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Experimental infection of sheep with ovine and bovine *Dichelobacter nodosus* isolates

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ABSTRACT

The aim of this study was, under experimental conditions, to investigate infection of Norwegian White sheep with ovine and bovine isolates of *Dichelobacter nodosus* of varying virulence. In addition, the efficacy of gamithromycin as a treatment for the experimentally induced infections was examined. The study was performed as a single foot inoculation using a boot. Four groups, each with six lambs, were inoculated with four different challenge strains (Group 1: benign bovine strain; Group 2: virulent bovine strain; Group 3: benign ovine strain; Group 4: virulent ovine strain). The main criterion to determine that infection was transferred was that *D. nodosus* isolate was obtained by culture. After the trial all lambs were treated with gamithromycin. Clinical symptoms of footrot developed in all groups, and when removing the boots two weeks after challenge, *D. nodosus* was isolated from 5 of 24 experimental lambs. All lambs tested negative for *D. nodosus* by PCR within six weeks after treatment with gamithromycin. This study strongly indicates that *D. nodosus* isolates from both sheep and cattle can be transferred to sheep under experimental conditions. The study also indicates that gamithromycin may be effective against *D. nodosus*.

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1. Introduction

In 2008, ovine footrot was diagnosed in Norway for the first time in 60 years (Melting and Ulvund, 2009). The outbreak was restricted to Rogaland, a county with a high density of farm animals where co-grazing of sheep and cattle is practised (Vatn et al., 2012). Ovine footrot is a debilitating disease causing lameness and reduced animal

welfare (Stewart, 1989; Dwyer and Bornett, 2004). *Dichelobacter nodosus*, a Gram negative anaerobic bacterium, is the main aetiological agent and the bacterium produces extracellular proteases, which are categorized based on their thermostability (Beveridge, 1941; Depiazzi et al., 1991). Benign isolates producing thermolabile proteases are associated with mild interdigital dermatitis which does not progress, whereas virulent isolates producing thermostable proteases tend to cause severe footrot where the keratinous part of the claw horn separates from its underlying tissues (Stewart, 1989). In addition to the virulence of the involved bacterial strain, development of disease depends on environmental conditions and differences in

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the susceptibility between breeds (Beveridge, 1941; Emery et al., 1984; Egerton and Raadsma, 1991; Depiazzi et al., 1998). Due to variations in DNA sequence of the fimbrial subunit gene *fimA*, *D. nodosus* is divided into ten serogroups (Claxton, 1989; Ghimire et al., 1998).

Antibiotics, such as oxytetracycline and enrofloxacin, are used to treat footrot (Kaler et al., 2012). The more recently introduced macrolid antibiotic gamithromycin has been used as flock treatment and even though highly effective in some areas *D. nodosus* was not eliminated from all sheep in all flocks even though (Forbes et al., 2014).

D. nodosus is commonly isolated from cows with interdigital or digital dermatitis (Laing and Egerton, 1978; Knappe-Poindecker et al., 2013). All isolates tested from cattle in Norway having no contact with sheep produce the heat labile protease and have been defined as benign (Gilhuus et al., 2013; Knappe-Poindecker et al., 2013). The epidemiological importance of these infections for the spread and control of ovine footrot is considered to be low, and Beveridge (1941) concluded that cattle are unlikely to be important as reservoirs for *D. nodosus*. However, two previous Norwegian studies have indicated that cross-infection of *D. nodosus* from sheep to co-grazing cattle did occur (Rogdo et al., 2012; Knappe-Poindecker et al., 2014). In the latter study, two cows stayed infected for at least one housing season, creating a reservoir for virulent *D. nodosus*. The bacterium has previously been transmitted both naturally and experimentally between cattle and sheep (Egerton and Parsonson, 1966; Wilkinson et al., 1970; Laing and Egerton, 1978), but more studies of the possible transfer of different strains from sheep and cattle in Norway are needed.

The aim of this study was, under experimental conditions, to investigate infection of Norwegian White sheep with ovine and bovine isolates of *D. nodosus* of varying virulence. In addition, the efficacy of gamithromycin as a treatment for the experimentally induced infections was investigated.

2. Materials and methods

2.1. Experimental animals

The trial was conducted on 27 weaned lambs of the breed Norwegian White sheep (NKS). The 15 ewe lambs and 12 ram lambs were chosen randomly from the flock belonging to the Norwegian University of Life Sciences. This flock is considered free of footrot based on clinical inspection of the claws in all sheep in the flock and bacterial samples from random sheep as part of the surveillance programme “Healthy Feet” (Vatn et al., 2012). Prior to the start of the trial, all 27 lambs tested negative for *D. nodosus* on PCR and FISH.

Each lamb had tags in both ears with a unique identification number. The lambs were aged 4–5 months at the start of the trial, and had a mean body weight of 44 kg (range 33–56 kg). During the trial, the lambs were kept in the closed animal unit at the Norwegian University of Life Sciences, Campus Sandnes, approved by the Norwegian Food Safety Authority (National Animal Research Authority) for infectivity studies in sheep. The stall contained nine boxes with rib mesh floor with no bedding and they were completely separated from each other (mean size of 3 m², range 2.1–3.6 m²). Each box housed three lambs of the same gender.

