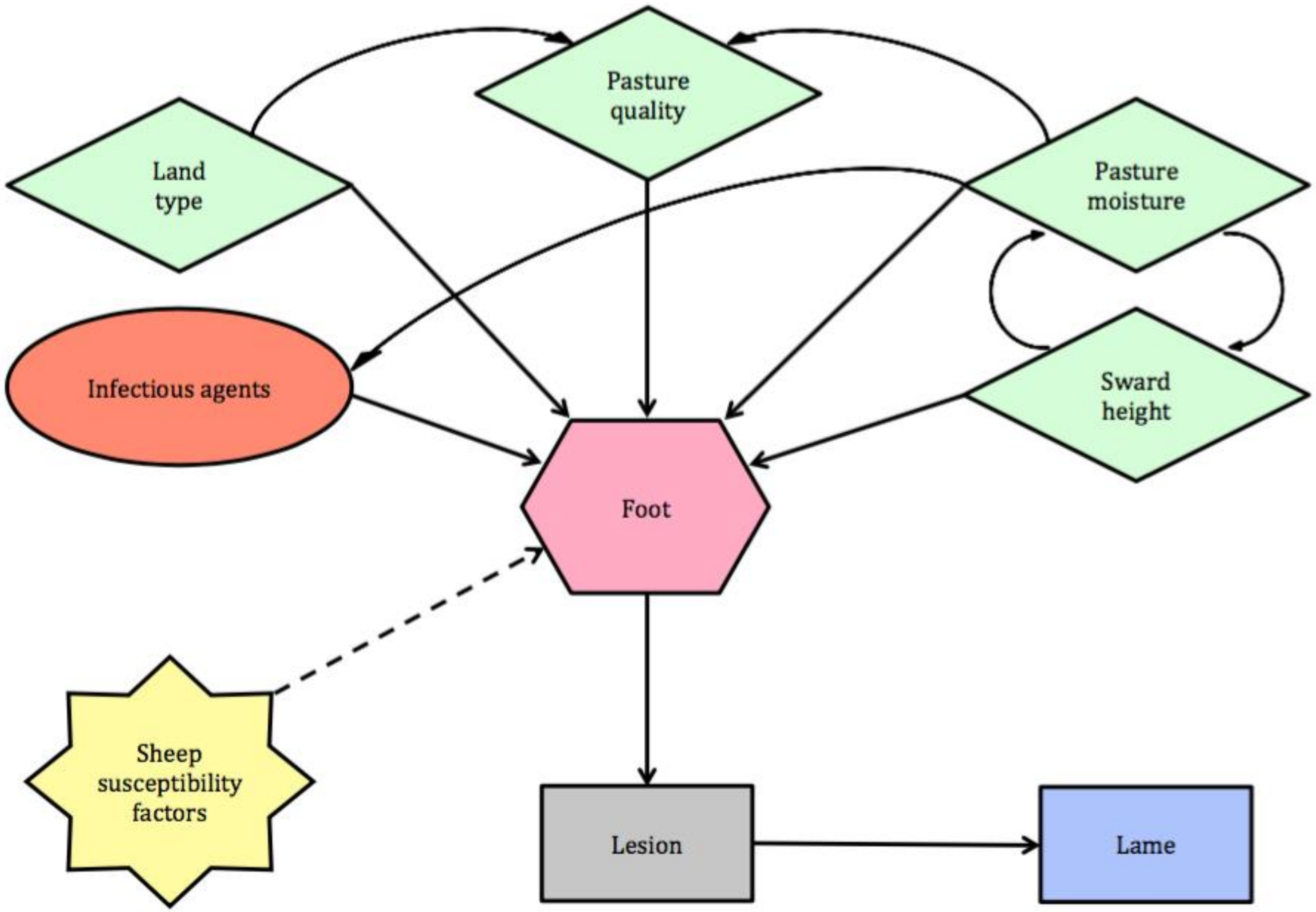

Figure 5: A theoretical causal diagram for lameness in sheep. Field level variables (green triangles) affect the foot (pink hexagon) to increase the susceptibility to infectious agents (red oval). The 'sheep susceptibility factors' (yellow star) represent unmeasured and unquantified determinants that make a sheep more susceptible to developing a foot lesion, be they genetic, nutritional or other factors. These host-pathogen-environment interactions lead to the development of a lesion (grey rectangle), which may cause pain and the behaviours observed as lameness (blue rectangle). Some factors are likely to interact (represented by curved arrows) e.g. longer swards are likely to hold greater moisture; greater moisture is likely to lead to longer swards; increased pasture moisture can affect pasture quality e.g. poaching; increased pasture moisture can assist the survival of bacteria; lowland land is likely to have better quality pasture.

Results

Study Population

All six farms were commercial sheep farms in North Wales. They ranged in size from 300 to 870 breeding ewes and from 68.8 to 360.2 Ha (Table 3).

Visits commenced on 14th June 2012 and were completed on 3rd October 2013. Five out of the six farms remained in the study for the full six visits; one dropped out after three visits due to farmer time constraints. In total, therefore 32 visits were made with 22,724 sheep presented for inspection of which 6,515 sheep were examined from all six farms giving a sampling proportion of 28.7% (95%CI: 28.1-29.3%).

Of the 6,515 examined sheep, 1,447 (22.2%; 95%CI: 21.2-23.2%) were lame (locomotion score >0) and 4,812 (73.9%; 95%CI: 72.8-74.9%) were not lame (locomotion score 0) giving an approximate ratio of 1:3, lame: not lame; 256 (3.9%; 95%CI: 3.5-4.4%) sheep had missing locomotion scoring data (Table 1).

Table 1 details the number of re-sampled sheep per visit. The proportions of those re-sampled that were lame, not-lame and had active CODD were approximately similar at each visit.

Farm	Land Type	Total size (Hectares)	Total number of breeding ewes (LU*)	Total number of cattle (LU*)	Stocking Density (SD): number of livestock units per hectare	Breeds
1	Hill	186.2	740 (44.4)	30 (19.5)	Total LU = 63.9 **SD: 0.34 LU/Ha**	Welsh Mountain (99.4% (95%CI: 96.0-99.9%)) Other breeds (0.6% (95%CI: 0.1-4.0%))
2	Lowland	360.2	500 (50.0)	50 (32.5)	Total LU = 82.5 **SD: 0.23 LU/Ha**	Scottish Blackface (21.4% (95%CI: 19.1-23.8%)) Mule (43.7% (95%CI: 40.8-46.5%)) Suffolk/Suffolk Cross (20.7% (95%CI: 18.4-23.2%)) Other breeds/ crossbreeds (14.2% (95%CI: 12.3-16.4%))
3	Lowland/Upland	111.3	870 (87.5)	150 (97.5)	Total LU = 185.0 **SD: 1.66 LU/Ha**	Lleyn/Lleyn Cross (31.3% (95%CI: 28.8-33.9%)) Easy Care/Easy Care Cross (63.2% (95%CI: 60.5-65.8%)) Other Lowland Crossbreeds (5.5% (95%CI: 4.3-6.9%))
4	Lowland	72.8	300 (33.0)	120 (78.0)	Total LU = 111.0 **SD: 1.52 LU/Ha**	Mule (70.5% (95%CI: 67.8-73.0%)) Charollais Cross (20.5% (95%CI: 18.3-22.9%)) Other Lowland Crossbreeds (9.0% (95%CI: 7.4-10.7%))
5	Lowland/Upland	68.8	460 (50.6)	240 (156.0)	Total LU = 206.6 **SD: 3.00 LU/Ha**	Suffolk Cross (43.4% (95%CI: 39.0-47.8%)) Mule (34.8% (95%CI: 30.6-39.2%)) Texel Cross (18.7% (95%CI: 15.5-22.4%)) Other Lowland Crossbreeds (3.1% (95%CI: 1.7-5.0))
6	Upland	97.1	400 (32.0)	0 (0.0)	Total LU = 33.8 **SD: 0.35 LU/Ha**	Lleyn/Lleyn Cross (85.1% (95%CI: 82.3-87.6%)) Mule (10.7% (95%CI: 8.6-13.1%)) Other Lowland Crossbreeds (4.2% (95%CI: 2.9-5.9%))

Table 3: Farm attributes of the six study farms in North Wales, as reported for June 2012.

* Livestock units (LU): Lowland ewes 0.11, Upland ewes 0.08, Hill ewes 0.06, Cattle 0.65 (DEFRA, 2010).

1) <u>Prevalence</u>

Due to the nature of the study, incidence could not be estimated, although obviously incidence will impact on prevalence. The mean estimated prevalence of lameness (locomotion score >0), over all 32 visits and all six farms, was 6.7% (95%CI: 5.0-8.4%). For the following lesions the on farm sheep prevalence was estimated: CODD 5.1% (95%CI: 3.3-6.8), FR 5.0% (95%CI: 3.2-6.8%), ID 13.3% (95%CI: 7.0-19.6%), WL 36.7% (95%CI: 28.7-44.7%), FA 1.9% (95%CI: 1.3-2.4%), OG 15.4% (95%CI: 9.2-21.6%), GR 0.4% (95%CI: 0.2-0.6%), IH 1.0% (95%CI: 0.3-1.7%), IN 0.2% (95%CI: 0.0-0.4%) and JI 0.1% (95%CI: 0.0-0.3%). The prevalence of CODD varied by farm and by visit (Figure 6).

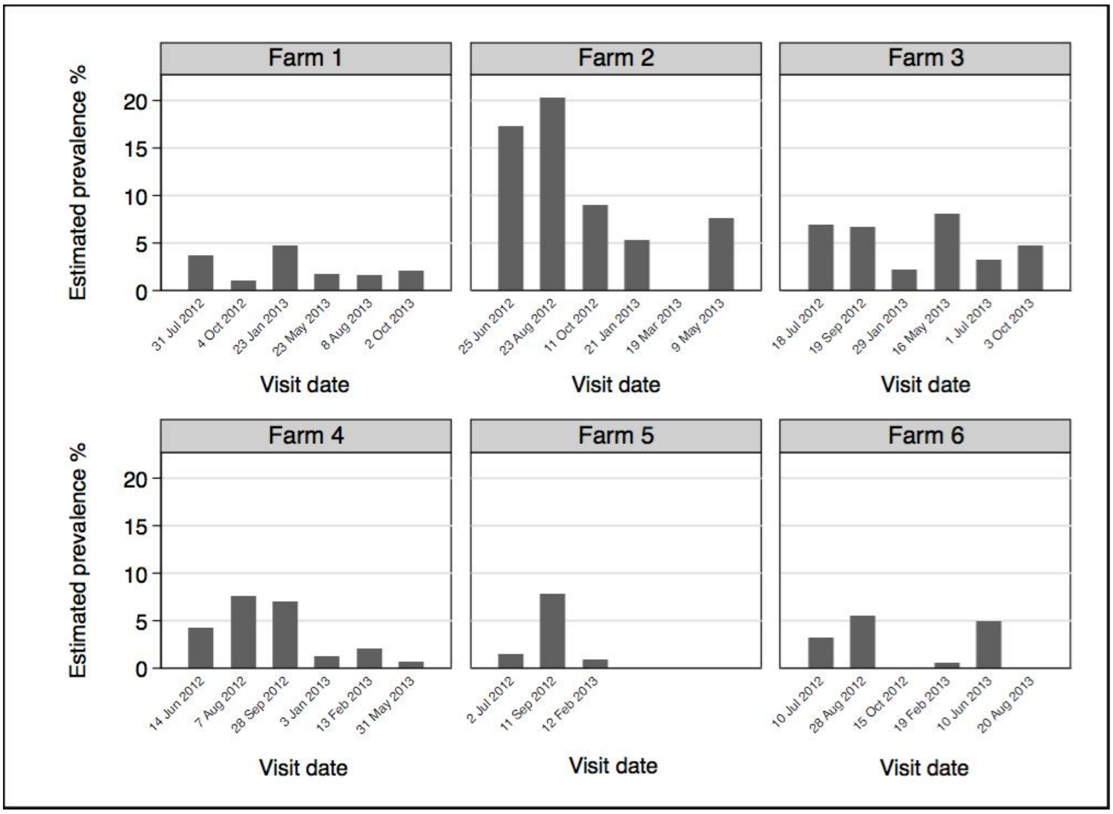

Figure 6: Estimated prevalence of active CODD by farm determined at each visit.

N.B. For Farm 2 visit 5, and Farm 6 visits 3 and 6, some of the data were missing preventing accurate prevalence estimates. For Farm 5 visits 4-6, all data were missing for these visits due to the termination of the study on that farm.

Distribution of lesions

The distribution of lesions varied significantly (P≤0.001) by age for CODD, FR, ID, GR, WL, FA, IH and OG (Table 4). The proportion of lesions in front and back feet varied significantly (P<0.001) for CODD, FR, ID, WL, IH, IN and OG (Table 5). The lesions CODD, ID, IH, JI and IN were observed more in back feet and FR, WL and OG were observed more in the front feet. Granulomas and FA were found equally between the front and back feet.

Disease	Total number of sheep with disease	Lamb (<1 year)			Yearling (1 year)			Adult (≥2 years)			P-value*
		n	%	(95% CI)	n	%	(95% CI)	n	%	(95% CI)	
CODD (active)	733	174	23.8	(20.8-28.0)	81	11.1	(9.0-13.6)	477	65.2	(61.6-68.5)	<0.001
Footrot	526	200	38.0	(34.0-42.3)	39	7.4	(5.5-10.0)	287	54.6	(50.3-58.8)	<0.001
Interdigital dermatitis	851	371	43.6	(40.3-47.0)	82	9.6	(7.8-11.8)	398	46.8	(43.4-50.1)	<0.001
White line	2,179	187	8.6	(7.5-9.8)	393	18.0	(16.5-19.7)	1599	73.3	(71.5-75.2)	<0.001
Foot abscess	164	16	9.8	(6.0-15.4)	18	11.0	(7.0-16.8)	130	79.3	(72.3-84.8)	<0.001
Granuloma	36	1	2.8	(0.4-18.7)	2	5.6	(1.3-20.8)	33	91.7	(76.1-97.4)	0.001
Interdigital hyperplasia	54	1	1.9	(0.02-12.7)	2	3.7	(0.09-14.2)	51	94.4	(83.6-98.3)	<0.001
Joint infection	14	5	35.7	(13.7-66.0)	2	14.3	(2.9-48.0)	7	50.0	(23.2-76.8)	0.512
Injury	19	6	31.6	(13.7-57.3)	1	5.3	(0.06-33.8)	12	63.2	(38.0-82.7)	0.361
Overgrown	894	65	7.3	(5.7-9.2)	126	14.1	(12.0-16.5)	703	78.6	(75.8-81.2)	<0.001
Total number of sheep in each age group		1,484			1,044			3,979			

Table 4: Foot lesions in sheep stratified by age. The percentages (95%CI) refer to the number of sheep in the age group with a given lesion; from the 6,515 sheep examined.

*Chi-squared test

Disease	Total Number of sheep with disease	Sheep with disease in FRONT feet			Sheep with disease in BACK feet			Sheep with disease in BOTH FRONT and BACK feet concurrently			P value*
		n	%	(95% CI)	n	%	(95% CI)	n	%	(95% CI)	
CODD (all grades)	1047	469	44.8	(41.8-47.8)	636	60.7	(57.8-63.7)	58	5.5	(4.3-7.1)	<0.001
CODD (active)	733	311	42.4	(38.9-46.0)	0468	63.8	(60.3-67.3)	46	6.3	(4.7-8.3)	<0.001
Footrot	526	354	67.3	(63.3-71.3)	248	47.1	(42.9-51.4)	76	14.4	(11.4-17.5)	<0.001
Interdigital dermatitis	851	432	50.8	(47.4-54.1)	652	76.6	(73.8-79.5)	233	27.4	(24.4-30.4)	<0.001
White line	2180	1641	75.3	(73.5-77.1)	1315	60.3	(58.3-62.4)	776	35.6	(33.6-37.6)	<0.001
Foot abscess	164	88	53.7	(45.9-61.4)	78	47.6	(39.8-55.3)	2	1.2	(0.00-2.9)	0.269
Granuloma	36	18	50.0	(32.8-67.2)	18	50.0	(32.8-67.2)	0	-	-	1.0
Interdigital hyperplasia	54	9	16.7	(6.4-26.9)	49	90.7	(82.8-98.7)	4	7.4	(0.2-14.6)	<0.001
Joint infection	14	4	28.6	(1.5-55.6)	10	71.4	(44.4-98.5)	0	-	-	0.023
Injury	19	5	26.3	(4.5-48.1)	14	73.7	(51.9-95.5)	0	-	-	0.004
Overgrown	894	667	74.6	(71.7-77.5)	457	51.1	(47.8-54.4)	230	25.7	(22.9-28.6)	<0.001

Table 5: Lesion distribution by front and back feet in affected sheep, from the 6,515 sheep examined.

*Z-test of proportions comparing the proportion of front feet affected with the proportion of back feet affected.

2) <u>CODD as an outcome</u>

Descriptive statistics for CODD

Of the 6,515 sheep examined, 1,047 had CODD (16.1% [95%CI: 15.2-16.9]), of which 733 had an active lesion (11.3% [95%CI: 10.5-12.0]). Of the 26,060 feet examined there were in total 1,143 feet with CODD (4.4% [95%CI: 4.1-4.6%]), of which 775 feet had an active CODD lesion (3.0% [95%CI: 2.8-3.2%]). The proportion of feet with CODD lesions varied by grade (Figure 7).

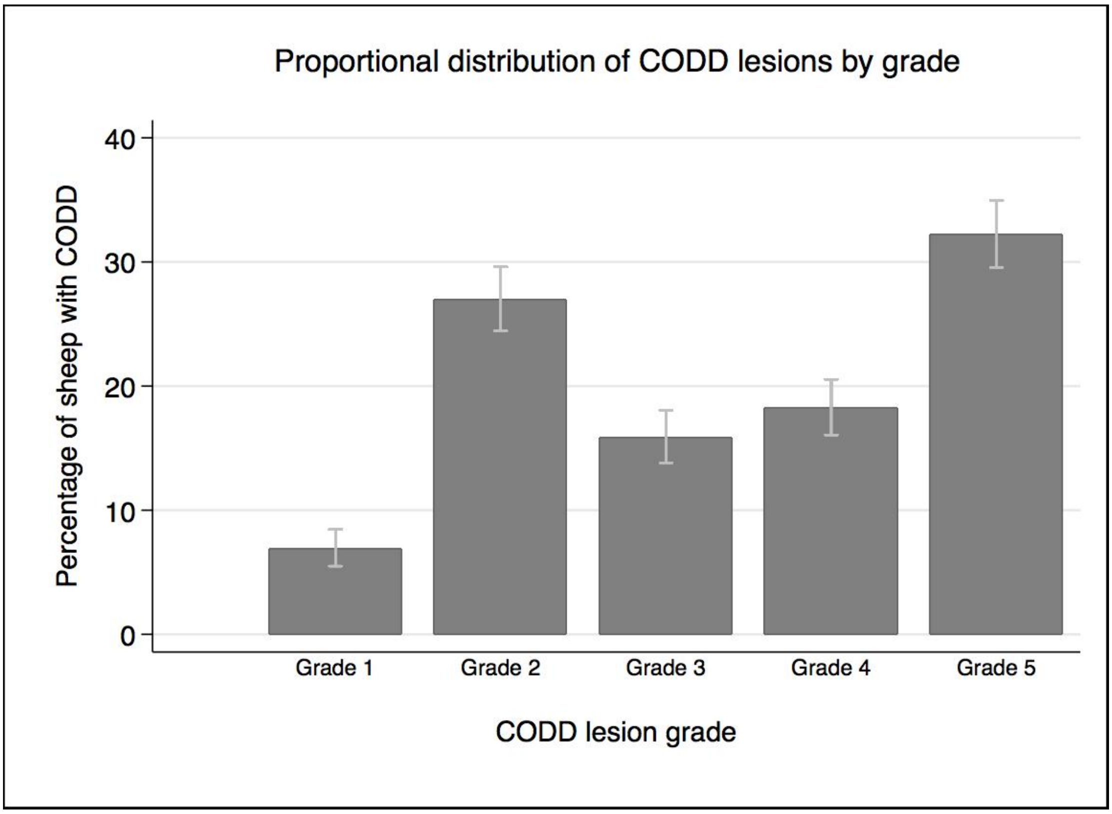

Figure 7: The proportion of CODD lesions of each grade, from 1,143 feet with CODD lesions. The capped spikes represent the 95% confidence intervals for each proportion.

For sheep over one year of age: 78 individuals were found to have active CODD in a foot at one visit and were also found to have active CODD in another foot on at least one other subsequent visit; 17 individuals were found to have healed CODD in a foot at one visit and then active CODD in another foot at a subsequent visit. No individual sheep was found to have active CODD in the same foot on two consecutive occasions. No sheep had active CODD in a foot previously classified as healed CODD.

Univariable analysis

The presence of active CODD at foot level varied significantly by farm flock size, farm land type, flock size at each visit, pasture moisture, pasture quality, pasture coverage and year (Table 6). No association was found for stocking density at each visit, sward height and perineal cleanliness.

