

Infection Reservoirs and Transmission of Digital Dermatitis in the Dairy Herd

Amy Gillespie and Nicholas Evans

Amy Gillespie BVMS MSc DipECBHM FHEA MRCVS
Nicholas Evans BSc PhD FHEA

Infection Biology, Institute of Infection and Global Health, University of Liverpool.

The authors would like to acknowledge support from AHDB Dairy for recent research on hygiene during foot-trimming.

The authors declare that there is no conflict of interest.

Abstract

This article discusses the aetiology of digital dermatitis (DD), summarises key research in the field, and emphasises more recent advances in terms of our understanding of infection reservoirs and transmission of disease. It also identifies areas for future development enabled by technological advances.

Keywords

Digital dermatitis, Infection reservoirs, Transmission, Hygiene, Treponeme

Key Points

- A wide variety of bacteria are identified in DD lesions however only *Treponema* spp. are consistently implicated in DD aetiology.
- Infection models have demonstrated that tissue damage is required for lesions to develop, as well as direct contact with fresh lesional material containing treponemes.
- As well as the lesions themselves, the environment and the gastrointestinal tract have been investigated as potential infection reservoirs.
- Foot-trimming provides an opportunity for fresh lesional material containing viable treponemes to be transferred between cows' feet, frequently offering these pathogens damaged tissue to colonise.
- Good biosecurity is essential to prevent entry of DD into uninfected herds. It is recommended to inspect feet for lesions before bought in animals contact the rest of the herd. Due to possible gastrointestinal carriage of treponemes, however, absence of DD lesions does not guarantee animals from endemic herds are not carrying causative bacteria.
- Improving our understanding of DD, including both pathobiology and host genetic resistance, should open options for novel prevention and treatment strategies. In the meantime, we must continue to apply practical solutions such as enhancing foot-trimming hygiene and reducing the slurry exposure of animals together with appropriate, regular footbathing.

