Detection of specific *Treponema* species and *Dichelobacter nodosus* from digital dermatitis (Mortellaro’s disease) lesions in Swiss cattle

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**Summary**

The aim of this study was to determine the prevalence of the three *Treponema* species as well as *D. nodosus* in Digital dermatitis (DD) and slurry of Swiss cattle using PCR. A total of 86 specimens from 24 farms were enrolled in the study. Slurry samples from 21 DD-affected and one unaffected farm were collected to assess the potential of environmental transmission. Nested and real-time PCR were performed from the specimens to detect *Treponema* species and *D. nodosus*, respectively. The DD-stages were positive for at least one or more of the DD-associated *Treponema* species in 50 of 61 cases (82.0%) and in 9 of 25 cases (36.0%) in unaffected animals. Infected animals with small focal active lesions showed a significantly lower prevalence (14.8%) compared to the other DD stages (67.2%; *P*=0.011). Most prevalent was *T. phagedenis* (65.1%). *D. nodosus* was detected in 51.8% of clinical DD lesions and 24.1% in unaffected cases, but its presence was not significantly associated with the various DD-stages. All samples positive for *D. nodosus* contained the acid protease gene aprB2 but were negative for aprV2, the latter associated with virulence in sheep foot rot. Control farms were negative for all DD-associated *Treponema* species while positive for aprB2 and negative for aprV2. The presence of aprB2 suggests it is ubiquitous in the animal environment. With respect to the slurry samples, three out of 21 specimens (14.3%) were positive for one or more of the DD-associated *Treponema* species and eleven out of 21 specimens (52.4%) were positive for aprB2 and negative for aprV2 of *D. nodosus*. In conclusion, an association was found between the presence of clinical DD and specific *Treponema* species, while for *D. nodosus* no such link with DD lesions could be observed.

**Keywords:** Cattle, *Dichelobacter nodosus*, *Treponema* spp., Digital Dermatitis, PCR

**Nachweis spezifischer *Treponema* Spezies und von *Dichelobacter nodosus* aus Läsionen von *Dermatitis digitalis* (Mortellaro’sche Krankheit) bei Rindern in der Schweiz**


Am häufigsten wurde *T. phagedenis* (65,1%) nachgewiesen. *D. nodosus* wurde in 51,8% der klinischen DD-Läsionen (M1 bis M4.1) und in 24,1% der M5 Proben (klinisch gesund) nachgewiesen. Das Vorkommen der verschiedenen Treponemen korrelierte nicht mit den DD-Stadien. Alle für *D. nodosus* positiv getesteten Proben waren aprB2-positiv und negativ für aprV2.

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M. Alsaaod et al.

Introduction

Digital dermatitis (DD) (also known as Hairy Foot Warts, Strawberry Foot Rot, Mortellaro’s Disease, Raspberry or Verrucose Dermatitis) is an infectious acute or chronic ulcerative foot disease, initially reported in Italy1. DD lesions typically develop on the plantar skin, proximal to the bulb of the heel, or occasionally, within the interdigital cleft. The disease has a big impact on the well-being of animals, as well as the productivity of dairy farms. Losses are caused by high treatment costs including the application of antibiotics, decreased milk production and reduced reproductive efficiency in affected cattle2,3. Compared to other foot diseases, DD causes the highest financial impact, as the incidence rate of the clinically relevant M2 stage within a herd is high4. The average costs per case of DD were estimated to amount in total to 133 USD, made up of treatment costs (42%), followed by the consequences of decreased fertility (31%) and milk loss (27%)3. A more recent study showed that even DD lesions smaller than 2 cm in diameter can cause lameness and production losses5.