The lambs were randomly allocated to four experimental groups, each with six lambs, and a control group with three ewe lambs. Each experimental group consisted of three ram lambs and three ewe lambs. Before the start of the trial, the selected lambs underwent a clinical examination by a veterinary surgeon. The claw health of each lamb was controlled and recorded. Swabs were taken from the interdigital skin on the right hind

Table 1
Origin, virulence and serogroup of the challenge strains.

| Group | Origin and breed | Virulence | Serogroup |
|------------------|-----------------------|-----------|-----------|
| 1 | Norwegian Red cattle | Benign | I |
| 2 | Norwegian Red cattle | Virulent | A |
| 3 | Norwegian White sheep | Benign | G |
| 4 | Norwegian White sheep | Virulent | A |
| 5 (Controlgroup) | – | – | – |

foot of all lambs, and tested for *D. nodosus* by PCR as described below. From this point, the lambs were isolated from other animals. Care was taken to prevent cross-contamination during handling and feeding and gloves were changed between every lamb at removal of the boots.

2.2. Preparation of the bacterial suspensions

Four different indigenous challenge strains were used in this study (Table 1). It was aimed for each experimental lamb to be inoculated with 10 ml of a bacterial suspension containing 10⁶–10⁷ bacteria/ml. The challenge strains were grown anaerobically for six days on 4% hoof agar (HA) with addition of 1% ‘Lab-Lemco’ powder (Oxoid, Basingstoke, UK) and 0.2% tryptose (Oxoid). Growth on each agar plate was checked for purity using phase contrast, flushed with 2 ml room-tempered saline and gently scraped with an L-shaped spreader to detach bacteria. The saline containing the bacteria was collected using a Pasteur pipette and gathered in a 15 ml falcon tube (Greiner bio-one, Frickenhausen, Germany). Additional saline was added to each falcon tube to a total volume of 11 ml. After gentle mixing, 1 ml of the suspension was removed and used to prepare ten-fold dilutions in double distilled water. The dilutions were boiled for 1 min and used as template in a real time PCR to detect *D. nodosus* (Frost et al., 2012). Using a 10 µl inoculation needle, undiluted bacterial suspension from the falcon tube was cultured on 2% HA and incubated anaerobically at 37° C. Two days later confluent growth of *D. nodosus* on the HA plates was confirmed from all the prepared broths used for inoculation of experimental lambs. By real-time PCR, the presence of *D. nodosus* was confirmed in the 10^{–7} dilutions from each inoculation broth.

The remaining 10 ml bacterial suspension in each falcon tube was immediately used to infect the experimental lambs.

2.3. The trial

Fig. 1 illustrates the timeline for the trial. On day 1, no events directly affecting the infection trial were performed, but measurements were done and samples collected to be used in an animal welfare study by Stubbsjøen et al. (submitted).

On day 2 of the trial, the claw health of all lambs was controlled. Biopsies were taken with a 3 mm biopsy punch (Miltex, Inc. USA) from the interdigital skin for histopathological evaluation and fluorescence *in situ* hybridization (FISH) for identification of *D. nodosus*. The right hind foot of each lamb was placed in Nordstrøms rubber boots®, and 10 ml tap water was added to create moist conditions. The boots were secured with an adhesive bandage and left on for seven days. The position of the boots was controlled daily, and the lambs were observed for signs of lameness. Lameness was scored after Morck et al. (1994) as 0 = no limp; 1 = slight limp; 2 = moderate limp and 3 = non-weight bearing.

On day 9 of the trial, the boots were removed and the feet examined and scored for footrot after Egerton and Roberts (1971), as described in Table 2. The boots were replaced and infused with 10 ml bacterial suspension, which was prepared as described above. In the control group, 10 ml tap water was added instead of bacterial suspension. The lambs were monitored daily for the following two weeks to assess lameness and pain, and the position of the boots was controlled. Pain was scored after Ahern et al. (2009) as described in Table 3. Lambs scoring >1 on lameness and/or pain received 0.5 mg/kg live weight meloxicam SC every other day until the pain resolved (Metacam; Boehringer Ingelheim vetmedica GmbH).

