

1 **Missing pieces of the puzzle to effectively control Digital Dermatitis**

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15 Summary

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

32 Introduction to the disease

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

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

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

64

65 Significance

66 DD is a significant concern for cattle producers and veterinarians for several reasons. The
67 clinical manifestation of lameness associated with DD poses a significant welfare concern for
68 cattle and represents a leading cause of culling in the dairy cattle industry throughout the world
69 (Cramer et al., 2009; Booth et al., 2004; Charfeddine and Perez-Cabal, 2017). However, the
70 impact of DD is not restricted to clinical disease, but includes financial losses associated with the
71 cost of treatment, decreases in both milk production and fertility, and losses due to increased
72 culling even in the absence of clinical symptoms (Argaez-Rodriguez et al., 1997; Gomez et al.,
73 2015b; Bruijnis et al., 2010; Cha et al., 2010; Relun et al., 2013).

74

75 Geographical distribution

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

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

81 Given the differences in herd size, housing and management across these different
82 geographic areas, it is safe to say that the disease is able to adapt and persist in a wide range of
83 ecologic and management settings. In New Zealand, where the dairy industry has been
84 historically pasture based, DD was reported only as sporadic cases until recent years when it has
85 been implicated as a growing concern for non-healing lesions of the sole (Vermunt and Hill,
86 2004; Van Andel M, 2012). The situation in New Zealand, as well as some other similar
87 observations in other countries has led to the hypothesis that DD becomes an increasingly
88 important issue when dairy cattle management changes from a more extensive pasture based
89 system to confinement freestall housing (Sogstad et al., 2005). In countries like the UK, where
90 cattle have housed and pasture seasons, the disease is almost restricted to the housing season
91 (Evans et al., 2016). There is a need to further test this hypothesis in well-designed studies along
92 with an effort to better understand the potential drivers of this disease progression. Herd stocking
93 density, moisture content and hydration of the foot and skin, increased herd introductions and
94 increased time on concrete have all been discussed and considered but there is at present little
95 definitive evidence to support any sort of relative prioritization of these based on evidence based
96 outcomes. It is important to acknowledge and recognize that emergence of the disease in
97 countries and production systems, like the North American pasture-based ranching system, that
98 have previously had little to no DD provide a rich research site for these critical studies to occur.
99 We have to, however, realize that underreporting and the disease going unnoticed might be the

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

102

103 Pathogens involved

104 Despite a significant number of studies focused on elucidating the etiology of DD, debate
105 remains regarding the exact etiology. Although fungal and viral etiologies have been considered,
106 the scientific community has largely agreed that these organisms are less likely to drive the
107 disease process, and the field has focused its attention on bacterial organisms (Rebhun et al.,
108 1980; Krull et al., 2014b; Zinicola et al., 2015; Brandt et al., 2011). For a detailed overview of
109 the findings of this body of knowledge, readers are directed to the review articles referenced at
110 the start of this manuscript; however, two consistent themes have emerged from these
111 studies. First, DD lesions are consistently associated with an abundant and diverse population of
112 multiple species of Treponemes (Zinicola et al., 2015; Krull et al., 2014b; Evans et al.,
113 2016). Second, these diverse treponeme populations exist as a portion of a much more diverse
114 and complex bacterial community that comprises the total microbiota of the DD lesions.
115 Furthermore, the non-treponemal constituents of the microbiota are not random and instead show
116 association with the stage of lesion development (Krull et al., 2014b, Zinicola et al., 2015). As
117 described in more detail by Krull et al. (2014b), non-affected animals showed an abundance of
118 *Staphylococcaceae*, *Streptococcaceae*, *Bacteroidaceae*, *Corynebacteriaceae* and
119 *Pasteurellaceae*, replaced by other bacterial families as lesions progressed. Whereas
120 *Spirochaetaceae* increased systematically from 0 to over 90% in chronic stages of the disease
121 (Krull et al., 2014c). With lesions classified as active and inactive, Zinicola et al. (2015)
122 identified *Firmicutes* and *Actinobacteria* as the predominant bacterial phyla of control animals,

