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Treponemes Detected in Digital Dermatitis Lesions in Brazilian Dairy Cattle and Possible Host Reservoirs of Infection

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The main pathogenic treponemes causing bovine digital dermatitis were identified from 17 infected herds in southern Brazil for the first time in this study using PCR. We did not find a relationship between treponeme phylogroup composition and clinical classification. *Treponema phagedenis* was present in all lesions. Rumen fluid was implicated as a reservoir location for these pathogens.

Although bovine digital dermatitis (BDD) has been known to occur in Brazil since the early 1990s (1), there have been no attempts to describe the possible pathogens involved, apart from reports of histopathological (2) and electron microscopic (3) findings. *Treponema* species are difficult to culture, so molecular approaches are useful for detecting and identifying these spirochetes (4, 5). The present study describes, for the first time, the frequencies of the major treponeme species in BDD lesions in Brazil. Similar results in Europe, North America, and Asia (6–11) confirm the polytreponemal aspect of this clinical condition.

Farms in southern Brazil where BDD infections are endemic ($n = 28$) were identified by consulting veterinary records. Previously identified dairy cows ($n = 200$) were individually reexamined and, if positive for BDD ($n = 22$), were photographed and their lesions classified according to clinical stage, from M1 to M4 (12–14). Samples ($n = 22$) were surgically excised, placed in phosphate-buffered saline (PBS) solution, and refrigerated (4°C to 8°C) until processing. The infected digit was then topically treated with antibiotics (oxytetracycline) and bandaged. Using a stomach tube, ruminal fluid (≥50 ml of fresh fluid) was collected from 15 cows from seven different BDD-positive herds, which were chosen by convenience. From one BDD-free herd with a history of lameness but without any clinical BDD lesions detected on previous examinations of the whole herd, ruminal fluid from 10 cows was sampled for use as a negative control. All samples underwent extraction of bacterial DNA, as previously described (12, 15). A nested-PCR method was used, as previously described (10). The treponeme-specific primers were called *Treponema* sp., *Treponema medium*/*T. vincentii*-like, *Treponema phagedenis*-like, and *Treponema denticola*/*T. putidum*-like (10). The research was approved by the Committee for the Ethical Use of Animals of Pontifícia Universidade Católica do Paraná (PUCPR) (registration no. 646) in 2011.

Of the herds, 17 (60.71%) had BDD lesions on the day of examination and were positive for subsequent molecular BDD detection. The lesions were in different clinical stages (14) and were classified as follows: 13.64% were M1, 45.45% were M2, 22.73% were M3, and 18.18% were M4. However, certain lesions classified as M2 or M3 contained areas that were M4 or M1. In comparison, the cows in the present study exhibited considerably more M2 lesions (45.45% versus 21.03% in the previous study) and fewer M4 lesions (18.18% versus 50.03% in the previous study) (Table 1). The reason for this observation or why the different forms can transition from one stage to another relatively quickly or in unexpected ways (14) or persist for months at the same score (16, 17) needs further investigation. Mixed M2 and M4 stages and mixed M3 and M1 stages were reported for the first time in Brazilian BDD cases.

As expected, all lesions and all samples of ruminal fluid were positive for the *Treponema* sp. primer. All treponeme groups were found in 81.82% of the lesions, whereas the *T. phagedenis*-like and *T. medium*/*T. vincentii*-like groups occurred together in 95.45% and *T. phagedenis*-like and *T. denticola*/*T. putidum*-like in 86.36% of the lesions. In 60% of the rumen fluid samples, we found at least one of the pathogenic phylogroups. In 15 rumen fluid samples, the *T. phagedenis*-like group was the most prevalent at 40%, followed by *T. medium*/*T. vincentii*-like at 33.33% and *T. denticola*/*T. putidum*-like at 26.67%. In two cows, all three phylogroups were identified in the rumen fluid. The *T. medium*/*T. vincentii*-like and *T. phagedenis*-like groups were identified together in two of the rumen fluid samples, whereas the *T. phagedenis*-like and *T. denticola*/*T. putidum*-like groups occurred alone in 13.33%, and the *T. medium*/*T. vincentii*-like group occurred in one cow (6.67%). None of the rumen fluid samples from the cows in the negative-control group were positive for pathogenic treponemes (Table 1). Although the phylogroups identified in the rumen fluid samples were also present in the lesions of the animals, not all phylogroups present in the lesions were identified in the rumen fluid. The phylogroups that were absent in the rumen fluid from BDD-positive cows were, in order of frequency, *T. denticola*/*T. putidum*-like

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TABLE 1 Treponemes detected in BDD lesions and ruminal fluid of lesional and nonlesional cows in Brazil

