Missing pieces of the puzzle to effectively control Digital Dermatitis

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Summary

Since the first report of bovine digital dermatitis (DD) in 1974, there is a large body of literature published; however, effective prevention and control of the disease remain elusive. Although many aspects of the pathogenesis of DD have been investigated, even some of the most basic questions such as the etiology of this disease remain under debate. *Treponema* spp. have been strongly associated with DD lesions and occur in abundance in advanced lesions; however, efforts to induce disease with pure cultures of these organisms have been largely underwhelming and inconsistent. Furthermore, although the disease has been present for several decades, there is limited scientific evidence regarding effective treatment of DD. Apparent discrepancies between effectiveness *in vitro* and *in vivo* has challenged the scientific community to identify new potential treatment options. With no treatment resulting in a 100% cure rate, the current expectation is manageable control, but prospects for the eradication of the disease are unlikely using current approaches. In order to develop more effective approaches to control DD on-farm, there is a critical need for a deeper understanding regarding the causation, ecology, transmission and treatment of this disease. In this article, we attempt to provide insights into specific research needs related to DD in order to assist the industry, researchers, pharmaceutical companies and research sponsors with decision-making and identified research gaps.

Introduction to the disease

Digital dermatitis (DD), a skin disorder of the feet that mainly affects cattle, was first described in 1974 in Italy (Cheli and Mortellaro, 1974). It is characterized by an inflammatory dermatitis of the skin most commonly located at the plantar aspect of the interdigital cleft, although alternative locations have been reported (Holzhauer et al., 2008). A typical lesion is a circumscribed, moist ulcerative erosive area that is painful to the touch. The raw-red granular appearance of the lesion resulted in one of its alternative names (i.e. Strawberry foot rot), although the disease is also known as footwart, hairy heel warts, raspberry heel, verrucose dermatitis, Mortellaro's disease, and papillomatous DD. Notwithstanding, DD is likely the most accurate and commonly used term.

The most important clinical presentation of DD is lameness (Blowey and Sharp, 1988; Bassett et al., 1990; Read and Walker, 1998), although a significant number of affected cattle lack obvious clinical signs. Lesions are painful upon palpation and prone to bleeding after their surfaces are touched. Clinically, DD presents itself as a dynamic process with morphologically distinct stages. A variety of classification systems used to describe the stages of DD development have been described (Vink, 2006; Laven, 1999; Manske et al., 2002; Krull et al., 2014a), with the most widely adopted being the M-stage scoring system developed by Döpfer et al. (1997) and amended by Berry et al. (2012). This is score identifies 5 categories where M0 is defined as normal digital skin with no evidence of dermatitis; M1 if a small (< 2 cm in diameter) circumscribed red to grey epithelial defect is present; M2 if an ulcerative active ≥ 2 cm in diameter with a red-grey surface; M3 (healing stage) after M2 lesion surface becomes firm and scar-like; M4 (chronic stage) if the lesion surface is raised with brown or black tissue, hyperkeratotic, scaly or proliferative; and M4.1 defined as small red circumscribed lesions

occurring within the boundaries of an existing M4 lesion (Berry et al., 2012; Döpfer et al., 1997). Consistency in scoring methodology would be much needed for scientific comparison of study results. A number of recent review articles have summarized the current understanding of the bacterial agents, epidemiology, therapy and treatment of digital dermatitis in detail in the last 2 years (Evans et al., 2016; Palmer and O'Connell, 2015; Plummer and Krull, 2017; Wilson-Welder et al., 2015a). The goal of this manuscript as part of the DISCONTOOLS collection, is to identify and discuss significant knowledge gaps that should be addressed by the research community in order to propel the field and to drive the development of novel and effective intervention strategies for controlling this disease.

Significance

DD is a significant concern for cattle producers and veterinarians for several reasons. The clinical manifestation of lameness associated with DD poses a significant welfare concern for cattle and represents a leading cause of culling in the dairy cattle industry throughout the world (Cramer et al., 2009; Booth et al., 2004; Charfeddine and Perez-Cabal, 2017). However, the impact of DD is not restricted to clinical disease, but includes financial losses associated with the cost of treatment, decreases in both milk production and fertility, and losses due to increased culling even in the absence of clinical symptoms (Argaez-Rodriguez et al., 1997; Gomez et al., 2015b; Bruijnis et al., 2010; Cha et al., 2010; Relun et al., 2013).

Geographical distribution

Digital dermatitis has been described as an endemic disease of dairy cattle in most parts of the world (van Amstel et al., 1995; Holzhauer et al., 2006; Rodriguez-Lainz et al., 1998; Wells

et al., 1999; Solano et al., 2016). In France, the PARABOV project aiming at describing the different lesions in cattle herds, reported that 16% of the feet and 70% of the herds were affected by DD lesions (Bleriot et al., 2013).

