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Author: N.J. Evans, R.D. Murray, S.D. Carter

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1 **Review**

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3 **Bovine digital dermatitis: Current concepts from laboratory to farm**

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6 N.J. Evans <sup>a,b,\*</sup>, R.D. Murray <sup>b,c</sup>, S.D. Carter <sup>a,b</sup>

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8 <sup>a</sup> *Department of Infection Biology, Institute of Infection and Global Health, University of*  
9 *Liverpool, L69 7ZJ, UK*

10 <sup>b</sup> *School of Veterinary Science, University of Liverpool, L69 7ZJ, UK*

11 <sup>c</sup> *Institute of Translational Medicine, University of Liverpool, L69 7ZJ, UK*

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16 \* Corresponding author. Tel.: +44 151 7944755.

17 *E-mail address:* [evansnj@liverpool.ac.uk](mailto:evansnj@liverpool.ac.uk) (N.J. Evans).

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## 19 **Highlights**

- 20 • Bovine digital dermatitis (DD), DD treponemes, DD risk factors and DD control  
21 strategies are reviewed.
- 22 • DD lesions, hoof trimming tools and bovine GI tract are DD infection reservoirs.
- 23 • Improved hygiene and foot trimming practice could help prevent DD transmission.
- 24 • Current antibiotic treatments are not the most effective against DD treponemes.
- 25 • New antibiotics, additional transmission prevention and/or effective vaccines are needed.

26

27

## 28 **Abstract**

29 Bovine digital dermatitis (DD) is a severe infectious disease causing lameness in dairy  
30 cattle worldwide and is an important ruminant welfare problem that has considerable economic  
31 issues. Bovine DD is endemic in many regions worldwide and it is important to understand this  
32 major disease so that effective control strategies can be identified. There is substantial evidence  
33 that specific treponeme phylotypes play an important causative role in bovine DD. This review  
34 considers current research, including DD *Treponema* spp. investigations, associated DD  
35 pathobiology, and current and potential treatment and control options. Epidemiological data,  
36 alongside new microbiological data, help to delineate important transmission routes and  
37 reservoirs of infection that allow effective interventions to be identified. Better on-farm housing  
38 hygiene, pasture access, routine footbathing and claw trimming with disinfected equipment need  
39 to be implemented to significantly reduce the incidence of DD. There is a paucity of peer

40 reviewed research into both commonly used and novel treatments. In vitro antimicrobial  
41 susceptibility studies of DD treponemes and effective treatment of human treponematoses clearly  
42 indicate that antibiotics frequently selected for DD treatments are not the most efficacious. Whilst  
43 there are understandable concerns over milk withdrawal times in dairy cattle, more needs to be  
44 done to identify, license and implement more appropriate antibiotic treatments, since continued  
45 overuse of less efficacious antibiotics, applied incorrectly, will lead to increased disease  
46 recurrence and transmission. More research is needed into methods of preventing DD that  
47 circumvent the use of antibiotics, including vaccination and transmission blocking studies, in  
48 order to reduce or hopefully eradicate DD in the future.

49

50 *Keywords:* Bovine digital dermatitis; Papillomatous digital dermatitis; Treponemes; Spirochaetes

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## 51 **Introduction**

52           Bovine digital dermatitis (DD) was first reported in the 1970s (Cheli and Mortellaro,  
53 1974) and is also called Mortellaro's disease in Europe, whereas papillomatous DD (PDD) or  
54 hairy foot/heel-warts (Read and Walker, 1998) is frequently used in North and South America  
55 and Australasia. The contagious nature of this severe disease and poor treatment response has  
56 resulted in a major worldwide problem in nearly all countries with dairy cattle. Substantial focal  
57 lesions on one or both rear feet of cattle are the typical presentation of DD, which is often  
58 extremely painful. The ensuing lameness is an important animal health and welfare concern,  
59 resulting in reduced milk yield and reproductive performance, with substantial costs of treatment  
60 and control. An effective prevention or treatment capable of eliminating bovine DD has yet to be  
61 identified. Spirochaetes and, more specifically, treponemes have been implicated as important in  
62 the aetiology of DD. This review discusses worldwide research that advances our understanding  
63 of current concepts in DD and the role of associated treponemes, with implications for best  
64 practices.

65

## 66 **Presentation, epidemiology and impact of the disease**

67           The main clinical feature of bovine DD is lameness resulting from an ulcerative lesion  
68 consisting of an extensive or localised superficial dermatitis immediately on or above the  
69 coronary band between the heel bulbs (Weaver et al., 1981; Blowey and Sharp, 1988). The  
70 majority of lesions (~80-90%) are typically found on hind feet, with far fewer occurring on the  
71 front feet (~10-20%) (Murray et al., 2002). Animals with DD are frequently severely lame and, as  
72 a result, may walk on their toes or shift their weight from foot to foot (Cheli and Mortellaro,  
73 1974; Read and Walker, 1998). Lesions adjacent to the interdigital space often extend locally to  
74 involve interdigital skin (Read and Walker 1994) and long standing lesions frequently (42%)

75 develop wart-like papillary keratotic proliferations (Read and Walker, 1998). A relevant scoring  
76 system for DD based on macroscopic lesion characteristics has been described (Dopfer et al.,  
77 1997) and recently adapted, now including six stages (Berry et al., 2012) (Table 1).

78  
79 In the 20 years following the initial report from Italy (Cheli and Mortellaro, 1974), bovine  
80 DD subsequently appeared across the world, being reported in the USA (Rebhun et al., 1980),  
81 UK (Blowey and Sharp, 1988) and Japan (Kimura et al., 1993). Bovine DD is now endemic in  
82 dairy cattle populations across several European countries, including Germany, the Netherlands,  
83 Denmark, France and the UK (Laven, 2001; Koenig et al., 2005; Holzhauer et al., 2006; Capion  
84 et al., 2008; Relun et al., 2013), and the USA, including California, Georgia, Iowa and Wisconsin  
85 (Read and Walker, 1998; Brown et al., 2000; Faust et al., 2001). Globally, the problem is now  
86 common and there have also been case reports of DD from Egypt, South Africa, Turkey, Chile,  
87 Brazil, Canada and New Zealand (van Amstel et al., 1995; Rodriguez-Lainz et al., 1998; Cruz et  
88 al., 2001; el-Ghoul and Shaheed, 2001; Demirkan and Güzel, 2004; Vermunt and Hill, 2004;  
89 Cramer et al., 2009).