Multivariable analysis

The final multivariable logistic regression model with robust standard errors, for the presence of active CODD at foot level (n=23,776 feet; 5,944 sheep from six farms) showed significant associations with farm identity, pasture coverage, FR, the absence of co-infection with ID, the absence of WL in the other feet of a sheep with CODD and time - including interaction with year (Table 7). Interactions considered but not retained included flock size interacting with land type.

Variable	N with CODD	% with CODD	Odds Ratio	95% CI	P value
Farm and environment					
Farm flock size (n=26,060)					
300 (n=4,856)	150	3.1	(Baseline odds = 0.003)		
400 (n=2,960)	50	1.7	1.4	0.5-3.4	0.5
460 (n=1,968)	39	2.0	1.1	0.5-2.8	0.8
500 (n=4,696)	234	5.0	6.1	2.9-12.7	<0.001
740 (n=6,304)	62	1.0	1.2	0.5-2.8	0.7
870 (n=5,276)	240	4.6	3.6	1.7-7.7	0.001
Farm land type (n=26,060)					
Hill (n=6,304)	62	1.0	(Baseline odds = 0.003)		
Upland/lowland mixed (n=10,204)	329	3.2	1.9	1.1-3.3	0.024
Lowland (n=9,552)	384	4.0	1.4	0.8-2.7	0.248
Flock size at visit (number of sheep) (n=25,332)					
210-390 (n=5,236)	60	1.2	(Baseline odds = 0.002)		
396-600 (n=6,296)	172	2.7	2.1	0.8-5.2	0.119
655-711 (n=3,696)	82	2.2	3.3	1.3-8.5	0.014
738-1227 (n=5,564)	222	4.0	5.1	2.1-12.2	<0.001
1405-1821 (n=4,540)	209	4.6	5.0	2.0-12.2	<0.001
Pasture moisture (n=25,932)					
Dry (n=3,392)	35	1.0	(Baseline odds = 0.003)		
Wet (n=22,540)	736	3.3	2.2	1.3-4.0	0.006
Pasture quality (n=24,332)					
Lush (n=2,568)	143	5.6	(Baseline odds = 0.002)		
Average (n=11,932)	359	3.0	3.5	2.4-5.2	<0.001
Rough (n=9,832)	241	2.5	2.5	1.7-3.7	<0.001
Pasture coverage (n=24,332)					
Good coverage (n=16,988)	516	3.0	(Baseline odds = 0.004)		
Patches (n=6,212)	189	3.0	1.7	1.1-2.6	0.021
Heavily poached (n=1,132)	38	3.4	6.9	3.7-12.8	<0.001
Time					
Year (n=26,060)					
2012 (n=9,496)	470	5.0	(Baseline odds = 0.01)		
2013 (n=16,564)	305	1.8	0.3	0.2-0.5	<0.001
Sheep variables					
Body condition score (n=25,148)					
1 (n=2,720)	117	4.3	(Baseline odds = 0.008)		
2 (n=11,512)	346	3.0	0.8	0.5-1.3	0.4
3 (n=9,628)	269	2.8	0.5	0.3-0.8	0.005
4 (n=1,272)	28	2.2	0.5	0.2-1.1	0.07
5 (n=16)	0	0	-	-	-
Age (n=26,028)					
Lamb (n=5,936)	190	3.2	(Baseline odds = 0.008)		
Yearling (n=4,176)	84	2.0	0.7	0.3-1.5	0.4
Adult (n=15,916)	500	3.1	0.6	0.4-0.8	0.005
Foot variables					
CODD (n=26,060)					
No other feet in the sheep with active CODD (n=23,832)	695	2.9	(Baseline odds = 0.005)		
Other feet in the sheep with active CODD (n=2,228)	80	3.6	3.9	2.3-6.7	<0.001
Footrot (n=26,060)					
No footrot in the same foot (n=25,333)	689	2.7	(Baseline odds = 0.005)		
Footrot present in the same foot (n=727)	86	11.8	7.3	3.9-13.7	<0.001
No other feet in the sheep with footrot (n=24,335)	715	2.9	(Baseline odds = 0.005)		
Other feet in the sheep with footrot (n=1,725)	60	3.5	2.7	1.4-5.3	0.003
Scald (n=26,060)					
No scald in the same foot (n=24,623)	770	3.1	(Baseline odds = 0.005)		
Scald present in the same foot (n=1,437)	5	0.4	0.1	0.03-0.2	<0.001
White line (WL) lesion (n=26,060)					
No other feet in the sheep with WL (n=18,362)	592	3.2	(Baseline odds = 0.006)		
Other feet in the sheep with WL (n=7,698)	183	2.4	0.5	0.3-0.8	0.001
Foot abscess (n=26,060)					
No other feet in the sheep with FA (n=25,564)	764	3.0	(Baseline odds = 0.005)		
Other feet in the sheep with FA (n=496)	11	2.2	3.5	1.0-12.3	0.047

Table 6: Univariable analyses of the associations between covariates and active CODD at foot level.

Variable	Odds Ratio	95% CI	P value
Farm and environment			
Farm 1	1.2	0.5-2.7	0.7
Farm 2	4.7	2.5-8.8	<0.001
Farm 3	4.4	2.2-8.8	<0.001
Farm 4 (Baseline)			
Farm 5	1.5	0.6-3.9	0.4
Farm 6	2.6	1.2-5.9	0.02
Pasture coverage (patches or heavily poached)	2.3	1.5-3.5	<0.001
Foot variables			
Co-infection with footrot in same foot	7.7	3.9-15.5	<0.001
Co-infection with interdigital dermatitis in same foot	0.04	0.02-0.1	<0.001
Other feet in the sheep with WL	0.5	0.3-0.9	0.01
Time			
Year 2013	$2.5e^{-6}$	$1.4e^{-11}$-0.5	0.04
X 1	$1.2e^{5}$	0.2-$6.7e^{10}$	0.09
Year × X 1	$1.5e^{-5}$	$3.0e^{-11}$-8.0	0.1
X 2	$1.4e^{5}$	1.5-$1.3e^{10}$	0.04
Year × X 2	$3.6e^{-6}$	$3.8e^{-11}$-0.3	0.03
X 3	0.7	0.2-2.9	0.6
Year × X 3	2.0	0.4-10.2	0.4
X 4	$2.6e^{2}$	0.9-$7.1e^{4}$	0.05
Year × X 4	$1.3e^{-3}$	$3.1e^{-6}$-0.5	0.03
Baseline odds (Farm 4, good coverage, no footrot no interdigital dermatitis and no other feet in the sheep with WL)	$2.4e^{2}$	$1.6e^{-3}$-$3.6e^{7}$	

Table 7: Multivariable logistic regression model with robust standard errors, including covariates associated with the probability of diagnosing active CODD at foot level. In this final model n=23,776 feet (5,944 sheep, of which 3,591 had been examined more than once (see Table 1)).

Time

The prevalence of feet with an active CODD lesion, across all six farms over time was predicted from a model containing the four time covariates with year included as an interaction term (Figure 8). This showed significant variation in the prevalence of CODD over the six farms over time. In 2012 a significant increase in prevalence was observed in late summer/early autumn. In 2013 an increase in prevalence was observed in spring and early summer, and it appears that a further increase in prevalence may also have occurred in late summer/early autumn in 2013, prior to the termination of the study.

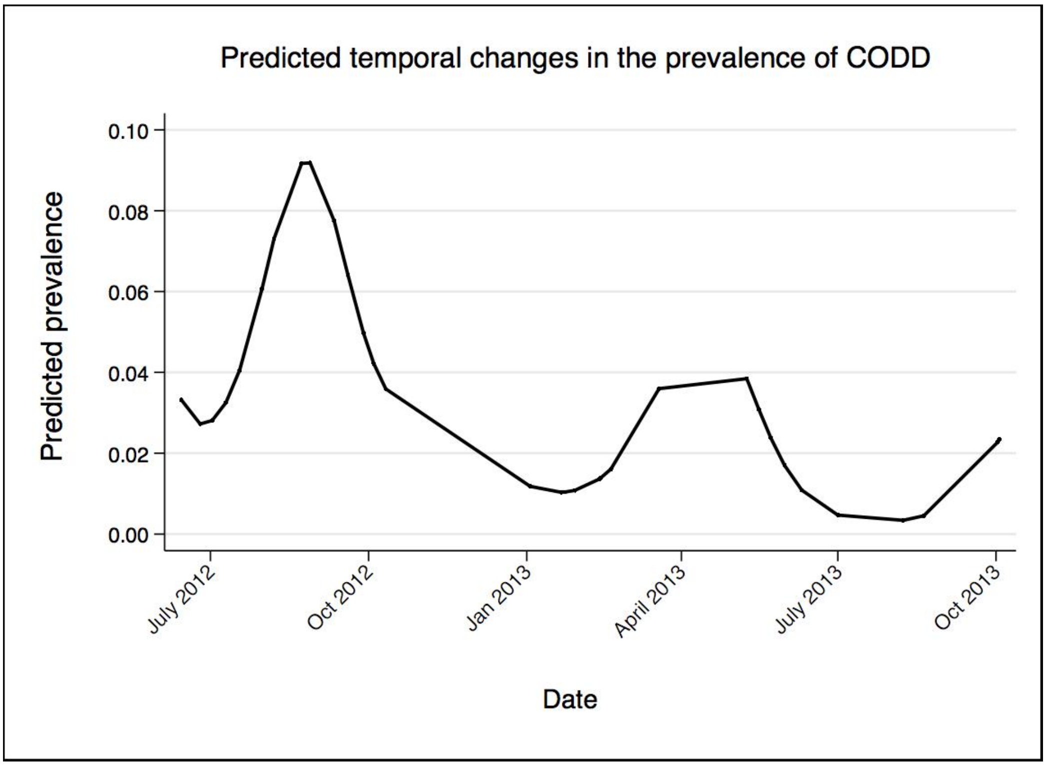

Figure 8: Predicted temporal changes in the prevalence of CODD.

3) Lameness as an outcome

Descriptive statistics for lameness

Of the 1,047 sheep with CODD, 664 (63.4% [62.0-64.9]) were recorded as being lame (locomotion score >0). The median locomotion score for sheep with active CODD was 2 (IQR: 1-3) (Figure 9). This was significantly greater (P<0.001) than that for sheep with FR: median locomotion score 1 (IQR: 0-2) or ID: mean locomotion score 0 (IQR: 0-1). There was no significant difference in median locomotion score between sheep with active CODD only and those with concurrent infection with CODD and ID: median locomotion score 2 (IQR: 1-3) (P=0.4), or between those with active CODD only and those with concurrent infection with CODD and FR: median locomotion score 2.0 (IQR: 1-3) (P=0.1).

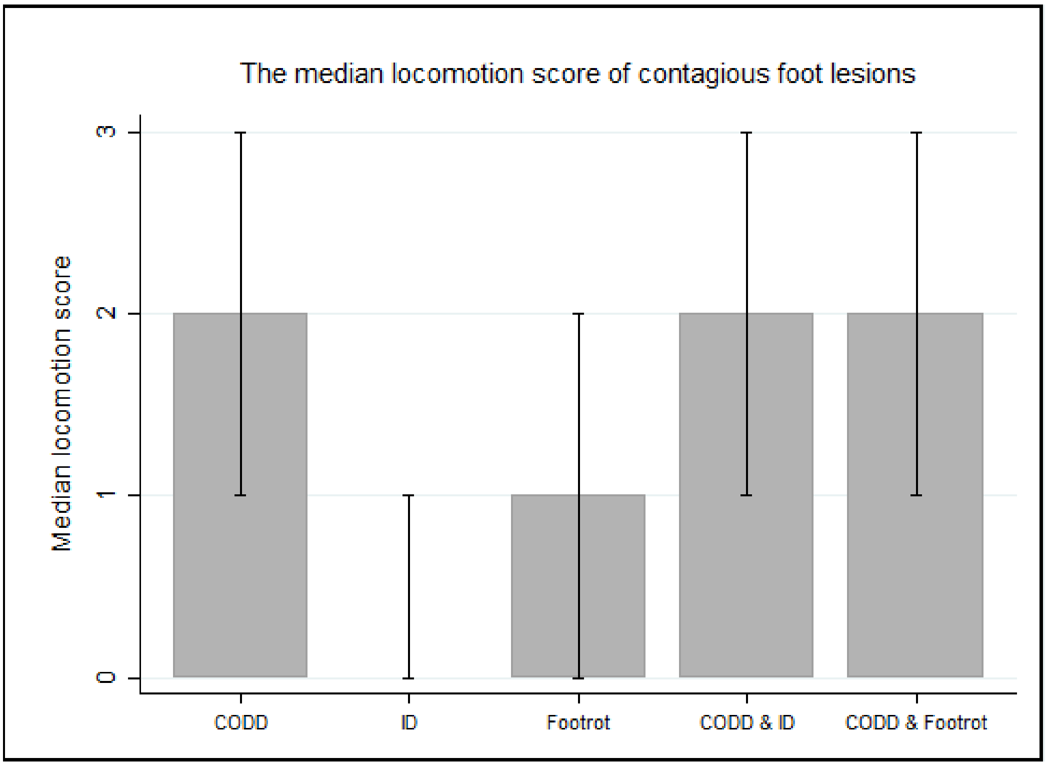

Figure 9: The median locomotion score for sheep with contagious foot lesions, from the 6515 sheep examined. The capped spikes represent the interquartile ranges for each median value. Cases of CODD in this figure are all active CODD (grades 1–4).

Univariable analyses

Lowland land, increased pasture moisture, rough pasture, a longer sward and age (lambs and adults, compared to yearlings) were all positively associated with an increased probability of a sheep being lame (Table 8). Of the foot lesions: CODD, FR, ID, GR, FA and IN were all positively associated with being lame and WL and OG were negatively associated with being lame. Active CODD was very strongly associated with lameness (OR 29.4, 95%CI: 23.8-36.3), and lesion grade was also significant with those sheep with lesions graded 2 or 3 most strongly associated with lameness (Table 8 and Figure 10). Sheep with more than one foot affected with CODD (OR 25.0, 95%CI: 12.5-49.9) were much more likely to be lame than sheep with just one foot affected (OR 10.0, 95%CI: 8.6-11.6). There was no association with flock size, BCS, perineal cleanliness, IH and JI.

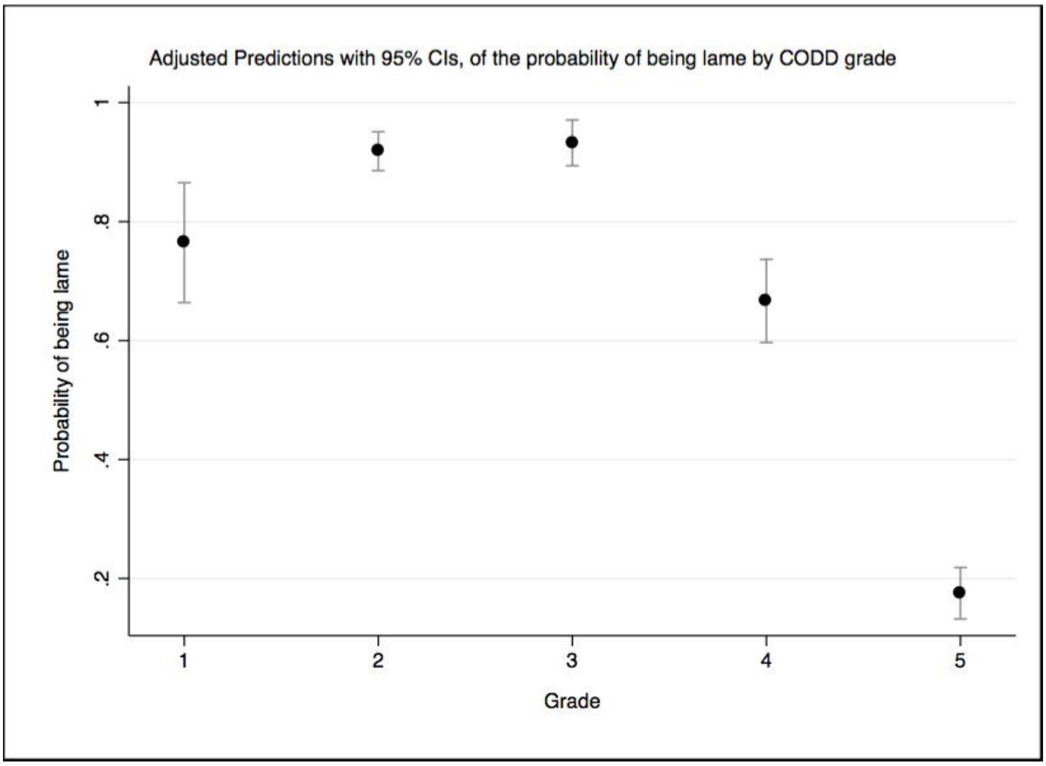

Figure 10: The adjusted marginal mean probability of being lame by CODD grade. The capped spikes represent the 95% confidence intervals for each probability.

Variable	N examined sheep lame	% examined sheep lame	Odds Ratio	95% CI	P value
Farm and environment					
Land type (Hill as baseline) (n=6,259))	1,447	23.1	1.3	1.2-1.4	<0.001
Pasture moisture (n=6,228)	1,382	24.8	3.6	2.7-4.7	<0.001
Pasture quality					
Lush (n=2,271)	456	20.1	(Baseline odds = 0.25)		
Average (n=2,920)	742	25.4	1.4	1.2-1.5	<0.001
Rough (n=637)	194	30.5	1.7	1.4-2.1	<0.001
Sward height (cm) (4,351)	1,085	24.9	1.2	1.1-1.2	<0.001
Sheep variables					
Age (n=6,256)					
Lamb (n=1,480)	401	27.1	1.8	1.5-2.2	<0.001
Yearling (n=1,011)	174	17.2	(Baseline odds = 0.21)		
Adult (n=3,765)	871	23.1	1.4	1.2-1.7	<0.001
Foot variables					
CODD all grades(n=1,026)	664	64.7	10.4	9.0-12.1	<0.001
CODD active (n=729)	612	84.0	29.4	23.8-36.3	<0.001
CODD grade					
Grade 1 (n=68)	52	76.5	18.5	10.5-32.5	<0.001
Grade 2 (n=270)	248	91.9	64.1	41.2-99.7	<0.001
Grade 3 (n=163)	152	93.3	78.5	42.4-145.5	<0.001
Grade 4 (n=174)	116	66.7	11.4	8.2-15.7	<0.001
Grade 5 (n=297)	52	17.5	1.2	0.9-1.6	0.234
CODD in more than one foot (6,259)					
No CODD (n=5,233)	783	15.0	(Baseline odds = 0.18)		
1 foot affected (n=972)	620	63.8	10.0	8.6-11.6	<0.001
2-4 feet affected (n=54)	44	81.5	25.0	12.5-49.9	<0.001
Footrot (n=516)	323	62.6	6.9	5.7-8.3	<0.001
Scald (n=835)	343	41.1	2.7	2.3-3.2	<0.001
White line lesion (n=2,046)	439	21.5	0.9	0.8-0.99	0.030
Overgrown (n=863)	169	19.6	0.8	0.7-0.9	0.008
Granuloma (n36)	23	63.9	6.0	3.0-11.8	<0.001
Foot abscess (n=164)	98	59.8	5.2	3.8-7.2	<0.001
Injury (n=18)	9	50.0	3.3	1.3-8.4	0.011

Table 8: Univariable analyses of the associations between covariates and a sheep being lame.

Time

Lameness improved markedly over the course of the study, 2013 OR 0.3 (95%CI: 0.2-0.5). There were seasonal peaks in late summer/early autumn in both 2012 and 2013 (Figure 11), although the peak in 2013 is much smaller than that in 2012.

