

**A DESCRIPTION OF THE BACTERIA AND MANAGEMENT PRACTICES
ASSOCIATED WITH DIGITAL DERMATITIS IN SASKATCHEWAN DAIRY CATTLE**

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ABSTRACT

Digital dermatitis (DD), a polybacterial skin infection of the bovine foot, is among the most common causes of lameness on Canadian dairy farms. The current prevention and treatment methods require constant attention and resources, with regular footbathing and topical treatment necessary to keep outbreaks under control. While a vaccine is desired by many in the dairy industry, the complete etiology of DD is not fully understood, making vaccine development currently unattainable. With the more recent recognition of painless preclinical lesions that develop before the painful clinical stages, an opportunity has presented itself to further study the bacteria present in preclinical lesions among dairy cows in different herds. The microbiota of preclinical lesion tissue is previously unstudied in a commercial dairy housing cows without any sign of clinical DD. In addition, while DD risk factors and herd prevalence have been studied in Ontario, Alberta, and internationally, no published research has indicated whether Saskatchewan dairy farmers perceive and manage DD in a similar manner.

The first objective of this research was to describe the known DD risk factors identified on six selected Saskatchewan dairies that are endemic for clinical DD and one dairy non-endemic for clinical DD. The surveys used to obtain these results also served to ensure that study herds were representative of other Canadian herds, and to recruit and further describe the participant dairies for part two of this project. The second objective was to describe the presence, abundance, and identity of DD-associated bacteria in heel skin tissue, fecal, and slurry samples from cows on these seven dairies.

A survey was used to recruit participant dairies in Saskatchewan who then completed an on-farm questionnaire at the time of sample collection. Samples were collected from cows with varying stages of DD and were subjected to 16S rRNA gene amplicon sequencing.

The survey data indicated that respondents were representative of other Saskatchewan dairies in terms of production and housing, and representative of Canadian dairies regarding record keeping, trimming practices, and perceived importance of hoof lesions causing lameness. The dairy non-endemic for clinical DD practiced more stringent biosecurity than the other participant dairies. The most critical findings of this study are that preclinical lesions were present on the dairy that was free of clinical DD, that these did not progress to clinical disease, and the near complete lack of the bacteria associated with the transition of preclinical lesions to the clinical stages on the dairy non-endemic for clinical DD. Continued stringent biosecurity appears necessary to keep

clinical DD-associated treponemes from proliferating in these tissues that are otherwise susceptible to clinical DD.

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LIST OF ABBREVIATIONS

DD	Digital dermatitis
OTU	Operational taxonomic unit
DIM	Days in milk
ADG	Average daily gain
AI	Artificial insemination
PCR	Polymerase chain reaction
DNA	Deoxyribonucleic acid
rRNA	Ribosomal ribonucleic acid
kg	Kilogram
g	Gram
d	Day
min	Minute
No.	Number
CI	Confidence interval
D	Dairy
T	Tissue
F	Fecal

1 INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

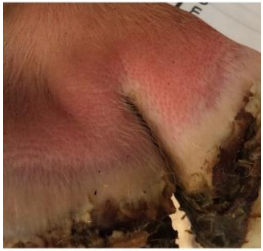
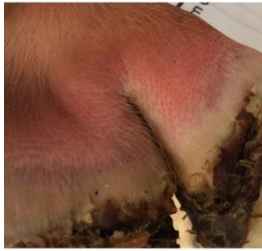


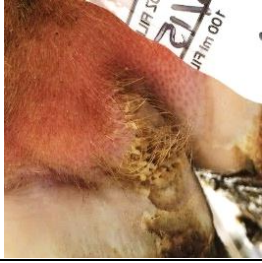



Digital dermatitis (DD) is the most common infectious cause of lameness in dairy cattle (Zuerner et al. 2007). This painful disease was first described in Italy by Cheli and Mortellaro in 1974 and typically develops in the caudal interdigital cleft at the hoof-epithelium interface (Sullivan et al. 2013; Brown et al. 2000), most commonly on the hind feet (Blowey and Sharp 1988). Etiology is considered to be polybacterial in origin with *Treponema* spp. being strongly associated with clinical lesions (Krull et al. 2014; Zinicola et al. 2015). Clinical presentation is a circumscribed red or grey, moist, ulcerative to papillomatous lesion with a distinct hyperkeratotic border between healthy and diseased epithelium (Read and Walker 1998). In its advanced clinical stages, DD causes lameness and anatomical changes of affected bovine feet (Gomez et al. 2015). Recognized lesion location has since expanded to include the proximal and distal interdigital space; these lesions are called interdigital dermatitis (Blowey et al. 1994; as referenced by Wilson-Welder et al. 2015). Digital dermatitis is prevalent globally with reports originating from Europe, Japan, Israel, New Zealand, North America, South America, and South Africa (Holzhauer et al. 2006; Evans et al. 2008; Yano et al. 2009; Bargai 2006; Yang et al. 2017; Cramer et al. 2009; Rodríguez-Lainz et al. 1999; van Amstel et al. 1995). Producers spend significant amounts of money, time, chemicals, and antibiotics in attempts to control DD (Dolecheck 2018) but the hypothesized agents of infection have not yet been proven to reliably induce clinical DD in an experimental infection model (Gomez et al. 2012).







Multiple attempts have been made to develop a vaccine against DD using both *Serpens* spp. and *Treponema* spp. as vaccine candidates. Research on a bacterin vaccine based on two *Treponema* spp. resulted in significant increases in antibody titres following vaccination but showed no significant difference in the occurrence of DD following vaccination (Ertze et al. 2006). Similar results were seen with the *Serpens* spp. vaccine. While it did induce a significant antibody response in vaccinated cows, it did not provide significant protection against DD (Fidler et al. 2012).

Past DD research has focused on a particular group of *Treponema* subtypes (Döpfer et al. 2012a) but recently, a more complex etiology has been proposed (Krull et al. 2014). This polybacterial etiology is an explanation for the ineffective protection provided by vaccines developed using only one or two species of bacteria. Krull et al. (2014) used 16S rRNA metagenomics and high-throughput sequencing techniques to reveal changes in the microbiota of DD lesions as the disease progressed. As part of that study, a new clinical scoring system, the Iowa DD scoring system, was developed and validated. The Iowa DD scoring system includes developing lesions in addition to the painful, clinical lesions recognized by the M-stage scoring system (**Table 1.1**) developed by Döpfer et al. (1997), modified by Berry et al. (2012), and used extensively in DD progression research to identify clinical, healing, and chronic lesions (Berry et al. 2012; Döpfer et al. 2012b; Klitgaard et al. 2013; Zinicola et al. 2015b; Solano et al. 2017a).

This research project was originally focused on preliminary vaccine development but with the publication of manuscripts illustrating the complex etiology of DD disease progression, our research efforts were redirected. Until a specific combination of pure strains that successfully and reliably induce clinical DD lesions is identified and an effective vaccine can be developed, the spread of disease must be controlled by managing risk factors. Due to the infectious nature of DD, risk factors are associated with exposure to infected animals, fomites, poor hygiene, and individual susceptibility concerning immune response, behaviour, and conformation. An evaluation of risk factors for DD in Canada has been performed on dairies in Alberta, Ontario, and Quebec (Solano et al. 2015) but not Saskatchewan. Canadian producers' perceptions of lameness were investigated by Higginson Cutler et al. (2017), but again, Saskatchewan dairies were not included in that analysis. The objectives of this literature review are to describe the appearance of DD, the various ways lesions are scored, previous efforts to characterize the bacteria associated with it, how DD is managed and prevented, and to outline the risk factors for increased DD prevalence in dairy cows.

Table 1.1 A visual comparison of the M-stage and Iowa digital dermatitis scoring systems.

M-Stage DD Scoring System			Iowa DD Scoring System		
Stage	Description (M0: Döpfer et al. 2012; M1-M4.1: Berry et al. 2012)	Photo	Stage	Description (Krull et al. 2014)	Photo
M0	<i>Normal skin</i>		0	<i>Normal skin</i>	
M1	<i>Small (<2 cm across) focal active state. Circumscribed lesion. Surface is moist, ragged, mottled red–grey with scattered small (~1mm diameter) red foci</i>		1	A1 <i>Non-proliferative dermatitis, +/- dermal pitting within the interdigital fold</i>	
				B1 <i>Focal or multifocal proliferative scabs on heel</i>	
M2	<i>Larger (>2 cm across) ulcerative active stage. Extensively mottled red–grey. Can be painful upon manipulation</i>		2	A2 <i>Advanced erosion and proliferation within the interdigital fold</i>	
				B2 <i>Diffuse proliferative scabs across the heel</i>	

M3	<p><i>Healing stage. Typically seen within a few days after antibiotic treatment. The ulcerated surface is now transformed to a dry brown, firm rubbery scab. No pain on manipulation</i></p>		3	<p><i>Focal area of hyperemic ulceration within area of A2 or B2 epidermal changes</i></p>	
M4	<p><i>Chronic stage. Surface is raised by tan, brown, black, rubbery, irregular, proliferative hyperkeratotic growths that vary from papilliform to mass-like projections</i></p>	 <p>Photo by Alberta Dairy Hoof Health Project Lesion Severity Guide</p>	4	<p><i>Chronic papillomatous lesions</i></p>	 <p>Photo by Krull, 2014.</p>
M4.1	<p><i>Chronic stage with small active painful M1 focus</i></p>	 <p>© Döpfer</p>	5	<p><i>Subset of [healing] biopsies taken exactly nine days post treatment with tetracycline</i></p>	 <p>Photo by Krull, 2014</p>

1.2 Appearance and Progression of Digital Dermatitis

1.2.1 Classic Digital Dermatitis Lesion Appearance

The common names for DD, hairy heel wart, strawberry heel, heel warts, and raspberry warts, all speak to the clinical presentation of DD. Clinical lesions present as inflamed, red or grey ulcerative masses at the hoof-epithelium interface (coronary band) or interdigital space, most often on the hind feet above the heel bulbs (Blowey and Sharp 1988). These clinical lesions can take on the appearance of a red, lumpy, painful “berry” (Choi et al. 1997) and may have a distinctive, foul odour (Somers et al. 2005). In the absence of treatment, lesions can progress to large, chronic, granulomatous masses with keratinous hair-like projections and long hair growth surrounding the lesion; thus, the name “hairy heel warts” is used despite the lack of wart virus involvement in DD etiology. A white epithelial margin often surrounds the ulcerative area, indicating a clear border between diseased and healthy tissue (Choi et al. 1997).

1.2.2 Scoring Digital Dermatitis Lesions

The ‘M’ scoring system recognizes DD in its clinical stages (Döpfer et al. 1997). An updated version of this scoring system, modified by Berry et al. 2012, has been used in a lot of previous research on DD-associated bacteria (Berry et al. 2012; Döpfer et al. 2012b; Klitgaard et al. 2013; Zinicola et al. 2015b; Solano et al. 2017a). With this scoring system, the recognized lesions range in severity from M1 to M2, while M3 lesions are healing and M4 lesions are chronic (**Table 1.1**). Healthy skin is given a score of M0 and a healed lesion is M5. The updated detailed macroscopic differences in M stages are described as follows. Originally, M1 lesions were 0.5 cm to 4 cm across (Döpfer et al. 1997), but are now limited to <2 cm measured across the lesion (Berry et al. 2012). M2 lesions are ≥ 2 cm across (Berry et al. 2012; Knappe-Poindecker et al. 2013) and are the stage commonly recognized as appearing berry-like. M2 lesions are painful and prone to bleeding upon palpation. Those lesions scored as M3 are considered “healing” and have a dark, horse-shoe shaped scab covering the lesion after topical treatment (Berry et al. 2012). There is no size restriction on M3 lesion size and they are not as painful as M2 lesions. Chronic DD lesions are scored as M4 and have a surface of raised white, tan, black, or brown hyperkeratotic growths varying from papilliform to hair-like and may be painless upon palpation (Berry et al. 2012). These chronic lesions are more likely to recrudescence to an active M1 or M2 lesion than return to M0 (Relun et al. 2013).

In 2014, an entirely new scoring system was developed that recognizes DD lesions in their preclinical, clinical, and chronic stages (Krull et al. 2014). Other studies had not previously described preclinical lesions, focusing only on clinical or chronic lesions. Preclinical lesions differ in that they do not cause noticeable pain upon palpation and may appear as a focal ulceration or diffuse scabs across the heel (Krull et al. 2014). Preclinical lesions have been found to progress to the classic clinical lesions described by Berry et al. (2012), regardless of their preclinical appearance (Krull et al. 2014). Similar to clinical lesions in the M scoring system, preclinical lesions are scored on severity and size. However, instead of limiting lesion size to a set measurement like the M system, the Iowa DD scoring system evaluates relative size based on how much of the plantar skin adjacent to the interdigital cleft is diseased. If small, localized focal or multifocal lesions are present but have not yet coalesced to involve the majority of skin adjacent to the plantar interdigital cleft, the hoof is given a score of 1 (Krull et al. 2016). Once the majority of the skin adjacent to the plantar interdigital cleft is evidently diseased but not yet clinical, the hoof is scored as stage 2 (Krull et al. 2016). Those lesions presenting as focal and ulcerative are categorized as ‘A’ type while those that present as multifocal or diffuse and crusted are newly recognized and have been categorized as ‘B’ type lesions (Krull et al. 2014). For example, a small, focal lesion with dermal pitting within the interdigital fold would be categorized as A1, while a hoof with diffuse, proliferative scabs across the heel and caudal interdigital cleft would be categorized as B2. Preclinical lesions are generally painless and can go unnoticed until progression to a clinical lesion causes noticeable lameness (Plummer and Krull 2017). A complete illustration of the Iowa DD scoring system can be found in Figure 1 of Krull et al. 2014 while a modified illustration is provided here in **Table 1.1** to illustrate how various preclinical and clinical lesions are classified by each of the scoring systems. To compare this scoring system to the M-scoring system, M1 and M2 would be equivalent to stage 3, while M4 would be stage 4. An M4 lesion with an active infectious area would be considered an M4.1 if the active area was <2cm and an M2 if it were \geq 2cm. The healing stage, M3, is not recognized in the Iowa scoring system but appears comparable to stage 5, clinical lesions that received topical antibiotic treatment (**Table 1.1**).

1.2.3 Bacterial Etiology of Digital Dermatitis

Treponema spp. have been regarded as the dominant bacterial genus in clinical DD lesions, causing significant inflammation and excessive keratinization of the epidermis (Döpfer et al. 1997). The involvement of *Treponema* spp. has been hypothesized two different ways: treponemes are

causal and primary invaders or they are opportunistic secondary invaders. It is largely undisputed that DD is of bacterial etiology, mainly due to the consistent findings of bacteria dominating affected tissue, but also due to its responsiveness to topical antibiotic treatment and insignificant amounts of fungal and viral DNA found in lesion microbiota (Krull et al. 2014).

1.2.3.1 *Digital dermatitis-associated bacteria*

The first organisms identified within DD lesions were spiral, filamentous *Spirochaete*-like bacteria (Blowey and Sharp 1988), later identified as species in the genera *Treponema* (Read et al. 1992; Walker et al. 1995). Since then, *Treponema* spp., of the *Spirochaetes* phylum, have been identified consistently in DD lesion tissue by numerous visual and molecular diagnostic techniques including bacterial culture, silver staining, fluorescence in situ hybridization (FISH), polymerase chain reaction (PCR), and metagenomics (Plummer and Krull 2017). Most early research on *Treponema* spp. in DD was conducted on lesions that were apparently clinical as preclinical DD was not yet recognized. In the earliest DD manuscripts, *Treponema* spp. were the most commonly identified specimen, likely due to their abundance in clinical lesions. An example of only clinical DD being studied in early manuscripts is given by Choi et al. (1997) who described early lesions as “granulomatous strawberry-like ulcerations,” a description that would now be classified as M2 or stage 3 clinical lesions. Similarly, Walker et al. (1995) described early lesions as “red, flat, and ulcerative,” another description of a clinical lesion. Previous research also indicated that DD usually led to lameness (Blowey and Sharp 1988). However, recent publications report that only 39% of cows with clinical DD have a locomotion score ≥ 3 on a scale of 1-5, where 1 indicates normal locomotion and 5 is severely lame (Frankena et al. 2009). Since the recognition of non-painful preclinical lesions, (Plummer and Krull 2017), it is now evident that prevalence estimates considering only cows with clinical DD underestimate DD prevalence in a herd. This demonstrates that early DD research likely focused on painful clinical lesions and did not yet recognize preclinical DD. Therefore, the first studies to suggest that *Treponema* spp. were causal in DD were based on clinical DD and subsequently, this literature review will focus on more recent studies on the bacteria associated with preclinical DD and its transition to clinical DD.

Since the initial observations of *Treponema* spp. in DD lesions, specific groups of treponemes (clusters) have been reported as clinically relevant in DD lesion development and persistence. Within these clusters, separate phylotypes were sequenced and assigned identifiers by Klitgaard et al. (2008) and later by Rasmussen et al. (2012). Phylotypes (PT) are defined as groups of often

unnamed species with 16S rRNA sequences that are $\geq 98\%$ similar to a known species' 16S rRNA gene sequence and $\geq 99\%$ similar to other members of their cluster (Klitgaard et al. 2013). The *Treponema* clusters of importance are: *T. denticola*/*T. pedis*-like (cluster 1), *T. phagedenis*-like (cluster 2), *T. refringens*-like (cluster 3), and *T. medium*/*T. vincentii*-like (cluster 4; Yano et al. 2010; Klitgaard et al. 2013). An additional 5th cluster, *Treponema brennaborensis*, was included when the original 4 were classified by Yano et al. in 2010, but recent studies suggest it was likely the product of fecal contamination or a secondary invader (Nordhoff et al. 2008; Klitgaard et al. 2008). Given that *T. brennaborensis* was identified in only 2 of 41 DD lesions by FISH analysis, and in those two cases, it was seen deep in the epidermis at the front of the diseased tissue (Klitgaard et al. 2008), *T. brennaborensis* may be clinically important to DD, but its presence could be associated with regional or geographical differences (Wilson-Welder et al. 2015). The relationship between these strains was illustrated in a phylogenetic tree by Klitgaard et al. in 2013 (**Figure 1.1**). The phylotypes of importance within cluster 1 are *T. pedis*, *Treponema* PT8, *T. putidum*, and *T. denticola*; cluster 2 contains *Treponema* PT6 and *T. phagedenis*; cluster 3 includes *Treponema* PT1-PT4 and *T. refringens*; phylotypes of importance in cluster 4 are *Treponema* PT5, *T. medium*, *T. medium* subspecies *bovis*, *T. vincentii*, and *Treponema* PT9 (Krull et al. 2014; Klitgaard et al. 2013).

Recently, these phylotypes were associated with specific stages of DD lesion progression (**Table 1.2**; Krull et al. 2014). The most abundant reads of *Treponema* phylotypes identified with 16S rRNA metagenomics in preclinical lesions (staged 1 and 2, A-type or B-type) taken from cows at Iowa State University were *Treponema* PT1-PT3, *T. phagedenis*, and *Treponema* sp. 44 (Krull et al. 2014). The most abundant reads in stage 1 lesions (including A1 and B1) were associated with *Treponema* PT2 and *Treponema* sp. 44, while in stage 2 lesions (A2 and B2), the most abundant reads were associated with *Treponema* PT1, *Treponema* PT3, and *Treponema phagedenis* (Krull et al. 2014).

Significant differences between A and B-type preclinical lesions were evident at a bacterial level. The A1 lesions were associated with *Treponema* PT2 and *Treponema* sp. 44, while the significant taxa associated with OTUs in A2 lesions were *T. phagedenis* and *Campylobacter ureolyticus* (phylum *Proteobacteria*; Krull et al. 2014). A significant association was not found with the B-type lesions and any *Treponema* OTUs, but rather with the families *Corynebacteriaceae* (phylum *Actinobacteria*) and *Tissierellaceae* (phylum *Firmicutes*) in B1 lesions and the family

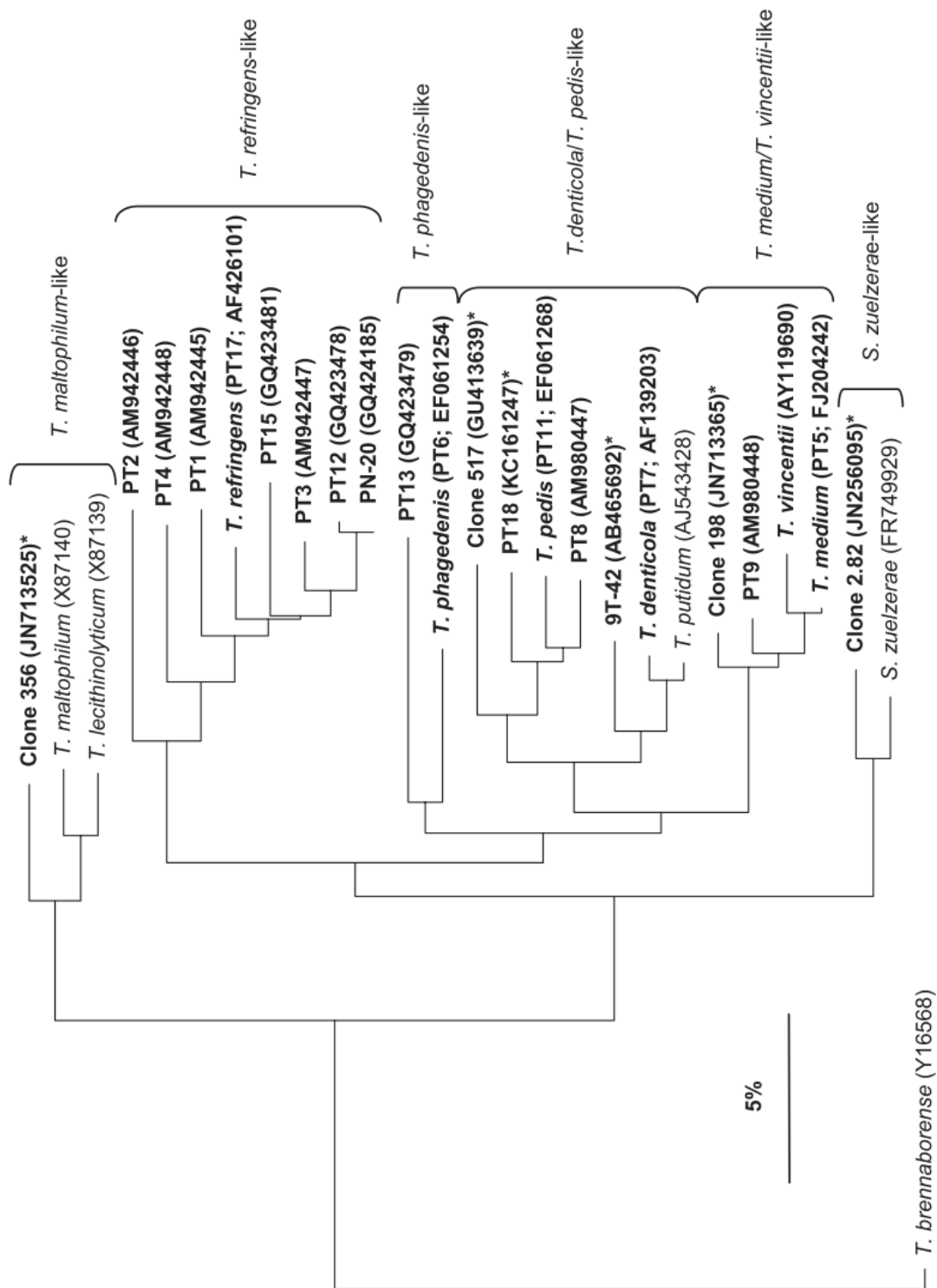


Figure 1.1 A phylogenetic tree of *Treponema* clusters based on the V3-V4 hypervariable region of the 16S rRNA gene sequence according to Klitgaard et al. 2013.

The scale bar represents a 5% difference in nucleotide sequences.

Aerococcaceae (phylum *Firmicutes*) in B2 lesions. It is not necessary for B-type lesions to be significantly associated with *Treponema* spp. to be considered preclinical, because they have been shown to progress into identical clinical lesions as A-type lesions (Krull et al. 2014). It was

observed that as lesions progressed to clinical stages (stages 3 and 4), the bacterial population, as indicated by OTU read counts, shifted, regardless of whether the initial lesion was A or B-type, to a profile dominated by *T. denticola*, *T. medium*, and *Treponema* PT8 in stage 3 with the significant addition of *T. pedis* in stage 4 (Krull et al. 2014).

In addition to the group of *Treponema* spp. associated with DD, *Dichelobacter nodosus*, *Fusobacterium*, *Gugenheimella*, *Porphyromonas*, *Bacteroides*, *Prevotella*, *Peptostreptococcus*, *Clostridium*, *Campylobacter*, *Mycoplasma*, *Cornybacterium/Actinomyces*, and Gram-positive aerobic cocci have also been associated with DD (summarized by Wilson-Welder et al. 2015). However, these bacteria are not consistently identified in all DD lesions. Bacteria of interest that play a potential pathogenic role in DD lesion initiation and development are *Porphyromonas*, *Fusobacterium*, and *D. nodosus* due to the reported antibody response in cattle with active or recent lesions (Moe et al. 2010; Knappe-Poindecker et al. 2013). A recent study on the bacteria related to DD in year-round grazing dairy cattle in Brazil reported that, using FISH analysis of 66 DD lesion samples, *Treponema* spp. were present in 97%, *P. levii* in 64.6%, *D. nodosus* in 48.5%, and *F. necrophorum* in 23.5% of these samples (Moreira et al. 2018). The spatial distribution of these bacteria indicated that *D. nodosus* may be working together with *Treponema* spp. to advance lesion development and that *P. levii* and *F. necrophorum* are secondary invaders. *Mycoplasma* has also been identified in clinical DD lesions (Nielsen et al. 2016) and found to make up >10% of the total OTU reads with 16S rRNA gene analysis (Krull et al. 2014). Preclinical lesions have a greater abundance of *Mycoplasma* associated OTU reads determined by 16S rRNA gene analysis than in samples of healthy skin (Krull et al. 2014).

Despite the association of these bacteria with DD lesions, the etiology has yet to be determined by fulfilling Koch's postulates. The relative abundance of the causal pathogen does not necessarily need to be the most prevalent within the tissue to cause disease. An example of this occurs in ovine foot rot in which the agent of disease, *D. nodosus*, makes up less than 2% of the sequence reads when 16S rRNA gene sequencing was carried out on infected tissue samples (Maboni et al. 2017). Further research on the importance and function of DD-associated bacteria is necessary to determine the focus of vaccine development, targeted treatment, and prevention of painful clinical lesions.

Table 1.2 A summary of the bacteria associated with the Iowa digital dermatitis stages

Stage	Significant Biomarker(s)	Other Abundant Bacteria
Stage 0	<i>Treponema</i> sp. 33	
	<i>Treponema</i> sp. 44	
Stage 1	<i>Treponema</i> PT2	
	<i>Treponema</i> PT1	
Stage 2	<i>Treponema</i> PT3	
	<i>T. phagedenis</i>	
Stage 3	<i>Treponema</i> PT8	
	<i>Mycoplasma</i>	
Stage 4	<i>T. pedis</i>	
Stage 5	<i>Bacteroidia</i>	
	<i>Gammaproteobacteria</i>	

Adapted from Krull et al. 2014 and Krull 2015

1.2.3.2 Characteristics of digital dermatitis-associated bacteria

Treponemes have been notoriously difficult to culture but growth and isolation has been successful under strict conditions. Treponemes have been successfully visualized in infected tissue using silver staining techniques (Döpfer et al. 1997) and FISH with phylotype-specific 16S rRNA gene targeting oligonucleotide probes (Klitgaard et al. 2008). All treponemes are anaerobic or microaerophilic with at least one periplasmic flagella (Edwards et al. 2003). These flagella are unique to *Spirochaetes* and give them the ability to swim through highly viscous media (Edwards et al. 2003). Wilson-Welder et al. (2013) used phase contrast, dark field, and electron microscopy to describe the morphology of 4 *Treponema* isolates from DD lesions in Iowa dairy cows. These isolates had 6-8 flagella on each end and ranged in length from 8.0 to 9.7 μm . Successful growth

of isolate 4A required anaerobic conditions, serum, volatile fatty acids, an optimal pH of 7, ranging from 6.8-8.5, and an optimal temperature of 40°C but within the range of 29°C-43°C. It was noted that this preference in temperature is much higher than the natural temperature of a cow's foot, 21 to 23°C. These isolates were capable of fermenting fructose, mannitol, pectin, mannose, ribose, maltose, and glucose, and resulted in formate, acetate, and butyrate as fermentation products. A complete genome sequence is not available for any *T. phagedenis* isolates, but comparison of assembled contigs with isolate 4A paired with the aforementioned morphology and metabolic characteristics of DD isolates indicated that these isolates were not dissimilar enough to be considered a separate species, and instead of being referred to as *T. phagedenis*-like, they should simply be called *T. phagedenis*. It was concluded that although *T. phagedenis* is a human genital commensal, it should also be considered a pathogen of the bovine digit. Whether the term "pathogen" is appropriate for *T. phagedenis* in bovine DD is questionable as the organism has not been proven able to cause disease in a healthy bovine host when introduced as pure isolate. It has however been proven to downregulate genes important to immune functions in the bovine host that would allow *T. phagedenis* to resist clearance by macrophages and impair wound repair functions (Zuerner et al. 2007).

