

High prevalence of treponemes in bovine digital dermatitis – a molecular epidemiology

Marcel Nordhoff, Annette Moter, Kirstin Schrank, Lothar H. Wieler

▶ To cite this version:

Marcel Nordhoff, Annette Moter, Kirstin Schrank, Lothar H. Wieler. High prevalence of treponemes in bovine digital dermatitis – a molecular epidemiology. Veterinary Microbiology, 2008, 131 (3-4), pp.293. 10.1016/j.vetmic.2008.04.019 . hal-00532416

HAL Id: hal-00532416 https://hal.science/hal-00532416

Submitted on 4 Nov 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Title: High prevalence of treponemes in bovine digital dermatitis – a molecular epidemiology

Authors: Marcel Nordhoff, Annette Moter, Kirstin Schrank, Lothar H. Wieler





Please cite this article as: Nordhoff, M., Moter, A., Schrank, K., Wieler, L.H., High prevalence of treponemes in bovine digital dermatitis – a molecular epidemiology, *Veterinary Microbiology* (2007), doi:10.1016/j.vetmic.2008.04.019

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1	<u>High prevalence of treponemes in bovine digital dermatitis –</u>
2	<u>a molecular epidemiology</u>
3	
4	Running title: Detection of different Treponema groups in digital dermatitis
5	Research paper
6	
7	
8	Marcel Nordhoff ¹ *, Annette Moter ² , Kirstin Schrank ¹ and Lothar H. Wieler ¹
9	
10	Institut für Mikrobiologie und Tierseuchen, FU Berlin ¹
11	Institut für Mikrobiologie und Hygiene, Charité-Universitätsmedizin Berlin ²
12	
13	
14	
15	
16	
17	
18 19 20 21 22 23 24	Correspondent address *Mailing address: Freie Universität Berlin, Fachbereich Veterinärmedizin Institut für Mikrobiologie und Tierseuchen, Philippstrasse 13, D-10115 Berlin, Germany Phone: +49 30 20936335; Fax: +49 30 20936067; e-mail: nordhoff.marcel@vetmed.fu-berlin.de
25	
26	
27	
28	Keywords: Treponema, digital dermatitis, molecular epidemiology, FISH

29 Abstract

30 To validate the epidemiology of Treponema spp. associated with digital dermatitis (DD) a 31 large number of DD samples (n = 56) were examined by DNA-DNA dot blot analyses using 32 oligonucleotide probes specific for phylogenetic group I – VII of oral treponemes and DD-33 associated phylotypes DDKL-4, DDKL-12 and DDKL-20 as well as for T. brennaborense 34 and T. socranskii. Positive hybridisation results were obtained for phylogenetic groups I, II 35 and IV and phylotypes DDKL-4 and DDKL-12. While phylotype DDKL-4 was detected in 36 100 % of the samples treponemes belonging to phylogenetic group TRE I, TRE II and 37 TRE IV were prevalent in nearly 80 % of the samples and phylotype DDKL-12 was detected 38 in 66.1 % of the samples.

39 Analysis of *Treponema* groups present concurrently in the same sample revealed that a 40 combination of TRE I - TRE II - TRE IV - DDKL-4 was most prevalent and could be 41 detected in up to 71 % of the samples. These data indicate that this combination of different 42 *Treponema* spp. seems to be the most important one in the pathogenesis of DD.

In contrast, *T. brennaborense* originally isolated from DD material this treponeme was not
detected in any of the samples clearly indicating that this species is not absolutely associated
with DD and therefore may represent only an incidental treponeme.

Fluorescence *in situ* hybridisation (FISH) obviously highlights the invasive character of DDassociated treponemes. Mainly treponemes belonging to phylogenetic group TRE I and phylotype DDKL-4 were detected in high numbers compared to the total number of bacteria and also in deeper layers of the epithelium at the transition of unaffected and affected tissue.

50 Our results confirm a high prevalence and diversity of *Treponema* spp. in DD lesions. In 51 addition, our data indicate that certain combinations of *Treponema* spp. are detected much 52 more frequently than others. Furthermore, *Treponema* spp. appear at the interface between 53 healthy and diseased tissue underlining their importance for the pathogenesis of DD.

