

Bacterial Causes of Digital Dermatitis (DD) in Dairy Cattle

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Summary and Implications

Bovine digital dermatitis (DD) is a leading cause of lameness in dairy cattle throughout the world. Despite 35 years of research, the definitive cause of the disease process is still unknown. Previous studies have demonstrated that multiple bacterial species are associated with lesions, with spirochetes being the most reliably identified organism. This study utilized total DNA sequencing of 48 staged DD biopsy specimens collected during a 3-year longitudinal study of disease progression in dairy cattle. Over 175 million sequences were obtained and used to identify the bacterial species that were present in the biopsies. There was no evidence of a fungal or DNA viral causes. The bacterial communities present in the biopsy specimens was found to progress through a systematic series of changes that correlate with the novel visual lesion scoring system developed as part of this project. Although *Treponema* spp. predominated in the advanced lesions, they were in relatively low abundance in the newly described early lesions that are associated with the initiation of the disease process. The results of this study support the hypothesis that DD is a polybacterial disease process and provide unique insights into the temporal changes in bacterial populations throughout lesion development. These insights are expected to be critical in the development of new treatment strategies and the potential development of effective vaccines. In addition, the development and validation of a lesion scoring system will assist with determining the prognosis of a lesion.

Introduction

Bovine Digital Dermatitis, also known as hairy heel warts, strawberry footwarts, Mortellaro's disease and papillomatous digital dermatitis is an important disease process of modern dairy production. BDD is the leading cause of lameness in dairy cattle and results in significant welfare concerns as well as economic cost to the dairy industry. Due to the success of topical antibiotics therapy, the lack of evidence for a viral or fungal cause and the routine isolation of *Treponema* spp. bacteria the disease has been assumed to be bacterial in origin. However, the use of

pure culture treponemes has been unable to reproduce the disease and vaccines developed against this organism have traditionally performed poorly in reducing disease development. This has led to disagreement regarding the causative organisms of BDD and has hampered the development of organisms specific control measures and interventions (ie. vaccines etc.). Given the importance and prevalence of this disease process to the dairy cattle industry there is a critical need for an improved understanding of what bacteria are responsible for initiating and progressing the lesion development.

Traditional culture methods have been attempted on BDD samples in the past and have resulted in consistent isolation of several bacterial species including the treponemes. Recent developments in molecular microbiology have however demonstrated that less than 10% of the bacterial species present on earth can be grown in the laboratory. This knowledge suggests that additional important bacteria may be present in BDD lesions that were not identified based on traditional culture methods. To evaluate for the presence of all bacterial species we utilized a technique known as "metagenomics" to sequence the total DNA in samples collected from biopsies. By sequencing all the DNA we are able to identify what bacteria are present in a "culture-independent" method. This allows us to make a less biased evaluation of the bacterial species present in the lesions.

Materials and Methods

BDD lesions develop slowly in cattle and we hypothesized that the bacterial species present in the lesions would change as the lesions developed. In order to test this hypothesis, we first needed to collect BDD lesion biopsies from varying stages of lesion development. In order to better understand how the lesions developed we performed a 3 year prospective trial on lesion development in 60 Holstein dairy cattle. Once enrolled cattle were placed in a foot trimming chute on a regular basis (every 2-3 weeks) and digital pictures and lesion characteristics were evaluated and recorded. During this period these cattle were not subjected to any additional BDD control measures (footbath, topical treatment etc.) Over the three-year period we collected upwards of 900 skin biopsies and 10,000 digital images of lesion development in these 60 cattle, from normal skin to severe active lesions. Using these pictures we developed a novel lesion scoring system that identifies earlier stage DD lesions than previously described. The novel scoring system also sub-classifies two distinctly different early stage lesion progressions.

Once the lesion staging systems were developed and validated on the database to consistently represent the normal progression of lesion development, we selected 48 representative biopsies from our database for DNA sequencing. These biopsies were selected due to their

characteristic appearance for a given stage of development as well as confirmation that the lesion stages prior and after the current stage were observed in the database. Total DNA was extracted from the biopsy samples and subjected to 16S based phylogenomic evaluation using the V3-V4 region of the 16S gene as a “nametag” for the bacterial isolate present. Following sequencing, the bacterial communities were evaluated and compared using Qiime bioinformatics software.

