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Interdigital dermatitis, heel horn erosion, and digital dermatitis in 14 Norwegian dairy herds

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ABSTRACT

The aim of this study was to assess infectious foot diseases, including identification and characterization of *Dichelobacter nodosus* and *Treponema* spp., in herds having problems with interdigital dermatitis (ID) and heel horn erosion (E) and in control herds expected to have few problems. We also wanted to compare diseased and healthy cows in all herds. The study included 14 dairy herds with a total of 633 cows. Eight herds had a history of ID and E, and 6 were control herds. All cows were scored for lameness, and infectious foot diseases on the hind feet were recorded after trimming. Swabs and biopsies were taken from the skin of 10 cows in each herd for bacterial analyses. In total, samples were taken from 34 cows with ID, 11 with E, 40 with both ID and E, and 8 with digital dermatitis (DD), and from 47 cows with healthy feet. Swabs were analyzed for identification and characterization of *D. nodosus* by PCR, culture, virulence testing, and serotyping. Biopsies were analyzed by fluorescent in situ hybridization regarding histopathology, identification, and characterization of *Treponema* spp., and identification of *D. nodosus*. Interdigital dermatitis was the most frequent foot disease, with a prevalence of 50.4% in problem herds compared with 26.8% in control herds. Heel horn erosion was recorded in 34.8% of the cows in problem herds compared with 22.1% in control herds. *Dichelobacter nodosus* was detected in 97.1% of the cows with ID, in 36.4% with E, in all cows with both ID and E, in all cows with DD, and in 66.0% of cows with healthy feet. All serogroups of *D. nodosus* except F and M were detected, and all isolates were defined as benign by the gelatin gel test. *Treponema* spp. were detected in 50.0% of the cows with ID, in 9.1% with E, in 67.5% with ID and E, in all cows with DD, and in 6.4% of those with healthy feet. In total, 6 previously described phylotypes (PT) of *Treponema* were detected: PT1, PT3, PT6, PT13, and PT15 in cows with ID, PT1 in a cow with E, and PT1, PT2, PT3, PT6, and PT13 in cows with both ID and E. One new phylotype (PT19) was identified. The epidermal damage score was higher but the difference in inflammatory response of the dermis was minor in cows with ID versus those with healthy feet. Fisher's exact test revealed an association between ID and *D. nodosus*, and between ID and *Treponema* spp. Logistic regression revealed an association between both ID and E and dirty claws (odds ratios = 1.9 and 2.0, respectively). Our study indicates that *D. nodosus*, *Treponema* spp., and hygiene are involved in the pathogenesis of ID.
Key words: interdigital dermatitis, heel horn erosion, *Dichelobacter nodosus*, *Treponema* spp.

INTRODUCTION

Infectious foot diseases are common in dairy herds, causing reduced animal welfare and financial losses (Kossaibati and Esslemont, 1997; Bruijnis et al., 2012). Foot disorders are the cause of approximately 90% of lameness cases in dairy cattle, and infectious foot diseases are increasing in many countries (Murray et al., 1996). In recent years, Norway has experienced a shift from tiestall to freestall housing of cattle. Combined with larger herds and more frequent trade of cattle, this change has increased the occurrence of most infectious foot diseases, including interdigital dermatitis (**ID**), heel horn erosion (**E**), and, to some extent, digital dermatitis (**DD**) and interdigital phlegmon (Sogstad et al., 2005; Rogdo et al., 2011).

Interdigital dermatitis frequently occurs in wet, unhygienic environments (Hultgren and Bergsten, 2001). *Dichelobacter nodosus* is commonly isolated from the lesions, but its prevalence varies greatly between herds (Laing and Egerton, 1978). *Dichelobacter nodosus* is a gram-negative anaerobic bacterium known to cause footrot in sheep (Beveridge, 1941). The bacterium produces extracellular proteases that are assumed to be responsible for the associated tissue damage (Thomas, 1964). A few studies have virulence-tested *D. nodosus* isolates from cattle and all isolates were categorized as benign (Stewart, 1979; Richards et al., 1980; Gilhuus

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et al., 2013). *Dichelobacter nodosus* is divided into 10 serogroups (A to I and M) based on fimbrial antigens (Claxton, 1989; Ghimire et al., 1998; Bhat et al., 2012).

A survey from 2002 stated that E was the most common infectious foot disease in Norwegian dairy cattle with a prevalence of 38% in freestall herds (Sogstad et al., 2005). A wet, unhygienic environment reduces the hardness of the claw and predisposes for E (Enevoldsen et al., 1991; Borderas et al., 2004). A strong association exists between E and ID (Manske et al., 2002), and the prevalence of both diseases is reduced by grazing (Holzhauer et al., 2012).

Digital dermatitis is a multifactorial disease with a great impact on animal welfare and production (Argáez-Rodríguez et al., 1997; Bruijnis et al., 2012). Even though different bacteria such as *D. nodosus, Fusobacterium necrophorum, Bacteroides* spp., and *Campylobacter* spp. have been identified in the lesions (Blowey and Sharp, 1988; Cruz et al., 2005; Rasmussen et al., 2012), studies have indicated that *Treponema* spp. is the main causative agent (Walker et al., 1995; Evans et al., 2008). Several different phylotypes (**PT**) have been identified (Pringle et al., 2008; Evans et al., 2009; Yano et al., 2010). The PT isolated from DD lesions is categorized into 6 phylogenetic clusters (Evans et al., 2009; Yano et al., 2010). Digital dermatitis is considered endemic in many countries in Europe as well as in the United States (Rebhun et al., 1980; Blowey and Sharp, 1988; Holzhauer et al., 2006). Characteristic lesions are rare in Norway, but *Treponema* spp. have been detected and the prevalence seems to be increasing (Forshell et al., 2001; Rogdo et al., 2011). A study from 2009 identified PT1 and PT3 in Norwegian dairy cattle (Rasmussen et al., 2012), and hitherto unknown phylotypes were also discovered (Rogdo et al., 2011).

Infectious diseases of the bovine foot are often recorded in the same herd, and some authors have included all of them in "the bovine digital epidermitis syndrome" (Read and Walker, 1998; Cruz et al., 2005). Studies have shown that ID and E predispose for DD, and that these diseases have similar causative mechanisms (Manske et al., 2002; Holzhauer et al., 2006). It has recently been reported that *D. nodosus* may interact synergistically with treponemes to contribute to the development of DD in some regions (Rasmussen et al., 2012).