1.2.4 Pathology of Digital Dermatitis Lesions

1.2.4.1 Skin physiology

The skin consists of three layers: the epidermis, dermis, and subcutaneous tissue. The outermost epidermal layer, the stratum corneum, consists of horny, keratinized, flat, dead, squamous cells that are continuously renewed. These cells originate at the deepest epidermal layer, the stratum basale. As basal stem cells divide, the resulting keratinocytes enter a differentiation process that causes their terminal migration to the surface (Lippens et al. 2009). During this migratory process, the nucleus degenerates and the cytoplasm fills with keratin. Once the keratinocytes die and become flat, these corneocytes act as the protective stratum corneum before shedding, at which time they are replaced by new keratinocytes. The keratinocytes undergo programmed cell death which is different from classical apoptosis as there is evidence that nuclear factor NF- κ B can reduce keratinocyte apoptosis under inflammatory conditions (Lippens et al. 2009). Below the stratum basal is the dermis. At this junction, rete ridges of the epidermis extend downward and between the papillae of the dermis. The dermis is composed of mostly fibroblasts,

macrophages, and adipocytes; this combination of connective tissue, host defence cells, and the cushioning nature of fat make the dermis an important protective cutaneous layer.

1.2.4.2 *Host-pathogen interactions*

Beneath the surface of a raw, ulcerated, clinical DD lesion, many pathogenic bacteria are at work, eliciting an immune response from the host. The host immune response has both innate and adaptive components. The innate immune system is the first line of defence and made up of all the physical and chemical barriers that respond immediately and mostly non-specifically to pathogen invasion (Mogensen 2009). This includes epithelial barriers, natural killer (NK) cells, and phagocytic- and antigen-presenting cells including granulocytes, macrophages, and dendritic cells (Mogensen 2009). The innate immune response to invading pathogens depends on pattern recognition receptors (PRRs) to recognize pathogen-associated molecular patterns (PAMPs). PAMPs are consistent across classes of pathogens and essential to pathogen survival, so they are a good target for host recognition. Host recognition of PAMPs is an evolutionarily conserved system (Mogensen 2009). Macrophages can release cytokines such as interleukin-1 (IL-1), IL-6, IL-12, and tumour necrosis factor-alpha (TNF- α). When macrophages present antigen proteins on their surface, T helper cells are activated (Alberts et al. 2002). The type of effector cell an activated T helper cell develops into dictates how the adaptive immune system is signalled (Alberts et al. 2002).

The adaptive immune system uses antigen-specific responses to identify, process, and attack invading antigens. Unlike innate immunity, adaptive immunity is developed, not genetically transferred through the germ-line (Iwasaki and Medzhitov 2010). This makes adaptive immunity very specific (Iwasaki and Medzhitov 2010). The humoral and cellular immune systems make up the adaptive immune response with differentiated lymphocytes including B cells, helper T cells and cytotoxic T cells. The release of cytokines by helper T cells and macrophages helps to direct adaptive responses.

Zuerner et al. (2007) used serial analysis of gene expression (SAGE) to quantify gene expression of bovine macrophages when exposed to a pure culture of sonicated *T. phagedenis*-like cells *in vitro*. The pure culture of *T. phagedenis*-like bacteria (GenBank accession no. AF546873, cultured by Trott et al. 2003) had a 16S rDNA sequence that was 99% identical to strains 2-1498 (GenBank accession no. L78126) and DDLK-4 (GenBank accession no. Y08894f), and 98% identical to *T. phagedenis* (GenBank accession no. L78126). Zuerner et al's focus was on the genetic expression of encoding proteins involved in immune stimulation, controlled cell death

(apoptosis), cytoskeletal structure, and wound healing. They reported that transcription of 255 genes increased and transcription of 291 genes decreased in sonicate-treated bovine macrophages. Treated macrophages released proinflammatory cytokines but transcription of some receptors and their accessory proteins were reduced or unchanged. Expression of genes transcribing proteins for I- κ B (NF- κ B inhibitor) and SIVA-1 (negatively regulates NF- κ B) were upregulated in treated macrophages. NF- κ B helps regulate cellular immune responses to infection. Wound healing, cytoskeletal structure, and antigen presentation associated genes were transcribed less in sonicate-treated macrophages. These changes in gene transcription of bovine macrophages, when stimulated with *T. phagedenis*-like antigens, indicate that the infiltration and proliferation of DD-associated bacteria in bovine tissue cause direct changes in the host's innate immune response and wound repair functions, potentially resulting in *Treponema* spp. proliferation due to inadequate pathogen clearing functions (Zuerner et al. 2007). It has also been demonstrated with histochemistry and immunohistochemistry that eosinophils, neutrophils, lymphocytes, and macrophages are present in clinical DD lesion tissue, and hyperplasia and hyperkeratosis of the epidermal and keratin layers occurs (Refaai et al. 2013). It was determined that the source of significantly higher IL-8 detected in diseased tissue was the keratinocytes due to both their increase in number and the increased expression of IL-8 genes (Refaai et al. 2013). Clinical DD lesions typically show general thickening of the epidermis and increased keratinization. It has been hypothesized that this response is beneficial to the survival of *Treponema* spp. due to their anaerobic nature (Döpfer et al. 2012).

1.2.4.3 *Histopathology of digital dermatitis lesions*

Some of the earliest accounts of detailed DD lesion histopathology reported acute inflammation with superficial necrosis and hyperkeratosis of the stratum corneum (Choi et al. 1997; Döpfer et al. 1997). In tissue samples of lesions staged with the M system, M1 lesions had nuclei retained in the stratum corneum resulting in parakeratosis. M1, M2, and M4 lesions showed up to 14 mitotic figures per 10 high power fields in the stratum basale (Döpfer et al. 1997), indicating increased cell division and keratinocyte production. M1, M2, and M4 lesions showed increased infiltration of inflammatory cells such as monocytes, neutrophils, eosinophils, and plasma cells aggregating around blood vessels (Döpfer et al. 1997). In M2 lesions, complete loss of the epidermis exposed the dermal papillae, resulting in a granulomatous appearance (Choi et al. 1997). Döpfer et al. reported many micro-abscesses in both the epidermis and dermis of M1 and M2 lesion tissue (Döpfer et al. 1997). All reports of DD lesion histology indicate that the presence of

treponemes and other pathogenic bacteria invading the epidermis cause inflammation and disrupt the healthy keratinocyte and squamous cell regeneration process (Döpfer et al. 1997).

1.3 Significance of Digital Dermatitis

Digital dermatitis is an infectious disease primarily affecting the dairy industry worldwide, but has also been observed to affect beef cattle in feedlots (Sullivan et al. 2013), sheep (Sullivan et al. 2015a), goats (Sullivan et al. 2015b), and wild elk (Clegg et al. 2015). The prevalence of DD-affected beef cattle at slaughter was 4% in 2000 (Brown et al. 2000) but has since been reported as high as 78% on an animal-level in a pen of steers (Kulow et al. 2017). The prevalence of lameness in free-ranging Roosevelt elk in southwest Washington has been reported at 36% of animals (Mansfield et al. 2011) with anecdotal reports of up to 90% of animals lame within a group (Clegg et al. 2015). Confirmation of the disease spreading from cattle to elk grazing in southwest Washington was done through histopathology and microbiome analysis, confirming the involvement of *Treponema* spp. genetically similar to those reported in bovine DD (Clegg et al. 2015).

Digital dermatitis lesions cause pain, can reduce production, and cost producers money (Green et al. 2002; Bruijnijis et al. 2012; Cha et al. 2010). Lameness is a serious issue for dairy and other production animal industries not only for its negative production effects but also because it compromises welfare. The next step towards reducing lameness due to DD is to identify the causative agent(s) of infection so that effective vaccines, specific prevention tactics, and improved treatments can be developed.

1.3.1 Prevalence and Incidence of Digital Dermatitis

Lameness is one of the most important causes of reduced longevity among cattle due to its high prevalence (Cook 2003). The 2007 National Animal Health Monitoring System (NAHMS) survey reported that DD was the primary cause of lameness in American dairy cattle, attributing to 61.8% of lameness in bred heifers and 49.1% in cows (USDA 2009). Most prevalence studies reviewed here used the M-scoring system, which does not recognize the Iowa scoring system's non-painful, preclinical lesions in stages 1 and 2 (Krull et al. 2014), so all prevalence values reported include only clinical DD unless stated otherwise.

The estimated prevalence of lameness within Canadian dairy herds is between 19 and 24% (Solano et al. 2015), while DD prevalence is reported as 15% of cows and 94% of herds (Solano

et al. 2016). In 2000, a prevalence study was carried out on culled adult dairy and beef cattle entering a slaughterhouse in the southeastern United States. It was found that 29% of dairy and 4% of beef cattle had gross clinical DD lesions (Brown et al. 2000). The average prevalence of combined M2 and M4 lesion scores in growing finisher steers has since been reported between 26 and 61% per study group at a feedlot operation in the Midwestern USA (Kulow et al. 2017). According to several Dutch studies, the prevalence of clinical DD increased from 17.6% in 1992 to as high as 30% in 2003 (Smits et al. 1992; Somers et al. 2003). Canadian studies report prevalence values similar to those reported in The Netherlands. In Ontario, DD was found to be the most common lesion in both tie-stall and free-stall herds at 9.3 and 22.9% of cows, respectively (Cramer et al. 2008). It was also found that 69.7 and 92.1% of herds were affected by DD in Ontario tie-stall and free-stall herds, respectively. The highest dairy cow-level prevalence reported in the literature was from a footbath efficacy study that had to reallocate cows from the control group to a weekly footbath regimen to control the >60% active DD lesion (M1 and M2 stages) prevalence that had developed during the study (Speijers et al. 2010).

Prevalence of DD in Alberta dairy herds was reported in the 2013 Alberta Dairy Hoof Health Project at an average of 20.8% of cows affected in each study herd. By 2016, herd-level prevalence was 94% (156 farms) with an overall cow-level prevalence of 21.8% (Solano et al. 2016a). It was found that in herds with partial-herd trims (<80% of the herd trimmed over one 1-3 day session), a greater prevalence of lesions was reported (25%), likely due to producers prioritizing lame cows for trimming (Solano et al. 2016a). When results from whole-herd trims (>80% of the herd trimmed over one session) were evaluated separately, a cow-level prevalence of 15% was reported (Solano et al. 2016a). While preliminary research focused on mature animals, recent prevalence reports of up to 9.3% have been reported in Alberta dairy young stock by using pen-walks as a method of detection (Jacobs et al. 2017).

Saskatchewan's dairy industry is more similar to Alberta's than Ontario's. According to Statistics Canada, the average herd size in Alberta was 142 cows in 2015 while the average in Saskatchewan was 166 cows (extrapolated from report D056 and D042 in the Agricultural Industry Market Information System dairy genetics database). Similar to Alberta, the vast majority of Saskatchewan dairy barns are the free-stall type, while in Ontario, the majority of barns are the tie-stall type. The 2015 average milk production per cow was 9,006 L in Alberta and 8,995 L in Saskatchewan. Considering 2015 dairy production in Saskatchewan was similar to that in Alberta

in terms of average herd size, barn type, and milk production (Canadian Dairy Information Centre, Statistics Canada, summarized in Progress. Dairym. 2015), one could expect that the prevalence reports from Alberta are more representative of the DD prevalence in Saskatchewan than those from Ontario.

1.3.2 Costs of Digital Dermatitis and Production Effects

The costs of lameness on a dairy are broken down into reduced milk yield, reduced fertility, culling costs, medicine costs, labour costs, and veterinary costs (Willshire and Bell 2009). The most costly of these categories are reduced milk production and fertility, making up 82% of the costs of lameness (Willshire and Bell 2009). Along with mastitis and fertility problems, lameness issues are among the most prevalent and costly health problems faced by dairy farmers (Bruijnis et al. 2010).

The cost of DD for the US dairy industry was calculated in 2018 as $\$137 \pm 36$ USD per case with a range in cost of $\$30 \pm 21$ USD for a mild case during 121-240 DIM of the first lactation to $\$399 \pm 116$ USD for a severe case during the early lactation of a multiparous cow (Dolecheck 2018). In that report, the cost of therapeutics, outside labour, on-farm labour, discarded milk, decreased milk production, extended days open, recurrence, and increased risk of culling all contributed to the total cost per case. The cost per case was differentiated according to parity, DIM, and severity of each case. This report being the most thorough and recent account of costs associated with DD so far.

In the USA, the economic impact was estimated at over \$190 million USD per year (Losinger 2006). That estimate used a cow-level prevalence of 17% and considered the costs of treatment, reduced reproduction, and decreased milk production due to DD. Considering herd-level prevalence is reportedly as high as 94% (Solano et al. 2016a) and recent cow-level prevalence has been reported between 20 and 25% (Solano et al. 2016a; Cramer et al. 2008), it can be inferred that the cost of DD in Canada may be significantly higher if costs were adjusted for the significant difference in industry size.

Unlike the American dairy industry, the Canadian dairy industry prices milk on a component basis and uses a quota system. Due to the differences concerning milk pricing and cost structure, the costs associated with lameness cannot be directly compared between Canadian and American dairy industry reports. Generally, costs associated with lameness in lactating dairy cows result from

reduced milk production, reduced reproductive performance, increased involuntary culling rates, and the cost of treatment (Green et al. 2002; Hernandez et al. 2002; Booth et al. 2004). No total cost estimates have been reported in the Canadian dairy industry, but 82% of the cost of lameness is due to reduced production and fertility (Willshire and Bell 2009) and the cost of DD to the Saskatchewan dairy industry based on lost milk production can be estimated (**Table 1.3**).

In the 2016-2017 Canadian dairy year, the average component prices in Saskatchewan were \$11.30/kg butterfat, \$8.30/kg protein, and \$1.21/kg other solids (Saskmilk 2017). From 161 Saskatchewan dairies, 260,951,549 total litres of milk were shipped (average 1,620,817 L/farm) with an average milk composition of 4.01% butterfat, 3.34% protein, and 5.72% other solids. The average butterfat production/cow/305d was 1.24 kg. Using published values, the estimated cost of DD in Saskatchewan due to reduced milk production in the 2016-2017 dairy year was calculated to be \$1,674,430 provincially and \$10,400 per dairy farm (**Table 1.3**).

Similarly to other causes of lameness, DD can reduce the performance of all cattle; lactating dairy cattle, replacement heifers, feedlot cattle (Kulow et al. 2017), and dairy bulls could all show reduced performance which would reduce efficiency and increase cost. Canadian dairies milk cows year-round at varying stages of lactation, rather than the structure of intensive finished beef production, where pens are fed and sold based on projected finishing weight and date of slaughter.

Steers with one case of an M2 lesion had reduced average daily gain (ADG) resulting in finished weights 10.06 kg less than their penmates ($P = 0.022$) and a reduced hot carcass weight (HCW) of 5.5 kg ($P = 0.043$) in a Midwestern feedlot study (Kulow et al. 2017). In March 2018 the average fed steer price in Canada was approximately \$165 per 100 lbs (CanFax 2018). If the projected finished weight of a pen of 250 beef steers was 650 kg/head and the prevalence of M2 lesions was a conservative 20% (Kulow et al. 2017), there would be a calculated loss of \$1830 in potential earnings for that pen.

Similar to feedlot steers, the growth of replacement dairy heifers may also be compromised by DD. However, unlike feeder steers, the primary goal of growing dairy heifers is to produce a calf and become a productive dairy cow replacement. Puberty starts when a heifer reaches 55% of her projected mature body weight and after first calving, she should be 85% of her projected mature weight (NRC 2001). Benchmarks of American Holstein heifers at breeding age include having a BCS near 3.0, weighing 340-365 kg, and measuring 127-132 cm tall at the hip by the target breeding age of 13-14 months (Heinrichs and Lammers 2008). Heifers that calve at >24 months of

age cost more to rear, produce less milk as adults, and more heifers will be required to match the herd's culling rate (Laven 2018). Digital dermatitis may impede projected growth and production of replacement dairy heifers in a similar manner to that of beef steers.

Table 1.3 Estimated total cost of digital dermatitis in Saskatchewan due to reduced milk production

Input	Value	Source
Reduced milk production/year in cows with lameness (kg/cow)	355	(Green et al. 2002)
Prevalence of CAD dairy cows with DD in 2013	22%	(Solano et al. 2016a)
No. SK dairy cows in 2017	27,600	(Statistics Canada 2017)
Mean blended price of milk in SK in 2017 (\$/hl)	\$80.01	(Saskmilk 2017)
No. SK dairy producers in 2017	161	(Saskmilk 2017)
Total estimated cost of lost production due to DD in SK in 2017	\$1,674,430	<i>Total Cost</i> = (lost production per cow)(cows with DD) $\frac{\$}{hl}$
Average estimated cost/dairy in SK in 2017	\$10,400	= $\frac{\textit{Total Cost}}{\textit{SK Dairies}}$

Costs of treatment and prevention rather than lost production potential are perceptible losses, particularly in the Canadian dairy industry due to the continuous structure of milk production. The most common control measure for DD used in Canadian dairies is regular footbathing with CuSO₄ or formalin. In Saskatchewan, one 22.7 kg (50 lb) bag of CuSO₄ costs approximately \$80 CAD (ProFarm, Saskatchewan, personal communication). On a 200-cow dairy, footbathing 6 milkings

per week with a 5% CuSO₄ solution in a 150 L footbath, that producer could expect to spend \$159 CAD per week on CuSO₄ alone. In a recent publication, recommendations on footbath length have been increased to accommodate a minimum of two submersions per foot (Cook 2017). The footbath design recommended by Cook is 3.0 to 3.7 m long, 0.6 m wide at the base, and would be filled to 10 cm deep. The volume of solution in a footbath this size would be 222 L. To fill it with a 5% CuSO₄ solution would take 11.1 kg of product and cost approximately \$39/bath or \$235/wk. It is recommended to start footbathing at a frequency of 4 milkings per week and adjust according to the outcome (Cook 2017). Following this recommendation, a producer milking 200 cows could expect to spend over \$8100 CAD per year on footbath chemical alone, not including labour, a footbath, mixing tank, chemical transfer pump, or the cost of water and additional wastewater management. In addition to the cost of footbathing chemicals, their handling is problematic. Formalin is a known carcinogen with malodourous fumes while CuSO₄ causes corrosion of cement and requires vigorous mixing before use. The dirty solution washed away after footbathing can also create problems. While formalin breaks down into non-toxic compounds, CuSO₄ does not. Used footbath solution is most often handled with the farm's wastewater and slurry then later spread on fields as fertilizer. Although CuSO₄ is less toxic to handle than formalin, it can build up in the soil of fields on which it is spread, potentially reducing their cropping potential.

1.3.3 Welfare and Physical Function Effects of Digital Dermatitis

Animal welfare can be evaluated by considering three points: 1) animals should have good physical function, health, and growth, 2) animals should be able to live natural lives in consideration of behaviour, socialization, and environmental aspects such as fresh air, and 3) animals should be free of negative affect and where possible, experience positive affect (Fraser 2008). Examples of negative affective states include hunger, pain, and heat stress, while an example of positive affective states includes the pleasure associated with grooming, exploring, and play behaviour (Fraser 2008). Characteristics of good animal welfare were also laid out in the Five Freedoms updated by the Farm Animal Welfare Council in 1992: 1) freedom from thirst and hunger; 2) freedom from discomfort; 3) freedom from pain, injury, and disease; 4) freedom to express normal behaviour; and 5) freedom from fear and distress (FAWC 1992).

Pain associated with clinical DD can be severe, resulting in lameness and the side effects associated with such stressors. Pain can negatively affect natural behaviour and welfare of dairy

cows (O'Callaghan et al. 2003; Ito et al. 2010; Bruijnis et al. 2012) in addition to limiting their production potential (Green et al. 2002). In the case of lactating cows managed inside a free-stall barn, lame cows show longer lying bouts (Solano et al. 2016) and reduced time feeding (González et al. 2008; Palmer et al. 2012). Time spent consuming feed is important as DMI establishes the quantity of nutrients available for milk production (NRC 2001). The transition diseases common to high producing dairy cows are often exacerbated by excessive negative energy balance (NEB), which is aggravated by reduced DMI, during the periparturient period. Lame cows, in particular, may be at a greater risk of transition-associated diseases due to their reduced DMI during the first 60 days of lactation ($P < 0.05$; Palmer et al. 2012). Therefore, in addition to welfare, production potential is also limited by lameness.

Examples of good physical function in dairy cows include high lifetime milk production and young age at onset of puberty. Because measures of physical function in terms of production are relatively easy to quantify, producers actively monitor these functions as part of herd management. It is recommended that Canadian Holstein breeders should aim to breed their heifers at 13-15 months of age, so they calve at 22-24 months of age. If heifers are delayed in reaching puberty, it would not only indicate reduced welfare but would also cost the producer money. Much of the same concept applies to dairy bulls; if they are not able to perform their breeding duties, they are costing the farm in potential pregnancies as well as feed, equipment maintenance, fuel, and labour.

Physical function is impaired by lameness. Cows experiencing a chronic stressor such as lameness can show reduced estrus expression behaviour such as mounting herd-mates or chin-resting (Walker et al. 2010). In addition to less intense estrus behaviour, fewer lame cows ovulated compared to their healthy herd-mates when their follicular phases were synchronized with gonadotrophin-releasing hormone (GnRH) followed by prostaglandin (PG; Morris et al. 2011). Reduced reproductive performance costs the producer in increased cost of synchronization hormones, veterinary fees, semen straws, and labour. In addition, cows that are still open in late lactation will cost the producer in feed and labour if she is kept in the milking herd and will likely experience a long dry period if she is not culled. During a long dry period, BCS can increase and put cows at a greater risk for periparturient metabolic diseases and retained placenta (Roche et al. 2013; McGuffey 2017). Ketosis, in particular, is a concern during the transition period of over-conditioned cows (Vanholder et al. 2015). Mature cows that experience ≥ 0.72 mEq/L serum non-esterified fatty acids (NEFA) or ≥ 10 mg/dL beta-hydroxybutyrate (BHBA) in early lactation

(excessive levels being indicators of NEB and ketosis, respectively) are at a greater risk for lower milk production and reduced fertility than cows that do not (Ospina et al. 2010).

1.3.4 Digital Dermatitis in Saskatchewan

The significance of DD in Saskatchewan dairies is previously unstudied, but anecdotal reports indicate that Saskatchewan producers experience DD prevalence in their herds similar to other Western Canadian dairies. In the undertaking of this project, it became evident through discussions with producers that estimates of 95-98% herd prevalence were likely consistent with the true herd prevalence. This project revealed a single herd free of clinical DD with housing methods and production levels consistent with those of other SK dairies. The rarity of this herd must be highlighted as it is the only dairy apparently free of clinical DD and housed in a similar manner to commercially relevant dairies to be reported in any DD-related literature.

1.4 Managing Digital Dermatitis

1.4.1 Risks Associated with Digital Dermatitis

1.4.1.1 Herd level risks

The risk of developing DD is commonly associated with exposure to wet, dirty conditions, maceration of the digital skin, and adequacy of the immune response (Blowey and Sharp 1988). Herd-level risk factors are housing, diet, environment, biosecurity measures, management practices, general hygiene, and transmission of pathogens (Wells et al. 1999). Cow-level risk factors include physiological, physical, and behavioural characteristics (reviewed by Palmer and O'Connell 2015) in addition to parity and stage of lactation (Holzhauer et al. 2006).

Examples of poor biosecurity practices leading to increased risk of DD transmission are: lack of washing hoof trimming equipment between cows, use of a professional hoof trimmer who trims at other operations (Wells et al. 1999), buying in new animals (summarized in Evans et al. 2016), and co-grazing dairy cows with contagious ovine digital dermatitis (CODD) infected sheep (Knappe-Poindecker et al. 2014). Potential risk-factors acting on the herd-level in addition to biosecurity are the geographic location (Read and Walker 1998), herd size, herd management, trimming practices, floor type, bedding type, and herd DD prevalence (Klitgaard et al. 2017).

Management factors such as stocking density, manure management, footbathing, and bedding management can influence the cleanliness of cows' feet and legs. Poor leg cleanliness is

associated with greater risk for DD (Relun et al. 2013b). Given this risk, it is somewhat surprising that when alleys were automatically scraped >7 times per day in Ontario free-stall herds, the risk for DD increased significantly ($P<0.01$, 95% CI=0.1–0.3; Cramer et al. 2009). The reason for increased risk with automatic scrapers running more frequently is likely associated with the resultant wave of slurry that cows are forced to walk through. If automatic scrapers are only run after fetching a milking group and before the cows return from the parlour, this repeated exposure to deep slurry can be avoided. Consistent with this theory, reduced risk for DD is seen in dairies which tractor scrape or have automatic scrapers with a slatted floor (Somers et al. 2005). In these cases, either the barn would be cleaned manually while cows are not in the pen, or the amount of slurry pushed by the automatic scraper would not accumulate to the same degree of a solid floored barn.

Flooring type has been associated with risk for DD (Barker et al. 2009; Frankena et al. 2009; Somers et al. 2005; Wells et al. 1999), with conflicting reports of texture effects but consistent reports of wetter surfaces resulting in greater DD. A greater risk for DD was associated with grooved concrete (OR=2.7, 95% CI=1.5-4.7) and smooth or slatted concrete (OR=1.8, 95% CI=1.0-3.1) compared to textured-concrete flooring (Wells et al. 1999). When slatted floors without an automatic manure scraper were used as the reference population, slatted floors with a manure scraper reduced the odds of DD (OR=0.57, $P=0.053$) but solid concrete floors had a numerically greater but statistically insignificant effect on risk (OR=1.19, $P=0.55$; Somers et al. 2005). An increased risk of DD with grooved concrete compared to solid non-grooved or slatted concrete (OR=11.31, 95% CI=5.04-25.42) was also reported by Barker et al. (2009).

The absence of hoof-trimming has been significantly associated with increased DD risk (Relun et al. 2013b). However, among both herds that trimmed and those that did not, those that used a professional trimmer that also trimmed at other dairies were at a greater risk of DD than those who used an in-house trimmer or did not trim at all in a 1999 study (OR=2.8, 95% CI=1.9-4.2; Wells et al. 1999). A recent report of a significant association between hoof trimmer and DD came out of Denmark in 2017. Oliveira et al. reported that using both a trained farm worker and a professional trimmer to manage DD resulted in less risk ($P<0.001$) than using one or the other alone (farm worker: OR=1.44, 95% CI=1.19-1.74; professional trimmer: OR=1.20, 95% CI=1.04-1.38; Oliveira et al. 2017b). The difference between these two studies may be that in the more recent one, effective treatment was delivered sooner in the herds which used both a professional

and a trained farm worker. It is worth noting that 100% of trimming tools sampled by Sullivan et al. had treponemal DNA on their surfaces (Sullivan et al. 2014) and biosecurity is gaining more importance in preventing DD (Oliveira et al. 2017b). Dairy herds housed in free-stall barns in Ontario experienced a greater prevalence of DD lesions if cows were trimmed in the spring rather than the summer or fall (OR=-0.2, $P<0.01$; Cramer et al. 2009). Although no reasons were given for this difference, one can speculate that recently trimmed hooves in the spring allow the soft tissues of the feet to be in closer contact to the wet, muddy surfaces common to spring in Canada.

