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Research Article

PREVALENCE OF DIGITAL DERMATITIS IN EUROPEAN BISON (*BISON BONASUS*) IN SWITZERLAND AND REFERENCE TO OTHER EUROPEAN COUNTRIES

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Abstract: In 2018, digital dermatitis (DD) associated with *Treponema* spp. was detected in a herd of European bison (EB, *Bison bonasus*) in Switzerland. This follow-up study was carried out in 2021–2022 to evaluate the DD prevalence in the Swiss EB population (n = 49) by taking photographs and biopsies of the interdigital cleft of 10 anesthetized and eight deceased animals from five out of the six Swiss EB herds. Further samples of captive animals were available from Germany (n = 4), France (n = 1), and Poland (n = 1), and of free-living individuals from Germany (n = 4) and Poland (n = 11). Except for the Polish specimens, of which only one front foot per animal was available, all four feet were examined. Overall, 120 feet from 39 animals were available. Biopsies were taken according to a standardized protocol and investigated by histopathology, *Treponema spp*. fluorescence in situ hybridization (FISH) and full-length 16S rRNA gene sequencing. In addition, a transmission electron microscopy (TEM) examination was performed on tissues of randomly selected animals (n = 7). For the Swiss population, a DD prevalence of 94.4% (72.7–99.9%) was determined. Overall, 89/120 (74.2%) feet presented with macroscopic lesions. Histopathologically, a perivascular and lymphoplasmacytic dermatitis was present in 86/119 (72.3%) of the feet. In TEM, helically coiled bacteria were identified within the tissue of four animals, whose morphology confirmed them to belong to the order *Spirochaetales*. FISH was positive in 60/116 (51.7%) samples. 16S rRNA gene sequencing revealed the presence of three *Treponema* phylotypes (PT1, PT12, and PT3), which were clearly distinct from previously reported *Treponema* spp. in cattle and other ruminant species. This study shows that DD associated with *Treponema* spp. is widespread in Swiss EB herds and in other European individuals. So far, the disease does not seem to have a significant clinical relevance for EB, as clinical signs, such as lameness were not evident.

INTRODUCTION

Digital dermatitis (DD) is an infectious foot disease that clinically presents as an inflammatory dermatitis of the foot skin, originally described in dairy cattle.[7] DD is prevalent in many countries with dairy farming due to its contagious nature and unsatisfactory responses to control programs. The within-herd prevalence is continuously increasing.[1,35] DD is currently regarded as a multifactorial disease driven by host, environmental and agent factors.[10,41] Anaerobic

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spirochetes, specifically *Treponema* spp., are consistently associated with the onset and progression of DD lesions, and several species of treponemes have been identified in DD lesions.[14,15,22] Histopathologically, DD lesions are characterized by epithelial changes including hyperplasia, degeneration and necrosis as well as by spirochetal colonization of the stratum corneum.[3,22]

Dermatitis associated with the presence of *Treponema* spp. may present in other species, such as sheep, in which it is termed contagious ovine digital dermatitis (CODD),[2,13,37] dairy goats, [38] in skin and tail lesions in pigs,[9] but also in free-ranging elk (*Cervus elaphus*),[17,42] which suggests that all cloven-hoofed animals are susceptible to DD.

In 2018, a newly discovered dermatitis of the interdigital cleft with different degrees of severity was diagnosed in all ten captive European Bison (*Bison bonasus*) also called wisent at Bern Animal Park.[18] The European bison is the largest European terrestrial mammal and has been extinct in the wild since 1927, and its reintroduction started in 1952 in Poland.[11] At present, the species is listed as near threatened according to the International Union for Conservation of Nature (IUCN) Red List, due to its general susceptibility to disease among other reasons.[33] Infectious diseases, such as bovine tuberculosis,[27] bluetongue virus (BTV)[26] infection, or malignant catarrhal fever (MCF)[40] are among the biggest threats to that species and need to be monitored carefully. European bison are conservation dependent, and rewilding of captive individuals is still regularly performed. Therefore, it is essential to rewild only healthy animals.[21] As *Treponema* spp. are known to cause DD in a wide range of ungulate species the consequences of pathogen spread through a new wildlife host species are unpredictable and must be avoided.[32]