The aim of this study was to assess ID, E, and DD, as well as the prevalence of *D. nodosus* and *Treponema* spp., both in cows in herds expected to have problems with these diseases and in cows expected to have healthy feet (control herds). We also aimed to characterize the bacterial species with respect to genotypes, phylotypes, and virulence, and to analyze the association between ID and the bacterial traits, between ID and E, and between both ID and E and the cleanliness of the claws.

MATERIALS AND METHODS

Study Design

The study was a cross-sectional study of infectious foot diseases in herds with an expected high prevalence of such diseases and in herds assumed to have few such problems.

Study Population and Selection of Herds

We wanted the study population to include 8 herds with a high incidence of infectious foot diseases and an approximately equal number of herds with low incidence in the most recent years. We aimed for an equal number of freestall and tiestall herds. The herds were chosen from members of the Norwegian dairy herd recording system (**NDHRS**). Only freestall herds and tiestall herds with at least 30 and 20 dairy cows, respectively, were evaluated for eligibility.

All herds with 15 or more cases of foot disease registered in NDHRS between July 2009 and June 2010 received a questionnaire regarding general management, housing, claw trimming routines, and interest in participating in the study. Ninety-three out of 112 farmers replied, and 89 were willing to participate. After consultation with the local claw trimmer, local veterinarian, or both, 4 freestall and 4 tiestall herds were chosen that were expected to have problems with ID and E.

Only herds with 0 or 1 recorded foot disease were considered as control herds. Selecting such herds based solely on NDHRS recordings was not feasible because NDHRS does not differentiate between missing data and no reported cases. Therefore, claw trimmers were asked to recommend possible herds with previously good claw health. The owners of 8 farms were contacted by phone and answered a questionnaire, and finally 3 tiestall and 3 freestall control herds were included in the study.

Study Sample

The study sample consisted of 14 herds and 633 cows. Three herds were located in the western region, 10 in the east, and one in central Norway. Eleven herds had only Norwegian Red animals (n = 591), whereas 3 herds had a small percentage (2.5 to 17.5%) of Norwegian Red and Holstein crossbreeds (n = 14) and a few purebred Holstein (n = 7). Seven herds were housed in freestalls (444 cows), and 7 herds (189 cows) were housed in tiestalls. All heifers in the last 2 mo of gestation and all cows in each herd were examined. Cows that were in labor and cows that had most recently calved were not examined (n = 3).

Recording of Locomotion, Infectious Foot Diseases, and Cleanliness

The study was conducted between October 2010 and May 2011, with all data collected by the first author on a single occasion during routine claw trimming of cows. Because hind feet are more affected by diseases than front feet (Murray et al., 1996; Manske et al., 2002), only lesions on the hind feet were recorded. The highest score was recorded independently of the number of feet affected. The locomotion scores (**LocS**) of all cows were assessed according to Sprecher et al. (1997), and lameness was defined as a LocS >2. The cows from freestall herds were assessed when walking in the alleys before trimming, and the cows from tiestall herds were assessed on their way to the stand-up trimming chute. The cleanliness of the cow, divided into abdomen and udder, hind quarters, extremities distal to the carpal or hock joint, and remaining body, was given a subjective score from clean (1) to very dirty (4), adapted from Schreiner and Ruegg (2002). By summing the 4 scores, a total cleanliness score of the whole body on a scale from 4 (clean) to 16 (very dirty) was created. The cleanliness of the abaxial claw walls and the plantar aspect and interdigital space on the right hind foot were recorded using the same scale, creating a total cleanliness score of the claws from 3 (clean) to 12 (very dirty). The cleanliness of the body was assessed before the cow entered the trimming chute, and the cleanliness of the claw wall and plantar foot was assessed in the chute before the foot was elevated from the ground. The cleanliness of the interdigital space was assessed directly after the foot was elevated. Heel horn erosion and dermatitis were both recorded as not present (0), mild (1), moderate (2), or severe (3). Dermatitis scores 1 and 2 were equal to ID and score 3 was equal to DD. Digital dermatitis lesions were also classified as M1 to M4 lesions, as in Döpfer et al. (1997).

Sampling for Bacterial Analyses

Ten animals from each herd were selected for bacterial analysis, which included swabs for identification and characterization of *D. nodosus* and biopsies for histopathological evaluation, identification, and characterization of *Treponema* spp., and identification of *D. nodosus*. The samples were collected from the first 3 cows with healthy feet, and from 7 cows with ID, E, or DD. If fewer than 3 cows had healthy feet in a herd, the number of samples from cows with symptoms was increased, ensuring that bacterial samples were obtained from a total of 10 cows. When the number of cows with symptoms was less than 7, the same practice was carried out and the number of samples from cows with healthy feet was increased.

Bacterial Sampling for D. nodosus: PCR, Culture, Virulence Testing, and Serotyping

After cleaning the skin thoroughly with tap water and drying off with paper towels, samples were taken from the plantar skin of the foot or the interdigital skin using 2 sterile swabs. Swabs for culturing were placed in Transystem Amies agar gel medium with charcoal (Copan, Brescia, Italy), and swabs for real-time PCR analysis were placed in tubes with sterile PBS containing 0.02 M EDTA. Samples were sent by overnight courier to the Norwegian Veterinary Institute (Oslo) for analysis.

Then, DNA was extracted from the swabs in PBS with EDTA using a nucliSENS easyMAG extractor (bioMèrieux, Boxtel, the Netherlands) following the manufacturer's instructions. The DNA from cultured isolates was obtained by diluting broth culture 1:5 in double-distilled water followed by boiling for 1 min. Extracted DNA was stored at –20°C. *Dichelobacter nodosus* was detected using a real-time PCR as described previously (Frosth et al., 2012).

Culture was performed on 4% hoof agar (**HA**) basically as described by Stewart and Claxton (1993), but with the addition of 1% Lab-Lemco powder (refined meat extract; Oxoid, Basingstoke, UK) and 0.2% tryptose (Oxoid) to the HA. When possible, at least 2 *D. nodosus* suspect colonies from each sample were subcultured onto 2% HA. An approximately 5- × 5-mm piece of agar with pure confluent bacterial growth was cut from the agar and transferred to HEPES-TAS (trypticase-arginine-serine) broth (Stewart and Claxton, 1993). The broth was incubated anaerobically at 37°C for 48 to 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. The remaining broth cultures were used for virulence testing by the gelatin gel (**GG**) test as described below. Isolates were also stored at –70°C in Bacto heart infusion broth (BD, Sparks, MD) with 15% glycerol.