Given the differences in herd size, housing and management across these different geographic areas, it is safe to say that the disease is able to adapt and persist in a wide range of ecologic and management settings. In New Zealand, where the dairy industry has been historically pasture based, DD was reported only as sporadic cases until recent years when it has been implicated as a growing concern for non-healing lesions of the sole (Vermunt and Hill, 2004; Van Andel M, 2012). The situation in New Zealand, as well as some other similar observations in other countries has led to the hypothesis that DD becomes an increasingly important issue when dairy cattle management changes from a more extensive pasture based system to confinement freestall housing (Sogstad et al., 2005). In countries like the UK, where cattle have housed and pasture seasons, the disease is almost restricted to the housing season (Evans et al., 2016). There is a need to further test this hypothesis in well-designed studies along with an effort to better understand the potential drivers of this disease progression. Herd stocking density, moisture content and hydration of the foot and skin, increased herd introductions and increased time on concrete have all been discussed and considered but there is at present little definitive evidence to support any sort of relative prioritization of these based on evidence based outcomes. It is important to acknowledge and recognize that emergence of the disease in countries and production systems, like the North American pasture-based ranching system, that have previously had little to no DD provide a rich research site for these critical studies to occur. We have to, however, realize that underreporting and the disease going unnoticed might be the

real reason for apparent freedom of disease. Once the disease becomes endemic, these studies become much more difficult, if not impossible, to test in anything other than a simulated system.

Pathogens involved

Despite a significant number of studies focused on elucidating the etiology of DD, debate remains regarding the exact etiology. Although fungal and viral etiologies have been considered, the scientific community has largely agreed that these organisms are less likely to drive the disease process, and the field has focused its attention on bacterial organisms (Rebhun et al., 1980; Krull et al., 2014b; Zinicola et al., 2015; Brandt et al., 2011). For a detailed overview of the findings of this body of knowledge, readers are directed to the review articles referenced at the start of this manuscript; however, two consistent themes have emerged from these studies. First, DD lesions are consistently associated with an abundant and diverse population of multiple species of Treponemes (Zinicola et al., 2015; Krull et al., 2014b; Evans et al., 2016). Second, these diverse treponeme populations exist as a portion of a much more diverse and complex bacterial community that comprises the total microbiota of the DD lesions. Furthermore, the non-treponemal constituents of the microbiota are not random and instead show association with the stage of lesion development (Krull et al., 2014b, Zinicola et al., 2015). As described in more detail by Krull et al. (2014b), non-affected animals showed an abundance of *Staphylococcaceae, Streptococcaceae, Bacterioidaceae, Corynebacteriaceae* and *Pasteurellaceae,* replaced by other bacterial families as lesions progressed. Whereas *Spirochaetaceae* increased systematically from 0 to over 90% in chronic stages of the disease (Krull et al., 2014c). With lesions classified as active and inactive, Zinicola et al. (2015) identified *Firmicutes* and *Actinobacteria* as the predominant bacterial phyla of control animals,

and *Spirochetes, Bacteroidetes* and *Proteobacteria* as highly abundant in DD-affected animals.

These themes are consistent with the vast majority of the published literature on the topic and can be agreed upon by most researchers in the field. Herein, however, lies a remaining uncertainty regarding the etiologic role that each of these organisms plays in the molecular mechanisms responsible for the development of DD. We will address the research needs related to etiology in three broad areas related to 1) the role of the treponemes, 2) the role of other bacterial members in the community, and 3) the role of the interaction between the community members.

First, while it is clear that *Treponema* spp. are consistently present in DD lesions and make up the majority of the bacterial community in advanced lesions, it is also clear that these populations represent a diversity of species instead of a single species (Klitgaard et al., 2013; Marcatili et al., 2016; Krull et al., 2014c; Yano et al., 2009; Evans et al., 2008). This in itself poses a problem with fulfilling Koch's postulates for this disease process. At a very minimum, one must acknowledge that if treponemes are the primary etiologic agents associated with DD, it is a polytreponemal process, and this hypothesis has been argued for in the literature (Evans et al., 2008). If this hypothesis is true, it still leaves the significant question of why does the disease require the presence of multiple treponemal species instead of one? Furthermore, how do these different treponemal species interact with each other, and what is the minimum treponema consortium required for inducing clinical disease? How does the polytreponemal community change during progression of the disease? An alternate hypothesis that emerges is that the diversity of Treponema species present in the lesions is more suggestive of an overgrowth of opportunists that find a unique niche for expansion during the induction of DD lesions (Edwards et al., 2003; Krull et al., 2014b; Wilson-Welder et al., 2015a). Indeed, there is now much

146 evidence that the DD-associated treponemes are promiscuous opportunistic invaders of
147 established skin lesions, particularly on feet (Evans et al., 2011), other limb skin tissues (Clegg et
148 al., 2016a) and have been identified in a particularly virulent udder disease, ischaemic teat
149 necrosis (Clegg et al., 2016b). This opportunistic nature of treponeme tissue invasion may also
150 account for their strong associations with DD lesions in UK sheep (Dhawi et al., 2005) and goats
151 (Sullivan et al., 2015b), skin lesions in UK pigs (Clegg et al., 2016d), and foot lesions in US wild
152 elk (Clegg et al., 2015). While the morphologic appearance of DD lesions is essentially identical
153 in beef cattle compared to dairy cattle, we have very limited information regarding the bacterial
154 communities present in beef cattle DD and how it compares to that of dairy lesions. When beef
155 cattle DD lesions were analyzed by PCR for the DD-associated *Treponema* spp., and also for
156 *Dichelobacter nodosus* and *Fusobacterium necrophorum*, Sullivan et al. reported that at least 1
157 of the known *Treponema* phylogroups associated with DD was present in all beef cattle DD
158 lesions (Sullivan et al., 2015a). This sudden emergence of new clinical phenotypes associated
159 with these specific bacteria is suggestive of genomic changes affecting treponeme physiology
160 and ability to transmit between tissues, animals and even species. As such, there is a need for
161 vigilance in case of further spread leading to new clinical phenotypes. Whether these are primary
162 or secondary infections, the treponemes represent an important bacterial community for which
163 there is need to better understand their physiology and ecology in lesions. In the current era of
164 bacterial genomics there is a significant need for the identification of "type strains" for each of
165 the species and for full genome sequencing of isolates from each of these strains. These
166 resources would allow for the continued development and refinement of research methodologies
167 focused on better evaluating the role that these organisms play in each stage of lesion
168 development and any significant interactions with other bacterial species. Genome sequences