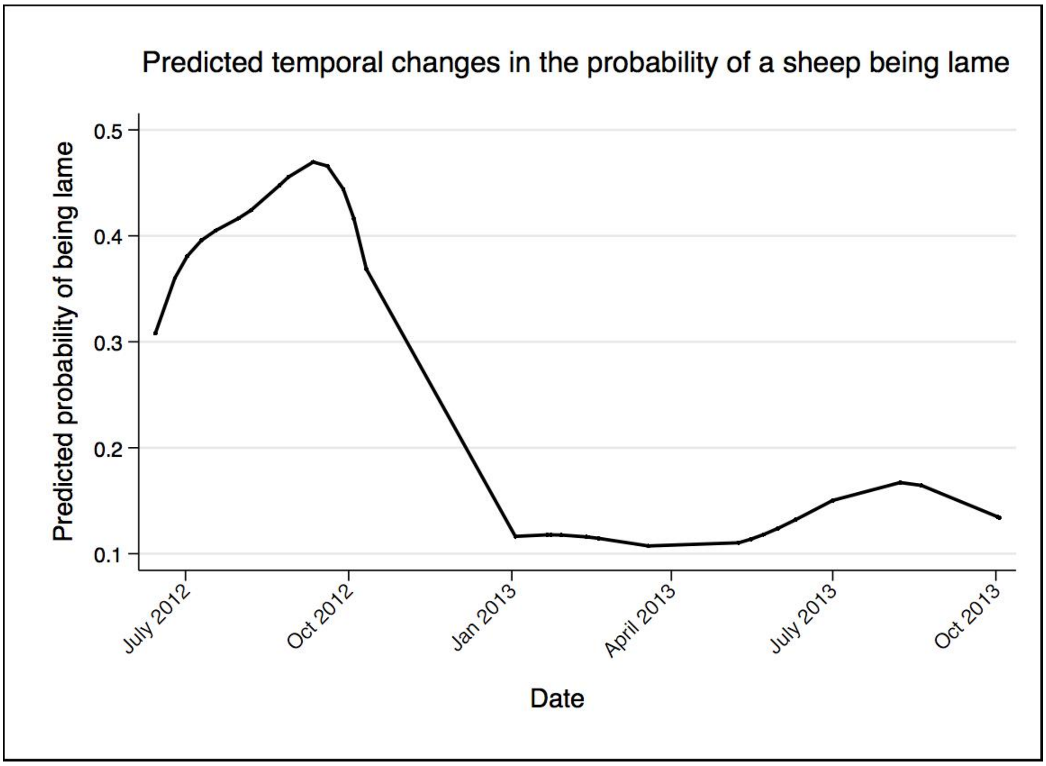

Figure 11: Predicted temporal changes in the probability of a sheep being lame.

Discussion

Study limitations

The sampling strategy of sampling all lame sheep but only a proportion of non-lame sheep was designed in order to identify the greatest number of sheep and feet with CODD at each visit within the practical constraints of the study. As such, given the dynamic changes within sheep flocks throughout the year this cross-sectional sampling strategy enabled sheep with and without CODD to be examined, whilst allowing for population changes within each flock. As such, at each visit there were a proportion of sheep re-sampled from previous visits and also a proportion examined that were not sampled previously, either due to the individual being a new entrant to the flock or simply for having not been sampled (Table 1). To account for seasonal variations over time harmonic regression was used. Probability weights were employed in order to account for the non-random sampling strategy but still obtain meaningful estimates of prevalence and risk factor impact. It would have been preferable to physically examine every sheep on the farm at each visit, but practically this was not possible. As a consequence, all prevalence values are estimates based on the observed data.

The results are clearly impacted by the particular study farms chosen. Due to financial and time constraints only six farms could be studied. Consequently in all the models, farm and environmental factors may be strongly influenced by the individual farms – as shown by the model in Table 7 - and as such these data should be interpreted cautiously. For example the farm land-type associations seen for both CODD and lameness could be simply a farm effect rather than a true association. Whilst this is an obvious weakness, the study is strengthened by the wide range of farms - in terms of farm type, location, breed, mixture of farm enterprises and production targets - and as such provide a reasonable cross-section of current UK sheep farming. Furthermore, the small sample size enabled a much more detailed examination of a large number of sheep feet over a prolonged time frame compared with other study designs, and allowed for the specific analysis of sheep and foot factors.

A further limitation is the impact of the observer effect. To ensure that the welfare of the sheep was not compromised sheep with foot lesions at examination were treated as per the farmer's normal routine. The reduction in lameness and CODD on all the farms over the study period may be due to this effect. In light of this the seasonal changes reported in this paper should be interpreted cautiously due to this possible observer effect.

1) Prevalence

Due to the nature of the study design, prevalence and not incidence is estimated, although prevalence will be impacted by the incidence rate. Contagious ovine digital dermatitis is widespread with reported between farm prevalence estimates of between 13% and 53% (Wassink et al., 2003a; Kaler and Green, 2008a; Angell et al., 2014). The present study shows wide variation between farms (mean prevalence: 2.5-11.9%) and within a farm over the year (e.g. for farm 2 range: 5.3-20.3%). Currently in the UK, national prevalence figures are used to describe levels of disease on farms and to provide targets for disease control (FAWC, 2011). These findings have implications for this approach to describe disease levels, in that point prevalence refers to one point in time, and prevalence can vary widely over a year. This begs the question – what is meant by “farm prevalence”. For example, farm 2 (Figure 6) demonstrated a wide range in disease point prevalence estimates. At one observation, prevalence may be at or below a particular target and at another outside it, and in using either figure independently, neither represents the true disease status of that flock – except at that point in time. Consequently, it is necessary for more meaningful measures to be developed in order to achieve reliable targets that may be used to reduce lameness in the national flock. In reality, only repeat surveys on farms over a minimum of 12 months with samples taken in different seasons would allow an accurate description of lameness on a farm.

Distribution of lesions

The distribution of lesions between the front and back feet varied for different lesions. In this study CODD was more prevalent in back feet compared with front feet, in agreement with Duncan et al. (2011); Duncan et al. (2012). In cattle hind foot lesions predominate, with the causes well documented (Read and Walker, 1998; Sogstad et al., 2005). However, the reason for this distribution in sheep is less obvious. Furthermore, the reverse distribution was found for FR, with front feet affected more, in contrast to Duncan et al. (2012). The biological significance of these observations is uncertain.

Age

In this study, yearling sheep showed fewest lesions from contagious foot conditions (CODD, FR and ID). This could be a reflection on the management practices on sheep farms. Yearling sheep tend to be replacement breeding ewes to replace those culled out of a flock, and it is likely that farmers will select ewe lambs that show no evidence of having been lame. These sheep also tend to be managed separately until first parturition, which may reduce the risk of becoming infected from the rest of the flock.

Both CODD and FR were found more in adult sheep than lambs, and this may reflect the more chronic nature of these diseases. However, ID was found to be evenly distributed between lambs and ewes, adding weight to theories whereby ewes and lambs may be considered to be potential sources of infection for each other (Kaler et al., 2010b). This has implications for control in that reducing disease in one group may help reduce the risk of infection in the other.

2) <u>CODD as an outcome</u>

Grade 1 CODD lesions were significantly under represented compared to other grades. This may be for a number of reasons: Firstly these lesions being less severe may result in lower locomotion scores hindering detection (Table 8). Secondly these lesions may be short-lived progressing to grade 2 lesions rapidly. This highlights the need for intensive longitudinal studies of individuals with natural infections of CODD in order to determine the time frames for lesion progression.

In this study individual sheep were observed to have active CODD more than once. However, we did not observe any re-infection or recrudescence at foot level. In cattle there is poor humoral immunity to *Treponema* spp. (Walker et al., 1997; Demirkan et al., 1999; Refaai et al., 2013) and the same is suspected in sheep (Dhawi et al., 2005). However, these data suggest that in sheep there may be adequate cellular immunity at foot level. Histopathology of affected feet may demonstrate cellular evidence to help understand this further.

Factors associated with CODD

In this study the biggest single factor associated with CODD in sheep was co-infection with FR (OR: 7.7 [95%CI: 3.8-15.5]). This corroborates findings by Duncan et al. (2012) who also found a strong association (OR: 3.83 [95%CI: 2.61-5.62]) between FR and CODD. It is not known whether there is a synergism between the two diseases, whether *D. nodosus* is required in the aetiology of CODD or whether the same environmental conditions lead to an increased susceptibility for both diseases. There is a need for controlled experimental studies to investigate this.

In this study, sheep were more likely to have CODD if they were part of larger flocks, on lowland pasture, lush pasture or poached pasture and despite the small sample size it is biologically plausible that all these factors may play a causal role in increasing the probability of sheep acquiring CODD. Increasing flock size has been associated with CODD in a previous study (Angell et al., 2014) and it is plausible that with larger flocks the ability to identify and manage individuals with disease may be impaired.

It is also feasible that those environmental conditions that favour FR may also favour CODD. Stewart (1989) reported that predisposing factors for FR included lush pasture and moisture, both of which maximise grass production, allowing higher stocking densities thereby favouring transmission. In this study, increased stocking densities *per se* were not associated with CODD however poaching which may be associated with increased stocking densities was associated with CODD.

In this study co-infection with ID was negatively associated with CODD OR: 0.04 (95%CI: 0.02-0.1). This is perhaps surprising given the strong associations between FR and ID (Stewart, 1989; Wassink et al., 2003a; Green and George, 2008). It is possible that early FR as ID is a predisposing factor for CODD, damaging the skin and allowing access for *Treponema* spp. but by the time CODD is diagnosed the ID lesion may have become obliterated.

Therefore, this study adds weight to the argument that on farm interventions for CODD should include a focus on FR control – for which effective control strategies already exist.

Seasonal changes in CODD prevalence

In this study an increase in CODD prevalence was noted in late summer/early autumn. This is consistent with numerous anecdotal observations by farmers and veterinary surgeons. One possible explanation is that at this time of year there is the greatest number and mass of sheep on the farm (all the ewes, many of the lambs and the lambs are at their biggest size) – resulting in an overall increased stocking density at farm level. It is also consistent with observed seasonal fluctuations seen with FR (Clements and Stoye, 2014) and with observed environmental risk factors for FR such as increased rainfall and warmer temperatures (Green and George, 2008).

A second (smaller) increase in prevalence was observed in the spring, and this is consistent with an increase in infection pressure following housing for lambing, a practice which occurred on five of the six farms. It is also consistent with observed increases in FR prevalence following housing (Whittington, 1995; Clements and Stoye, 2014), and consistent with farmers' perceptions that housing leads to an increase in CODD prevalence (Angell et al., 2014).

3) Lameness as an outcome

In this study CODD, FR and ID were all associated with lameness (Table 8). However, the median locomotion score for sheep with CODD was much greater than that for sheep with FR or ID (Figure 9). This suggests that whilst these contagious diseases are all associated with lameness, CODD has the greatest impact on the welfare of individuals, which is in agreement with Phythian et al. (2013) who observed that whilst 59.0% of sheep with FR and 61.5% of sheep with ID were lame, 83.9% of sheep with CODD were lame.

This severe welfare impact is likely to be due to the extensive pathology seen in affected feet (Angell et al., 2015a). This is supported by this present study that shows strong associations between lameness severity and CODD lesion grade.

Sheep with partial and complete under-running of the hoof horn (grades 2 and 3) had greater odds of being lame compared to those with grade 1 lesions or healing lesions (grade 4) (Table 8). Furthermore, as expected sheep with more than one foot affected were more likely to be lame than those with just one foot affected.

In this study, the lesions WL and OG were both not associated with lameness. Traditionally these lesions would have been treated using foot paring. These data would therefore support recent opinions suggesting that the paring of feet with these lesions is at best unnecessary (Kaler et al., 2010b; Smith et al., 2014). Indeed GR - which are caused by over trimming (Winter, 2004a) - whilst at low prevalence on these farms, were strongly associated with lameness (OR: 6.0 [95%CI: 3.0-11.8]).

Seasonal changes in lameness

The probability of being lame reduced significantly throughout the study. It is likely that due to the farms being studied, the very act of doing so helped reduce the overall burden of infection. In this study two of the farms had large areas of rough extensive grazing such that observation of lame individuals at pasture was on frequent occasions impossible. Furthermore to gather sheep on one of these areas took on average 3-4 hours and was only possible in the right weather conditions. To expect farmers to be able to identify lame individuals in these situations, promptly catch them and treat them is highly impractical and unrealistic. An alternative strategy might be planned repeated gathering in order to treat affected individuals, as occurred by default in the present study.

Conclusions

Sheep with CODD present a considerable welfare concern, particularly as there remains very little robust evidence for effective treatment and control. Within farm prevalence is variable and there may be seasonal effects that could aid targeted intervention strategies. Furthermore, this study demonstrated that point prevalence is highly inaccurate in determining the overall disease and associated welfare situation on a farm.

Footrot is consistently observed to be significantly associated with CODD although it is unknown if it is an interactive or causal association. Indeed given the 'bacterial soup' in diseased feet it might be more helpful to consider the conditions CODD, FR and ID under the umbrella of 'contagious foot disease' and adopt control and intervention strategies in a microbiologically broader and less individualistic way.

The intervention of studying these commercial farms, had a surprisingly positive impact on the overall lameness of the flocks and as such challenge the accepted advice of the prompt treatment of lame individuals with antibiotic therapy as the 'best' option for all farms. Those farms with more extensive grazing – particularly hill and mountain farms – may benefit from a different approach such as the planned regular gathering of sheep for the purpose of inspection and treatment, an idea that requires further investigation.

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Chapter 7

In vitro susceptibility of contagious ovine digital dermatitis associated *Treponema* spp. isolates to antimicrobial agents in the UK

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Abstract

Background

Contagious ovine digital dermatitis (CODD) is an important cause of infectious lameness in sheep in the UK and Ireland and has a severe impact on the welfare of affected individuals. The three treponemal phylogroups *Treponema medium/Treponema vincentii*-like, *Treponema phagedenis*-like and *Treponema pedis* spirochaetes have been associated with clinical CODD lesions and are considered to be a necessary cause of disease. There is scant data on the antimicrobial susceptibility of the treponemes cultured from CODD lesions.

Objective

The aim of this study was to determine *in vitro* the MIC/MBC of antimicrobials used in the sheep industry for isolates of the three CODD associated treponeme phylogroups *T. medium/T. vincentii*-like, *T. phagedenis*-like and *T. pedis*.

Animals

Twenty treponeme isolates from 19 sheep with clinical CODD lesions.

Methods

A microdilution method was used to determine *in vitro* the MIC/MBC of ten antimicrobial agents for 20 treponeme isolates (five *T. medium/T. vincentii*-like, ten *T. phagedenis*-like and five *T. pedis*). The antimicrobials tested were penicillin G, amoxicillin, oxytetracycline, tilmicosin, lincomycin, spectinomycin, tylosin, tildipirosin, tulathromycin and gamithromycin.

Results

The treponeme isolates tested showed low MICs and MBCs to all ten antimicrobials tested. They were most susceptible to gamithromycin and tildipirosin (MIC90: 0.0469 mg/L), and were least susceptible to lincomycin, spectinomycin and oxytetracycline (MIC90: 48 mg/L, 24 mg/L and 3 mg/L respectively).

Conclusions

These data are comparable to *in vitro* antimicrobial susceptibility data for treponemes cultured from bovine digital dermatitis lesions. Dependent on local licensing, penicillin and tilmicosin appear the best candidates for future *in vivo* studies.

Introduction

Contagious ovine digital dermatitis (CODD) is a cause of infectious lameness in sheep in the UK and Ireland and has been shown to have a severe impact on the welfare of affected individuals (Duncan et al., 2014). Recent surveys have shown that CODD may affect approximately 35% of flocks in the UK; while on farm prevalence is typically low, it may affect up to 50% of the flock at any one time (Duncan et al., 2014).

Information about the microbial flora of CODD lesions is limited, although the bovine digital dermatitis (BDD) associated treponemes *Treponema medium/T. vincentii*-like, *Treponema phagedenis*-like and *Treponema pedis* are currently considered to be a necessary cause of disease (Duncan et al., 2014). The recent characterization of treponemes associated with CODD demonstrated the presence of at least one BDD phylotype present in all 58 lesions studied whereas these were totally absent from all healthy sheep foot tissues (Sullivan et al., 2015).

There have been a wide range of empirically chosen treatments employed in clinical cases e.g. parenteral oxytetracycline and topical tylosin (Judson, 2010), with only one randomised controlled trial conducted comparing parenteral amoxicillin and simultaneous topical chlortetracycline with topical chlortetracycline alone (Duncan et al., 2011).

As with CODD, the successful treatment of BDD has remained problematic with many farms adopting management and control strategies 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, a greater understanding is required of the susceptibility of the treponemes found in CODD lesions to antimicrobials currently available for use in sheep.

The aim of this study was to determine *in vitro,* the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of a panel of antimicrobials for representatives from each of the three treponeme phylogroups cultured as pure isolates from clinical CODD lesions.

Materials and Methods

Bacterial isolates

Twenty treponeme isolates from CODD lesions from 19 sheep from six farms in England, Wales and Northern Ireland were used (Table 1). Included were five isolates from the *T. medium/T. vincentii*-like group, ten isolates from the *T. phagedenis*-like group and five isolates from the *T. pedis* group.

Strain no.	Strain	Isolation date	UK Region	Nearest related organism[a]
1	G1F7C5	07/2013	Conwy	*Treponema medium/Treponema vincentii*
2	G1F9C27	07/2013	Conwy	*Treponema medium/Treponema vincentii*
3	G1OV11	08/2009	Gloucester	*Treponema medium/Treponema vincentii*
4	G2S2R	02/2009	Cheshire	*Treponema medium/Treponema vincentii*
5	ST27	07/2013	Conwy	*Treponema medium/Treponema vincentii*
6	3F2	02/2014	Anglesey	*Treponema phagedenis*
7	C2F	06/2009	Gloucestershire	*Treponema phagedenis*
8	G2S4F	02/2009	Cheshire	*Treponema phagedenis*
9	G2F3C12	07/2013	Conwy	*Treponema phagedenis*
10	G13F3	02/2014	Denbighshire	*Treponema phagedenis*
11	G2SL1	05/2014	Anglesey	*Treponema phagedenis*
12	G23F1	02/2014	Anglesey	*Treponema phagedenis*
13	S3R2	03/2009	Cheshire	*Treponema phagedenis*
14	G2ST24	07/2013	Conwy	*Treponema phagedenis*
15	C2R	06/2009	Gloucestershire	*Treponema phagedenis*
16	Ovine (G179)	2000	Northern Ireland	*Treponema pedis*
17	G3ST1	07/2014	Shrewsbury	*Treponema pedis*
18	G3S45	07/2014	Shrewsbury	*Treponema pedis*
19	G3T1	07/2014	Shrewsbury	*Treponema pedis*
20	G3T7	07/2014	Shrewsbury	*Treponema pedis*

Table 1: Treponemes tested for susceptibility to antimicrobial agents

[a]As determined by 16S rRNA gene phylogenetic analysis.

In vitro antimicrobial susceptibility testing

The MIC/MBC for each antimicrobial was determined using a broth microdilution method as previously described (Evans et al., 2009a). One small adjustment was made to the method such that prior to inoculation, bacterial counts were assessed by determining the optical density (OD) of the cultures using spectrometry with wavelength set at 540 nm. The *T. medium/T. vincentii*-like cultures had an OD of 0.25; the *T. phagedenis*-like cultures had an OD of 0.43 and the *T. pedis* cultures had an OD of 0.37. This corresponds to 8.75 x 10^7, 1.14 x 10^8 and 2.69 x 10^8 treponemal organisms/ml, respectively (Evans et al., 2009a). At this time point, cultures were assessed by phase contrast microscopy to determine that the cultures were alive, of the correct morphology and lacking contaminants. The antimicrobials and their test ranges are listed in Table 2.

Determination of MICs

The MIC for each antimicrobial was taken as the lowest concentration of antimicrobial that prevented growth in the wells observed at the same time points (Evans et al., 2009a). Cell growth was determined by comparison of the absorbance measurement immediately after inoculation with the absorbance measured at the late exponential/early stationary phase. All of the absorbance measurements were at 540 nm 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.

Determination of MBCs

The MBC for each antimicrobial was determined as described (Evans et al., 2009a).

Determination of MIC_{90} and MBC_{90}

The cumulative inhibitory/bactericidal concentration for each antimicrobial tested across all the treponeme isolates was expressed as the concentration at which 90% of CODD associated treponemes were inhibited from growing (MIC_{90}) or killed (MBC_{90}).

Statistical analysis

Differences in MICs between the three different treponeme phylogroups were assessed using the Kruskal-Wallis test. The Kruskal-Wallis test and the non-parametric equality-of-medians test were used to compare the MICs for penicillin, oxytetracycline, lincomycin and spectinomycin with previous data (Evans et al., 2009a) and for amoxicillin and gamithromycin (Evans et al., 2012). All statistical analyses were conducted using Stata IC 13 (Stata Corp; TX, USA) and statistical significance was set at $P < 0.05$.

Study validation

The MIC microdilution method described in this study was validated by comparing the results produced from four antimicrobials (penicillin, oxytetracycline, lincomycin and erythromycin) incubated with two control microorganisms *T. phagedenis* biotype Reiter and *T. phagedenis*-like T320A against results previously obtained using a macrodilution method (Abramson and Smibert, 1971) and also results obtained using a similar microdilution method (Evans et al., 2009a; Evans et al., 2012). The data were also compared statistically using linear regression.

Results

Antimicrobial susceptibilities of CODD associated treponemes

The individual MIC/MBCs of the antimicrobial agents to each treponeme isolate are summarised in Tables 2 and 3; in this study all isolates showed low MIC/MBCs to all the antimicrobials tested. Using Table 2, all treponeme groups were most susceptible to gamithromycin and tildipirosin, and least susceptible to lincomycin, spectinomycin and oxytetracycline. The MIC_{90} for the other five antimicrobials were all relatively low, being <1.0μg/ml. No bimodal distributions were identified.

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

Table 2: Minimum inhibitory concentrations (MIC) of ten antimicrobial agents tested against contagious ovine digital dermatitis associated treponemes.