55 Introduction

56 Although digital dermatitis (DD) was first described by Cheli and Mortellaro nearly 30 years

57 ago the exact aetiological cause of this disease still remains unclear (Cheli and Mortellaro,

58 1974; Dhawi et al., 2005).

DD is associated with unfavourable conditions in animal husbandry (Cruz et al., 2005;
Rodriguez-Lainz et al., 1999; Wells et al., 1999) and several anaerobic bacterial species have
been detected in DD lesions so far including *Porphyromonas* spp., *Prevotella* spp., *Fusobacterium* spp. and *Guggenheimella bovis* (Nattermann et al., 1998; Nordhoff and
Wieler, 2005; Schlafer et al., 2008; Wyss et al., 2005).

In addition *Treponema* (*T*.) spp. are thought to play an important role in the pathogenesis of DD (Dhawi et al., 2005; Grund et al., 1995; Logue et al., 2005). In particular FISH analyses of DD revealed that treponemes are of prime importance in the pathogenesis of DD showing high numbers of treponemes deep within the tissue (Moter et al., 1998b). Several virulence factors have been described so far for bovine and ovine isolates (Edwards et al., 2003a) and recent cell culture assays indicate that bovine *T. phagedenis*-like spirochetes impair the innate immune system as well as wound repair functions (Zuerner et al., 2007).

Culture independent 16S rRNA gene analyses of DD samples revealed five different *Treponema*-phylotypes (Choi et al., 1997). While phylotypes DDKL-3 and DDKL-13 are closely related to *T. denticola and T. vincentii*, respectively, phylotypes DDKL-12 and DDKL-20 have no known close relatives of oral treponemes. Phylotype DDKL-4 is nearly identical to *T. phagedenis*, a non-pathogenic treponeme in humans originally isolated from the urogenital tract.

Furthermore, several bovine *Treponema* isolates assigned to the *T. phagedenis*-like, *T. denticola*-like and *T. vincentii/T. medium*-like group have been cultivated from DD lesions
repeatedly while *T. brennaborense* was isolated only once (Demirkan et al., 1999; Schrank et al., 1999; Stamm et al., 2002; Walker et al., 1995).

- To examine the prevalence and diversity of different *Treponema* spp. and their role in the pathogenesis of DD a large number of acute DD lesions (n = 56) from affected cows were screened for treponemes by DNA-DNA dot blot hybridisation and FISH analyses.
- 84

85 Material and methods

86 Collection of DD biopsy samples

Biopsies were taken from cows suffering from acute digital dermatitis. A total of 56 biopsies were collected from typical DD-lesions as previously described (Schlafer et al., 2008). In brief, samples were taken from typical DD lesions of 56 affected dairy cows (Holstein Friesian breed (n = 47), Red Holstein breed (n = 5), Fleckvieh (n = 4)) originating from different farms in Saxony, Brandenburg, Berlin and Bavaria, and transported to the laboratory immediately.

93 Before sample collection DD lesions were washed softly with water to remove dirt attached to94 the skin's surface.

95

96 DNA extraction from biopsies

Biopsies were cut in to small pieces and put directly into lysis buffer (500 mM Tris-HCl, pH 98 9.0, 20 mM EDTA, 10 mM NaCl, 1% sodium dodecylsulfate [SDS]) and stored at -20°C. For 99 subsequent DNA extraction samples were thawed and proteinase K was added in a final 100 concentration of 500 μ g / ml. Incubation was performed at 56°C until lysis was completed. 101 DNA was purified by a standard phenol-chloroform extraction. After precipitation with 102 ethanol DNA was dissolved in TE buffer (10mM Tris-HCL, 1 mM EDTA, pH 7.4) and stored 103 at -20°C.