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

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

131 First, while it is clear that *Treponema* spp. are consistently present in DD lesions and
132 make up the majority of the bacterial community in advanced lesions, it is also clear that these
133 populations represent a diversity of species instead of a single species (Klitgaard et al., 2013;
134 Marcatili et al., 2016; Krull et al., 2014c; Yano et al., 2009; Evans et al., 2008). This in itself
135 poses a problem with fulfilling Koch's postulates for this disease process. At a very minimum,
136 one must acknowledge that if treponemes are the primary etiologic agents associated with DD, it
137 is a polytreponemal process, and this hypothesis has been argued for in the literature (Evans et
138 al., 2008). If this hypothesis is true, it still leaves the significant question of why does the disease
139 require the presence of multiple treponemal species instead of one? Furthermore, how do these
140 different treponemal species interact with each other, and what is the minimum treponema
141 consortium required for inducing clinical disease? How does the polytreponemal community
142 change during progression of the disease? An alternate hypothesis that emerges is that the
143 diversity of *Treponema* species present in the lesions is more suggestive of an overgrowth of
144 opportunists that find a unique niche for expansion during the induction of DD lesions (Edwards
145 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

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

175 Second, as alluded to above, constituents of the non-treponemal bacterial communities
176 that are present in the DD lesions vary by lesion stage, but are amazingly consistent within a
177 given stage of lesion development (Krull et al., 2014c; Zinicola et al., 2015). This finding
178 suggests that their presence is not merely coincidental or due to background from the dairy
179 environment, but instead suggests that there is a driving force behind the development and
180 transition of this complex microbiota shift. There is a clear need to better understand what is
181 driving this transition and how this transition is involved in the development, maintenance and
182 response to therapy of digital dermatitis. Given that several of these organisms are known
183 pathogens in other disease processes of the foot of ruminants (for example, *Dichelobacter*
184 *nodosus*, *Fusobacterium necrophorum* and others) it is important that hypotheses are developed
185 and tested regarding their specific role in DD. Interestingly, many of these “known” pathogens
186 are present in low relative abundance and this fact has been used to argue that they may not be
187 relevant to the disease process (Moe et al., 2010; Collighan and Woodward, 1997). However,
188 recent evidence from other disease processes has demonstrated that relative abundance in
189 phylogenomic studies needs to be interpreted with caution. This is particularly important because
190 abundance is not necessarily commensurate with pathogenicity. Neither does it controvert or
191 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

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

220

221 The hosts

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

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

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

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

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

272

273 Immune responses to infection

274 Local dermal tissue and inflammatory response to DD infection has been evaluated using
275 several approaches. There is a general dermal thickening in lesion development that is
276 accompanied by varying degrees of infiltration of inflammatory cells (neutrophils and
277 eosinophils) and changes in local cytokine concentrations (Refaai et al., 2013). Similarly, gene
278 expression in skin biopsies from 5 bovine DD lesions and 5 healthy bovine feet were compared
279 using RNA-Seq technology (Scholey et al., 2013). They demonstrated changes in cytokine
280 expression (especially interleukin 1 β being upregulated in DD lesions) and changes in expression
281 of several other keratin or keratin associated genes. Interestingly, they detected evidence of poor
282 local immune and inflammatory reactions to the bacterial infection present in lesions, possibly
283 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

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

311

312 Transmission

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

330 DD lesions) than in environmental samples collected from dairy environments (Evans et al.,
331 2012b; Klitgaard et al., 2017; Rock et al., 2015). However, low numbers of DD-associated
332 *Treponema* spp can be identified in dairy farm slurry on farms with endemic DD when using
333 deep sequencing based phylogenomic approaches (Rock et al., 2015; Klitgaard et al., 2017).
334 Likewise, there is evidence from multiple groups that foot trimming equipment can be
335 contaminated with treponemes and may act as a source of infection between animals and farms
336 (Sullivan et al., 2014; Rock et al., 2015). While there is a growing body of evidence that
337 treponemes can be identified in samples beyond active DD lesions, the relative role of these
338 sources as primary reservoirs of infection remains unclear. It is possible that these organisms are
339 simply transient members of the bacterial community that are continuously shed in the
340 environment from lesions but survive for very short periods; a hypothesis that may be more
341 likely given the apparent affinity of treponemes for host environments. Alternatively, it is
342 possible that the organisms are able to survive off the host for sufficient periods of time to allow
343 disease transmission. Consequently, there is a need to better understand how these organisms
344 adapt to the non-host environment and how long they are able to persist in the absence of host
345 tissue and nutrients. Further complicating the issue of reservoirs of infection is the complex
346 etiology (either polytreponemal or polybacterial) of the disease process, which results in a
347 situation where one must potentially consider reservoirs for each of the species and the fact that
348 there is potential that those could be different. The work thus far has focused on reservoirs of
349 treponemes due to their known association with the disease process; however, this may be an
350 over simplification.