[a] Isolates 1-5 are *Treponema medium/Treponema vincentii*-like BDD spirochaetes with antibiotic test ranges (μg/L) of: penicillin G 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 (Sigma-Aldrich, Dorset, UK); tildipirosin 0.75-0.0059 (Zuprevo; MSD Animal Health, Milton Keynes, UK); tulathromycin 9.375-0.0732 (Draxxin; Zoetis UK Limited, London, UK); gamithromycin 0.188-0.0015 (Merial LLC, Duluth, Georgia, USA); and tylosin 0.375-0.0029 (Sigma-Aldrich, Dorset, UK).

Isolates 6-15 are *Treponema phagedenis*-like CODD spirochaetes and isolates 16-20 are *Treponema pedis* CODD spirochaetes with test ranges the same as those for *Treponema medium/Treponema vincentii*-like BDD spirochaetes.

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

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	0.0375	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

Table 3: Minimum bactericidal concentrations (MBC) of ten antimicrobial agents tested against contagious ovine digital dermatitis associated treponemes.

[a] 1-5, *Treponema medium/Treponema vincentii*-like; 6-15, *Treponema phagedenis*-like; 16-20, *Treponema pedis*.

[b] Cumulative susceptibility results across all treponemes tested are expressed as MBC_{90}, the concentration at which 90% of CODD associated treponemes were killed.

Variation in MIC across the different treponeme phylogroups

There was no significant difference in MIC values between the three different phylogroups for five of the seven macrolides (P=0.2), with phylogroup differences for lincomycin and spectinomycin approaching significance (P=0.05). Whilst there was no significant difference between phylogroups in the case of penicillin MIC (P=0.1), in the case of amoxicillin and oxytetracycline, *T. phagedenis*-like bacteria were more susceptible compared to *T. medium/T. vincentii*-like and *T. pedis* (P=0.002 and P=0.001 respectively).

Comparisons with data from previous studies

The MICs for penicillin, oxytetracycline, lincomycin, spectinomycin, amoxicillin and gamithromycin for the 20 isolates investigated here, were not significantly different to those previously reported (Evans et al., 2009a; Evans et al., 2012).

Study validation

The comparison described matched the previous results in all cases except for one antimicrobial (oxytetracycline), which was different by one serial dilution when compared with the macrodilution method (Abramson and Smibert, 1971). Linear regression for these comparisons showed strong correlations (R=0.99 P<0.004) indicating the efficacy and reproducibility of this microdilution method.

Discussion

Study validation and comparisons with data from previous studies

The methodological validations described reinforce the comparable nature of these current data with previous studies. Comparisons of these current data for penicillin, oxytetracycline, lincomycin and spectinomycin with previous studies do not reveal any statistically significant differences (Evans et al., 2009a; Evans et al., 2012). Therefore, these current data make a valuable contribution to the available data on the *in vitro* antimicrobial susceptibility of these treponemes.

Antimicrobial use in sheep with CODD

All the isolates in this study were susceptible (*in vitro*) to all the antimicrobials tested, with gamithromycin, tildipirosin, penicillin, tylosin and tilmicosin demonstrating the lowest MICs and MBCs. This susceptibility however may not necessarily be reflected *in vivo*. To date, there have been very few robust *in vivo* studies examining effective treatment. In two clinical studies (Duncan et al., 2011; Duncan et al., 2012) systemic amoxicillin together with topical chlortetracycline was found to have a clinical cure rate in clinical cases of CODD of approximately 80%. Anecdotally, systemic tilmicosin was also proposed to be an effective treatment for sheep with CODD (Watson, 1999) and systemic oxytetracycline together with a tylosin footbath were considered to be an effective preventative method (Judson, 2010).

Currently, no antimicrobial product has a license for CODD in the UK. The antimicrobials studied here were selected to include antimicrobials that already have a license for sheep (penicillin, amoxicillin, oxytetracycline and tilmicosin) together with those that in the authors' experience are already used (off label) in the sheep industry. Therefore, given this context and these data as a whole, penicillin and tilmicosin would appear to be the most likely candidates for future *in vivo* studies.

This study provides the first detailed examination of the *in vitro* antimicrobial susceptibilities of all three associated phylogroups of treponemes cultured from CODD lesions to antimicrobials. As such, these data provide important *in vitro* information on antimicrobials currently used to treat this infection and should help inform researchers planning further *in vivo* studies when considering which products to include.

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Chapter 8

Whole-flock, metaphylactic tilmicosin use failed to eliminate contagious ovine digital dermatitis and footrot in sheep: a cluster randomised trial

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Abstract

The aim of this study was to evaluate the clinical success of whole-flock systemic tilmicosin in eliminating active CODD from sheep flocks for one year.

The trial was a two-armed, cluster-randomised controlled trial. A sample of 30 farms were recruited in the UK and randomly allocated to receive either treatment as usual or a whole-flock treatment with tilmicosin, together with isolation and extended treatment of clinically affected individuals. All farms were visited once at the beginning of the trial to examine all sheep. Sheep with foot lesions on the control farms were treated as per the farmer's normal routine. On the intervention farms, all sheep were treated with tilmicosin 10mg/kg subcutaneously, and those with clinical lesions were isolated and treated again 14 days later. Any new entrants to the intervention flocks during the study period were isolated and treated as well. One year later all the sheep were re-examined to determine the presence/absence of clinical lesions. The primary outcome was the clinical elimination of CODD from flocks. Secondary outcomes were the reduction in prevalence of CODD at flock level, the clinical elimination of footrot and the reduction in prevalence of footrot.

The final analysis included 11 control farms and 13 intervention farms, with initially 3,460 and 4,686 sheep respectively.

For CODD: at follow-up, in the intervention group 6/13 (46.2% (95%CI:19.2-74.9%)) farms had a prevalence of zero compared to 1/11 (9.1% (0.2-41.3%)) in the control group. Fishers exact test of these two proportions showed no evidence of a difference (P=0.12). There was also no significant difference in the change in prevalence of CODD between control and treatment farms

For footrot: at follow-up, no farms had a prevalence of zero.

Therefore the intervention is not recommended for use for the elimination/reduction in prevalence of CODD or footrot.

Introduction

Contagious ovine digital dermatitis (CODD) is now common, and causes severe lameness and pathological changes (Angell et al., 2014; Angell et al., 2015a). Clinical lesions have been associated with *Treponema* spp. phylogenetically identical to those associated with bovine digital dermatitis (BDD) (Sullivan et al., 2015) and as such are currently considered a necessary cause of disease (Duncan et al., 2014).

To date there have been few reports detailing attempts at treating CODD. In Duncan et al. (2011); Duncan et al. (2012) cure rates of approximately 70-80% were achieved with systemic amoxicillin and topical chlortetracycline. A case report by Watson (1999) indicated that a single injection of tilmicosin, together with topical oxytetracycline led to the clinical recovery of a severe ovine foot disease (clinically resembling CODD) in a group of approximately 100 lambs. Furthermore, Judson (2010) reported that systemic oxytetracycline together with a tylosin footbath prior to housing prevented the development of CODD in groups of lambs from an infected flock.

To date there has only been one *in vitro* antibiotic sensitivity study of *Treponema* spp. isolates cultured from CODD lesions, which demonstrated low minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) for penicillin and macrolide antibiotics (Angell et al., 2015b).

Recently, Sawyer (2010) reported the use of a whole flock treatment with tilmicosin at a dose of 5 mg/kg in 15 flocks affected by footrot and/or CODD. However, the author did not examine the sheep prior to treatment, there were no details of lesion prevalence/incidence data before or after treatment and there were no control data hampering evaluation of the study. However, despite this, there are widespread anecdotal reports of veterinarians across the UK using a similar regime.

Given the severe welfare impact of CODD, and the lack of robust clinical treatment studies, there is clearly a need to identify efficacious treatments for sheep with CODD. The aim of this study was to evaluate the clinical success of using whole flock systemic tilmicosin, together with isolation and repeated treatment of clinical cases, and treatment of purchased sheep, in eliminating active CODD from sheep flocks for one year.

Currently there are no treatments licensed for CODD, therefore the choice of treatment is empirical. In considering a particular antibiotic to investigate as a therapeutic agent tilmicosin (Micotil; Elanco Animal Health) was considered for the following reasons:

1) Tilmicosin demonstrated low MIC and MBC data in an *in vitro* study (Angell et al., 2015b).
2) Tilmicosin already has a UK license for the treatment of footrot in sheep, so could be justified in terms of the UK Veterinary Medicines Cascade (DEFRA, 2013b).

3) Given the deep pathology associated with the disease (Angell et al., 2015a) systemic antibiosis was preferred over topical application.

4) Due to the lack of knowledge in treating CODD, current therapeutic strategies for other treponemal diseases were considered including BDD and syphilis. For BDD topical strategies aimed at control are preferred to systemic antibiosis due to the economic costs associated with milk withholds (Laven and Logue, 2006).

   In the treatment of syphilis, the objective is for a clinical cure and treatment can be required for up to 28 days (Kingston et al., 2008).

   In the case of CODD, tilmicosin could be expected to persist within infected tissues (Scorneaux and Shryock, 1999; Naccari et al., 2001), and furthermore a repeat dose could be given safely after 14 days (Elanco Animal Health, personal communication) thereby potentially achieving a 28-day treatment period.

5) The responsible use of macrolides in veterinary medicine is imperative given that they are considered by the World Health Organisation to be critically important for human medicine (WHO, 2011). In the UK, tilmicosin injection can only be administered by a veterinary surgeon (Elanco, 2013), which may ensure more accurate and responsible use.

6) Tilmicosin was also chosen for this study, despite its status as 'critically important', on the basis that successful elimination would potentially result in lower overall use of antimicrobials.

7) Anecdotally there are good clinical responses in treating CODD with tilmicosin (Watson, 1999).

8) A pilot study[11] examining the clinical efficacy of tilmicosin showed excellent results. A cohort of 58 affected individuals, with 62 active CODD lesions were treated at a dose of 10mg/kg, given subcutaneously twice 14 days apart. The sheep were then re-examined 4 and 6 weeks after the first injection. At these visits, all the active lesions had healed although the horn was thinner and soft in many cases, and there were no new cases or reversions to active CODD.

Furthermore, due to the apparently widespread use of this whole flock approach, advocated by the manufacturer, and based on a poorly designed study (Sawyer, 2010) it was considered desirable to subject the intervention to a robust randomised controlled trial on a suitable number of farms with the desired outcome being elimination of CODD. Also, given the high welfare impact of CODD, the inherent difficulties in individual animal treatment of lame sheep, coupled with the apparent lack of a true carrier status as demonstrated by absence of evidence of treponemes in healthy and grade 5 (healed lesions) (Angell et al., 2015d) and the observation that a large proportion of lesioned sheep are not actually lame (Angell et al., 2015e), the prospect of elimination via a single mass treatment is attractive. The present study was designed to test the hypothesis that a single flock treatment could eliminate CODD from a flock.

[11] See Appendix

Materials and methods

Study design

The null hypothesis was: a single whole flock treatment with tilmicosin, together with isolation and repeated treatment of cases and treatment of purchased sheep would result in the elimination of CODD for one year based on clinical examination and lesion scoring. To test this hypothesis, a cluster randomised controlled trial design was used, with flocks as the cluster units, and control flocks continuing with treatment as usual (TAU).

Study population

A convenience sample of 30 flocks was recruited (Table 1), the inclusion criteria being: having active CODD (lesion grades 1-4 (Angell et al., 2015a)) in the flock as diagnosed by the authors, having a flock-size of approximately 300 ewes and being willing to fulfill the requirements of the study. A flock-size of 300 ewes was chosen to reflect the average flock-size in the UK (excluding smallholders) for 2013 (DEFRA, 2013a) and from experience this number of sheep would be feasible to gather, examine and treat in one study day. Twenty-one flocks were located in Wales, four in Devon, two in Lancashire and one each in Derbyshire, Cheshire and Yorkshire. Each farmer participated voluntarily, provided informed consent and was able to leave the study at any time. No financial payments were made to any of the farmers, but all treatments in both groups were provided free of charge as a gesture of good will. In addition, those flocks randomly allocated to receive TAU were given the option to receive the intervention at the end of the trial provided they had remained in the study for the duration.

Interventions and follow-up

Flocks were randomised to the control or intervention arms after recruitment. All the flocks were visited once at the beginning of the study to establish baseline prevalence data and administer the intervention, and then on one further occasion a year later to record the end prevalence data. In addition, they were visited a further three times evenly spaced in the interim period (at approximately three month intervals) in order to monitor the health of the ewes and to retain contact with the farmers.

For the initial visit and final visit, each flock was visited on a single day at a point approximately half way through pregnancy. This was considered the safest time to examine pregnant sheep, and also when fewest sheep would be present on the farm – mainly ewes and rams - ensuring the minimum amount of antibiotic used. Furthermore, it was considered that CODD prevalence might be at a lower level at this time than at other times of year (Angell et al., 2015e). At the initial visit, every sheep on the farm was individually examined and data recorded.

For the control group any foot lesion observed was treated as per the farmers' normal routine as agreed with their own veterinary surgeon. For the intervention group, the intervention comprised both treatment of all animals together with extended treatment and isolation of all clinical cases. All sheep received a single dose of tilmicosin (Micotil, Elanco Animal Health) at a dose of

10mg/kg administered subcutaneously over the left shoulder via an automatic injector through a ¾ inch X 18g needle. In addition, any sheep observed to have clinically active CODD (lesion grade 1-4 (Angell et al., 2015a)) was marked and isolated in a separate group on the farm. A second dose of tilmicosin was then administered to these individual sheep 14 days later in exactly the same manner as the first dose, except that it was given over the right shoulder. The farmer was then instructed to continue the isolation of these individuals for a further 14 days i.e. affected sheep were isolated for 28 days post initial treatment. Furthermore, throughout the year farmers were instructed to isolate any sheep moved on to the farm e.g. replacement ewes or rams. Those sheep were then inspected by the author and treated with a single dose of tilmicosin at this point, and the farmers were then instructed to keep those new animals in isolation for 14 days.

Outcomes

The primary outcome was the clinical elimination of active CODD at the final visit. A further secondary outcome was the reduction in prevalence of active CODD. A flock was deemed to have active CODD if any single foot was observed with a lesion in that flock. Lesions graded 5 (healed) could be present. With regard to active CODD, any lesion deemed to be ambiguous (n=2) was biopsied and the tissue tested by nested PCR assays specific for any of the three CODD associated treponeme phylogroups: *T. medium/T vincentii*-like, *T. phagedenis*-like and *T. pedis* (Sullivan et al., 2015) to confirm the diagnosis.

Due to the data available, the same analyses could also be done for footrot. In this case the primary outcome was the clinical elimination of footrot at the final visit and a secondary outcome was the reduction in prevalence of footrot.

Sample size calculation

Sample size calculations were carried out for a cluster-randomised design (Hayes and Bennett, 1999). At the end of the study, the expected prevalence of CODD in the control group was assumed to be 4% (mean obtained from (Angell et al., 2014)) and in the intervention group 0.5%; the cluster size was 300. The intra-class correlation coefficient was unknown but a coefficient of variation was estimated to be 0.25, power was set at 80% and alpha 0.05. This calculation estimated the inclusion of 3 clusters per arm, however, according to Kirkwood and Sterne (2003) cluster trials with few clusters are not recommended and so in order to account for the expected intra-cluster correlation, and for an expected 10% drop out of flocks, balanced with the ethical considerations of including enough flocks but no more, 15 farms were recruited to each arm.

Statistical methods

Outcome: Elimination of CODD from flocks

The proportion of flocks in the intervention arm that had a CODD prevalence of zero at the final visit was compared to the proportion in the control arm using Fisher's exact test.

Outcome : Change in prevalence of active CODD and footrot

For the outcomes: active CODD and footrot, the prevalence (calculated as the number of sheep as a percentage of the total flock size) at the initial visit (p1) and at the final visit (p2) were calculated for each farm directly. The change in prevalence (p_{diff}) was calculated as p1-p2 and displayed graphically. The mean pdiff for the intervention farms and that for the controls farms was calculated together with a 95%CI; a comparison of the 95%CI was used to assess the presence of a meaningful difference.

Results

Recruitment and participant flow

Thirty flocks were enrolled in the study during October and November 2013 (Table 1 and Figure 1). Two flocks (29 and 30) dropped-out from the control group shortly before their first visit and prior to being informed of their treatment allocation. Farmer 29 chose not to take part for practical reasons and farmer 30 was concerned regarding the stress to his animals due to their poor condition. For all flocks the initial visit occurred between 3rd December 2013 and 29th January 2014. All flocks remaining in the study for the duration were visited for the final time (visit 5) between 21st November 2014 and 13th February 2015.

Two control flocks (16 and 19) were excluded from the analysis as the follow-up period had to be terminated two months early. Two intervention flocks (4 and 11) were excluded from the analysis due to inadequate follow-up data; farmer 4 failed to present all the sheep for examination at the final visit and farmer 11 left the study after eight months due to a change of business.

As such, in the final analysis there were 11 control flocks and 13 intervention flocks. At the initial visit the number of sheep examined in total on the 11 control flocks was 3,460 and on the 13 intervention flocks 4,686. At the final visit there were 4,354 and 5,098 sheep examined respectively.

Farm	County	Land Type	Total size (Hectares)	Total number of breeding ewes (LU*)	Total number of cattle (LU*)	Stocking Density: number of livestock units per hectare	Breeds (%)
1	Powys	Lowland	28.3	175 (19.3)	64 (41.6)	2.2	Mules (90.9) Other cross breeds (9.1)
2	Denbighshire	Lowland	60.7	510 (56.1)	82 (53.3)	1.8	Texel X (31.1) Welsh mule (22.7) Mule (20.0) Suffolk X (13.5) Other cross breeds (12.7)
3	Conwy	Lowland	78.9	351 (38.6)	120 (78.0)	1.5	Mule (67.2) Suffolk X (20.5) Texel X (7.4) Other cross breeds (4.9)
4	Denbighshire	Upland	80.9	696 (55.7)	90 (58.5)	1.4	Beulah Speckled-Face (96.3) Other cross breeds (3.7)
5	Denbighshire	Upland	40.5	211 (23.2)	51 (33.2)	1.4	Mule (92.6) Texel (5.5) Other cross breeds (1.9)
6	Devonshire	Lowland	64.8	463 (50.9)	48 (31.2)	1.3	Suffolk (71.4) Mule (21.2) Texel X (3.9) Other cross breeds (3.5)
7	Powys	Lowland	76.9	381 (27.1)	118 (76.7)	1.3	Texel X (30.2) Mule (27.8) Welsh Mountain X (27.8) Charollais X (8.1) Other cross breeds (6.1)
8	Yorkshire	Hill	40.5	350 (27.7)	0	0.7	Swaledale (61.7) Mule (17.7) Texel X (16.0) Texel (3.4) Other cross breeds (1.2)
9	Lancashire	Upland	131.5	535 (58.9)	52 (33.8)	0.7	Texel X (56.3) Mule (42.2) Other cross breeds (1.5)
10	Devonshire	Lowland	80.9	441 (48.5)	55 (35.8)	1.0	Mule (38.3) Romney X (26.1) Lleyn X (18.8) Suffolk X (11.1) Other cross breeds (5.7)
11	Powys	Lowland	22.3	168 (18.5)	140 (91.0)	4.9	Texel X (67.9) Mule (22.6) Suffolk X (7.1) Other cross breeds (2.4)
12	Denbighshire	Lowland/Upland	80.9	414 (42.0)	40 (26.0)	0.8	Aberdale (79.5) Welsh Mountain (16.9) Other cross breeds (3.6)
13	Conwy	Hill	121.4	248 (14.9)	74 (48.1)	0.5	Welsh Mountain (100.0)
14	Derbyshire	Upland	32.4	228 (23.9)	0	0.7	Texel/Texel X (69.7) Mule (19.7) Swaledale (10.5)
15	Denbighshire	Upland	52.6	379 (30.4)	0	0.6	Welsh Mountain X (99.5) Charollais X (0.5)
16	Powys	Hill/Upland	64.8	250 (27.5)	0	0.4	Texel X (45.6) Suffolk X (24.4) Lleyn X (22.0) Mule (6.4) Charollais X (1.6) Border Leicester (1.0)
17	Denbighshire	Lowland	48.6	359 (38.1)	10 (6.5)	0.9	Suffolk X (24.2) Texel X (21.7) Mule (19.5) Welsh Mountain X (12.8) Charollais X (11.7) Other cross breeds (10.1)
18	Cheshire	Lowland	40.5	315 (34.7)	0	0.9	Texel X (68.2) Cheviot X (28.7) Other cross breeds (3.1)
19	Conwy	Lowland	32.4	343 (37.7)	0	1.2	Texel X (62.1) Suffolk X (30.6) Mule (7.3)

20	Conwy	Upland	44.9	259 (28.5)	60 (39.0)	1.5	Texel X (100)
21	Conwy	Upland	80.9	384 (38.9)	75 (48.8)	1.1	Mule (65.9) Welsh Mountain X (14.8) Welsh Mountain (8.3) Texel X (7.3) Other breeds (3.7)
22	Denbighshire	Lowland	70.8	410 (43.9)	55 (35.8)	1.1	Cheviot X (37.8) Texel X (19.7) Mule (15.2) Romney X (13.3) Welsh Mountain X (9.6) Other cross breeds (4.4)
23	Devonshire	Lowland	48.6	116 (12.8)	40 (26.0)	0.8	Suffolk X (73.0) Mule (20.0) Lleyn X (3.5) Other cross breeds (3.5)
24	Denbighshire	Lowland	121.4	338 (37.2)	100 (65.0)	0.8	Lleyn X (97.0) Other breeds (3.0)
25	Devonshire	Lowland	64.8	317 (34.9)	10 (6.5)	0.6	Mule (100)
26	Conwy	Upland	123.4	287 (31.6)	70 (45.5)	0.6	Lleyn X (43.2) Suffolk X (21.6) Polled Dorset (16.0) Mule (14.3) Texel X (3.5) Other breeds (1.4)
27	Conwy	Upland	121.4	491 (37.4)	53 (34.5)	0.6	Welsh Mountain (53.4) Texel X (31.0) Welsh Hill Speckled Face (14.3) Other breeds (1.3)
28	Powys	Lowland	32.4	184 (20.2)	150 (97.5)	3.6	Texel X (100)
29	Lancashire	Lowland		600	This farm dropped out before the first visit due to personal reasons.		
30	Conwy	Upland		400	This farm dropped out before the first visit as the ewes were in poor condition.		