90  
91 The impact of bovine DD is considerable. Bovine DD can cause severe pain, making it an  
92 important animal welfare issue (Bruijnjs et al., 2012), especially considering the number of  
93 animals worldwide with this disease. Furthermore, bovine DD is an important worldwide  
94 economic issue, since it results in reduced milk yields and reproductive performance (Argaez-  
95 Rodriguez et al., 1997). The economic impact resulting from milk production losses in the USA  
96 has been calculated at US\$190 million<sup>1</sup> per year (Losinger, 2006). In the UK, the cost of bovine

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<sup>1</sup> US\$1.00 = UK£0.61 = €0.78 at 25 September 2014.

97 DD has been estimated at UK£99.00 per case (GB Cattle Health and Welfare Group, 2014),  
98 whilst in The Netherlands, the cost per dairy holding of 65 cows was estimated at  
99 US\$1,517.00/year including milk loss and treatment costs (Bruijnjs et al., 2010). Another study  
100 reported the cost as US\$132.96 per case of DD consisting of US\$35.41 for milk loss, US\$41.37  
101 for decreased fertility and US\$56.18 for treatment costs (Cha et al., 2010). Therefore bovine DD  
102 has substantial economic implications.

103

#### 104 **Aetiology**

105 Epidemiological evidence, such as geographic spread and healing after antibiotic  
106 treatment, has implicated bovine DD as an infectious disease caused by bacteria (Read and  
107 Walker, 1998). Initially, the aetiology of DD was unclear, mainly due to the plethora of  
108 microorganisms present on the lesion surface as a result of the slurry environment that many  
109 dairy cows stand in. Various types of bacteria have been identified within bovine DD  
110 manifestations, including spirochaetes, *Bacterioides* spp., *Guggenheimella bovis*, *Campylobacter*  
111 spp., *Fusobacterium* spp. and *Peptococcus* spp. (Blowey and Sharp, 1988; Sabo et al., 1988;  
112 Read et al., 1992; Koniarova et al., 1993; Blowey et al., 1994; Dopfer et al., 1997; Schlafer et al.,  
113 2008). A recent systematic microbiological study of DD anaerobes and aerobes additionally  
114 reported *Porphyromonas levii*, *Mycoplasma* spp., *Prevotella* spp. and spirochaetes as lesion  
115 associated (Berry et al., 2010). However, when considering all studies in entirety, the  
116 spirochaetes and specifically treponemes are the only microorganisms where there is substantial  
117 evidence of an aetiological association.

118

119 An initial study involving cloning and sequencing of bacterial 16S rRNA genes from  
120 bovine DD lesions in Germany identified five phlotypes of treponemes within lesions (Choi et

121 al., 1997). Subsequent immunohistochemistry with molecular detection methods demonstrated  
122 both large numbers of treponemes within bovine DD lesions (Dopfer et al., 1997; Demirkan et  
123 al., 1998; Moter et al., 1998). Eight spirochaetes were isolated from bovine DD lesions in the  
124 USA, with seven forming a distinct phenotypic group (Walker et al., 1995); subsequently, a  
125 German BDD spirochaete (*Treponema brennaborensis*) was reported to be quite different  
126 (Schrank et al., 1999). A genetic typing study of six isolates from the USA identified three  
127 phylotypes (Walker et al., 1995; Stamm et al., 2002).

128  
129 In a further study, four USA bovine DD spirochaetes were identified as similar to  
130 *Treponema phagedenis* (Trott et al., 2003). A spirochaete isolated from a UK BDD lesion was  
131 identified as similar to USA bovine DD isolates (Demirkan et al., 2006). Most recently, a larger  
132 number ( $n = 23$ ) of treponemes from bovine DD lesions were isolated in the UK and were  
133 characterised both genotypically and phenotypically, demonstrating three distinct taxonomic  
134 groups analogous to USA DD phylotypes (Evans et al., 2008). In line with previous studies, these  
135 were designated as *Treponema medium*/*Treponema vincentii*-like, *Treponema phagedenis*-like  
136 and *Treponema putidum*/*Treponema denticola*-like spirochaetes. These phylogroups were very  
137 similar to human treponemes and therefore described as ‘-like’ (pending further taxonomic  
138 propositions) and by 16S rRNA gene phylogenetics were identical to 3/5 phylotypes identified in  
139 bovine DD lesions in the initial German study (Choi et al., 1997). Following additional  
140 characterisation, one isolated bovine DD treponeme phylogroup, the *T. putidum*/*T. denticola*-like,  
141 was designated as a new species, *Treponema pedis* (Evans et al., 2009b); the other two isolated  
142 phylogroups still require further taxonomic appraisal.

143



144 Immunohistochemistry and PCR assays targeting the three isolated phylotypes in the UK,  
145 along with German and Danish fluorescent in situ hybridisation (FISH) studies of bovine DD  
146 lesions, have identified multiple unique treponeme phylotypes together in bovine DD lesions,  
147 suggesting that the disease is polytreponemal rather than more broadly polymicrobial (Klitgaard  
148 et al., 2008; Nordhoff et al., 2008; Evans et al., 2009c). Subsequently, this hypothesis of multiple  
149 treponemal infection has been substantiated by further molecular metagenomic studies from  
150 Europe, Japan and the USA (Yano et al., 2010b; Santos et al., 2012; Klitgaard et al., 2013).  
151 Whilst similar DD treponemes have been isolated in the UK and USA; there have been some  
152 differences identified in the treponeme phylotypes highly associated with DD lesions between  
153 European countries such as Denmark and the UK suggesting regional differences in DD aetiology  
154 (Klitgaard et al., 2008; Evans et al., 2009).

155  
156 In hand with the considerable molecular evidence supporting specific treponeme  
157 phylotypes as playing an important causative role in DD, some workers have partially fulfilled  
158 Koch's postulates towards definitively demonstrating a treponemal aetiology for bovine DD.  
159 Transmission has been previously demonstrated using foot inoculation of 4-month-old naive  
160 calves with lesional material (Read and Walker, 1996) and this was repeated recently using the  
161 same protocol on yearling cattle. In the latter experiment, lesional material achieved a lesion  
162 success rate of 4/6 animals, whereas a single treponeme isolate produced an incipient DD lesion  
163 in 1/4 animals (Gomez et al., 2012), suggesting that, if the experimental conditions were more  
164 optimal, a full-blown DD lesion may have developed. When experimental lesions were studied  
165 pathologically treponemes were the primary, predominant and the deepest invaders (Read et al.  
166 1998) suggesting they play an important pathogenic role in DD. In the above described  
167 experiments, cows' feet were wrapped to reproduce conditions of prolonged moisture

168 (maceration) and reduced access to air before and after application of infectious material. Such  
169 conditions were required for transmission and could be considered to mimic the cows' feet  
170 environment in a typical dairy farm unit.