On day 23, two weeks after inoculation, the boots were removed and the claws were examined for symptoms of footrot and blisters. Signs of lameness were recorded. Swabs from the interdigital space for culturing and PCR regarding *D. nodosus* were collected. Because a viscid material had formed in the boot, the skin was first wiped with moist paper towels before being dried off with a paper towel. Biopsies were taken and analyzed as described below. Lambs with signs of footrot, lameness or

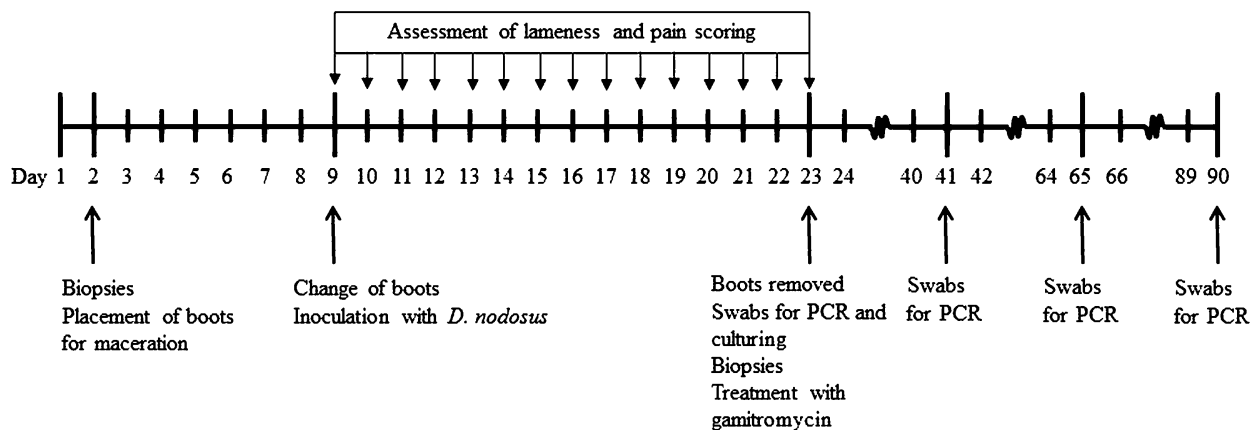


Fig. 1. Timeline for the trial where 24 lambs were inoculated with four different strains of *Dichelobacter nodosus*.

Table 2

Scoring system adapted from Egerton and Roberts (1971) to describe the foot lesions in the experimental lambs.

| Score | Definition |
|-------|--|
| 0 | Normal interdigital space and claw horn |
| 1 | Limited mild interdigital dermatitis |
| 2 | More extensive interdigital dermatitis |
| 3 | Severe interdigital dermatitis and underrunning of the horn of the heel and sole |
| 4 | As 3, but the underrunning has extended to the walls of the claw |

blisters were treated with 0.5 mg/kg live weight meloxicam SC (Metacam; Boehringer Ingelheim vetmedica GmbH). Afterwards, all lambs were treated once with 6 mg/kg live weight gamithromycin (Zactran; Merial) SC as recommended by the manufacturer. The lambs were monitored daily until fully clinically recovered.

Over the following three weeks, the appetite and habitus of the lambs were recorded daily. To test the effect of gamitromycin new swabs for PCR were taken on day 41, 65 and 90 of the trial.

2.4. Bacterial sampling for *D. nodosus* – culturing, virulence testing, PCR analysis and serogrouping

The skin was wiped with paper towels before samples were taken from the interdigital skin using two sterile swabs. Culture swabs were placed in Transystem Amies agar gel medium with charcoal (Copan, Brescia, Italy). When swabbing for real-time PCR, the wooden end of the cotton swabs was used and were placed in tubes with sterile phosphate buffered saline (PBS) containing 0.02 M EDTA. Samples were sent by overnight courier to the Norwegian Veterinary Institute in Oslo for analysis.

DNA was extracted from the swabs in PBS with EDTA using a nucliSENS easyMAG extractor (bioMérieux, Boxtel, The Netherlands) following the manufacturer's instructions. DNA from cultured isolates was obtained by

Table 3

The pain assessment scoring system after Ahern and others (2009) used in a trial where 24 lambs were infected with four different isolates of *Dichelobacter nodosus* and three control lambs also monitored for signs of pain.

| Variable | 0 | 1 | 2 | 3 |
|-------------------|------------------|-------------------------|---------------------------------|------------------------------|
| Mental assessment | Normal and alert | NC | NC | Signs of depression |
| Respiratory rate | Normal | NC | Abnormal (slow or panting) | NC |
| Recumbency | Normal | Slightly delayed rising | Requires encouragement to stand | Unwilling or unable to stand |
| Shifting weight | Normal | Mildly or occasional | Moderately | Constantly |
| Appetite | Normal | Mildly reduced interest | Moderately reduced interest | Inappetent |
| Palpation of foot | No signs of pain | Mild signs of pain | Moderate signs of pain | Severe signs of pain |

NC = No criteria applicable for this category

diluting broth culture 1:5 in double-distilled water followed by boiling for one minute. Extracted DNA was stored at -20°C . *D. nodosus* was detected using a real-time PCR as described previously (Frosth et al., 2012).