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 with thermostable proteases (virulent) and AC 6466 ST 305 with thermolabile proteases (benign). Culture broths of virulent and benign control strains were included on each gel.

To allocate the isolates to serogroups A to 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. Distilled water was included as negative control.

For isolates that did not yield a positive band for any of the 9 serogroups, *fimA* was amplified by PCR using the primer combination *fimA*-u1 and *fimA*-d1 or *fimA*-u1 and *fimA*-d2 (Zhou and Hickford, 2001), or the primer combination PTC830 and PTC5 (Cox, 1992). All primer combinations amplify the variable region of *D. nodosus* class-I *fimA*, which includes serogroups A, B, C, E, F, G, I, and M (Mattick et al., 1991; Ghimire et al., 1998). The amplification mixture contained 0.2 μmol/L of each primer, 0.05 U/μL of Taq DNA polymerase (Qiagen GmbH, Hilden, Germany) and 0.2 mmol/L of dNTP mix (Finnzymes Oy, Espoo, Finland). Amplification was performed on an MJ Research DNA Engine Dyad (Bio-Rad, Hercules, CA) with an initial denaturation step of 95°C for 2 min followed by 30 cycles of 95°C for 1 min, 55°C for 1 min, 72°C for 2 min, and a final extension of 72°C for 7 min. The PCR products were analyzed by electrophoresis in a 1% agarose gel, and stained with GelRed (Biotium, Hayward, CA).

Selected PCR products were purified using ExoSAP-IT (GE Healthcare, Uppsala, Sweden) and sequenced at GATC Biotech (Constance, Germany). Sequences were aligned using CLC Main Workbench 6.7 (CLC Bio, Aarhus, Denmark) and trimmed to include only the coding region of *fimA*. The identity of the serogroup was determined using a BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Serotypes and serogroups were defined according to previously suggested criteria for *fimA* sequences (Mattick et al., 1991; Bhat et al., 2012): ≤5 AA changes within a serotype, 8 to 15 changes between serotypes, and ≥35 changes between serogroups.

Sampling, Histopathology, and Analyses of Biopsies by Fluorescence In Situ Hybridization

Biopsies were taken on the border between healthy and diseased skin with a 6-mm biopsy punch (Miltex Inc., York, PA). Each biopsy punch was only used once. On the cows with healthy feet, the biopsies were taken from the plantar aspect of the foot approximately 1 to 2 cm proximal to the interdigital space. 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 specimens were stained with hematoxylin and eosin and by the Ayoub-Shklar method for visualization of keratin and prekeratin. For fluorescence in situ hybridization (**FISH**) analysis, serial sections were cut (4 μm) and mounted on SuperFrost+ slides (Menzel-Gläser, Braunschweig, Germany). All biopsies were histopathologically evaluated in hematoxylin-eosin sections. The degree of epidermal damage and the inflammatory response of the dermis were scored from 0 to 3. Score 0 was defined as normal epidermis or dermis, score 1 (mild epidermal damage) 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). The cellular, inflammatory response in the dermis was defined as score 1 (mild) characterized by only a few lymphocytes and mononuclear cells, score 2 (moderate) with some lymphocyte or mononuclear cells infiltrations around small vessels, or score 3 (severe) with perivascular dermatitis. In correctly orientated sections, the thickness of epidermis from the stratum disjunction to the tip of the epidermal pegs was measured using an Axio Imager M1 microscope equipped with the software AxioVision (Zeiss, Oberkochen, Germany).

The 16S rRNA targeting oligonucleotide probes used in this study have previously been published and includes probes targeting PT1 to PT17 (Klitgaard et al., 2008; Rasmussen et al., 2012). Moreover, a *Treponema* group probe and a probe for *D. nodosus* were applied (Klitgaard et al., 2008; Rasmussen et al., 2012). The oligonucleotide probes were 5′ labeled with fluorescein isothiocyanate or Cy3, and hybridization was carried out at 46°C as previously described (Rasmussen et al., 2012). For epifluorescence microscopy, an Axioimager M1 epifluorescence microscope equipped with an AxioCAM MRm version 3 FireWire monochrome camera (Zeiss) was used.

For each of the *Treponema* phylotypes, the prevalence in biopsy specimens (PT prevalence score) was scored from 0 to 3 according to Klitgaard et al. (2008): 0 = no hybridization, 1 = sparse hybridization (up to 5% of the total number of bacteria), 2 = moderate hybridization (between 5 and 10% of the total number of bacteria), and 3 = strong hybridization (more than 10% of the total number of bacteria). The presence of *D. nodosus* was scored as 0 = no hybridization, or 1 = positive hybridization.

For identification of possible new phylotypes, purified DNA from 5 cows infected with yet unidentified treponemes (3 cows had ID and 2 cows had ID and E) was pooled for PCR amplification of the 16S rRNA gene as previously described (Rasmussen et al., 2012). The bacterial DNA was amplified using primers Trep-346-F: 5′-GGG AGG CAG CAG CTA AGA A-3′ and

Trep-705-R: 5′-ATC TAC AGA TTC CAC CCC TA-3′ as described by Klitgaard et al. (2013) and cloned.

Statistical Analysis

Data recorded on the farm were transferred to Stata (Stata SE/11, Stata Corp., College Station, TX) for statistical analysis. Estimates are given with 95% CI in parentheses whenever possible. Because few cows had DD, these cows were excluded from the statistical analyses. A small number of the sampled cows were diagnosed solely with E, and no statistical analyses regarding the association between E alone and *D. nodosus* or *Treponema* spp. were performed. The association between ID and *D. nodosus* and between ID and *Treponema* spp. was analyzed by Fisher's exact test and presented in frequency tables.

Dirty claws were defined as those with a cleanliness score of ≥ 7. The association between ID and E and between both ID and E and the cleanliness of the claws was analyzed using logistic regression.