In recent years, DD has become an emerging issue in dairy herds worldwide with increasing prevalence in many countries6,7. A cross-sectional study conducted during routine claw-trimming of 1,449 Swiss dairy cows from June 2010 until February 2011 estimated the prevalence of DD to be 29.1% at the cow and 73.1% at herd level8. Poor environmental conditions of housed cattle are likely to result in increased risk of contracting DD, as continuous exposure of feet to moisture and poor hygiene conditions are considered predisposing factors for DD9,10. DD is characterized by an inflammatory dermatitis of the skin with necrosis of infected tissue11. Histopathologically, DD lesions show hyperplasia of the epidermis with hyperkeratosis, loss of the stratum corneum and/or granulosum, necrosis of the epidermis leading to ulceration, colonies of spirochaetal bacteria, and a mixed inflammation of varying severity in the dermis with exocytosis into the epidermis2,12-14. The etiology of DD is still not completely resolved. Studies on the pathogenesis of DD support the hypothesis that bacteria from the genus Treponema appear to be the most commonly identified organisms present and involved in DD lesions14,15. Treponema species are gram-negative anaerobic spirochaetes that are very difficult to culture16. Attempts to isolate Treponema from DD lesions are still mostly unsuccessful due to their fastidious nature and the high level of bacterial contamination of specimens17. However, a limited number of Treponema species from DD lesions have been successfully isolated as shown in earlier studies in the United Kingdom14,16. Isolates from DD lesions could be attributed to three distinct phylogenetic groups represented by the species Treponema pedis, Treponema phagedenis, and Treponema medium17-19. The presence of one or any combination of these three species is associated with clinical symptoms of DD as documented by several PCR-based investigations18,20.

Other bacteria, including Fusobacterium necrophorum, Porphyromonas species, Bacteroides species, Campylobacter species, Guggenheimella species, Borrelia species, and Dichelobacter nodosus, have also been found in DD lesions, suggesting a polymicrobial etiology of Treponema species and other microbes21,22.
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M. Alsaaod et al.

*D. nodosus* is the main pathogen involved in the multifactorial disease of ovine foot rot. We recently established a competitive real-time (rt)PCR distinguishing between the protease genes *aprV2* and *aprB2*, thereby allowing the direct detection and differentiation of ovine virulent and benign strains of *D. nodosus*, respectively.

Identification of the *Treponema* species associated with DD lesions in Switzerland would enable better understanding of the epidemiology of DD, facilitate efficient treatment campaigns and subsequently control DD. In the present study, we determined the prevalence of *T. pedis*, *T. phagedenis*, and *T. medium* in healthy and DD-affected animals as well as slurry from the environment of DD-positive farms in different regions of Switzerland using PCR-based methods. Farms without clinical findings of DD were included as a control group and examined using the same methodology.

**Animals, Material and Methods**

**Ethics statement**
The study protocol was approved by the animal experimentation committee of the Swiss cantons of Aargau, Basel, Bern, Fribourg, Graubünden, Jura, Luzern, Nidwalden, St. Gallen, Schwyz, Solothurn, Thurgau, Vaud, and Zürich (permission BE 62/15+).

**Collection of swab samples and DD lesion scoring**
A total of 22 farms with clinical DD (14 free-stall and 8 tie-stall) and two farms without clinical DD (one free-stall and one tie-stall) were enrolled in the study (Fig. 1) and sampled between November 2015 and June 2018. Twenty two dairy farms and 2 beef cattle farms consisting of different breeds were involved in this study. The cows were allowed daily access to pasture during the grazing season (April to October) and weekly access to an outside yard (mastic asphalt) during the winter season (November to March).

The DD lesions were scored by a trained and experienced study author (MA) according to Döpfer et al. and extended by Berry et al. Briefly, M1 (“M” refers

**Fig.1:** Geographical distribution of farms with clinical Digital Dermatitis (DD) (black squares) and farms without clinical DD (white circles) in Switzerland. The map was created with QGIS v2.8.1 (https://www.qgis.org/de/site/) using postal codes of farm locations. Postal code 6166 represents 3 farms and 4466 corresponds to 2 farms.
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M. Alsaaod et al.

A total of 86 samples were collected (M1, n=15; M2, n=19; M3, n=9; M4, n=2 and M4.1, n=16 and M5, n=25) using sterile, dry cotton swabs. The individual samples were taken by rubbing the swab over the lesion (M1 to M4.1) and from the interdigital space in cases of clinically unaffected tissue (M5).