Table 1: Farm attributes of the 30 study farms in England and Wales, as reported at the first visit at the commencement of the study.

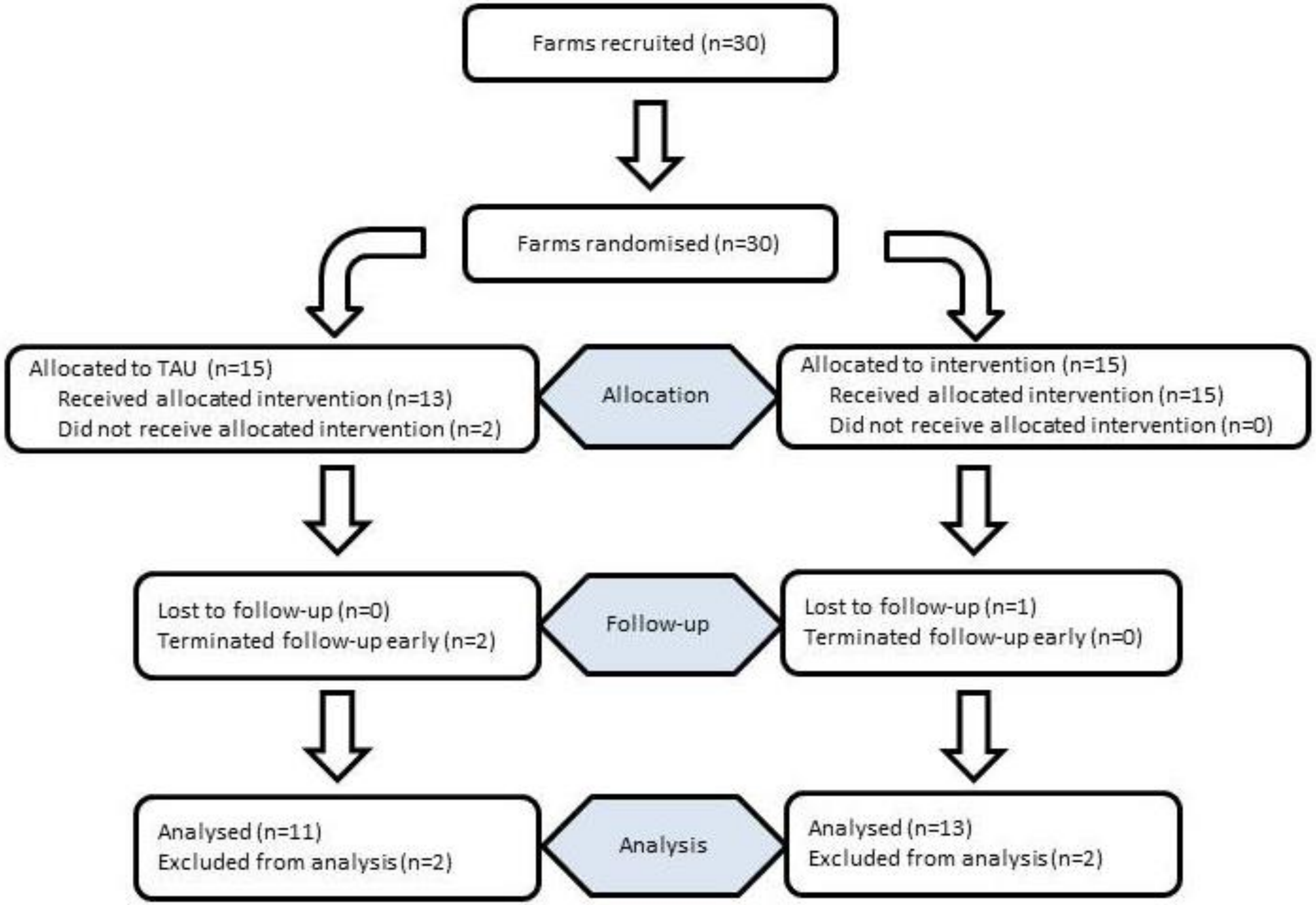

Figure 1: Participant flow diagram detailing the farms included at each stage of the trial.

Interventions

Control group

At the initial visit for the contagious foot diseases CODD, footrot and interdigital dermatitis (ID), each flock used slightly different combinations of treatments. Nine flocks used long acting oxytetracycline injection (Alamycin LA; Norbrook), three flocks used long acting amoxicillin injection (Betamox LA; Norbrook), eight used oxytetracycline spray (Alamycin; Norbrook) topically, one used lincomycin and spectinomycin (Lincospectin; Zoetis Animal Health) in a hand-held sprayer, and one used a tylosin (Tylan; Elanco Animal Health) footbath. For all flocks, feet were only trimmed if there was obvious impingement of soft tissues by loose horn or in some cases of white line disease (WL).

Intervention group

At the initial visit, all flocks received the intervention as described.

Baseline data showing the clinical characteristics of each group (Table 2) reveal significant differences between the intervention and control flocks for nearly all the variables except for foot abscess.

Variable (number of sheep)	Control (n=3,460 sheep)			Intervention (n=4,686 sheep)			
	N sheep	%	95% CI	N sheep	%	95% CI	P value*
Foot lesions							
CODD active (n=198)	99	2.86	2.35-3.47	99	2.11	1.74-2.57	0.03
CODD healed (n=416)	200	5.78	5.05-6.61	216	4.61	4.04-5.25	0.02
Footrot (n=1,837)	420	12.10	11.09-13.27	1,417	30.24	28.93-31.57	<0.001
Scald (n=917)	427	12.34	11.28-13.48	490	10.46	9.61-11.37	0.008
Granuloma (n=27)	18	0.52	0.33-0.82	9	0.19	0.10-0.37	0.01
White-line disease (n=2,317)	794	22.95	21.58-24.38	1,523	32.50	31.17-33.86	<0.001
Foot abscess (n=16)	7	0.20	0.10-0.42	9	0.19	0.10-0.37	0.9
Interdigital hyperplasia (n=46)	28	0.81	0.56-1.17	18	0.38	0.24-0.61	0.01
Overgrown (n=128)	76	2.20	1.76-2.74	52	1.11	0.85-1.45	<0.001
Farm variables							
Farm Size (Ha)(n=8,146)							
Small (28.3-52.6)(n=2,576)	1,233	35.64	34.06-37.25	1,343	28.66	27.38-29.97	<0.001
Medium (60.7-78.9)(n=2,432)	727	21.01	19.69-22.40	1,705	36.38	35.02-37.77	<0.001
Large (80.9-131.5)(n=3,138)	1,500	43.35	41.71-45.01	1,638	34.96	33.60-36.33	<0.001
Land-type (n=8,146)							
Hill (n=598)	0	0	-	598	12.76	11.84-13.75	<0.001
Upland (n=3,188)	1,421	41.07	39.44-42.72	1,767	37.71	36.33-39.11	<0.001
Lowland (n=4,360)	2,039	58.93	57.28-60.56	2,321	49.53	48.10-50.96	<0.001
Cattle (n=6,874)	3,145	90.90	89.94-91.85	3,729	79.58	78.42-80.73	<0.001
Lame (n=8,146)	318	9.19	8.23-10.15	624	13.32	12.34-14.29	<0.001
Breed (n=7,876)		(n=3,196 sheep)			(n=4,680 sheep)		
Upland (n=2,482)	1,242	38.86	37.18-40.56	1,240	26.50	25.25-27.78	<0.001
Lowland (n=5,394)	1,954	61.14	59.44-62.82	3,440	73.50	72.22-74.75	<0.001
Age (n=8,131)		(n=3,450 sheep)			(n=4,681 sheep)		
Lamb (n=1,096)	255	7.39	6.56-8.31	841	17.97	16.89-19.09	<0.001
1 year (n=1,216)	499	14.46	13.32-15.68	717	15.32	14.31-16.38	0.9
Adult (n=3,123)	2,696	78.14	76.73-79.49	3,123	66.72	65.35-68.05	<0.001

Table 2: Baseline characteristics of the control (n=11) and intervention (n=13) flocks as recorded at visit 1.
* Chi-squared test comparing the proportion of sheep in the control group with that in the intervention group.

CODD

Outcome: Elimination of CODD from flocks

The null hypothesis was: a single whole flock treatment with tilmicosin, together with isolation and repeated treatment of cases and treatment of purchased sheep would result in the elimination of CODD for one year based on clinical examination and lesion scoring. Of the control flocks (n=11), one flock (flock 25) (9.1% (95%CI: 0.2-41.3%)) had a prevalence of active CODD of zero at the final visit. Of the intervention flocks (n=13) six flocks (46.2% (95%CI: 19.2-74.9%)) had a prevalence of zero at the final visit. Fishers exact test of these two proportions showed no evidence of a difference (P=0.12). None of the flocks that had received the intervention and had a final prevalence of zero at the final visit had any observed clinical cases throughout the year following the initial visit.

Outcome: Change in prevalence of CODD

The null hypothesis was that there would be no difference between the p_{diff} of active CODD in the control flocks compared to the intervention flocks. Figure 2 shows that at the final visit the prevalence was reduced on 11/13 (84.6 % (95%CI: 54.6-98.1%)) of the intervention flocks with no meaningful change in prevalence occurring in 2 flocks (7 and 10) (15.4% (95%CI: 1.9-45.4%)). However, for the control flocks, seven (63.6% (95%CI: 30.8-89.1%)) showed a reduction in prevalence whilst four (36.4% (95%CI: 10.9-69.2%)) showed an increase in prevalence. The mean p2 for the control flocks was 3.04% (95%CI: 1.22-4.86) and for the intervention flocks was 0.54% (95%CI: 0.13-0.95). The mean p_{diff} for the control flocks was 0.26% (95%CI: -1.58-2.09) and for the intervention flocks was 1.52% (95%CI: 0.84-2.21). A comparison of the 95%CIs for these two proportions shows no evidence of a significant difference.

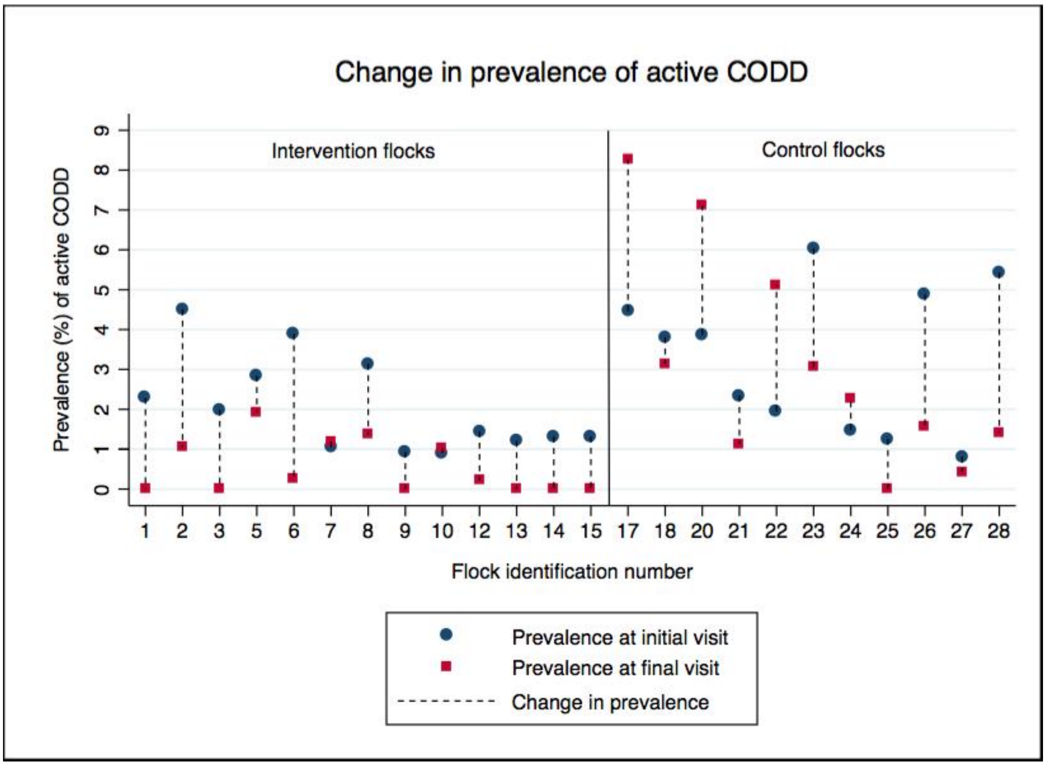

Figure 2: Change in prevalence (p_{diff}) of active CODD for each flock between the initial visit and the final visit one year later. Intervention flocks: 1-15, control flocks 17-28.

Footrot

Outcome: Elimination of footrot from flocks

No flocks in either group had a prevalence of footrot of zero at the final visit.

Outcome: Change in prevalence of footrot

The null hypothesis was that there would be no difference between the p_{diff} of footrot on the control farms compared to the intervention farms. Figure 4 shows that all the intervention flocks except flock 2 had a reduced prevalence at the final visit compared to the initial visit. However, of the control flocks, six (54.5% (95%CI: 23.4-83.3%)) showed a reduction in prevalence whilst five (45.5% (95%CI: 16.7-76.6%)) showed an increase in prevalence. The mean p2 for the control flocks was 16.59% (95%CI: 8.26-24.91) and for the intervention flocks was 5.73% (95%CI: 2.67-8.79). The mean p_{diff} for the control flocks was -2.91% (95%CI: -9.93-4.12) and for the intervention flocks was 26.05% (95%CI: 11.27-40.84). A comparison of the 95%CIs for these two proportions shows evidence of a significant difference.

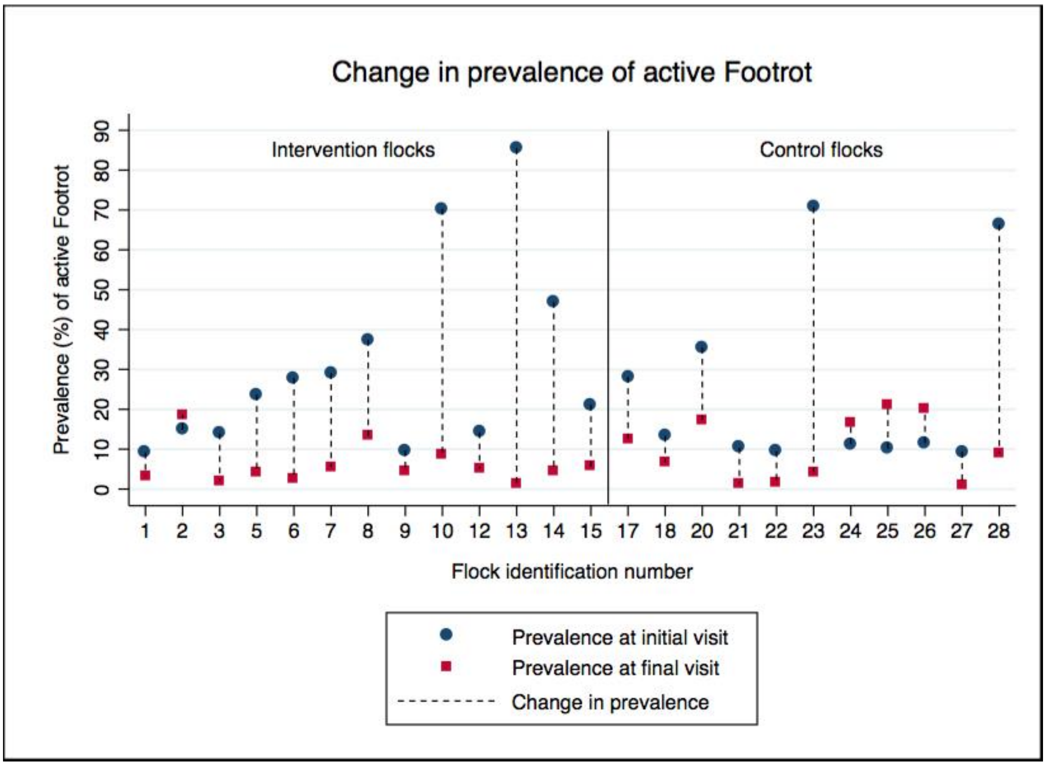

Figure 4: Change in prevalence (p_{diff}) of footrot for each flock between the initial visit and the final visit one year later. Intervention flocks: 1-15, control flocks 17-28.

Harms

During the study two sheep died shortly after being injected with tilmicosin, although it is not known whether they died as a result of tilmicosin toxicity. It is feasible that inadvertent intravenous delivery of the drug occurred as a result of the use of an automatic injector, which is a known risk. No other sheep suffered any injury or death associated with the study. There were no reported pregnancy failures following examination or treatment of any of the sheep.

Discussion

Study design and limitations

Table 2 demonstrates that there were some significant differences between the intervention and control flocks, despite randomisation. In particular, CODD prevalence was higher in control flocks (2.86% (95%CI: 2.35-3.47%) vs 2.11% (95%CI: 1.74-2.57%), P=0.03) whilst footrot prevalence was higher in the intervention flocks (30.24% (95%CI: 28.93-31.57%) vs 12.1% (95%CI: 11.09-13.27%), P<0.001). This was considered to be due to chance due to the small number of flocks included in the study and is a known risk with cluster randomised trials with small numbers of clusters (Kirkwood and Sterne, 2003).

The study was also weakened by a lack of blinding, which was not possible due practical constraints. As such this may lead to bias in that farmers in the intervention arm may manage their flocks differently due to being in the intervention arm, and the observer might have been less likely to diagnose a lesion/record lameness in an intervention flock at follow-up. To try and reduce this bias, rigorous observer training was employed with ambiguous CODD lesions biopsied for nested PCR analysis for CODD associated treponemes.

Further bias is likely to be present due to the convenience sample necessary for this study, in that relatively interested farmers were more likely to be included. However, the study flocks were all commercial flocks with a variety of different breeds, location and land type, and as such, this should strengthen the generalisability of the results.

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CODD

Contagious ovine digital dermatitis was eliminated from only six of the 13 intervention flocks, and one of the control flocks. Furthermore, a comparison of the proportion of flocks that eliminated CODD between the two trial arms was not significant. Where clinical elimination occurred it was not possible to say whether infection was *eradicated*; however elimination seems possible since no clinical cases were observed for one year and the observed prevalence at the final visit was zero. Therefore, whilst the elimination of clinically active CODD at flock level seems possible, there was a high failure rate, and in this study, was no more likely for the intervention farms and was also possible without whole flock treatment (e.g. farm 25, Figure 2).

Given the need for veterinary practitioners to use antimicrobials responsibly - in particular macrolides - categorised as critically important for human medicine (WHO, 2011), together with the financial costs of such an intervention, then on the basis of this study metaphylactic administration cannot be justified for the elimination of CODD from flocks. Furthermore, the change in prevalence was not significantly different between the intervention and control farms, suggesting that any initial improvement seen was short-lived and thus this intervention would not be suitable as a control measure.

As demonstrated, even with regular contact and monitoring only six of the 13 intervention farms remained clinically free from active CODD after one year. It is not known why these failures occurred, however detailed investigation of the intervention farms that failed to eliminate CODD revealed biosecurity problems and management issues that made failure more likely. It is a well recognised that farmers may not always follow advice, even if given with the aim of helping them (Kaler and Green, 2013; Garforth, 2015). All the farmers in this study were repeatedly reminded about the need for rigorous biosecurity measures e.g. maintaining adequate fencing, isolating and inspecting purchased animals etc. However, even with good intentions failings may occur for a variety of reasons.

Given the high clinical cure rates observed in the pilot study (100%), tilmicosin may be considered suitable for the individual treatment of lesioned sheep.

However, a long acting amoxicillin preparation together with topical chlortetracycline also achieved high cure rates (approximately 70-80%) in two other studies (Duncan et al., 2011; Duncan et al., 2012) and would provide a suitable alternative. Given that a significant proportion of sheep with lesions do not show lameness (Phythian et al., 2013; Angell et al., 2015e), an approach inspecting every foot on the farm, together with the treatment and isolation of individuals using one of these two antibiotics may be more appropriate.