Results and Discussion

The Iowa State Digital Dermatitis Lesion Scoring System developed as part of this project is summarized in figure 1. Briefly, stage 0 represents normal skin. Early BDD lesions are divided into two distinct “pre-lesion” progressions with the “A” type lesions representing a non-proliferative dermatitis that progresses to an erosive lesion of the interdigital fold. Type “B” lesions produce conspicuous proliferative scabs that are more diffusely spread across the heel. Both pre-lesions progress to converge into a shared stage 3 lesion that is the classic and active erosive DD lesion of the interdigital fold. Progression of these lesions is characterized by the development of papillomatous dermal pegs associated with stage 4 lesions. An additional stage 5 lesion was utilized in this study for simplicity and represents BDD lesions that were subjected to topical tetracycline therapy 9 days prior to biopsy. This lesion scoring system allows for the earlier identification of BDD lesions compared to previous scoring systems and has been validated on a large database of lesions as being representative of the true progression of DD lesions.

Sequencing of the DNA from the 48 biopsies confirmed the presence of complex bacterial communities composed of upwards of 100 bacterial species present in the lesions. The relative abundance of the top 12 bacterial species for each lesion stage is demonstrated in figure 2.

These results confirmed the hypothesis that the BDD lesions are not caused by a single bacterial species (*Treponema*) but are much more complex. Early lesions have minimal populations of *Treponema* spp. (labeled as Spirochaetaceae in figure 2), however these populations

grow significantly in the more advanced and active lesion stages. In contrast, early lesions are composed of much more complex bacterial communities suggesting that the early lesions may develop as a result of the concurrent presence of several bacterial species at the same time (polybacterial disease). The implications of these findings for the control of digital dermatitis in dairy cattle is that vaccination strategies may need to be developed to protect against multiple bacterial organisms instead of a single species.

These findings suggest that the lesion progression is not associated with the infection and proliferation of a single species of treponemes. These results fit with the hypothesis that the treponemes represent an opportunistic organism that colonizes pre-existing lesions of the foot of dairy cattle. Based on the fact that the treponema species typically fail to have significant genetic capabilities to break down the skin barrier, they likely colonize the hospitable niche associated with skin lesions induced by other bacterial organisms (perhaps those associated with early stage lesions) or physical trauma to the foot (scratch, lacerations, etc). This finding is consistent with the fact that treponema species have also been associated with non-DD lesions of the bovine hoof like sole ulcers and toe necrosis where they prevent healing of the lesion.

Collectively these results provide novel insights into the cause and progression of bovine digital dermatitis lesions. Further work is underway to develop novel control strategies, develop evidence based medicine measures of control efficacy and to develop vaccine candidates for use in controlling the disease process.

Acknowledgments

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The full results of this research have been published and are available in Infection and Immunity 2014 82:8.



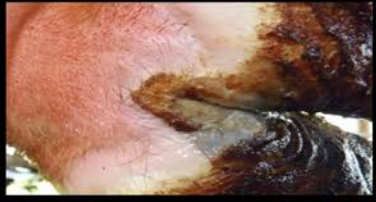













<p>Stage 0 Normal Skin</p>		
<p>Stage A1 Non-proliferative dermatitis, +/- dermal pitting, within the interdigital fold</p>		
<p>Stage A2 Advanced erosion and proliferation within the interdigital fold</p>		
<p>Stage B1 Focal or multifocal proliferative scabs on heel</p>		
<p>Stage B2 Diffuse proliferative scabs across the heel</p>		
<p>Stage 3 Focal are of hyperemic ulceration within area of A2 or B2 epidermal changes</p>		
<p>Stage 4 Chronic papillomatous lesions</p>		
<p>Stage 5 Subset of biopsies taken exactly nine days post treatment with tetracycline</p>		

Figure 1: Iowa State Digital Dermatitis Lesion Scoring System (Copyright American Society of Microbiology, Infection and Immunity 2014 82:8)