also allow for more informed generation of hypotheses related to the virulence and ecologic adaptation abilities that each strain possesses and how these functions interact in a central disease process. Currently, large scale genomic analyses are hampered by culture techniques struggling to isolate pure single species cultures with consistency and representing all *Treponema* species that have been demonstrated in DD lesions by metagenomic studies (Krull et al., 2014c; Zinicola et al., 2015).

Second, as alluded to above, constituents of the non-treponemal bacterial communities that are present in the DD lesions vary by lesion stage, but are amazingly consistent within a given stage of lesion development (Krull et al., 2014c; Zinicola et al., 2015). This finding suggests that their presence is not merely coincidental or due to background from the dairy environment, but instead suggests that there is a driving force behind the development and transition of this complex microbiota shift. There is a clear need to better understand what is driving this transition and how this transition is involved in the development, maintenance and response to therapy of digital dermatitis. Given that several of these organisms are known pathogens in other disease processes of the foot of ruminants (for example, *Dichelobacter nodosus*, *Fusobacterium necrophorum* and others) it is important that hypotheses are developed and tested regarding their specific role in DD. Interestingly, many of these "known" pathogens are present in low relative abundance and this fact has been used to argue that they may not be relevant to the disease process (Moe et al., 2010; Collighan and Woodward, 1997). However, recent evidence from other disease processes has demonstrated that relative abundance in phylogenomic studies needs to be interpreted with caution. This is particularly important because abundance is not necessarily commensurate with pathogenicity. Neither does it controvert or confirm etiology. For example, recent metagenomic data derived from ovine footrot, a disease

192 process with a well-known and Koch's postulates confirmed etiology of *Dichelobacter nodosus*,
193 demonstrated that the relative abundance of that organism was between 0.5-1.9% in active
194 lesions (Maboni et al., 2017). In contrast and as a reference point, the relative abundance of
195 *Treponema* spp. in those same samples of ovine footrot averaged 14%. In order to address these
196 issues and research needs, there is a need for additional genomic information and the
197 identification of type strains for these non-treponemal species associated with DD lesions. In
198 addition, the sensitivity to detect low abundant species involved in the pathogenicity of DD
199 lesions needs to be increased.
200 Not surprisingly, the third area of research needs related to the etiology of DD, focuses
201 on the interface of the two issues discussed above. The literature suggests that in other
202 treponeme-associated diseases, such as periodontal disease in humans, the association of
203 treponemes and other organisms extends beyond simply co-isolation and is associated with direct
204 molecular interaction or nutritional symbiosis of the organisms (Grenier, 1992b; Grenier, 1992a;
205 Hashimoto et al., 2003; Ito et al., 2010; Nilius et al., 1993; Simonson et al., 1992; Yao et al.,
206 1996). Despite the fact that these organisms are very closely genetically related to the species
207 found in DD, these types of interactions have not yet been addressed in DD research. Likewise,
208 we must also consider the possibility that regardless of potential interaction between the bacterial
209 species themselves, the presence of these multiple species could impact the immune response of
210 the host, particularly by polyclonal activation of the lymphoid system and induction of
211 immunological dysregulation (Montes et al., 2007). Alternatively, expression of virulence factors
212 such as proteases or leukotoxins by some organisms may alter the ecological adaptation and
213 virulence potential of other organisms in the same niche (Smalley and Olczak, 2017; Lohinai et
214 al., 2015; Castro et al., 2017). Although these interactions have the potential to be extremely

complex and time consuming to study, it is likely that this broader systems approach to the complex pathobiology of DD holds potential for more fully understanding the mechanisms and roles that each of these organisms may play in the disease process. Without a clear understanding of DD etiology, development of effective vaccines for disease control as well as targeted treatments could be hampered.

The hosts

In contrast to an almost 40-year history of recognition of the importance of DD in dairy cattle, DD in beef cattle has been emerging as an increasingly recognized disease in recent years. After an initial case report from the UK (Sullivan et al., 2013), there have been several reports of DD in the North American feedlot industry (Campbell, 2014; Orsel and Schwartzkopf-Genswein, 2015). Deeper exploration of the literature suggests that DD-like lesions have been recognized in the US in beef cattle even prior to their description in dairy cattle, which may point to the potential for the disease being unrecognized (Lindley, 1974; Barthold et al., 1974). A number of questions still remain and deserve attention with regards to the growing importance of DD in beef cattle worldwide. Additional questions remain regarding what epidemiologic, environmental and management factors and changes are driving the recent emergence of DD as a recognized disease of feedlot cattle. Further efforts to understand how the disease differs from that of dairy cattle, and what knowledge can be gained from comparison of this disease across these very divergent management systems may prove fruitful in improving our understanding of the disease in both systems.