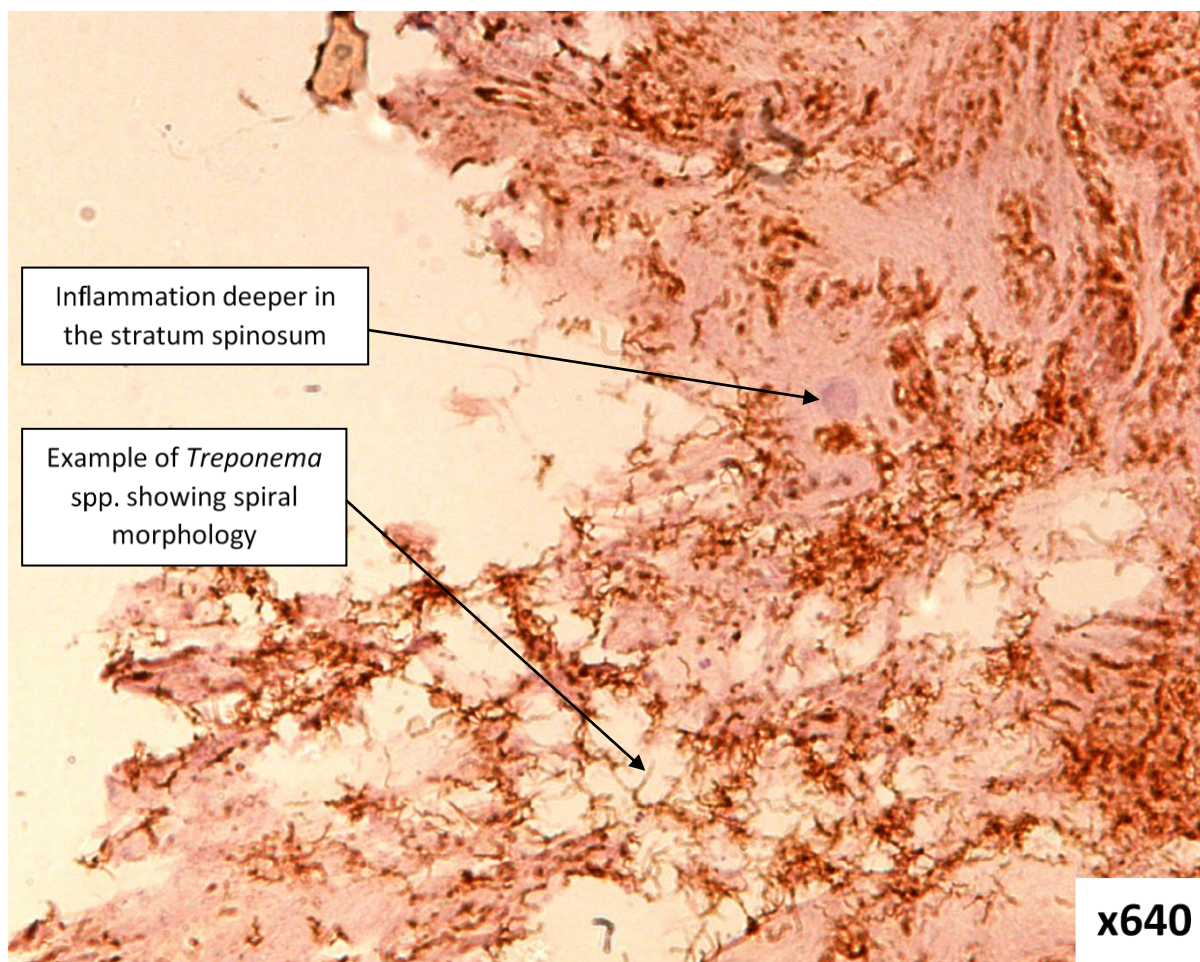
Introduction

Digital Dermatitis (DD) is a multifactorial disease, both in terms of the pathogens that can inhabit lesions, and in terms of the environmental and management conditions which are presumed to cause damage to the stratum corneum and changes to the foot-skin microbiome, thus allowing pathogens to cause lesion development. Gaps in our understanding of the complex pathophysiology of DD alongside practical problems with implementing control measures are hindering our efforts to eliminate disease.

A wide variety of bacteria have been detected from DD lesions, including *Fusobacterium* spp., *Bacteroides* spp., *Guggenheimella bovis*, *Campylobacter* spp. and *Peptococcus* spp; and more recently *Porphyromonas levii*, *Mycoplasma* spp. and *Prevotella* spp.(1)

. However, it is currently understood that there are specific *Treponema* phylogroups that are important in causing DD and they are found in combination (2). More detailed genotypic and phenotypic characterisation carried out on UK isolates demonstrated three distinct taxonomic groups, which were designated as *Treponema medium/ vincentii-like*, *Treponema phagedenis -like*, and *Treponema putidum/ denticola- like*(3). More recently the latter phylogroup has been re-classified as *Treponema pedis*(4); further taxonomic scrutiny of the other two groups is still needed to distinguish them from the human treponemes they were found to be similar to. Only treponemes have been found deep in the epidermis (Figure 1), with tissue invasion considered a major virulence trait (5–7). The importance of treponemes in DD is supported by successful development of infection models (8–10), however skin needs to be macerated to allow infection to become established; and the use of tissue homogenate prepared from fresh lesion material is more effective than inoculation with pure *Treponema* spp. cultures.

Figure 1. Histopathology of the epidermis affected by an active DD lesion, showing erosion of the stratum corneum (completely irregular surface), and deeper infection in the stratum spinosum prompting an inflammatory response (presence of polymorphonuclear leukocytes). Treponemes are abundant and visible in the eroded areas, showing their spiral morphology highlighted in brown due to immunohistochemical detection of *Treponema* spp. antigens.



Infection Reservoirs

The most recently calculated reproduction ratio (R_0) for DD is 2.36, meaning that in the absence of control measures it is expected that each case will give rise to 2.36 further cases (11). When R_0 is reduced below one the number of new cases will reduce, and the lower the value, the more likely it is that eradication can be achieved. To prevent spread of DD, infection reservoirs for the pathogenic *Treponema* phylogroups need to be identified and controlled.

The Lesions

It is widely considered that the most important source of infection are the lesions themselves, particularly M4 lesions (Figure 2). The lesion scoring system proposed by Dopfer et al in 1997 (7) and adapted by Berry et al in 2012 (12) describes the development of disease from small focal active lesions (M1), through the larger ulcerative active stage (M2), then on to a healing stage (M3), and the development of a chronic lesion with a hyperkeratotic scab which is still considered infectious (M4) (Figure 2). Treponemes have an encysted form that is suspected of lying dormant in lesions deep in bovine skin, contributing to the chronic form of the disease (13). For reasons not fully understood, M4 lesions can “reactivate” giving rise to chronic lesions with new focal active lesions superimposed (M4.1). Although there is little difference in transmission rate depending on lesion class, as 70% of the infectious time is spent at M4, this class contributes 88.5% to R_0 (11). Prevention of progression of active lesions to the M4 form is crucial to reducing cases.