An interval >7 months between two herd trimmings has been positively associated with DD (OR 1.87, $P<0.005$) compared to intervals <7 months (Somers et al. 2005). A study with opposing results reported that cows trimmed >12 months before a study began had a lower risk for DD than cows trimmed at shorter intervals (Holzhauer et al. 2006). In the latter study, herds on a more frequent trimming schedule before the study began may have developed that protocol as a means to mitigate high prevalence of DD and other hoof lesions. The presence of DD is associated with interdigital hyperplasia and interdigital dermatitis (Holzhauer et al. 2006).

Tie-stall housed herds had a greater prevalence of DD if they had year-round access to an exercise yard (prevalence ratio=2.1, $P=0.04$, CI=1-4.1; Cramer et al. 2009). Similarly, Holzhauer et al. (2006) reported a greater risk of DD (OR=1.6, $P<0.05$, CI=1.3-2.0) when cows had >8 hours of pasture access per day compared to those with no pasture access. Contrasting results were observed in that study, where longer access to pasture slightly decreased the risk for DD in cows also afflicted by interdigital hyperplasia (Holzhauer et al. 2006). Although the risk of DD was not evaluated in a recent American study, lameness was found to be significantly associated with outside access and the characteristics of surface accessed (Adams et al. 2017). A higher prevalence of severe lameness was observed on dairies where cows had outside access to concrete compared to those where cows had access to open/dry lots ($P=0.002$) or no outside access ($P=0.004$; Adams et al. 2017). Dairies on which the surface accessed outside was wet half the time had a lower prevalence of lameness compared to those with dry ($P=0.016$) or almost always wet ($P=0.023$) outside surfaces (Adams et al. 2017). In that study, where lameness but not the specific causes of lameness were observed, it appears that hard, abrasive, and/or slippery surfaces increased lameness. It is frequently observed by the author that concrete flooring where cows are housed becomes slippery when the thin layer of manure left behind dries after alley scraping. This observation is in agreeance with Adams et al (2017) who reported greater lameness prevalence on dairies where fans

are used to cool cows in the absence of misters/soakers ($P < 0.0001$) and increased lameness prevalence on dairies where the outside surface accessed is dry. It is likely in these cases that the cause of lameness would be traumatic rather than infectious in nature. Notably, where outside surfaces were almost always wet, increased lameness prevalence was seen; a known risk factor for DD. Therefore, it is likely that the type of surface accessed and its characteristics such as slippery, rough, and/or wet are associated with the type of lesion causing the lameness in question.

The risk for DD was higher when concentrate fed was rapidly increased to the maximum amount by two weeks after calving compared to a concentrate increase over 2-3 weeks (Somers et al. 2005). It was theorized that in the rapid step-up group, greater metabolic stress was experienced and susceptibility to disease increased. A Finnish study reported reduced instances of infectious claw disorders when a total mixed ration (TMR) was fed in comparison to a partial mixed ration and a ration with concentrates and roughage fed separately (Häggman and Juga 2015). Of note, the main dairy breed in Finland is Ayrshire, while the main breed in Canada is Holstein (Häggman and Juga 2015); Holsteins are at a higher risk of developing DD than other dairy breeds (Holzhauer et al. 2006).

1.4.1.2 *Animal level risks*

Within herds affected by a high prevalence of DD, some cows will not develop DD, despite inevitable exposure to the agents of infection. Given that cows housed in the same conditions and fed the same diet would be exposed to the same herd-level risks as their herd-mates, differences may lie in each cow's individual susceptibility to DD infection. Risk-factors at the cow-level are physical, physiological, behavioural, and influenced by lifecycle and parity. Potential physical risk-factors include hoof conformation, heel depth, toe length (Laven 2007), width and depth of interdigital space (Gomez et al. 2015), quality of epidermal barrier (Palmer et al. 2013), previous incidents of DD (Gomez et al. 2015), concurrent heel horn erosion (HHE, Capion et al. 2009) and parity (Holzhauer et al. 2006; Solano et al. 2016; Gomez et al. 2015). Some of these are heritable and influenced by breed (Rodríguez-Lainz et al. 1999).

Immune function differs between cows and chronic or intermittent stressors lead to reduced immune efficacy and increased risk of disease (Proudfoot et al. 2012). This increased risk of infectious disease with chronic or intermittent stress may be exaggerated in animals with low social status and reactive coping styles due to a potential relationship between these behavioural characteristics, relative immune response, and increased risk of infection (Proudfoot et al. 2012).

The stressful effects of low social status are increased with overstocking and frequent group changes (Proudfoot and Habing 2015). There is evidence that, with the large group sizes common on modern free-stall dairies, individuals lose the ability to recognize and remember herd-mates, leading to less antagonistic behaviour and less competition for resources and social status (Zwald and Shaver 2018).

Behavioural differences between cows may influence their risk of DD infection due to differences in predisposition to spending increased time in higher risk environments. For example, free-stall housed cows that spend more time in passageways and standing perched with their hind feet in the alley have a greater risk of developing DD (Palmer et al. 2012). This behaviour may be a response to short and/or uncomfortable stalls, concurrent lameness that negatively affects the ease of lying, or a large range in cow size in a single group, reducing the likelihood that the producer is able to accommodate the largest cow. Comfortable stalls may have a preventative effect on DD due to reduced perching; thereby the rear feet spend less time in the manure alley. Cows that are lame tend to have longer lying bouts, increased lying times, and greater variability in the duration of lying bouts compared to sound cows (Ito et al. 2010). The comfort of the stall surface influences how a lame cow changes her lying behaviour compared to her sound herd-mates (Ito et al. 2010). Behavioural differences can also be the effect of social hierarchy and high stocking density. In regards to lifecycle, the greatest likelihood of developing DD is experienced by primiparous cows while DD is less prevalent in dry cows (Somers et al. 2005). The odds of developing DD were greatest for primiparous cows in mid (100-199 DIM) to late lactation (>200 DIM) in one study (Solano et al. 2016b), while the risk for DD was reportedly reduced after 180 DIM in another (Barker et al. 2009). If a heifer experienced one or more DD events during the rearing period, her risk of developing DD in her first lactation and developing it sooner than her peers who did not experience a DD event in the rearing period is increased (Gomez et al. 2015). Alternatively, Holzhauser et al. (2006) reported cows having greater odds for DD during peak lactation (30-60 DIM) and in the third parity compared to cows >60 DIM, but also reported decreasing risk with increasing parity. The reduced prevalence among dry cows may be the result of drier feces from the high forage ration provided during this period (Somers et al. 2005) and the lower metabolic demand of dry cows compared to lactating cows.

1.4.2 Prevention and Control of Digital Dermatitis

1.4.2.1 *Detection of digital dermatitis*

The prevalence of DD is under-estimated by producers when evaluating their own herds (Oliveira et al. 2017a). Considering the infectious nature of DD, prevention and treatment can only be executed effectively if lesions are accurately detected. The location of typical DD lesions, proximal to the heels and distal to the dew-claws of rear feet, is not easily accessed or noticed if lameness is not evident (Solano et al. 2017a). Diagnosing DD with the use of a trim chute is considered the gold standard (Cramer et al. 2018). Other observation methods for lesion detection have been evaluated in the milking parlour (Relun et al. 2011), headlocks, alleyways (Cramer et al. 2018), and young stock pens (Jacobs et al. 2017). High specificity and sensitivity of DD-detection programs along with easy implementation would result in sufficiently frequent detection required to control DD (Cramer et al. 2018).

The sensitivity and specificity of scoring DD lesions with the M system in milking parlours, pens, management rails, and headlocks compared to inspection in a trimming chute were evaluated (Cramer et al. 2018). Although these alternative scoring methods had a high specificity (>93%) for the presence or absence of DD at the foot-level, sensitivity was relatively low (<70%). The milking parlour (Se, 69.6; Sp, 96.2) and headlocks (Se, 60.6; Sp, 95.8) provided the second-best detection settings for detection of lesions on hind feet after the trimming chute. Although the highest prevalence of DD is found in the milking herd, young stock are also susceptible (Jacobs et al. 2017). Using pen walks, Jacobs was able to estimate the prevalence of DD affecting hind feet of group-housed young stock on 28 Alberta dairy farms without washing the feet first (Jacobs et al. 2017). This method only evaluated presence/absence of DD but had a sensitivity of 98% and specificity of 65% at the animal level.

A reliable method of detection second to using a trimming chute is cleaning the feet with water and using a headlamp/flashlight and mirror in the parlour (Solano et al. 2017a). Producers and farm workers may be reluctant to spray cows' feet in the parlour due to compromising udder hygiene and the tendency of cows to defecate during this practice. It has been speculated that regularly spraying the feet in the parlour may decrease the startle response through behavioural conditioning and thereby decrease defecation (Oliveira et al. 2017c). A method of parlour DD-detection was tested by Oliveira et al. (2017b) in which feet were not washed before scoring. Flashlights were used in both methods, but mirrors were not mentioned. Scoring with and without

washing the feet first gave highly correlated results, but cow-level prevalence of DD affecting the hind feet was higher by a median of 32% with washing indicating that not washing first may substantially underestimate herd prevalence (Oliveira et al. 2017c).

1.4.2.2 *Prevention of digital dermatitis*

Given the endemic status of DD in North American dairy herds, prevention is of utmost importance in controlling its incidence. Prevention can be broken down into seven categories of control: flooring surface, concurrent claw trauma, nutrition, detection and treatment, heifer management, environmental hygiene, and biosecurity (Potterton et al. 2012).

The most common prevention tactic used by dairy producers is the use of disinfecting footbaths. Solano et al. found that of Canadian free-stall dairies that do not footbath, 95% were smaller than 100 cows ($P < 0.001$), indicating that most large dairies realize the importance of footbathing (Solano et al. 2015). In managing footbath efficacy, concentration and contamination level of the solution in addition to sufficient coverage of all four feet is paramount to success (Cook 2017). Common footbath solutions used in North America are prepared with CuSO_4 , formalin (37% formaldehyde w/v), and to a lesser degree, ZnSO_4 as active ingredients. Copper sulphate has been used in concentrations of 2.0% (Laven and Logue 2006) to 10% (Teixeira et al. 2010) while formalin has been studied at 2.5% (Laven and Logue 2006) to 5% (Teixeira et al. 2010). Reports of zinc sulphate footbaths have been somewhat anecdotal, suggesting a solution of 5 to 20% (Tomlinson et al. 2014). None of these products is a perfect solution due to side effects of their use; disposal of slurry with high levels of copper or zinc sulphate from footbathing can be detrimental to the fields it is spread on, and formaldehyde is a known human carcinogen (Relun et al. 2012). Efficacy of acidified CuSO_4 footbaths is of interest due to the potential for decreasing the amount of CuSO_4 necessary to have a preventative effect on DD (Cook 2017). The bioactive form of copper, Cu^{2+} , requires a pH of 3.5 to 4.0 to best react with the target organisms' thiol groups so reducing the footbath solution pH theoretically improves this desired action (Cook 2017). Acidification of footbath solutions should be done with caution, as over-acidification can damage skin and promote lesion proliferation (Cook 2017). Formalin has a natural pH of 3 to 5 and further acidification is unnecessary. However, it is recommended that formalin use is limited to temperatures above 18°C due to possible polymerization and reduced efficacy below 15°C (Cook 2017). Addition of methanol may limit polymerization to some extent. There are anecdotal reports of bleach, detergents, and chlorine used in footbath solutions. Some farmers have claimed to use

one of these on alternating days between more traditional footbath solutions. Organic dairy producers and those concerned with the above footbath chemicals may consider alternatives with tea tree oil, copper chelates, or acidified ionized copper (Pinedo and Velez 2017; Cook 2017). A 3% tea tree oil and organic acid footbath product was compared to 5% CuSO_4 and they were not statistically different ($P=0.59$) in efficacy, but both significantly reduced the proportions of M1 and M2 lesions by the end of the study according to McNemar's test statistic ($P<0.01$; Smith et al. 2014).

Footbaths need to be well designed and managed; those requiring excessive labour such as portable or poorly situated footbaths are inconvenient and may not be frequently used. One of the key factors affecting the likelihood of follow-through by farm workers is the time and effort required for task execution (Relun et al. 2013a). If possible, producers should consider installing automatic footbaths situated sufficiently far away so as not to prevent cows from exiting the parlour in a timely fashion. The flow of cattle should be able to move straight through the footbath with no turns immediately before or after to encourage smooth passage. A footbath design by Cook recommends a minimum length of 3.0 m, step-in height of 0.25 m, 0.6 m wide at the base, and have walls sloping up from either side of the base to a width and height of 0.9 m and 0.9 m, respectively (Cook 2017). This length would ensure that all four feet are immersed 2 times each for 95% of cows (Cook et al. 2012). If the bath is only long enough for each foot to be immersed once, it is common for one hind foot to miss the bath entirely. The sloped side-walls allow the use of a narrower bath while eliminating the cow's ability to step on the edge or outside of the footbath.

A standardized footbathing protocol was tested by Solano et al. on 9 farms over 22 weeks in Alberta and significantly positive effects were observed in herd-level active lesion prevalence (Solano et al. 2017b). The standardized footbath regimen implemented in this study consisted of a 3 m long, 0.50 m wide bath with separate baths divided by a grate and automatically filled with 0.15 m of 5% CuSO_4 solution. The footbath was used for 4 consecutive milkings over 2 consecutive days on a weekly basis and solution was changed automatically before 200 cow passes or 24 hours, whichever came first. Both a decrease in active lesions and an increase in healing lesions was observed to be significant ($P < 0.05$) in herds with a high baseline DD prevalence pre-trial ($\geq 15\%$).

1.4.2.3 *Controlling risk for digital dermatitis*

To effectively control DD, one must consider all aspects of the disease triangle. This model is used in epidemiological studies to illustrate the relationship between pathogen, environment, and

host to the disease in question. Concerning DD in Canadian dairies, the bovine host is most commonly Holstein and lives in a relatively intensively managed environment. The identity of the pathogens in question is not entirely clear but it is generally agreed upon that various bacteria including *Treponema* spp. work in concert to cause clinical DD. When considering DD, prevention should focus on reducing the introduction and proliferation of DD pathogens on the farm in addition to limiting risks in the environment that increase the likelihood of exposure to DD pathogens and limiting host inability to combat disease. Comorbidity of a transition cow disease with an introduction to an environment with a greater pathogen load is an example of reducing the host's ability to combat DD infection.

Examples of biosecurity tactics associated with reduced risk include keeping a closed herd, keeping all young and dry stock on the same yard as the milking herd, providing boots for visitors (Oliveira et al. 2017b), and ensuring the hoof trimmer disinfects tools between farms (Evans et al. 2016). A significantly higher risk for DD was seen on farms where replacement heifers were purchased (Rodríguez-Lainz et al. 1999). If purchasing young stock is unavoidable, a quarantine pen, footbathing, and treatment upon arrival should be employed. These methods of improving farm biosecurity limit the introduction of DD pathogens from endemic herds.

If DD pathogens are already present, the focus should be on reducing the risk of transmission from chronic to naïve animals. It is in this part of DD prevention that footbaths and early treatment play a key role. Dirty feet are associated with increased risk of DD and may also limit the efficacy of footbath performance by contaminating the solution before the maximum recommendation of 200 cow passes (Cook 2017). A management focus on improving cow cleanliness is thereby important for reducing the risk of DD and enabling efficient footbath use. A greater risk for DD has been associated with low heels and long toes (Laven 2007), conformation traits that would inevitably increase heel exposure to slurry and physical abrasion. Regular trimming reduces the risk of DD (Somers et al. 2005). Producers should consider a barn design that allows easy, efficient sorting of cattle for trimming, and an indoor trimming area so appointments are not postponed during disagreeable weather.

Management decisions that mitigate stress can affect the bovine host's susceptibility to infection. Stress has been positively associated with increased risk of disease due to its immune suppressive effect (Proudfoot and Habing 2015). Common stressors include regrouping, calving, estrus, rough handling, transportation, and improper nutrition. Producers can reduce the effects of

these stressors by keeping first lactation heifers separate from multiparous cows, providing stable social environments during parturition, grooving concrete floors to provide traction, moving animals in a calm manner with appropriate stockmanship, and giving recently transported animals palatable feed and clean water in a separate pen upon arrival.

Until an efficacious vaccine is developed, risk can only be managed by reducing exposure to DD pathogens in the environment and giving cattle the best chance to mount an effective immune response.

1.4.3 Treatment of Digital Dermatitis

Although footbaths are a key to preventing DD spread, they are not considered a treatment except in the rare instance of antibiotic footbaths. Due to the nature of DD bacteria's ability to thrive deep in the tissue under a thickened layer of keratinocytes (Döpfer et al. 2012b), regular disinfecting footbaths only kill superficial pathogens. Antibiotic footbaths are rare due to the risk they pose in contaminating milk with antibiotic residues, the cost of dumping potentially contaminated milk, and because their use may promote bacterial resistance. The most common mode of treatment is to apply an antibiotic powder or paste topically to the lesion, with or without a bandage. Treatment bandages are normally removed after 1 to 3 days, at which time lesions are in the healing stage (M3). It has been suggested that due to the frequent reoccurrence of clinical lesions after treatment, true lesion resolution is not achieved by only one treatment (Berry et al. 2012). The antibiotics used commonly in research and anecdotally on farm are oxytetracycline and lincomycin applied topically (Blowey and Sharp 1988; Laven and Logue 2006; Berry et al. 2010); this method is off-label. As of 2011 in the UK, only cefquinome is licensed for parenteral administration to cattle with DD (Potterton et al. 2012). A recently developed chlortetracycline hydrochloride topical spray is now available for DD treatment in Canada (Cyclo Spray®, Bimeda Ireland). In-vitro susceptibility studies of DD-associated treponemes have indicated these antibiotics are only moderately effective, while penicillin, penicillin derivatives, erythromycin, azithromycin, and gamithromycin are much more effective (Evans et al. 2016, 2009a).

Systemically administered penicillin has been used with success in 7 out of 7 clinically infected cows (procaine penicillin G, 18,000 units/kg intramuscularly twice daily for 3 days; Read and Walker 1998), but it is not practical for use in lactating cows due to the long milk withdrawal period required (Evans et al. 2012). Similar cure rates were seen with 5g of topical oxytetracycline

powder in the same study (4 out of 4 cured; Read and Walker 1998), while a recent study reported a cure rate of 75% (of 68 M2 lesions; Holzhauser et al. 2017). In the latter study, a cure was defined as a transition of an M2 lesion to an M0 or M4, which were considered inactive stages. Continued use of moderately effective antibiotics is not a sustainable practice considering the increasing numbers of antibiotic-resistant bacteria. Syphilis and yaws, human diseases caused by treponemes, have been treated successfully with long-acting intramuscular penicillin and oral azithromycin (Giacani and Lukehart 2014). However, *in-vitro* studies alone are not enough to determine the treatment efficacy of an antibiotic.

The typical site of DD affects the epidermis and dermis of the feet, which may experience comparatively less blood flow, a key factor in delivering antibiotics to the infected tissue. One study investigated the milk, plasma, and synovial fluid concentrations of tetracycline hydrochloride administered via the jugular vein and through regional intravenous antibiotic (IVRA; Rodrigues 2010). Peak synovial fluid concentrations were higher and plasma concentrations were lower in the group of animals which received IVRA compared to those who received jugular IV tetracycline ($P < 0.05$). In that study, neither IVRA nor IV administration of tetracycline hydrochloride resulted in improvement of lesion or locomotion scores, indicating that despite remaining above 0.25 $\mu\text{g/mL}$, either the drug or the minimum inhibitory concentration (MIC) was not high enough to be therapeutically effective against the pathogens of DD. Notable downfalls of that study include the complete neglect to mention treponemes as agents of DD, the failure to consider the MIC of these important pathogens, and the lack of any pathology work done to measure antibiotic levels in tissues harbouring DD-associated bacteria. Further research is necessary to improve treatment of DD with both systemically and topically administered antibiotics. Penicillin and its derivatives may play a useful role in treating chronically infected cows at dry off but reports of this practice were not found in the literature. It is evident that more efficacious antibiotics are necessary to control DD and to further impede antibiotic resistance.

1.5 Literature Review Conclusions

Digital dermatitis is a highly prevalent disease among high-producing dairy cattle in Canada and around the world. The lesions associated with clinical disease are painful and negatively affect welfare, DMI, production, immunity, fertility, and dairy efficiency. The lost production associated with DD is costly in addition to the treatment and preventative management associated with its

control. A variety of DD management practices are reportedly employed by Canadian dairy producers in Ontario and Alberta, but research focused on Saskatchewan dairy producers is lacking. Since no efficacious vaccine is available at present due to the variety of bacterial strains associated with DD, further research focused on detailed bacterial etiology may prove useful in developing more effective management practices, vaccines, and improved antibiotic treatments.

1.6 Thesis Objectives

Dairy producer management practices for controlling DD and other causes of lameness on Saskatchewan dairies has not been previously studied. In addition, critical information on the heel skin microbiota is previously unstudied in a commercial dairy housing cows without any sign of clinical DD. The first objective of this research on bovine DD was to describe the known DD risk factors identified on selected Saskatchewan dairies that are endemic and non-endemic for clinical DD. The surveys used to obtain these results also served to ensure the study herds were representative of other Canadian herds and to recruit and further describe the participant dairies for part two of this project. The second objective was to describe the presence, abundance, and identity of DD-associated bacteria in heel skin tissue and fecal samples from cows with preclinical DD, as well as slurry samples from one dairy non-endemic for clinical DD and from 6 dairies endemic for clinical DD in Saskatchewan.

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2 ON-FARM QUESTIONNAIRE AND RISK FACTORS FOR DIGITAL DERMATITIS

2.1 Introduction

Digital dermatitis (DD) is a painful, ulcerative lesion, polybacterial in nature (Krull et al. 2014) that develops proximal to the caudal interdigital cleft of ruminant and ungulate species (Clegg et al. 2015; Blowey and Sharp 1988). Lameness is a common consequence of DD. The negative effects of DD on welfare, fertility, and production, in addition to the treatment and preventive tactics used, cost producers money. Prolonged exposure of the feet to wet, unhygienic conditions weaken the epidermis (Palmer et al. 2013), making it more susceptible to pathogen invasion. Digital dermatitis-associated bacteria have been identified in slurry and its management appears to be important in controlling DD incidence (Klitgaard et al. 2014). Digital dermatitis lesions are often treated with topical antibiotics such as oxytetracycline (Berry et al. 2010), tetracycline (Milinovich et al. 2004), chlortetracycline (Speijers et al. 2010), lincomycin (Berry et al. 2012), chlortetracycline (Relun et al. 2013a), or pastes of copper and zinc chelates (Holzhauer et al. 2011). Although these treatments are generally effective in temporarily reducing the severity of a DD lesion, DD lesions often return to a clinical stage post-treatment (Krull 2015). Preventative tactics in closed herds may differ compared to open herds due to the level of exposure to risks experienced by cows in each environment. In a closed herd, regular preventative trimming may be prioritized over regular footbath use because of lower infection pressure. However, in an open, endemic herd, footbathing regularly may be prioritized due to the exposure to DD pathogens via newly introduced, infectious herd-mates.

Increased prevalence of DD is associated with biosecurity, housing, management, and traits of the potential host animals in question. Previous studies have found that breakdowns in biosecurity, such as buying in replacement heifers (Wells et al. 1999; Rodríguez-Lainz et al. 1999), use of a professional hoof trimmer who trims at other dairies, lack of cleaning trimming tools between cows (Wells et al. 1999), and not providing boots for visitors are associated with an increased odds of DD (Oliveira et al. 2017b). Housing considerations such as flooring type, free-stall size, and whether the lactating herd is housed in free-stalls, tie-stalls, in a pack barn, or seasonally at pasture also affect the odds of higher DD prevalence (Somers et al. 2003; Cramer et

al. 2009). Footbath routine, re-bedding schedule, stocking density, and grouping techniques are examples of management factors that could influence hygiene and exposure to active lesion pathogens. Unhygienic, wet conditions are associated with an increased risk for DD (Rodríguez-Lainz et al. 1996) and management tactics that prevent these conditions can reduce DD risk.

Producers have often asked what they could do besides footbathing and trimming their cattle's feet regularly to prevent and manage DD. This questionnaire serves as a starting point to answer that question in a practical manner, by providing measures of risk factors on Saskatchewan (SK) dairies and discussing their pertinence to the control of clinical DD. The objective was to describe known DD risk factors of the selected participant herds endemic and non-endemic for clinical DD in Saskatchewan dairies. The risk factors studied include housing, management, hoof health, and biosecurity practices known to be associated with greater DD risk.

2.2 Materials and Methods

2.2.1 Participant Recruitment

A 12-question survey to determine the perceptions Saskatchewan (SK) dairy producers had on lameness was delivered to all 163 SK dairy producers anonymously through SaskMilk, the provincial marketing board, in June 2015. From the 25 survey participants who indicated their willingness to participate in further studies, a convenience sample of 6 dairies endemic for clinical DD was selected to be compared to a clinically non-endemic dairy. The non-endemic dairy was identified as being free of clinical DD through a review of veterinary records by the author. This non-endemic dairy (dairy 7) completed the same survey as the endemic dairies (dairies 1 through 6) and indicated their willingness to participate in further research. These 7 dairies were visited between August 12th and September 29th of 2015 to coincide with regularly scheduled hoof trimmer visits. Dairies are identified by 'D' then a number, signifying the order in which each dairy was visited for the remainder of this chapter. For example, the first dairy visited was D1, while the third dairy was D3. The seventh, and last, dairy visited was the dairy non-endemic for clinical DD, D7.

2.2.2 Data Collection

During each farm visit, producers were interviewed with a structured questionnaire, detailing herd demographics, housing conditions, hoof trimming, footbath use, and lameness management.

The aim of this questionnaire was to determine housing and management practices of the selected participant herds to compare to known risk factors. A total of 62 questions were asked by a research team member (Appendix B). Some questions were open-ended while others prompted numerical and categorical answers. Contingency questions were used to determine whether a section topic was relevant to that producer. For example, “do you use footbaths?” guided further questioning on footbath usage for those producers who replied “yes.”

As part of the farm visit, general observations were recorded pertaining to footbath dimensions, bedding material, parlour design, and flooring in parlour and alleys. The aim of recording these observations was to acquire as much information as possible about the participant dairies so comparisons with published research on Canadian dairies concerning DD-associated risks could be made. The results of this study yielded a report of management practices utilized by Saskatchewan dairy producers to mitigate the risk of DD in their herds.

2.2.3 Statistical Methods

To determine if trimmer 1, who trimmed at 6 of the 7 participant dairies (all but D2), evaluated clinical DD in agreement with the author, Cohen’s kappa statistic was calculated. First, 84 hind feet were scored independently by the trimmer and the author during a routine trim session at D4 in fall 2016. The trimmer used Hoof Supervisor® and the author used a pen and paper. Feet were evaluated on a binary basis; they either had a clinical DD lesion, or they did not (APPENDIX B) Then, the calculation, $\left[\kappa = \frac{P_o - P_e}{1 - P_e}\right]$, was used to determine the inter-rater agreement between trimmer 1 and the author. The standard error of κ was defined as $\left[SE(\kappa) = \sqrt{\frac{P(1-P)}{n(1-P_e)^2}}\right]$. All other statistics performed were descriptive in nature. Quantitative results are presented as sample mean \pm standard deviation (min-max). Qualitative results are presented as the percentage of the sampled population which responded positively to the category in question.

2.3 Results

Participants completed a questionnaire at the time of the farm visit in which preclinical lesion biopsy specimens were collected. Dairies 1-6 had an average milking herd size of 292 while the non-endemic herd, D7, had 110 cows in milk (**Table 2.1**). Dairies 1, 5, and 6 cleaned their alleys

with a skid-steer or tractor while cows were milked but all other dairies used automatic scrapers that ran an average of 10.7 times/day (**Table 2.2**).

Table 2.1 Background information of participating farms [mean \pm SD (min-max)] including herd size, mean group size (not including special needs group), mean animals purchased per year, and knowledge of purchased animals' exposure to digital dermatitis.