171

## 172 **Similar disease manifestations in cattle**

173 New forms of bovine foot lesions have been described that previously were considered to  
174 be non-infectious, including toe necrosis, sole ulcers and white line disease. The new forms are  
175 chronic lesions that appear refractory to conventional treatment, are patently infected with  
176 bacteria, have a raised, red stippled appearance (granulation tissue) and the same pungent smell  
177 as bovine DD lesions. These non-healing foot lesions have a high association with DD  
178 treponemes (Blowey, 2008; Evans et al., 2011a), are particularly destructive and may have an  
179 even greater economic and animal welfare cost than DD itself. Ulcerative mammary dermatitis  
180 (UMD) also has an association with DD treponemes (Read et al., 2003; Stamm et al., 2009;  
181 Evans et al., 2010) although the association does not appear to be strong, suggesting that the  
182 disease may be more polymicrobial (Evans et al., 2010).

183

## 184 **'Digital dermatitis in non-bovine species'**

185 DD has now also emerged in sheep, causing contagious ovine DD (CODD), which was  
186 first reported in the UK as severe virulent ovine footrot (Harwood et al., 1997) and, after  
187 confirmation of treponeme involvement, reclassified as CODD (Davies et al., 1999). Treponemes  
188 have been implicated in CODD through serological and molecular studies (Dhawi et al., 2005;  
189 Moore et al., 2005; Sayers et al., 2009) although *Dichelobacter nodosus* may also have a role in  
190 this disease (Moore et al., 2005; Duncan et al., 2012). Furthermore a CODD-like infection has  
191 now been reported in dairy goats in the UK (Sullivan et al., 2015) and a similar manifestation has

192 been reported in Wild Elk in Washington State, USA (Clegg et al., 2015) suggesting an even  
193 greater economic, animal welfare and global cost of this disease.

194  
195 Treponeme phylotypes involved in DD have recently been implicated in porcine ear  
196 necrosis and shoulder ulcers in Sweden (Pringle et al., 2009; Karlsson et al., 2013). It appears  
197 that DD treponemes are continually emerging into new hosts and disease presentations,  
198 suggesting these bacteria pose a much greater threat than first conceived.

199

### 200 **Host genetics, immune and inflammatory responses**

201 There has been growing interest in host genetics of bovine DD in recent years.  
202 Heritability of bovine DD has been reported to be as low as 0.039 in one study (Onyiro et al.,  
203 2008) and as high as 0.4 in another (Oberbauer et al., 2013), lending support for further  
204 heritability studies as well as genetic studies to identify loci contributing to DD susceptibility.  
205 Another study linked eight single nucleotide polymorphisms (SNPs), on three chromosomes,  
206 with dairy cattle DD susceptibility (Scholey et al., 2012). Larger studies are needed to further  
207 identify important loci and there is a need to develop diagnostic screening and targeted breeding  
208 to reduce the prevalence of DD.

209

210 Pathological and immunohistochemical investigations of bovine DD lesions are described  
211 in Table 1. Lesions consistently have large numbers of spirochaetes invading the superficial skin  
212 layers, along with necrosis (Bassett et al., 1990; Read and Walker, 1994, 1998; Dopfer et al.,  
213 1997). Infection with DD treponemes generates a strong but ineffective immune response for a  
214 relatively short duration (Demirkan et al., 1999; Trott et al., 2003), which neither resolves disease  
215 nor offers future protection.

216

217           In terms of inflammatory host responses, after in vitro exposure of bovine macrophages to  
218 bovine DD treponeme constituents, there is decreased expression of many genes associated with  
219 wound repair and immunity (Zuerner et al., 2007). Subsequent tissue culture studies have  
220 demonstrated that skin fibroblasts, but not keratinocytes, are responsive to BDD treponemes,  
221 producing macrophage elastase and RANTES, potentially important inflammatory mediators,  
222 which are also upregulated in human psoriasis (Evans et al., 2014). A host transcriptomics study  
223 of whole host lesion tissues reiterated an absence of innate immune responses in lesions. There  
224 was also increased expression of  $\alpha$ 2-macroglobulin-like 1, a protein potentially involved in  
225 bacterial immune evasion and bacterial survival, as well as increases in keratin 6A and  
226 interleukin 1 $\beta$  (Scholey et al., 2013).

227

### 228 **Molecular pathogenesis of digital dermatitis**

229           Recent work has isolated and compared commensal bovine gastrointestinal tract  
230 treponemes with bovine DD treponemes and identified these microorganisms as belonging to two  
231 large separate phylogenetic clusters (Evans et al., 2011b). Bovine DD treponemes belong to a  
232 proteolytic, serum-dependent cluster whose members have a gene encoding tissue attachment  
233 machinery and demonstrated haemolytic ability, whilst the bovine gastrointestinal treponemes  
234 belonged to a saccharolytic cluster which do not require or were inhibited by serum, do not have  
235 a gene encoding tissue attachment machinery and do not exhibit haemolysis. A previous review  
236 has detailed likely pathogenic mechanisms through comparison with human oral treponemes  
237 (Edwards et al., 2003a). These include a large number of shared cell surface proteins important  
238 for binding the host extracellular matrix (ECM). In a study of two DD treponeme strains, the

239 bacteria bound to a panel of mostly skin ECM proteins, including fibronectin, laminin, collagen  
240 type I, gelatin and keratin (Edwards et al., 2003b).

241  
242 Inflammatory dysregulation is also considered to make a key contribution to treponemal  
243 pathogenesis (Radolf et al., 2006). As aforementioned, the treponemes appear to allow immune  
244 evasion by downregulating key inflammatory markers in macrophages (Zuerner et al., 2007)  
245 whilst causing increased expression of substantial inflammatory markers in fibroblasts (Evans et  
246 al., 2014). Interestingly the treponeme-mediated increase in macrophage elastase production by  
247 fibroblasts should enable degradation of elastin, a key ECM protein responsible for skin integrity  
248 (Evans et al., 2014).