Figure 2. M4 lesion before and after removal of the overlying hyperkeratotic scab, showing ulcerated tissue where invasion of pathogens has caused severe damage to the epidermis.



Broadly, our options are individual or collective treatments (footbathing). Early individual treatment is important for promoting healing from M2, whilst effective footbathing slows the transition from M4 back to M2 (14). Current industry advice for individual treatment is to use licensed topical oxytetracycline or thiamphenicol spray (15), however it is possible that non-antibiotic alternatives have similar efficacy (16). For footbathing, there is little evidence to prompt recommendation of one product in favour of another. The most recent review of published footbathing trials found only 5% copper sulphate used at least four times per week could be superior both to no footbath and to water only (17). It is certainly no longer acceptable to use antibiotics in this fashion (18) due to mass exposure of animals to products which may lead to an increase in antimicrobial resistance, and also soil exposure to the same selection pressure if slurry (which typically includes footbath waste) is spread on agricultural land.

Industry recommendations advocate the use of footbaths 3-3.7 metres long and 0.5-0.6 metres wide to ensure all four feet are submerged twice, and at least 12cm deep to ensure the whole hoof is covered by solution to the coronary band, even at the cranial aspect of the hoof (19). Footbath volume and disinfectant used should be measured each time to ensure correct concentrations and contact times are achieved. Footbath contamination levels resulting in product inactivation is largely unknown, although EU Regulation 528/2012 specifies biocides have to be effective against *Enterococcus hirae*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* at contamination levels of 20g/L organic matter (20). Laboratory testing of a range of disinfectants showed that minimum bactericidal concentrations for a *Treponema phagedenis*-like isolate in the presence of 20g/L organic matter were below recommended working concentrations (21). Footbaths frequently become contaminated above this level, and footbaths often deplete below the recommended depth, leading to poor foot coverage (20). The use of automatic footbaths may solve these practical problems with minimal time investment, however their benefits for reducing DD incidence have not been proven in peer-reviewed literature.

The Environment

Environment muddiness has been identified as a risk for DD (22). Furthermore good animal hygiene reduces the risk of DD (23) whilst increases in DD cases during housing under conditions of poor hygiene suggest faeces as a source of treponemes (24). Interestingly, detecting treponemes from environmental slurry samples and individual fresh faecal samples using PCR assays was unsuccessful (25). Metagenomic studies of slurry, however, did identify small numbers of *Treponema* spp. in DD-infected herds and their absence in healthy herds, suggesting that slurry may be a vehicle for spread, but not the primary infection reservoir (26).

The Gastrointestinal Tract

Sampling of 44 sites from six cows from a DD endemic herd (3 healthy animals and 3 affected by DD) to investigate the bovine gastrointestinal tract (GIT) as an infection reservoir using PCR found *T. phagedenis*-like DD spirochaetes in one DD-affected cow in oral gingival tissue, the rumen dorsal sac and the reticular pillar. The recto-anal junction from a healthy cow was also positive for *T. medium*-like DD spirochaetes. Further testing of oral gingival samples found 1/8 positive for *T. pedis*, and additional testing of recto-anal junction samples found DD spirochaetes in 4/21 samples (25). In a more recent metagenomic study *Treponema* spp. found in DD lesions have been found in rumen and faecal microbiomes of cattle from DD infected farms (27). The importance of GIT carriage of treponemes in terms of transmission dynamics between and within farms is unknown, and more work is required to clarify the involvement of this infection reservoir in disease transmission.

The Role of Foot Trimming in Transmission

An epidemiological study published in 1999 associated the use of a primary hoof trimmer who trims cows' hooves at other farms, and lack of washing of hoof trimming equipment between cows being trimmed, with increased incidence (>5%) of DD in herds (28). A 2018 study of pasture-based herds in New Zealand supported these findings, and the authors concluded that farms with DD should ensure