Disease Status	No. of Dairies	Herd Size	Group Size	Animals Purchased	Know DD status
Endemic	6	292 \pm 136 (160-510)	101 \pm 26 (65-127)	19 \pm 13 (0-40)	50%
Non-Endemic	1	110	15	0	100%

Table 2.2 Bedding and walkway alley maintenance schedule. Alley cleaning and stall cleaning are per day, bedding frequency is per week and bedding replaced is on a per year basis.

Dairy Abbreviation	Disease Status	Alley Cleaned	Stalls Cleaned	Bedding Added	Bedding Replaced
D1	Endemic	3	N/A	7	5
D2		12	3	2	0
D3		10	2	0.5	26
D4		10	3	2	0
D5		2	N/A	7	6
D6		3	3	4	0
D7	Non-Endemic	8	3	7	52

When asked to list the three most problematic foot lesions on their dairy, not every producer named three. A total of 18 answers were given and 33% of those were DD, 22% were foot rot, and 17% were sole ulcers (**Table 2.3** and Appendix B).

Risk assessing questions resulted in answers regarding replacement heifer/cow purchasing practices, housing management, lameness management, and trimming (**Table 2.4**). All producers hired a hoof trimmer that regularly trimmed at other dairies. Hoof trimming equipment was pressure washed between dairies in all cases but never washed between cows (**Table 2.4** continued). Dairies 1, 2, and 6 treated DD with an antibiotic but D7 did not treat for DD with antibiotic or other treatment (**Table 2.4** continued). Although D7 did not experience clinical DD

nor the treatments associated with it, all lame cows with other lesions were seen to promptly by a practicing veterinarian experienced in ruminant hoof care and trimming. The complete questionnaire and its results can be found in Appendix B.

Table 2.3 Foot lesions identified as the top three problematic lesions on participant dairies

Most problematic foot lesions	% of the time top three most problematic foot lesion	N
DD	33%	6
Foot rot	22%	4
Sole ulcers	17%	3
Heel erosion	6%	1
Corns	6%	1
White line disease	6%	1
Abscesses	6%	1
Corkscrew toe	6%	1

DD scoring agreement between the author and trimmer 1, who trimmed at 6 of the 7 study dairies, was evaluated with Cohen’s kappa statistic ($\kappa \pm 95\% [CI = 0.736 \pm 0.187]$), which showed good agreement (>0.70). Clinical DD was scored as either present or absent (**Table B.1**).

Table 2.4 Risk assessing questions for participant producers

Risk Assessing Question:	Dairy 1	Dairy 2	Dairy 3	Dairy 4	Dairy 5	Dairy 6	Dairy 7
Average no. animals purchased /year	20	35	25	40	25	0	0
Most recent year animals were purchased from another herd	2015	2015	2015	2015	2014	NA	2009
Disease status of herds purchased from	Not known	DD +	Not known	Not known	Not known	DD +	DD -
Alley cleaning frequency (x/day)	3	12	10	10	2	3	8
Cows with outside access	2 Milking groups, dry cows	NA	Dry cows	NA	NA	Dry cows	Dry cows
Trim entire herd or select animals at a time	Entire herd	Select	Entire	Select	Entire	Select every 3 weeks, entire herd 2-3x/year	Entire
Hoof trimming frequency	3 times/year	1 time/month	1 time/year	Select animals every 3 weeks	3 times/year	Every 3 weeks and 2.5 times/year	2 times/year
Timing of heifers' first trim	After 1st calving	Before 1st calving	After 1st calving	Before 1st calving	After 1st calving	After 1st calving	Before 1st calving

Table 2.4 continued

Risk Assessing Question:	Dairy 1	Dairy 2	Dairy 3	Dairy 4	Dairy 5	Dairy 6	Dairy 7
Method of cleaning trimming equipment	Pressure washes equipment between dairies	Pressure washes equipment between dairies	Pressure washes equipment between dairies	Pressure washes equipment between dairies	Pressure washes equipment between dairies	Pressure washes equipment between dairies	Pressure washes and disinfects equipment between dairies
Number of cow passes before footbath solution changed	160	110	250	200	160	250	66
Treat feet with disinfecting spray in the parlour	Yes	No	Yes	Yes	Yes	No	No
Type of foot spray used	Quick-Hit 1:1	NA	Repiderma	Quick-Hit 1:1	37% Formalin	NA	NA
How great of a problem producer thinks DD is	Neutral	Somewhat of a problem	Neutral	Somewhat of a problem	Large problem	Somewhat of a problem	No problem at all
Use of antibiotic for treatment	Yes, by the farmer, not by the trimmer	Oxytetracycline and wrap	Trimmer uses Heelsol paste and wraps	No	No	Tetra-cycline and wrap	No
Most problematic foot lesions	DD and Corns	DD, Sole ulcers, White line disease	Foot rot, DD, Heel erosion	DD, Sole ulcers, Foot rot	DD	DD, Abscesses, Foot rot	Sole ulcers, Foot rot, Corkscrew toe

2.4 Discussion

A study by Wells et al. reported an association between herds with >200 cows in milk and a greater incidence of DD (1999). In the present study, the mean herd size of the endemic participant dairies was 292 (\pm 136) cows, while the herd size of D7 was 110 (**Table 2.1**). A greater proportion of bred heifers affected by DD was seen by Wells et al. in large herds (>200) compared to small (<100) or marginal (100-199) herds. Prevalence rates of DD in the lactating herd and replacement heifers were not evaluated as part of this study. However, the risk of heifers experiencing DD in their first lactation is reportedly increased if they have experienced DD once (Adj. OR [95% CI] = 5.6 [3.23 - 8.29], $P < 0.01$) or multiple times (Adj. OR = 12.5 [7.52 - 21.1], $P < 0.01$) before calving (Gomez et al. 2015b). The absence of hoof-trimming has been associated with increased DD risk (Relun et al. 2013b). Heifers that are older when they calve have increased odds of DD infection at calving (OR = 2.02 over a period of 30d, $P = 0.026$; Capion et al. 2012). On dairies where heifers are trimmed before calving, the date of first trim is not entirely dependent on calving date and there is the opportunity for successful treatment before heifers enter the milking herd. In this study, most endemic dairies trimmed their heifers after their first calving (**Table 2.4**). The dairy non-endemic for clinical DD, D7, employed the practice of trimming heifers before calving.

Most endemic herds purchased animals within the two years prior to the farm visits (**Table 2.1** and **Table 2.4**). An incidence of DD >5% has been associated with the proportion of the milking herd born off the farm, with >25% born elsewhere resulting in greater odds of DD (OR = 7.9 [95% CI = 4.9 - 13.0]; Wells et al. 1999). Over 65% of endemic dairies did not know the DD status of herds from which they purchased replacements. In every case where the exposure history of replacements purchased by endemic dairies was known, those animals came from a DD positive herd (**Table 2.4**). The farm manager for the non-endemic dairy, D7, explained that during a herd expansion, some animals had been purchased in 2009 from a known DD-free herd. Dairy 7 is now a closed herd in which no cows, heifers, or bulls are purchased or housed in the same facilities as cows or heifers.

The odds of DD infection in cows has been reported at nearly three times higher in herds which purchase replacement heifers compared to that of cows in closed herds (Rodríguez-Lainz et al. 1999). There are 66 Hutterite colonies in Saskatchewan (<http://www.hutterites.org/directory/>) and most own dairies in which artificial insemination is not used so bulls are purchased. One of the endemic dairies in this study was owned by a Hutterite colony and likely purchased bulls in addition

to replacement heifers from other dairies. Because of the very high herd prevalence rate in Western Canada, the animals of unknown exposure history purchased by endemic dairies in this study were likely purchased from an endemic herd.

Cramer et al. found an association between the prevalence of DD and how often automatic floor scrapers were run over a solid concrete floor (2009). In the present study, alleys were cleaned with varying frequency (2-10 times/day). Dairies 1, 5, and 6 cleaned their alleys with a skid-steer or tractor at each milking while all other dairies used automatic scrapers which ran 8-12 times/day. While the open pack-barn housed cows at D1 and D5 were bedded with long straw, D2, D4, and D6 were bedded with undigested manure solids, D3 had water beds with a thin layer (<2cm) of chopped straw on top, and D7 used rubber mats with a thin layer (<2cm) of chopped straw. Somers et al. evaluated the association of bedding type and depth with DD but only bedding depth was evaluated due to bedding type having a moderate correlation ($r > |0.5|$) with other management factors (2005). In that study, bedding deeper than 5 cm resulted in a numerically lower odds ratio (OR = 0.6) for DD at the end of the housing period, but without significance ($P = 0.105$; Somers et al. 2005). D2, D4, and D6 used a moderate (5 cm) to deep (>10 cm) undigested manure fibre bedding. Bedding deeper than 2 cm has also been associated with reduced lameness in Canadian Holstein-Friesian free-stall herds (OR = 0.74 [95% CI = 0.55 - 0.99], $P = 0.05$; Solano et al. 2015).

Previous research has found that the type of outside access provided was associated with the risk of DD, regardless of indoor housing system used. Increased DD risk was identified at dairies that allowed daily outside access to a dry lot during winter compared to daily access to pasture (OR = 4.3 [95% CI = 1.9 - 9.7]; Wells et al. 1999). Solano et al. reported that the prevalence of DD was two times greater in free-stall, tie-stall, and deep bedded pack housed herds with access to an exercise area compared to those with no access (2016a).

Two of the dairies in the present study housed their cows in cold barns deep-bedded with a straw pack. One of these, D1, housed one or more of their milking groups outside year-round. The type of surface cows spend most of their standing and walking time on also influences the risk for DD. As reported by Solano et al., only 35% of herds housed in deep-bedded pack barns were positive for DD, while 92-100% of herds housed on concrete flooring were DD positive (Solano et al. 2016b). A herd was considered DD positive if it had at least one cow with clinical DD.

In the present study, producers with endemic herds differed in their reports of most frequent causes of lameness compared to D7 (**Table 2.4**). D7 only listed sole ulcers and foot rot as the most

frequent causes of lameness while endemic dairies most commonly reported DD, foot rot, and sole ulcers. Previously, DD has been reported as the most frequent cause of lameness along with sole ulcers and white line disease in UK dairies (Amory et al. 2008). The prevalence of DD lesions was reported at 14.7%, sole ulcers at 5.7%, and white line disease at 4.4% of cows during whole-herd trims in Alberta free-stall housed cows (Solano et al. 2016a). The prevalence of foot rot was reported in combination with heel erosion and interdigital dermatitis for a combined 2% of cows affected during whole herd trims. Green et al. reported that the most common lesions identified on the feet of lame cows were sole ulcers, white line disease, interdigital necrobacillosis (foot rot), and DD (2002). The cow-level prevalence of foot lesions in Ontario free-stall dairy herds in 2004-2005 was 22.9% for DD, 9.3% for sole ulcers, and 0.2% for foot rot (Cramer et al. 2008). The cow-level prevalence of all lame cows with DD was 12.6%, with sole ulcers was 20.8%, and with foot rot was 8.9% in a 2004 study on two New York State dairy herds (Booth et al. 2004). In that study, the authors combined all cases of interdigital dermatitis and interdigital hyperplasia with foot rot cases and analyzed them together under the title, “foot rot.” It is clear that in that study, foot rot was over-reported. In the present study, producers reported DD, foot rot, and sole ulcers as the top three causes of lameness (**Table 2.3** and Appendix B).

Foot rot and DD are both infectious lesions so the endemic producers who reported these among the most frequent causes of lameness on their dairies may have environments suitable to the spread of infectious lesions. Because D7 reported foot rot as the second most common cause of lameness, their housing environment is likely to be conducive to the spread of infectious lesions, indicating a superior housing environment is unlikely to be responsible for their non-endemic status.

Two trimming routines were employed by the study dairies. Either the majority of their cows were trimmed twice a year or else $\leq 25\%$ of the herd was selected once every three weeks for trimming. Neither of the trimmers cleaned tools between cows but both claimed to pressure wash equipment between dairies. Reportedly, dairies that hired trimmers who also trimmed at other dairies were more likely to experience a greater incidence of DD than dairies who did not (Wells et al. 1999). Although D7’s trimmer trimmed at other dairies and did not disinfect tools between endemic dairies, he did report washing and chemically disinfecting his tools before visiting D7.

All dairies claimed to use a footbath and the active ingredients used were either CuSO_4 or formaldehyde. In a 2015 study by Solano et al. on lameness prevalence and associated risk factors,

lameness prevalence was not associated with footbathing routine nor footbath size (Solano et al. 2015). It was suggested that the lack of association may be due to certain footbathing practices increasing lameness (e.g. a tall step-in height of bath edge or high speeds of cows trafficking through the bath resulting in injury) and that high lameness prevalence can increase the footbathing frequency at farm level (Solano et al. 2015).

Of the endemic dairies, 4 of 6 sprayed feet with a disinfecting solution in the parlour at milking time. Cramer et al. used a cross-sectional observational study and reported that DD was 2.0 times more prevalent in herds where cows' feet were sprayed in the parlour compared to dairies that did not spray feet in the parlour (2009). This does not necessarily indicate that spraying feet in the parlour is a cause of DD but is likely rather a response to high DD prevalence by the farmer.

Across the results of previous studies, producers and herd managers underestimate the percent of lame cows compared to trained investigators (Espejo et al. 2006). In the case of DD, lameness can be prevented if lesions are detected and treated early. The most reliable diagnosis of DD necessitates a trim chute, but none of the producers in this study used this method to diagnose DD in their herd. Instead, the hoof trimmer was relied on to diagnose all lesions during trimming. DD scoring agreement between the author and trimmer 1 was considered good (>0.70) through evaluation with Cohen's kappa statistic. Clinical DD was scored as either present or absent.

We did not evaluate whether the producers fed a mineral mix intended to control DD. Gomez et al. used organic Zn, Mn, Cu, and Co in Holstein steer diets and found a reduced incidence and severity of DD (2014). A more recent study in 2017 evaluated the prevalence of DD in beef feedlot cattle and found it was significantly higher ($P < 0.05$) in the control group fed a diet with strictly inorganic trace minerals compared to one with a unique formulation of organic and inorganic trace minerals (Kulow et al. 2017). One limitation of the present study is the lack of nutritional information collected for the diets fed at each farm.

Treatment methods differed between the participant dairies. Of the endemic dairies, only 50% (3/6) used an antibiotic when treating clinical DD while the others used a mineral paste and a wrap. D7 did not use any antibiotics or wraps for preclinical lesions or other hoof lesions prior to biopsy collection. DD lesions on Danish dairy cows were treated with salicylic acid under a bandage in a 2012 study by Thomsen et al. and was reportedly better at resolving DD lesions than chlortetracycline spray according to a study by Schultz and Capion (2013). Although salicylic acid

is not an antibiotic and is considered safe for the environment, its use requires bandaging, a laborious and costly practice (Schultz and Capion 2013).

2.5 Conclusions

Management and biosecurity characteristics of dairies endemic for clinical DD were more often those associated with greater risk for clinical DD compared to those of D7, a dairy non-endemic for clinical DD. While D7 was a closed herd with less than 200 cows, all endemic dairies evaluated were open herds which had bought replacement animals in the last two years, a practice associated with increased DD prevalence. D7 also required their hoof trimmer to use a disinfecting solution on his trim chute and purchase new blades, the cost of which they reimbursed before he came to trim twice yearly. All cows at D7 that became lame between trimmer visits were reportedly treated promptly by a veterinarian specializing in ruminant hoof care, while most endemic dairies relied on their hoof trimmer to treat lesions during regularly scheduled visits. The type of management-associated risks evaluated were both responsive to DD and preventative in nature. While preventative footbathing frequency was similar at all dairies, only endemic dairies sprayed feet in the parlour, a response to obvious clinical DD. Endemic dairies generally focused their management tactics on reducing severity of DD in their herds by footbathing and treating DD with topical antibiotics or a mineral paste, while D7 focused on prevention with biosecurity measures such as housing only animals free of clinical DD on the dairy and surrounding grounds, not purchasing replacement heifers, breeding with AI only, and requiring hoof-trimmer equipment to be disinfected. It is recommended that dairies looking to grow their herd consider biosecurity measures such as knowing the DD status of purchased animals, footbathing all new animals on arrival, treating new animals with DD infections, and providing their hoof-trimmers with disinfectant to use on tools between endemic cows and dairies.

2.6 Transition Statement

The on-farm questionnaire revealed that Saskatchewan dairy producers manage their herds in a way representative of other Canadian dairies. These dairies are most often endemic for clinical DD and their responsive rather than preventative management styles reflect this. While dairy producers can manage the severity of DD with footbathing and timely topical treatment, once prevention and treatment tactics are ceased, lesions often recrudescence along with their production limiting effects. Although milk production remains a top priority of dairy producers, the importance

of good animal welfare and the implementation of ProAction in Canada has brought a greater focus to lameness prevention and management. Because infectious causes of lameness require constant management and treatment yields inconsistent lesion resolution, the desire for an effective vaccine is strong. Previous attempts at vaccine development have been unsuccessful and thought to be due to the abundance and variety of bacteria detected in DD lesions at various stages of development. This complex etiology, paired with the recognition of preclinical lesions hosting different bacteria than clinical lesions (Krull et al. 2014), warrants further investigation into which organism or combination of organisms are most important in the transition from the painless preclinical to the painful clinical stages of DD. Within this study, 6 of 7 dairies were endemic for clinical DD and one had cows with preclinical lesions but no clinical DD. The existence of a clinically non-endemic dairy is rare and although this study is not extensive enough to determine truly significant differences between the microbiome of preclinical lesions sampled at these 7 different dairies, a valuable opportunity has been presented to describe the bacteria in these environments. A better understanding of the microbiome of preclinical lesions would direct future research into the key bacteria responsible for the transition to clinical DD, aiding vaccine development efforts.

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3 DETECTION OF BACTERIA IN APPARENT EARLY DIGITAL DERMATITIS LESIONS

3.1 Introduction

Digital dermatitis (DD) is a painful, infectious disease affecting the feet of dairy cattle and costs American producers an estimated US\$132.96 per case (Cha et al. 2010). Lesions occur commonly between the heel bulbs of the hind feet and resemble a hairy, strawberry-like wart in their painful, clinical stages. The agents of the disease have not been confirmed, but *Treponema* spp. of the *Spirochaetaceae* family have been consistently identified in clinical DD lesions (Krull et al. 2014; Döpfer et al. 2012). Treatment with topical oxytetracycline (Blowey and Sharp 1988; Shearer and Hernandez 2000) and lincomycin antibiotics (Berry et al. 2012) has shown moderate success in reducing lesion severity (Berry et al. 2010), but lesion location affects topical treatment efficacy (Hernandez and Shearer 2000). In a small study from 1998, systemic treatment with penicillin showed successful lesion resolution in 9 out of 9 clinical lesions (Read and Walker 1998), but milk-withdrawal makes this an unattractive choice for use in lactating dairy cows (Evans et al. 2012). Systemic treatment of DD has not been studied as extensively as topical treatments, likely due to inconsistent lesion resolution with systemic approaches. Recent cure rates of 75% were reported in M2 lesions treated with a topical spray containing oxytetracycline as the active ingredient (Engemycin Spray, MSD B.V. Boxmeer, The Netherlands) while the other treatment, thiamphenicol spray (TAF SPRAY, Dechra Veterinary Products; MAH Eurovet Animal Health B.V., Bladel, The Netherlands), a product unavailable in Canada, had a cure rate of 89% (Holzhauer et al. 2017). In that study, a lesion was considered cured if it was an M0 or inactive M4 lesion on d28. Clinical DD lesions that do not completely heal to an M0 stage following treatment will likely return to a clinical lesion in the absence of preventative tactics (Krull et al. 2016). Recrudescence of clinical lesions causes deformation of hoof conformation and increased susceptibility to additional claw lesions (Gomez et al. 2015). In addition, the antibiotics used most commonly, of the tetracycline drug class, are used in human medicine and are known to be associated with the evolution of antibiotic-resistant bacteria (Chopra and Roberts 2001). The cost to the producer, the detrimental effects on animal welfare, the risk of increasing antibiotic

resistance by overuse of tetracycline antibiotics, and the complex etiology of DD give sufficient reason to characterize the microbiome of lesion initiation rather than its clinical stages to better develop successful treatment and prevention strategies.

Digital dermatitis research has not yet fulfilled Koch's postulates regarding *Treponema* spp. as the cause of DD (Krull et al. 2016). It may be that *Treponema* spp. are opportunistic pathogens infecting pre-existing lesions and are not solely responsible for lesion initiation (Krull et al. 2016; Nielsen et al. 2016). No previous research has described the microbiome of preclinical DD lesions in an endemic herd alongside the heel tissue of cows from a herd negative for clinical DD and managed under commercial conditions. Only one previous study on DD-associated bacteria has compared results to a herd free of DD, but the DD-free herd consisted of 7 mixed-breed, pasture-managed cows (Klitgaard et al. 2014). In Canada, the most common dairy breed is Holstein and the seasonal climate ensures all dairy cows are housed and managed intensively for at least part of the year.

The first objective of this study was to determine the DD status of and describe Saskatchewan dairies from which a sample was recruited for participation. The second objective was to describe the bacteria detected in tissue and fecal samples collected from cows with preclinical DD lesions as well as slurry samples from 7 different Saskatchewan dairies. A province-wide survey was used to fulfil the first objective. Information gathered from this survey will serve to bridge the gap in what is known about how Saskatchewan dairy producers manage DD in comparison to Alberta and Ontario, the other Canadian provinces with published DD management data. This research is unique in that one of the participant dairies housed 110 lactating Holstein cows in an intensive environment, like that of most Canadian dairies, yet clinical disease was not apparent. However, irregular dermal pitting on heel skin was evident on the non-endemic dairy that is similar to preclinical DD lesions described by previous work (Krull et al. 2014). Unlike previous research that used mixed breed, extensively managed cows, in which neither the environment nor host was comparable to that of the diseased population of interest (Klitgaard et al. 2014), this research compares the tissue and feces of intensively managed Holstein cows exposed to the agents of disease to intensively managed Holsteins that, given their clinically DD-free status, are hypothesized not to be exposed to the agents of clinical disease. The research hypothesis was that the bacteria associated with clinical DD would not be detected in preclinical tissue samples from

the dairy non-endemic for clinical DD but would be detected in preclinical tissue samples from the dairies endemic for clinical DD.

3.2 Materials and Methods

All experimental procedures were conducted in accordance with guidelines of the Canadian Council for Animal Care and were approved by the University of Saskatchewan Animal Care Committee (project number: 20140031).

3.2.1 Saskatchewan Producer Lameness Survey

A 12-question survey was prepared to determine the DD status of Saskatchewan (SK) dairies and to gauge the size of the operations. Included were questions on the size of milking herd, average cow weight and milk production, trimming practices, infectious and non-infectious causes of lameness, how often claw lesions were treated, whether there was DD in their herd, and how long DD had been in their herd. The survey (Appendix A) was sent to every registered dairy farm in Saskatchewan (n=163), Canada by the provincial milk board (SaskMilk) to maintain producer anonymity.

3.2.2 Participant Recruitment

From the 25 producers willing to participate in further studies, a convenience sample of 6 dairies endemic for clinical DD was selected to be compared to the clinically non-endemic dairy. These dairies were selected because they had scheduled hoof trimmer visits for the fall of 2015, their hoof trimmer was willing to participate, they had primarily Holstein cows, and they were in a 150 km proximity of Saskatoon. The non-endemic dairy was identified as being free of clinical DD through a review of veterinary records. This non-endemic dairy (dairy 7) completed the same survey as the endemic dairies (dairies 1 through 6) and indicated their willingness to participate in further research. All selected dairies were contacted with the details they provided and farm visits were planned. These 7 dairies were visited between August 12th and September 29th of 2015 to coincide with regularly scheduled hoof trimmer visits.

3.2.3 Sample Collection

Up to 10 cows from each of the 7 participant dairies were selected for lesion biopsy and fecal collection. All cows selected for lesion biopsy also had a fecal sample collected. On dairies 1

through 6, cows were selected from those being trimmed during a farm visit and were biopsied if they displayed a lesion in any one of the following stages: A1, A2, B1, B2, or 3. Preclinical DD lesions are A1, A2, B1, and B2 and appear as dermal pitting and scabs on the skin adjacent to the caudal interdigital cleft. Stage 3 lesions are clinical, acute, ulcerative, and bleed upon palpation (Plummer and Krull 2017). Considering the clinical DD-free status of dairy 7 (D7), there the selection process for biopsy collection was not based on visual appearance of the cows' heels but randomly selected from cows receiving a foot trim. This method was decided upon before biopsies were collected because D7 claimed to be completely free of DD, not recognizing the preclinical stages of the disease. Every second cow that entered the trim chute from the two different housing systems on D7 was selected, whether a preclinical lesion was visible or not. Seven of the selected cows on D7 were housed in free-stalls, three were housed in tie-stalls.

Heels were washed with tap water and a clean towel then photographed. A line block was then administered above the intended biopsy site with 3 ml of 2% neat lidocaine, before a 3 mm biopsy punch, forceps, and a scalpel were used to extract a biopsy specimen. All lesions selected were biopsied from the edge of the lesion area, where causal bacteria are most likely to be active. Immediately, the biopsy specimen was put in a sterile, labelled cryovial and dropped into a tank of liquid nitrogen until sample collection was complete for that day. All samples were then transported to the laboratory and stored in a freezer (-80°C).

While a selected cow was still in the trimming chute, a fecal sample was obtained from the rectum with a clean palpation glove then stored on ice until sample collection was complete for that day, then all samples were stored in a freezer (-80°C) until further processing. A slurry sample was collected from the scraper alley of dairies 1, 4, 5, and 7 and treated the same as fecal samples. Slurry samples were not collected at every farm due to a protocol failure.

Photographs of lesions taken pre-biopsy were scored by 6 blinded observers using the Iowa DD scoring system. The principal investigator was the only one who selected lesions for biopsy. Upon comparing lesion scores given to identical photos by different observers, a final score was assigned by the principal investigator. Only samples from cows with lesions scored A1, A2, B1, or B2 were considered preclinical lesions and used in the final analysis of 16S rRNA gene amplicon sequencing results. Additional tissue samples were collected from stage 3 lesions at dairy 1 (D1) and two healthy skin biopsies were collected at D7 through random selection. A summary of all the biopsy samples taken of each DD score can be seen in **Table 3.1**. All samples were named

according to first the farm and then the sample type, in the sequence in which they were collected. For example, the first preclinical tissue sample collected from dairy 3 was named D3-T1, while the third fecal sample collected from dairy 3 was named D3-F3. A summary of preclinical tissue samples, fecal samples, and slurry samples collected at each dairy can be seen in **Table 3.2**.

Table 3.1 Summary of biopsies and lesion stages.

DD Stage	No. of Samples
0	2
A1	32
A2	13
B1	3
B2	1
3	8
Sum	59

Table 3.2 Summary of samples collected at participating dairies from cows with preclinical lesions.

Dairy ID	Disease Status	No. Preclinical Tissue Samples	No. Fecal Samples	Slurry Sampled
Dairy 1	Endemic	2	2	Yes
Dairy 2	Endemic	8	8	No
Dairy 3	Endemic	9	9	No
Dairy 4	Endemic	8	8	Yes
Dairy 5	Endemic	10	10	Yes
Dairy 6	Endemic	4	4	No
Endemic Total		41	41	50%
Dairy 7	Non-Endemic	8	8	Yes

3.2.4 Participant Farm Management Practices

As part of chapter 2, a separate questionnaire was conducted while visiting each participant dairy for sample collection. The questionnaire covered herd demographics, general herd management, and lameness management. Although the results of that questionnaire will not be

discussed in this chapter, those data did indicate the importance of controlling for “dairy” during results analysis.

3.2.5 Extraction of Bacterial gDNA

Biopsy specimens were thawed for one min in a 56°C water-bath and processed using the Qiagen DNeasy® blood and tissue kit (Qiagen, Toronto, ON), according to the manufacturer’s instructions. Slurry and fecal samples were thawed overnight at 4°C then 184-223 mg of wet fecal matter was processed using the Qiagen DNeasy® stool kit according to the manufacturer’s instructions. Genomic DNA yield was quantified by UV absorbance using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Mississauga, ON). Extracted gDNA specimens were stored at -20°C until further processing. All gDNA samples containing more than 25 ng/µl were diluted and aliquoted accordingly with the elution buffer used in extraction. Of the fecal and slurry samples, 66 of 67 contained less than 25 ng/µl gDNA while only 9 of 59 tissue samples contained less than 25 ng/µl. One sample, D4-T2, had less than 5 ng/µl with 4.1 ng/µl.