249

#### 250 **Infection reservoirs and risk factors**

251 In a comprehensive PCR study of the presence of bovine DD treponemes in the dairy  
252 farm environment, in bovine tissues and gastrointestinal tract content, DD treponemes were  
253 occasionally present in two non-pedal bovine regions, the oral cavity (14.3% of cattle tested) and  
254 the rectum (14.8% of cattle tested) (Evans et al., 2012b). Interestingly, single phylotypes were  
255 detected in the oral cavity, whilst two rectal tissues yielded DNA from multiple DD treponeme  
256 phylotypes. In contrast, all farm environmental samples, including faeces, together with insects  
257 and gastrointestinal tract content samples, were negative using bovine DD treponeme PCR  
258 assays. Since DD treponemes were present in non-pedal tissues in only a small number of  
259 samples and animals, they do not appear to be part of the typical treponeme microbiota of the  
260 bovine gastrointestinal tract. Interestingly, there was a significant association between rectal  
261 presence of *T. phagedenis*-like DD treponemes and the housing period. Given the housing  
262 association for both carriage at the rectoanal junction and the occurrence of DD (Murray et al.,

1996; Somers et al., 2005), similarities could be drawn with *Brachyspira* spp., pathogenic spirochaetes responsible for pig dysentery which are spread by the faecal oral route, with increased infection prevalence resulting from group housing (Haggman et al., 2013; Weber et al., 2013). Further studies are required to characterise the contribution of oral and rectal carriage of DD treponemes to BDD transmission.

Given parallels with the non-venereal human skin treponematosis, yaws, where touch is implicated in transmission (Antal et al., 2002), direct skin-to-skin contact may be a major route of transmission of DD treponemes. Recently, foot trimming equipment has been implicated as an important transmission route for DD treponemes, which were identified on the knife blade both before and after disinfection in some cases (Sullivan et al., 2014). Since hoof trimming provides both a direct method of contact, as well as exposure to potentially infected cattle foot tissues, this may well be an important mechanism by which the DD treponemes gain easy access to host tissues and are able to initiate disease. The recently identified chronic necrotic horn lesions might be considered to be a direct cause of such transmission (Sullivan et al., 2014).

Another newly described transmission route may be M4.1, a chronic stage of the DD lesion with an active painful M1 focus. It has been suggested that healed M4 lesions can revert to this state because the lesion was not treated effectively in the first instance and then the infectious agents can be passed from animal to animal again (Berry et al., 2010, 2012).

These newly identified infection reservoirs give a biological basis for many of the well-established risk factors identified from epidemiology based studies (Table 2). Importantly, these infection reservoirs validate previously identified important risk factors and allow a more

287 thorough argument for better preventative farm management strategies. Indeed, these identified  
288 transmission routes can be readily targeted through farm management practices such as better  
289 hygiene and thus the results need to be heeded and utilised.

290

## 291 **Treatment and control**

292         Given the plethora of data now supporting treponemes as causal of DD, it would be  
293 reasonable to propose that treponeme targeted antibiotics, vaccines or transmission blocking may  
294 allow for reduction or even eradication of this disease.

295

### 296 *Towards effective antibiotic treatment*

297         In an excellent review of DD treatment strategies that explained both relevant research  
298 and implementation difficulties, Laven and Logue (2006) identified a lack of peer reviewed  
299 published articles describing controlled clinical trials of DD treatment and that relevant research  
300 was urgently needed (Laven and Logue, 2006). To assist decisions regarding antibiotic choice on  
301 farms, in vitro antimicrobial susceptibility data can be most useful and there have now been  
302 several such studies on DD treponemes (Evans et al., 2009a, 2012a; Yano et al., 2010a). These  
303 studies clearly identified the most effective antibiotics for use against DD treponemes as  
304 penicillin, penicillin derivatives (such as amoxicillin and ampicillin) and the macrolides  
305 erythromycin, azithromycin and gamithromycin. Treponemes were least susceptible to  
306 sulphamethoxazole, trimethoprim, cefalexin and colistin, and those antibiotics with intermediate  
307 susceptibility values included lincomycin, spectinomycin, oxytetracycline, ceftiofur and  
308 gentamicin (Evans et al., 2009a, 2012a; Yano et al., 2010a).

309

310 Since many commonly used/licensed DD antibiotic treatments have only intermediate  
311 treponeme antimicrobial susceptibility values, whilst some healing does occur likely resulting  
312 from limited activity against treponemes and destruction of secondary invaders, it is unsurprising  
313 that DD frequently recurs. Indeed the newly described DD disease stage (M4.1) is specifically  
314 associated with the topically applied, frequently used, antibiotics oxytetracycline and lincomycin  
315 (Berry et al., 2010, 2012) with intermediate DD treponeme susceptibilities (Evans et al., 2009a).  
316 Thus, M2 lesions treated with such products can result in M4 lesions which can reactivate to  
317 M4.1 lesions (Berry et al., 2010, 2012).

318  
319 In Tables 3 and 4, antibiotic clinical trials are compared with DD treponeme antimicrobial  
320 susceptibilities and treatments of human treponematoses. In agreement with DD treponeme  
321 antimicrobial susceptibility data, penicillin and azithromycin have been used effectively to treat  
322 the human treponematoses, syphilis and yaws, with nearly 100% clinical cure and little  
323 recurrence. Long acting penicillin was used to nearly eradicate yaws by the world health  
324 organisation (WHO) in the 1960s and now azithromycin is being used by the WHO to finally  
325 achieve eradication (Giacani and Lukehart, 2014).

326  
327 Given the success of human treponeme treatments with antibiotics, it has been proposed  
328 that comparable clinical trials with these antibiotics could effectively cure DD (Evans et al.,  
329 2012a). As shown in Tables 3 and 4, some known treatment approaches for human  
330 treponematoses have been repeated in cattle, although either with different application methods,  
331 shorter treatment durations or substantially reduced antibiotic quantities. These sub-optimal  
332 bovine treatment trials were likely to have been driven by growing concerns about antibiotic  
333 overuse in farm animals and to minimise the quantity of milk that needs to be discarded



334 according to relevant legislation. However, given that these treatments are very effective against  
335 human trepanematoses with little or no disease recurrence (Tables 3 and 4), the data suggests it is  
336 highly likely they should be efficacious in cattle.

337  
338 For human treponematoses, only antibiotics with the highest treponemal susceptibilities  
339 are commonly used either by systemic injection or oral administration. For DD the most common  
340 antibiotic treatment is topical with antibiotics of intermediate susceptibilities. The time at/above a  
341 microorganism's inhibitory concentration is a key pharmacodynamic parameter for  $\beta$ -lactam  
342 activity against another pathogenic spirochaete, *Borrelia burgdorferi* (Wormser and Schwartz,  
343 2009). In treatment of human treponematoses, the aim is to maintain a minimum serum penicillin  
344 concentration of 0.03 U/mL for 7-10 days in early infections (and 15-20 days in latent infections)  
345 (WHO, 1982). Aqueous procaine penicillin G (APPG) provides appropriate penicillin serum  
346 levels in humans when 600,000 U (~10,000 U/kg) a day are administered; hence, a 10 day course  
347 for syphilis is used (Table 3). However, penicillin preparations with slower absorption rates have  
348 been used preferentially in humans, as they only require single doses.