Slurry samples (n=21) from DD-affected farms and one unaffected farm with free-stall accommodation were collected with sterile, dry cotton swabs and pooled from different locations on the floor: (i) from the floor of the drinking area, (ii) from below the cow brush, (iii) from below the concentrate feeding area and (iv) from the milking robot in cases where an automatic milking system was present. In tie-stall farms (data available only as a negative control and the corresponding positive field samples were used as positive controls. PCR products were visualized by gel electrophoresis on submarine 1% agarose gels after staining with ethidium bromide. Positive reactions were determined by the presence of bands of the appropriate sizes using 100 bp ladder as a molecular size marker.

The nested species-specific *Treponema* PCRs were done using 29 µl master-mix and 1 µl PCR product as a template from the initial reaction of 16S rRNA gene amplification (see above). Temperature cycling entailed an initial denaturation step at 95°C for 3 min, followed by 40 cycles of 95°C for 30 s, annealing for 30 s either at 68°C for *T. medium* and *T. pedis* primers, or at 64°C for *T. phagedenis* primers and extension at 72°C for 1 min. A final elongation step at 72°C for 7 min was included.

The individual swabs were immediately placed into an Eppendorf tube containing 1 ml SV lysis buffer (4 M guanidine thiocyanate, 0.01M Tris–HCl, 1% β-mercaptoethanol) for 2 min with gentle stirring. The swab was then discarded and the remaining lysate transferred to the laboratory for DNA extraction within 1 week of sampling. DNA extraction was performed from 500 µl lysate according to an adapted protocol of Stäuble et al. using a semi-automated extraction robot (KingFisher™ Duoprime, Thermo Fisher Scientific). The DNA was eluted in 60 µl H2O and stored at −20°C until further processing.

PCR assays

General *Treponema* and species-specific *Treponema* PCR.

A general *Treponema* PCR, as well as specific nested PCR assays for *T. medium*, *T. phagedenis*, and *T. pedis*, were performed according to Evans et al. with the exception of the 16S rRNA gene PCR which was performed utilizing primers 16SUNI-L and 16SUNI-R as published elsewhere. PCR master-mix contained 1× FIREPol® Master Mix Ready to load with 12.5 mM MgCl₂ (Solis Biodyne) and 0.4 mM of each primer. For general *Treponema* PCR and the initial 16S rRNA gene amplification, 2 µl DNA template was added to 28 µl master-mix. Temperature cycling for these two PCRs entailed an initial denaturation step at 95°C for 3 min followed by 35 cycles of 95°C for 30 s, annealing for 30 s at 53°C and extension at 72°C for 90 s. A final elongation step at 72°C for 7 min was included.

Detection of *D. nodosus*:

Detection and virulotyping of *D. nodosus* was done using the competitive rtPCR according to Stäuble et al. This rtPCR distinguished between the presence of the gene *aprV2* encoding the thermostable protease AprV2, and the gene *aprB2* coding for the thermostable protease AprB2 of *D. nodosus*. All rtPCR reactions were analyzed in duplicate, with a mean threshold cycle (Ct) value of <40 rated positive.

Statistical analysis

Chi-square test (with Pearson’s Chi-Square) was used to investigate associations between the presence of *Treponema* species, *aprB2*-positive *D. nodosus* and the DD status of the animals. In all analyses, an associated probability (p-value) of 0.05 was considered significant. All the data were analyzed using the software package NCSS (NCSS LLC, Kaysville, UT).
Results

DD presence on farms

In the DD positive farms (n=22), 11 farmers were aware of the disease, 8 were completely unaware of the presence of clinical DD on their farm, 2 suspected the presence of clinical DD, but were not sure and information from 1 farm was not documented and is therefore missing.