3.2.6 Selection of Preclinical Lesion Biopsies for PCR

Targeted PCR was done with previously published methods and primers (Evans et al. 2009b) with the nested portion of the procedure removed. From the collection of tissue biopsy specimens, a representative group of 10 gDNA samples from preclinical lesions was selected for PCR based on their macroscopic similarity in photographs to the 10 preclinical tissue samples collected from D7 (**Table 3.3**). The 10 tissue samples from D7 were each matched with an endemic tissue sample that came from an identically scored lesion. In the case that a sample could not be matched by score, the sample with the next closest lesion severity was selected.

3.2.7 PCR of Selected Biopsy Specimens

Ten gDNA samples from preclinical lesions were divided and analyzed with both 16S rRNA gene sequencing and targeted PCR (**Table 3.3**). PCR was executed using published primer sequences (Evans et al. 2009) targeting three previously characterized DD-associated *Treponema* phylogroups: *T. phagedenis*-like, *T. vincentii/T. medium*-like, and *T. putidum/T. denticola*-like (Integrated DNA Technologies, Coralville, IA; Evans et al. 2009). The *T. phagedenis*-like, *T. vincentii/T. medium*-like, and *T. putidum/T. denticola*-like PCR products were 400, 475, and 475 bp each, respectively. Amplicons were stained with ethidium bromide and viewed on 1.5% agarose

gels under long-wavelength ultraviolet light following electrophoresis. Bacterial gDNA from a clinical, stage 3, DD lesion positive for the tested sequence was used as a positive control in each gel. The master mix containing all necessary reagents except gDNA was used as a negative control. A different positive control and master mix were used for each PCR test. Capillary EZ-sequencing of the resulting PCR products was carried out by Macrogen (Seoul, Rep. of Korea) and results were matched with the most similar sequence ($\geq 98\%$ sequence homology) identified with a nucleotide BLAST search (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Table 3.3 Selected non-endemic and endemic tissue samples subjected to PCR.

Non-Endemic Sample ID	Score	Score	Endemic Sample ID
D7-T1	A1	A1	D1-T5
D7-T2	0	A1	D6-T1
D7-T3	B1	B2	D2-T8
D7-T4	A2	3	D4-T7
D7-T5	A1	A1	D3-T5
D7-T6	A2	A2	D3-T10
D7-T7	B1	A2	D2-T6
D7-T8	A1	A1	D4-T5
D7-T9	0	A1	D5-T10
D7-T10	B1	A2	D5-T2

3.2.8 Phylogenetic Tree of Treponemal PCR Amplicons

The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei, 1993). The tree with the highest log likelihood (-910.67) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 14 nucleotide sequences. There were a total of 426 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al., 2018).

3.2.9 16S rRNA Gene Sequencing of Bacterial gDNA

Genomic DNA samples collected from alleyway slurry (n=4), cows with pre-clinical lesions (n=98; **Table 3.2**), and fecal and tissue samples from both clinically affected and non-affected cows (stage 0 and 3 tissue=10, fecal samples from stage 0 and 3 cows=10; **Table 3.1**) were processed for sequencing. The V3-V4 hypervariable region of the bacterial 16S rRNA gene was amplified in these 122 DNA samples using a universal 16S forward primer (515F) and 122 unique Golay barcoded reverse primers (806R) with methods described by (Krull 2015). After 35 cycles of PCR amplification, PCR product was confirmed by visualization of an approximately 300-bp band on an agarose gel (0.8%). Every sample and primer combination were processed with a negative control. The absence of amplification was verified on agarose gels. Sample library DNA concentrations were quantified with a Qubit fluorometer (Life Technologies, Grand Island, NY) and samples were pooled with equal amounts of DNA. The pooled libraries were cleaned up and samples diluted to 2nM according to previously published methods (Krull et al. 2014). Illumina PhiX Control v3 was added at a proportion of 20% before loading on the MiSeq platform (Krull et al. 2014). Two runs, each with n=61 three hundred-bp paired-end sequences were run on an Illumina MiSeq. Only samples from cows with preclinical lesions scored A1, A2, B1, or B2, were used in the final analysis of 16S rRNA gene amplicon sequencing results.

Using QIIME 1.7, the forward and reverse reads from paired-end sequencing were merged using the fastq-join script. Demultiplexing and quality filtering were performed using the split_libraries_fastq.py script. The pick_reference_otus_through_otu_table.py script was used for operational taxonomic unit (OTU) calling and the taxonomic assignment of reads was based on the Greengenes 16S rRNA gene database. Results were sorted by OTU, taxa (if identified), and sample ID then imported to Microsoft Excel. An appropriate depth of sequencing was verified with an alpha-rarefaction curve in Qiime 1.7. Bray-Curtis dissimilarity results were obtained with Qiime 1.7.

3.3 Results

3.3.1 Saskatchewan Producer Lameness Survey Results

Of all 163 Saskatchewan dairy producers contacted, 38 returned the survey (23% response rate). Of these, 25 included personal contact information, indicating their willingness to participate in further studies (**Table 3.4**). The complete survey can be found in Appendix A. Not all producers

completed every section of the survey. Out of the anonymous responders, only one claimed to have a herd free of DD. However, their herd size was the smallest of all responders (n=50 cows), they were the only producer that did not get their cows' hooves trimmed, and an average milk production was not reported. Due to that producer choosing to remain anonymous, no follow up could be completed and their results were not included in **Table 3.5**. The one identified non-endemic dairy was confirmed as being free of clinical DD through a review of veterinary records. Of the self identified population of survey responders, 75% (18/24) identified DD as a major cause of lameness on their farm while in the anonymous population, 62% (8/13) identified DD as a major cause of lameness (**Table 3.4**). Of those that reported having at least one case of DD on their farm, it was identified on average just over a decade before the survey was distributed in 2015 (**Table 3.4**).

Table 3.4 Digital dermatitis status of survey respondents.

Type of Survey Responder	No. of Responders	Herd endemic for DD (n)	Identify DD as a top cause of lameness (n)	Years ago DD was first identified if present
Self-Identified	25	96% (24)	75% (24)	12.4 ± 5.2
Anonymous	13	92% (12)	62% (13)	10.5 ± 5.2

Table 3.5 Mean ± SD (minimum-maximum) herd size (number of lactating cows) and milk production (kg/cow/year) of participating dairies per disease status.

Disease Status	Dairies	Herd Size	Milk Production
Endemic	36	187 ± 147 (60-700)	10,565 ± 1,112 (8,235-13,725)
Non-Endemic	1	110	12,200

The 7 dairies selected for sample collection all had their cows' hooves trimmed on a regular basis by a professional bovine hoof trimmer. Dairies 1 and 3-7 all used the same trimmer who recorded foot lesions electronically with Hoof Supervisor® and made them available to the producer as requested. Dairy 2 used a hoof trimmer who did not keep individual cow records for lesions, only the number of cows trimmed and wraps applied.

Dairies 1-2 and 4-6 reported DD as the most common cause of lameness while D3 and D7 reported sole ulcers as the most common cause. Dairies 1-6 first noticed DD an average of 13 years ago and reportedly treated 17 cases of DD/month on average. The other survey respondents treated an average of 8 and 4 cases of DD/month in the identified and anonymous populations, respectively.

Table 3.6 Lameness management practices of survey respondents.

Type of Survey Responder	Keep lameness records	Trim hooves		Timing of trimming			
		Professional trims	Producer trims	Regular schedule	When lame		
					Yes	Other	
Self-Identified	80%	100%	96%	4%	100%	0%	0%
Anonymous	54%	92%	92%	0%	77%	15%	8%

Cows from D7 were only footbathed one day per week but on that day, the cows went through a fresh footbath at each of the three milking times and thus were footbathed a similar number of times per week as cows in the other dairies. In many ways, D7 was managed in a way representative of the wider industry. Some unique aspects of D7's herd management are that the herd was closed to outside animals, all milking and close-up cows were kept on rubber floors, and all cows displaying possible foot issues were seen to immediately by a veterinarian or bovine hoof-trimming professional. During the sampling period, it was recommended that the hoof-trimmer continue his usual course of action in treating animals showing early staged lesions with a non-antibiotic topical paste of his choice. Because he had never seen a lesion progress past the preclinical stages at D7, he had never treated cows of this herd with a wrap like he would at other dairies.

3.3.2 PCR and Amplicon Sequencing Results

Amplicons from primer pairs targeting three different *Treponema* spp. were stained with ethidium bromide and visualized on an agarose gel under long-wavelength ultraviolet light. Primers targeting *Treponema phagedenis*-like spp. resulted in bands from 9 of 10 selected endemic samples, while primers targeting *T. medium*/*T. vincentii*-like bacteria resulted in bands from 5 of 10, and those targeting *T. denticola*/*T. putidum*-like bacteria resulted in bands from 3 of 10 selected

endemic samples (**Table 3.7**). Of the 8 preclinical biopsy samples and 2 healthy skin samples from D7, 6 preclinical and 1 healthy sample amplified *T. phagedenis*-like PCR products as viewed on an agarose gel, while no bands were evident with *T. medium*/*T. vincentii*-like or *T. denticola*/*T. putidum*-like primers (**Table 3.8**).

Table 3.7 *Treponema* PCR products from preclinical digital dermatitis lesion samples from dairies 1 through 6 viewed on an agarose gel.

Dairies 1 to 6				
Sample ID	DD Stage	<i>T. phagedenis</i> -like	<i>T. medium</i> / <i>T. vincentii</i> -like	<i>T. denticola</i> / <i>T. putidum</i> -like
D1-T5	A1	+	+	+
D3-T5	A1	-	-	-
D4-T5	A1	+	-	-
D5-T10	A1	+	+	-
D6-T1	A1	+	+	+
D2-T6	A2	+	+	+
D3-T10	A2	+	+	-
D5-T2	A2	+	-	-
D2-T8	B2	+	-	-
D4-T7	3	+	-	+
		9/10 (+)	5/10 (+)	4/10 (+)

Table 3.8 *Treponema* PCR products from preclinical digital dermatitis lesion samples from dairy 7 viewed on an agarose gel.

Dairy 7				
Sample ID	DD Stage	<i>T. phagedenis</i> -like	<i>T. medium</i> / <i>T. vincentii</i> -like	<i>T. denticola</i> / <i>T. putidum</i> -like
D7-T2	0	+	-	-
D7-T9	0	-	-	-
D7-T1	A1	-	-	-
D7-T5	A1	+	-	-
D7-T8	A1	+	-	-
D7-T4	A2	+	-	-
D7-T6	A2	+	-	-
D7-T3	B1	-	-	-
D7-T7	B1	+	-	-
D7-T10	B1	+	-	-
		7/10 (+)	0/10 (+)	0/10 (+)

The best sequence match for *T. phagedenis*-like amplicons from D7 samples was different than the best match for the selection of endemic samples taken from dairies 1-6. The best sequence match in GenBank for *T. phagedenis*-like amplicons from endemic (dairies 1-6) samples was *T. phagedenis* 1498med (accession number KR025851). Of the 7 PCR products amplified with *T. phagedenis*-like primers from D7 samples, 5 had good quality DNA for sequencing and were included in the phylogenetic tree (**Figure 3.1**). The sequences most similar to those amplified from D7 samples were partial sequences of *Treponema* PT3, PT12 and *Treponema* sp. clone PN-20 of the 16S rRNA gene, as identified with BLAST. The sequence for *Treponema* PT3 was submitted to GenBank by Klitgaard et al. in their 2008 publication. *Treponema* PT12 was submitted to GenBank by Rasmussen et al. in 2012. The sequence for *Treponema* sp. clone PN-20 was submitted to GenBank by Yano et al. in 2010 and its closest match in GenBank was *Treponema* PT3 at the time of this report. As can be seen in **Figure 3.1**, the sequences amplified with primers targeting *T. phagedenis*-like DNA from D7 heel tissue clustered separately from those of dairies 1-6.

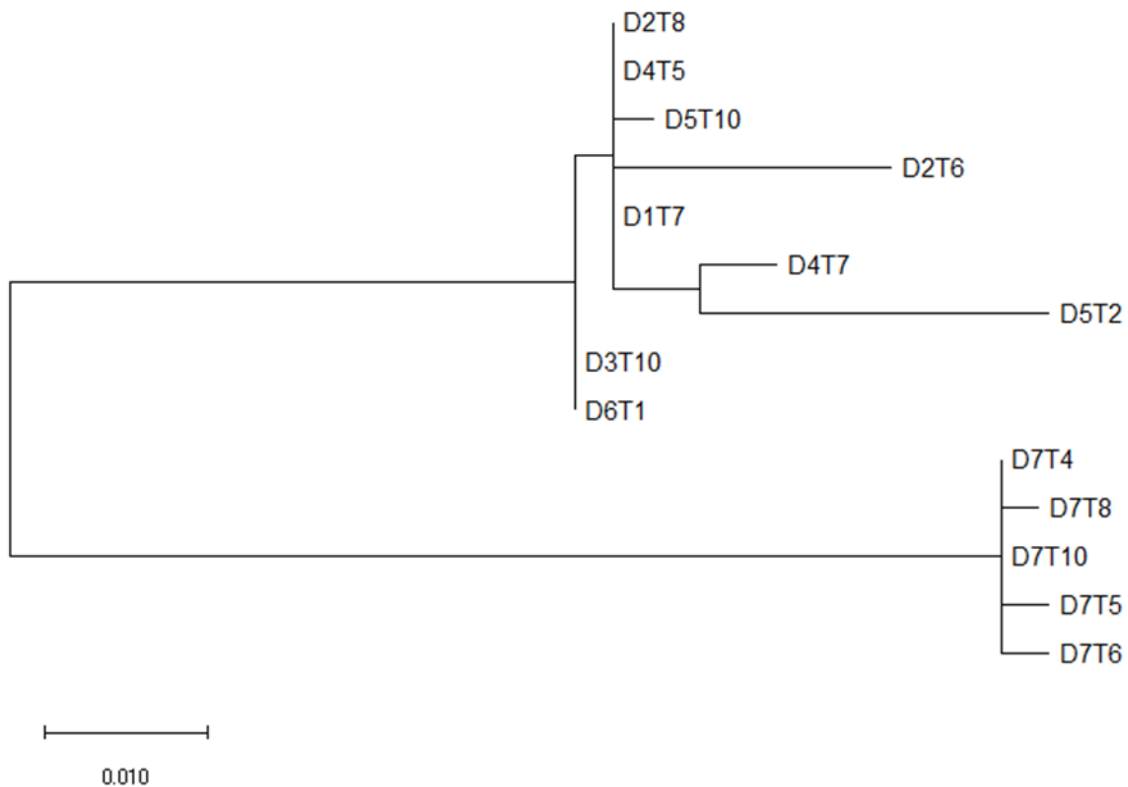


Figure 3.1 A phylogenetic tree of 14 *T. phagedenis*-like PCR amplicons sequenced from heel tissue samples of cows from environments endemic and non-endemic for clinical DD.

3.3.3 16S rRNA Gene Amplicon Sequencing Results

16S rRNA gene amplicon sequencing yielded 7,846,867 total reads of 11,874 unique OTUs after quality filtering using QIIME's default settings. The mean number of total reads per sample was 77,993 with a minimum of 12,973 (D7-F8) and a maximum of 197,574 (D6-F4). The tissue samples generated a mean of 89,441 reads/sample and the fecal samples generated a mean of 68,447 reads/sample. An unimportant number of total reads was recorded for sample D2-F3 (reads=1), as can be seen by the blank D2-F3 columns of **Figure 3.4** and **Figure 3.7**. Any reads that were not associated with an OTU were not included in further analysis. An appropriate depth of sequencing was achieved as illustrated by the alpha-rarefaction curves of each dairy farm reaching an asymptote (**Figure 3.2**). Bray-Curtis dissimilarity on preclinical lesion sample

microbiota revealed that statistically significant differences exist between dairies of the same disease status and also between those of different disease statuses (**Figure 3.3**).

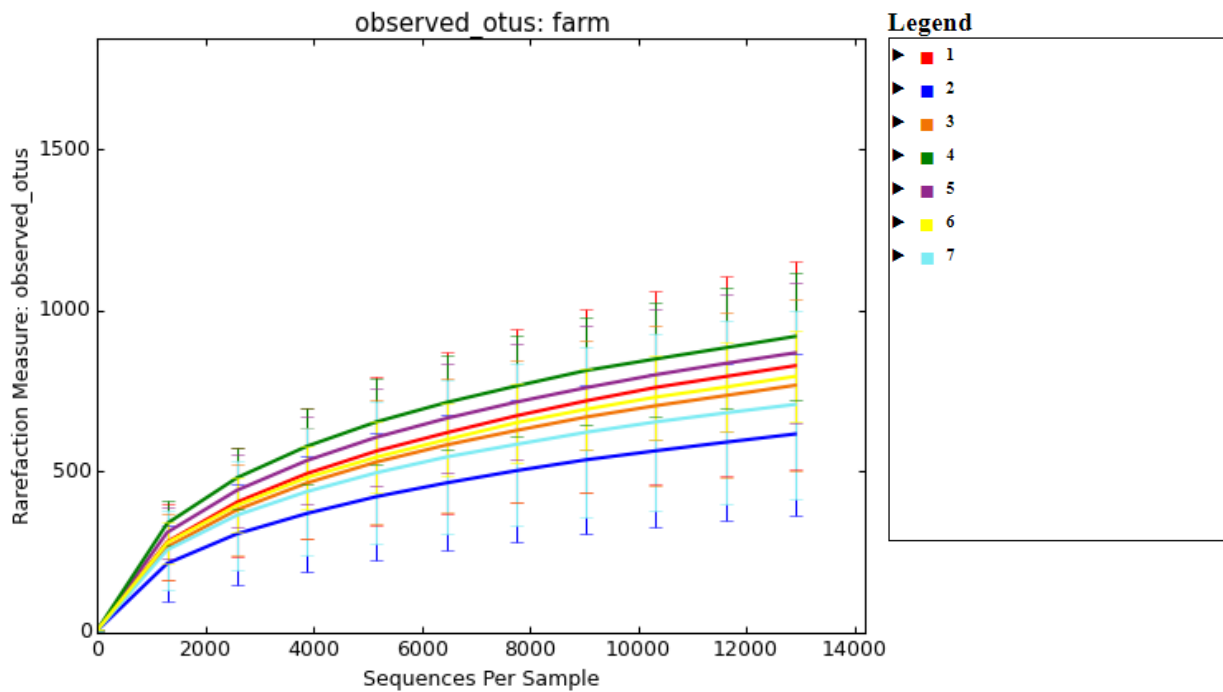


Figure 3.2 Alpha-rarefaction curves of observed operational taxonomic units in preclinical lesion tissue for dairies 1-7.

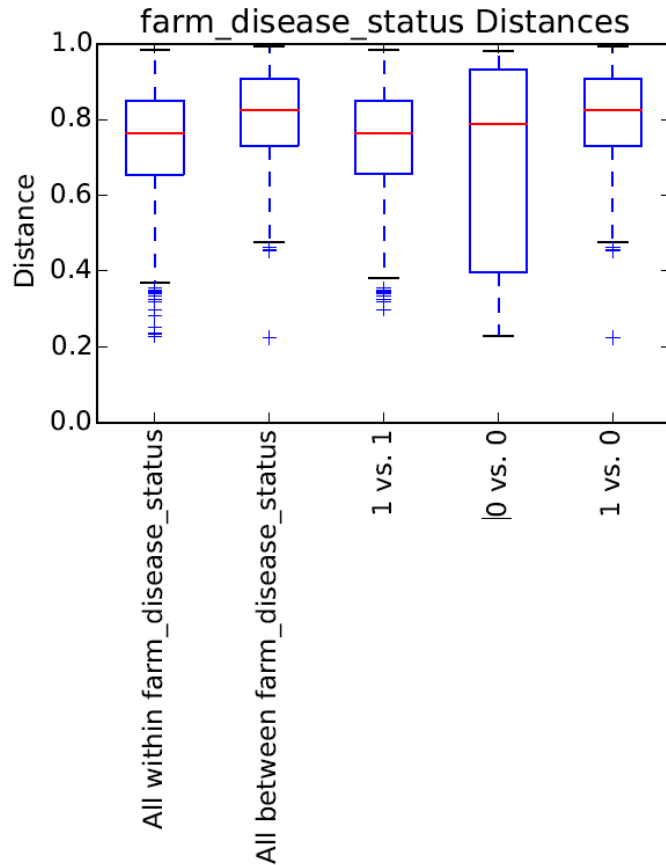


Figure 3.3 Bray-Curtis dissimilarity box-plot of significant differences in beta-diversity (of preclinical lesion samples) between and within dairies of disease status 0 (clinically non-endemic) or 1 (clinically endemic).

3.3.3.1 Phyla-associated OTUs in slurry, fecal, and preclinical lesion samples

Every dairy had at least one preclinical lesion sample where the most prevalent reads were *Spirochaete*-associated OTUs (**Figure 3.4**), while in all fecal and slurry samples, the most prevalent reads were almost equally *Bacteroidetes* and *Firmicutes*-associated OTUs (**Figure 3.5** and **Figure 3.6**). *Spirochaete*-associated OTUs only made up 1.9% of the reads in all fecal samples with 64,886 reads total. The number of *Spirochaete*-associated reads in fecal samples (**Figure 3.5**) ranged from 52 to 9,472 with the following mean reads per dairy: D1, 367; D2, 599; D3, 2,078; D4, 930; D5, 422; D6, 5,270; and D7, 990. The preclinical lesion samples from all dairies had the following relative abundance (%) and mean (min-max) prevalence of *Actinobacteria*-associated reads (**Figure 3.4**): D1, 0.4%, 518 (226-810); D2, 0.8%, 704 (170-3,491); D3, 1.6%, 1,324 (63-4,806);

D4, 5.6% 5,801 (513-27,546); D5, 1.6%, 1,424 (39-3,795); D6, 1.0%, 829 (364-1,805); and D7, 3.5%, 3,211 (95-11,975).

Spirochaete-associated reads were more abundant in tissue samples than fecal or slurry samples and within the tissue samples, were the most prevalent phylum detected in 27/49 preclinical lesion samples. Interestingly, in 3 of 4 B-type lesions, the least abundant reads were associated with the *Spirochaete* phylum [D2-T8, 880 reads (B2); D7-T7, 1425 reads (B1); and D7-T3, 186 reads (B1)]. D4 and D5 each had only two and three preclinical lesions, respectively, with *Spirochaete* as the most abundant phylum. In all the other D4 and D5 preclinical lesions but one [D4-T1, *Tenericutes* (A2)], the most reads were associated with *Firmicutes* (**Figure 3.4**). It is worth noting that D4's preclinical lesions all had the darkened, crusted appearance of feet that had regularly gone through a disinfecting CuSO₄ footbath, and with the M-stage scoring system, may have been scored as M3. Although D5's preclinical lesions did not appear darkened and crusted in appearance, the farmer reportedly used 37% formaldehyde as a foot spray in the parlour. D7's preclinical lesions were not characterised by any notable differences at the phylum level in spite of their originating from a dairy non-endemic for clinical DD.

Phyla-Associated Reads in Preclinical Lesions

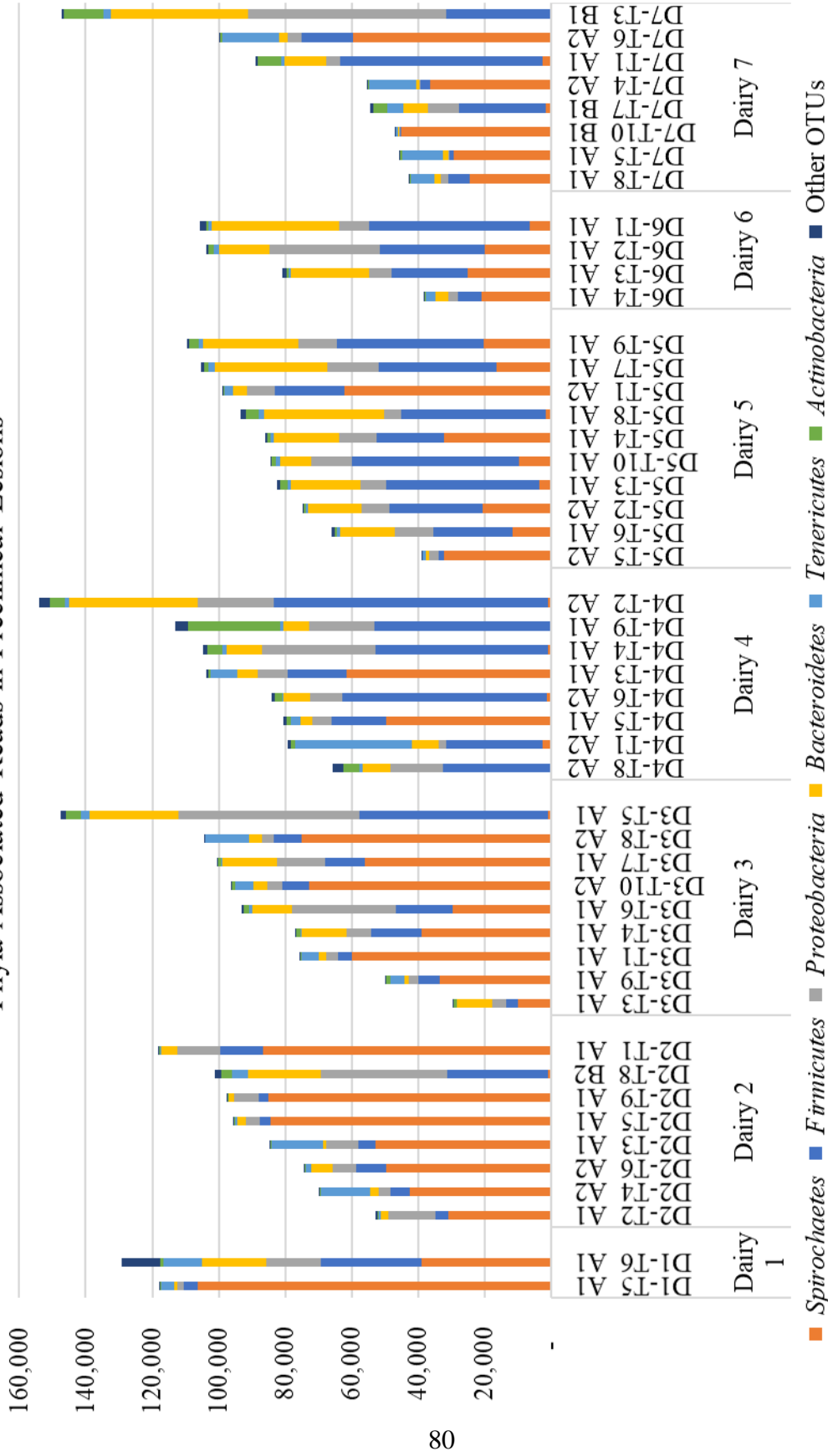


Figure 3.6 Abundance of operational taxonomic unit reads associated with phyla sequenced from preclinical digital dermatitis lesion tissue.