RESULTS

Locomotion and Cleanliness

In both problem and control herds, 3.1% of the cows had LocS 3, but the confidence interval was narrower in the problem herds (95% CI: 1.4 to 5.8%) than in the control herds (95% CI: 1.1 to 6.6%). Table 1 shows the mean LocS and the mean cleanliness scores of body and right hind claw for all cows in problem and control herds in tiestalls and freestalls. No cows had LocS 4 or 5, or a total cleanliness score of the claws >9, or a total cleanliness score of the body >12. Only 0.8% of the cows chosen for bacterial sampling had a LocS >2.

Infectious Foot Diseases

Table 1 shows the mean prevalence for ID, E, and DD for the problem and control herds in tiestalls and freestalls. Interdigital dermatitis was recorded in 50.4% (95% CI: 45.4 to 55.4%) of the cows in the problem herds compared with 26.8% (95% CI: 21.2 to 33.0%) in the control herds, and E was recorded in in 34.8% (95% CI: 30.2 to 39.7%) of the cows in the problem herds compared with 22.1% (95% CI: 16.9 to 28.0) in the control herds. Of the 9 cows with DD, 8 had M1 lesions and 1 had an M2 lesion. Table 2 shows the prevalences of these diseases among the cows chosen for bacterial sampling in problem and control herds in tiestalls and freestalls.

Dichelobacter nodosus

The prevalence of *D. nodosus* was 94.5% (95% CI: 86.6 to 98.5%) in problem herds and 66.1% (95%

Table 1. Number of herds, number of cows, mean cleanliness score of the body and claw, locomotion score (LocS), and the prevalence of interdigital dermatitis (ID), digital dermatitis (DD), and heel horn erosion (E) of 633 cows in 14 Norwegian dairy herds[1]

Stall type/previous claw health status	Herds (no.)	Cows (no.)	Cleanliness score: body (CI)	Cleanliness score: claw (CI)	LocS >2 (%) (CI)	ID (%) (CI)	DD (%) (CI)	E (%) (CI)
Freestall								
Problem herds	4	306	5.6 (5.5–5.7)	5.7 (5.6–5.8)	1.5 (0.1–4.3) (n = 198)	49.7 (43.9–55.4)	2.0 (0.7–4.3)	33.9 (28.6–39.5)
Control herds	3	138	5.6 (5.4–5.8)	5.8 (5.6–5.9)	2.9 (0.8–7.3)	32.1 (24.4–40.6)	0.7 (0.1–4.0)	33.6 (25.7–42.1)
Tiestall								
Problem herds	4	95	5.7 (5.4–6.0)	5.2 (4.9–5.5)	6.3 (2.3–13.2)	52.6 (42.1–63.0)	2.1 (0.3–7.4)	37.9 (28.1–48.4)
Control herds	3	94	5.4 (5.2–5.7)	4.7 (4.4–5.0)	3.5 (0.4–12.1) (n = 57)	19.2 (11.8–28.6)	0 (0–3.8)[1]	5.3 (1.8–12.0)
Total	14	633	5.6 (5.5–5.7)	5.5 (5.4–5.6)	3.1 (1.7–5.0)	41.7 (37.9–45.7)	1.4 (0.7–2.7)	30.2 (26.6–33.9)

[1]One-sided 97.5% CI.

Table 2. Number of cows with healthy feet, with interdigital dermatitis (ID), digital dermatitis (DD), and heel horn erosion (E) in sampled cows in problem and control herds in freestalls and tiestalls

Stall type/previous claw health status	Cows (no.)	Healthy feet (no. of cows)	ID			E			ID and E
			Score 1	Score 2	DD	Score 1	Score 2	Score 3	
Freestall									
Problem herds	40	10	2	9	5	2	0	0	12
Control herds	30	9	6	0	1	1	6	0	7
Tiestall									
Problem herds	40	12	1	6	2	0	1	0	18
Control herds	30	16	10	0	0	1	0	0	3
Total	140	47	19	15	8	4	7	0	40
%	100	33.6	13.6	10.7	5.7	2.9	5.0	0	28.6

CI: 52.6 to 77.9%) in control herds. Of the 140 cows sampled, 116 tested positive by PCR (82.9%), 73 by culture (52.1%), and 47 by FISH (33.6%). By the FISH method, *D. nodosus* organisms were found within the superficial layers of the epidermis, in keratinized as well as degenerated tissue. A total of 124 *D. nodosus* isolates were obtained from 73 cows in 12 of the 14 herds. In 1 of the 2 remaining herds, the only PCR-positive cow was negative by culture, and in the other herd, *D. nodosus* was not detected. All isolates were benign, as defined by the GG test.

Results from PCR, culture, and serogrouping for 121 cows (cows diagnosed with DD or E alone were not included) are presented in Table 3, and results from the *fim*A PCR and sequencing of all serogrouped samples in each herd are shown in Figure 1. Among the 11 cows diagnosed with E alone, 4 were positive for *D. nodosus* by PCR and 1 was positive by culture and was of serogroup C. It was possible to determine the serogroup for 110 of the 124 *D. nodosus* isolates, with serogroup A (16.1%) and B (16.1%) being the most frequent.

Twenty isolates from 4 farms were negative by the multiplex PCR for serogroups A to I. In total, 6 isolates from the 4 farms were therefore tested by the class I *fimA* PCR. Two of the isolates from 2 farms yielded a positive band using the primer combination PTC830 and PTC5, whereas 4 isolates from 3 farms were positive using the primers *fimA*-u1 and *fimA*-d1. The primer combination *fimA*-u1 and *fimA*-d2 yielded no product for any of the isolates. The PCR products were sequenced; 2 of the sequences showed 97% identity with the *fimA* coding region of *D. nodosus* serogroup B strain 183 (GenBank ID: M92190.1). The predicted AA sequences differed by 9 and 10 AA from the reference and were thus categorized as serogroup B. The remaining 4 of the sequences showed 94% identity to the *fimA* coding region of *D. nodosus* serogroup G strain VCS1703A (GenBank ID: ABQ13217.1). The predicted AA sequences differed by 15 AA from the reference and were thus categorized as serogroup G.

Treponema spp.

The prevalence of *Treponema* spp. was 48.8% (95% CI: 37.4 to 60.2%) in problem herds and 28.3% (95% CI: 17.5 to 41.4%) in control herds. In 60 clones from 30 different biopsies, treponemes were identical to known phylotypes, whereas the remaining treponemes did not bind to the PT-specific probes and were classified as yet unidentifiable.