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

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

366

367 Experimental models

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

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

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

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

403

404 Lesion detection

405 Key to any DD control program is the efficient and consistent identification of lesions.
406 Given a relatively distinct clinical presentation of the disease, diagnosis of DD is usually based
407 on visual inspection of the foot. This process can be labor-intensive, and since the location of the
408 lesion is not always easily accessed, small lesions can be easily missed (Solano et al., 2017a).
409 Most commonly, animals are inspected in a chute that allows for safe lifting of the foot and
410 thorough cleaning before inspection and this method of evaluation is considered the gold
411 standard for diagnosis. To facilitate a more efficient and less labor-intensive inspection
412 alternative means of observation in the parlor, headlocks and alleyways have been systematically
413 compared to chute observations (Stokes et al., 2012; Winders et al., 2015; Solano et al., 2017a;
414 Relun et al., 2011), also in young stock using pen walks (Jacobs et al., 2017). The consensus of
415 these studies is that the highest agreement between chute and alternate observation methods
416 occurs when the lesion status is condensed to a dichotomous presence or absence. In this
417 situation sensitivity of lesion detection ranged from 65-100% while specificity ranged from 80-
418 99% (Stokes et al., 2012; Winders et al., 2015; Solano et al., 2017a). When efforts are made to
419 evaluate more precise lesion characteristics (color, erosiveness, proliferation) or score the lesions
420 on a standardized severity scoring system the sensitivity and specificities consistently decrease to
421 a slight to moderate level of agreement with chute evaluation (Relun et al., 2011; Winders et al.,

422 2015; Solano et al., 2017a). The presence of DD lesions at sites in the interdigital space or dorsal
423 aspect of the foot further drops sensitivity. As might be expected, parlor observation of washed
424 feet performed better than headlocks and pen, with pen observation showing the lowest
425 sensitivity and specificity (Winders et al., 2015). Therefore, although DD scoring in the milking
426 parlor as a routine practice should facilitate early detection, prompt treatment interventions, and
427 herd monitoring, it was not sufficiently reliable to replace definitive identification of lesions
428 done in the trimming chute. In addition, it is noteworthy that milking parlor scoring has not been
429 implemented as a routine method of DD diagnostics and alternatives should be developed for
430 early disease detection in automated milking systems.

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

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

447

448 Treatment

449 Given the endemic nature of DD, many field studies have been performed to identify
450 effective treatments. With the most commonly accepted pathogenesis being based on a bacterial
451 origin, treatments have focused on this aspect of the disease. Treatment with systemic penicillin
452 has been shown to be efficacious but is not widely used due to the necessity of withholding milk
453 and costs (Laven and Logue, 2006). Systemic antibiotic therapy with other antibiotics routinely
454 used in US dairy cattle milking herds did not increase or decrease DD lesion scores (Krull et al.,
455 2016b), and due to cost, is rarely used (Laven and Logue, 2006). Conversely, topical treatment,
456 usually with antibiotic preparations, is the most common method employed by veterinarians and
457 foot trimmers for the treatment of advanced lesions (Apley, 2015). There is still uncertainty and
458 disagreement regarding the actual efficacy of treatment outcomes with topical therapy. Success
459 rates as low as 9% and as high as 73% have been reported (Krull et al., 2016b; Cutler et al.,
460 2013; Berry et al., 2010; Nishikawa and Taguchi, 2008; Shearer and Hernandez, 2000; Laven
461 and Hunt, 2001). There is a pressing need for good comparative field studies using robust study
462 designs (ideally prospective randomized controlled trials) to determine the most efficacious
463 treatment approach. Design of these studies needs to consider and normalize the stage of lesions
464 development, as the treatment response may vary by lesion severity. Likewise, prolonged
465 durations of post treatment observation (upwards of 120 days) are required to confirm that
466 lesions fully heal and do not recrudescence (Krull et al., 2016b), while shorter observation periods
467 may allow for observation of improvement of lameness.