It has become increasingly apparent that other mammalian species, including small ruminants (sheep and goat) and wildlife (e.g. elk) can be affected with lesions of the hoof and

skin that have significant similarities to DD (Duncan et al., 2014; Clegg et al., 2015; Han and Mansfield, 2014; Crosby-Durrani et al., 2016). Interestingly, despite the presence of very similar organisms being isolated from these various hosts, the clinical manifestations of these diseases vary across the hosts as was eluded to before. For instance, classic bovine DD lesions are confined to the skin (hence the term dermatitis), although in cattle with DD, severe horn heel erosion are 46% more commonly reported (Gomez et al., 2015a). When treponemes are associated with non-healing sole lesions in cattle, it is primarily believed to be the result of secondary infection of pre-existing sole lesions such as sole ulcers, white line disease, toe necrosis and puncture wounds (Clegg et al., 2016a; Clegg et al., 2016c; Clegg et al., 2016d). In contrast, contagious ovine digital dermatitis, treponeme associated hoof lesions in dairy goats (Crosby-Durrani et al., 2016; Sullivan et al., 2015b) and treponeme associated hoof lesions in elk (Clegg et al., 2015; Han and Mansfield, 2014) typically present with dermatitis along with under running of the sole, and in severe cases complete avulsion of the hoof capsule. The propensity for development of these primary sole lesions in these host species raises questions regarding the difference in disease manifestation based on the host. Potential hypotheses include: 1) intrinsic differences in the host anatomy or genetics allows for differences in disease manifestation, 2) despite similarities in the treponemal species isolated, the clones involved in these diseases differ in their genetics or virulence attributes, and 3) the presence of the treponemes in these cases is more of an opportunistic infection with other organisms in the bacterial consortium driving the lesion pathogenesis. These differences in host response to the organisms along with the development of disease induction models in both cattle (Gomez et al., 2012; Krull et al., 2016a) and sheep (Wilson-Welder et al., 2015b) provide a good foundation for experimental approaches designed to address and test these hypotheses. By utilizing similar inoculums in both species and

observing the differences in clinical disease combined with multi-omic approaches, we can start to dissect the importance of host differences in the disease process.

The role of host genetics in DD lesion susceptibility has also been evaluated and has clearly demonstrated a genetic role for disease susceptibility or resistance (Scholey et al., 2012; Schopke et al., 2015). In addition, genetic parameters and breeding values have been identified for most hoof lesions and their relationships with feet and leg traits (Chapinal et al., 2013). With large variations in sire estimated breeding value for resistance to hoof lesions, the authors concluded there were long-term opportunities for genetic selection. Further research is required to determine the influence of susceptibility factors, identify the genetic basis of variation, clarify heritability of DD susceptibility and determine how host-related factors are correlated with production and health traits currently used in breeding programs (Palmer and O'Connell, 2015).

Immune responses to infection

Local dermal tissue and inflammatory response to DD infection has been evaluated using several approaches. There is a general dermal thickening in lesion development that is accompanied by varying degrees of infiltration of inflammatory cells (neutrophils and eosinophils) and changes in local cytokine concentrations (Refaai et al., 2013). Similarly, gene expression in skin biopsies from 5 bovine DD lesions and 5 healthy bovine feet were compared using RNA-Seq technology (Scholey et al., 2013). They demonstrated changes in cytokine expression (especially interleukin 1β being upregulated in DD lesions) and changes in expression of several other keratin or keratin associated genes. Interestingly, they detected evidence of poor local immune and inflammatory reactions to the bacterial infection present in lesions, possibly indicating a suppressed host response to DD. It has been speculated that local innate immune

284 responses may contribute to the proliferative, inflammatory conditions that perpetuate DD
285 lesions (Wilson-Welder et al., 2015a).
286 In general, there is a limited body of knowledge in the literature regarding host innate or
287 adaptive immune responses to DD infection. Several studies have evaluated the systemic
288 humoral immune response of cattle and have consistently demonstrated that, despite the
289 restricted presentations of clinical signs, systemic immune responses to treponemal antigens and
290 some other DD-associated organisms can be identified using serology (Demirkan et al., 1999;
291 Gomez et al., 2014a; Vink et al., 2009). However, use of these assays has not been widely
292 implemented in diagnostic or prognostic studies, in large part due to uncertainty regarding how
293 to utilize the outputs to effectively monitor disease in the farm. In large part, this lack of clear
294 diagnostic serology is considered to be due to the endemic nature of disease and persistence of
295 the DD-associated treponemes in farm environments, rendering most animals seropositive to one
296 degree or another. Even less is known about the cell-mediated immune responses to DD and their
297 role, if any, in disease. Future studies that evaluate both arms (humoral and cell mediated) of the
298 immune response are warranted and have the potential to provide insights important for disease
299 control and lesion healing. Field experience demonstrates that the majority of cattle do not
300 develop a protective immune response that results in spontaneous lesion healing, although
301 spontaneous healing of M1 and M2 lesions has been described (Relun et al., 2012). Efforts to
302 compare the “typical” immune response of cattle with active DD lesions, to those of cattle that
303 are able to clear the lesions (either spontaneously or following treatment) may provide insights
304 into specific immune responses that are beneficial. Furthermore, these efforts need to extend
305 across a diversity of DD-associated organisms (including multiple species of treponemes). It is
306 likely that the greatest return on investment related to continued efforts to understand DD

immune responses focuses on improving our understanding of the antigenic targets, whether a TH1 or TH2 immune response predominates and which is most likely to be protective. All of the above will be essential information to boost immunity, possibly by enabling development of an effective vaccine.