Cow no. or name	Result with PCR primer used for:									
	Lesions					Ruminal fluid				
	16S	*Treponema* sp.	*T. medium*/*T. vincentii*-like	*T. phagedenis*-like	*T. denticola*/*T. putidum*-like	*Treponema* sp.	*T. medium*/*T. vincentii*-like	*T. phagedenis*-like	*T. denticola*/*T. putidum*-like	M stage
Lesional cows										
355 a	+	+	+	+	−	+	−	−	−	4
821	+	+	+	+	+	+	+	+	−	4
318 a	+	+	+	+	+	NA[a]	NA	NA	NA	4
1685	+	+	+	+	+	NA	NA	NA	NA	4
764	+	+	+	+	+	+	−	−	−	3
765	+	+	+	+	+	+	−	−	−	3
779	+	+	+	+	+	+	+	+	−	3
665	+	+	+	+	+	NA	NA	NA	NA	3
2703	+	+	+	+	+	NA	NA	NA	NA	3
2645	+	+	+	+	+	+	−	−	−	2
2669	+	+	+	+	+	+	−	−	−	2
1127	+	+	+	+	+	+	−	−	+	2
2677	+	+	+	+	+	+	−	−	+	2
2683	+	+	+	+	+	+	+	+	+	2
2665	+	+	+	+	+	+	+	+	+	2
2716	+	+	+	+	+	+	+	−	−	2
2696	+	+	+	+	+	NA	NA	NA	NA	2
2700	+	+	+	+	+	NA	NA	NA	NA	2
2596	+	+	+	+	+	NA	NA	NA	NA	2
39 m	+	+	+	+	−	+	−	−	−	1
811 m	+	+	−	+	+	+	−	+	−	1
Frida	+	+	+	+	−	+	−	+	−	1
Nonlesional cows ($n = 10$)	NA	NA	NA	NA	NA	+	−	−	−	0

[a] NA, not available.

(73.33%), *T. medium*/*T. vincentii*-like (66.67%), and *T. phagedenis*-like (60%). No association was established between the clinical classification of the lesion and the presence of a specific group of pathogenic treponemes.

We successfully detected the three main treponeme phylogroups implicated in BDD from the ruminal fluid of BDD-affected animals. The testing of ruminal fluid in the present study was based on a previous study (18) in which the *T. phagedenis*-like phylogroup was isolated from the dorsal sac and reticular pillar of the rumen; however, it was successfully isolated from the rumen fluid in only one case (18). Better yields were obtained (18) from the oral cavity and rectoanal junction, leading to a hypothesis of these regions being potential contributors to the transmission of BDD beyond direct lesion-to-skin contact. We hypothesized that the liquid and rich medium of the ruminal fluid, which is in constant contact with the oral cavity during rumination, might represent the actual vehicle of transmission, particularly considering that during rumination and even eructation, particles of ingesta may fall out of the oral cavity or be lost by drooling and contaminate the bovine environment. Initial infection of the oral cavity and ruminal fluid may occur by licking a BDD wound, during which a massive amount of bacteria can easily be debrided from the wound and superficial and deeper skin layers. Different types and amounts of treponemes would then be ingested according to the temporal microbiota changes during the development of BDD lesions (19). In addition, ingested treponemes are able to invade the epithelial cells of the crypts of the cecum and colon (20), with possible exfoliation into forming stool, which may explain the isolation of treponemes at the rectoanal junction (18).

Of the BDD-positive cows from which ruminal fluid was sampled, 10 had all three main phylogroups in the lesion, but only two had the three phylogroups together in the ruminal fluid, which can possibly be explained by the fact that survival of treponemes in the ruminal fluid is time limited and that treponemes populating the superficial layers are more likely to be ingested, thereby influencing the types of treponemes entering the ruminal fluid. The ruminal environment may favor the survival of certain treponemes, because an environment rich in volatile fatty acids favors *T. phagedenis* (21). Further, the ability of treponemes to colonize different environments depends, among other factors, on the presence of periplasmic flagella for better propulsion through highly viscous or viscoelastic material (22), such as ruminal fluid and humid claw skin.

None of the three main phylogroups were present in the ruminal fluid samples from healthy animals of a noninfected herd. A future study investigating these aspects is warranted to further elucidate the epidemiology of BDD.

Three treponeme phylogroups, *T. medium*/*T. vincentii*-like, *T. phagedenis*-like, and *T. denticola*/*T. putidum*-like, were identified in Brazilian dairy-cattle herds. The polytreponemal nature of bovine dermatitis digitalis lesions was reinforced in this study, and lesions of different clinical stages can coexist in the same cow. A possible reservoir of the treponemes other than the skin lesion is the ruminal fluid.

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