that hoof trimming equipment is disinfected effectively between cattle (29). DD treponeme DNA was detected on 17/17 hoof knives following foot trimming of clinical DD cases, and 7/8 gloves worn by a foot trimmer to trim feet of DD positive cows (30). In addition an isolate belonging to the *T. phagedenis*-like spirochaetes was cultivated from a knife after trimming a DD positive cow (31) and they can be isolated in culture for up to three days from gloves contaminated during handling of sheep feet affected by the analogous disease contagious ovine DD (32). Overall this data suggests hoof knives and gloves to be major control points for preventing transmission. It has also been shown that DD treponemes are culturable for at least two hours after application to hoof knife blades implicating hoof knives as fomites for carrying treponemes between cows during foot trimming (33). An evidence-based disinfection protocol has been developed with AHDB Dairy to mitigate the risk of DD transmission during foot trimming (33). The protocol is available from the AHDB Knowledge Library at <https://ahdb.org.uk/reducing-spread-of-DD>.

Several potential infection reservoirs for DD have been identified, but the relative importance of each remains unknown. DD infection models have shown that for lesions to develop, existing tissue damage is required plus direct contact with a fresh lesional material containing viable polytreponemal bacterial load (8–10). The existing paradigm is that infection is spread from M2 and M4 lesions via the environment, however treponemes have not been isolated in culture from environmental sources or detected by PCR (25). They have been detected in slurry, the rumen and faeces by metagenomic analyses (26,27,34,35), however sequencing methods are capable of detecting very small numbers of organisms, are less specific, and give no indication of viability and true ability to transmit and cause disease. Hence questions remain about the importance of slurry and the environment in transmission. The epidemiological association of poor environmental hygiene with increased DD prevalence could be due mainly to the detrimental effect on the condition of the foot skin. Table 1 rationalises the possibilities and makes the argument that foot-trimming is the only event during which it is known that viable treponemes contact the feet of other cows in the herd (assuming that effective disinfection is not practised). It is also more likely that there will be existing tissue damage, at least for lame cows

being treated, that could make them more susceptible to acquiring the pathogens needed to cause DD lesions during foot-trimming, fulfilling the infection model criteria.

Table 1. Identified sources of infection on farm, fulfilment of infection model criteria and associated risk factors and microbiological evidence.

<i>Source of Infection</i>	<i>PERCIEVED LIKELIHOOD OF DIRECT CONTACT</i>	<i>VIABLE BACTERIAL LOAD</i>	<i>Associated risk factor evidence</i>	<i>Tissue damage/ Infection reservoir evidence</i>
Foot-trimming tools	HIGH	YES	Not washing hoof trimming tools in between animals=increased DD risk (28). Use of hoof trimmer who works on multiple farms=increased risk of DD (28,29).	Tools will make direct contact with damaged feet. DD is highly contagious The DD treponemes are identified frequently on hoof trimming equipment and can be cultured and can survive for up to 2 hours (31,33).
Foot trimming hands/gloves	HIGH	YES	Use of hoof trimmer who works on multiple farms=increased risk of DD (28,29).	Hands/gloves will make direct contact with damaged feet. DD is highly contagious. The DD treponemes are identified frequently on hands/gloves and can be cultured (30,32).
Farm slurry	HIGH	?	Increased environment slurry= increased DD risk (22). Increased hygiene = reduced DD risk (23).	Exposure of skin to moisture (such as moist slurry environment) needed for DD transmission (8–10). DD treponemes in oral and rectal cavities suggests faecal shedding (25), although cultivable treponemes not isolated from slurry.
Oral or rectal cavity	LOW	YES	Increased hygiene = reduced DD risk.	DD treponemes in oral and rectal cavities (25) and can be cultured from ruminant rectal tissue (36).
Lesions	LOW	YES	Mathematical model shows M4 to be the most significant lesion stage for transmission (11).	DD treponemes culturable from lesions (3).

Biosecurity

With infection reservoirs in mind, biosecurity measures must be considered both between herds, and within herds. Bringing infected animals to a farm is an important route of introduction (22,28) and must be avoided. Where buying in cattle from such a source is unavoidable, it is recommended to inspect all four feet carefully before animals contact the rest of the herd. Even so, because histological changes can occur in the foot-skin before morphological changes become apparent (37), the risk from this route cannot be ruled out. Furthermore, now that the gastrointestinal tract has also been identified as a possible infection reservoir, it is possible that any cow brought from a DD-infected herd could carry the disease (25).