Transmission

Although the exact route of transmission for DD is not fully elucidated, DD presents itself as a highly infectious disease, consistent with the experimental model of Krull et al. (2016a), in which the negative controls could be infected by being comingled with experimentally infected animals despite the feet of both animals being completely wrapped in bandages for the duration of the study. Another experimental model was used by the Liverpool research team, using sheep affected with DD lesions to induce DD in healthy animals by just mixing and intermingling in a normal farm environment with standard herd management and then chronic lesion development over time (SD Carter, personal communication). This attempt at an infection model resulted in over 50% of the naïve sheep developing contagious ovine digital dermatitis lesions, with the full range of severity, from small lesions to complete hoof evulsion requiring euthanasia (SD Carter, personal communication). The outcomes of these studies clearly demonstrate that transmission can occur when susceptible animals are housed in the same environment as those with active DD lesions. However, the fact that transmission occurred in the presence of foot wraps could suggest that direct physical contact with lesions is not required (Krull et al., 2016a). The literature has also evaluated the role that early or active host-associated DD lesions play as a primary reservoir of infectious organisms. Multiple studies have demonstrated that the quantitative levels of DD-associated treponemes are higher in host-associated tissues (including rectum, gingiva, rumen,

DD lesions) than in environmental samples collected from dairy environments (Evans et al., 2012b; Klitgaard et al., 2017; Rock et al., 2015). However, low numbers of DD-associated *Treponema* spp can be identified in dairy farm slurry on farms with endemic DD when using deep sequencing based phylogenomic approaches (Rock et al., 2015; Klitgaard et al., 2017). Likewise, there is evidence from multiple groups that foot trimming equipment can be contaminated with treponemes and may act as a source of infection between animals and farms (Sullivan et al., 2014; Rock et al., 2015). While there is a growing body of evidence that treponemes can be identified in samples beyond active DD lesions, the relative role of these sources as primary reservoirs of infection remains unclear. It is possible that these organisms are simply transient members of the bacterial community that are continuously shed in the environment from lesions but survive for very short periods; a hypothesis that may be more likely given the apparent affinity of treponemes for host environments. Alternatively, it is possible that the organisms are able to survive off the host for sufficient periods of time to allow disease transmission. Consequently, there is a need to better understand how these organisms adapt to the non-host environment and how long they are able to persist in the absence of host tissue and nutrients. Further complicating the issue of reservoirs of infection is the complex etiology (either polytreponemal or polybacterial) of the disease process, which results in a situation where one must potentially consider reservoirs for each of the species and the fact that there is potential that those could be different. The work thus far has focused on reservoirs of treponemes due to their known association with the disease process; however, this may be an over simplification.

Other routes of fomite-associated transmission should be considered, including contact with contaminated equipment, as *Treponema* spp. has been identified on hoof knives and other

trimming equipment (Sullivan et al., 2014; Rock et al., 2015). Transmission through insect vectors is not likely, as no vectors tested for presence of *Treponema* spp. DNA were positive (Evans et al., 2012b). However, it is reported that in a portion of dairy farms, non-lactating heifers are also affected by DD (Jacobs et al., 2017; Holzhauer et al., 2012). If undetected and untreated these animals are a continuous source of DD-affected animals for the lactating herd. It is not clear though what portion of the prevalence of DD in adult cows can be attributed to young stock entering the lactating herd after calving. There is a need for significant effort related to better understanding the relative importance of all of these potential routes of transmission on the overall epidemiology of this disease on dairy farms. Efforts in this area should consider the potential for a multi-species etiology and need to evaluate the ecologic fitness and survivability of these organisms in non-host environments. With limited knowledge regarding the key reservoir of the *Treponema* phylogroups and the role of other bacteria in pathogenesis as well as uncertainty about route of transmission, control of DD could well be hampered.

Experimental models

Robust and efficient experimental models of infection are critical to research efforts focused on better understanding the pathogenesis and etiology of DD. Several induction models have been described for use in the induction of DD lesions in both cattle and sheep (Gomez et al., 2012; Krull et al., 2016a; Wilson-Welder et al., 2015b). The most obvious benefit of an experimental model would be to evaluate the etiology of the disease; however, efforts to use the models in this manner have thus far been underwhelming. Both bovine models have attempted to induce lesions using pure culture of DD-associated *Treponema phagedenis*-like bacteria (Gomez et al., 2012; Krull et al., 2016a). While both studies observed some degree of lesion formation,

the size and severity of the lesions was considerably less than observed when macerated lesion material was used as the inoculum (Gomez et al., 2012). Additionally, in both studies, inoculations of pure growth treponeme isolates were performed on one foot of animals that had macerate used to induce lesions on another foot, meaning that while the one foot was only exposed to a single organism there were other organisms used in the pen and even on the same animal. This design is particularly problematic to the interpretation of the data with regards to etiology because one of the studies showed that negative control animals (i.e. animals that had their feet wrapped and inoculated with media alone) housed in the pens with animals that were induced with macerate had an induction rate and lesion severity essentially identical to those induced with pure growth organisms, whereas negative control animals that were housed in isolation remained uninfected (Krull et al., 2016a). Knowing this information, along with the experience gained in these studies, allows for the development of more robust study designs that can be effectively used to further probe the question of etiology. Considerations that need to be included in that approach include animal housing with regards to cross contamination, use of pure cultures of single organisms versus consortia of multiple pure growth organisms, the role of individual animal immunity, and the potential confounders of pre-existing immunity in animals sourced from an industry that has high endemic rates of disease and consequently a high risk of previous exposure to the disease.