Footrot

The point prevalence of footrot on some farms was very high, e.g. 85% in flock 13 at the initial visit. This was surprising given the average reported prevalences from other studies e.g. 8.3% in Grogono-Thomas and Johnston (1997), 9.4% in Wassink et al. (2003a) and 3.1% in Winter et al. (2015). In the present study, footrot was not eliminated from any farms and whilst there was a significantly reduced prevalence on the intervention farms compared to the control farms the intervention cannot be recommended for this effect. In the report by Sawyer (2010) it was reported that many farms 'reported no sign of footrot'. However, it is difficult to believe that there were no cases at all given that the author was relying on farmer reports and did not examine sheep at follow-up. Currently, there are well-researched and established effective treatment and control methods for footrot e.g. Green et al. (2007b); Wassink et al. (2010b); Clements and Stoye (2014), and therefore the use of whole flock tilmicosin for treating or controlling footrot cannot be justified or recommended, for similar reasons as those for CODD.

Conclusions

The whole flock treatment intervention as described cannot be recommended for the elimination of CODD or footrot. The elimination of CODD from affected farms in the UK appears possible and a number of approaches using targeted treatment of clinical cases may achieve this. Given the real risk of introducing or reintroducing CODD, effective biosecurity measures are essential to maintain elimination and/or control of the disease in a flock. Other effective measures are already available for controlling footrot in the UK and should be employed instead.

Acknowledgements

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Chapter 9

Concluding discussion

Introduction

The aim of this thesis was to further the current knowledge of CODD as prior to the studies reported here there has been relatively little scientific knowledge of CODD available, and the disease has caused severe welfare problems to individuals and flocks in the UK. So far a lack of understanding has hampered prevention, treatment and control strategies, and thus building on previous work within the University of Liverpool, a number of methodological disciplines were used including epidemiology, pathology, microbiology and pharmacology, in order to answer fundamental questions of the nature and management of this disease.

In order to strengthen the reporting of the data in this thesis, where applicable, the appropriate reporting guidelines were used. The STROBE guidelines (STROBE, 2009) were used for the observational studies reported in Chapter 2, Chapter 4 and Chapter 6. In Chapter 8 the CONSORT guidelines for intervention studies including the cluster trial extension (CONSORT, 2010) were used for the cluster randomized trial reported in Chapter 8. These guidelines are designed to increase transparency, clarity, consistency and accuracy in the reporting of epidemiological studies and data, and whilst not perfect provide a sound basis upon which to base the structure of a study report.

The study of lame sheep

In general this thesis highlights the complexity and enormous practical difficulty in studying lameness in sheep. In particular, a key problem emerged with identifying and re-identifying lame individuals, as well as identifying lesioned sheep that were not lame. Further problems were identified with the use of questionnaires to farmers on the subject of lameness or lesions in sheep, in particular problems with response rates and the misclassification of lesions by farmers. Together with these issues, the general trend throughout research carried out into lameness in sheep in using *prevalence* to describe disease levels has raised questions over whether this is in fact a suitable measure, and indeed whether it adequately describes what is actually a dynamic situation.

Locomotion scoring

The only remote indicator we have to identify a sheep with a locomotor problem are the behaviours associated with lameness, which are then applied in locomotion scoring systems. The problem then occurs that on the basis of their status as a prey species, sheep can reduce pain associated behaviours when handled (Fitzpatrick et al., 2006; Phythian et al., 2013) and so in a field situation sheep may appear more lame when observed from a distance than when gathered and handled. Furthermore, in most situations, re-identifying an individual sheep once it has been gathered into a handling facility is extremely difficult due to their visual similarity. During this project various attempts were made to circumvent this problem including identifying individuals with numerical paint/pitch markings on the fleece. Whilst visible over large distances with binoculars, these still proved inadequate due to the sheep rubbing their fleeces on one another and on field furniture thereby distorting the numbers. After a few days many of the markings were unidentifiable (Figure 1). Together with this the farmers were uncomfortable having the fleece marked in such an unusual way.

Figure 1: A few days after application fleece markings become difficult to identify even with binoculars.

In addition, in Chapter 3 when testing the locomotion scoring system developed in this project, both veterinarians and farmers found it difficult to consistently identify mildly lame sheep (locomotion score 1). This is comparable with the results of Kaler et al. (2009) who reported that 'the discrepancy between observers' scores was mainly for scores 0 and 1' – score 1 being 'uneven posture and/or short stride on one leg compared with others'. In other studies farmers have reported that they can easily recognise lame sheep when a head-nod becomes present (King and Green, 2011). However, these workers describe a head-nod being present when lameness has already become quite pronounced, and thus a head-nod does not appear to be an indicator of early or mild lameness. These studies together, illustrate the consistent difficulties in detecting mildly lame sheep. Consequently, the interpretation of studies reporting lameness scores in whatever form are difficult, in that there are likely to be observer differences in identifying individuals, and the problems with identifying mildly lame sheep imply that many lameness estimates are likely to be too low.

Furthermore, it was shown in Chapter 4 that sheep with all grades of CODD could demonstrate sound gait or severe lameness, and in Chapter 6 the mean locomotion scores for sheep with CODD, footrot and ID lesions were all under 2.0, i.e. many were mildly lame, and thus within the threshold of being difficult to detect. This creates a number of problems. Firstly, our only remote indicator of a locomotor problem and thus potentially a foot lesion are the behaviours associated with lameness, and many sheep with a foot lesion, even a severe one such as a grade 2/3 CODD lesion fail to demonstrate these behaviours. Indeed in Chapter 6 only 63.4% (95%CI: 62.0-64.9%) of sheep with CODD lesions were lame (locomotion score >0). Consequently, if we are relying on using lameness to identify sheep with a lesion then many are going undetected. Secondly, this not only has implications for research, but also for current advice on treating lame sheep, which includes advice to farmers to catch and treat *mildly* lame sheep (FAWC, 2011). As stated, farmers (and veterinarians) struggle to identify lame sheep and thus – even conscientiously - may not be able to carry this out as recommended.

Training and practice improve the repeatability of observational tests (Vasseur et al., 2013). The studies by King and Green (2011) and Phythian et al. (2012), demonstrate the need for specific training and practice to help observers detect subtle changes indicating lameness. Two shrewd comments by observers involved in Chapter 3 appear helpful: 'mildly lame sheep appear a bit uncomfortable and may be 'a bit tender' on their feet', and 'if you are in a hurry you don't see any lame sheep' – that is to detect early and subtle signs of alterations in locomotion an observer needs time, patience and practice to make an accurate observation.

In general, through locomotion scoring approximately 57,000 sheep during this period of study, whilst it has its place as a general indication of locomotor pain, with regards to lesions, only by handling and examining the feet of sheep can an accurate assessment be made as to their presence or absence (Figure 2).

Figure 2: The use of a turn over crate, like this Harrington Turn Over Crate (Yorkshire, UK) facilitates the examination of all four feet – particularly the hind feet - more easily than traditional turning and examination on the floor.

The locomotion scoring system developed in Chapter 3 is an improvement on previous studies in that it simplifies more complex systems, yet still provides an indication of severity compared to a binary system. Furthermore, it formalises what anecdotally, and in my own experience many within the industry would do intuitively. However, given this it remains flawed both as a means of detecting lesioned sheep and as a tool for measuring lameness associated welfare deficits, due to the as yet insurmountable difficulties discussed, and these flaws must be borne in mind when interpreting lameness data.

Questionnaires as a tool to investigate lameness in sheep

In this study, to gain background evidence and to identify potential risk factors associated with CODD, a questionnaire was carried out (Chapter 2). In carrying out this survey two related problems with using questionnaires as a tool to study lameness in sheep emerged: 1) response rates amongst farmers and 2) the validity of reported data.

The questionnaire in Chapter 2 had a disappointing response rate (28.3% (95%CI: 26.3-30.3%)), but was comparable to other surveys on lameness in sheep e.g. 27% in Kaler and Green (2008a) and 31.5% in Winter et al. (2015). Indeed, even in the survey carried out by Wassink et al. (2003a) that utilised a pre-identified group of farmers who were interested in being involved in research, a response rate of only 53.3% was achieved. In research into the validity of the questionnaire as an investigative tool within the field of human health, response rates of under 60% could be seriously biased (White et al., 2008). Consequently, all surveys on lameness in sheep are likely to be biased. Furthermore it is difficult to know if they are subject to the same biases and are thus comparable. Various methods of assessing the level and extent of bias can be used, such as following up a number of non-responders and determining if their responses would have been significantly different from those who did respond. This was not done in the present study due to cost issues and I am not aware that this has been done in any of the other surveys on lameness in sheep.

Researcher administered questionnaires may garner a greater number of responses over postal, self-administered questionnaires, particularly if the questions involve open, complex or branching forms. However, appropriate training, and – possibly as important with regard to farmers – an appropriate person are required to ensure reporting bias does not creep in, and in order to hold the trust of the respondent (White et al., 2008). Increasingly in veterinary science, internet based questionnaires are being used with a number of advantages over the traditional postal method, in that they are extremely cheap to administrate and the data is organised and recorded with little effort on the part of the researchers. Such a method was considered in administering the questionnaire in Chapter 2. However, in light of my considerable personal experience of many farmers who either do not have any computer access, or who only use one for certain specific tasks such as the recording of animal movements, compounded by significant poor rural internet provision, and a significant proportion of more elderly farmers – who may be considered more likely to be computer illiterate, the traditional paper method was employed.

With regard to surveys on lameness in sheep, farmers with a particular problem, e.g. CODD in their flock, may be more motivated to respond to a questionnaire on foot lesions than those with relatively few lameness problems. This selection bias may be compounded by those with in general more interest and proactive engagement with the industry potentially more likely to respond.

Further difficulties are likely to arise through reporting bias. It is well established that the majority of sheep farmers in the UK keep few if any meaningful records on the health aspects of their sheep (Kaler and Green, 2013), and are likely to be guessing at best in their responses to questions on numbers or proportions of sheep with lameness behaviours or lesions. As discussed, this is also likely to be compounded by the difficulties in identifying lame sheep, and the problem that not all sheep with a lesion demonstrate lameness. Due to the similar methodologies and potentially similar problems encountered by previous surveys on lameness in sheep, these problems are likely to be found in all postal questionnaires on lameness in sheep, and not restricted to the reports in this thesis, and thus it is possible that the data from these questionnaires are to some extent comparable.

In the questionnaire in Chapter 2, it was also apparent that a number of farmers did not know what CODD was before completing the questionnaire, and this hampered their ability to provide reliable estimates of disease and lameness prevalence. This problem has been highlighted by Kaler and Green (2008a) whereby of those who answered all questions, only 23% were correctly able to identify all six foot lesions studied. Footrot and ID were readily identified (by 85% and 83% of respondents respectively) but CODD was poorly identified (36%). This factor has possibly led to underestimation of the levels of disease in all questionnaires on lameness in sheep; indeed in the most recent questionnaire on lameness in sheep only 51% of farmers were correctly able to identify CODD (Winter et al., 2015). To try to surmount this problem, large clear photographs of footrot, ID and CODD were supplied alongside the questionnaire to assist farmers [12], and so that they could still answer the questionnaire even when initially unaware of CODD.

For the future, serious consideration should be given to funding researcher-administered questionnaires, with an appropriate person, equipped with the

[12] See Appendix A

right training and given ample time and opportunity to conduct it. In addition supplying clear unambiguous photographs, with descriptions should help reduce reporter bias and may help reduce selection bias through farmers not responding if not knowing the name of a lesion.

Prevalence

In Chapter 2 CODD was shown to be widespread with a between farm prevalence estimate of 35% (95%CI: 31.0-39.3%) in Wales, and surveys by Wassink et al. (2003b); Kaler and Green (2008a); Winter et al. (2015) confirm this, reporting prevalences in England of between 13% and 53%. In Chapter 6 there was wide variation between farms (mean prevalence: 2.5-11.9%) and within a farm over the year (e.g. for farm 2 in that study the prevalence ranged from 5.3% to 20.3%). Currently in the UK, national prevalence figures are used to describe levels of disease on farms and to provide targets for disease control (FAWC, 2011). The findings in this thesis have implications for this approach to describing disease levels, in that point prevalence refers to one point in time, and prevalence can vary widely over a year. This is further likely to be confounded in farmer reported surveys with the vast majority of farmers supplying their best estimates of disease prevalence rather than accurate observed figures.

This may be made even more confusing by the way this information is sought. In the survey reported in in Chapter 2 the question was phrased 'If you know the cause of lameness in your flock, can you estimate, when lameness is at its worst what percentage of your sheep are affected by CODD.', and for example in Winter et al. (2015) and Kaler and Green (2008a) the question was phrased 'Did you see this lesion (referring to a photograph) in your flock during [specific time period]; *if yes*, what percentage of ewes had this lesion?___________%'. These differences could potentially alter prevalence estimates in that for the latter two papers, if for example lambs were affected (as was reported by 27.4% of farmers in Chapter 2) then at a *flock* level these figures will be under estimated. Or if the reported value was an average estimate of lesion prevalence then this does not provide information with regard to the size of temporal changes in prevalence, which might be potentially useful.

This begs the question – what is meant by 'farm prevalence'. For example, farm 2 in Chapter 6 demonstrated a wide range in disease point prevalence estimates. At one observation, prevalence may be at or below a particular target and at another above it, and in using either figure independently, neither represents the true disease status of that flock – except at that point in time. Consequently, it is necessary for more meaningful measures to be developed in order to achieve reliable targets that may be used to reduce lameness in the national flock. One possibility could be that following appropriate training, farmers could record lameness or lesions in their sheep using individual ear tag recording technology or their smartphones, as has been developed as part of the Ease of Data Collection work stream as part of the Technology Agriculture and Greater Efficiencies (TAG) project in Wales (Menter a Busnes, 2015). Whilst still a flawed system, heavily dependent on farmers identifying and examining lame sheep (and not all sheep with a lesion may be lame), as well as requiring technological confidence, it may go some way to provide improved estimations of these figures.

The most useful figures would be incidence rates, allowing the number of new cases (lesions or lameness) over different time periods to be investigated. However, due to the rapid spread, and sometimes resolution of some foot lesions, a farmer recorded system is still likely to record under-estimates or on occasion over-estimates. Lesion/lameness incidence would be possible to observe in a group kept solely for research purposes, but due to the practicalities of farming and the need to examine every foot, it would not be possible to do this on a regular basis at flock level on a commercial farm, except over short periods of time. In reality, only repeat surveys on farms over a minimum of 12 months would allow an accurate description of lameness on a farm. Indeed in dairy cattle, Farm Assurance schemes e.g. Red Tractor Farm Assurance (2013) now require repeated observations of cow mobility as part of the overall monitoring process. This begs the question - should a similar protocol be adopted on sheep farms?

Contagious ovine digital dermatitis

Identifying CODD

Through the questionnaire, and as was quickly identified through field work, there was a lack of a clear standardised case definition available in the literature, and a supply of clear standardised photographs to which clinicians and researchers could refer when identifying a lesion as CODD. To this end a detailed clinical description of CODD was proposed (Chapter 4), and the lesions subdivided into 'lesion grades' detailing a proposed progression of disease in order to facilitate more precise communication, and to highlight the variety of lesions. Chapter 5 revealed the histopathological changes associated with these clinical lesions grades and thus as a result of this thesis, CODD may be defined as a severe, infectious disease of the ovine digit, with a broad range of clinical signs. Lesions typically start as an erosive/ulcerative dermatitis commencing at the coronary band, progressing to underrun the hoof horn dorsally and abaxially, through to a circumferential underrunning resulting in hoof horn avulsion in some cases.

The aetiology/aetiopathogenesis of CODD

This thesis has identified a complex aetiology of CODD and identified areas of research necessary to determine a specific aetiopathogenesis. Since the earliest reported outbreaks of CODD, spirochaetes, identified as *Treponema* spp. have been associated with disease (Naylor et al., 1998; Collighan et al., 2000; Moore et al., 2005b). Recently, using surgical tissue biopsies of lesions (as opposed to swabbing lesions), Sullivan et al. (2015) demonstrated by PCR one or more of the three BDD associated *Treponema* spp. phylogroups in 100% of 58 CODD lesions sampled. Moore et al. (2005b) also surveyed CODD lesions for *Treponema* spp. by PCR, but only found them in 70% of 50 lesions examined. It is possible however, that the swabbing technique and storage of swabs used in that study yielded insufficient DNA to be detected. In studies examining treponemes in BDD lesions, higher DNA detection rates have been obtained using surgical tissue biopsies (which are then stored in transport medium containing foetal calf serum) compared to swabbing (Evans et al., 2009c).

Footrot has clearly been demonstrated to be caused by *D. nodosus* (Beveridge, 1941), but its role in the aetiopathogenesis of CODD has been a matter of debate since the initial failure to detect it in the first identified outbreaks (Davies et al., 1999). Since then investigations have failed to conclusively state whether *D. nodosus* is involved in CODD or not. For example in Moore et al. (2005b) 74% of lesions were positive for *D. nodosus* by PCR and in Sullivan et al. (2015) 59% of lesions were positive by PCR.

Several studies have identified strong epidemiological associations between FR and CODD. In Duncan et al. (2012) sheep with footrot were more likely to have CODD at a subsequent visit than those without, OR: 3.83 (95%CI: 2.61-5.62, P<0.001), and a multivalent FR vaccine containing formalin-killed strains of *D. nodosus* had an efficacy of 32% against CODD. In the questionnaire in Chapter 2, of the 43 farmers that responded to the question, 81.4% (95%CI: 66.6-91.6%) considered that they observed CODD to be concurrent with footrot or ID. Then in Chapter 6, after controlling for confounders, co-infection with FR *in the same foot* was shown to be strongly associated with the presence of CODD, OR: 7.7 (95%CI: 3.9-15.5, P<0.001).

As such, *D. nodosus* does not seem *necessary* for the development of a CODD lesion, however a number of hypotheses to explain these positive associations seem plausible:

1. Footrot and CODD are caused by different infectious agents but are influenced by similar/the same environmental and management risk factors and thus *appear* associated.

2. *Dichelobacter nodosus* may cause damage to the skin barrier and induce tissue necrosis thereby providing a favorable environment for *Treponema* spp. to become established. The *Treponema* spp. are the *necessary cause* and thus associated with footrot as a *risk factor*.

Various epidemiological risk factors have been identified as being associated with CODD. In Chapter 2 farmers with larger flocks were more likely to report CODD, OR: 1.4 (95%CI: 1.2-1.7, P<0.001), and flock size was also positively associated with CODD in Chapter 6. This is consistent with many other contagious diseases. For example in cattle, BDD (Ettema et al., 2009) and tuberculosis (Skuce et al., 2012) are both more likely in larger herds, and in sheep, Bluetongue (Gaire et al., 2014) and coxiella burnetii seropositivity (Meadows et al., 2015) are both more likely in larger flocks. Practically, farmers with larger flocks may be more at risk due to a greater number of animal movements e.g. purchasing greater numbers of replacement ewes and rams, and therefore need to be more vigilant for the disease entering or re-entering their flock. Greater numbers of sheep – indeed greater numbers *per farm worker* specifically, may make it more difficult to identify individuals with locomotion disorders in general, and grade 1 CODD lesions in particular, due to the less severe lameness caused, as demonstrated in Chapter 4.

Also in Chapter 2, farmers that farmed both cattle and sheep were no more likely to report CODD than those that just farmed sheep, however those reporting BDD in their cattle were more likely to report CODD than those that did not have BDD in their cattle, OR 3.1 (95%CI: 1.9-5.1, P<0.001). This intriguing piece of evidence is possibly the first time an epidemiological link has been established between BDD and CODD, however due to it being based on farmer reports (together with all the methodological issues previously discussed) it is less robust than a controlled experimental study. As such, whilst the association is plausible if the treponemal aetiopathogenesis hypothesis is borne out, it is currently too weak a piece of evidence to make strong industry recommendations in terms of grazing or management practices.

In Chapter 6 a seasonal effect was observed, with higher prevalences recorded in early Autumn on six study farms. Possible explanations for this include that this may be the time when the greatest number and mass of sheep are present on sheep farms, with all the ewes and rams, together with a large number of lambs and when lambs are at their greatest size. However, this effect may also corroborate well with seasonal increases in footrot following a typical warm wet summer (Ridler et al., 2009).

Thus, from the studies presented in this thesis, in the wider context of the current understanding of these diseases and the interplay between them, it is possible that both the suggested hypotheses may be true - simultaneously. Footrot and CODD do share similar risk factors, for example both are associated with housing as reported by farmers for CODD in Chapter 2 and for footrot by Wassink et al. (2003a) and trauma to the feet from poor pasture as shown for CODD in Chapter 6 and for footrot in Wassink et al. (2003a). However, cases of CODD can occur without concurrent footrot as demonstrated in Chapter 6, and *D. nodosus* may not always be detected in clinical cases, although *Treponema* spp.

are when tissue biopsies are used (Sullivan et al., 2015) (compared to swabs of lesions (Moore et al., 2005b)).