349  
350 Two preparations, procaine penicillin G in oil with aluminium monostearate (PAM) and  
351 benzanthine penicillin G (BPG), provide the correct prolonged serum penicillin dosage with  
352 single injection for treatment of the human treponematoses. Whilst PAM is no longer widely  
353 available, BPG has become the antibiotic of choice for human syphilis (2.4 million units,  
354 effective levels 3-4 weeks) and yaws (1.2 million units, effective levels, 1 week) (WHO, 1982). If  
355 these treatments and criteria are then compared with those of  $\beta$ -lactam field trials against DD, the  
356 studies of Read and Walker (1998), which showed good cure rate but substantial recurrence rate,  
357 used too short a treatment, only using 3 days of APPG or ceftiofur. It might be anticipated that, if

358 used at an appropriate concentration for longer periods (7-10 days), these treatments might  
359 provide a more optimal cure with little recurrence. In that study, the superior results of APPG  
360 over ceftiofur are in agreement with reported in vitro data, where ceftiofur requires a higher  
361 concentration to be bactericidal (Evans et al., 2012a). Further evidence supporting prolonged  
362 penicillin use is a study using cefquinome, another  $\beta$ -lactam, where a 5 day rather than 3 day  
363 treatment resulted in a more effective treatment of DD (Laven, 2006) with the authors suggesting  
364 further injectable antibiotic studies were needed and a subsequent case report which reported  
365 potential eradication with long acting ceftiofur (Bell, 2011).

366  
367 For macrolide DD studies (Table 3), systemic erythromycin (injection) was comparable  
368 with the efficacy of footbath erythromycin (Laven, 2006). However, whilst considered  
369 efficacious, erythromycin footbaths resulted in significant improvement of DD lesions in only  
370 60% of animals (Laven and Proven, 2000). In human beings, a minimum of 20 g erythromycin  
371 distributed over 10 days orally were required for effective cure of syphilis (Fernando, 1969).  
372 Subsequently, a recent study has shown azithromycin in single oral dose at 30 mg/kg has an  
373 excellent cure rate against yaws. Unfortunately, this highly efficacious dose is a three-fold  
374 increase per kg on the only erythromycin systemic trial in cattle thus far (Laven, 2006). It is clear  
375 that further trials are needed for reappraisal of antibiotic selection, application, dose, treatment  
376 time and licensing.

377  
378 If treatments are not effective on all animals on a farm, then lesion recurrence might  
379 represent reinfection or treatment failure. In syphilis and yaws, all patient contacts are treated,  
380 which is interesting if considering housed dairy cattle, where all animals might be considered  
381 contacts, especially given the high stocking densities on many farms. For treatment of yaws, the

382 WHO treats the entire population if the active case prevalence is  $> 10\%$  (WHO, 1982). Given that  
383 DD is endemic in many countries and the prevalence is typically 20-30% in each herd (Holzhauer  
384 et al., 2006; Capion et al., 2008; Barker et al., 2009; Cramer et al., 2009), this suggests whole  
385 herd treatment is required on many farms regionally to allow effective treatment and to stop  
386 reinfection. Furthermore, in human treponematoses, both clinical and serological cure are  
387 generally used in treatment criteria/outcomes (Brown, 1985; Parkes et al., 2004).

388  
389 When DD was first reported in the UK and USA, there was clear serological distinction  
390 between infected and naïve animals, even on the same farms (Walker et al., 1997; Demirkan et  
391 al., 1999). However, more recent studies suggest that all the animals in a herd are frequently  
392 exposed and seroconvert (Vink et al., 2009). Thus, if we only treat active clinical manifestations,  
393 microorganisms may be present in other animals subclinically and their serological status may  
394 indicate if treatment is recommended.

395  
396 In most studies on topical antibiotic treatments, cure rates after 1 month for topical  
397 antibiotic treatments of DD tend to be ~60-70% (Holzhauer et al., 2011). One recent study  
398 reported no significant difference between DD treatment with lincomycin or oxytetracycline (73  
399 and 68% cure, respectively) (Berry et al., 2010). Whilst this could be considered efficacious, this  
400 is not comparable to success rates in treating human treponematoses. Furthermore, rates of  
401 recurrence of ~50% have been reported in cattle with DD during 12 month follow up (Berry et  
402 al., 2012). In addition, the underlying tissue pathology of many considered cured lesions at 1  
403 month after treatment were suggestive of disease reinitiation (Berry et al., 2010).

404

405           Clearly, current antibiotic use with only intermediate susceptibilities from topical (not  
406 systemic) applications will only maintain DD lesions, since they are not properly treated and are  
407 likely to result in maintenance of an M4/M4.1 infection reservoir and therefore increase disease  
408 spread. Historically, it is understandable how topical antibiotics have come to be commonly used,  
409 as they demonstrate some efficacy, are not as environmentally polluting as footbaths and, at least  
410 for oxytetracycline, are not considered to enter the animal's milk (Britt et al., 1999). However,  
411 given the general failure of current treatments to reduce DD on farms, resulting in the disease's  
412 endemic status in many countries globally, clinical trials are needed to demonstrate whether the  
413 common treatments for human treponematoses may work, even if they do not immediately  
414 translate to actual farm use due to milk withdrawal times. From such a benchmark, there could  
415 then be development of novel antibiotics not entering the animal's milk supply that are effective  
416 against DD. Given the continual use of relatively ineffective antibiotic treatments and how much  
417 DD costs financially, if an eradication program farm by farm or region by region could be  
418 considered, this might be the best way to control this disease and, in the long term, would be  
419 more beneficial for environment, animal welfare and economically. However, given the WHO  
420 includes macrolides as a 'highest priority' critically important antimicrobial (CIA) for human  
421 medicine and penicillin as a CIA also (WHO, 2011), the veterinary antimicrobial prescriber  
422 currently faces a substantial challenge in terms of the routine treatment of DD with these most  
423 relevant antimicrobials.

424

#### 425 *Non-antibiotic control strategies*

426           A bacterin vaccine was developed based on a two treponeme phylotypes, which showed  
427 initial promise but subsequently produced poorer results and was withdrawn from market (Keil et  
428 al., 2002; Ertze et al., 2006). As we now know, DD lesions typically contain several ( $\geq 3$ -5)

429 treponeme phylotypes (Klitgaard et al., 2008; Nordhoff et al., 2008; Evans et al., 2009c), so it is  
430 maybe not surprising that a dual phylotype vaccine failed. Given that some phylotypes remain  
431 uncultivable, the use of targeted gene sequencing and recombinant vaccine production could  
432 allow for a multivalent vaccine to be produced in the near future that is representative of multiple  
433 treponeme phylotypes and therefore is more efficacious. However, given that it has been shown  
434 that four *T. phagedenis*-like isolates from DD lesions varied in virulence and antigenicity *in vivo*  
435 (Elliott et al., 2007) further studies are needed to characterise such diversity to ensure that any  
436 vaccine candidate identified would allow for effective DD protection.