468 In order to evaluate a larger diversity of antibiotics and to address the issue of potential
469 antibiotic resistance, several DD treponeme studies have used *in vitro* minimum inhibitory
470 concentration (MIC) based approaches (Hartshorn et al., 2013; Evans et al., 2009; Evans et al.,
471 2012a). However, it is important to recognize that the Clinical and Laboratory Standards Institute
472 (CLSI) does not have a validated methodology or bacterial MIC cut-off points established for
473 DD-associated bacteria. This consequently complicates clinical interpretation and utility of *in*
474 *vitro* derived MIC data and represents an area where additional research and the development of
475 validated cut-off points could benefit the field. Caution should be exercised when interpreting the
476 outcomes of *in vitro* MIC data, since the pharmacokinetic and pharmacodynamic differences
477 between drugs can greatly influence the dosage of the drug delivered to the lesion. As a result,
478 simply comparing which drug has the lowest MIC fails to address the clinical complexity of
479 treatment efficacy and pharmacology. For instance, topical administration of several grams of
480 oxytetracycline directly to a lesion may result in local drug concentrations far above an MIC that
481 could not be achieved in the same location using systemic administration. Continued efforts to
482 better understand the potential presence of antibiotic resistance should focus on identification of
483 genetic resistance determinants to important classes of antibiotics used in DD control. Likewise,
484 evaluation of genetic mechanisms of resistance to heavy metals (such as copper commonly used
485 in footbaths) is warranted.

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

491 related spirochete), and have been hypothesized to play a role in persistent infection as has been
492 hypothesized for *Borrelia* (Murgia and Cinco, 2004). Additional work to fully demonstrate the
493 roll of these morphologically variable cells in *in vivo* infections is needed, as the role of these
494 forms in chronic Lyme disease is hotly debated (Merilainen et al., 2016; Murgia and Cinco,
495 2004; Merilainen et al., 2015; Lantos et al., 2014). To date, very little information is available in
496 the peer-reviewed literature that definitively identifies and details their presence in the tissue of
497 DD lesions. Efforts to understand the biochemical and genetic drivers of cellular morphology
498 change along with improving our understanding of the metabolic activity of these cells would aid
499 in understanding their importance. Likewise, efforts to definitively demonstrate their significance
500 in active lesions and the underlying molecular mechanisms related to the potential for their role
501 in persistence of disease may allow for the identification of novel control targets for this endemic
502 disease.

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

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

516

517 Prevention and control

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

525 Footbaths

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

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

547 Questions have been asked about the safety for human and environmental health as
548 related to large quantities of chemicals and minerals being used for footbaths (Laven and Logue,
549 2006). In Canada, there is a wide variety of products in numerous combinations as well as
550 concentrations (Solano et al., 2015). Although risks to human health due to formaldehyde have
551 been explored (Doane and Sarenbo, 2014), it was concluded to not exceed public health
552 guidelines. Based on frequent questions regarding antimicrobial use, environmental and health
553 impacts, future directions should focus on early interventions and potential use of
554 environmentally friendly products.

555 Control

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

560 to achieve a manageable state of disease, but no strategy was identified to eradicate DD (Dopfer,
561 2009). While DD eradication at herd or even country level would be the ideal objective, the
562 literature suggests that in most cases this is extremely difficult if not impossible given the current
563 tools available and the global nature of this disease. The combination of biosecurity, various
564 footbaths and antimicrobials has patently not been effective in preventing disease spread or
565 reduce severity. Consequently, we need an approach that takes a different line and preferably has
566 more potential for prevention and control. Efforts to develop vaccines that were effective in
567 limiting disease prevalence or severity would have significant economic and welfare benefit for
568 the industry. The development of effective vaccines for the control of similar disease processes,
569 such as ovine footrot, gives hope that one day these might be an option. The current research
570 gaps identified in this manuscript, including an uncertain and complex etiology, minimal
571 understanding of the disease transmission dynamics the significant lack of knowledge regarding
572 the nature of protective immunity of this disease will provide challenges for vaccine
573 development efforts in the short-term. However, we are rapidly developing a better
574 understanding of the infective nature of DD and post-genomic technologies, such as reverse
575 vaccinology offer hope that vaccine candidates, based on treponeme genomes, may be developed
576 in the near future.

577

578 Role of the dairy producer in control of digital dermatitis

579 There is considerable variation in producers' mindsets towards an issue like DD on their
580 farm, leading to variation in behaviors to address DD (Garforth, 2012). The perception of risk in
581 general for example, can vary greatly based on information source (Lam, 2007). When a
582 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 (Bruijnjs 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; Bruijnjs 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

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

607

608 Conclusions

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

622

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