Phylotype 1, the most frequent, was identified in 42.9% (95% CI: 29.7 to 56.8%) of the biopsies in which known treponemes were identified, followed by PT13 with a prevalence of 23.2% (95% CI: 13.0 to 36.4%). The mean number of phylotypes present in each biopsy was 2.0. In biopsies that scored 3 for epidermis damage, large numbers of treponemes were found deep in the tissue on the border between dead and vital epidermis, whereas the presence of other bacteria was negligible. If more than one phylotype was identified, the treponemes were seen to be closely intermingled. Epidermis damage score 2 biopsies were usually characterized by a less severe treponemal infection, with colonization of only the more superficial layers of degenerated and nonkeratinized epidermis. Occurrence of other bacteria was negligible.

It was not possible to determine the phylotypes in the very few positive samples from cows with healthy feet. In these samples, the treponemes were found only on the epithelial surface, in the stratum disjunction, or both. Figure 2 illustrates the prevalence of all phylotypes present, including unidentified ones, in each herd. The prevalence of *Treponema* spp. and phylotypes detected in problem and control herds in tiestalls and freestalls is presented in Table 4 (cows with DD and cows diagnosed with E alone are not included).

After DNA purification and PCR amplification using previously described primers, the 46 clones that were randomly picked from a clone library and sequenced were all identical, thus representing only one phylotype. This new phylotype displayed 97% sequence similarity
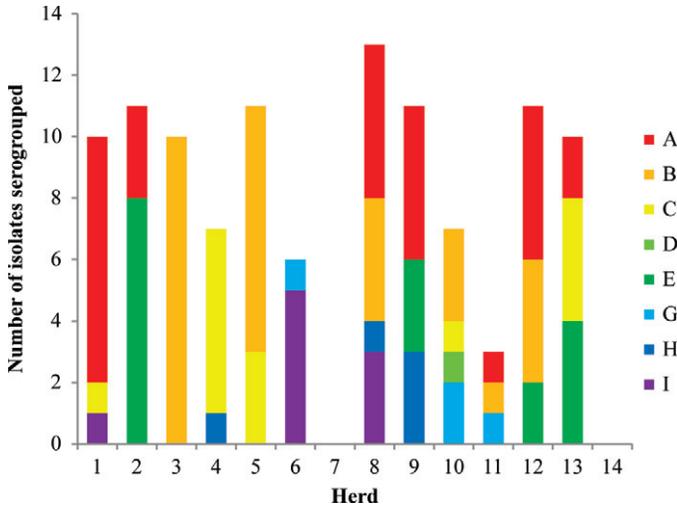
Figure 1. Number of selected *Dichelobacter nodosus* isolates (A to I) serogrouped in the 14 Norwegian dairy herds.

to PT1, with 824 bp being identified. This phylotype will henceforth be referred to as PT19. An oligonucleotide probe specific for PT19 was designed (5′-CAT CCC AGT GTC ATT CCC-3′) and applied on a few selected biopsies. Phylotype 19 colonized the epidermis in the same way as the other phylotypes. The sequence of this putative *Treponema* phylotype has been deposited at GenBank under accession number KC250001.

Histopathological Evaluation

The dermis and epidermis scores for cows with ID, cows with both ID and E, and cows with healthy feet
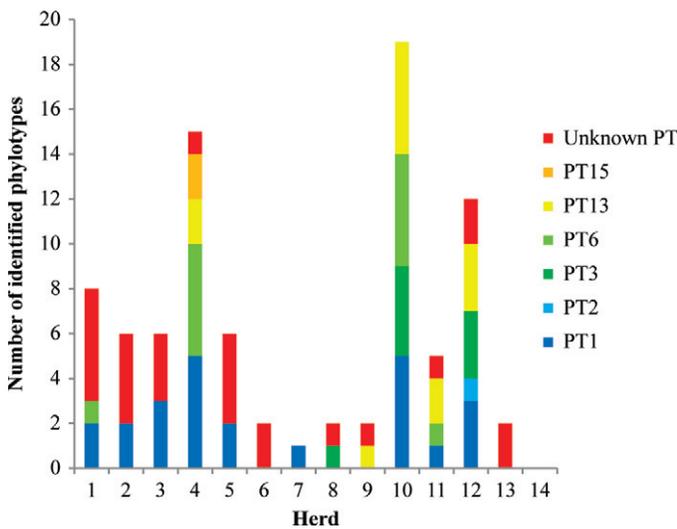

Figure 2. Number of *Treponema* phylotypes (PT) identified by fluorescent in situ hybridization in the 14 Norwegian dairy herds.

Table 3. Prevalence of *Dichelobacter nodosus* (no./total) detected by PCR, culture, and fluorescence in situ hybridization (FISH) in cows with interdigital dermatitis (ID), ID and heel horn erosion (E), and in cows with healthy (H) feet with the detected serogroups (A to I) in 121 sampled cows

Stall type/previous claw health status	PCR			Culture			FISH			Serogroup		
	ID	ID and E	H	ID	ID and E	H	ID	ID and E	H	ID	ID and E	H
Freestall												
Problem herds	11/11	12/12	7/10	8/11	10/12	0/10	9/11	6/12	0/10	A, E, I	A, B, C, E, H	—
Control herds	6/6	7/7	7/9	5/6	7/7	1/9	4/6	4/7	2/9	G, I	B, C	I
Tiestall												
Problem herds	6/7	18/18	12/12	5/7	13/18	5/12	3/7	5/18	0/12	A, B, E, H, I	A, B, E, G, H	A, C, D, I
Control herds	10/10	3/3	5/16	9/10	3/3	0/16	7/10	3/3	0/16	A, B, C, E	A, B	—
Total	33/34	40/40	31/47	27/34	33/40	6/47	23/34	18/40	2/47	A, B, C, E, G, H, I	A, B, C, E, G, H	A, C, D, I
% positive	97.1	100	66.0	79.4	82.5	12.8	67.6	45.0	4.3			

Table 4. Histopathological evaluation (mean lesion score), mean *Treponema* spp. score, and *Treponema* phylotypes detected by fluorescent in situ hybridization (FISH) from 121 sampled cows with interdigital dermatitis (ID), ID and heel horn erosion (E), and in cows with healthy feet (H)