The aim of this study was to estimate the prevalence of DD in captive European bison from Switzerland, and to compare the results with samples from captive and free-living animals from other European countries. Another important goal was to evaluate the *Treponema* species and hence the risk of disease transmission to other domestic and free-living ungulates and vice versa. The correlation between macroscopical appearance, histopathological findings, detection of *Treponema* spp. by 16S rRNA-based metagenomic analysis and fluorescent in situ hybridization (FISH) as well as by transmission electron microscopy was assessed in biopsies.

MATERIALS AND METHODS

Ethical statement

The study protocol was approved by the animal experimentation committee of the cantons of Bern, Schwyz, Vaud, and Basel-Stadt (BE10/21, 33227) in Switzerland.

Animals

Switzerland

Samples were collected in 2021 and 2022 and were available from all four feet of 18 animals out of the total population of 49 animals (37%). Five of the six Swiss European bison herds were included. All herds consisted of a breeding male and two to four breeding females and their offspring. The animals were of mixed sex and age (eight months up to 22 years) and belonged to both European bison genetic lines (Lowland-Caucasian and Lowland). Supplemental Table 1 provides detailed individual demographic, husbandry and sample collection information.

Other European countries

Samples were available from a total of 21 individuals, including captive animals from three different German animal herds (n = 4), a single French (n = 1) and a single Polish animal herd (n = 1), as well as from free-living animals in Poland (n = 11, Bieszczady Mountains) and Germany (n = 4, Wittgenstein). Samples were taken from all four feet, except for the specimens from Poland (n = 12), where only one front foot per animal was sampled. Additional sample collection information is provided in Supplemental Table 1.

Preparation

All living animals (n = 11) were observed one day prior and one day after sampling by sight to check their general and body condition as well as locomotion. Only clinically healthy animals were anesthetized for sampling. If the facility settings allowed so, the animals were isolated and fasted overnight before anesthesia was induced as described earlier.[18] As soon as the behavior and condition allowed it, the animal was released to the herd (within 15 to 30 minutes after antagonization). Two animals (No. 17A and No. 18A, Supplemental Table 1) were anesthetized using a different protocol that was consistent with the facility veterinarian's standard procedures. In case of deceased animals (n = 28), the feet were cut off immediately after death, stored and transported at +4°C to Bern, where the biopsy

Figure 1. Macroscopic appearance of the dorsal interdigital cleft in European bison (*Bison bonasus*). **A.** no lesion, score 0; **B.** focal lesion on the dorsal aspect of the digital skin, location score 1, size score 1 (<2 cm); **C.** extensive lesion involving the dorsal aspect of the digital skin and the interdigital cleft, location score 2, size score 2 (>2 cm).

samples were taken at a maximum of six days postmortem. All samples from Poland were stored in Warsaw at—20°C for a maximum of 5 months until biopsy samples were taken. Those feet were thawed for 24 hours at room temperature (21°C) before the biopsy samples were taken. The area of interest was clipped on each foot followed by cleaning of the area with tap water and drying with disposable paper. Afterwards the site to be biopsied was disinfected by application of a 70% ethanol-soaked gauze.

Clinical foot examination and classification of macroscopic lesions

Scoring of the macroscopic lesions was performed on blinded photographs by the authors (MA, SJ, and SH) using the following two scores describing the location of the lesion: (0) no lesion; (1) focal lesion on the dorsal aspect of the digital skin; (2) extensive lesion involving the dorsal aspect of the digital skin and the interdigital cleft, and the size of the lesion: (0) no lesion; (1) maximum length <2 cm; (2) maximum length ≥2 cm (Figure 1). Only the dorsal area of the digital and interdigital skin was evaluated due to insufficient quality of palmar/plantar interdigital photographs. If at least one foot of an animal reached a score of ≥1 in both macroscopical evaluation criteria the animal was defined as DD affected [macroscopical score: not affected (0) and affected (1)].