Culturing was performed on 4% HA basically as described by Stewart and Claxton (1993), but with the addition of 1% 'Lab-Lemco' powder (Oxoid, Basingstoke, England) and 0.2% Tryptose (Oxoid) to the HA. When possible, at least two *D. nodosus* suspect colonies from each sample were subcultured onto 2% HA. An approximately 5 mm x 5 mm piece of agar, with pure confluent bacterial growth, was cut from the agar and transferred to HEPES-TAS broth (Stewart and Claxton, 1993). The broth was incubated anaerobically at 37°C for 48–72 h. Purity of the broths was checked by phase contrast microscopy, and the presence of *D. nodosus* was confirmed using real-time PCR as described above. Remaining broth cultures were used for virulence testing using the gelatin gel test (GG-test) as described below. Isolates were also stored at -70°C in Bacto Heart Infusion Broth (BD, Sparks, MD) with 15% glycerol. A lamb was considered positive when an isolate was obtained.

Isolates were categorized as virulent or benign based on their ability to secrete thermostable or thermolabile proteases, respectively, as shown by the GG-test. The test was performed as described by Palmer (1993) with previously described modifications (Gilhuus et al., 2013). Control strains of *D. nodosus* were AC 6465 ST 198, a virulent strain producing thermostable proteases, and AC 6466 ST 305, a benign strain producing thermolabile proteases. Culture broths of virulent and benign control strains were included on each gel.

In order to allocate the isolates to serogroups A–I, the variable region of the gene encoding the fimbrial subunit *fimA* was amplified by multiplex PCR (Dhungyel et al., 2002) with previously described modifications (Gilhuus et al., 2013). DNA from the Australian *D. nodosus* prototypes for serogroups A to I were included as positive controls. Double-distilled water was included as negative control.

2.5. Sampling, histopathological evaluation and analyses of biopsies for *D. nodosus* by fluorescent in situ hybridization (FISH)

Biopsies were taken from the caudal part of the interdigital space with a 3 mm biopsy punch (MilteX, Inc. USA). In lambs with symptoms of footrot, the biopsies were taken on the border between healthy

Table 4

Results from culturing, serogrouping, PCR, footrot score and prevalence of lameness in the 27 lambs at removal of the boots on day 23 of the trial, two weeks after inoculation in the four experimental groups and in the control group.

| Group | Origin | Virulence | N | Culturing + | Serogroup | PCR + | Footrot score | | | | Lameness score | | | |
|-------|----------------|----------------|----|-------------|-----------|-------|---------------|----|----|---|----------------|---|---|---|
| | | | | | | | 0 | 1 | 2 | 3 | 0 | 1 | 2 | 3 |
| 1 | C ¹ | B ³ | 6 | 1 | I | 3 | 0 | 1 | 5 | 0 | 4 | 1 | 0 | 1 |
| 2 | C | V ⁴ | 6 | 1 | A | 2 | 1 | 2 | 2 | 1 | 5 | 1 | 0 | 0 |
| 3 | S ² | B | 6 | 0 | – | 4 | 0 | 2 | 4 | 0 | 6 | 0 | 0 | 0 |
| 4 | S | V | 6 | 3 | A | 6 | 0 | 2 | 4 | 0 | 6 ⁵ | 0 | 0 | 0 |
| 5 | – | – | 3 | 0 | – | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 0 |
| Total | | | 27 | 5 | | 15 | 1 | 10 | 15 | 1 | 24 | 2 | 0 | 1 |

¹Cattle.

²Sheep.

³Benign.

⁴Virulent.

⁵ One of these lambs had lameness score 2 on day 17, but the lameness resolved.

and diseased skin. The biopsies were immediately fixed in 10% neutral buffered formalin and sent to the National Veterinary Institute, Technical University of Denmark, Copenhagen, for analysis.

The biopsies were processed routinely for histopathology and embedded in paraffin wax. Sections from all biopsies were stained with haematoxylin–eosin for histopathological evaluation. The degree of epidermal damage was defined as score 0 (normal epidermis), score 1 (mild) as mild epithelial proliferation and hyperkeratosis, score 2 (moderate) as severe epithelial proliferation and hyperkeratosis (parakeratosis with increasing degeneration and mal-keratinization) and score 3 (extensive to diffuse) as severe epithelial proliferation with exudation, erosion or necrosis of the dermal papilla according to Rasmussen et al. (2012). For FISH analysis, sections were cut (4 µm) and mounted on Super-Frost + slides (Menzel-Gläser, Germany). For detection of *D. nodosus* and bacteria in general, FISH was performed with previously published 16S rRNA targeting oligonucleotide probes (Rasmussen et al., 2012). The oligonucleotide probes were 5' labelled with fluorescein isothiocyanate or Cy3 and hybridization was carried out at 46° C. For light and epifluorescence microscopy an Axioimager M1 microscope equipped with AxioCAM MRc and MRm cameras, and equipped with the software AxioVision (Zeiss, Oberkochen, Germany) was used.

3. Results

3.1. *Dichelobacter nodosus*

Altogether 5 out of 24 experimental lambs had *D. nodosus* isolated on day 23, when the boots were removed. The results from the culturing and PCR in each group are presented in Table 4. *D. nodosus* was not detected by FISH in any of the challenged sheep or in the control sheep.