Phyla-Associated Reads in Fecal Samples from Cows with Preclinical Lesions

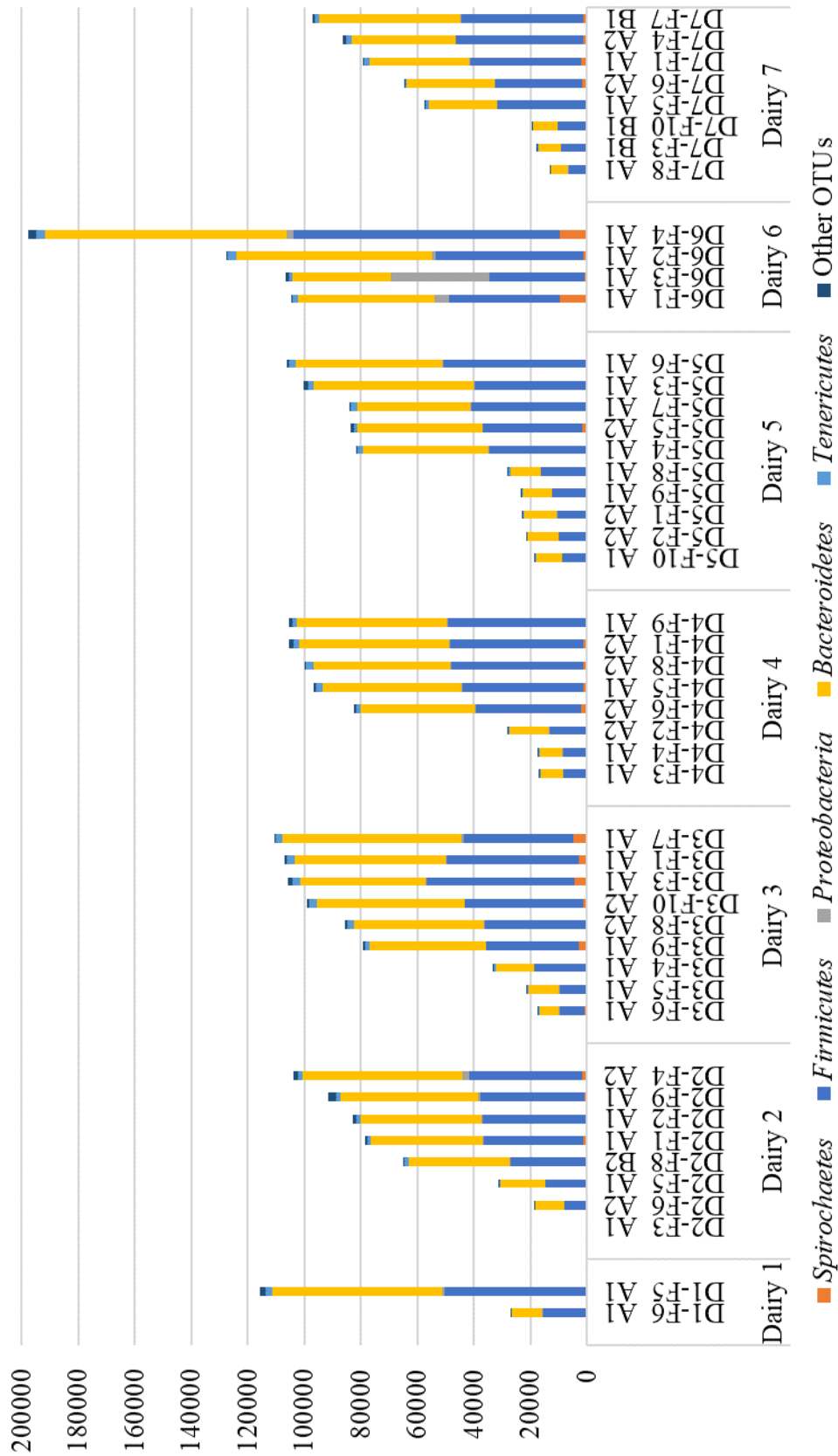


Figure 3.9 Abundance of operational taxonomic unit reads associated with phyla sequenced from fecal samples from cows with preclinical digital dermatitis lesions.

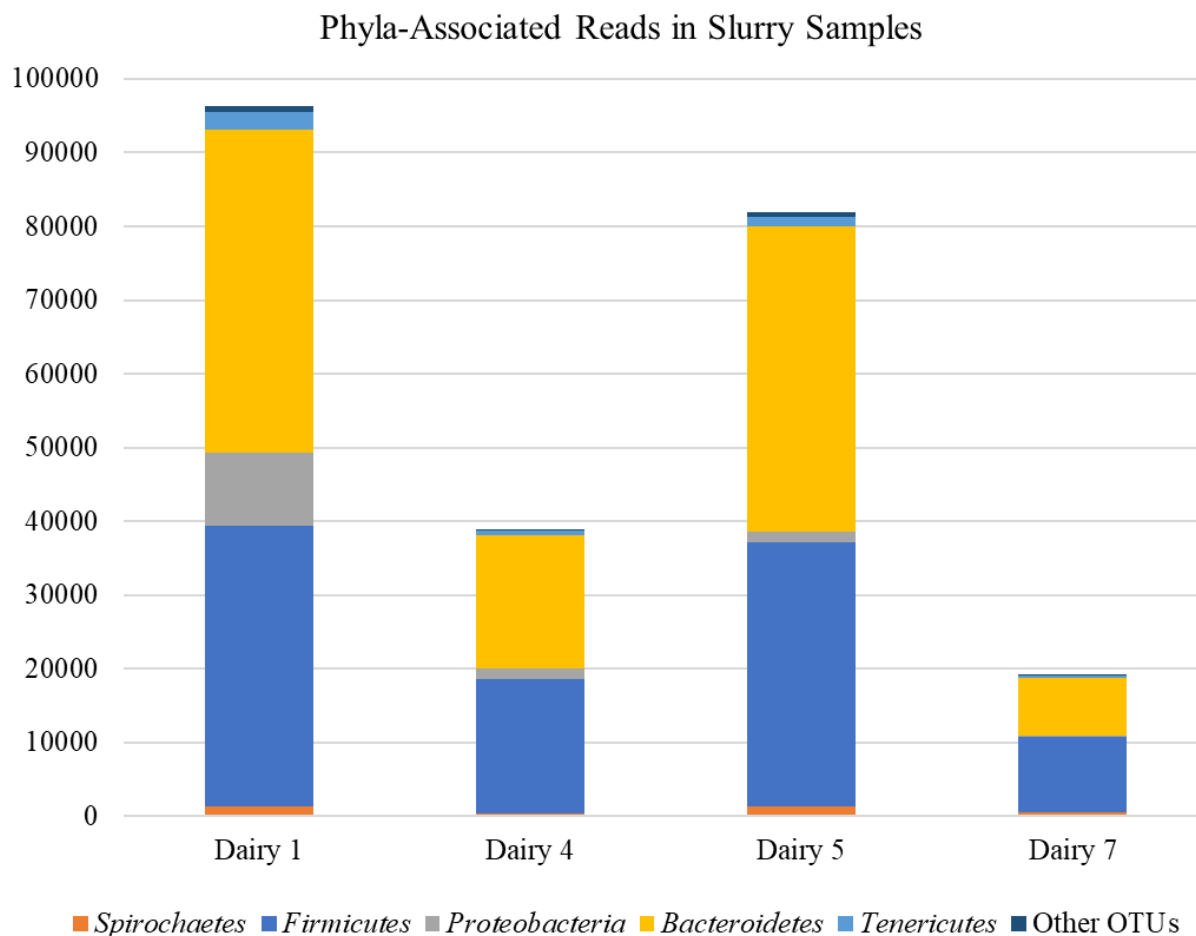


Figure 3.12 Abundance of operational taxonomic unit reads associated with bacterial phyla sequenced from slurry samples.

3.3.3.2 Species-associated OTUs in slurry, fecal, and preclinical lesion samples

The 16S rRNA gene amplicon sequencing results also delivered reads down to the species/phylolevel (Figure 3.7, Figure 3.8, Figure 3.9, Figure 3.10, Figure 3.11, and Figure 3.12). The *Treponema* spp.-associated reads most abundant in all classes of fecal samples were associated with unspecified *Treponema* spp. (Figure 3.8 and Figure 3.9). The preclinical lesion samples from all dairies contained *Treponema* spp.-associated reads that have been previously identified in preclinical DD lesions.

3.3.3.2.1 *Treponema*-associated OTUs

Some samples yielded very few *Treponema*-associated reads. Ten preclinical tissue samples with the lowest *Treponema*-associated reads were D2-T8, D3-T5, D4-T2, D4-T8, D4-T9, D5-T8,

D7-T3, and D7-T7 with a range of 66 to 1495. The 10 fecal samples with the lowest *Treponema*-associated reads (not including D2-F3, with 0 *Spirochaete* reads) were D1-F6, D2-F5, D2-F6, D2-F8, D4-F2, D4-F4, D5-F1, D5-F8, D5-F9, and D5-F10 with between 54 and 197 total *Treponema*-associated reads. Both the least and greatest number of *Treponema*-associated reads were in fecal samples.

Preclinical lesion tissue from each dairy yielded a variety of *Treponema*-associated reads (**Figure 3.7**), with the most prominent phylotypes in samples from dairies 1, 2, and 4 being *T. phagedenis*, *Treponema* sp44 and *Treponema* PT3 while D3 samples yielded mostly *Treponema* PT3, *Treponema* sp44, and *Treponema* PT1 associated reads. Preclinical lesion samples from D5 and D6 yielded an assortment of reads with *Treponema* sp44, *Treponema* PT2, and *T. phagedenis* being generally the most abundant. The *Treponema* spp.-associated reads that made up the top 99% of D7's preclinical lesion sample treponemes were *Treponema* PT2 (62.7%, mean: 15,561 reads/sample), *T. refringens* (20.5%, mean: 5,102 reads/sample), and *T. sp44* (16.1%, mean: 3,991 reads/sample). Consistently, the most abundant treponeme reads in D7 preclinical lesion samples were associated with *Treponema* PT2 (**Figure 3.7**), a *T. refringens*-like phylotype previously associated with stage 1 preclinical DD lesions (Krull et al. 2014). Even though *T. phagedenis*-associated reads were associated with all stages of DD by Krull et al., *T. phagedenis*-associated reads measured ≤ 7 (range: 2-7 reads) per D7 tissue sample. Likewise, the sum of DD-associated treponemes that fell into the "other treponemes" category with the other *Treponema*-associated reads that made up less than 5% of reads in **Figure 3.7**, *T. medium*, *T. denticola*, *T. sp22*, *T. PT8*, and *T. pedis*, were associated with only 1 to 6 total reads per D7 sample. That means that less than 0.015% (29 total reads in all D7 preclinical samples) of all *Treponema* spp.-associated OTU reads in D7 preclinical lesion samples were associated with clinical DD-associated treponemes. The use of negative controls negated the possibility of cross contamination skewing these results.

These results were in agreeance with the targeted PCR results from section 3.3.2, where no sequences were amplified with primers targeting *T. medium*/*T. vincentii*-like or *T. denticola*/*T. putidum*-like phylotypes. Although targeted PCR for *T. phagedenis*-like did result in 5 tissue samples from D7 with quality DNA for sequence analysis (D7-T3, T4, T5, T6, T8, and T10), these samples happened to have the most abundant reads associated with *T. refringens*-like phylotypes using 16S rRNA gene amplicon sequence analysis among all other D7 tissue samples with between 13,492 and 39,441 reads associated with *Treponema* PT2, between 2,998 and 14,951 reads

associated with *T. refringens*, and between 2,747 and 10,223 associated with *Treponema* sp44. The same analysis resulted in only 2 to 7 total reads associated with *T. phagedenis* from these same samples.

Krull et al. reported that *Treponema* PT2 and *Treponema* sp44 made up 77.4% of the *Treponema* population in stage 1 lesions (2014). In the present study, *Treponema* PT2 and *T. sp44* made up only 1% of the *Treponema*-associated reads in D1's stage 1 lesions, between 7 and 91% (mean: 34.6%) of D2's, 0.1 to 91% (mean: 16.5%) of D3's, 3 to 24% (mean: 12.6%) of D4's, 2 to 74% (mean: 47.1%) of D5's, 34 to 72% (mean: 51.2%) of D6's, and 65 to 89% (mean: 77.4%) of D7's stage 1 lesions.

Krull et al. reported stage 2 lesions with *Treponema* PT1, *Treponema* PT3, and *T. phagedenis* comprising 82.6% of the *Treponema* spp. population (2014). In the present study, reads associated with these three treponemes made up 19-69% (mean: 50.2%) of D2's stage 2 lesions, 39-59% (mean: 48.9%) of D3's, 4-66% (mean: 24.8%) of D4's, 41-59% (mean: 48.9%) of D5's, and 0.2-0.6% (mean: 0.4%) of D7's stage 2 lesions.

The 16S rRNA sequencing yielded OTU reads associated with *T. phagedenis*, *T. medium*, and *T. putidum*, the species targeted by PCR in results section 3.3.2. Although targeted PCR resulted in amplicons from D7's heel tissue samples using *T. phagedenis*-like primers, 16S rRNA gene amplicon sequencing indicated that D7 tissue samples yielded between 2 and 7 total reads associated with *T. phagedenis*.

Treponema-Associated Reads in Preclinical Lesion Samples

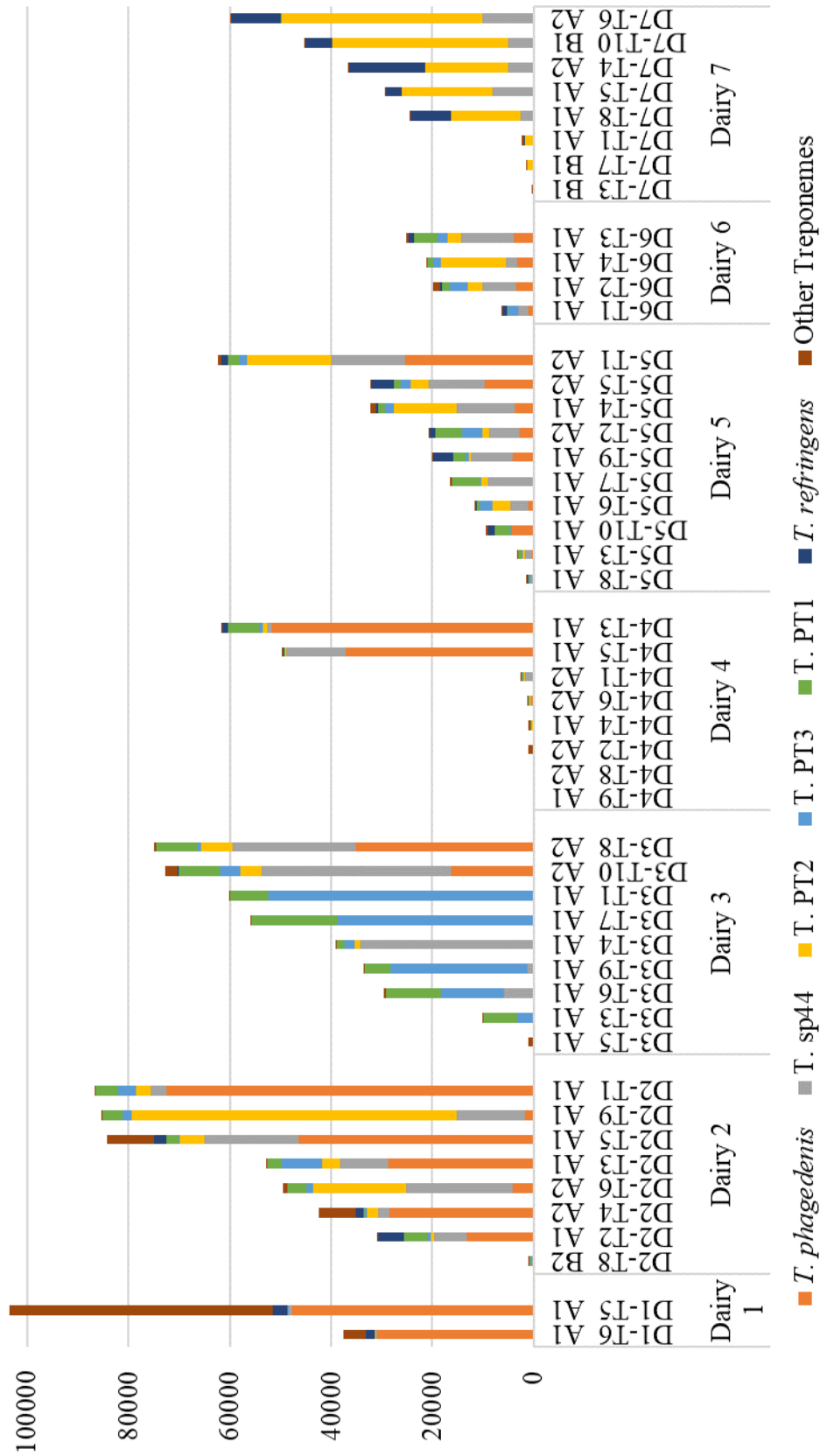


Figure 3.14 Abundance of operational taxonomic unit reads associated with *Treponema* species sequenced from preclinical lesion tissue.

Treponema-Associated Reads in Fecal Samples from Cows with Preclinical Lesions

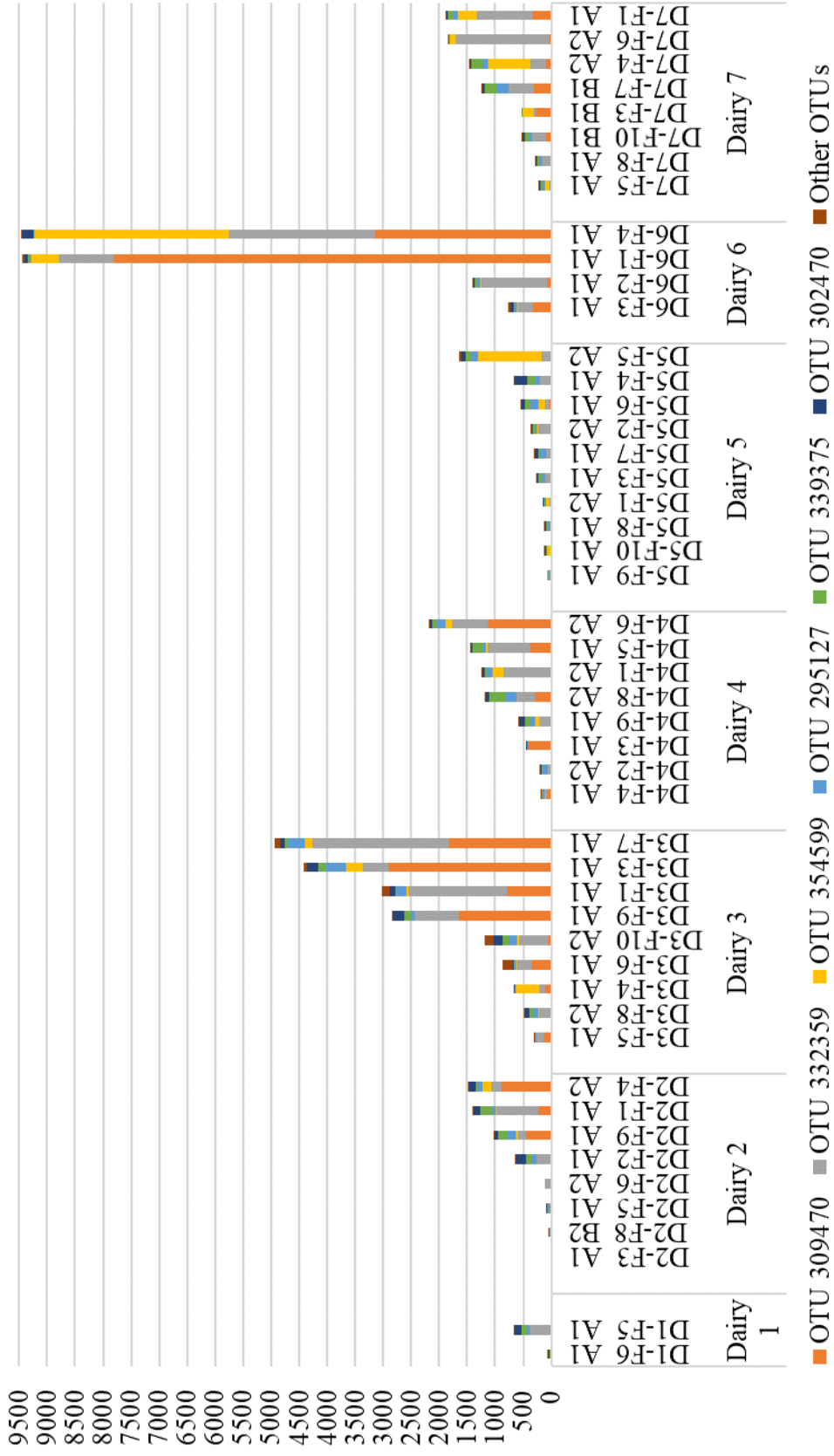


Figure 3.15 Abundance of operational taxonomic unit reads associated with *Treponema* in fecal samples collected from cows with preclinical digital dermatitis.

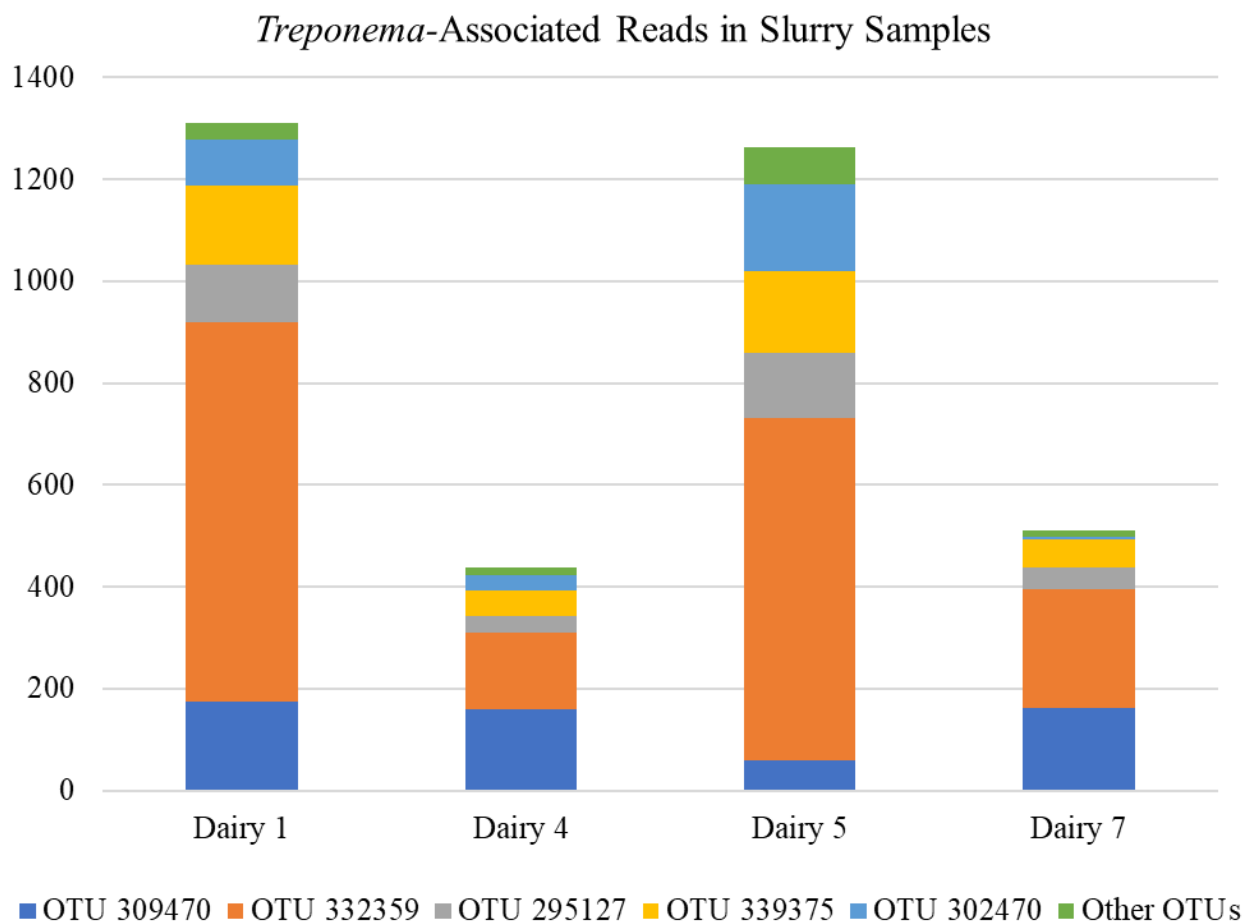


Figure 3.16 Abundance of operational taxonomic unit reads associated with *Treponema* in slurry samples.

3.3.3.2.2 Non-*Treponema*-associated OTUs

Every dairy had at least one preclinical lesion sample with *D. nodosus*-, *C. ureolyticus*-, or *Porphyromonas*-associated reads (**Figure 3.10**). The dairy with the greatest mean reads/sample of *C. ureolyticus*- and *D. nodosus*-associated OTUs (**Figure 3.10**) was D2 with a mean of 3,639 and 3,680 mean reads, respectively. D4 contained the preclinical lesion sample with the greatest *D. nodosus*-associated reads [D4-T4, 18,598 (A1)] while D2 contained the sample with the most *C. ureolyticus*-associated reads [D2-T1, 10,160 (A1)]. D6 had the highest mean reads/sample of *Porphyromonas* spp.-associated OTUs with 11,014 reads but D7 had the sample with the most *Porphyromonas* spp.-associated reads [D7-T3, 36,996 (B1)].

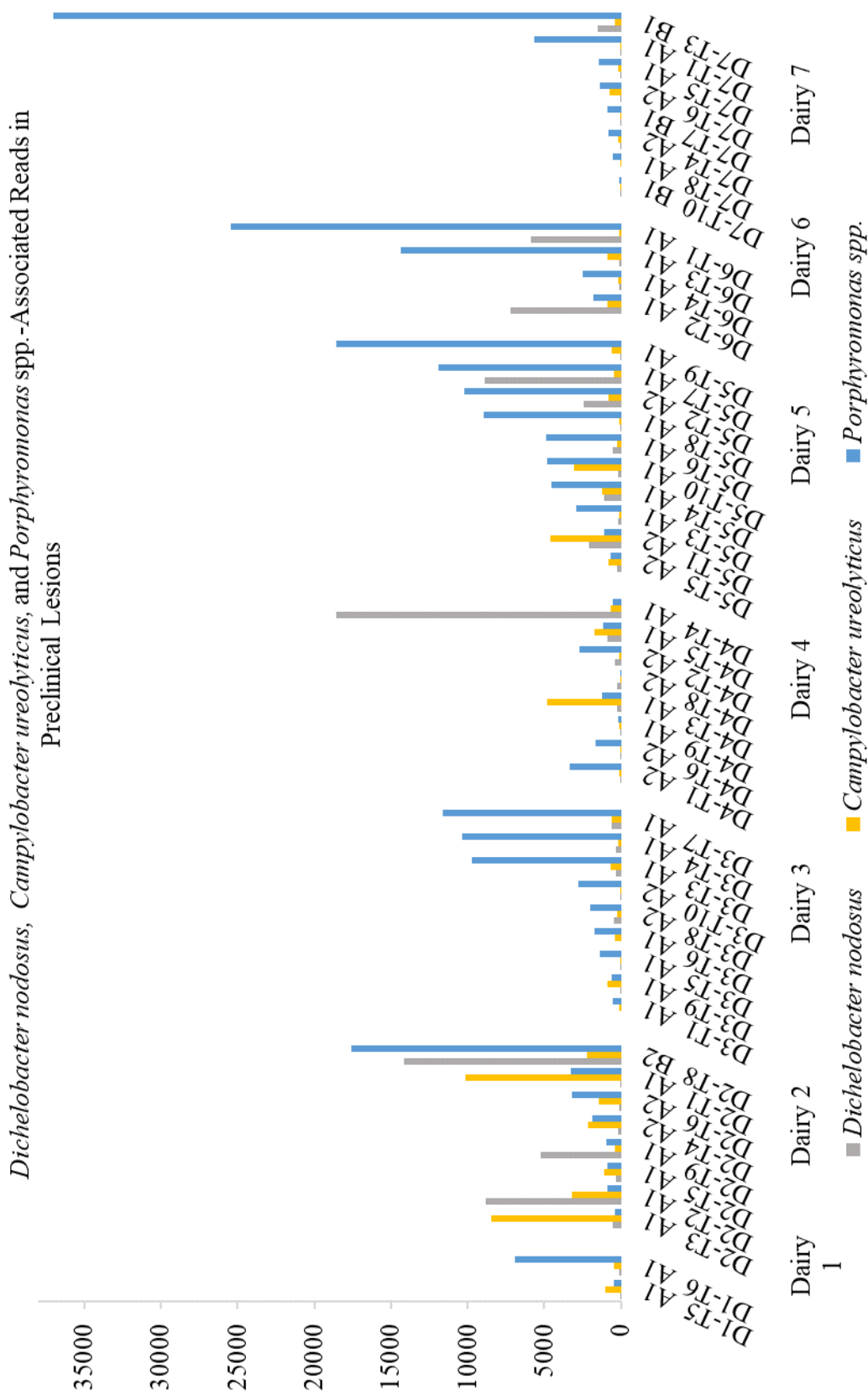


Figure 3.17 Abundance of operational taxonomic unit reads associated with *Dichelobacter nodosus*, *Campylobacter ureolyticus*, and *Porphyromonas* species sequenced from preclinical digital dermatitis lesions.