Sample collection and treatment

A total of 120 biopsy samples were collected from the center of the lesion (or in case of no lesion from the dorsal aspect of the digital skin) using a sterile biopsy punch (4 mm in diameter with a maximum depth of 7 mm) and transferred to a sterile petri dish. Each individual biopsy was longitudinally dissected into three parts using sterile forceps and #11 sterile scalpel blades. Each part was immediately placed in a different tube for 16S rRNA gene sequencing (native), histopathological (in 10% neutral buffered formalin), and electron microscopical (in 2.5% glutardialdehyde) analysis. In living animals, povidone-iodine ointment (Mundipharma, Basel 4020, Switzerland) was applied around the biopsied area, and a loose bandage was attached that dropped off within a few hours to days to prevent bacterial infection at the biopsy site. In five animals, a firm bandage was applied, and those individuals were further treated with keratolytic salicylic acid paste as described earlier.[19]

Histopathological scoring

For histological examination, biopsies were fixed in 10% neutral buffered formalin for 24 h, then trimmed, embedded in paraffin, sectioned at 4 μm and mounted on glass slides. All sections were routinely stained with hematoxylin and eosin (HE) and with Warthin-Starry stain (WS-stain) for visualization of spirochetes. The histopathological scoring of sections was performed as described by Read and Walker and modified by Klitgaard et al.[22] to allow for classification of epidermal and dermal changes. Histopathological changes commonly include focally circumscribed hyperplastic epidermis with or without parakeratotic papillomatous proliferation, loss of the stratum granulosum, and/or dermal inflammation. Scoring also took respective degrees of keratinolysis and chronic dermatitis into account. Keratinolysis was graded as follows: 0 = none; 1 = focal, 2 = multifocal, 3 = extensive. Chronic dermatitis was classified as follows: 0 = no changes present; 1 = mild perivascular, chronic, lymphoplasmacytic dermatitis; 2 = moderate perivascular,

chronic, lymphoplasmacytic dermatitis; 3 = severe perivascular, chronic, lymphoplasmacytic dermatitis. The number of spirochetes was semi-quantitatively categorized as: 0 = none visible, 1 = minimal number, 2 = moderate number, 3 = high number of spirochetes. One sample (15 S, right front) could not be evaluated due to incomplete biopsy tissue.

FISH Scoring

For blinded FISH analysis, formalin-fixed, paraffin-embedded biopsies were prepared and serial 4-μm sections were mounted on glass slides and hybridized as previously described.[34] The oligonucleotide probe used in this study included a probe specific for the genus *Treponema*[22], 5'-labeled with the isothiocyanate derivative Cy3 (Eurofins Genomics, Ebersberg, Germany). The hybridization signal was scored from 0 to 3 according to Klitgaard et al[22]: 0 = no hybridization, 1 = sparse hybridization, 2 = moderate hybridization, and 3 = strong hybridization. Four samples (5L, left hind; 11R, left front; 15S, right front; TP5, left front) had to be removed from analysis due to incomplete biopsy tissue.

Transmission electron microscopy (TEM)

Biopsies were fixed in a solution of 2.5% glutaraldehyde and 0.1 M cacodylate buffer pH 7.4 (Merck KGaA, Darmstadt 64293, Germany), washed with 0.1 M cacodylate buffer and post-fixed in 1% osmium tetroxide (Polysciences Europe GmbH, Eppelheim 69214, Germany) for two hours. An ascending series of ethanol was used for dehydration. Samples were then embedded in EPON resin with DMP 30 (Polysciences Europe GmbH) as catalyst and placed five days at 60°C for polymerization. Semi- and ultrathin sections were obtained with a Reichert-Jung Ultracut E ultra-microtome (Leica, Wetzlar 35578, Germany). Semithin sections (0.5 μm) were stained with 1% toluidine blue for 40 seconds, dried at + 60°C on an electric hotplate, and examined to select regions for ultrathin sections by light microscopy. Ultrathin sections (80 nm) were mounted on copper grids, contrasted with lead citrate and uranyless (EMS, Hatfield PA 19440, USA), and examined under the electron microscope (Philipps CM12 FEI, Eindhoven 5600, The Netherlands). A subset of randomly selected animals (n = 7) was evaluated, including a German sample (1D) and six Swiss samples from four different herds.