437  
438         Towards using farm management to control DD, when taking risk factors (Table 2) into  
439 consideration, many issues need to be addressed, including better on-farm housing hygiene,  
440 access to pasture, a balanced diet, regular footbathing and hoof trimming with appropriately  
441 disinfected equipment. Purchasing new stock from herds with no history of DD or ensuring  
442 bought animals have no evidence of DD, whether active or healed, would help to reduce the risk.  
443 Further characterisation of infection reservoirs and transmission routes in the future may allow  
444 for even better farm management of DD or even removal of specific infection reservoirs.

445  
446         Worries about antibiotic use in farm animals means that footbaths have been developed to  
447 offer an alternative and their use is associated with a reduced risk of DD (Rodriguez-Lainz et al.,  
448 1999). Given the lack of tissue penetration, it is unlikely footbaths can be used to eradicate DD;  
449 however, together with other farm management practices, they may help to reduce transmission  
450 and allow some healing. Table 5 shows a selection of commonly used footbath solutions with  
451 associated efficacies. Copper sulphate is widely used and does show some efficacy. In one study,  
452 copper sulphate was more effective than formalin (Teixeira et al., 2010), whilst another

453 demonstrated a comparable cure rate for both, but copper sulphate reduced risk of development  
454 of new lesions (Holzhauer et al., 2012). More frequent treatment with copper sulphate has been  
455 reported to increase efficacy (Speijers et al., 2012), as does treatment for a longer duration  
456 (Logue et al., 2012).

457  
458         Unfortunately, the widely used footbath chemicals copper sulphate and formalin are  
459 environmentally damaging and carcinogenic respectively, with legislative bodies beginning to  
460 ban/limit their use for these reasons, suggesting that they may have limited future capability. As a  
461 result, several alternative products have been developed, some of which are comparable in  
462 efficacy to copper sulphate (Teixeira et al., 2010; Smith et al., 2014). There is little data provided  
463 on recurrence of DD with the use of footbaths (Table 5) and continual regular use is required to  
464 keep the prevalence of DD as low as possible (Blowey, 2010). Interestingly, an in vitro method  
465 has been developed to assay footbath solutions against DD treponemes (Hartshorn et al., 2013).  
466 This study assesses the interaction of footbath solutions with both faeces and microbes, and could  
467 be a useful tool in future product development. Interestingly, the efficacy of copper sulphate was  
468 severely diminished in the presence of 20% manure, with several alternatives performing better  
469 under this condition.

470  
471         A variety of non-antimicrobial topical treatments other than footbath solutions have been  
472 discussed in a previous relevant review, with many performing no better than tap water (Laven  
473 and Logue, 2006) and the most efficacious being very labour intensive; for example triplicate  
474 application for 21 days (Britt et al., 1996). Obviously, for any practical treatment, minimal labour  
475 is required. Table 5 details recent studies showing the efficacy of several solutions with  
476 corresponding regimens. Several treatments now give comparable or better results than topical

477 antibiotic spray. Unfortunately, follow up studies are not described for the majority of studies and  
478 recurrence may be considerable given tissue penetration problems with topical applications.  
479 Whilst most topical treatments involve antiseptics (Table 5), more recently there have been novel  
480 approaches, such inducing sloughing of necrotic skin with salicylic acid (Schultz and Capion,  
481 2013) or increasing the general hygiene of cows with specifically designed washing systems  
482 (Thomsen et al., 2012).

483

#### 484 **Conclusions**

485 Bovine DD has been with us for at least 30 years and, unless drastic changes to treatment  
486 and control can be made, it is here to stay. Given associated animal welfare issues and substantial  
487 costs to farmers and the wider economy, substantially more research is needed into this severe  
488 infectious disease towards eradication. Studies into appropriate systemic antibiotic use might  
489 allow for eradication and stop the continued overuse of less efficacious antibiotics and  
490 environmentally damaging footbath solutions. Further studies are needed worldwide to allow for  
491 more comprehensive investigations of infection reservoirs and transmission studies. Genomics  
492 and proteomics studies are needed to identify relevant vaccine candidates. In the future,  
493 hopefully, we may be able to finally prevent this disease by vaccines, good farm practice and/or  
494 effective treatment.

495

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500

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502           None of the authors of this paper has a financial or personal relationship with other people  
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504

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1056 **Table 1**  
 1057 Current bovine digital dermatitis scoring system with pathological descriptions.  
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Risk factors	Macroscopic description	Underlying pathology
M1	A small focal active red/grey circumscribed lesion < 2 cm wide with 1 mm wide red foci (Dopfer et al., 1997)	Partial epithelium loss; tissue degradation with fibrin islands; hyperplastic stratum corneum; acanthotic stratum spinosum; dermal perivascular infiltration with neutrophils and mononuclear cells located in the dermis and epidermis (Read and Walker, 1994, 1998; Dopfer et al., 1997)
M2	A painful large ulcerative, red/grey active lesion > 2 cm wide (Dopfer et al., 1997)	Stratum corneum now absent; haemorrhages at lesion edge; increased degradation and acanthosis; rete ridge formation; microabscesses; increased dermal perivascular infiltration; in the epidermis there are now mostly eosinophils and neutrophils (Read and Walker, 1994, 1998; Dopfer et al., 1997)
M3	A healing, painless brown scab; typically seen after treatment (Dopfer et al., 1997)	Not determined
M4	A chronic stage presenting as dyskeratosis or irregular proliferative hyperkeratotic overgrowths (Dopfer et al., 1997)	Highly proliferative epidermis with rete ridge formation and pronounced stratum corneum hyperplasia, acanthosis of the stratum spinosum; numerous horny columns surrounded by haemorrhages/cell detritus; stratum granulosum shows empty vacuoles, more neutrophils than mononuclear cells in epidermis, many plasma cells in dermis (Read and Walker, 1994, 1998; Dopfer et al., 1997)
M4.1	A chronic stage with active, painful M1 focus (Berry et al., 2012)	Not determined
M5 or M0	Healthy skin with no evidence of previous lesion (Berry et al., 2012)	Not determined