104

105

107 PCR amplification of 16S rRNA gene

108 For PCR amplification of approximately 550 bp of 16S rRNA gene the spirochete-specific 109 forward primer SPU1 (5'- GTYTTAAGCATGCAAGTCG-3', position 46-64 in E. coli 16S 110 rRNA gene) and the universal reverse primer RTU-3 (5'-GWATTACCGCGGCKGCTG-3', 111 position 518-535 in E. coli 16S rRNA gene) were used. Amplification of the partial 16S 112 rRNA gene was conducted in a final volume of 50 µl containing 2,5 mM MgCl₂, 0,2 mM 113 dNTP's, 25 pmol of each primer and 2,5 U Taq DNA polymerase (Invitrogen, Germany). 114 Samples were preheated at 94 °C for 5 min followed by amplification with 94 °C for 30 s, 56 115 °C for 30 s and 72 °C for 45 s. A total of 30 cycles was carried out with a final elongation step 116 at 72 °C for 10 min. Successful amplification was verified by agarose gelelectrophoresis.

117

118 Dot blot analysis

Dot blot analyses were carried out as previously described (Moter et al., 1998a). In brief, PCR products were spotted and cross-linked on a nylon membrane. Oligonucleotide probes (TRE I-VII) specific for the phylogenetic groups I - VII of oral treponemes were used as well as probes specific for DD-associated phylotypes DDKL-4, DDKL-12, DDKL-20 and for *T. brennaborense* and *T. socranskii*. Probes were labelled nonisotopically using the DIG labelling system (Boehringer Mannheim, Germany).

<u>/</u>(

All hybridisation steps were performed at temperatures between 54 °C to 59 °C depending on the probe used. Stringency washes were performed with washing buffer containing 5x SSC and 0.1% SDS, 2x SSC and 0.1% SDS or 0,1x SSC and 0.1% SDS at temperatures ranging from 56 °C to 62 °C. Chemiluminescence detection was done with alkaline phosphatase labelled anti-DIG antibody and CSPD according to the manufacturer's instructions. After exposure to X-film (X-OMAT AR, Kodak, Germany) from 2 to 12 h the membrane was stripped with buffer containing 0,2 M NaOH and 0,1% SDS.

To assess specificity PCR amplification products of the 16S rRNA gene obtained fromcontrol strains listed below were included in all dot blot analyses.

Additionally, PCR products from a 16S rRNA gene clone library resembling uncultivated *Treponema* phylotypes groups I – VII of oral treponemes and DD-associated phylotypes
DDKL-4, DDKL-12 and DDKL-20 were included as controls as described previously (Choi
et al., 1997; Choi et al., 1994; Moter et al., 2006).

138

139 Fluorescence in situ hybridisation (FISH)

For FISH applications probes TRE I, TRE II, TRE IV and DDK-4 were used as previously described (Moter et al., 1998b). In brief, biopsies were fixed in 4% formaldehyde in PBS, pH 7.4 and embedded in Technovit 8100 (Heraeus Kultzer, Germany) according to the manufacturer's instructions. Histological sections (4 μ m) were mounted on slides silanized with 3-aminopropyltrimethoxysilane (Sigma) and stored at 4°C.

145 FISH probes were synthesized commercially (Thermo Fisher Scientific, Germany) and 5'-146 labelled with Cy3 (indocarbocyanine) for the treponeme specific oligonucleotide probes TRE 147 I, TRE II, TRE IV and DDK-4 while FITC (fluorescein isothiocyanate) was used for labelling 148 the eubacterial probe EUB 338 as well as TRE I. FISH was performed at 46 °C in a humid 149 chamber for 4 h using 20 µl hybridisation buffer for each section (20% deionised formamide, 150 0,9 M NaCl, 20 mM Tris-HCl pH 7.4, 0,01% SDS) and 100 ng of the probes. After incubation sections were washed with distilled water and mounted with ProLong® Antifade reagent 151 152 (Molecular Probes, The Netherlands). 153 Fluorescent microscopy was performed using either an epifluorescence microscope (DMBL,

Leica) or confocal laserscanning microscope (TCS SP2, Leica) for high resolution microscopy.