Experimental induction models also represent a useful tool for evaluating a variety of other important issues. These include but are not limited to, experimental approaches focused on adaptive immune responses (both humoral and cell mediated), therapeutic interventions, and vaccine evaluation and development. The availability of multiple induction models allows researchers to determine which models best test their hypothesis while providing the needed

controls. A significant downside of current bovine models is that they tend to be quite expensive and labor intensive, so the development of a small ruminant model provides some potential cost benefits while allowing for comparison across species as described in the host portion of this manuscript.

Lesion detection

Key to any DD control program is the efficient and consistent identification of lesions. Given a relatively distinct clinical presentation of the disease, diagnosis of DD is usually based on visual inspection of the foot. This process can be labor-intensive, and since the location of the lesion is not always easily accessed, small lesions can be easily missed (Solano et al., 2017a). Most commonly, animals are inspected in a chute that allows for safe lifting of the foot and thorough cleaning before inspection and this method of evaluation is considered the gold standard for diagnosis. To facilitate a more efficient and less labor-intensive inspection alternative means of observation in the parlor, headlocks and alleyways have been systematically compared to chute observations (Stokes et al., 2012; Winders et al., 2015; Solano et al., 2017a; Relun et al., 2011), also in young stock using pen walks (Jacobs et al., 2017). The consensus of these studies is that the highest agreement between chute and alternate observation methods occurs when the lesion status is condensed to a dichotomous presence or absence. In this situation sensitivity of lesion detection ranged from 65-100% while specificity ranged from 80-99% (Stokes et al., 2012; Winders et al., 2015; Solano et al., 2017a). When efforts are made to evaluate more precise lesion characteristics (color, erosiveness, proliferation) or score the lesions on a standardized severity scoring system the sensitivity and specificities consistently decrease to a slight to moderate level of agreement with chute evaluation (Relun et al., 2011; Winders et al.,

2015; Solano et al., 2017a). The presence of DD lesions at sites in the interdigital space or dorsal aspect of the foot further drops sensitivity. As might be expected, parlor observation of washed feet performed better than headlocks and pen, with pen observation showing the lowest sensitivity and specificity (Winders et al., 2015). Therefore, although DD scoring in the milking parlor as a routine practice should facilitate early detection, prompt treatment interventions, and herd monitoring, it was not sufficiently reliable to replace definitive identification of lesions done in the trimming chute. In addition, it is noteworthy that milking parlor scoring has not been implemented as a routine method of DD diagnostics and alternatives should be developed for early disease detection in automated milking systems.

Alternatively, detection of cows affected with DD could focus on detection of lameness. However, not all stages of DD result in visible lameness, and conversely, not all lameness results from DD. The use of locomotion score was very inconsistent in its ability to accurately identify cows with DD (Krull et al., 2016b). In fact, cows with the most severe changes in locomotion score were more likely to have other claw-horn lesions than DD, and the majority of cattle with DD failed to show high locomotion scores. These findings are consistent with the findings of Frankena et al. (2009) in which only 39% of the cows with severe DD lesions showed lameness . Therefore, DD detection is still either labor intensive as feet need to be lifted or only low to moderately sensitive based on simplified assessment methodologies. Notwithstanding, an overall lameness control program would facilitate identification of cows that need individual attention. Given that the primary welfare concern associated with DD involves induction of lameness, the field would benefit from a better understanding of the drivers of lameness as it relates to DD lesions. Clearly, the presence of a lesion alone is probably not sufficient to induce lameness, despite the fact that the lesions are universally sensitive to pressure. Likewise, the fact that

lameness typically improves markedly within several days following topical treatment suggest that the underlying mechanisms of pain can be minimized even in the presence of unhealed skin.

Treatment

Given the endemic nature of DD, many field studies have been performed to identify effective treatments. With the most commonly accepted pathogenesis being based on a bacterial origin, treatments have focused on this aspect of the disease. Treatment with systemic penicillin has been shown to be efficacious but is not widely used due to the necessity of withholding milk and costs (Laven and Logue, 2006). Systemic antibiotic therapy with other antibiotics routinely used in US dairy cattle milking herds did not increase or decrease DD lesion scores (Krull et al., 2016b), and due to cost, is rarely used (Laven and Logue, 2006). Conversely, topical treatment, usually with antibiotic preparations, is the most common method employed by veterinarians and foot trimmers for the treatment of advanced lesions (Apley, 2015). There is still uncertainty and disagreement regarding the actual efficacy of treatment outcomes with topical therapy. Success rates as low as 9% and as high as 73% have been reported (Krull et al., 2016b; Cutler et al., 2013; Berry et al., 2010; Nishikawa and Taguchi, 2008; Shearer and Hernandez, 2000; Laven and Hunt, 2001). There is a pressing need for good comparative field studies using robust study designs (ideally prospective randomized controlled trials) to determine the most efficacious treatment approach. Design of these studies needs to consider and normalize the stage of lesions development, as the treatment response may vary by lesion severity. Likewise, prolonged durations of post treatment observation (upwards of 120 days) are required to confirm that lesions fully heal and do not recrudesce (Krull et al., 2016b), while shorter observation periods may allow for observation of improvement of lameness.