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1061 **Table 2**  
 1062 Identified risk factors for bovine digital dermatitis (DD), with underlying pathobiological basis.  
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Risk factors	Association with DD presence	Underlying pathobiological basis
Foot hygiene	Increased muddiness of environment associated with an increased risk of DD compared with less muddy environment (Rodríguez-Lainz et al., 1996a; Rodríguez-Lainz et al., 1996b).	Required maceration (continual exposure to moisture) of skin required to transmit DD in experimental model (Gomez et al., 2012; Read and Walker, 1996). Presence of digital dermatitis treponemes in the bovine GI tract suggests faecal shedding (Evans et al., 2012b)
	Increased animal hygiene associated with a reduced risk of DD (Hultgren and Bergsten, 2001) Footbathing reduces the risk of DD compared to farms that do not footbath (Rodríguez-Lainz et al., 1999)	As above Decreases exposure of feet to unhygienic conditions. As above
Claw trimming	Lack of washing hoof trimming equipment between cows increased the risk of DD (Wells et al., 1999)	Disease considered highly contagious and transmitted through hoof trimming equipment (Wells et al., 1999). Presence of DD treponemes identified frequently on hoof trimming equipment (Sullivan et al., 2014a)
	Use of a primary hoof trimmer who trimmed cows hooves on other operations increases the risk of DD (Wells et al., 1999)	Disease considered highly contagious and transmitted through hoof trimming equipment (Wells et al., 1999). Presence of DD treponemes identified frequently on hoof trimming equipment even after disinfection in some cases (Sullivan et al., 2014a)
Housing and land access	Regular claw trimming twice a year reduces the risk of DD compared with longer durations between trimming (>7 months) (Somers et al., 2005)	Hoof trimming considered important for ruminant foot health since it reduces mechanical pressures (Toussaint Raven, 1985)
	Cows kept on pasture have a decreased risk of DD than cows housed indoors (Blowey and Sharp, 1988; and Rodríguez-Lainz et al., 1996b; Onyiro et al., 2008). Cows with daily access to pasture in winter have a decreased risk of DD compared to those animals which do not (Wells et al., 1999)	Increases exposure of cows to poor hygiene and moist foot conditions, which are a risk factor as above Increases exposure of cows to poor hygiene and moist foot conditions, which are a risk factor as above
Heifer replacement	Buying in new heifers increases the risk of DD (Rodríguez-Lainz et al., 1996; Wells et al., 1999)	Since DD is considered to be a contagious infectious disease, it can be introduced with affected heifers (Read and Walker, 1998). DD asymptomatic animals harbouring DD treponemes in rectal tissues may shed DD treponemes and therefore transmit infection (Evans et al., 2012b). Macroscopically healed lesions may reactivate and cause subsequent spread (Berry et al., 2012; Berry et al., 2010)
Flooring type	Grooved concrete > Smooth/slatted concrete > Textured concrete for increased risk of DD (Wells et al., 1999)	Abrasiveness or slipperiness of concrete (Wells et al., 1999)
	Increased risk of DD from solid floor compared to use of rubber-slatted flooring (Hultgren and Bergsten, 2001) Increased risk of DD from solid floor compared to use of slatted flooring with scraper system (Somers et al., 2005)	Improved hygiene at the rear end of cows due to optimal flooring allowing drainage of urine and faeces (Hultgren and Bergsten, 2001) Regular removal of manure reduces unhygienic moist conditions (Somers et al., 2005)
Nutrition	Cows receiving maximum concentrate supplement 2 weeks after calving are at increased risk of DD compared to those receiving their maximum concentrate supplement at 3 weeks (Somers et al., 2005)	Excessive concentrate shortly after calving may enhance the postnatal metabolic imbalance. Higher metabolic stress increases susceptibility to diseases and therefore DD (Enevoldsen et al., 1994)
	Feeding of by-products (Somers et al., 2005)	By-products are typically protein-rich and may cause an inappropriate diet with excessive protein intake identified as a potential risk factor for DD previously (Somers et al., 2005)
Parity	Animals with a body condition score too high or low have a higher risk of developing DD (Schopke et al., 2013)	Inappropriate diet weakens immune system, resulting in an increased susceptibility to infectious diseases (Schopke et al., 2013)
	First parity cows have increased risk of DD (Read and Walker, 1998; Rodríguez-Lainz et al., 1999; Somers et al., 2005)	Primiparous cows exposed to severe changes in nutrition and environment in the period surrounding calving (Somers et al., 2005) and considered to have less immunity (Blowey et al., 1994; Read and Walker, 1998)
Lactation	Increased risk of DD during lactation than dry period (Read and Walker, 1998; Murray et al., 2002; Somers et al., 2005)	Lactating cows frequently shed more liquid faeces due to diet, whereas dry cows excrete solid faeces, since they are fed a higher proportion of roughage. Liquid faeces are associated with more unhygienic and moist floor conditions (Somers et al., 2005)

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1067**Table 3**

Comparison of bovine digital dermatitis (DD) treatment clinical trials, DD treponeme antibiotic in vitro susceptibility data and known effective treatments for human trepanematoses: Systemic treatment.

DD Treatment	Response	Recurrence	MBC <sub>90</sub> <sup>a</sup>	References	Human treponematoses treatment <sup>b</sup>	Response	Recurrence	References
Aqueous procaine penicillin G, IM, 3 days, 18,000 U/kg, twice daily	Highly responsive, all (100%) healed	Yes, in 25% of cases, 7-12 weeks after healing	0.1875	Read and Walker (1998)	Early syphilis: Procaine penicillin IM 600,000 units/day once daily for 10 days	Highly responsive (100% cured)	None	Idsoe et al. (1972)
					Early syphilis: Penicillin G benzathine 2.4 MU, single injection	Highly responsive (97% cured)	1% after 2 years	Smith et al. (1956)
					Yaws: Penicillin G benzathine 1.2 MU, single injection	Highly responsive (96% cured)	1.5% after 6 months	Grin et al. (1954)
Ceftiofur sodium, IM, 3 days, 2 mg/kg, daily	Highly responsive, with 87% healed	Yes, in 27% of cases, 7-12 weeks after healing	24	Read and Walker (1998)	-	-	-	-
Cefquinome, IM, either 3 days or 5 days 1 mg/kg	5 days cefquinome lesion scores better than 3 day use or erythromycin footbath	Not reported	Not reported	Laven (2006)	-	-	-	-
Erythromycin, IM, 10 mg/kg, day 1	Lesion scores comparable to erythromycin footbath but not as good as cefquinome	Not reported	0.1875	Laven (2006)	Alternative to penicillin for early syphilis: Erythromycin, oral, 500mg per 6 hours for 10 days (total 20g)	Highly responsive (100% cured)	None	Fernando (1969)
					Penicillin alternative for early syphilis: Azithromycin, oral, 1 g initially, then 500 mg daily for 8 days	Highly responsive (100% cured)	None	Gruber et al. (2000)
					Also note recently reported penicillin alternative for yaws: Azithromycin, oral, 30 mg/kg, day 1	Highly responsive (96% cured compared to 95% in benzathine Penicillin G group)	None	Mitja et al. (2012)

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1072<sup>a</sup> Reported using in vitro susceptibility testing of DD treponemes as described by (Evans et al., 2009a, 2012a) whereby in those studies the cumulative susceptibility results across all BDD spirochaetes tested are expressed as MBC<sub>90</sub>, the concentration at which 90% of digital dermatitis associated spirochaetes are killed.<sup>b</sup> Average weight of a mature Holstein Friesian cow is 680 kg and average weight of a human being is 62 kg.