3.3.3.3 Phyla and species-associated OTUs in healthy skin and clinical lesion samples

In addition to the preclinical DD lesions, two stage 0 (healthy skin) samples and 8 clinical DD samples (stage 3 or 4) were also sequenced on the MiSeq platform. The most abundant *Treponema* reads in each of the two healthy tissue samples, D7-T2 and D7-T9, were associated with *Treponema* PT1 (42%, 211 reads) and *Treponema* PT2 (59%, 7,561 reads), respectively (**Figure 3.11**). While the reads associated with *Treponema* PT2 were the second most abundant species or phylotype-associated OTU in sample D7-T9, *Treponema* PT1 came much further down the list of abundant species/phylotype-associated reads in sample D7-T2 (102nd most abundant) with only 0.22% of total reads. The most abundant species or phylotype-associated reads in sample D7-T2 were associated with OTU 1047041 of the genus *Corynebacterium* (4%, 3,855 reads) and OTU 720093 of the *Ruminococcaceae* family (3%, 2,988 reads). All other reads totalled less than 2% of the species/phylotype-associated reads in sample D7-T2. The most abundant reads on a phyla level in sample D7-T2 were associated with *Firmicutes* (45%, 43,872 reads), *Proteobacteria* (19%, 18,867 reads), *Bacteroidetes* (18%, 17,555 reads), and *Actinobacteria* (14%, 13,448 reads). *Spirochaetes* only made up 1% with a total of 501 associated reads in D7-T2. The most abundant species or phylotype-associated reads in sample D7-T9 were attributed to *Acinetobacter lwoffii* (5%, 8,744 reads), *Treponema* PT2 (4%, 7,561 reads), and OTU 720093 of the *Ruminococcaceae* family (4%, 6,465 reads). At the phyla level, sample D7-T9 yielded an abundance of *Firmicutes* associated reads (38%, 65,294 reads) but the next most abundant were associated first with *Bacteroidetes* (32%, 54,963 reads), then *Proteobacteria* (18%, 31,257) and *Spirochaetes* (7%, 12,771 reads). These samples were both considered healthy, normal heel skin.

At the phyla level, *Spirochaetes*, *Tenericutes*, *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* consistently made up the top 5 most abundant families of clinical lesions with *Spirochaetes* dominating the microbiome at 68-92% of reads. At the family level, reads associated with *Spirochaetaceae* and *Mycoplasmataceae* had the greatest relative abundance in D1-T1, D1-T2, D1-T3, D2-T7, D3-T2, and D4-T7. The abundance of reads associated with *Treponema* at the species/phylotype level in clinical lesions is illustrated in **Figure 3.12**. The clinical DD lesions biopsied at D1 were samples D1-T1, D1-T2, D1-T3, D1-T4, and D1-T7. Reads associated with *Treponema* PT8 were the most abundant of all species or phylotype-associated reads in samples D1-T2 (52%, 52,162 reads), D1-T3 (25%, 28,561 reads), and D1-T7 (44%, 36,697 reads), while reads associated with *T. phagedenis* were most abundant in sample D1-T4 (41%, 42,913 reads) and

OTU 669126 was associated with the most abundant reads in sample D1-T1 (26%, 11,874 reads). The 16S rRNA gene amplicon sequence of OTU 669126 matched 100% with the complete GenBank sequence of accession number AF023032, a sequence originating from a human mouth sample and grouped in a phylogenetic tree between *T. denticola* and sequence DDKL-3, a phylotype closely related to *T. denticola* (Nordhoff et al. 2008). Other notable abundant species or phylotype associated reads in D1's clinical lesion samples were associated with *T. medium* (2-25% of reads), *Treponema* sp22 (3-14% of reads), OTU 124932 (0.4-22% of reads), *T. refringens* (0-5% of reads), and *C. ureolyticus* (0.1-3.8% of reads). In the stage 3 lesion from D2, sample D2-T7, the most abundant species or phylotype-associated reads were associated with OTU 669126 (28%, 33,200 reads), OTU 124932 (17%, 20,855 reads), *T. phagedenis* (15%, 18,252 reads), OTU 1537706 (12%, 14,785 reads), *T. medium* (11%, 12,702 reads), *T. denticola* (5%, 5,930 reads), and *Treponema* PT8 (5%, 5,825 reads). All the aforementioned OTUs were associated with *Treponema* except for OTU 1537706, which is an unnamed sequence associated with *Bacteroidetes* (family: *Marinilabiaceae*). OTU 124932 is a *Treponema* spp. whose sequence matched with GenBank accession number DQ0032624, a partial sequence of the 16S rRNA gene sampled from a human mouth. The most abundant reads on a species/phylotype level sequenced from D3's stage 3 lesion, sample D3-T2, were associated with *Treponema* PT8 (38%, 39,516), *T. phagedenis* (21%, 21,761), OTU 669126 (10%, 9,918), and OTU 124932 (8%, 8,549), *T. medium* (7%, 7,052), and *T. pedis* (3%, 3,052). In the stage 3 lesion collected at D4, sample D4-T7, the most abundant species or phylotype-associated reads were associated with *Treponema* PT8 (34%, 15,508 reads), *T. phagedenis* (24%, 10,953 reads), OTU 669126 (12%, 5,310 reads), and *Mycoplasma haemobos* (4%, 1,737 reads).

In Krull et al.'s stage 3 and 4 lesions, the *Treponema* phlotypes that increased significantly compared to stage 1 and 2 lesions were *Treponema* PT8, *T. denticola*, *T. pedis*, and *T. medium*, while *T. phagedenis* remained at a substantial level throughout lesion progression (Krull et al. 2014). These 4 *Treponema* spp. increased in abundance to comprise 68.1% of *Treponema* reads in Krull et al.'s stage 3 lesions and 69.3% of stage 4 lesions while *T. phagedenis* remained at 24.9% in stage 3 and 23.4% in stage 4 lesions (Krull et al. 2014). In the present study, reads associated with these 4 *Treponema*-associated OTUs made up the following percentages of the total *Treponema* population in clinical lesions: 15% of D1-T4, 42% of D1-T3, 47% of D1-T1, 68% of D1-T7, 70% of D1-T2, 25% of D2-T7, 54% of D3-T2, and 52% of D4-T7. In these same lesions,

T. phagedenis-associated reads made up 44% of D1-T4, 3.2% of D1-T3, 0.7% of D1-T1, 8% of D1-T7, 1% of D1-T2, 19% of D2-T7, 23% of D3-T2, and 33% of D4-T7's *Treponema* spp.-associated reads. These 5 phylotypes made up 44-84% of *Treponema*-associated reads in stage 3 lesions in this study.

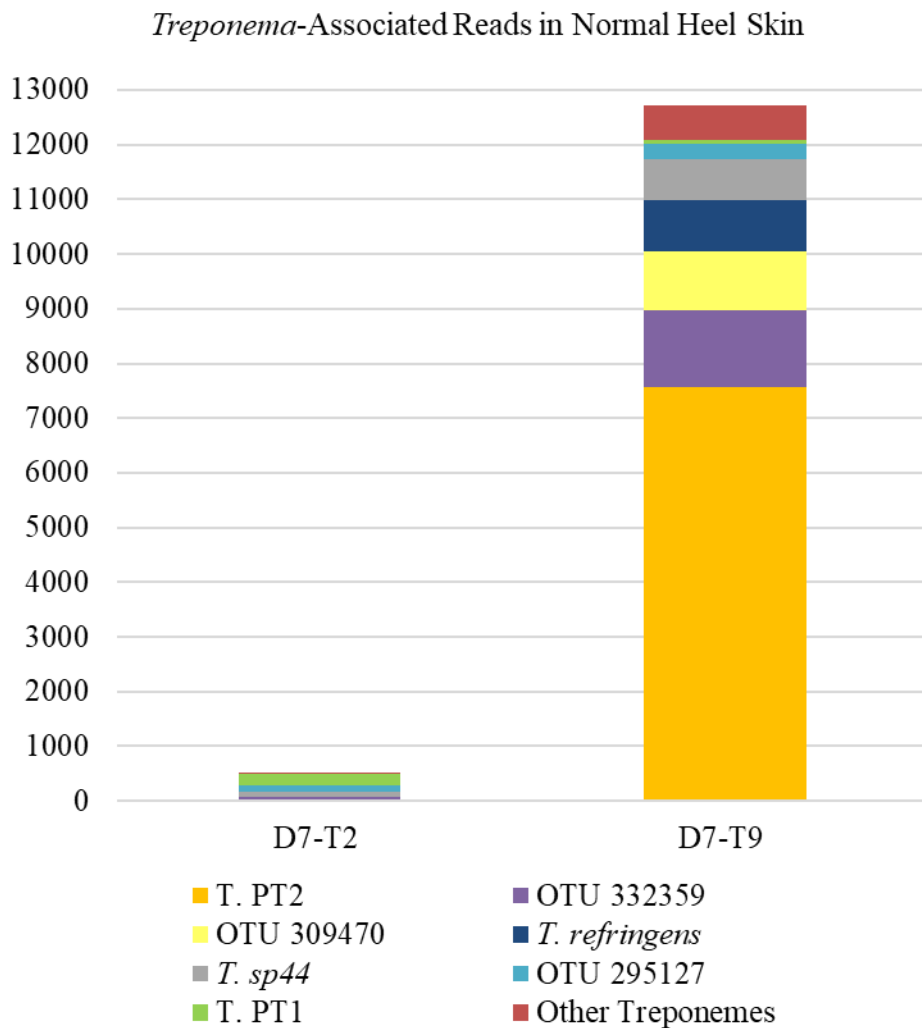


Figure 3.18 Abundance of operational taxonomic unit reads associated with *Treponema* sequenced from normal (score 0) heel skin from dairy 7.

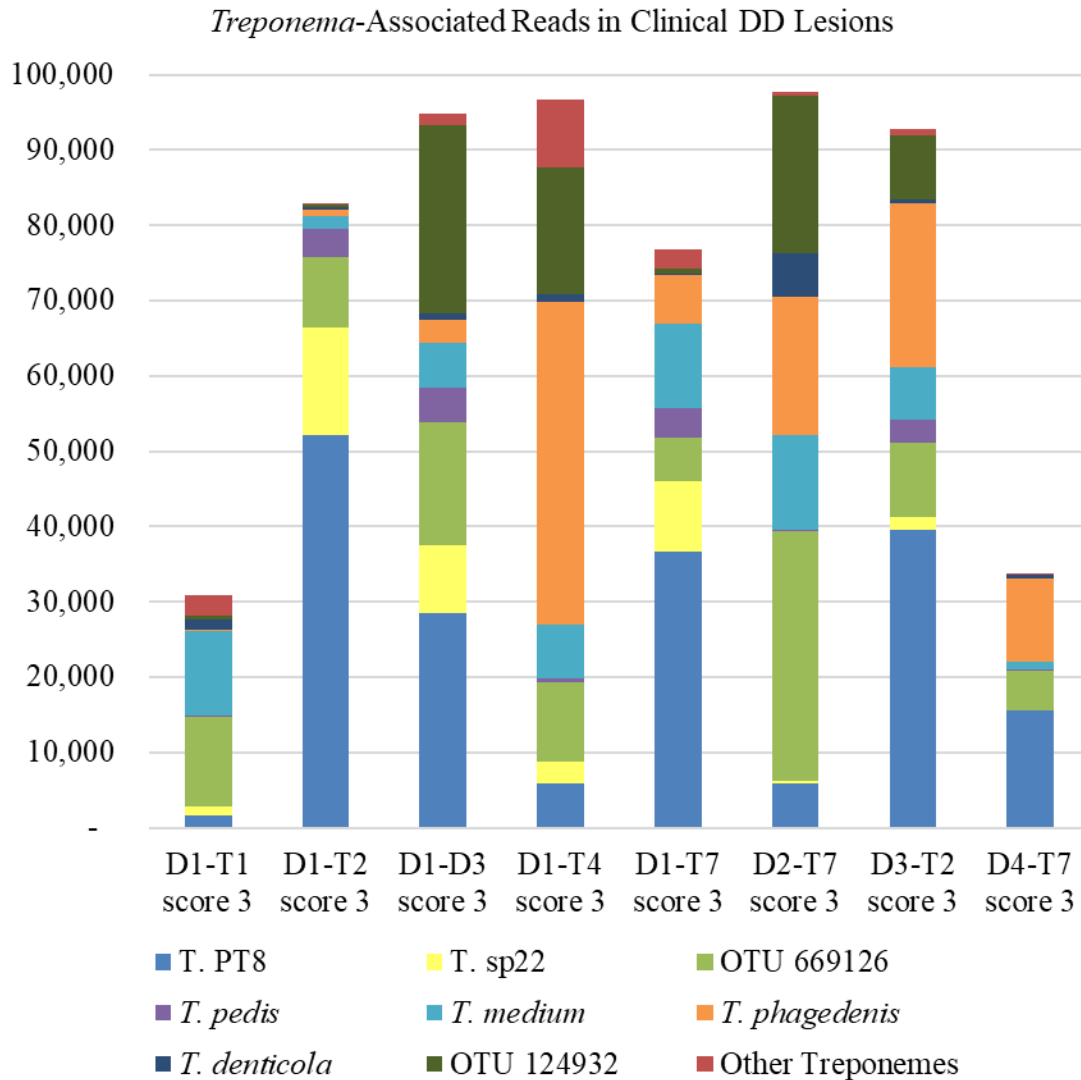


Figure 3.19 Abundance of operational taxonomic unit reads associated with *Treponema* sequenced from clinical digital dermatitis lesions.

3.4 Discussion

3.4.1 Saskatchewan Dairy Producer Lameness Survey

This survey was used to determine the DD status of and describe Saskatchewan dairies from which a sample was recruited for participation in further research. Although the response rate was a disappointing 23%, the results indicate those who did respond had dairies comparable to an average SK dairy. Other Canadian dairy producer surveys have had response rates between 9% (Denis-Robichaud et al. 2018) and 41% (Tse 2016). In 2015, average SK dairy herd size was 165

cows while the herd size of the average survey respondent endemic for clinical DD was 187 ± 147 (**Table 3.5**) and the herd size of the respondent non-endemic for clinical DD was 110 (D7; **Table 3.5**). The average 305-day milk production per cow in SK was 31.6 kg/day in 2015 (CanWest DHI, Guelph, Ontario, Canada) while the average for all respondents was 34.3 kg/day (extrapolated from **Table 3.5**). The selected participants all housed their cows in free-stalls or open pack barns, like most SK dairies. In 2015, 79.3% of SK dairies registered with CanWest DHI (n=93) housed their milking cows in free-stalls (CanWest DHI, Guelph, Ontario, Canada).

Of respondents that self-identified, 80% claimed to keep records of lameness (**Table 3.6**) while only 54% of anonymous respondents did. An average 67% of all respondents claimed to keep lameness records. The average of these results is similar to those of a recent study by Higginson Cutler et al. (2017) in which 60% of the 237 Canadian dairy producers interviewed reported keeping lameness records. In that study, producers were interviewed on lameness management in person, a practice that is not without potential bias (Choi and Pak 2005). Record keeping is inherently a good practice and it is likely that producers would feel more comfortable reporting if they chose to remain anonymous. It is likely that self-reporting bias occurred in the self-identified population of the present study, resulting in a greater report of record keeping. Overall, the number of producers who reported keeping records of lameness is similar to the Canadian average.

The final SK producer lameness survey question that suggests the present study population was representative of the Canadian dairy herd was, “what is the most common cause of lameness in your herd?” Many respondents gave more than one answer, but the most commonly reported was DD (54%, n=26/48, **Table 3.4**). The next most popular answers were sole ulcers (19%) and foot rot (17%). The cow-level prevalence of foot lesions in Ontario free-stall dairy herds in 2004-2005 was 22.9% for DD, 9.3% for sole ulcers, and 0.2% for foot rot. The cow-level prevalence of all lame cows with DD was 12.6%, with sole ulcers was 20.8%, and with foot rot was 8.9% in a 2004 study on two New York State dairy herds (Booth et al. 2004). In that study, the authors included all cases of interdigital dermatitis and interdigital hyperplasia with the foot rot cases, indicating that foot rot was over-reported. Judging by the fact that foot rot is commonly reported by participants in the present study, one may speculate that producers are misdiagnosing this relatively rare foot disease. However, foot rot is known to cause severe lameness and it is proven that dairy producers vastly underestimate lameness in their herd due to missing moderately lame

cases (Espejo et al. 2006). If a producer were only considering severe cases of lameness, they may find that foot rot is the most common cause on their farm. With these considerations in mind, it is likely that the study population's perception of lameness causing lesions was representative of other Canadian dairy producers.

Lameness management practices reported also differed between self-identified and anonymous respondents (**Table 3.6**). All the respondents that self-identified claimed to trim on a regular basis, while 15% of those that remained anonymous used lameness as a motivator for trimming. The presence of lameness is intrinsically negative, so the difference in these survey respondent populations may be again due to bias. In contrast, one could argue that those respondents who self-identified are proud of their lameness management, so may have been more likely to self-identify than their anonymous counterparts. Regular, preventive trimming has been associated with reduced DD prevalence (Somers et al. 2005) but in this study, anonymous respondents reported treating less DD lesions per month than the self-identified respondents (3.9 and 8.3 cows/month, respectively; Appendix A). Dairies 1-6 claimed to treat on average more cases of DD (17) per month than the average survey respondent of both identified and anonymous populations (8.3 and 3.9 cases/month, respectively; Appendix A). The pool of identified survey respondents from which D1-D7 participants were selected may have contained an over-representation of producers with an increased incidence of DD, therefore being more invested in the present study's results.

The SK producer lameness survey provided herd parameters and hoof lesion management tactics of dairies endemic and non-endemic for clinical DD. The results of this survey indicated the respondents compared to previously studied Canadian dairy producers in production parameters, lameness record keeping, perceived causes of lameness in their herds, and general trimming routines. This survey successfully recruited representative participants for the second objective of this research project.

3.4.2 PCR and Amplicon Sequencing

Despite the fact that the *T. phagedenis*-like group was identified on dairies both with and without clinical DD, the separate clustering in a phylogenetic tree of sequences from dairy 7, the dairy free of clinical DD, suggests a significant difference between dairy 7 and the endemic dairies, dairies 1-6. The DNA amplified from D7 preclinical lesion samples by these primers was more similar to *T. refringens* than *T. phagedenis*. These products were best matched with GenBank

sequences from *Treponema* phylotype 3 (PT3, *T. calligyrum*/*T. refringens*-like) and clone PN-20, while preclinical lesion samples from D1-D6 resulted in amplicon sequences best matched with *T. phagedenis*.

Treponema PT3 has been previously identified in bovine DD lesions (Yano et al. 2010; Klitgaard et al. 2008) and has greater than 99% similarity to DDKL-20, a clone isolated from a clinical bovine DD lesion by Choi et al. in 1997. *Treponema* PT3 and PN-20 are included in cluster 3 along with DDKL-20 (Choi et al. 1997) and associated with *T. refringens* (Krull 2015). *Treponema* PT3 was found frequently and in great abundance in early lesions by Klitgaard et al. and is mostly associated with the superficial layers of the epidermis (2008). A later study by Rasmussen et al. identified *Treponema* PT3 as the only *Treponema* phylotype in a subclinical DD lesion affecting a Norwegian cow (Rasmussen et al. 2012). They also identified *Treponema* PT12, another member of *T. refringens*-like cluster 3, in all 6 study herds but only once was it among the most abundant treponemes in the junction between healthy and diseased epidermis (Rasmussen et al. 2012). That study found *Treponema* PT3 in many clinical DD lesions and even a case of CODD in which it was the only *Treponema* sp. identified among severe infiltration of *Fusobacterium necrophorum* and *Dichelobacter nodosus* (Rasmussen et al. 2012). The results of the present study paired with the findings of previous studies allow speculation that *Treponema* PT3, *Treponema* PT12, and PN-20 are primarily associated with early lesions, an observation consistent with that of Krull et al. (2014). The most commonly amplified gDNA sequences were more similar to *T. refringens*-like than *T. phagedenis*-like sequences in D7 tissue samples. These results also indicate that the primers used were not sufficiently specific for *T. phagedenis*-like sequences that phylotypes more similar to *T. refringens* would not be amplified.

The *Treponema* phylotype most similar to those sequences amplified with *T. phagedenis*-like primer pairs in preclinical lesion samples from D1 through D6 was 2-1498med, a *T. phagedenis*-like phylotype isolated from a DD lesion in California (Walker et al. 1995). The 16S-23S rDNA intergenic spacer region of 2-1498med is 99.5% similar to *T. phagedenis* (Stamm et al. 2002), adding confidence to the successful amplification of *T. phagedenis*-like DNA in preclinical lesion samples from D1-D6.

3.4.3 16S rRNA Gene Amplicon Sequencing

The microbiome of DD lesions is known to harbour an abundance of *Treponema* spp. (Evans et al. 2008; Borgmann et al. 1996; Nordhoff et al. 2008; Choi et al. 1997; Döpfer et al. 2012a) in

addition to reports of other Gram-negative, anaerobic, and microaerophilic organisms. Among *Treponema* spp., these taxa have also been found in clinical DD lesions: *Mycoplasma* spp. (Berry et al. 2010), *Porphyromonas* spp. (Moe et al. 2010), *Fusobacterium necrophorum* (Moe et al. 2010; Klitgaard et al. 2008), *Dichelobacter nodosus* (Rasmussen et al. 2012), *Campylobacter* spp. (Zinicola et al. 2015a; Döpfer et al. 1997), and *Firmicutes* spp. (Santos et al. 2012). Various taxa of *Firmicutes* have been associated with healthy skin, preclinical DD, and inactive lesions. Among those are *Streptococcus* (Klitgaard et al. 2008), *Peptostreptococcus* (including species previously classified as *Peptococcus*), *Tissierellaceae*, *Aerococcaceae*, *Ruminococcus*, *Corynebacteria*, *Moraxellaceae* (Krull 2015), and *Guggenheimella* spp. (Schlafer et al. 2008; Wyss et al. 2005). It may be that a number of different bacteria are necessary for epidermal barrier breakdown, colonization, lesion development, and chronicity.

Fecal and slurry samples yielded very few OTUs associated with DD-associated *Treponema* phylotypes. Although Zinicola et al. (2015a) reported these phylotypes as present when they contributed much less than 0.005% of the *Treponema* species in fecal samples, those fecal samples generated 105,904-159,024 total sequences per sample. The present study's fecal samples only generated 12,973 (D7-F8) to 197,574 (D6-F4) total sequence reads per sample with an average of 68,447 reads. In the present study, a relative abundance of 0.005% would mean a species were present even if less than 10 reads per sample were attributed to them.

In other 16S rRNA gene sequencing studies, the bacteria associated with the greatest prevalence of reads in clinical DD lesions have been distinctly different from those of preclinical lesions and healthy skin (Krull et al. 2014 and Zinicola et al. 2015a). The microbiome of healthy skin has been reportedly dominated by *Firmicutes* and *Actinobacteria*, while *Spirochaetes* dominate the active stages and *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* are the most abundant in chronic or inactive stages (Zinicola et al. 2015b). This study described the microbiome of normal healthy skin samples (n=2), preclinical lesions staged as A1 (n=32), A2 (n=13), B1 (n=3), and B2 (n=1), and stage 3 clinical lesions (n=8) using 16S rRNA gene amplicon sequencing analysis. Although 6 of the dairies were endemic for clinical DD and 1 was non-endemic for clinical DD, the beta diversity analysis of preclinical lesions from these two dairy types revealed that these dairies were different both within themselves and between each other. As such, the results of each dairy's amplicon sequence analysis were described in a non-comparative manner

The relative abundance of OTUs related to *Treponema* spp. associated with stage 1 lesions, *Treponema* sp44 and *Treponema* PT2, was lower on average in this study compared to Krull et al.'s value of 77.4% (2014). Although the range in relative abundance was not reported in that study so no comparison can be done, the relative abundance in this study had a wide range in values among samples within and between dairies (range: 0.1-89.4%). In many cases where the relative abundance of stage 1 *Treponema*-associated reads was lower than anticipated, the relative abundance of *T. phagedenis*-associated reads was higher than the anticipated range of 3.8% to 48.8% reported by Krull et al. (2014). The exception to this was with D7's preclinical lesions, which yielded almost negligible values of *T. phagedenis*-associated reads.

The relative abundance of OTUs associated with stage 2 *Treponema* phylotypes, *T. phagedenis*, *Treponema* PT3, and *Treponema* PT1, was again lower than the 82.6% reported by Krull et al. (2014). The average relative abundance of OTUs associated with these treponemes among all *Treponema* spp.-associated reads in stage 2 lesions from dairies 1 through 6 ranged between 24.8 and 50.2%. A glaring exception to this was evident in all of D7's tissue samples. *Treponema*-associated OTUs related to stage 2 lesions made up <0.4% of all *Treponema*-associated reads in D7's stage 2 lesions and <0.5% in D7's stage 1 and 2 lesions combined.

When the microbiome of D7's preclinical lesions was investigated further, it became evident that almost negligible quantities of *Treponema* spp.-associated reads of phylotypes known to be related to clinical DD were identified in these lesions. The abundance of reads associated with *T. phagedenis* was very low (range: 2-7 reads) in D7's preclinical lesion samples, even those that tested positive for *T. phagedenis*-like with targeted PCR using Evans et al.'s (2009) primers. The sum of OTUs associated with *Treponema* PT8, *T. medium*, *Treponema* sp22, *T. pedis*, and *T. denticola* made up less than 0.014% of all *Treponema* spp.-associated reads in D7's preclinical lesions with 29 total reads. Krull et al. (2014) reported that *T. phagedenis* contributed to more than 3.8% of the *Treponema* population at every stage of lesion development. The preclinical lesions of D7 do not appear to follow this reported trend.

Eight clinical lesion samples were subjected to 16S rRNA gene amplicon sequencing and the relative abundance of the most prevalent taxa at the phyla, family, and species/phylotype level were reported. Clinical lesions are considered "active" lesions and in other studies, were reportedly dominated by the phyla *Spirochaetes*, *Firmicutes*, *Bacteroidetes*, *Tenericutes*, and *Proteobacteria* (Zinicola et al. 2015a). The most abundant reads were consistently associated with *Spirochaetes*

(77-92% of phyla-associated reads) followed by either *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, or *Tenericutes* in this study's clinical lesions with the addition of *Actinobacteria* among the fifth to sixth most abundant. In Zinicola's study (2015a), *Actinobacteria* was among the most prevalent in inactive lesions, however, the present study's results indicate reads associated with *Actinobacteria* can contribute up to 2.2% of the reads in clinical lesions on a phyla level. On a species/phylolevel, the most abundant reads were associated with those that had been identified in clinical lesions before. *Treponema* PT8, *T. phagedenis*, *T. denticola*-like, *T. medium*, *Treponema* sp22, *T. refringens*, *T. pedis*, *C. ureolyticus*, and in one case, a *Bacteroidetes* OTU related to the *Marinilabiaceae* family, were identified among the most abundant species-associated OTUs in the clinical lesions sampled from dairies 1-4. Krull et al. (2014) reported that OTUs associated with *Treponema* PT8, *T. denticola*, *T. pedis*, and *T. medium* made up 68.1-69.3% of the *Treponema*-associated reads in clinical lesions but in this study, OTUs associated with these four treponemes comprised 15-70% of the *Treponema*-associated reads. In sample D1-T4, where these four phylotypes made up less than expected of the *Treponema*-associated reads, *T. phagedenis* made up more than expected (44%). An OTU identified as *Mycoplasma haemobos* made up 4% of all reads at the species/phylolevel in sample D4-T7. Although this species has not been associated with clinical DD before, it is known to be associated with bovine haemoplasmosis, anaemia, and depression in cows and may have immunosuppressive effects (McFadden et al. 2016). Sample D4-T7 did not stand out from the other clinical tissue samples in any other way. The collection of clinical lesion samples presented here appeared to yield a good representation of taxa previously associated with active lesions but like in the case of the normal tissue samples collected, more samples would have delivered greater confidence in this observation by allowing statistical analysis.