In order to evaluate a larger diversity of antibiotics and to address the issue of potential antibiotic resistance, several DD treponeme studies have used *in vitro* minimum inhibitory concentration (MIC) based approaches (Hartshorn et al., 2013; Evans et al., 2009; Evans et al., 2012a). However, it is important to recognize that the Clinical and Laboratory Standards Institute (CLSI) does not have a validated methodology or bacterial MIC cut-off points established for DD-associated bacteria. This consequently complicates clinical interpretation and utility of *in vitro* derived MIC data and represents an area where additional research and the development of validated cut-off points could benefit the field. Caution should be exercised when interpreting the outcomes of *in vitro* MIC data, since the pharmacokinetic and pharmacodynamic differences between drugs can greatly influence the dosage of the drug delivered to the lesion. As a result, simply comparing which drug has the lowest MIC fails to address the clinical complexity of treatment efficacy and pharmacology. For instance, topical administration of several grams of oxytetracycline directly to a lesion may result in local drug concentrations far above an MIC that could not be achieved in the same location using systemic administration. Continued efforts to better understand the potential presence of antibiotic resistance should focus on identification of genetic resistance determinants to important classes of antibiotics used in DD control. Likewise, evaluation of genetic mechanisms of resistance to heavy metals (such as copper commonly used in footbaths) is warranted.

The potential for various morphotypes of *Treponema* spp. has been raised as an explanation for the discrepancy of *in vitro* susceptibilities and limited effectiveness *in vivo*. During *in vitro* growth of *Treponema* spp. isolated from DD, morphological variability was observed (Döpfer et al., 2012), indicating the presence of a spiral form and a round body form. The round body forms are morphologically similar to those observed in *Borrelia burgdorferi* (a

related spirochete), and have been hypothesized to play a role in persistent infection as has been hypothesized for *Borrelia* (Murgia and Cinco, 2004). Additional work to fully demonstrate the roll of these morphologically variable cells in *in vivo* infections is needed, as the role of these forms in chronic Lyme disease is hotly debated (Merilainen et al., 2016; Murgia and Cinco, 2004; Merilainen et al., 2015; Lantos et al., 2014). To date, very little information is available in the peer-reviewed literature that definitively identifies and details their presence in the tissue of DD lesions. Efforts to understand the biochemical and genetic drivers of cellular morphology change along with improving our understanding of the metabolic activity of these cells would aid in understanding their importance. Likewise, efforts to definitively demonstrate their significance in active lesions and the underlying molecular mechanisms related to the potential for their role in persistence of disease may allow for the identification of novel control targets for this endemic disease.

Due to global concerns regarding prudent antibiotic use, and the inconsistent response of DD lesions to antibiotic treatment, alternative approaches to the use of antimicrobials for control of DD are desired and have been considered. For example, the impact of altered trace mineral nutrition was evaluated in a randomized efficacy study to evaluate the effect of a premix containing concentrations of organic trace minerals and iodine (HOTMI). This study showed a reduction in the incidence of active DD lesions acquired naturally or induced by an experimental infection challenge model (Gomez et al., 2014b). The mineral premix tended to reduce the total DD infection rate and the average size of the experimentally induced lesions, although the results failed to reach the level of statistical significance. Additional work utilizing larger sample sizes are warranted to determine if the effect is real. Likewise, the mechanistic reasons for the improvement should be thoroughly evaluated in order to provide insights into the cellular

pathways that benefit lesion prevention. There is also a need for an improved understanding of the broader role of nutrition in DD prevention.

Prevention and control

As reported by Potterton et al. (2012), between 2000-2011, 62 scientific papers could be identified focusing on prevention of digital dermatitis, with the seven distinct areas of interest being, standing time on concrete, claw trauma, diets and feeding, detection and treatment, heifer breeding, environmental hygiene and biosecurity. In more detail Holzhauer et al. (2012) reported the importance of prevention of transmission of disease to young stock as housed on the same farm. With DD having high within-herd prevalence, herd-level interventions are warranted to try to decrease the prevalence.

Footbaths

The most commonly used herd-level intervention is a footbath, primarily used to prevent new cases through increased hygiene, but sometimes perceived important for treatment of clinical cases. Proper footbath design has been evaluated and is based on dimensions (Logue et al., 2012; Cook et al., 2012), frequency of use, product used and appropriate concentration of solution (Speijers et al., 2010; Speijers et al., 2012; Teixeira et al., 2010; Relun et al., 2012). When used, the footbath must be managed to ensure sufficient solution is consistently available to achieve full immersions of hooves of all 4 feet (Cook et al., 2012). Furthermore, fecal contamination is known to interfere with effectiveness of most footbath solutions. With copper sulphate, a common choice in North America, the pH of the concentration is critical to keep copper soluble and efficacious (Laven and Hunt, 2002; Speijers et al., 2010; Speijers et al., 2012; Teixeira et al., 2010). Optimizing footbath management according to scientific knowledge

reduces the prevalence of active DD lesions. On farms where footbathing practices do not meet recommendations, an automatic footbath may provide benefit (Solano et al., 2017b). With most footbath products having adverse legislative, health and safety and environmental effects, *in vitro* models have been developed to screen new footbath products. The assays designed allow for determination of minimum inhibitory concentration and minimum bactericidal concentration of disinfectants for *Treponema* spp. Additionally, manure contamination, potentially resulting in inhibition of the solution, was also mimicked. This assay was useful to categorize disinfectants, based on effects of exposure and manure concentration regarding their ability to inhibit *Treponema* spp. growth (Hartshorn et al., 2013). Despite the large body of literature, no footbath studies had acceptable efficacy in control of DD.