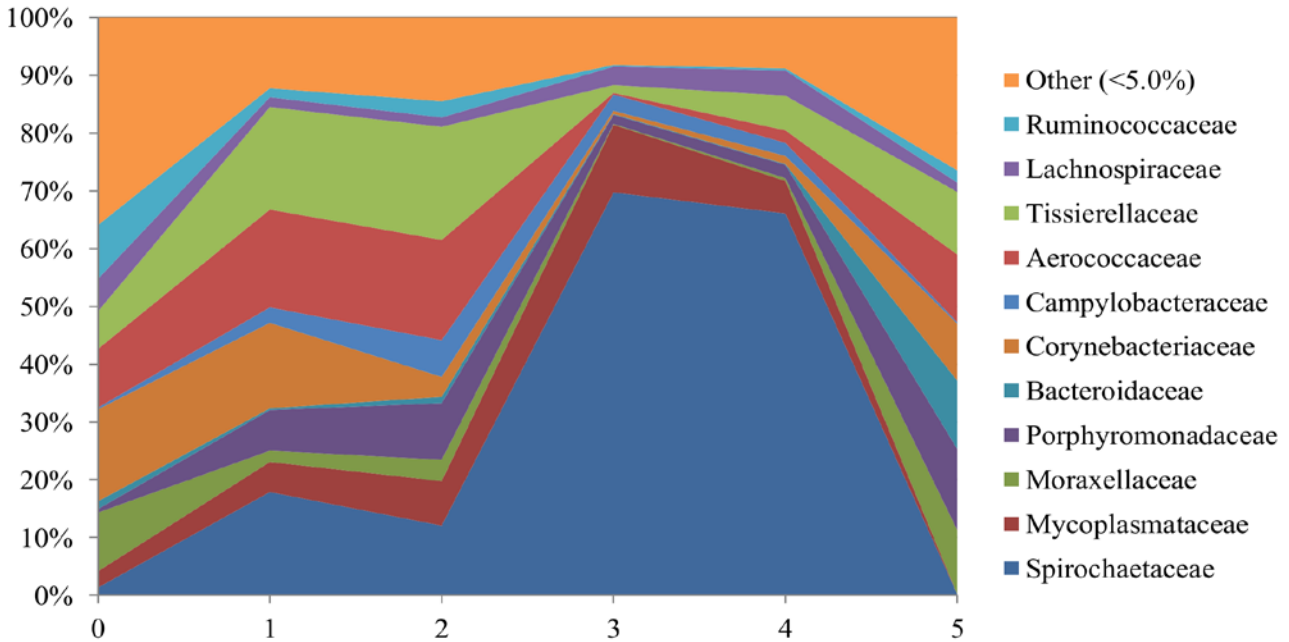


Figure 2: Bacterial community composition of 48 BDD stages lesions using 16S based metagenomics. (Copyright American Society of Microbiology Infection and Immunity 2014 82:8)

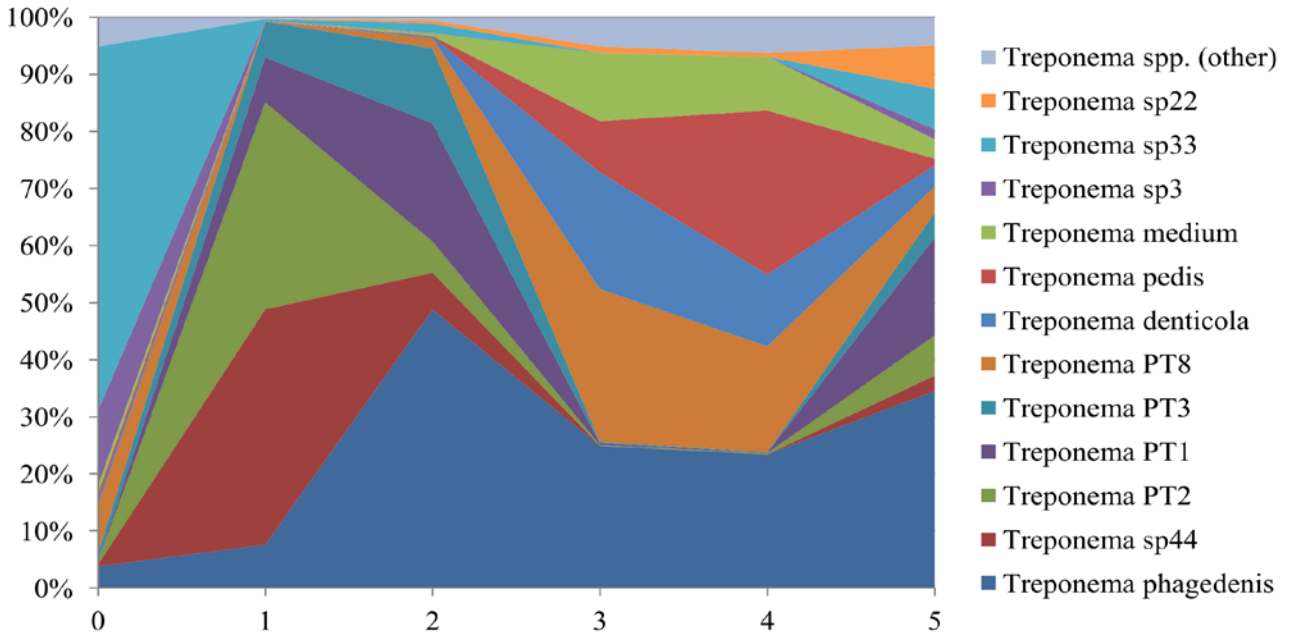


Figure 3: Relative abundance of different *Treponema* spp. based on 16S sequence during the development of bovine digital dermatitis lesions. (Copyright American Society of Microbiology, Infection and Immunity 2014 82:8)