16S rRNA gene sequencing, bioinformatics and phylogenetic analysis

The biopsy specimens were immediately transferred to Eppendorf tubes and stored at –20°C until further processing. Whole DNA was extracted from numbered biopsy aliquots using a commercial DNeasy Blood and Tissue kit (Qiagen, Hilden 40724, Germany). Negative controls (n = 2) were used in which the DNA was replaced by water and three different positive controls were used throughout the entire process: Zymobiomics Microbial Community DNA Standard, Zymobiomics Microbial Community DNA Standard II (log distribution) and Zymobiomics HMW DNA Standard (Zymo Research, Freiburg 79110, Germany). PCR amplification of bacterial full-length 16S rRNA genes (V1—V9 regions) was done according to the PN 101-599-700 procedure and checklist document from PacBio, UK. The following primers were used: 27F[25] (5′-GCATC/barcode/AGRGTTYGATYMTGGCTCAG-3′) and 1492R[36] (5′-GCATC/barcode/RGYTACCTTGTTACGACTT-3′). The barcodes consisted of 16 nt. The barcoded amplicons were quality checked and pooled equi-volume. A SMRT bell library was generated using the SMRTbell prep kit 3.0 procedure and checklist document PN 102-359-000 from PacBio following the primer-indexed sample workflow. The libraries were sequenced by PacBio Sequel IIe with the Sequel Sequencing kit 3.0. to generate highly accurate single molecule reads (HiFi reads). All steps were performed at the Next Generation Sequencing Platform, University of Bern, Switzerland.

Hifi full-length 16S read analysis was performed with the pb-16S-nf nextflow pipeline (available at https://github.com/PacificBiosciences/HiFi-16S-workflow). In brief, reads were processed into high-quality amplicon sequence variants (ASV) using QIIME2[5] and DADA2[6] with a rarefaction depth of 10'000 reads and were taxonomically classified by the Naive-Bayes classifier function within DADA2. Taxonomical data were then manually curated in Excel (Microsoft) spreadsheets. A sample was considered *Treponema* spp. positive with at least one *Treponema*-specific read being detected.

Phylogenetic analysis of full-length 16S genes of *Treponema* phylotypes was conducted after sequence alignment with MAFFT (vers. 7.490)[20] and by the Maximum Likelihood method and Tamura-Nei model in MEGA11[39] with 1000 bootstraps.

Statistical analysis

The proportion of macroscopic lesions on animal level and the respective 95% confidence interval (CI) were calculated for the Swiss European bison population. Chi-square test (with Pearson's Chi-Square) was used to evaluate associations between the macroscopic lesions (absent versus present) and the different diagnostic tools at foot level. Wilcoxon signed rank tests (data not normally distributed, Shapiro–Wilk test, $P = 0.91$) were used to determine the differences of macroscopic scores [not affected (0) and affected (1)] between front and hind feet. The threshold level for statistical significance was set to $P < 0.05$, and $P < 0.1$ and $P > 0.05$ were interpreted as trends. All the data were analyzed using the software package NCSS 2022 (NCSS LLC, Kaysville 84037, USA).

RESULTS

Macroscopic findings

For the Swiss population, macroscopic lesions were found in 17/18 animals, leading to a prevalence of 94.4% (72.7–99.9%). Macroscopic lesions were found in 8/9 (88.9%) animals from the other investigated European herds of which all four feet were available.

At foot level, 89/120 (74.2%) feet presented with macroscopic lesions: 50/120 (41.7%) with score 1 and 39/120 (32.5%) with score 2. The size of the lesion was evaluated with score 1 in 34/120 (28.3%) and score 2 in 55/120 (45.8%) of the feet, respectively. The lesions (mean ± SD) were significantly more severe in terms of location (score 2: 2.5 ± 1.3 and 1.8 ± 1.4; $P = 0.004$) and size (score 2: 2.9 ± 1.5 and 2 ± 1.6; $P = 0.0008$) in the hind feet when compared to the front feet, whereas overall lesion occurrence was not significantly different between hind and front feet (0.9 ± 0.3 and 0.8 ± 0.4; $P = 0.08$).