156

158 Control strains

- 159 The following strains were included as controls for dot blot hybridisation and FISH analyses:
- 160 T. vincentii ATCC 35580, T. denticola (ATCC 33521^T), T. maltophilum (ATCC 51939^T),
- 161 T. lecithinolyticum (ATCC 700332^T), T. amylovorum (ATCC 700288^T), T. phagedenis
- 162 (Biotype Reiter), T. brennaborense (DSM 12168^T), T. socranskii ssp. socranskii (ATCC
- 163 35536^T), T. socranskii ssp. buccale (ATCC 35534^T), T. socranskii ssp. paredis (ATCC
- 164 35535^{T}), *T. pectinovorum* (ATCC 33768^T).
- 165

166 <u>Oligonucleotide sequences</u>

Sequences of probes TRE I – VII, DDK-12, DDK-20 and TBREN and TSOC, specific for *T. brennaborense* respectively *T. socranskii*, used in this study for dot blot and FISH analyses
have been described previously and have been deposited in ProbeBase, an online resource for
16S rRNA-targeted oligonucleotide probes where probe difference alignments are available
(Loy et al., 2003; Moter et al., 1998a; Schrank, 2000).

172 As previously described probe DDK-4 matches at position 1235 (in E. coli numbering) this 173 probe was not applicable for screening PCR products generated by SPU1 and RTU-3 174 amplifying only the first 550 bp of the 16S rRNA gene. For dot blot hybridisation analyses it 175 was therefore necessary to design a probe specific for T. phagedenis-like spirochetes 176 matching within the first 550 bases of the 16S rRNA gene. Specificity of this new designed 177 probe (5'-TCATCAAGGACGCATTCCCTCA-3', position 460-481 in E. coli 16S rRNA 178 gene) for T. phagedenis-like spirochetes, designated DDK-4-B in the following, was checked 179 against all 16S rRNA gene sequences entries available in the Ribosomal Database Project II 180 (RDP) and the Genbank database using the software tool "Probe Match" which is part of the 181 RDP environment. Specificity of DDK-4-B in dot blot hybridisation analyses was ensured 182 additionally including representative 16S rRNA clones of above mentioned phylogenetic

- 183 groups of oral treponemes and DD associated phylotypes as well as several *Treponema*184 control strains
- 185
- 186 <u>Results</u>

187 Dot blot hybridization

Using the newly designed probe DDK-4-B specific for *T. phagedenis*-like spirochetes in dot blot hybridization analyses positive results were obtained only for *T. phagedenis* and phylotype DDKL-4 while the other control strains remained negative proving specificity of this probe.

192 Using primer SPU1/RTU3 amplification of the partial 16S rRNA gene was successful in all 193 samples resulting in PCR products of about 550 bp. Dot blot analyses with probes specific for the phylogenetic groups I, II and IV of oral treponemes gave positive results in up to 84 % of 194 195 the samples while probes specific for phylogenetic groups III, V, VI and VII of oral 196 treponemes and specific for T. socranskii remained negative in all samples. When 197 oligonucleotide probes specific for T. phagedenis-like spirochetes and DD-associated 198 phylotypes DDKL-12 and DDKL-20 as well as specific for T. brennaborense were applied, 199 positive results were obtained only for T. phagedenis-like spirochetes and phylotype DDKL-200 12 while phylotype DDKL-20 and T. brennaborense could not be detected by dot blot hybridization. 201

T. phagedenis-like spirochetes were most prevalent und could be detected in 100 % of the
samples. DDKL-12, in contrast, was detectable only in 66.1 %. Somewhat more prevalent
was phylogenetic group I with 83.9 %, phylogenetic group II with 80.4 % and phylogenetic
group IV of oral treponemes with 82.1 % (Fig.1).

Most of the samples were found positive for several phylogenetic groups and phylotypes simultaneously, summed up as treponemal groups in the following (Tab. 1). In 46.4 % of the samples five different treponemal groups were discovered simultaneously, while in 34.0 % of

209 the samples four different treponemal groups are still detectable. To a much lesser extent only 210 three respectively two treponemal groups are present in 7.2 % respectively 12.4 % of the 211 samples.