Detection of *Treponema* species and *D. nodosus*

**Feces samples**

The results of specific *Treponema* species PCR assays of DD stages M1 to M5 are shown in Table 1. The DD-stages M1 to M4.1 (affected skin) and M5 (healthy skin) were positive for at least one or more of the DD-associated *Treponema* species (50/61; (81.97%)) and (9/25; (36%)), respectively (P< 0.0001). *Treponema phagedenis* was the most prevalent species detected (65.12%). When only evaluating samples taken from clinically affected skin, M1 showed lower prevalence (14.7%) when compared to the other DD stages (M2, M3, M4, and M4.1) (67.2%) (P=0.011).

Chi-square analysis showed that the three DD-associated *Treponema* species were unequally distributed among the DD stages. Positive samples for *aprB2* of *D. nodosus* were not significantly associated with DD stages. Positive samples for at least one or more of the DD-associated *Treponema* species (2/21 [9.5%] for *T. medium* and 1/21 [4.8%] for *T. phagedenis*), while 52.4% (11/21) were positive for *aprB2* and all were negative for *aprV2* of *D. nodosus*. DD *Treponema* species and *aprB2* positive *D. nodosus* were not identified in the slurry of the unaffected DD farm.

**Slurry samples**

Three out of 21 slurry specimens (14.3%) were positive for at least one or more of the DD-associated *Treponema* species (2/21 [9.5%] for *T. medium* and 1/21 [4.8%] for *T. phagedenis*), while 52.4% (11/21) were positive for *aprB2* and all were negative for *aprV2* of *D. nodosus*. DD *Treponema* species and *aprB2* positive *D. nodosus* were not identified in the slurry of the unaffected DD farm. Of the total 107 samples tested, 87 (81.3%) showed consistent results with the general *Treponema* PCR and the specific *Treponema* PCRs. In 10 cases (4 from the same farm with stage M5, 5 from slurry samples and one from a stage M2 animal), the general PCR was positive while all specific *Treponema* PCRs were negative. In another 10 cases (all clinical stages of DD but no slurry samples were represented) at least one of the specific PCRs was positive whereas the general *Treponema* PCR was negative.

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**Discussion**

Digital dermatitis is most commonly diagnosed by clinical foot examination. Laboratory investigations are often not conclusive due to the unclear etiology of DD and difficulties when culturing *Treponema*. Fluorescent in situ hybridization (FISH) has already been applied to visualize and localize *Treponema* species. Results of the FISH analysis indicated that *Treponema* species were the predominant bacteria in the deep part of the lesions at border between affected and healthy tissue.

The use of PCR-based techniques has helped to overcome these problems and certain *Treponema* species have recently been shown to be involved in the etiology of DD. To the best of our knowledge, our study is the first to investigate the prevalence of three *Treponema* species and *D. nodosus* in DD lesions and environmental samples in Switzerland.

In addition, the present study found only two control farms without any clinical signs of DD lesions. This demonstrates that DD prevalence on farms is underestimated. Eight out of 21 farmers were not aware of the presence of clinical DD on their farm. This can be explained by the findings of Berry et al. who reported that only certain stages of DD cause pain and thus early growth stages of DD do not always present with lameness. Furthermore, detection of slight lameness is challenging for farmers.

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**Table 1: PCR detection of *T. medium*, *T. phagedenis*, *T. pedis* and *aprB2*-positive *D. nodosus* in DD specimens swabbed from lesions in Swiss cattle.**

<table>
<thead>
<tr>
<th>DD-stage</th>
<th><em>T. medium</em></th>
<th><em>T. phagedenis</em></th>
<th><em>T. pedis</em></th>
<th>Presence of at least one DD-associated species</th>
<th><em>aprB2</em>-positive <em>D. nodosus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>2/15 (13.3%)</td>
<td>9/15 (60%)</td>
<td>8/15 (53.3%)</td>
<td>9/15 (60%)</td>
<td>13/15 (86.7%)</td>
</tr>
<tr>
<td>M2</td>
<td>13/19 (68.4%)</td>
<td>15/19 (79%)</td>
<td>13/19 (68.4%)</td>
<td>16/19 (84.2%)</td>
<td>11/19 (57.9%)</td>
</tr>
<tr>
<td>M3</td>
<td>5/9 (55.6%)</td>
<td>8/9 (88.9%)</td>
<td>4/9 (44.4%)</td>
<td>8/9 (88.9%)</td>
<td>8/8 (100%)</td>
</tr>
<tr>
<td>M4</td>
<td>2/2 (100%)</td>
<td>2/2 (100%)</td>
<td>2/2 (100%)</td>
<td>2/2 (100%)</td>
<td>2/2 (100%)</td>
</tr>
<tr>
<td>M4.1</td>
<td>12/16 (75%)</td>
<td>15/16 (93.8%)</td>
<td>13/16 (81.3%)</td>
<td>15/16 (93.8%)</td>
<td>9/14 (64.3%)</td>
</tr>
<tr>
<td>M5</td>
<td>4/25 (16%)</td>
<td>7/25 (28%)</td>
<td>6/25 (24%)</td>
<td>9/25 (36%)</td>
<td>20/25 (80%)</td>
</tr>
</tbody>
</table>