The study by Duncan et al. (2012) however implies that footrot can in some cases precede CODD and thus colonisation by *D. nodosus* may act as the traumatic incident necessary for colonisation by *Treponema* spp. subsequently. Coupled with this, in Chapter 5, whilst immunolabelling for *D. nodosus* was not carried out, the immunolabelling of the *Treponema* spp. antigen was strongly positive at the leading edge of the pathological processes, particularly in the active lesion grades (grades 2 and 3) compared to the healing and healed lesions (grades 4 and 5).

In conclusion, the evidence presented in this thesis, together with other reports, it appears that the BDD associated *Treponema* spp. are a necessary cause of disease, but *D. nodosus*, and environmental and management risk factors associated with footrot also increase the risk of sheep developing CODD.

In this thesis a variety of risk factors were identified as being associated with CODD, either at sheep/foot level or at flock level. Most of these have not been tested as specific interventions but are deduced from observational studies and thus future experimental work may strengthen or refute them.

In Chapter 6 individual sheep were observed to have active CODD more than once - although no re-infection or recrudescence was observed at foot level. However, in Chapter 4, Figure 3 clearly shows a foot where the left-hand digit is healed whilst the right-hand digit has an active lesion. Thus whilst uncommon in these studies, reinfection may occur at sheep and at foot level. Therefore whilst no specific immunological investigations were carried out, it could be expected that sheep may have poor humoral immunity to *Treponema* spp., as is the case for cattle (Walker et al., 1997; Demirkan et al., 1999; Refaai et al., 2013).

The welfare of sheep with CODD

The development of the locomotion scoring tool in Chapter 3 enabled a systematic estimate of the severity of locomotion abnormalities in sheep for this thesis. As such, CODD can and frequently does cause severe lameness. Phythian et al. (2013) reported that whilst 59.0% of sheep with footrot, and 61.5% of sheep with ID were lame, 83.9% of sheep with CODD were lame. In Chapter 6, 664 (63.4% (95%CI: 62.0-64.9%)) of the 1047 sheep with CODD were lame and on average those with CODD were more lame, demonstrating significantly greater locomotion scores compared with those with ID or footrot. Also, when an individual was co-infected with CODD and ID, or CODD and footrot, these sheep demonstrated similarly severe locomotion scores as those with CODD alone. Therefore, in an individual sheep, CODD causes a more severe lameness than ID or footrot, and this is made no worse when co-infection with ID or footrot is present, and thus the presence of a CODD lesion forms the critical element of the welfare comprise of such an individual.

When equating locomotion score with pain, it was shown in Chapter 6 that the severity of lameness varies predictably by severity of the lesion present, in that those lesions with underrunning of the hoof horn (grades 2 and 3) result in an increase in locomotion score compared to earlier lesions (grade 1), and healing or healed lesions (grades 4 and 5). Interestingly, as shown in Chapter 4, despite the clinical resolution of many sheep with CODD (those demonstrating grade 5 lesions), and usually a reduction in locomotion score (and thus lameness severity), some of these sheep remained lame. This was also reported previously by Scott (2010), who suggested that a prolonged duration of disease may cause hyperasthesia and allodynia due to chronic inflammation of nerve endings (Lumb and Jones, 1996).

In Chapter 4 the radiography of digits with CODD lesions demonstrated osteophytosis and in some cases demineralisation of the distal phalanx, together with extensive damage to the soft tissue structures more superficially. Further investigation using histopathology in Chapter 5 revealed severe chronic inflammatory changes within affected digits, significantly over and above the background inflammation present in the normal ovine digit, together with granulation tissue reaction, osteophytosis and demineralisation of the distal phalanx. These findings corroborate well those of Scott (2010) and it is not difficult to imagine that such severe pathological changes will cause severe pain for prolonged periods. As to the neurophysiological phenomena – hyperaesthesia and allodynia - suggested as explanations for the poor locomotion, whilst it is plausible that nerve endings are affected in this way with such pathology, there is as yet no specific evidence for this.

Winter et al. (2015) reported that on a flock basis, the *farmer reported* prevalence of CODD typically remains low at around 2.0%, which supports observations made in Chapter 2. However prevalences of up to 50% were also reported, and from the observations made in Chapter 6, a point prevalence of 20.3% (95%CI: 16.9-23.7%) was recorded, implying that those reported high prevalences are possible. Consequently, on the majority of farms CODD typically causes a small number of sheep extreme suffering, and on a small proportion of farms the disease affects a larger proportion of sheep resulting in a severe welfare problem.

Treatment, control or elimination on farms with CODD

Previous research indicated that a single systemic dose of long acting amoxicillin together with topical chlortetracycline achieved a clinical resolution in approximately 70-80% of sheep with CODD (Duncan et al., 2011; Duncan et al., 2012). In this thesis, based on a range of factors, tilmicosin was investigated as a possible therapeutic agent. Both in the cluster randomised controlled trial in Chapter 8 and in a pilot study of 58 sheep for that trial (Appendix), tilmicosin given twice 14 days apart, subcutaneously and at a dose of 10mg/kg was *clinically* effective in 100% of clinical cases. No bacteriological follow-up was carried out on any of these cases and so it is possible that treponemal bacteriological sterilisation did not occur in some or all cases. However, given the lack of recrudescence in many cases for one-year, bacteriological sterilisation may be possible using this method. This corroborates anecdotes from other veterinarians using single doses where less rigorous follow-up was employed (Watson, 1999; Winter, 2004b; Sawyer, 2010).

Ethically, veterinarians choosing to use tilmicosin over amoxicillin for example, need to consider in balance the need to address the immediate welfare of individual sheep concerned, together with their judgment of the ability of the farmer to comply with their advice. Together with this there is also a professional obligation to act responsibly when prescribing all antimicrobials, particularly those considered to be critically important for human medicine (WHO, 2011; Royal College of Veterinary Surgeons, 2015). Consequently it is not possible to be prescriptive about the specific antimicrobial agents or products used by individual veterinarians to treat individual clinical cases, or what to advise their clients, as the factors highlighted may lead to different prescribing outcomes under varying circumstances.

The evidence provided in Chapter 2, Chapter 5 and Chapter 8 would suggest that typically CODD exists at a low (2-5%) prevalence on the majority of farms in the UK, which is corroborated by other farmer reports e.g. Winter et al. (2015). Adopting similar strategies to those advocated in the Five Point Control Plan for footrot in terms of the prompt clinical treatment of cases, isolation of cases, avoiding spread in fields, at gathering and in handling areas are all likely to aid in the maintenance of disease to a low level. For example, in Chapter 8, one of the control farmers (number 25) was able to reduce the clinical prevalence of CODD to zero within a year using these kinds of measures, treating clinical cases with long acting amoxicillin.

Figure 3: Extensively grazed hill ewes. Attempting to identify individual lame sheep and treat them is impossible in such an environment.

However, for many farmers, some of the methods advocated are highly impractical and impossible in some situations. For example in Chapter 6, Farmer 1 grazed 740 ewes for a large part of the year on a 300 acre mountain. To access this mountain took at least 30 minutes by quad bike and 90 minutes on foot (when weather conditions were too severe for the quad bike) and trying to identify individual sheep on such a scale, repeatedly and consistently, and then catch them for prompt treatment was impossible (Figure 3). Together with this even gathering sheep (which took on average 3-4 hours) was a large undertaking and only possible when the weather allowed with cloud, fog, snow and heavy rain rendering such a task impossible and dangerous (Figure 4)[13]. For some farms – particularly, but not exclusively hill farms - the planned repeated gathering of sheep may be the best method of controlling CODD, and indeed other foot lesions such as footrot. Indeed Farmer 1 in Chapter 6 adopted this as a control method following the cessation of the study.

Figure 4: Gathering sheep is a skilled art on terrain like this, and is impossible in bad weather.

[13] For a more in depth description of the experiences of one shepherd farming in a mountainous region, the reader is referred to the popular book: *The Shepherd's Life: A Tale of the Lake District* by James Rebanks (2015), published by Allen Lane ISBN-10: 1846148545. This book gives detailed experiential insights into the difficulties of farming in such an environment, including gathering sheep and the impact of the weather.

According to the reports from farmers in Chapter 2, together with the likely biosecurity breakdowns that occurred in the cluster randomised trial in Chapter 8, which were considered among the reasons for clinical elimination failure, it would appear that a key element to maintaining a low or zero prevalence of CODD, with whatever approach chosen, would be the isolation and manual inspection of foot lesions of all new entrants into a flock. Together with this, it would appear important to ensure all clinical cases in these new entrants are further isolated and treated appropriately. An alternative approach could be the metaphylaxis of all new entrants with tilmicosin as in the cluster randomised trial in Chapter 8, to ensure clinical/sub-clinical disease does not go unchecked, although with rigorous inspection this is probably unnecessary. The time in quarantine for this to be effective cannot be determined from the current evidence, however that for footrot is estimated at over three weeks, and a period of one month may be an easily communicated message to farmers (Winter et al., 2015). Further research should examine the rate and methods of transmission of CODD amongst sheep, together with the time necessary for effective quarantine procedures.

From the work in this thesis, together with two previous reports by Duncan et al. (2011); Duncan et al. (2012), CODD may effectively be treated in clinically affected individual sheep using systemic amoxicillin or tilmicosin. Building on the data presented in this study, particularly the *in vitro* work presented in Chapter 7, future *in vivo* studies using different antimicrobials, e.g. penicillin, may produce similarly effective results. Together with this, as already described, those farms that tackle footrot effectively are likely to see a lower prevalence of CODD, and are likely to be able to control CODD more easily as a result.

In terms of the eradication of CODD, The World Health Organization defines eradication as 'achievement of a status whereby no further cases of a disease occur anywhere, and continued control measures are unnecessary.' (Porta et al., 2008). The cluster-randomised trial in Chapter 8 is the only study examining clinical elimination on UK farms, and demonstrated that the *clinical elimination* of CODD from affected farms was possible for one year, with and without a whole flock metaphylactic approach (Chapter 8). However, in order to achieve *eradication* of a disease the risk of re-introduction must also be eliminated, and this clearly was not achieved. The quasi-experimental study by Sawyer (2010) using tilmicosin with a whole flock metaphylactic approach serves to corroborate this due to the annually repeated use reported.

Given the need for veterinary practitioners and farmers to use antimicrobials responsibly - in particular macrolides - categorised as critically important for human medicine (WHO, 2011), together with the financial costs of metaphylactic administration, then metaphylactic administration cannot be justified for the elimination of CODD, or the control of CODD in flocks. However, it is feasible, that in certain exceptional circumstances a single metaphylactic administration may be necessary from a welfare perspective, for example on larger farms with an exceptionally high prevalence of disease.

Alternative approaches to flock level control or elimination worthy of future investigation could be a similar whole flock examination (including detailed examination of *every foot*) and isolation of affected animals as occurred in the cluster randomised trial in Chapter 8, together with the treatment of infected individuals.

Furthermore, given the growing body of evidence linking the digital dermatitises of dairy cattle, beef cattle, goats, sheep and now elk, and the identification of common treponemal pathogens, interspecies transmission should be investigated in more detail. On many farms in the UK sheep and cattle are present providing a suitable opportunity for such an investigation. Should the presence of interspecies transmission occur, this may highlight potential problems or opportunities for the integration of treatment and control programmes on affected farms.

Throughout the studies in this thesis, together with extensive personal experience and published reports, biosecurity practices on sheep farms are in the main woefully inadequate or non-existent. Indeed, even the very structure of the industry is fundamentally flawed when trying to maintain biosecurity on farms. For example, for closed flocks (perceived to be more biosecure than flying flocks) rams still need to be purchased in order to introduce new genetics and to maintain the flock. The majority of rams bought and sold are still traded through specialist ram sales very close to the commencement of the breeding season, and as such many are immediately introduced to the breeding ewes following purchase. Appropriate quarantining is impossible with such a structure, and is a significant breech in biosecurity, difficult to overcome without a fundamental change in the way rams (and to some extent breeding females) are traded.

Holding ram sales in the summer months, would allow many farmers to make a purchase and still allow plenty of time to quarantine, inspect and apply other biosecurity measures such as vaccinations or treatment of any emerging diseases. The industry can ill afford to not adopt the quarantining of new entrants to a flock, and needs to consider changing sale dates or adopting other strategies such as purchasing stock direct from source farms. Such changes would also allow rams to adapt to a new environment, as well as be properly prepared for the breeding season in terms of nutrition as well as health.

Conclusion

Contagious ovine digital dermatitis is a severe and debilitating disease that is widespread within the ovine population in the UK. It shares a similar association with *Treponema* spp. as BDD, Elk DD and Caprine DD and is still under recognised by farmers within the UK. It can now be successfully treated, and clinical elimination from flocks is possible, although the lack of routinely strict biosecurity practices for sheep flocks in the UK may make this difficult. Implementation of the findings from this study should make a positive difference to affected sheep and flocks.

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Appendix A

Supplementary material relating to Chapter 2

The questionnaire sent out to farmers, including the pictorial guide to assist farmers in their classification of foot diseases.

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HOLIADUR FFERMWYR AR GLOFFNI DEFAID

Annwyl Ffermwr,

'Rwyf yn un o grwp o filfeddygon fferm yn Ysgol Milfeddygol Lerpwl sy'n ymchwilio cloffni mewn defaid, ac yn arbennig Contagious Ovine Digital Dermatitis (CODD), cyflwr cymharol newydd sy'n achos cloffni.

Fel rhan o'r astudiaeth, 'rydym eisiau casglu gwybodaeth cefndir am gloffni a CODD gan y rhai sydd a mwyaf o brofiad ohono – Y CHI! fel ffermwyr defaid. Buasem yn gwerthfawrogi eich cymorth drwy lenwi'r holiadur amgaedig. Hoffem ddysgu oddiwrthych chi pa mor gyffredin yw gwahanol achosion o gloffni mewn defaid, pa ffactorau sy'n cyfrannu tuag at y broblem, a'ch barn chi ar driniaeth. Bydd y wybodaeth yma, ynghyd ac astudiaethau cloffni sy'n cymeryd lle ar ffermydd defaid, o gymorth i wella ein gwybodaeth a'n dealltwriaeth o gloffni - CODD yn arbennig, ac yn rhoi gwell cyngor i ffermwyr ar sut i'w reoli.

Ymddiheurwn am anfon copi Saesneg yn unig o'r holiadur. Gan mai corff elusennol sy'n noddi'r gwaith, ac fod cyllid yn brin, 'rydym yn ceisio gostwng costau argraffu. Fodd bynnag, os hoffech lenwi holiadur drwy gyfrwng y Gymraeg, cysylltwch â ni ar bob cyfri' ac mi anfonwn un i chi.

Y Gymdeithas Milfeddygol Brydeinig (British Veterinary Association – BVA) sy'n noddi'r astudiaeth a dim ond ffermwyr sy'n aelodau o FAWL (Farm Assured Welsh Lamb) sydd yn cael gwahoddiad i gymeryd rhan. Fe berchir cyfrinachedd unrhyw wybodaeth a gesglir, a bydd y gwybodaeth yna yn ddi-enw. Ni enwir y chi na'ch fferm o gwbwl. Mae'n bosib y cyhoeddir canlyniadau'r astudiaeth (ond heb enwi y chi, na'ch fferm na manylion eich diadell) mewn cylchgronnau amaethyddol neu wyddonol, ac mewn pwyllgorau.

Os hoffech fwy o wybodaeth, cofiwch gysylltu â ni drwy ddefnyddio'r manylion uchod. Hoffem bwysleisio nad oes rheidrwydd arnoch i dderbyn y gwahoddiad yma i gymeryd rhan, eich dewis chi yw bod yn rhan o'r prosiect a'i peidio. Cewch roi'r gorau i'r astudiaeth unrhyw bryd pe dymunwch a gallwch ofyn i unrhyw ddata gael ei ddileu.

'Rydym yn hynod ddiolchgar i chi am roi o'ch amser i gyflawni'r holiadur. Os yn fodlon ystyried cymeryd rhan yn y prosiect estynedig, cofiwch nodi eich manylion cyswllt ar yr holiadur. Os yn nodi eich manylion, ni gysylltir â chi am unrhyw reswm arall ond i drafod y prosiect yma.

Os y penderfynwch nodi eich manylion cyswllt, cynhwysir eich enw mewn raffl i ennill un o ddwy wobr o £100 yr un.

Yn gywir,

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FARMER QUESTIONNAIRE ON LAMENESS IN SHEEP

Dear Farmer,

I am one of a group of farm vets at Liverpool Vet School, who are investigating lameness in sheep, and in particular, a relatively new cause of lameness - Contagious Ovine Digital Dermatitis (CODD).

As part of the study we want to gather as much background information that we can on lameness and CODD from those who have most experience of it - YOU! the sheep farmers. We would really appreciate your help by filling in the enclosed questionnaire. We hope to be able to learn from you how frequent different causes of lameness in sheep are, what factors contribute to the problems, and your views on treatment. This information together with studies of lameness taking place on sheep farms, will help to improve our knowledge and understanding of lameness, and in particular CODD, and provide better advice to farmers in how to control it.

We must apologise for sending a copy of the questionnaire only in English. This is in an attempt to reduce printing costs as our sponsor is a charitable organisation and funds are limited. However, if you would prefer to fill in a questionnaire written in Welsh please do not hesitate to contact us and we will be happy to send one to you.

The study is sponsored by the British Veterinary Association and only sheep farmers who are members of Farm Assured Welsh Lamb (FAWL) have been asked to take part in the study. Information collected will be treated completely confidentially and is anonymous. You or your farm will not be identified to anyone. The results of the studies, (although no details of you, your farm or your sheep) may be published in farming or scientific journals and meetings.

Please feel free to contact us using the details above if you would like more information or if there is anything you do not understand. We would like to stress that you do not have to accept this invitation and should only agree to take part in the study if you wish to. If you do take part in the study you are free to withdraw and request your data be removed.

We are extremely grateful to you for taking the time to complete this questionnaire. If you would be willing to consider taking part in the wider project please provide your contact details on the questionnaire. You will only be contacted with regards to this project if you supply your details and you will not be contacted for any other reason.

If you DO provide your contact details you will be entered into a prize draw for one of two prizes of £100 each.

Yours faithfully

Joseph Angell BVSc MRCVS

Questionnaire on Lameness in sheep

Details of your farm

1. Please enter your FAWL number in the box below

Answer:

2. Please state the number of acres of land farmed that you would describe as hill/mountain, upland or lowland

Type of land	Acres farmed
Hill/Mountain	
Upland	
Lowland	

3. Please fill in the following table detailing the number and breed of sheep on your farm **LAST YEAR** on the 1st June 2011

Category	Breeds	Total Number
Number of breeding ewes over 1 year old		
Number of lambs on the farm under 1 year old		
Number of store lambs aged between 1 and 2 years		
Number of breeding rams		

4. What date did you start and finish lambing **LAST YEAR - 2011**? (Please indicate if you do not lamb at all)

START:
FINISH:

5.

a) Do you lamb your sheep inside or outside? (Please circle ONE answer)

Inside

Outside

Both

b) If **BOTH** please state approximately the percentage of ewes lambed inside e.g. 80% lambed inside

Answer:

6.

a) How many times a year do you gather your **MAIN FLOCK** (excluding growing/fattening lambs) i.e. for dipping, shearing, vaccinating, dosing etc?

Answer:

b) Roughly how many times a year do you gather your **GROWING/FATTENING** lambs i.e. for dipping, shearing, vaccinating, dosing etc?

Answer:

7.

a) Do you carry out supplementary feeding of your sheep **OUTSIDE** during the year? (This would include giving extra forage, concentrates or providing buckets). (Please circle ONE answer)

Yes (please answer Q 7b)

No (please go to Q8)

b) Consider when you are providing supplementary feed to your sheep **OUTSIDE** and please place a tick in the box next to those statements which are true for your farm (tick as many as are true)

Statement	
I provide supplemental feed most years	
I routinely feed ewes around lambing time	
I routinely feed sheep due to a lack of grass in winter	
Silage/hay/straw is used	
Silage/hay/straw is fed off the ground	
Silage/hay/straw is fed from ring feeders	
Ring feeders are moved before being topped up	
Ring feeders are sometimes moved	
Ring feeders are not moved	
Mineral/Energy buckets are used	
Concentrate feed is used	
Concentrate feed is fed off the ground	
Concentrate feed is fed from troughs	
Troughs are moved every day	
Troughs are moved occasionally	
Troughs are never moved	

8.

a) Do you **EVER** keep cattle on your farm? (circle ONE answer)

YES (please answer 8 b) AND 8 c) AND 8 d) AND 8 e) AND 8 f))

NO (please answer 8 e) AND 8f))

b) Are they dairy cattle, beef cattle or both?

DAIRY

BEEF

BOTH

c) APPROXIMATELY how many cattle (include all age groups) are there on your farm at any one time?

Answer:

d) Do your cattle **EVER** get Digital Dermatitis (also known as 'Digi')?