212 These treponemal groups detected in DD lesions could be assigned to ten different 213 combinations of treponemal groups (Tab.1). Among these combinations phylotype groups I, 214 II, IV and phylotypes DDKL-4 and DDKL-12 or the combination of phylotype groups I, II, IV and phylotype DDKL-4 were most frequently detected in 46.4 % respectively 25.0 % of 215 216 the samples. Based on these data, it is obviously that at least phylotype groups I, II, IV and 217 phylotypes DDKL-4 are most common in DD being detectable in nearly 71 % of the samples. 218 For visualizing the spatial distribution of treponemes in DD affected tissue five biopsies 219 already tested positive by dot blot hybridization for phylotype groups I, II and IV and 220 phylotype DDKL-4 were examined by FISH analyses (Fig. 2). As shown by these specific 221 oligonucleotide probes in combination with the eubacterial probe EUB338 Treponema spp. 222 represent a high proportion of the bacterial mass.

Furthermore, treponemes were not only detectable in superficial layers of the epidermis but also in deeper tissue layers. Mainly phylotype DDKL-4 and phylotype group I tend to spread in great numbers even into deeper and healthy layers of the epidermis while treponemes of phylotype group IV and II occurred to a lesser extent within the epidermis. FISH also revealed that treponemes invade the tissue penetrating yet uninfected tissue via intracellular junctions (Fig. 2)

229

230 Discussion

Recent investigations based on 16S rRNA sequence analyses revealed a high diversity of treponemes associated with DD (Choi et al., 1994; Trott et al., 2003). As cultivation of treponemes is highly sophisticated routine bacteriological techniques are presently insufficient for identification of treponemes in diagnostic samples.

Therefore dot blot hybridisation assays with a panel of treponeme specific probes were used to assess most comprehensive the treponemal epidemiology by screening a large number (n=56) of DD samples. For detection of *T. phagedenis*-like spirochetes a new probe, designated DDK-4-B, was designed and evaluated in this study for use in dot blot hybridization analysis showing specificity only for *T. phagedenis*-like treponemes.

240 By this approach we could identify several treponemal groups in DD samples. Most 241 frequently treponemes belonging to the T. phagedenis-like group (100 %) were identified 242 followed by the phylogenetic groups TRE I (83.9 %), TRE IV (82.1 %), TRE II (80.4 %) 243 while phylotype DDKL-12 was detected only in 66.1 % (Fig.1) being consistent with recent 244 studies reporting successful cultivation and characterization of T. vincentii-like and T. 245 *denticola*-like treponemes which are cultivable representative species of the phylogenetic 246 groups TRE I and TRE II as well as the frequent isolation of T. phagedenis-like treponemes 247 from DD-lesions (Demirkan et al., 1999; Stamm et al., 2002; Walker et al., 1995).

248 Additionally, in this study treponemes of group TRE IV and the DD associated phylotype 249 DDKL-12 were detected in a large number of samples (82.1 % respectively 66.1 %) by dot 250 blot hybridization. So far, no strain of both groups was successfully isolated from DD lesions 251 by cultivation. Only previously studies reported the detection of these treponemes by 252 cultivation-independent methods (Choi et al., 1994; Moter et al., 1998b). Theses findings are 253 most probably due to the fastidious character of treponemes during cultivation and the 254 difficulty in obtaining single Treponema isolates from samples associated with a mixed 255 polymicrobial flora like those associated with DD samples.

As the combination of TRE I - TRE II - TRE IV –DDKL-4 was present in up to 71 % of all samples investigated, this microbial community seems to be the most important one for the development of DD. Similar to recent findings that different bovine strains of *T. phagedenis*like spirochetes are not only antigenetic but also pathogenetically different it is also conceivable that certain combinations of *Treponema* sp. might be more pathogenic than

others and are therefore favoured in the course of infection maybe due to synergistic effects of
yet rarely known virulence or fitness mechanisms of these treponemes (Elliott et al., 2007).
This underlines the complexity of DD and the need to rule out further virulence traits of
bovine *Treponema* strains as it already has done in recent studies (Edwards et al., 2003b;
Elliott et al., 2007; Zuerner et al., 2007).
In contrast to those treponemes mentioned above, the DD associated phylotype DDKL-20 and

267 T. brennaborense, previously isolated from a DD sample, could not be detected in any of 268 these DD samples. One reason for these negative dot blot results might be that phylotype 269 DDKL-20 as well as T. brennaborense occur only in very low numbers in DD lesions below 270 the detection limit of the techniques used. It is also possible that these treponemes only occur 271 very sporadically in DD lesions and are rather environmental species like *T. bryantii* which is 272 an apathogenic treponeme belonging to the normal bacterial flora of the bovine rumen 273 (Stanton and Canale-Parola, 1980). Consequently, the significance of these treponemes in the 274 pathogenesis of DD remains questionable.