Stall type/previous claw health status	Dermis			Epidermis			FISH					
							Mean *Treponema* spp. score			*Treponema* phylotypes detected		
	ID	ID and E	H	ID	ID and E	H	ID	ID and E	H	ID	ID and E	H
Freestall												
Problem herds	1.2	1.0	0.7	2.4	2.4	0	1.9	1.9	0	1, 6, 15	1, 6, 13	—
Control herds	0.3	1.6	0.8	2.2	1.7	1.0	0.2	1.1	0.4	—	1	—
Tiestall												
Problem herds	0.6	0.5	0.4	2.3	2.1	0.2	0.1	1.4	0.1	—	1, 3, 6, 13	—
Control herds	1.1	1.5	0.6	2.3	2.0	0.2	0.7	2.7	0	1, 3, 13	1, 2, 3, 13	—
Total	0.9	0.9	0.6	2.3	2.1	0.3	0.9	1.6	0.1	1, 3, 6, 13, 15	1, 2, 3, 6, 13	—

from problem and control herds in tiestalls and freestalls are presented in Table 4. No cows had a severe inflammatory response of the dermis (score 3). In 11 biopsies (7.9%), the amount of dermis present was too small to score. Cows diagnosed with E alone had a mean dermis score of 0.6 and a mean epidermal score of 1.0.

The epidermis was correctly oriented for measurement in 54 biopsies, 42 of which were from cows with no symptoms of ID, E, or DD. The healthy skin had a mean epidermal thickness of 427 μm (95% CI: 385 to 469 μm). The epidermal thickness was measured in too few cows with ID, E, and DD to perform any analyses.

Association Between ID and Dichelobacter nodosus and Treponema spp.

The associations between ID and *D. nodosus* and *Treponema* spp. are presented in Table 5, and the association between healthy feet, feet with ID or DD, and *D. nodosus* is presented in Figure 3. The association between healthy feet, feet with ID or DD, and different scores of *Treponema* spp. is presented in Figure 4. Figure 5 presents the association between *D. nodosus* detected by different methods according to the prevalence of different scores of *Treponema* spp. in all sampled cows.

Association Between ID, E, and Claw Cleanliness

The analyses revealed associations between ID and E with an odds ratio (**OR**) of 1.8 (95% CI: 1.3 to 2.6, $P = 0.001$), between ID and dirty claws with an OR of 1.9 (95% CI: 1.3 to 2.7, $P = 0.002$ for a cleanliness score of ≥ 7), and between E and dirty claws with an OR of 2.0 (95% CI: 1.3 to 2.9, $P = 0.001$). The association between ID and cleanliness of the right hind claw is presented in Figure 6.

DISCUSSION

General Considerations

Because 8 out of the 14 herds were selected based on previously high prevalence of ID or E, this study is not representative of Norwegian dairy herds in general. The study, however, gives valuable information on both the prevalence of foot diseases and on *D. nodosus* and *Treponema* spp. and their characteristics in feet from Norwegian dairy herds expected to have high prevalence of ID and E and in feet in control herds expected to be healthy.

All clinical recordings, including locomotion scoring, assessment of cleanliness, and diagnosis of foot lesions were performed by the first author, ensuring conformity of the data. The claw cleanliness recording was per-
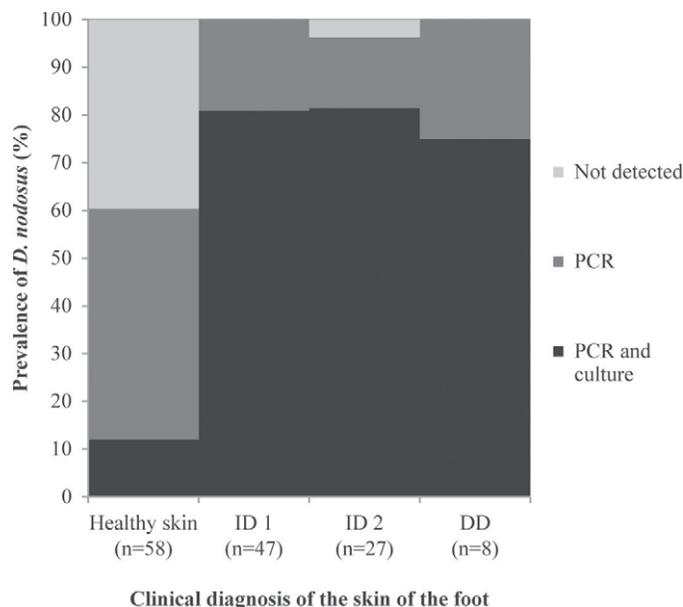
Figure 3. Prevalence of *Dichelobacter nodosus* detected by different methods (PCR, culture, or both) among cows with healthy skin and cows with different scores of interdigital dermatitis (ID) and digital dermatitis (DD) (n = 140).

formed before and directly after the foot was elevated to avoid being biased by the foot lesion assessment.

Extensive bacterial analyses of *D. nodosus* and *Treponema* spp., including culture as well as multiple molecular methods (PCR, histopathological evaluation, and FISH), have to the best of our knowledge not been performed in cattle with foot diseases. For epidemiological reasons, and in contrast to most studies, we included for bacterial analyses cows with symptoms of infectious foot disease as well as cows with healthy feet.

Locomotion and Cleanliness

Several factors influence the risk of foot diseases and lameness, and prevalence can vary greatly between
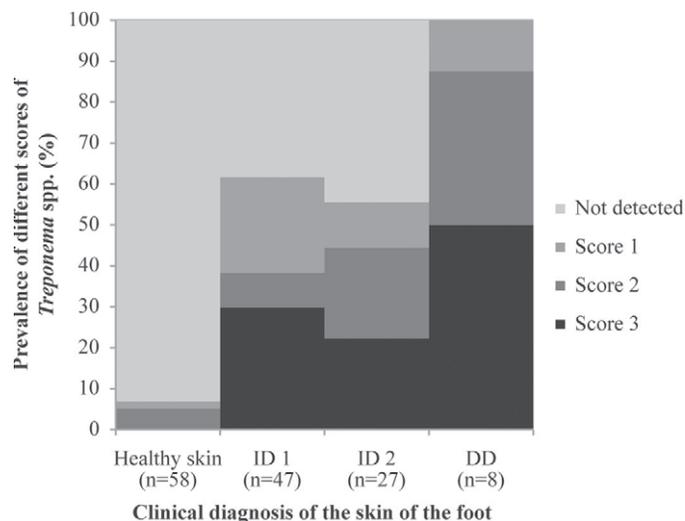

Figure 4. Prevalence of different scores of *Treponema* spp. diagnosed by fluorescent in situ hybridization among cows with healthy skin and cows with different scores of interdigital dermatitis (ID) and digital dermatitis (DD) (n = 140).

herds (Dippel et al., 2009). In total, 52.9% of the 140 cows selected for bacterial sampling in the current study had ID and only 0.8% had LocS >2. These findings support previous studies that have shown that ID usually does not cause lameness and they explain the lack of difference in LocS between our problem and control herds (Manske et al., 2002).