Histopathological findings

For the Swiss population, histopathological changes were found in all animals (18/18, 100%), keratinolysis in 7/18 (38.9%) and spirochetes were detected in 11/18 (61.1%). Of the animals from the other European countries, histopathological changes were present in 7/9 (77.8%) animals, keratinolysis in 4/9 (44.4%) and spirochetes in 6/9 (66.7%), respectively.

At foot level, 86/119 (72.3%) biopsies presented lesions and were evaluated as follows (one sample not available for keratinolysis score, three samples not available for spirochete score): 56/119 (47.1%) with score 1, 25/119 (21%) with score 2, 5/119 (4.2%) with score 3. Keratinolysis was mostly absent (score 0 in 102/118 (86.4%)). 12/118 (10.2%) biopsies yielded score 1, 3/118 (2.5%) score 2, 1/118 (0.9%) score 3. In 74/116 (63.8%) no spirochetes were detected by WS-staining, 26/116 (22.4%) presented with score 1, 15/116 (12.9%) with score 2, and a single biopsy (0.9%) with score 3.

FISH

In situ hybridization using the oligonucleotide probe for *Treponema* spp. revealed treponemes infiltrating the epidermis in all Swiss individuals (18/18, 100%) and in 5/9 animals (55.6%) from the other European countries. At foot level, 60/116 (51.7%) samples were FISH positive: 8/116 (6.9%) yielded score 1, 23/116 (19.8%) score 2, and 29/116 (25%) score 3 (three samples were not available for scoring).

Transmission electron microscopy

Helically coiled bacteria were found in four out of seven investigated samples, belonging to animals of three different Swiss collections. Morphological characterization of these bacteria revealed a homogeneous population among the examined animals. Bacteria presented a typical spirochetal appearance (Figure 2A), consisting of an outer membrane, a coiled protoplasmic cylinder and endoflagella characteristically located in the periplasmic space (Figure 2B). Round cytoplasmic fibrils (body fibrils) could be identified in the cytoplasm, supporting the assignment of these bacteria as belonging to the *Treponema* genus.

Bacterial populations investigated by full-length 16S rRNA gene sequencing and phylogenetic analysis

All Swiss animals (n = 18) were positive for *Treponema* spp. by 16S rRNA gene sequencing. 16S rRNA gene sequencing was also positive for *Treponema* spp. in all nine animals where we had access to all feet from the other European countries. At foot level, 94/120 (78.3%) were *Treponema* positive. The proportion of 16S rRNA gene reads per genus in samples presenting with (n = 89) or without (n = 31) macroscopical lesions of DD are presented in Supplemental Table 2. In both groups *Treponema* spp. reads were the most frequently classified bacteria with a proportion of 33% and 17.3% of all reads, respectively. Sequence analysis of the full-length 16S gene reads showed that most samples presented reads with close

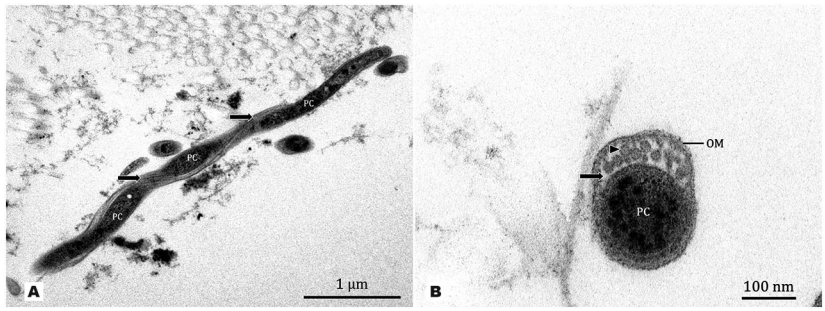

Figure 2. **A.** Ultrastructural morphology of a spirochete longitudinal section. Periplasmic endoflagella (arrows) can be seen wrapping around the coiled protoplasmic cylinder (PC). Transmission electron microscopy (TEM, magnification: 25.000 x). **B.** Transverse section of a spirochete. The endoflagella (arrowhead) are characteristically located in the periplasmic space (arrow) between the outer membrane (OM) and the protoplasmic cylinder (PC). (TEM, magnification: 140,000 x).