Similarly, phylogenetic groups III, V, VI and VII as well as *T. socranskii* could not be detected in our study indicating that these treponemes that were originally derived from the human oral cavity are simply not present in DD lesions or they are also not detectable by dot blot analyses due to very low numbers remaining their function in the pathogenesis of DD questionable.

Regarding dot blot and FISH results the most important *Treponema* spp. among the different *Treponema* groups detected in DD lesions seem to be the *T. phagedenis*-like group and treponemes belonging to the phylogenetic group I of oral treponemes. By FISH analyses these treponemes can not only be found in a high number of samples but also in deeper tissue layers at the transition of healthy and diseased tissue. FISH revealed that these treponemes are also present in very high numbers compared to the total bacterial count as demonstrated by the use of the universal bacterial probe EUB338. Our results underline that these treponemes have a

major pathogenic function in the aetiology of DD, also supported by recent findings suggesting that bovine *T. phagedenis*-like spirochetes impair the innate immune response and wound repair functions and resist clearance from infected tissue (Elliott et al., 2007).

Further treponemes frequently detected by dot blot hybridisation are those belonging to phylogenetic group TRE II and TRE IV. FISH analyses revealed that these treponemes occur mostly only in lower numbers similarly to results from Moter et al. (1998b). Therefore these treponemes are most likely not as important as treponemes of group TRE I and the *T*. *phagedenis* –like group.

Although phylotype DDKL-12 can be detected in 63.8 % of the samples its function in the aetiology of DD remains undetermined. As no cultivable *Treponema* sp. is available to serve as a validate control strain for evaluating FISH conditions such analyses have not been performed so far. Therefore no data about the spatial distribution and the quantity in DD lesion exist remaining the rule of this phylotype unclear.

Screening a large number of samples our results clearly indicate that DD is usually characterized by a high prevalence and diversity of *Treponema* spp. associated with DD. Furthermore, our dot blot hybridization and FISH analyses clearly suppose *Treponema* spp. to be among the most important agents in the pathogenesis of DD. Therefore future work has to be focused on the epidemiology of *Treponema* spp. associated with DD, how infection by *Treponema* spp. is initiated and on virulence traits being important for causing and maintaining DD.