Freestalls, in general, are wetter and dirtier than tiestalls, which explains why the claws in the current study were considerably cleaner in tiestall herds than in freestall herds. A similar association regarding body cleanliness was not, however, found in our study.

Infectious Foot Diseases

The prevalence of ID in this study was much higher than that in a previous Norwegian study (Sogstad et

Table 5. Associations between interdigital dermatitis (ID), heel horn erosion (E) and *Dichelobacter nodosus* and *Treponema* spp. (n = 132)

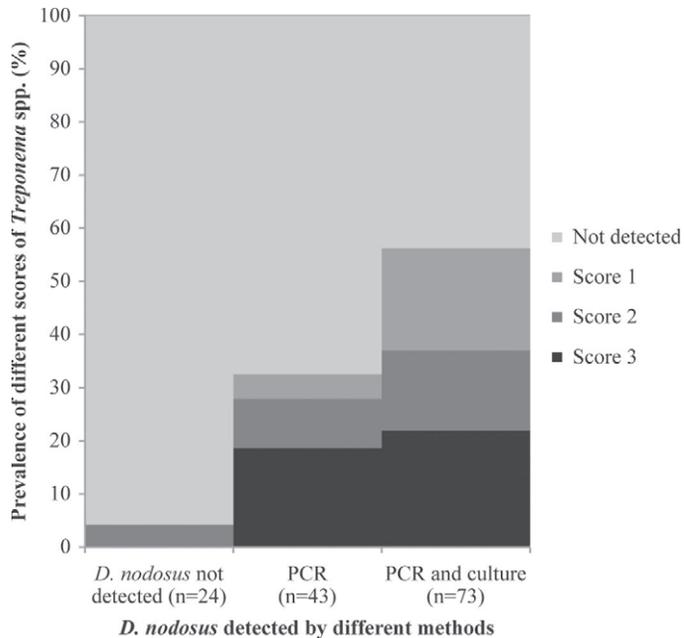| Group	Infection status	ID −	ID +	P-value
Cows with ID only				
D. nodosus	−	23	1	0.008
	+	75	33	
Treponema spp.	−	67	17	0.065
	+	31	17	
Cows with ID only and cows with ID and E				
D. nodosus	−	23	1	<0.001
	+	35	73	
Treponema spp.	−	54	30	<0.001
	+	4	44	

Figure 5. Prevalence of different scores of *Treponema* spp. diagnosed by fluorescent in situ hybridization according to *Dichelobacter nodosus* detected by different methods (PCR, culture, or both) (n = 140).

Figure 6. The association between prevalence of interdigital dermatitis and cleanliness score of the right hind claws (n = 620).

al., 2005). That study was considered representative of Norwegian dairy cows, whereas our study had a preponderance of herds with ID, E, and DD. The recordings were also performed by claw trimmers, who have a tendency to not record a claw disease or to use a lower score than the reference value, especially regarding ID (Manske, 2003), which might have led to an underestimation of the prevalence of foot diseases in general in the study of Sogstad et al. (2005).

The prevalence of ID and E in tiestalls was lower in the control herds than in the problem herds. The mean prevalence of ID in freestalls was also lower in the control herds than in the problem herds. The mean in this case, however, consisted of 2 control herds with high prevalences of ID and 1 herd with a prevalence of zero. In contrast to that in tiestalls, the prevalence of E in freestalls was almost equal in problem and control herds. These results show that even with information from the farmer, the claw trimmer, and the NDHRS recordings, the prevalence of these diseases is hard to predict in freestall herds. That the farmer may have implemented preventive measures before the visit should, however, also be considered. The prevalence of ID and mild and moderate E was high, but as these diseases usually do not cause lameness, they can be difficult for the farmer to discover. This unpredictability suggests that claw trimming chutes and other equipment, in addition to being washed, should be disinfected between every herd.
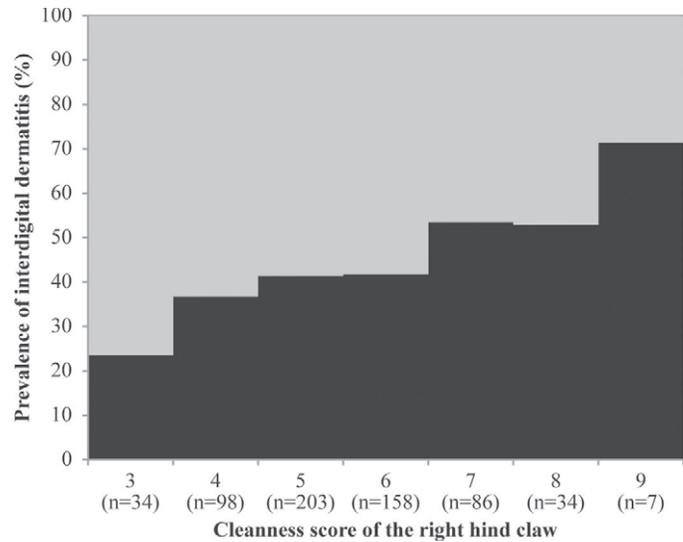
Only 1.4% of the 633 cows in this study had characteristic DD, a much lower prevalence than has been recorded in other Scandinavian countries (Manske et al., 2002; Capion et al., 2008). Differences between breeds may contribute to the lower prevalence in Norway than in other countries. Ødegård et al. (2013) reported a genetic hereditability of 0.2 for ID in Norwegian Red cattle. Freestall housing was introduced later in Norway than in the rest of Europe, which, together with a low average temperature, may have contributed to a slower spread of the bacteria (Sogstad et al., 2005). The smaller Norwegian herds may have a lower risk of DD compared with the larger herds in Denmark and Sweden, which can probably be explained by a lesser exposure to pathogens in the small herds (Holzhauer et al., 2006).