Within herds, cases can be decreased by reducing infection pressure. This can be achieved by early treatment of active M2 lesions and preventing transition of M4 lesions to M2 using effective footbathing (14). Practising good environmental hygiene will decrease any potential for slurry and walking surfaces to act as infection reservoirs and cause damage to foot skin. Disinfection of foot-trimming equipment, particularly hands and hoof knives, would also serve to reduce infection pressure. Finally, treatment and prevention of DD in youngstock must not be overlooked as heifers that are affected prior to parturition are a source of infection to the main herd (38).

The Future

Recent advances in metagenomic sequencing have made it possible to study the entire bacterial population of DD lesions, which has given insights into how these change in relation to lesion morphology (27,34,35). Studying molecular interactions between bacteria residing in lesions may reveal important pathological processes that cause transitions between lesion stages. Such systems approaches to understanding DD pathobiology holds potential for development of novel treatments which could be key to eradication (39).

It has been confirmed that there is a genetic component to DD susceptibility (40,41). Quantitative Trait Loci associated with susceptibility to claw- horn lesions have been identified (42), and similar associations likely exist for susceptibility to DD. Recent work has shown that reducing DD prevalence by selective breeding is a promising strategy due to substantial heritable variation in susceptibility (43,44).

Conclusion

Recent research has improved our understanding of infection reservoirs for DD in the dairy herd and highlighted some of the challenges with eliminating disease. As new avenues towards achieving eradication are explored, we need to continue to work with farmers to apply existing recommendations on farm. In most herds substantial improvements could be made by addressing practical problems such as increasing environmental hygiene, ensuring correct footbath use, and improving hygiene during foot-trimming.

References

1. Berry SL, Read DH, Walker RL, Famula TR. Clinical, histologic, and bacteriologic findings in dairy cows with digital dermatitis (footwarts) one month after topical treatment with lincomycin hydrochloride or oxytetracycline hydrochloride. *J Am Vet Med Assoc* [Internet]. 2010 Sep 1 [cited 2019 Aug 1];237(5):555–60. Available from: <http://avmajournals.avma.org/doi/abs/10.2460/javma.237.5.555>
2. Evans NJ, Murray RD, Carter SD. Bovine digital dermatitis: Current concepts from laboratory to farm. *Vet J*. 2016;211:3–13.
3. Evans NJ, Brown JM, Demirkan I, Murray RD, Vink WD, Blowey RW, et al. Three unique groups of spirochetes isolated from digital dermatitis lesions in UK cattle. *Vet Microbiol*. 2008;130(1–2):141–50.
4. Evans NJ, Brown JM, Demirkan I, Murray RD, Birtles RJ, Hart CA, et al. *Treponema pedis* sp. nov., a spirochaete isolated from bovine digital dermatitis lesions. *Int J Syst Evol Microbiol*. 2009;59(Pt 5):987–91.
5. Blowey RW, Carter SD, White AG, Barnes A. *Borrelia burgdorferi* infections in UK cattle: a possible association with digital dermatitis. *Vet Rec*. 1994 Dec;135(24):577 LP – 578.
6. Moter A, Leist G, Rudolph R, Schrank K, Choi BK, Wagner M, et al. Fluorescence in situ hybridization shows spatial distribution of as yet uncultured treponemes in biopsies from digital dermatitis lesions. *Microbiology*. 1998;144(9):2459–67.