| P-value | 0.00005 | 0.00009 | 0.0038 | 0.00045 | 0.1179 |

1 Macroscopic classification of bovine digital dermatitis lesions according to Döpfer et al. and extended by Berry et al.
Multiple studies have associated *Treponema* species with DD lesions in cattle\textsuperscript{12,25,19}. Evans et al.\textsuperscript{14} reported that *T. phagedeni*-like, *T. medium*/*T. vincentii*-like and *T. puti-dium*/*T. dentacole*-like were present in DD lesion biopsies with a prevalence of 98%, 96.1% and 74.5%, respectively. In our study, we used swab specimens and demonstrated the presence of at least one of the DD-associated *Treponema* species in DD lesions (82.0%) and healthy tissue (36%) using the same PCR-based approach. Swab sampling unlike the biopsy technique, does not require local anesthesia, but may only be sensitive to *Treponema* that are present on superficial tissues. *Treponema* species like *T. medium* and *T. phagedeni* have been reported to be found deep inside lesions\textsuperscript{31}. This may explain negative PCR results even in the presence of typical DD lesions as bacteria in the deep part of the lesions cannot be sampled by the swab method. Moter et al.\textsuperscript{34} reported that the presence of certain *Treponema* species may correlate with the invasiveness of the disease. More recently, Krull et al.\textsuperscript{23} reported an increase in *Treponema* prevalence from 0.0% in healthy foot skin to 94.3% in DD lesions using deep sequencing analysis and compared to a novel scoring system based on lesion morphology. Sullivan et al.\textsuperscript{32} reported that in beef cattle, at least one of the three DD-associated *Treponema* species were positive in all isolated DD lesions, while no DD-associated *Treponema* DNA was amplified from healthy foot tissues. Similar to our study, there was at least one *Treponema* species in a statistically significant proportion of DD lesions (M1 to M4.1) as compared to healthy foot tissues (M5).

Finally, the prevalence was lower in swabs of M1 lesions as compared to the other lesion stages (M2 to M4.1). This is also in agreement with Krull et al.\textsuperscript{23}, who reported relatively low abundance of *Treponema* species in the early stages of the lesions as compared to the advanced lesions, and the presence of certain *Treponema* species may correlate with the invasiveness of the disease\textsuperscript{31}.

There was good consistency between the general and the specific *Treponema* PCR with results showing more than 80% agreement. The fact that some samples representing all clinical stages were only positive with the specific but not the general *Treponema* PCRs is most probably due to the higher sensitivity of the nested specific PCRs compared to the conventional one-step general PCR. On the other hand, samples solely positive with the general *Treponema* PCR could indicate the presence of other *Treponema* species than the ones covered by the specific PCR in those samples. As these included mainly samples from slurry and stage M5 animals from a single farm, it suggests that these *Treponema* are most probably not associated with DD lesions.