307 308

309

- 312
- 313
- 314
- 315
- 316 317

318 319	References
320 321	Cheli, R., Mortellaro, C.M., 1974. La dermatite digitale del bovino. In: Int. Conf. Diseases of Cattle, Milan, Italy, pp. 208-213.
322 323 324	Choi, B.K., Nattermann, H., Grund, S., Haider, W., Gobel, U.B., 1997, Spirochetes from digital dermatitis lesions in cattle are closely related to treponemes associated with human periodontitis. Int J Syst Bacteriol 47, 175-181
325 326	Choi, B.K., Paster, B.J., Dewhirst, F.E., Gobel, U.B., 1994, Diversity of cultivable and uncultivable oral spirochetes from a patient with severe destructive periodontitis.
327 328 329	Cruz, C.E., Pescador, C.A., Nakajima, Y., Driemeier, D., 2005, Immunopathological investigations on bovine digital epidermitis. Vet Rec 157, 834-840.
330 331	Demirkan, I., Carter, S.D., Hart, C.A., Woodward, M.J., 1999, Isolation and cultivation of a spirochaete from bovine digital dermatitis. Vet Rec 145, 497-498.
332 333 334	Dhawi, A., Hart, C.A., Demirkan, I., Davies, I.H., Carter, S.D., 2005, Bovine digital dermatitis and severe virulent ovine foot rot: a common spirochaetal pathogenesis. Vet J 169, 232-241.
335 336	Edwards, A.M., Dymock, D., Jenkinson, H.F., 2003a, From tooth to hoof: treponemes in tissue-destructive diseases. J Appl Microbiol 94, 767-780.
337 338 339	Edwards, A.M., Dymock, D., Woodward, M.J., Jenkinson, H.F., 2003b, Genetic relatedness and phenotypic characteristics of Treponema associated with human periodontal tissues and ruminant foot disease. Microbiology 149, 1083-1093
340 341	Elliott, M.K., Alt, D.P., Zuerner, R.L., 2007, Lesion formation and antibody response induced by papillomatous digital dermatitis-associated spirochetes in a murine abscess model.
342 343 344	Grund, S., Nattermann, H., Horsch, F., 1995, [Electron microscopic detection of spirochetes in dermatitis digitalis of cattle]. Zentralbl Veterinarmed [B] 42, 533-542.
345 346	Logue, D.N., Offer, J.E., Laven, R.A., Ellis, W.A., 2005, Digital dermatitisthe aetiological soup. Vet J 170, 12-13.
347 348 349	 Loy, A., Horn, M., Wagner, M., 2003, probeBase: an online resource for rRNA-targeted oligonucleotide probes. Nucleic Acids Res 31, 514-516. Moter A. Hoenig C. Choi B.K. Riep B. Gobel U.B. 1998a Molecular epidemiology of
350 351 352 353	 oral treponemes associated with periodontal disease. J Clin Microbiol 36, 1399-1403. Moter, A., Leist, G., Rudolph, R., Schrank, K., Choi, B.K., Wagner, M., Gobel, U.B., 1998b, Fluorescence in situ hybridization shows spatial distribution of as yet uncultured treponemes in biopsies from digital dermatitis lesions. Microbiology 144, 2459-2467.
354 355 356 357	Moter, A., Riep, B., Haban, V., Heuner, K., Siebert, G., Berning, M., Wyss, C., Ehmke, B., Flemmig, T.F., Gobel, U.B., 2006, Molecular epidemiology of oral treponemes in patients with periodontitis and in periodontitis-resistant subjects. J Clin Microbiol 44, 3078-3085.
358 359 360	Nattermann, H., Grund, S., Ehrlich, M., Zucker, B., Choi, BK., Göbel, U.B., 1998. Zur Ätiologie und Immunprophylaxe der Dermatitis digitalis des Rindes. In: Proc. 10th Middle-European Buiatrics Congress, Siófok, pp. 74-84.
361 362 363	 Nordhoff, M., Wieler, L.H., 2005, [Incidence and significance of treponemes in animals]. Berl Munch Tierarztl Wochenschr 118, 24-36. Rodriguez-Lainz, A., Melendez-Retamal, P., Hird, D.W., Read, D.H., Walker, R.L., 1999,
364 365	Farm- and host-level risk factors for papillomatous digital dermatitis in Chilean dairy cattle. Prev Vet Med 42, 87-97.
366 367 368	U.B., Moter, A., 2008, Involvement of Guggenheimella bovis in digital dermatitis lesions of dairy cows. Vet Microbiol 128, 118-125.