Questions have been asked about the safety for human and environmental health as related to large quantities of chemicals and minerals being used for footbaths (Laven and Logue, 2006). In Canada, there is a wide variety of products in numerous combinations as well as concentrations (Solano et al., 2015). Although risks to human health due to formaldehyde have been explored (Doane and Sarenbo, 2014), it was concluded to not exceed public health guidelines. Based on frequent questions regarding antimicrobial use, environmental and health impacts, future directions should focus on early interventions and potential use of environmentally friendly products.

Control

Monitoring herds with endemic disease for changes in lesion prevalence or severity and classifying cattle based on lesion monitoring has been described as one means to provide insights into on-farm management decisions making. These approaches allow producers to potentially identify higher risk animals that might need intervention or culling. The goal of this approach is

to achieve a manageable state of disease, but no strategy was identified to eradicate DD (Dopfer, 2009). While DD eradication at herd or even country level would be the ideal objective, the literature suggests that in most cases this is extremely difficult if not impossible given the current tools available and the global nature of this disease. The combination of biosecurity, various footbaths and antimicrobials has patently not been effective in preventing disease spread or reduce severity. Consequently, we need an approach that takes a different line and preferably has more potential for prevention and control. Efforts to develop vaccines that were effective in limiting disease prevalence or severity would have significant economic and welfare benefit for the industry. The development of effective vaccines for the control of similar disease processes, such as ovine footrot, gives hope that one day these might be an option. The current research gaps identified in this manuscript, including an uncertain and complex etiology, minimal understanding of the disease transmission dynamics the significant lack of knowledge regarding the nature of protective immunity of this disease will provide challenges for vaccine development efforts in the short-term. However, we are rapidly developing a better understanding of the infective nature of DD and post-genomic technologies, such as reverse vaccinology offer hope that vaccine candidates, based on treponeme genomes, may be developed in the near future.

Role of the dairy producer in control of digital dermatitis

There is considerable variation in producers' mindsets towards an issue like DD on their farm, leading to variation in behaviors to address DD (Garforth, 2012). The perception of risk in general for example, can vary greatly based on information source (Lam, 2007). When a preferred source, e.g. a veterinarian, addresses or informs the producer of a potential issue or

583 risk, it is important that they are also aware of the individual beliefs of that producer. If
584 recommendations to improve a risk factor leading to DD on farm coincide with what the
585 producer believes, the producer will be more motivated to change and improve that issue. To
586 motivate producers to implement changes on farm, it is also important that they believe that the
587 issue at hand is, in fact, truly a significant matter (Ritter et al., 2017). Therefore, DD diagnostics
588 are important to keep the producer informed about within-herd prevalence of DD. Increasing
589 knowledge in the area of interest will likely inspire farmers to want to make changes and
590 improvements (Bruijnis et al., 2013). For example, in the UK, DairyCo launched the DairyCo
591 Healthy Feet Programme in 2011, with a goal to reduce lameness on farms. The program
592 increased producer' understanding and knowledge of lameness lesions. The more accurate
593 perceptions of lameness levels on farms increased, the greater was producers enthusiasm to
594 reduce lameness and motivation to make essential changes (Atkinson and Fisher, 2012). As seen
595 in the UK, veterinarians and farmers attitudes towards DD have been considerably influenced by
596 the knowledge that the DD-associated treponemes are implicated in the etiopathogenesis of many
597 lesions outside of cattle feet. Consequently, any effective treatments or control measures for
598 bovine DD are likely to have additive beneficial effects (Evans et al., 2016).
599 Another part of producer' motivation is driven by real or perceived economic impacts of
600 DD control. If a published economic impact is presented as decreased milk production or
601 increased risk of culling, there might be limited external validity of the study, and difficult to
602 compare to local situations or had limited validity in the country of farm origin (Gomez et al.,
603 2015b; Bruijnis et al., 2010). Therefore, locally applicable impact measures should be available
604 for decisions making. Unfortunately, with many gaps in our knowledge of treatment and control

of DD, producers' motivation might be limited and the problem not adequately and consequently addressed.

Conclusions

With the identified gaps in knowledge, it has become clear that effective prevention and control of the disease is still hampered. Although several aspects of the pathogeneses of the disease have been identified, the causal agent is still under debate. Indeed, the role of *Treponema* spp. in the development of lesions is still to be clarified. Efforts to definitively determine the consortium of organisms (either polytreponemal or polybacterial) necessary for disease induction should be a top priority, but will be costly and challenging. Without knowing what specific bacterial organisms are necessary and sufficient for disease induction, all other efforts focused on better understanding organism ecology, immunity and treatment have the potential to focus on the wrong bacteria. Additional priorities for research efforts should include an improved understanding of the ecology and reservoirs of the causal agents as well as a better understanding of the immune response to those organisms and how it improves or exacerbates lesion formation. Through filling these gaps in knowledge, the most effective intervention strategy can be developed.

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