7. Döpfer D, Koopmans A, Meijer FA, Szakall I, Schukken YH, Klee W, et al. Histological and bacteriological evaluation of digital dermatitis in cattle, with special reference to spirochaetes and *Campylobacter faecalis*. *Vet Rec*. 1997;140(24):620–3.
8. Gomez A, Cook NB, Bernardoni ND, Rieman J, Dusick AF, Hartshorn R, et al. An experimental infection model to induce digital dermatitis infection in cattle. *J Dairy Sci*. 2012;95(4):1821–30.
9. Krull AC, Cooper VL, Coatney JW, Shearer JK, Gorden PJ, Plummer PJ. A Highly Effective Protocol for the Rapid and Consistent Induction of Digital Dermatitis in Holstein Calves. *PLoS One* [Internet]. 2016 [cited 2019 Aug 1];11(4):e0154481. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/27119564>
10. Read D, Walker R. Experimental transmission of papillomatous digital dermatitis (footwarts) in cattle. *Vet Pathol*. 1996;33:607.
11. Biemans F, Bijma P, Boots NM, de Jong MCM. Digital Dermatitis in dairy cattle: The contribution of different disease classes to transmission. *Epidemics*. 2018;23.
12. Berry SL, Read DH, Famula TR, Mongini A, Döpfer D. Long-term observations on the dynamics of bovine digital dermatitis lesions on a California dairy after topical treatment with lincomycin HCl. *Vet J*. 2012;193(3):654–8.
13. Döpfer D, Anklam K, Mikheil D, Ladell P. Growth curves and morphology of three *Treponema* subtypes isolated from digital dermatitis in cattle. *Vet J*. 2012 Sep;193(3):685–93.
14. Döpfer D, Holzhauser M, Boven M van. The dynamics of digital dermatitis in populations of dairy cattle: Model-based estimates of transition rates and implications for control. *Vet J*. 2012 Sep;193(3):648–53.
15. Holzhauser M, Ploegmakers-van Deventer R, Smits D, Swart W. Comparing the curative efficacy of topical treatment with thiamphenicol and oxytetracycline on digital dermatitis lesions in dairy cattle. *Vet Rec* [Internet]. 2017 May 20 [cited 2020 Jan 30];180(20):500. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/28465327>
16. Manning A. Treatment of Digital Dermatitis in Dairy Cattle [Internet]. 2016 [cited 2020 Jan 30]. Available from: <https://www.cattle-lameness.org.uk/critically-appraised-topics/>
17. Jacobs C, Beninger C, Hazlewood GS, Orsel K, Barkema HW. Effect of footbath protocols for prevention and treatment of digital dermatitis in dairy cattle: A systematic review and network meta-analysis. *Prev Vet Med*. 2019 Mar 1;164:56–71.
18. Bell NJ, Atkinson O, Blowey R, Cobner A, Green M, Huxley JN, et al. Moving on from antibiotic foot baths for the control of digital dermatitis. Vol. 181, *Veterinary Record*. British Veterinary Association; 2017. p. 51.
19. Cook NB, Rieman J, Gomez A, Burgi K. Observations on the design and use of footbaths. *Vet J* [Internet]. 2012 [cited 2020 Jan 30];193(3):669–73. Available from: <http://dx.doi.org/10.1016/j.tvjl.2012.06.051>
20. Ariza JM, Bareille N, Oberle K, Guatteo R. Current recommendations for footbath solutions renewal rates in dairy cattle: the need for adaptation? *Animal* [Internet]. 2019 Jun [cited 2020 Jan 30];13(6):1319–25. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/30392490>
21. Hartshorn RE, Thomas EC, Anklam K, Lopez-Benavides MG, Buchalova M, Hemling TC, et al. Short communication: Minimum bactericidal concentration of disinfectants evaluated for bovine digital dermatitis-associated *Treponema phagedenis*-like spirochetes. *J Dairy Sci*.

