

# Metagenomic evaluation of the dairy farm environment and facilities for evidence of digital dermatitis-associated bacteria

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## Introduction

Bovine digital dermatitis is a common polybacterial disease process and a leading cause of lameness in dairy and beef cattle. Mature lesions are associated with a very high abundance of *Treponema* spp organisms, while developing lesions have a larger diversity with different populations of organisms. This study was designed to utilize 16S-based phylogenomics to evaluate a variety of environmental samples obtained from various areas of a dairy farm for the presence of bacterial organisms associated with digital dermatitis lesions.

## Materials and Methods

In total, 204 different samples were collected from a single digital dermatitis-positive dairy farm, and an additional 9 samples were collected from alley flush water or manure lagoons of other farms known to have digital dermatitis. Samples were collected from various anatomic locations on cattle (rectal, udder sores, gingiva), bedding, feeding, heavy equipment, hoof-trimming equipment, and a variety of farm worker environments (offices, computers, etc.). The samples were processed for sequencing of the V3-V4 region of the 16S ribosomal gene and each sample was barcoded with a unique DNA tag. The samples were multiplexed and run on a single lane of an Illumina MiSeq next generation sequencer.

## Results

In total, just over 10 million joined reads were obtained, of which 9.6 million passed initial quality filtering. Following

demultiplexing and OTU calling, there were just over 7 million unique reads representing 206 samples (7 samples failed to amplify). In order to normalize the data, the samples were rarified to a count of 9,000 reads and compared. Of the 17,882 taxonomies identified in environmental samples, 139 taxonomies were identified that overlapped with digital dermatitis lesions from metagenomic studies performed on this same dairy. Digital dermatitis associated taxa were identified in a large variety of environmental sampling types. With regard to *Treponema* spp, 141 of the samples were identified to contain at least 1 read from a *Treponema* spp that has previously been identified in digital dermatitis lesions. However when one considers the top 13 *Treponema* spp that are most abundant in digital dermatitis lesions, only 24 of the samples contained 1 or more of these *Treponema* spp. *Treponema*-positive samples were typically found in higher numbers and in a larger prevalence from animal-associated samples and hoof trimming equipment, whereas samples derived from animal bedding, equipment, and the farm worker environments were much less likely to have DD-associated *Treponema*. Several non-treponemal species that are closely associated with digital dermatitis lesions showed similar trends.

## Significance

The results of this study provide unique insights into the ecology of digital dermatitis-associated bacteria and help identify potential reservoirs of infection that can be targeted in disease control measures.