3.2. Pain and lameness

No lambs showed signs of pain or lameness at the start of the trial. During the trial, only one lamb was lame or showed other symptoms of pain. This lamb, belonging to group 4, had lameness score 2 on the right hind limb and was lying more than normal on day 17 of the trial, eight days after inoculation. The symptoms resolved without reemerging after one injection with meloxicam. On day 23, when the boots were removed, this lamb had footrot score 2, but was not lame. No lambs showed signs of depression, decreased appetite, were shifting weight or had pain by palpation of the foot during the trial. Upon removal of the boots, three lambs were lame. Group specific data are presented in Table 4.

3.3. Foot lesions

When examined prior to the start of the trial, none of the lambs had symptoms of footrot or other claw diseases. The boots remained in the correct position throughout the trial. When the boots were removed after the seven-day maceration period, the claw health was unremarkable in 26 lambs. In the last lamb, a ram from group 1, the interdigital space was slightly hyperaemic, but the wool and smell were normal and there were no signs of exudate. The conditions in all boots were moist. There were no signs of blisters in any of the sheep. Symptoms of footrot developed in all experimental groups and the recordings on day 23, when the boots were removed, are presented in Table 4. Six lambs had blisters upon removal of the boots on day 23. Table 5 shows the prevalence of *D. nodosus* among sheep with different footrot scores.

3.4. Histopathology and fluorescent in situ hybridization

The skin biopsies taken on day 2 of the trial were microscopically found to be within normal anatomical range with the exception of 4/27 sheep. Three of these lambs (from group 1, 2 and 3, respectively) had minor changes in the epidermis, and one lamb (from group 1) had moderate changes. When the boots were removed on day 23, only 7/27 biopsies were found to be within normal range apart from increased thickness of stratum disjunction, whereas evident histopathological changes were present in the other 20/27 biopsies, including two of the three control sheep. Although the severity of the changes varied, they

Table 5

Prevalence of *D. nodosus* detected by culturing among challenged sheep with different footrot scores.

| <i>D. nodosus</i> | Footrot score | | | |
|-------------------|---------------|---|----|---|
| | 0 | 1 | 2 | 3 |
| Culture | | | | |
| + | 0 | 1 | 3 | 1 |
| – | 1 | 6 | 12 | 0 |
| PCR | | | | |
| + | 0 | 3 | 11 | 1 |
| – | 1 | 4 | 4 | 0 |

all related to the derma-epithelial junction especially in the tips of the dermal papilla. Eight sheep, including one control animal, showed oozing of neutrophil granulocytes along the superficial papillary dermis and formation of micro-abscesses in the tips of the dermal papilla. Multiple small pustules could be seen in all layers of the epidermis. Slight lymphocytic perivascular dermatitis was commonly seen in the deeper layers of the dermis.

The five lambs which had *D. nodosus* isolated had a mean epidermis score of 1.2 while the three lambs in the control group had a mean epidermis score of 0.3 on day 23.

3.5. Follow-up of experimental lambs and treatment with gamithromycin

When the claw health was controlled on day 41, 18 days after removal of the boots and treatment with gamithromycin, 14/27 lambs had proliferative lesions of various sizes up to 2 cm in diameter in the interdigital space. The lesions occurred in lambs in all groups, including lambs from the control group. Six lambs were still positive by PCR, 20 lambs were negative, and the test was inconclusive in one lamb. On the follow-up examination of all lambs on day 65, the lesions were healed, and all lambs tested negative for *D. nodosus* by PCR. All lambs also tested negative by PCR on day 90 of the trial.

4. Discussion

D. nodosus was isolated from altogether 5/24 lambs from groups 1 (bovine benign), 2 (bovine virulent) and 4 (ovine virulent), but the proportion of infected lambs was lower than observed in other studies and the symptoms were mainly mild (Ghimire et al., 1999; Morck et al., 1994). Depiazzi et al. (1991) found that benign isolates are more likely to cause mild footrot, and virulent isolates are more likely to cause severe footrot and in agreement with previous studies, the lambs in our study successfully infected with benign *D. nodosus* developed mild footrot (Wilkinson et al., 1970; Laing and Egerton, 1978). Other studies have shown some, but not complete correlation between virulence and severity of the lesions which also was the case in the lambs infected with virulent strains in our study where only 1/6 lambs infected with the bovine virulent strain and none of the six sheep infected with the ovine virulent strain developed severe footrot (Moore et al., 2005). There is a known difference in natural susceptibility between breeds, and Merino sheep, on which the other studies were performed, are known to be highly susceptible to *D. nodosus* (Egerton et al., 1972; Skerman et al., 1982; Emery et al., 1984). There are indications that Norwegian White Sheep have some natural resistance to footrot, at least compared to Norwegian Pelt (Vatn et al., 2013), which may have reduced the number of successfully infected lambs.