369	Schrank, K., 2000. Diversity of treponemes from Dermatitis digitalis of cattle- description of
370	novel species Treponema brennaborense. PhD thesis. Freie Universität, Berlin.
371	Schrank, K., Choi, B.K., Grund, S., Moter, A., Heuner, K., Nattermann, H., Gobel, U.B.,
372	1999, Treponema brennaborense sp. nov., a novel spirochaete isolated from a dairy
373	cow suffering from digital dermatitis. Int J Syst Bacteriol 49 Pt 1, 43-50.
374	Stamm, L.V., Bergen, H.L., Walker, R.L., 2002, Molecular Typing of Papillomatous Digital
375	Dermatitis-Associated Treponema Isolates Based on Analysis of 16S-23S Ribosomal
376	DNA Intergenic Spacer Regions, J Clin Microbiol 40, 3463-3469.
377	Stanton, T.B., Canale-Parola, E., 1980, Treponema bryantii sp. nov., a rumen spirochete that
378	interacts with cellulolytic bacteria. Arch Microbiol 127, 145-156.
379	Trott, D.J., Moeller, M.R., Zuerner, R.L., Goff, J.P., Waters, W.R., Alt, D.P., Walker, R.L.,
380	Wannemuehler, M.J., 2003, Characterization of Treponema phagedenis-like
381	spirochetes isolated from papillomatous digital dermatitis lesions in dairy cattle. J Clin
382	Microbiol 41, 2522-2529.
383	Walker, R.L., Read, D.H., Loretz, K.J., Nordhausen, R.W., 1995, Spirochetes isolated from
384	dairy cattle with papillomatous digital dermatitis and interdigital dermatitis. Vet
385	Microbiol 47, 343-355.
386	Wells, S.J., Garber, L.P., Wagner, B.A., 1999, Papillomatous digital dermatitis and associated
387	risk factors in US dairy herds. Prev Vet Med 38, 11-24.
388	Wyss, C., Dewhirst, F.E., Paster, B.J., Thurnheer, T., Luginbuhl, A., 2005, Guggenheimella
389	bovis gen. nov., sp. nov., isolated from lesions of bovine dermatitis digitalis. Int J Syst
390	Evol Microbiol 55, 667-671.
391	Zuerner, R.L., Heidari, M., Elliott, M.K., Alt, D.P., Neill, J.D., 2007, Papillomatous digital
392	dermatitis spirochetes suppress the bovine macrophage innate immune response. Vet
393	Microbiol.
394	
395	
396	
397	
398	
399	
400	
401	
402	
405	
404	
405	
407	
408	
409	
410	
411	
412	
413	
414	
415	
416	
417	
418	
410	

- 420 Fig. 1: Percentage of positive samples (total number = 56) detected by dot blot hybridization
 421 using *Treponema* specific oligonucleotide probes.
- 422 423
- 424 Tab.1: Detailed distribution patterns of *Treponema* groups in biopsies. In most of the samples
 425 at least four different *Treponema* groups can be detected simultaneously indicating a
 426 very high diversity of *Treponema* spp. in digital dermatitis.
- 427 428
- 429 Fig. 2: FISH analyses of DD-bioptates using *Treponema*-specific oligonucleotides.
- Fluorescence *in situ* hybridisation of DD sections at high magnification with (a)
 DDK-4 (yellow) and the (b) universal bacterial probe EUB338 (green) respectively at
 (c) magnification. Hybridisation with (a, c) DDK-4 (yellow) and (d) TRE-I (yellow)
 show large amounts of treponemes invading deep layers of the epidermis.
- 434 In contrast treponemes (yellow) belonging to phylogenetic group II (e) and 435 phylogenetic group IV (f) are only present in lower numbers as indicated by asterisks.
- 436 Simultaneous hybridisation with (g) DDKL-4 (yellow) and (h) TRE-I (green).
- 437 Arrows indicate treponemes located at the interface of healthy and affected tissue.
- 438 Bar: 20 μm.
- 439
- 440
- 441 442





number of different treponemal groups	detailed composition of. <i>Treponema</i> spp.	prevalence (n = 56)	
5 treponemal groups:	TRE I - TREII - TREIV- DKL-4 - DDKL -12	46.4 %	
	total	: 46.4 %	
4 treponemal groups:	TRE I - TRE II - TRE IV - DDKL-4	25.0 %	
	TRE I - TRE IV - DDKL-4 - DDKL-12	3.6 %	
	TRE I - TRE II - DDKL-4 - DDKL-12	3.6%	
	TRE II - TRE IV - DDKL-4 - DDKL-12	1.8 %	
	tota	l: 34,0 %	
3 treponemal groups:	TRE I - TRE IV - DDKL-4	3.6 %	
	TRE IV - DDKL-4 - DDKL-12	1.8 %	
	TRE I - DDKL-4 - DDKL-12	1.8 %	
	tota	l: 7.2 %	
2 treponemal groups:	DDKL-4 - DDKL-12	8.8 %	
2 treponemal groups:	DDKL-4 - DDKL-12 TRE II - DDKL-4	8.8 % 3.6 %	