similarity to *Treponema* phylotype PT1 (n = 93), followed by phylotype PT12 (n = 56) and phylotype PT 3 (n = 1). Phylogenetic analysis showed that all three detected *Treponema* phylotypes are clearly distinct from previously reported *Treponema* spp. in cattle (Figure 3).

Macroscopical scores in relation to histopathological evaluation, FISH and 16S RNA gene sequencing

Chi-square analysis showed that macroscopic lesions were significantly associated with histopathological changes ($P = 0.032$), but not with

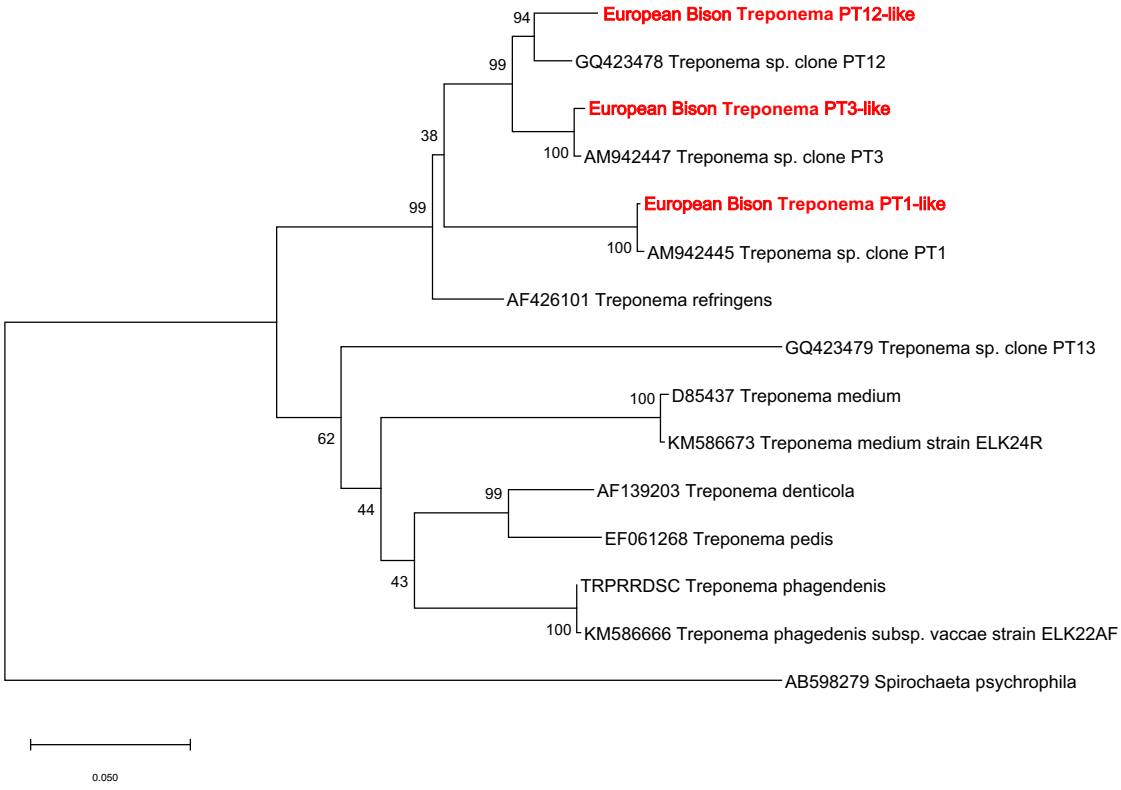

Figure 3. Phylogenetic comparison of European bison digital dermatitis associated *Treponema* 16s gene sequences. *Treponema* found in European bison samples cluster with *Treponema* phylotypes PT12, PT3 and PT1. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Genbank accession numbers are indicated for 16S full-length genes available on GenBank.