*D. nodosus* was recently shown to cause interdigital dermatitis in cattle and is considered to be a major player in the pathogenesis and polymicrobial character of DD lesions\textsuperscript{22,31}. We did not detect a difference in *D. nodosus* prevalence in DD lesions versus healthy foot skin specimens, which challenges the current view on the relevance of this species in DD pathogenesis. The lack of a statistical difference of *D. nodosus* in healthy and DD lesions is most likely due to its presence in all stages of lesions. This is in agreement with Krull et al.\textsuperscript{23} and Zinicolà et al.\textsuperscript{34} who found no difference in the prevalence of *D. nodosus* between DD lesions and healthy skin specimens. Sullivan et al.\textsuperscript{32} reported that *D. nodosus* were present in 68% and 26% of beef cattle DD lesions and clinically healthy feet of beef cattle, respectively, comparable with our findings (51.8% and 24.1% in DD and healthy skin specimens, respectively). The established real-time PCR by Stäuble et al.\textsuperscript{24} allowed the virulotyping of *D. nodosus* and confirmed the absence of acid protease AprV2-positive *D. nodosus* in all samples collected in our study.

The slurry samples showed a low prevalence of DD-associated *Treponema* species (14.3%). Similarly, a work by Klitgaard et al.\textsuperscript{35}, using high-throughput sequencing, identified a small amount of bacterial DNA from DD-associated *Treponema* species in environmental samples (e.g., manure slurry) collected from dairy farms. Although the DD *Treponema* species were present in very low abundance in environmental samples, their presence may act as a source of infection\textsuperscript{35}. Evans et al.\textsuperscript{36} used quantitative PCR to test environmental and animal associated samples for the three *Treponema* species identified in DD lesions. In their study, they were unable to identify the three DD-associated *Treponema* species in environmental or fecal samples; however, several samples from the bovine rectal mucosal junction and gingiva were positive.

**Conclusions**

This study clearly demonstrated an association between DD lesions and specific *Treponema* species (*T. pedis*; *T. medium* and *T. phagedeni*), while *D. nodosus* did not have such a close link, as compared to healthy feet. In addition, acid protease AprV2-positive *D. nodosus* were not identified in any sample investigated. DD *Treponema* species were present in a low number of slurry samples, where they may act as a potential reservoir of DD treponemes. Further investigations are needed to isolate and identify various species of *Treponema* within DD lesions.

**Acknowledgments**

We thank our collaborators at the Swiss Bovine Health Service for their help during sample collection.
La Dermatite digitée (DD) chez les bovins est une maladie infectieuse podale multifactorielle, qui est devenue un problème émergent pour le bien-être animal et pour l’économie au niveau mondial. Trois espèces de *Treponema*, *T. pedis*, *T. medium* et *T. phagedenis*, sont associées avec la DD. Cependant, leur prévalence est inconnue en Suisse. Il a également été rapporté que *Dichelobacter nodosus* pouvait contribuer au développement de la DD. Le but de cette étude a été de déterminer la prévalence des trois espèces de *Treponema* ainsi que de *D. nodosus* dans des lésions de DD et du lisier de vaches suisses, en utilisant des techniques basées sur la PCR. Vingt-deux exploitations avec de la DD clinique et deux exploitations sans signes cliniques de DD ont été incluses dans l’étude. Un total de 86 échantillons de cas de DD ont été prélevés (M1, n=15; M2, n=19; M3, n=9; M4, n=2, M4.1, n=16 and M5, n=25) en utilisant des coton-tiges secs et stériles. De plus, afin d’évaluer le potentiel de transmission par l’environnement, des échantillons de lisier ont été prélevés sur des exploitations atteintes de DD (n=21) et sur une exploitation à stabulation libre exempte de DD. La PCR nichée et la PCR en temps réel ont ensuite été utilisées sur l’ADN extrait des échantillons de lisier et de l’environnement. Les associations entre la présence d’espèces de *Treponema* et de *D. nodosus* respectivement, les associations entre la présence des espèces de *Treponema* et de *D. nodosus* avec le statut DD des animaux ont été évaluées avec le test Pearson’s Chi-Square. Les stades de DD (M1 à M4.1) et M5 (pauvre sain) étaient positifs pour au moins une ou plusieurs des espèces de *Treponema* associées à la DD dans 50 de 61 cas (82.0%) et 9 de 25 cas (36.0%), respectivement. Les lésions M1 ont montré une prévalence nettement inférieure (14.8%) comparé aux autres stades de DD (M2, M3, M4 et M4.1; 67.2%; P=0.011). *T. phagedenis* était prédominant (65.1%). *D. nodosus* a été détecté dans 51.8% des lésions cliniques de DD (M1 à M4.1) et 24.1% des échantillons M5, mais sa présence n’était pas associée significativement avec les divers stades de DD. Tous les échantillons positifs pour *D. nodosus* contenaient le gène de la protéase acide aprB2, mais étaient négatifs pour aprV2, un gène associé à la virulence dans le piétin des moutons. Les exploitations de contrôle étaient négatives pour toutes les espèces de *Treponema* associées à la DD, mais positives pour aprB2 et négatives pour aprV2. La présence du gène aprB2 suggère qu’il est ubiquitaire dans l’environnement des animaux et n’est pas une association en soi avec le piétin des moutons. En ce qui concerne les échantillons de lisier, trois des 21 échantillons (14.3%) étaient positifs pour au moins 