Inoculation of *D. nodosus* in sheep using a boot has previously been used and has the advantage that the sheep are inoculated with a known number of *D. nodosus* and avoids the risk of lameness in several feet which is present when using wet mats for inoculation (Morck et al., 1994). When using a boot in the trial, the possibility of false positive results occurs as a consequence of residual bacteria

from the inoculant, but this possibility was reduced when the skin was wiped clean before swabbed. In addition, *D. nodosus* is documented to survive for no more than 14 days and often shorter without claw material (Cederlöf et al., 2013). Consequently, the chances for living bacteria surviving in the boot without infecting the lamb are unlikely. We consider the chance of infection to be considerably greater than the risk of residual bacteria in the boot being cultured, even though this risk not can be excluded. This consideration is supported by the higher mean footrot score and mean epidermis score in the five lambs, from which *D. nodosus* was isolated, than in the control lambs, also indicating that the lambs were successfully infected.

Even though anaerobic and moist, the environment in the boots may have been suboptimal for the subsequent culturing because there was also some unexpected growth from other bacteria in some of the samples. In addition, *D. nodosus* is fastidious and demanding to culture, and the growth of these other bacteria may have disturbed the growth of *D. nodosus*, leading to false negative results. Wiping the skin with a moist paper towel before the bacterial samples were taken was necessary because the feet were covered in an unexpected viscid material after wearing a boot for several weeks. The cleaning may have wiped away *D. nodosus* leading to false negative results, but the bacterium is normally found infiltrating the epidermis of infected animals and should thereby be protected (Rasmussen et al., 2012; Knappe-Poindecker et al., 2013). When swabbing for PCR the wooden end of cotton swabs were used which also may have collected some of these protected bacteria and not only bacteria on the surface. Consistent with a previous study, three times as many lambs tested positive for *D. nodosus* by PCR than by culture (Frosth et al., 2012). However, PCR detects DNA from both living and deceased bacteria, which opens for the possibility of false positive results if residual bacteria from the inoculant are present.

Only 1/27 lambs showed signs of pain including lameness during the study, and another three lambs were lame when the boots were removed. It is possible that slight lameness may have been undiscovered because the size of the pens was smaller than ideal for lameness assessment. The shaping of the boxes was according to the guidelines of the Council of Europe (2006). Severe footrot is associated with lameness (Stewart, 1989), but the low prevalence of lameness and few signs of pain are in agreement with the mild symptoms developed, and that only 5/24 experimental lambs were successfully infected.

The three control lambs tested negative for *D. nodosus*, but all three had lesions score 1 at the end of the trial. Score 1 is considered a limited mild dermatitis, and in this case, we assume that the hyperaemia and the cellular reaction in the derma-epithelial junction found histopathologically were caused by irritation to the skin as a result of the maceration, even though scald cannot be excluded. The diagnosis interdigital dermatitis, as caused by maceration, is supported by Egerton et al. (1969), who found histopathological alterations in the interdigital skin after only four days of maceration. The hyperaemia in the interdigital space of one lamb in group 1 on day 7 of the trial is also assumed to be caused by the boots. The mild dermatitis

at the time of inoculation could, however, have made this lamb more susceptible to infection compared to the other experimental lambs.

As the proliferative lesions, which were found at the examination on day 41, 18 days after boot removal, were equally distributed in all groups including the control group, there is no reason to believe that they developed as a result of *D. nodosus* infection. It can, however, not be excluded that the wounds from the biopsies could have made the skin more susceptible to infections.

Experimental groups of six lambs are sufficient to provide indications, but ideally the experimental groups, as well as the control group, should have been larger. Because footrot can cause severe lameness and pain, only one foot on each lamb was inoculated taking account of animal welfare issues. Using a facility especially built for trials with infectious agents, the risk of both contamination and spreading the disease was minimized. *D. nodosus* has in previous studies been identified in skin biopsies from footrot lesions (Egerton et al., 1969), but with a lower sensibility than by PCR (Knappe-Poindecker et al., 2014). In their study, mainly severely affected sheep were included, whereas only one sheep in the present study developed severe symptoms of footrot.

In this study, and consistent with previous studies, gamithromycin seemed effective in the treatment of footrot (Angen, 2012; Stamphøj, 2013). The *D. nodosus* detected by PCR in 6/24 lambs on day 45 were probably deceased because the symptoms had healed, and this hypothesis is supported by the finding that all sheep tested negative on day 65 and 90. However, a study only treating lame sheep with gamithromycin cured almost all sheep from footrot, but was not able to eliminate the bacterium from all the treated sheep (Strobel et al., 2014). The dosage and frequency of treatment in our study can, based on the effect of the treatment, be considered adequate. However, the use of antibiotics is restricted in Norway, and the withdrawal time of 64 days can be impractical for the farmer. If gamithromycin is to be used in the elimination of footrot, treatment should be performed only after thorough examination of the flock and careful consideration of possible contribution to development of antibiotic resistance.

5. Conclusion

The present study strongly indicates that virulent *D. nodosus* isolated from Norwegian Red cattle and both virulent and benign *D. nodosus* isolated from Norwegian White sheep can be transferred to Norwegian White sheep under experimental conditions. The study also indicates that gamithromycin may be effective against *D. nodosus*.