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1076**Table 4**

Comparison of bovine digital dermatitis (DD) treatment clinical trials, DD treponeme antibiotic in vitro susceptibility data and known effective treatments for human trepanematoses: Topical treatment.

DD Treatment	Response	Recurrence	MBC <sub>90</sub> <sup>a</sup>	References	Human treponematoses treatment <sup>b</sup>	Response	Recurrence	References
Oxytetracycline, single treatment with bandage, 5 mL applied at 100 g/L	Cure rate at day 29 was 13·8% for primiparous and 38·7% in multiparous cows	Not described	6	Nishikawa and Taguchi (2008)	Oxytetracycline, 2 g orally for 5 days	Highly responsive, Clinical cure at 3 months	Not described	Loughlin et al. (1951)
Oxytetracycline, day 1 with bandage, 5 mL applied at 100 g/L, treatment repeated day 6 and new bandage removed 2 days later	Cure rate 87% at 32 days after treatment	Not described	6	Manske et al. (2002)	As above			
Lincomycin (10 g) or oxytetracycline (10 g) paste, single treatment with bandage (day 1), bandage removed day 4	Cure rates 73% at day 14 and 68% at day 30; no significant difference between antibiotics	Not described but 50% of cured lesions at day 30 were histologically considered active or incipient	48 or 6	Berry et al. (2010)	-	-	-	-
Lincomycin (10 g) paste, single treatment with bandage (day 1), bandage removed day 4, cows checked and retreated monthly for 1 year	-	High recurrence rate (54%) during 1 year follow up	48	Berry et al. (2012)	-	-	-	-
Lincomycin 0.5 g/L and spectinomycin 1.0 g/L (50:50), spray once daily for 10 days	Only 1.0 g/L showed significant improvement day 12 and significant cure compared to control (66%) at days 30 and 90	Not described	48/48 and a resistant isolate (> 12,288)	Berry and Maas (1997)	-	-	-	-
Erythromycin footbath (0.035 g/L), day 1	Assessed day 4 and lameness and several lesion scores were significantly better than control	Not described	0.1875	Laven and Proven (2000)	See oral erythromycin and azithromycin above.	-	-	-

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1079 **Table 5**  
 1080 Comparison of recent non-antibiotic bovine digital dermatitis (DD) treatment clinical trials.  
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DD Treatment	Response	Recurrence <sup>a</sup>	Mode of action	References
<u>Footbath</u>				
Copper sulphate, 2% (w/v), daily for 7 days	Comparable to 2 days erythromycin footbath treatment in reducing lesion score	Not described	Bactericidal antiseptic/disinfectant	Laven and Hunt (2002)
Copper sulphate, 5% and 2% (W/V), hypochlorite 2% (W/V) weekly	Copper sulphate 5% (W/V) resulted in significantly less DD when compared with 2% (W/V) copper sulphate, hypochlorite or control	Not described	Bactericidal antiseptic/disinfectant	Speijers et al. (2010)
Formalin, 2.5% (W/V), daily for 7 days	Comparable to 2 days erythromycin footbath treatment in reducing lesion score	Not described	Bactericidal antiseptic/disinfectant	Laven and Hunt 2002)
Peracetic acid, 1% (V/V), daily for 7 days	Comparable to 2 days erythromycin footbath treatment in reducing lesion score	Not described	Bactericidal antiseptic/disinfectant	Laven and Hunt 2002)
Acidified copper sulphate, 4%, twice daily, 5 days a week; formalin 4%, 1 day each fortnight	Cure rates between treatments not different but risk of new lesions was three times less for copper sulphate	Not described	Bactericidal antiseptic/disinfectant	Holzhauser et al. (2012)
Dragonhyde, 5% (V/V), twice weekly for 4 weeks	Dragonhyde is comparable to copper sulphate and better than formalin when comparing the number of DD lesions in each group	Not described	Bactericidal antiseptic/disinfectant	Teixeira et al. (2010)
Commercial footbaths containing glutaraldehyde, organic acids or quaternary ammonium compounds, twice weekly for 8 weeks	Difference between treatment and control (in terms of cure and prevention) were not significantly different for any of the three treatments applied	Not described	Bactericidal antiseptic/disinfectant	Thomsen et al. (2008)
Organic acid footbath solution with tea tree oil, 3% (V/V), daily, 5 days a week for 9 weeks	Organic acid footbath solution with tea tree oil is comparable to copper sulphate 5% (V/V) in decreasing the proportion of M1/M2 lesions	Not described	Bactericidal antiseptic/disinfectant	Smith et al. (2014)
<u>Direct topical treatment</u>				
Salicylic acid, 10 g applied with bandage, day 1	Increased lesion healing and improvement when compared to chlortetracycline spray as a control	Not described	Sloughing of necrotic skin	Schultz and Capion 2013)
Protexin Hoof-Care (containing metallic salts and organic acids), days 1 and 4	Reduced lameness effectively; comparable to oxytetracycline spray	Not described	Bactericidal antiseptic/disinfectant	Kofler et al. (2004)
Water-based gel with activated copper and zinc chelate, 5 g applied on days 1, 3 and 7	Cure rate of DD lesions treated with gel chelate was significantly better than lesions treated with chlortetracycline	At day 7 all M2 cured but recurrence/reinfection at days 21 (2%) and 28 (8%)	Bactericidal antiseptic/disinfectant	Holzhauser et al. (2011)
Non-antimicrobial cream, day 1	Reduced DD significantly compared to control; comparable to lincomycin spray	Yes (percentage not clarified)	Bactericidal antiseptic/disinfectant	Moore et al. (2001)
Automated hoof washing with water and 0.4% soap solution, daily, for 64 days	Reduced DD significantly compared to no washing	Not described	Tackling general foot hygiene of animals	Thomsen et al. (2012)

<sup>a</sup> Recurrence in this review is whether lesions recur after treatment had finished (not during).