Other taxa such as *Campylobacter ureolyticus* and *Dichelobacter nodosus* have been associated with DD and may play a role in lesion progression (Krull et al. 2014; Knappe-Poindecker et al. 2014). *C. ureolyticus* was found to be statistically associated with A2 lesions along with *T. phagedenis* (Krull 2015). In a DD lesion induction protocol tested by Krull et al. on immunologically naïve calves, the addition of *D. nodosus* pure culture to macerated clinical lesion tissue inoculum from infected cows did not increase the rate of positively infected test subjects compared to only the inoculation of macerated clinical lesion tissue to the palmer interdigital space of healthy calves' hooves ($P > 0.5$, Krull 2015). That study also evaluated the effectiveness of pure

cultures of *D. nodosus*, *Bacteroides* spp., *Porphyromonas levii*, *T. phagedenis*, and *C. ureolyticus* in inducing DD and reported an induction rate of only 3% while just macerated lesion tissue inoculant resulted in a 95% infection rate. The presence of these bacteria was evaluated in the present study, with the abundance of *D. nodosus*, *Porphyromonas* spp. and *C. ureolyticus* illustrated in **Figure 3.10**. The D2 preclinical lesions had the most *D. nodosus* and *C. ureolyticus*-associated OTU reads/sample on average compared to all other dairies, while D6 had the highest average reads/sample of *Porphyromonas* spp.-associated OTUs. *C. ureolyticus* has been associated with specifically stage A2 and *Porphyromonas* spp. has been associated with preclinical and healing lesions, while *D. nodosus* has not been associated with any specific stage (Appendix of Krull 2015) but was previously identified in 29% of DD lesion samples (Capion et al. 2012). At least one of these bacteria yielded >10 associated reads in every preclinical lesion sample and no obvious patterns could be detected without further research on their abundance in additional preclinical lesion samples. Even though OTUs associated with stage 2 treponemes were generated at an extremely low level in the present study, *C. ureolyticus* and *D. nodosus*, suspects in DD lesion progression, were still present in D7's preclinical lesions, albeit in low abundance. The role of *C. ureolyticus* and *D. nodosus* in stage 2 lesions should be further studied.

Although the relative abundance of *Spirochaete*-associated OTUs compared to other taxa in preclinical lesion samples was comparable across all dairies, the *Treponema*-associated phylotypes most abundant in D7's preclinical lesions were those associated with stage 1, not stage 2 DD lesions, even in lesions scored visually as stage 2.

3.5 Conclusion

The dairies in this study are representative of the wider population of Western Canadian dairies. The primers targeting *Treponema phagedenis* successfully amplified *T. phagedenis*-like DNA product from endemic preclinical lesion samples but amplified DNA more similar to *Treponema refringens*-like DNA from D7's preclinical lesion samples. With 16S rRNA gene amplicon sequencing, the relative abundance of bacterial phyla detected in preclinical lesion tissue was similar across all dairies. However, of the *Treponema* spp.-associated OTU reads from D7's preclinical lesions, 99% were associated with *Treponema* PT2, *T. refringens*, and *T. sp44* and less than 0.015% of total reads were associated with *T. phagedenis*, *Treponema* PT3, *Treponema* PT1, *T. medium*, *Treponema* PT8, *T. denticola*, *T. sp22*, and *T. pedis*. Although the sample size of this study is small and only one clinically non-endemic herd was included, the abundance of stage 1

associated treponemes paired with the extremely low abundance of treponemes associated with clinical DD suggest that D7's preclinical lesions are indeed early DD lesions, but may be in the absence of stage 2, 3, and 4 pathogens necessary to facilitate clinical DD.

3.6 References

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4 GENERAL DISCUSSION AND CONCLUSIONS

The Saskatchewan producer lameness survey indicated that respondents were representative of average Saskatchewan dairies in terms of herd size, production level, and housing type and representative of Canadian dairies regarding record keeping, trimming practices, and perceived importance of hoof lesions causing lameness. The 6 endemic self-identifying respondents that were selected for part two of this study had larger milking herds than average SK dairies and a greater perceived prevalence of DD compared to anonymous respondents, thus likely volunteering to participate due to their interest in DD prevention and control. Dairy 7 practiced more stringent biosecurity than the other participant dairies by not purchasing any dairy cattle in the previous 5 years and providing their hoof trimmer with disinfectant and new blades to use before trimming at their dairy.

Interestingly, both non-endemic and endemic dairies reported foot rot as a frequent cause of lameness. This disease is infectious, indicating that both farm types had housing and management practices conducive to the spread of pathogenic bacteria. The phyla identified in preclinical lesions were more similar in relative abundance to DD lesion samples than healthy skin samples in other studies. The types of bacteria identified in preclinical lesions from all dairies were similar at the phyla level, but differences became apparent at the species/phylolevel. Dairies 1-6 had preclinical lesion samples with varying abundances of *Treponema* spp. that have been previously associated with stage 1, 2, and 3 lesions, while D7's preclinical lesions were characterized by stage 1-associated *Treponema* spp. This was the most contrasting result among preclinical lesion samples, as differences in *C. ureolyticus*, *Porphyromonas* spp., and *D. nodosus* were less apparent.

Further research on the importance of *Treponema* spp. associated with stage 2 and 3 lesions would shed light on their roles in lesion progression. Pathogens important to the etiology of lesion progression between stage 1, 2, and 3 may be candidates for vaccine development, as halting DD at a painless stage would prevent lameness and the negative effects associated with it.

The most critical findings of this study are the near complete lack of stage 2, 3, and 4-associated *Treponema* OTUs identified in D7 preclinical lesions and the biosecurity practices of this dairy. The preclinical lesions identified on D7 should be considered true preclinical DD, but

continued stringent biosecurity appears necessary to keep clinical DD-associated treponemes from proliferating in these tissues that otherwise appear susceptible to clinical DD.

APPENDIX A

In the following results, let \bar{X} = sample mean, SD = standard deviation of a sample of the population, n = sample size of a category, and N = the total sample size. Standard deviation of a

sample was calculated using the equation: $SD = \sqrt{\frac{\sum(X-\bar{X})^2}{N-1}}$. These results are given in the format:

\bar{X} (\pm SD, min-max).

SASKATCHEWAN DAIRY PRODUCER LAMENESS SURVEY

Please answer the following questions by either circling your choice or filling in the blank provided.

1. For how many years have you continuously owned a milk quota?

➤ _____

Identified: 29 years (\pm 12; 3-41); n = 25

Anonymous: 24 years (\pm 14; 1-41); n = 12

Combined: 28 (\pm 13; 1 - 41); N = 37

2. What is the size of your milking herd?

➤ _____

Identified: 183 (\pm 124; 60-600); n = 25

Anonymous: 187 (\pm 186; 50-700); n = 13

Combined: 185 (\pm 146; 50 - 700); N = 38

3. What is your average cow weight?

➤ _____ **lb** or **kg** (please circle one)

Identified: 661 kg (\pm 64; 544-816); n = 24

Anonymous: 652 kg (\pm 90; 500-862); n = 13

Combined: 658 kg (\pm 73; 500 - 862); N = 36

4. What is your average milk production per cow?

➤ _____ **kg/cow/year** or **kg/cow/day** (please circle one)

Identified: 10,663 kg/cow/year (\pm 1,099; 9,150-13,275); n = 25

Anonymous: 10499 kg (\pm 1,231; 8,235-12,200); n = 12

Combined: 658 kg (\pm 73; 500 - 862); N = 37

5. Do you keep records for which animals are treated for lameness?

- Yes
- No

Identified: 80% Yes; 20% No; n = 25

Anonymous: 64% Yes; 36% No; n = 11

Combined, not including NA: 75% Yes; 25%; N = 36

6. What is the most common cause of lameness in your herd?

- **Foot rot/foul foot**

Identified: 16%; 5/31

Anonymous: 18%; 3/17

Combined: 17%; 8/48

- **Heel erosion**

Identified: 3%; 1/31

Anonymous: 6%; 1/17

Combined: 4%; 2/48

- **Sole ulcers**

Identified: 16%; 5/31

Anonymous: 24%; 4/17

Combined: 19%; 9/48

- **Overgrown feet**

Identified: 0%; 0/31

Anonymous: 0%; 0/17

Combined: 0%; 0/48

- **Hairy heel warts/strawberry heel/Mortellaro's disease/Digital dermatitis**

Identified: 58%; 18/31

Anonymous: 47%; 8/17

Combined: 54%; 26/48

- **Hock/knee abscesses**

Identified: 3%; 1/31

Anonymous: 6%; 1/17

Combined: 4%; 2/48

- **Other**_____

Identified: 3%; 1/31

Anonymous: 0%; 0/17

Combined: 2%; 1/48

7. Do your cows get their feet trimmed?

- **Yes**

Identified: Yes 100%; 25/25

Anonymous: Yes 92%; 12/13

Combined: Yes 97%; 37/38

➤ **No**

Identified: No 0%; 0/25

Anonymous: No 8%; 1/13

Professional Hoof Trimmer	Combined N = 38	Identified n = 25	Anonymous n = 13
Trimmer #1	37%	36%	38%
Trimmer #2	16%	20%	8%
Trimmer #3	24%	28%	15%
Producer or Farmworker trims	5%	4%	8%
Trimmer #5	5%	4%	8%
Trimmer #6	3%	4%	0%
Trimmer #7	3%	0%	8%
Trimmer #8	5%	4%	8%

Combined: No 3%; 1/38

8. Who trims your cows' feet?

- I trim them myself
- I hire a foot trimmer (name: _____)

9. When do your cows get their feet trimmed?

- **On a regular schedule to prevent lameness**
Identified: 86%; 24/28
Anonymous: 73%; 11/15
Combined: 81%; 35/43
- **When cows are lame**
Identified: 7%; 2/28
Anonymous: 13%; 2/15
Combined: 9%; 4/43
- **When cows develop overgrown feet**
Identified: 7%; 2/28
Anonymous: 7%; 1/15
Combined: 7%; 3/43
- **Other** _____
Identified: 0%; 0/28
Anonymous: 7%; 1/15
Combined: 2%; 1/43

10. Do you have digital dermatitis (hairy heel warts/strawberry heel/Mortellaro's disease) in your herd?

- **Yes**
Identified: 96%; 24/25
Anonymous: 92%; 12/13
Combined: 95%; 36/38
- **No**
Identified: 4%; 1/25
Anonymous: 8%; 1/13
Combined: 5%; 2/38
- **I'm not sure**
Identified: 0%; 0/25
Anonymous: 0%; 0/13
Combined: 0%; 0/38

11. If you have digital dermatitis in your herd, how long has it been since you first noticed it?

- _____ **months/years** (please circle one)
Identified: 12 years \pm 5 (3-20); n = 24
Anonymous: 10 years \pm 5 (5-20); n = 10
Combined: 12 years \pm 5 (3-20); N = 34

12. How many cows/month do you treat for:

- **Heel erosion:** _____ cows/month
Identified: 2 \pm 3 (0-10); n = 14
Anonymous: 1 \pm 1 (0-2); n = 4
Combined: 2 \pm 3 (0-10); N = 18
- **Sole ulcers:** _____ cows/month
Identified: 3 \pm 4 (0-15); n = 17
Anonymous: 3 \pm 2 (1-5); n = 4
Combined: 3 \pm 3 (0-15); N = 21
- **Foot rot:** _____ cows/month
Identified: 1 \pm 1 (0-4); n = 17
Anonymous: 2 \pm 1 (1-3); n = 6
Combined: 1 \pm 1 (0-4); N = 23
- **Digital dermatitis:** _____ cows/month
Identified: 8 \pm 10 (0-30); n = 20
Anonymous: 4 \pm 2 (2-6); n = 6
Combined: 7 \pm 9 (0-30); N = 26

13. If you are willing to participate in the larger study, please provide your name and contact information below.

Land location: _____ Email: _____
Address: _____ Ph. #: (____) _____ - _____
_____ Postal Code: _____

APPENDIX B

PARTICIPANT FARM QUESTIONNAIRE

In the following results, let \bar{X} = sample mean, SD = standard deviation of a sample of the population, n = sample size of a category, and N = the total sample size. Standard deviation of a sample was calculated using the equation: $SD = \sqrt{\frac{\sum(X-\bar{X})^2}{N-1}}$. Where question structure deems appropriate, quantitative results are given in the format: $\bar{X} \pm SD$ (min-max). Where qualitative results are given as percentages, let N = the total number of responses given for all categories and n = the numerator of the responses for each category's answer choice. For example, in question 23, "If you select individual animals to be trimmed, what criteria are used to select the animals to be trimmed?" there were 10 responses in the "Endemic" category, of which 50%, or n = 5, were "presence of lameness." In that same question, the "Non-Endemic" category had only 1 response, in which 100% was "depending on hoof condition" so both the category's denominator and numerator (n) was 1. The "N" for question 23 is N = 11 because a total of 11 answers were given for all categories of respondents.

Background:

1. How many dairy cattle do you currently have in milk? _____

Endemic:

292 \pm 136 (160 – 510) cows, n = 6

Non-Endemic:

110 cows, n = 1

N = 7

2. How many pens are the animals housed in? _____

Endemic:

2.8 \pm 0.8 (2-4) pens, n = 6

Non-Endemic:

7.0 pens, n = 1

N = 7

3. How many cows on average are housed in each pen? _____

Without including special needs pens:

Endemic:

101 ± 25 (65 – 125)/pen, n = 6

Non-Endemic:

15 ± 13 (8 – 44)/pen, n = 1

N = 7

4. What breed of dairy cattle do you have? _____

Endemic and Non-Endemic:

All dairies milked primarily Holstein cows.

N = 7

5. How many replacement heifers do you keep on average from your own herd each year?

Endemic and Non-Endemic:

All respondents kept all their heifer calves as replacement animals.

N = 7

6. How many animals on average do you purchase each year to add to your herd? (note: if you do not buy in animals for your herd put NA) _____ (If none purchased skip to question 9).

Endemic:

24 ± 14 (0 – 40)/year, n = 6

Non-Endemic:

0/year, n = 1

N = 7

7. When approximately was the first year you brought in animals from another herd?

Endemic:

12 ± 3 (8 – 15) years ago, n = 4

Non-Endemic:

67 years ago, n = 1

N = 5

8. What was the most recent year that you brought in animals from another herd?

Endemic:

2015 ± 0.45 (2014 – 2015), n = 5

Non-Endemic:

2009, n = 1

N = 6

9. Do you know whether the animals you brought in came from a herd that has had Digital Dermatitis?

- They came from a herd with Digital Dermatitis
- They did not come from a herd with Digital Dermatitis
- I am not sure

Endemic:

They came from a herd with DD: 33%, n = 2

I am not sure: 66%, n = 4

Non-Endemic:

They did not come from a herd with DD: 100%, n = 1

N = 7

Barn Characteristics:

10. How frequently do you clean the alleys in the barn? _____ times per day

Endemic:

7 ± 4 (2 – 12)/day, n = 6

Non-Endemic:

8/day, n = 1

N = 7

11. How frequently do you clean the stalls in the barn? _____ times per week

Endemic:

3 ± 1 (2 – 3)/week, n = 4

Non-Endemic:

3/week, n = 1

N = 5; inapplicable question for 2 endemic pack barn dairies.

12. How often do you add new bedding to the stalls/pack? _____ (per week)

Endemic:

4 ± 3 (1 – 7)/week, n = 6

Non-Endemic:

7/week, n = 1

N = 7

13. How often do you change the bedding completely in the stalls/pack? _____ (times per year)

Endemic:

6 ± 10 (0 – 26)/year, n = 6

Non-Endemic:

52/year, n = 1

N = 7

Access to Outside:

14. Do your animals have access to the outside at any point in the year?

- Yes
- No (If no skip to next section)

Endemic:

Yes: 17%, n = 1

No: 83%, n = 5

Non-Endemic:

Yes: 100%, n = 1

N = 7

15. How much time on average do your dairy cattle spend outside?

_____ hours per day _____ days per week

D1 - 2 groups are housed outside all day except during milking. The third group is never housed outside. Cows rotate through groups based on milk production. The highest producers are housed inside then they move outside when their production drops.

D2 - NA

D3 - Cows are housed outside for the dry period.

D4 - NA

D5 - NA

D6 - Cows are housed outside for the dry period.

D7 - Cows and replacement heifers are housed outside for the far-off dry period

N = 4

16. Are your cows let out to pasture or corrals? _____ (if pasture skip question 17)

Endemic:

Corrals: 100%, n = 3

Non-Endemic:

Corrals: 100%, n = 1

N = 4

17. If your cattle have access to corrals, how often do you clean the corrals?

_____times per year

Endemic:

$3 \pm 2 (2 - 5)/\text{year}, n = 3$

Non-Endemic:

2/year, n = 1

N = 4

Hoof Trimming:

18. Do you regularly have the hooves of your dairy cattle trimmed?

- Yes
- No (If no then move to the next section)

Endemic:

Yes: 100%, n = 6

Non-Endemic:

Yes: 100%, n = 1

N = 7

19. Do you trim the entire herd or select individual animals?

- Entire herd
- Individual animals
- Other _____

Endemic:

Entire herd: 50%, n = 3

Individual animals: 50%, n = 3

Non-Endemic:

Entire herd: 100%, n = 1

N = 7

20. If you trim the entire herd, how often do you get the hooves trimmed?

- More than 3 times a year
- 3 times a year
- 2 times a year
- Once a year
- Less than once per year

Endemic:

2.3 ± 1.2 (1.0 - 3.0) times/year, n = 4

Non-Endemic:

2 times/year, n = 1

N = 5

21. What month(s) of the year do you typically get the hooves trimmed? (if no specific month is used then put NA) _____

NA

22. If you select individual animals to be trimmed, what criteria are used to select the animals to be trimmed?

- Presence of lameness
- Age
- Hoof condition
- Status of animal (ex/ milking, dry, post-parturition)
- Other _____

Endemic:

Presence of lameness: 50%, n = 5

Hoof Condition: 30%, n = 3

Status of animal: 20%, n = 2

Non-Endemic:

Depending on hoof condition: 100%, n = 1

N = 11

23. When do you routinely trim hooves?

- Dry
- Lactating
- Whenever an animal comes up lame without a specific lifecycle point
- Other:

Endemic:

Dry: 11%, n = 1

Lactating: 67%, n = 6

Whenever an animal comes up lame without a specific lifecycle point: 22%, n = 2

Non-Endemic:

Dry: 50%, n = 1

Lactating: 50%, n = 1

N = 11

24. When do you trim heifers' hooves?

- Never
- Before first calving
- After first calving when in lactation
- Only when an animal comes up lame without a specific lifecycle point
- Other _____

Endemic:

Before first calving: 33%, n = 2

After first calving: 67%, n = 4

Non-Endemic:

Before first calving: 100%, n = 1

N = 7

25. Who does the hoof trimming on your dairy farm?

- Professional hoof trimmer coming to your farm
- Vet coming to your farm
- Vet at a vet clinic
- Farm Worker
- Farm Manager
- Other _____

Endemic:

100% use a professional trimmer, n = 6

33% do in-house trimming in addition to using a professional trimmer, n = 2

Non-Endemic:

100% use a professional trimmer only, n = 1

N = 9

26. How long has the current hoof trimmer been trimming hooves on your farm? _____
years

Endemic:

5 ± 3 (1 – 10) years, n = 6

Non-Endemic:

5 years, n = 1

N = 7

27. Does the hoof trimmer for your farm trim hooves at other farms?

- Yes
- No

Endemic and Non-Endemic:

All of the professional trimmers hired also trim at other farms.

N = 7

28. Does the hoof trimming equipment get cleaned between farms?

- Yes
- No (If no skip next question)
- I don't know

Endemic and Non-Endemic:

Yes: 100%

N = 7

29. What is the protocol for cleaning equipment between farms?

Endemic:

The trimmers pressure wash the equipment with water between dairies, n = 6

Non-Endemic:

The trimmer disinfects equipment and buys new grinder blades before visiting, n = 1

N = 7

30. Does the hoof trimming equipment get cleaned between individual cows on your farm?

- Yes
- No (if no skip next question)
- Only if the previous animal had a hoof lesion
- I don't know

Endemic and Non-Endemic:

No: 100%

N = 7

31. What is the protocol for cleaning equipment between animals on the farm?

N/A

Footbaths:

32. Do you use footbaths?

- Yes
- No (if no skip to next section)

Endemic and Non-Endemic:

Yes: 100%

N = 7

33. What season do you use footbaths in?

- Spring
- Summer
- Fall
- Winter
- All year
- Multiple seasons _____ specify
- Only use footbaths when lameness issues present
- Other _____

Endemic and Non-Endemic:

All year: 100%

N = 7

34. How frequently do you use footbaths? _____ times per week

Endemic:

3 ± 1 (2 – 4) times per week, n = 6

Non-Endemic:

3 times on Mondays, n = 1

N = 7

35. How often do you change the footbath solution? _____ frequency

Endemic:

After 188 ± 56 (110 – 250) cow-passes, n = 6

Non-Endemic:

After 66 cow-passes, n = 1

N = 7

36. Do you clean the footbath between fillings?

- Yes
- No (if no skip next question)

Endemic and Non-Endemic:

Yes: 100%

N = 7

37. How do you clean the footbath? _____

Endemic and Non-Endemic:

Rinse with water: 100%

N = 7

38. What product is used in the footbath? _____

Endemic:

Healmax: 10%, n = 1

Formalin: 40%, n = 4

CuSO₄: 50%, n = 5

Non-Endemic:

CuSO₄: 100%, n = 1

86% of herds use CuSO₄ either alone or in rotation with other products (n = 6). D2 only uses formalin.

N = 11

39. What concentration of product is used in the footbath? _____

Endemic:

Healmax: 2.1%, n = 1

CuSO₄: 9.3 ± 4 (6.6 – 12)%, n = 2

Formaldehyde: 0.81 ± 0.51 (0.4 – 1.2)%, n = 2

Non-Endemic:

CuSO₄: 5%

N = 6

40. Do the hooves of the cattle get cleaned before entering the footbath?

- Yes
- No (if no skip next question)

Endemic:

Yes: 50%, n = 3

No: 50%, n = 3

Non-Endemic:

No: 100%, n = 1

N = 7

41. What is used to clean the hooves of the cattle before entering the footbath?

Endemic:

Water: 100%, n = 3

N = 3

Hoof Spraying:

42. Do you spray the hooves of your dairy cattle?

- Yes
- No (if no skip to next section)

Endemic:

Yes: 67%, n = 4

No: 33%, n = 2

Non-Endemic:

No: 100%, n = 1

N = 7

43. How frequently do you spray the hooves? _____ times per week

Endemic:

2 ± 1 (1 – 3) times per week

N = 4

44. What do you use to spray the hooves? _____

Quick Hit: 50%, n = 2

Repiderma: 25%, n = 1

Formalin: 25%, n = 1

N=4

45. What concentration of product do you use? _____

Quick Hit: 50%, n = 2

Repiderma: 100%, n = 1

Formaldehyde: 37%, n = 1

N = 4

46. How long do the feet get sprayed for in a single session? _____seconds

4 ± 4 (1 – 10) seconds

N = 4

Lameness:

47. What do you consider to be the 3 most problematic foot lesions on your farm?

Endemic (n = 15):

DD: 40%, n = 6

Foot rot: 20%, n = 3

Sole ulcers: 13%, n = 2

Corns: 7%, n = 1

White line disease: 7%, n = 1

Heel erosion: 7%, n = 1

Abscesses: 7%, n = 1

Corkscrew toe: 0%

Non-Endemic (n = 3):

Sole ulcers: 33%, n = 1

Foot rot: 33%, n = 1

Corkscrew toes: 33%, n = 1

N = 18

48. How do you monitor for lameness? _____

Cows are watched for lameness on all dairies, most commonly during milking.

Endemic:

During milking: 67%, n = 6

Non-Endemic:

At parlour milking and by monitoring robot visit behaviour, n = 1

N = 7

49. What additional practices do you use to control for lameness?

Endemic:

No other additional practices besides footbathing, trimming, and using foot spray in the parlour were used.

Non-endemic:

All surfaces cows walk on are rubber and good stockmanship is encouraged.

N = 7

50. Do you consider hoof health in selecting replacement animals?

- Yes
- No
- Sometimes
- I don't know

Endemic:

Yes: 80%, n = 4

No: 20%, n = 1

Non-Endemic:

No: 100%, n = 1

N = 6

51. Has Digital Dermatitis been identified in the dairy animals in your herd previously?

- Yes
- No (if no skip to next section)
- I am not sure

Endemic:

Yes: 100%, n = 6

Non-Endemic:

No: 100%, n = 1

N = 7

52. Approximately how many years ago was the first case of DD identified on your farm?

Endemic:

14 ± 2 (10 – 15) years ago, n = 6

Non-Endemic:

NA

N = 6

53. Do you consider Digital Dermatitis to be a problem on your farm?

- A large problem
- Somewhat of a problem
- Neutral
- Not too much of a problem
- Not a problem at all

Endemic (n = 6):

A large problem: 17%, n = 1

Somewhat of a problem: 50%, n = 3

Average/Neutral: 33%, n = 2

Not much of a problem: 0%

No problem at all: 17%, n = 1

Non-Endemic:

Not a problem at all: 100%, n = 1

N = 7

54. How do you monitor for Digital Dermatitis?

- Check all cows at hoof trimming
- Examine cows during milking
- Examine only lame animals
- Other _____

Endemic (n = 11):

Check all cows at hoof trimming: 45%, n = 5

Examine cows during milking: 45%, n = 5

Examine only lame animals: 0%

Other: Watch as cows walk: 9%, n = 1

N = 11

Non-Endemic:

NA

55. Who diagnoses Digital Dermatitis on your farm? If multiple, please select all.

- Hoof trimmer
- Farm worker
- Farm manager
- Vet
- Other _____

Endemic (n = 14):

Hoof trimmer: 45%, n = 5

Farmworker: 36%, n = 4

Farm manager: 45%, n = 5

N = 14

Non-Endemic:

NA

56. Do you treat for Digital Dermatitis on your farm?

- Yes
- No (If no skip to question 58)

Endemic:

Yes: 100%, n = 6

No: 0%

N = 6

Non-Endemic:

NA

57. Who treats Digital Dermatitis on your farm? If multiple, please select all.

- Hoof trimmer
- Farmworker
- Farm manager
- Vet
- Other

Endemic:

Hoof trimmer: 50%, n = 5

Farmworker: 10%, n = 1

Farm manager: 40%, n = 4

N = 10

Non-Endemic:

NA

58. What is the protocol for treating Digital Dermatitis on your farm? _____

D1: Topical tetracycline under a wrap if done by the farm manager. Trimmer uses Heelsol paste under a wrap. Remove wraps after 2-3 days.

D2: Oxytetracycline under a wrap

D3: Trimmer uses Heelsol paste under a wrap

D4: Mark leg and spray with Quick Hit or Repiderma in the parlour. Bad cases wrapped when trimmer came.

D5: Footbath and spray in the parlour once a week.

D6: Tetracycline under a wrap.

D7: NA

N = 6

59. Do you keep individual treatment records for lameness?

- Yes
- No

Endemic:

Yes: 83%, n = 5

No: 17%, n = 1

Non-Endemic:

Yes: 100%, n = 1

N = 7

60. How many times on average do you have to treat a case of Digital Dermatitis before it gets better? _____

D1: once

D6: once

N = 2

61. During which lactation on your farm are you most likely to identify new cases of Digital Dermatitis? _____

1st lactation: 40%, n = 2

3rd lactation: 20%, n = 1

Every lactation: 20%, n = 1

Anytime throughout lactation: 20%, n = 1

N = 5

62. How many days in milk on your farm are you most likely to identify new cases of Digital Dermatitis? _____

50 days: 50%, n = 2

225 days: 25%, n = 1

Anytime: 25%, n = 1

N = 4

Table B.1 Digital dermatitis scoring agreement between the author and trimmer #1

M. F.			
Trimmer	DD+	DD-	Total
DD+	13	3	16
DD-	4	64	68
Total	17	67	84