Conflict of interest

The author wise to confirm that there is no conflict of interest associated with this article.

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References

- Ahern, B.J., Soma, L.R., Boston, R.C., Schaer, T.P., 2009. Comparison of the analgesic properties of transdermally administered fentanyl and intramuscularly administered buprenorphine during and following experimental orthopedic surgery in sheep. *Am. J. Vet. Res.* 70, 418–422.
- Angen, Ø., 2012. Ondartet klovsyge hos får. *Dansk Vet. Tidsskr.* 13, 42.
- Beveridge, W.I.B., Bulletin No. 140, 1–56 1941. Foot-rot in Sheep: A Transmissible Disease Due to Infection with *Fusiformis nodosus* (n.sp.). Studies on its Cause, Epidemiology and Control. Council for Scientific and Industrial Research, Melbourne.
- Cederlöf, S.E., Hansen, T., Klaas, I.C., Angen, Ø., 2013. An evaluation of the ability of *Dichelobacter nodosus* to survive in soil. *Acta Vet. Scand.* 55, 4.
- Claxton, P.D., 1989. Antigenic classification of *Bacteroides nodosus*. In: Egerton, J.R., Yong, W.K., Rifkin, G.G. (Eds.), *Footrot and Foot Abscesses of Ruminants*. CRC Press, Boca Raton, FL, pp. 155–165.
- Council of Europe, European Convention, 2006. ATS no. 123, Appendix A. <http://conventions.coe.int/Treaty/EN/Treaties/PDF/123-Arev.pdf> (accessed 25.10.13).
- Depiuzzi, L.J., Richards, R.B., Henderson, J., Rood, J.I., Palmer, M., Penhale, W.J., 1991. Characterisation of virulent and benign strains of *Bacteroides nodosus*. *Vet. Microbiol.* 26, 151–160.
- Depiuzzi, L.J., Roberts, W.D., Hawkins, C.D., Palmer, M.A., Pitman, D.R., McQuade, N.C., Jelinek, P.D., Devereaux, D.J., Rippon, R.J., 1998. Severity and persistence of footrot in Merino sheep experimentally infected with a protease thermostable strain of *Dichelobacter nodosus* at five sites. *Aust. Vet. J.* 76, 32–38.
- Dhungyel, O.P., Whittington, R.J., Egerton, J.R., 2002. Serogroup specific single and multiplex PCR with pre-enrichment culture and immunomagnetic bead capture for identifying strains of *D. nodosus* in sheep with footrot prior to vaccination. *Mol. Cell. Probes* 16, 285–296.
- Dwyer, C.M., Bornett, H.L.I., 2004. Chronic stress in sheep: assessment tools and their use in different management conditions. *Anim. Welfare* 13, 293–304.
- Egerton, J.R., Morgan, I.R., Burrell, D.H., 1972. Foot-rot in vaccinated and unvaccinated sheep. I. Incidence, severity and duration of infection. *Vet. Rec.* 91, 447–453.
- Egerton, J.R., Parsonson, I.M., 1966. Isolation of *Fusiformis nodosus* from cattle. *Aust. Vet. J.* 42, 425–429.
- Egerton, J.R., Raadsma, H.W., 1991. Breeding sheep for resistance to footrot. In: Owen, J.B., Axford, R.F.E. (Eds.), *Breeding for Disease Resistance in Farm Animals*. CAB International, Wallingford, pp. 347–370.
- Egerton, J.R., Roberts, D.S., 1971. Vaccination against ovine foot-rot. *J. Comp. Pathol.* 81, 179–185.
- Egerton, J.R., Roberts, D.S., Parsonson, I.M., 1969. The aetiology and pathogenesis of ovine foot-rot. I. A histological study of the bacterial invasion. *J. Comp. Pathol.* 79, 207–216.
- Emery, D.L., Stewart, D.J., Clark, B.L., 1984. The comparative susceptibility of five breeds of sheep to foot-rot. *Aust. Vet. J.* 61, 85–88.
- Forbes, A.B., Strobel, H., Stamphøj, I., 2014. Field studies on the elimination of footrot in sheep through whole flock treatments with gamithromycin. *Vet. Rec.* 174, 146.