Table 1. Association between macroscopic lesions and different diagnostic tools (histopathological evaluation including keratinolysis and the amount of spirochetes, fluorescent in situ hybridization (FISH) and 16S RNA gene sequencing) from biopsies of European bison at foot level (n = 120). Chi-square tests (with Pearson's Chi-square) were used to investigate associations between the macroscopical lesions (absent versus present) and the different diagnostic tools.

		Macroscopic lesion		
Evaluation method	Score[a]	Absent n (%)	Present n (%)	*P*-value
Histopathological changes	0	4 (3.4)	29 (24.4)	0.032
	1	27 (22.7)	59 (49.6)	
Keratinolysis	0	28 (23.9)	72 (61.5)	0.371
	1	3 (2.6)	14 (12)	
Amount of spirochetes	0	23 (19.8)	51 (44)	0.159
	1	8 (6.9)	34 (29.3)	
Treponema spp. FISH	0	20 (17.2)	36 (31)	0.035
	1	11 (9.5)	49 (42.2)	
Treponema 16S rRNA gene sequencing	0	13 (10.8)	18 (15)	0.0015
	1	13 (10.8)	76 (63.3)	
Phylotype PT1	0	13 (10.8)	18 (15)	0.0026
	1	14 (11.7)	75 (62.5)	
Phylotype PT3	0	31 (25.8)	0 (0)	0.55
	1	88 (73.3)	1 (0.8)	
Phylotype PT12	0	21 (17.5)	10 (8.3)	0.062
	1	43 (35.8)	46 (38.3)	

[a] Histopathological evaluation and fluorescent in situ hybridization (FISH) scores were evaluated as absent (0) and present (1).

keratinolysis ($P = 0.371$) and the presence of spirochetes ($P = 0.159$). Furthermore, a significant association was present between macroscopic lesions and the presence of *Treponema* spp. as evaluated by FISH ($P = 0.032$) and by 16S rRNA sequencing ($P = 0.015$, Table 1).

Treponema phylotypes PT1 and PT12 were detected in both feet with and without macroscopic lesions, however, the proportion of PT1 positive feet was significantly higher in feet with lesions than in those without lesions ($P = 0.0026$, Table 1).

DISCUSSION

A representative number (18/49, corresponding to 37%) of European bison was available to assess the prevalence of DD in Switzerland. Our results show that DD associated with *Treponema* spp. is widespread in Switzerland and also frequent in other European countries. To the best of our knowledge, this is the first report of DD-associated *Treponema* spp. detection in European bison in European countries other than Switzerland.

In the routine healthcare of cattle, but also in research, DD is usually diagnosed by a clinical-macroscopic examination of the feet. PCR-based techniques and FISH have helped to determine the anaerobic spirochetes of the genus *Treponema* spp. in DD lesions.[3,16,18] Treponemes are associated with infectious foot disease in livestock and wild ruminants. In cattle, clinical manifestation is mainly present in the heel area of the feet and is classified into stages using M scores (M0–M4.1) according to the macroscopical appearance, chronicity and response to treatment.[4] In European bison, foot lesions were located in the dorsal and/or interdigital skin and varied macroscopically from focal to extensive. All the lesions detected in our study showed different macroscopic manifestation in comparison to bovine DD,[24] CODD in sheep[37,39] and DD in wild North American Elk (*Cervus elaphus*)[8] but presented very similar to our previous report in European bison.[18] Another important species difference was absence of lameness in all investigated European bison, whereas this has been commonly reported in the species mentioned above. Recently, various treponemes associated with clinical manifestation in other ruminant species have been detected in the skin of the feet of tundra caribous (*Rangifer tarandus*) without any lesions,[12] highlighting knowledge gaps about the pathogenic relevance and species differences of treponemes in various ruminants.