- 2013;96(5):3034–8.
22. Rodriguez-Lainz A, Hird DW, Carpenter TE, Read DH. Case-control study of papillomatous digital dermatitis in southern California dairy farms. *Prev Vet Med.* 1996;28(96):117–31.
 23. Hultgren J, Bergsten C. Effects of a rubber-slatted flooring system on cleanliness and foot health in tied dairy cows. *Prev Vet Med.* 2001 Nov 2;52(1):75–89.
 24. Blowey RW, Sharp MW. Digital dermatitis in dairy cattle. *Vet Rec.* 1988;122(21):505–8.
 25. Evans NJ, Timofte D, Isherwood DR, Brown JM, Williams JM, Sherlock K, et al. Host and environmental reservoirs of infection for bovine digital dermatitis treponemes. *Vet Microbiol.* 2012;156(1–2):102–9.
 26. Klitgaard K, Strube ML, Isbrand A, Jensen TK, Nielsen MW. Microbiota Analysis of an Environmental Slurry and Its Potential Role as a Reservoir of Bovine Digital Dermatitis Pathogens. *Appl Environ Microbiol* [Internet]. 2017 [cited 2020 Jan 30];83(11). Available from: <http://www.ncbi.nlm.nih.gov/pubmed/28363959>
 27. Zinicola M, Lima F, Lima S, Machado V, Gomez M, Döpfer D, et al. Altered microbiomes in bovine digital dermatitis lesions, and the gut as a pathogen reservoir. *PLoS One.* 2015;10(3):e0120504.
 28. Wells SJ, Garber LP, Wagner BA. Papillomatous digital dermatitis and associated risk factors in US dairy herds. *Prev Vet Med.* 1999;38:11–24.
 29. Yang DA, Laven RA, Heuer C, Vink WD, Chesterton RN. Farm level risk factors for bovine digital dermatitis in Taranaki, New Zealand: An analysis using a Bayesian hurdle model. *Vet J.* 2018;234:91–5.
 30. Blowey R, Sullivan L, Evans N, Carter SD. Detection of Bovine Digital Dermatitis on Hoof Knives and Hands and their Removal by Disinfection. In: Whay, B., Hockenhull J (University of B, editor. 27th International Symposium and 9th International Conference on Lameness in Ruminants. 2013. p. 181.
 31. Sullivan LE, Blowey RW, Carter SD, Duncan JS, Grove-White DH, Page P, et al. Presence of digital dermatitis treponemes on cattle and sheep hoof trimming equipment. *Vet Rec.* 2014;175(8):201.
 32. Angell JW, Clegg SR, Grove-White DH, Blowey RW, Carter SD, Duncan JS, et al. Survival of contagious ovine digital dermatitis (CODD)-associated treponemes on disposable gloves after handling CODD-affected feet. *Vet Rec* [Internet]. 2017 Jul 22 [cited 2019 Aug 6];181(4):89. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/28642342>
 33. Gillespie A, Carter SD, Blowey RW, Evans N. Survival of bovine digital dermatitis treponemes on hoof knife blades and the effects of various disinfectants. *Vet Rec.* 2019;186:67.
 34. Krull AC, Shearer JK, Gorden PJ, Cooper VL, Phillips GJ, Plummer PJ. Deep Sequencing Analysis Reveals the Temporal Microbiota Changes Associated with the Development of Bovine Digital Dermatitis. *Infect Immun.* 2014;82(8):3359–73.
 35. Zinicola M, Higgins H, Lima S, Machado V, Guard C, Bicalho R. Shotgun Metagenomic Sequencing Reveals Functional Genes and Microbiome Associated with Bovine Digital Dermatitis. *PLoS One.* 2015;10(7):e0133674.
 36. Sullivan LE, Carter SD, Duncan JS, Grove-White DH, Angell JW, Evans NJ. The gastrointestinal tract as a potential infection reservoir of digital dermatitis-associated treponemes in beef cattle and sheep. *Appl Environ Microbiol.* 2015;81(21):7460–9.

37. Rasmussen M, Capion N, Klitgaard K, Rogdo T, Fjeldaas T, Boye M, et al. Bovine digital dermatitis: Possible pathogenic consortium consisting of *Dichelobacter nodosus* and multiple *Treponema* species. *Vet Microbiol.* 2012;160(1–2):151–61.
38. Capion N, Thamsborg SM, Enevoldsen C. Prevalence and severity of foot lesions in Danish Holstein heifers through first lactation. *Vet J.* 2009;182(1):50–8.
39. Orsel K, Plummer P, Shearer J, De Buck J, Carter SD, Guatteo R, et al. Missing pieces of the puzzle to effectively control digital dermatitis. *Transbound Emerg Dis.* 2018;65(May 2017):186–98.
40. Scholey RA, Blowey RW, Murray RD, Smith RF, Cameron J, Massey JP, et al. Investigating host genetic factors in bovine digital dermatitis. *Vet Rec.* 2012 Dec;171(24):624–624.
41. Schöpke K, Gomez A, Dunbar KA, Swalve HH, Döpfer D. Investigating the genetic background of bovine digital dermatitis using improved definitions of clinical status. *J Dairy Sci.* 2015;98(11):8164–74.
42. Sánchez-Molano E, Bay V, Smith RF, Oikonomou G, Banos G. Quantitative Trait Loci Mapping for Lameness Associated Phenotypes in Holstein-Friesian Dairy Cattle. *Front Genet [Internet].* 2019 [cited 2019 Dec 16];10:926. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/31636655>
43. Biemans F, de Jong MCM, Bijma P. Genetic parameters and genomic breeding values for digital dermatitis in Holstein Friesian dairy cattle: host susceptibility, infectivity and the basic reproduction ratio. *Genet Sel Evol [Internet].* 2019 Dec 20 [cited 2020 Jan 21];51(1):67. Available from: <https://gsejournal.biomedcentral.com/articles/10.1186/s12711-019-0505-3>
44. Biemans F, de Jong MCM, Bijma P. A genome-wide association study for susceptibility and infectivity of Holstein Friesian dairy cattle to digital dermatitis. *J Dairy Sci.* 2019 Jul 1;102(7):6248–62.