- Frosth, S., Slettemeås, J.S., Jørgensen, H.J., Angen, Ø., Aspán, A., 2012. Development and comparison of a real-time PCR assay for detection of *Dichelobacter nodosus* with culturing and conventional PCR: harmonisation between three laboratories. *Acta Vet. Scand.* 54, 6.
- Ghimire, S.C., Egerton, J.R., Dhungyel, O.P., 1999. Transmission of virulent footrot between sheep and goats. *Aust. Vet. J.* 77, 440–453.
- Ghimire, S.C., Egerton, J.R., Dhungyel, O.P., Joshi, H.D., 1998. Identification and characterisation of serogroup M among Nepalese isolates of *Dichelobacter nodosus*, the transmitting agent of footrot in small ruminants. *Vet. Microbiol.* 62, 217–233.
- Gilhuus, M., Vatn, S., Dhungyel, O.P., Tesfamichael, B., L'Abée-Lund, T.M., Jørgensen, H.J., 2013. Characterisation of *Dichelobacter nodosus* isolates from Norway. *Vet. Microbiol.* 163, 142–148.
- Kaler, J., Wani, S.A., Hussain, I., Beg, S.A., Makhdoomi, M., Kabli, Z.A., Green, L.E., 2012. A clinical trial comparing parenteral oxytetracycline and enrofloxacin on time to recovery in sheep lame with acute or chronic footrot in Kashmir, India. *BMC Vet. Res.* 8, 12.
- Knappe-Poindecker, M., Gilhuus, M., Jensen, T.K., Klitgaard, K., Larssen, R.B., Fjeldaas, T., 2013. Interdigital dermatitis, heel horn erosion, and digital dermatitis in 14 Norwegian dairy herds. *J. Dairy Sci.* 96, 7617–7629.
- Knappe-Poindecker, M., Gilhuus, M., Jensen, T.K., Vatn, S., Jørgensen, H.J., Fjeldaas, T., 2014. Cross-infection of virulent *Dichelobacter nodosus* between sheep and co-grazing cattle. *Vet. Microbiol.* 170, 375–382.
- Laing, E.A., Egerton, J.R., 1978. The occurrence, prevalence and transmission of *Bacteroides nodosus* infection in cattle. *Res. Vet. Sci.* 24, 300–304.
- Meling, S., Ulvund, M.J., 2009. Flock health visits in 17 sheep flocks in Rogaland. In: *Proceedings of the 7th International Sheep Veterinary Congress, Stavanger*, 12–16, June, pp. 148–149.
- Moore, L.J., Wassink, G.J., Green, L.E., Grogono-Thomas, R., 2005. The detection and characterisation of *Dichelobacter nodosus* from cases of ovine footrot in England and Wales. *Vet. Microbiol.* 108, 57–67.
- Morck, D.W., Gard, M.S., Olson, M.E., 1994. Experimental evaluation of a commercial footrot vaccine against native Canadian strains of *Dichelobacter nodosus*. *Can. J. Vet. Res.* 58, 122–126.
- Palmer, M.A., 1993. A gelatin test to detect activity and stability of proteases produced by *Dichelobacter (Bacteroides) nodosus*. *Vet. Microbiol.* 36, 113–122.
- Rasmussen, M., Capion, N., Klitgaard, K., Rogdo, T., Fjeldaas, T., Boye, M., Jensen, T.K., 2012. Bovine digital dermatitis: possible pathogenic consortium consisting of *Dichelobacter nodosus* and multiple *Treponema* species. *Vet. Microbiol.* 160, 151–161.
- Rogdo, T., Hektoen, L., Slettemeås, J.S., Jørgensen, H.J., Østerås, O., Fjeldaas, T., 2012. Possible cross-infection of *Dichelobacter nodosus* between co-grazing sheep and cattle. *Acta Vet. Scand.* 54, 19.
- Skerman, T.M., Erasmuson, S.K., Morrison, L.M., 1982. Duration of resistance to experimental footrot infection in Romney and Merino sheep vaccinated with *Bacteroides nodosus* oil adjuvant vaccine. *N. Z. Vet. J.* 30, 27–31.
- Stamphøj, I., 2013. Eradicating footrot with gamithromycin and the PCR test. In: *Proceeding of the 8th International Sheep Veterinary Congress, Rotorua*, 18–22, February, p. 140.
- Stewart, D.J., 1989. Footrot in sheep. In: Egerton, J.R., Yong, W.K., Riffkin, G.G. (Eds.), *Footrot and Foot Abscess of Ruminants*. CRC Press, Boca Raton, FL, pp. 5–45.
- Stewart, D.J., Claxton, P.D., 1993. Ovine footrot. Clinical diagnosis and bacteriology. In: Corner, L.A., Bagust, T.J. (Eds.), *Australian Standard Diagnostic Techniques for Animal Diseases*. CSIRO, Melbourne, pp. 1–27.
- Strobel, H., Lauseker, M., Forbes, A.B., 2014. Targeted antibiotic treatment of lame sheep with footrot using either oxytetracycline or gamithromycin. *Vet. Rec.* 174, 46.
- Vatn, S., Hektoen, L., Høyland, B., Reiersen, A., Kampen, A.H., Jørgensen, H.J., 2012. Elimination of severe footrot from the Norwegian sheep population – a progress report. *Small Rumin. Res.* 106, 11–13.
- Vatn, S., Hektoen, L., Fredriksen, B., Høyland, B., Reiersen, A., Jørgensen, H.J., 2013. *Proceeding of the 8th International Sheep Veterinary Congress, Rotorua*, 18–22, February, p. 149.
- Wilkinson, F.C., Egerton, J.R., Dickson, J., 1970. Transmission of *Fusiformis nodosus* infection from cattle to sheep. *Aust. Vet. J.* 46, 382–384.