La dermatite digitale (DD) èra un malattia infettiva multifattoriale del piede bovino che è diventata un problema economico e di benessere animale emergente a livello mondiale. Tre specie di *Treponema*, *T. pedis*, *T. medium* e *T. phagedenis* sono associate alla DD, ma la prevalenza in Svizzera rimane ancora sconosciuta. Inoltre è stato messo in evidenza che *Dichelobacter nodosus* è implicato nella patogenesi della DD. Lo scopo di questo studio era quindi quello di determinare la prevalenza delle tre specie *Treponema* e *D. nodosus* nelle lesioni di DD e nei liquami di bovini svizzeri utilizzando tecniche basate sulla PCR. Sono state arruolate nello studio 22 aziende con DD clinica e 2 aziende in cui la DD era assente. Sono stati raccolti 86 campioni di casi di DD (M1, n=15; M2, n=19; M3, n=9; M4, n=2, M4.1, n=16 e M5, n=25) utilizzando tamponi di cotone sterilizzati e ascelli. Inoltre, per valutare il potenziale di trasmissione ambientale, sono stati raccolti campioni di liquami provenienti da aziende agricole affette da DD (n=21) e da un’azienda agricola a stabulazione libera non affetta. Sono state poi effettuate “nested PCR” e “real-time PCR” sul DNA estratto dai campioni per rilevare rispettivamente le specie di *Treponema* e *D. nodosus*. Il test Chi-Quadro di Pearson è stato utilizzato per studiare le associazioni tra la presenza di specie di *Treponema* e *D. nodosus* e lo stato DD degli animali. Gli stadi DD (M1 a M4.1) e M5 (cute sana) erano positivi per almeno una o più delle specie di *Treponema* associate alla DD in 50 dei 61 casi (82.0%) e 9 dei 25 casi (36.0%), rispettivamente. Le lesioni M1 hanno mostrato una prevalenza significativamente più bassa (14.8%) rispetto agli altri stadi di DD (M2, M3, M4 e M4.1; 67.2%; P=0.011). La specie predominante era *T. phagedenis* (65.1%). *D. nodosus* è stato rilevato in 51.8% delle lesioni cliniche di DD (M1 a M4.1) e nel 24.1% dei campioni prelevati da lesioni M5, ma la sua presenza non era associata in modo significativo ai vari stadi di DD. Tutti i campioni positivi per *D. nodosus* contenevano il gene della proteasi acida aprB2, ma erano negativi per aprV2, quest’ultimo associato a virulenza nella Zoppina della pecora. Gli allevamenti di controllo sono risultati negativi per tutte le specie di *Treponema* associate alla DD, mentre sono risultati positivi per aprB2 e negativi per aprV2. La presenza di aprB2 suggerisce che questo gene sia ubiquitario nell’ambiente animale e non un’associazione di per sé con la Zoppina ovina. Per quanto riguarda i campioni di liquami, 3 dei 21 campioni (14,3%) sono risultati positivi per aprB2 e negativi per aprV2 di
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References


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