Dichelobacter nodosus

In agreement with the findings of Rogdo et al. (2012), the majority of the tested herds were positive for *D. nodosus*. Our results agree with those reported in a study of ovine footrot, which found the sensitivity to be 3 times higher when detecting *D. nodosus* by PCR than by culture (Frosth et al., 2012). Surface swabs of a relatively large area were used for PCR, whereas the FISH analyses were done on vertical sections of the skin; thus, the surface area tested by FISH was much smaller than that tested by PCR. On the other hand, *D. nodosus* organisms were identified by FISH not only on the skin surface but also invading the superficial layers of epidermis.

Dichelobacter nodosus has previously been associated with ID (Laing and Egerton, 1978; Kasari and Scanlan, 1987), and the results from this study support this association: *D. nodosus* was detected in 98.7% of the cows with ID. It is interesting, however, that *D. nodosus* was also detected in 60.3% of the cows with healthy skin. Laing and Egerton (1978) also investigated the prevalence of *D. nodosus* in healthy feet and revealed a prevalence of between 1.5 and 18.9% in smears. Based on those results, they considered severe lesions to be an exceptional sequela under normal conditions.

All the *D. nodosus* isolates tested were defined as benign by the GG test, which agrees with other studies where *D. nodosus* isolates from cattle were virulence tested (Stewart, 1979; Richards et al., 1980; Gilhuus et al., 2013). The isolates also showed great serogroup diversity, as all serogroups except F and M were detected. This supports the findings of Gilhuus et al. (2013), who found high serogroup diversity among benign *D. nodosus* isolates from cattle, sheep, and goats in Norway.

Treponema spp.

Even though characteristic DD lesions were observed in only 1.4% of the cows, *Treponema* spp. were detected frequently. *Treponema* spp. were, however, detected in only a few clinically healthy cows, but the prevalence increased to more than 60% in cows with ID and was, as expected, identified in the 8 sampled cows with DD. These results indicate that the risk of introducing new *Treponema* spp. strains into a herd increases even with mild ID.

The identification of only 6 previously described phylotypes and the low number of phylotypes in each herd are different from reports of foreign studies (Nordhoff et al., 2008; Rasmussen et al., 2012). Rasmussen et al. (2012) revealed 12 to 15 phylotypes at the herd level, and a mean number of *Treponema* spp. varying between 7.1 and 10.1 in the biopsies within the herds. Considering the mild clinical course with high prevalence of ID and few cows with DD in these Norwegian herds compared with studies in Denmark and Germany, the prevalence of treponemes in this study was higher than expected (Nordhoff et al., 2008).

Evans et al. (2009) suggested that DD-associated treponemes can be organized in phylogenetic clusters and that the distribution varies in different regions. Later studies have supported this theory (Yano et al., 2010). Phylotypes from cluster 3 have been proposed as appearing mainly in Germany and Denmark (Nordhoff et al., 2008; Rasmussen et al., 2012). In agreement with Rogdo et al. (2012), the present study revealed that the phylotypes from cluster 3 were the most prevalent in Norway, followed by PT6 from cluster 2. The finding of

the previously unknown PT19 also supports the theory that regional differences exist. Based on studies from different countries, Rasmussen et al. (2012) suggested that the total amount of treponemes is important for the outcome of disease and that the presence of specific phylotypes is of less importance.

Histopathological Evaluation

Epidermal damage was moderate in cows with ID (and cows with ID and E), usually without exudation, erosion, or necrosis from the dermal papilla, which is milder than the damage found in DD lesions (Rasmussen et al., 2012). The dermal inflammatory response was mild and none was scored as severe, which is lower than the abovementioned study of DD, where the inflammatory response in the dermis was moderate or severe in 88% of the cows. The dermal histopathological results agree with the less severe clinical lesions in our study. Interdigital dermatitis is considered a more superficial infection than DD, and the limited difference from healthy cows was expected (Cruz et al., 2005). The present findings also agree with the low prevalence of lameness, which probably is associated with erosive dermatitis lesions, and the low biodiversity of treponemes.

Associations Between ID and Dichelobacter nodosus and Treponema spp.

In agreement with Laing and Egerton (1978), our study showed a strong association between ID and *D. nodosus*. Almost all cows with ID, regardless of score, were infected with *D. nodosus*.

We also observed a strong association between ID and *Treponema* spp. when all cows with ID were included in the analysis and, in contrast to *D. nodosus*, *Treponema* spp. were almost exclusively detected in cows with ID or DD. The detection of *Treponema* spp. in the 8 sampled cows with DD is in agreement with previous studies (Nordhoff et al., 2008; Brandt et al., 2011) and the infrequent occurrence in cows with healthy feet (Evans et al., 2009; Brandt et al., 2011; Rasmussen et al., 2012).

Associations Between ID, E, and Claw Cleanliness

The strong association between ID and E agrees with other European studies (Hultgren and Bergsten, 2001; Manske et al., 2002). The association of ID and E with poor hygiene is also well elucidated (Hultgren and Bergsten, 2001; Somers et al., 2005; Relun et al., 2013). These results and the high prevalence of *D. nodosus* in healthy feet indicate that *D. nodosus* alone is less likely

to cause disease but is important in combination with an unhygienic environment.

CONCLUSIONS

Both *D. nodosus* and *Treponema* spp., as well as poor hygiene, are important for the development of interdigital dermatitis. Our analysis showed a strong association between heel horn erosion and cleanliness of the feet. *Treponema* spp. were widespread in these herds despite very few lesions characteristic of digital dermatitis. *Treponema* spp. were frequently detected in cows with interdigital dermatitis and hardly at all in cows with healthy feet. In contrast, *D. nodosus* was detected in almost all cows with interdigital or digital dermatitis, as well as in more than half of the cows with healthy feet. The high prevalence of *D. nodosus* in cattle, combined with the high serogroup diversity of isolates, indicates that *D. nodosus* may have been present in the Norwegian cattle population for a long time.

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