YES

NO

DON'T KNOW

e) Do cattle **EVER** graze with sheep at the same time on your farm? (please circle ONE answer)

YES

NO

f) Do cattle **EVER** occupy the same sheds as sheep at the same time on your farm? (Please circle ONE answer)

YES

NO

PLEASE TURN TO THE NEXT PAGE

Sheep Lameness Details: for the year January 2011 to December 2011

9. Do you have lame sheep on your farm? (please circle ONE answer)

YES

NO

10. Using the help sheet (included at the end of the questionnaire), which of the diseases Footrot, Scald and CODD do you see on your farm? (Circle as many as are relevant)

Footrot

Scald

CODD

DON'T KNOW

11. How much of a problem do you consider lameness to be on your farm? (circle ONE answer)

No problem Minor problem Moderate problem Severe problem

12. Do you trim your sheep's feet (Please circle all answers that are relevant)

Never

When lame

When overgrown

Routine trimming once per year

Routine trimming more than once per year

13. What time of year do you get **MOST** problems with lameness? (Circle as many as are relevant)

Jan Feb March April May June July Aug Sept Oct Nov Dec

DON'T KNOW

14. Do you think lameness on your farm is worse when the sheep are housed? (Please circle ONE answer)

YES

NO

DON'T KNOW

15. Which group/groups of sheep do you notice to be most **SEVERELY** affected with lameness (Circle as many as are relevant)

Lambs (New born to weaning)

Growing Lambs (weaning to 1 year old)

Ewe replacements (Ewe lambs up to 2 years of age)

Breeding Ewes (2 years and older)

Breeding Rams

DON'T KNOW

16.

a) If you are unsure of which disease is causing lameness in your sheep, do you consult your vet for a diagnosis? (Please circle ONE answer)

Always Sometimes Rarely Never

b) If you are unsure of the best treatment for treating lame sheep do you consult your vet for assistance? (Please circle ONE answer)

Always Sometimes Rarely Never

PLEASE TURN TO THE NEXT PAGE

THIS SECTION IS ABOUT FOOTROT

17. If you know the causes of lameness in your flock can you estimate, when lameness is at its worst, what percentage of your sheep are affected with **FOOTROT.**

Answer:

18. If you know that you have **FOOTROT** on the farm, what time of year do you get most problems? (Circle as many as are relevant)

Jan Feb March April May June July Aug Sept Oct Nov Dec

DON'T KNOW

19. If you know you have **FOOTROT** on your farm, do you think lameness on your farm is worse when the sheep are housed? (Please circle ONE answer)

YES

NO

DON'T KNOW

20. If you know you have **FOOTROT** on your farm, which group of sheep do you notice to be most severely affected with it? (Circle as many as are relevant)

Lambs (New born to weaning)

Growing Lambs (weaning to 1 year old)

Ewe replacements (Ewe lambs up to 2 years of age)

Breeding Ewes (2 years and older)

Breeding Rams

DON'T KNOW

THIS SECTION IS ABOUT CODD

21. If you know the causes of lameness in your flock can you estimate, when lameness is at its worst what percentage of your sheep are affected with **CODD.**

Answer:

22. If you know that you have **CODD** on the farm, what time of year do you get most problems? (Circle as many as are relevant)

Jan Feb March April May June July Aug Sept Oct Nov Dec

DON'T KNOW

23. If you know you have **CODD** on your farm, do you think lameness on your farm is worse when the sheep are housed?

YES

NO

DON'T KNOW

24. If you know you have **CODD** on your farm, which group of sheep do you notice to be most severely affected with it? (Circle as many as are relevant)

Lambs (New born to weaning)

Growing Lambs (weaning to 1 year old)

Ewe replacements (Ewe lambs up to 2 years of age)

Breeding Ewes (2 years and older)

Breeding Rams

DON'T KNOW

THIS SECTION IS ABOUT SCALD

25. If you know the causes of lameness in your flock can you estimate, when lameness is at its worst, what percentage of your sheep are affected with **SCALD**

Answer:

26. If you know that you have **SCALD** on the farm, what time of year do you get most problems? (Circle as many as are relevant)

Jan Feb March April May June July Aug Sept Oct Nov Dec

DON'T KNOW

27. If you know you have **SCALD** on your farm, do you think lameness on your farm is worse when the sheep are housed?

YES

NO

DON'T KNOW

28. If you know you have **SCALD** on your farm, which group of sheep do you notice to be most severely affected with it? (Circle as many as are relevant)

Lambs (New born to weaning)

Growing Lambs (weaning to 1 year old)

Ewe replacements (Ewe lambs up to 2 years of age)

Breeding Ewes (2 years and older)

Breeding Rams

DON'T KNOW

THIS SECTION IS RELEVANT ONLY IF YOU KNOW THAT YOU HAVE CODD (please go straight to question 37 if you do not have CODD)

29. When did you first recognize CODD on your farm? (please just give a year)

Answer:

30. How do you think it arrived on your farm? (please circle ONE answer)

Bought in ewes

Bought in rams

Bought in others e.g. stores

Close contact with neighboring farm/animals

Vet/scanner/contractor/walkers etc

Manure from other farms

With cattle coming on to the farm

DON'T KNOW

31.

a) Do you think CODD affects any breed/breeds of sheep in particular?

YES (Please answer Q 31 b))

NO (Please go straight to Q 32)

DON'T KNOW (Please go straight to Q 32)

b) Please state the breed/breeds you think CODD affects the most.

Answer:

32. When CODD is affecting your flock and it is at its worst, APPROXIMATELY what percentage of the **WHOLE FLOCK** is affected?

Answer:

33. Do you observe CODD to affect any group of sheep more than others? (Please circle ONE answer)

YES: Lambs (New born to weaning)

YES: Growing Lambs (weaning to 1 year old)

YES: Ewe replacements (Ewe lambs up to 2 years of age)

YES: Breeding Ewes (2 years and older)

YES: Breeding Rams

NO (please go straight to Q 34)

DON'T KNOW (please go straight to Q 34)

34. When CODD is affecting your sheep and it is at its WORST approximately what percentage of **THIS GROUP** are affected?

Answer:

35. When CODD is affecting your sheep and it is at its WORST approximately when would this be? (Circle as many as relevant)

Jan Feb March April May June July Aug Sept Oct Nov Dec

DON'T KNOW

36.

a) When you have CODD in your sheep does it usually occur by itself? (Please circle ONE answer)

YES I usually just see CODD alone (please go straight to Q36 c))

NO I usually see CODD with other diseases (please answer Q 36 b))

b) Please state the other disease/diseases that occur at the same time as CODD.

Other FOOT disease:	**Other disease: e.g. pneumonia, worms**

c) In your experience of dealing with CODD what do you feel is the most effective treatment?

Answer:

THIS SECTION IS RELEVANT TO ALL FARMS

37. How do you treat lame sheep when you are not sure of the cause?

Treatments (Tick all that apply)	
Formalin/Zinc Sulphate/or other non antibiotic footbath	
Antibiotic footbath	
Antibiotic spray on to feet	
Non-antibiotic spray on to feet	
Trim affected feet	
Inject with antibiotics	
Inject with Footvax vaccine	
Isolate affected sheep	
Take to the vet/seek advice	

38. Please indicate how you would TREAT each of the following:

Disease	Treatments (Tick all that apply)	
Footrot	Formalin/Zinc Sulphate/other non antibiotic footbath	
	Antibiotic footbath	
	Antibiotic spray on to feet	
	Non-antibiotic spray on to feet	
	Trim affected feet	
	Inject with antibiotics	
	Inject with Footvax vaccine	
	Isolate affected sheep	
Scald	Formalin/Zinc Sulphate/other non antibiotic footbath	
	Antibiotic footbath	
	Antibiotic spray on to feet	
	Non-antibiotic spray on to feet	
	Trim affected feet	
	Inject with antibiotics	
	Inject with Footvax vaccine	
	Isolate affected sheep	

CODD	Formalin/Zinc Sulphate/other non antibiotic footbath	
	Antibiotic footbath	
	Antibiotic spray on to feet	
	Non-antibiotic spray on to feet	
	Trim affected feet	
	Inject with antibiotics	
	Inject with Footvax vaccine	
	Isolate affected sheep	

39. How do you treat lame sheep when there are a number of different conditions present at the same time?

Treatments (Tick all that apply)	
Formalin/Zinc Sulphate/other non antibiotic footbath	
Antibiotic footbath	
Antibiotic spray on to feet	
Non-antibiotic spray on to feet	
Trim affected feet	
Inject with antibiotics	
Inject with Footvax vaccine	
Isolate affected sheep	
Take to the vet/seek advice	

40.

a) Do you buy in any sheep during the year? (Please circle correct answer)

YES (please answer Q40 b) and c) and d))

NO (Please go to Q41)

b) Which sheep do you buy in during the year?

Group	Tick any that apply
Lambs at foot (New born to weaning i.e. pet lambs, couples etc)	
Fattening lambs (Weaned to 1 year of age i.e. stores)	
Ewe replacements (Ewe lambs up to 2 years of age)	
Breeding Ewes (2 years and older)	
Rams	

c) Do you isolate your bought in sheep?

YES (please answer Q 40 d))

NO (Please go to Q 41)

d) Where do you isolate your bought in sheep?

In a separate field

In a shed completely on their own

In a separate pen located in a shed with other sheep

41. There are many sources of information available to farmers today, from the list below please write a number next to all those that you use, please order with 1 being the source you use the most.

Other farmers

Farmers Weekly

Local Vet

Farmers Guardian

Gwlad

Society briefings such as from the National Sheep Association (NSA), The Sheep Veterinary Society (SVS) etc

Websites i.e. Hybu Cig Cymru, DEFRA etc

Local meetings i.e. feed companies, ADAS meetings etc

Other (please state)…………………………………………………………………

PLEASE ADD ANY FURTHER COMMENTS YOU MIGHT HAVE REGARDING LAMENESS IN GENERAL AND CODD IN PARTICULAR (Please continue on to the next page if the box below is not big enough)

END OF QUESTIONNAIRE

As indicated in the cover letter this questionnaire is part of a much larger study into CODD in sheep. We are extremely grateful to you for taking the time to complete it and if you would be willing to consider taking part in the wider project please provide your contact details in the space below. You will only be contacted with regards to this project if you supply your details and you will not be contacted for any other reason.

<u>If you DO provide your contact details you will be entered into a prize draw for one of two prizes of £100 each.</u>

Please circle one of the following statements:

YES I would be willing to be involved in further studies

NO I would not be willing to be involved in further studies

Name, address and phone number:

BVA ANIMAL WELFARE FOUNDATION

A - Footrot

Characteristic damage to the sole

Cheesy rotten sole

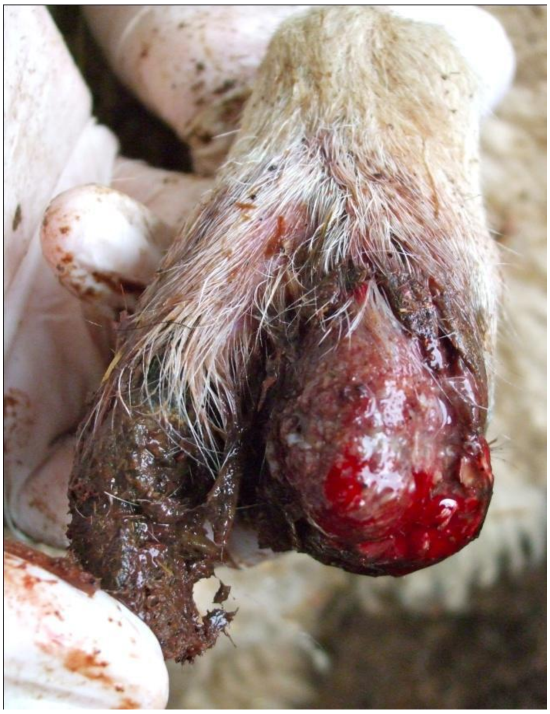
B - Scald

Sore and red in between cleats

18

C - Contagious Ovine Digital Dermatitis (CODD)

Moist and sore at the top of the hoof (coronary band), loss of hair here as well



This can worsen causing the hoof to come off

Appendix B

Supplementary material relating to Chapter 4

Listed on the next few pages are dorso-medial palmaro-lateral oblique radiographs corresponding to the lateral views presented in Chapter 4.

Figure 1: Dorso-medial palmaro-lateral oblique radiograph of a digit with a grade 1 contagious ovine digital dermatitis lesion. No radiographic abnormalities are evident.

Figure 2: Dorso-medial palmaro-lateral oblique radiograph of a digit with a grade 2 contagious ovine digital dermatitis lesion. The lesion at the coronary band is evident as a decrease in radiodensity in the soft tissue structures at this point (1). There are hair-like radiolucencies evident within the soft tissues and the surface of the soft tissue appears roughened (2). The separation of the hoof wall from the underlying phalanx is clearly evident as a radiolucent line (3). The surface of the lamellae appears disrupted. There is some mild osteophytic change to the dorsal aspect of the distal phalanx (4) and there is evidence that a radiolucency that extends from the dorsal aspect of the sensitive lamellae through the lamellae to the surface of the distal phalanx (5).

Figure 3: Dorso-medial palmaro-lateral oblique radiograph of a digit with a grade 3 contagious ovine digital dermatitis lesion. A radiolucent tract is visible from the coronary band to the dorsal aspect of P3 (1). There is mild osteophytic reaction to the dorsal aspect of P3 (2). The hoof horn appears avulsed (3).

Figure 4: Dorso-medial palmaro-lateral oblique radiograph of a digit with a grade 4 contagious ovine digital dermatitis lesion. The soft tissue surrounding the distal aspect of P3 appears to have reduced radiodensity (1). The dorsal and distal aspects of P3 demonstrate extensive demineralization (2) to the extent that there appears to be a section missing from the dorsal and distal aspect of P3 (3).

Figure 5: Dorso-medial palmaro-lateral oblique radiograph of a digit with a grade 5 contagious ovine digital dermatitis lesion. The soft tissue structures surrounding the distal aspect of the foot generally appear of more uniform soft tissue density compared to the grade 4 foot and the distance between the outer margin and P3 appears reduced when compared to the grade 1 foot (2).

Appendix C

Supplementary material relating to Chapter 8

Tilmicosin treatment in 58 sheep with contagious ovine digital dermatitis

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Keywords Contagious ovine digital dermatitis; treatment; tilmicosin; sheep

Abstract

Contagious ovine digital dermatitis (CODD) is now common in sheep, and causes severe lameness and pathological changes in the foot. Clinical lesions have been associated with treponemes, which are currently considered a necessary cause of disease. The aim of this study was to evaluate the clinical success of systemic tilmicosin in treating sheep with active CODD lesions.

A cohort of 58 sheep were treated twice, two weeks' apart, using tilmicosin given by injection. The sheep were isolated in a field during the study and followed-up for 7 weeks.

All 58 sheep demonstrated clinical resolution of the lesions and there were no new cases or recrudescence of disease during follow-up.

In conclusion, tilmicosin as used in this study was an effective clinical treatment for sheep with active CODD lesions.

Introduction

Contagious ovine digital dermatitis (CODD) is now common, and causes severe lameness and pathological changes (Angell et al., 2014; Angell et al., 2015a). Clinical lesions have been associated with treponemes phylogenetically identical to those associated with bovine digital dermatitis (BDD) (Sullivan et al., 2015) and are currently considered a necessary cause of disease (Duncan et al., 2014).

There are very few studies on treating CODD. In Duncan et al. (2011); Duncan et al. (2012) cure rates of approximately 70-80% were achieved with systemic amoxicillin with topical chlortetracycline. A report by Watson (1999) indicated that a single injection of tilmicosin, with topical oxytetracycline was successful in treating a severe ovine foot disease - clinically resembling CODD - in a group of lambs. Judson (2010) reported that systemic oxytetracycline together with a tylosin footbath prior to housing prevented the development of CODD in groups of lambs from an infected flock.

An *in vitro* antibiotic sensitivity study of treponeme isolates cultured from CODD lesions demonstrated low minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) for penicillin and macrolide antibiotics (Angell et al., 2015b).

The aim of this study was to evaluate the clinical success of systemic tilmicosin in treating sheep with CODD.

Materials and Methods

The study protocol was approved by The University of Liverpool ethics committee (VREC 13) on 24th August 2011.

Study design

Fifty-eight sheep were recruited from a single farm at week 1. Sheep were included if they fitted the clinical case description of having active CODD (one or more feet with a CODD lesion graded 1-4 (Angell et al., 2015a)).

Treatment protocol

All 58 sheep were injected at week 1 with tilmicosin (Micotil 300; Elanco Animal Health) at a dose of 10mg/kg, given subcutaneously over the left shoulder. A second injection was given at week 3 over the right shoulder (ensuring any possible reaction associated with the first injection was undisturbed). All the sheep were re-examined at weeks 5 and 7.

Throughout the study the sheep were kept isolated in a field next to the examination pens, which were cleaned and disinfected after use.

Health and safety

Accidental self-injection with tilmicosin may lead to serious injury or death in humans (Veenhuizen et al., 2006). To minimise this risk an automatic injector was used and set to 1.0ml (an expected sub-lethal dose) and cut resistant gloves were worn (Arco PU Cut Control System 5 Gloves; Arco 2014). The needle was kept detached until injection and the sheep were contained in a narrow race with the administering veterinary surgeon standing behind each sheep.

Data collection

Individual sheep data were collected at week 1, including: ear number, age (Spence and Aitchison, 1986), breed (as determined by the farmer), sex, locomotion score (Angell et al., 2015c), body condition score (Russel, 1984) and foot lesion including CODD lesion grade (Angell et al., 2015a).

At week 3 (2nd injection), sheep were re-identified by their ear number but no further data were collected. At weeks 5 and 7 all sheep were turned over and their feet inspected and graded for CODD lesions.

Results

The baseline characteristics of the 58 sheep are detailed in Table 1. Four sheep had active CODD in two feet; the other 54 had active CODD in one foot only.

At weeks 5 and 7 all the lesions (n=62) that previously had been graded 1-4 were now grade 5, however the horn was thinner and still soft in many cases. There were no new cases or reversions to active CODD.

Variable	Number of sheep	Percentage of sheep (n=58)
Breed		
North Country Cheviot	2	3.5
Scottish Black Face	4	6.9
Texel Cross	7	12.1
Suffolk Cross	20	34.5
Mule	25	43.1
Age		
Lamb	17	29.3
Yearling	14	24.1
Adult	27	46.6
Sex		
Male	11	19.0
Female	47	81.0
Body Condition Score		
1	20	34.5
2	34	58.6
3	4	6.9
Locomotion Score		
0	1	1.7
1	6	10.3
2	19	32.8
3	32	55.2
Variable	**Number of feet**	**Percentage of feet (n=232)**
CODD (by grade)		
1	4	1.7
2	7	3.0
3	27	11.6
4	24	10.3
5	1	0.4
Footrot	14	6.0
Interdigital dermatitis	5	2.2
White line	4	1.7
Overgrown	2	0.9

Table 1: Baseline characteristics of the 58 sheep at enrollment into the study at week 1.

Discussion

This study demonstrates the clinical resolution of CODD in 58 sheep with active lesions. This is an improvement on Duncan et al. (2011); Duncan et al. (2012) where cure rates of approximately 80% were observed. In those studies no animal that was recorded as having clinically recovered, developed a new lesion during the study period, as was observed here. This current study also corroborates the report by Watson (1999) on the use of tilmicosin in the treatment of CODD. It is important to note that this study does not provide information on a bacteriological cure for these animals, however the duration of follow-up was considered sufficient to allow recrudescence to occur.

Treatment justification

Currently there are no treatments licensed for CODD, therefore the choice of antibiotic was based on the following factors:

1) Tilmicosin demonstrated low MIC and MBC data in an *in vitro* study (Angell et al., 2015b).
2) Tilmicosin already has a UK license for the treatment of footrot in sheep, so could be justified in terms of the UK Veterinary Medicines Cascade (DEFRA, 2013b).
3) Given the deep pathology associated with the disease (Angell et al., 2015a) systemic antibiosis was preferred over topical application.
4) Due to the lack of knowledge in treating CODD, current therapeutic strategies for other treponemal diseases were considered including BDD and syphilis. For BDD topical strategies aimed at control are preferred to

systemic antibiosis due to the economic costs associated with milk withholds (Laven and Logue, 2006).

In treating syphilis the objective is for a clinical cure and treatment can be required for up to 28 days (Kingston et al., 2008). Tilmicosin was expected to persist within infected tissues (Scorneaux and Shryock, 1999; Naccari et al., 2001), and it was expected that a repeat dose could be given safely after 14 days.

5) The responsible use of macrolides in veterinary medicine is imperative given that they are considered by the World Health Organisation to be critically important for human medicine (WHO, 2011). Tilmicosin is only able to be administered by a veterinary surgeon (Elanco, 2013), which may ensure more accurate and responsible use.
6) Anecdotally there are good clinical responses in treating CODD with tilmicosin (Watson, 1999).

Conclusion

Two doses of tilmicosin given 2 weeks apart were an effective treatment for active CODD in sheep.

Acknowledgements

Thanks to the farmer for providing his sheep for the study and to Leigh Sullivan for helping with data collection.