In cattle, DD lesions are typically located at the hind feet (~80–90%) and to a lesser degree at the front feet (~10–20%).[30] In this study, the lesions were significantly more severe in terms of location and size at the hind feet when compared to the front feet, whereas overall lesion occurrence was not significantly different between hind and front feet. This may be related to the drier and less dirty

environment around the front feet than around the hind feet as hypothesized for cattle.[43]

All samples were collected by using biopsies. Biopsy, unlike swab collection requires local, or in the case of European bison, even general anesthesia. However, in terms of sensitivity, it is the method of choice to detect *Treponema* spp. that are located in superficial and deep skin tissue. As an example, *Treponema* spp. like *T. medium* and *T. phagedenis* have been found deep inside the lesions and may correlate with the invasiveness of the disease.[29]

Multiple studies have associated *Treponema* species with DD lesions in cattle. Evans et al.[14] reported that *T. phagedenis*, *T. medium,* and *T. denticola* were present in DD lesion biopsies with a prevalence of 98%, 96.1% and 74.5%, respectively. In European bison, phylotypes PT3 and PT13 have been detected in digital lesions by partial 16S gene PCR and sequencing, which was confirmed by FISH.[18] In the present study, PT1, PT12, and PT3 were detected by full-length 16S rRNA sequencing. The finding that the phylotypes detected in this study differed from those in the earlier study were anticipated on the grounds that the PCR used in that study constitutes a target DNA amplification method, which only requires the presence of a few target molecules to generate a positive result, while the present full length 16S rRNA amplification is more precise to distinguish between different *Treponema* spp. The phylotypes detected in this study clustered together and are clearly distinct from previously reported *Treponema* spp. in cattle and other ruminant species, which may also explain the different clinical presentation of skin lesions in European bison. 16S rRNA sequencing proved to be the most sensitive method for the detection of *Treponema* spp., followed by FISH and WS-staining.

Krull et al.[23] reported an increase in *Treponema* prevalence in cattle from 0.0% in healthy foot skin to 94.3% in advanced DD lesions using deep sequencing analysis. Similar to our study, a significant association was present between macroscopic lesions and the presence of *Treponema* spp. as evaluated by 16S rRNA sequencing. However, this separation was not that clear and *Treponema* spp. were detected also in feet without lesions, which may represent a contamination only. Although an overall higher abundance of *Treponema* spp. reads in animals presenting lesions argues in favor of a causal relationship between infection and lesions, our data do not allow to draw any further conclusions on this.

The phylotype PT1 was most often detected and statistically associated with the presence of macroscopic lesions in our study, which was not the case for PT12 and PT3. *Treponema* PT1 shows a high similarity to *T. refringens* in GenBank with a sequence identity of 93% and has been associated with an early stage of the infection process of DD in cattle.[22]

The presence of *Treponema* spp. has been shown to be associated with dermatitis and necrosis of affected tissue.[31] FISH has already been applied to visualize and localize *Treponema* spp. in biopsy specimens of DD lesions in cattle[3,22,34] and in European bison.[18,19] In our study, 29 lesions were scored 3 in FISH (strong hybridization). In this case, *Treponema* spp. infiltrated deep into the epidermis. Similarly, massive and deep colonization of treponemes was found in deep parts of lesions with severe epidermal damage (score 3) at the border of healthy and affected tissue using FISH in bovine DD.[28]

CONCLUSIONS

This study showed that DD associated with *Treponema* spp. is widespread in the Swiss European bison herds and in other European individuals. The bacteriological results showed that three *Treponema* phylotypes (PT1, PT12, and PT3) are present which are clearly distinct from previously reported *Treponema* spp. in cattle and other ruminant species. Therefore, evidence of disease transmission from other ruminant species including livestock and vice versa seems unlikely. Currently, the disease does not seem to have any significant clinical relevance for this species, as clinical signs, such as lameness, were not evident in affected animals. However, due to the contagious nature of the disease, we advise controlling foot health in every European bison herd and taking measures to prevent the spread of the disease from diseased to healthy stock.

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Supplemental Table 1. Detailed individual demographic, husbandry and sample collection information of European bison.

Supplemental Table 2. Proportion of full-length 16S rRNA gene reads per bacteria genus in samples of European bison with and without macroscopical foot lesions.