High throughput quantitative PCR to measure prevalence and gene expression of the three major groups of treponemes associated with Digital dermatitis in dairy cows.

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High throughput quantitative PCR to measure prevalence and gene expression of the three major groups of treponemes associated with Digital dermatitis in dairy cows

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Objective
To develop a fast and efficient method for measuring prevalence and gene expression of representatives from the three major groups of Treponema, most frequently identified in DD biopsies from cattle.

Introduction
Bovine digital dermatitis (DD), which causes lameness in cattle (Figure 1), is the most serious foot disorder of dairy cows from economic and welfare perspectives. Recent research point towards a polytreponemal etiology of infections, with Treponema phagedenis-like, Treponema medium/Treponema vincentii-like, and Treponema denticola/Treponema pedis-like phylotypes being highly associated with the disease2-4. Still the complex interplay between infecting Treponema species and their relative contributions to pathogenesis is largely unknown for this disease and must be elucidated.

Methods
From NCBI sequence data (http://www.ncbi.nlm.nih.gov/) we designed a high-throughput quantitative PCR (qPCR) assay to differentiate between T. denticola-like, T. medium/vincentii-like, and T. phagedenis-like phylotypes. Samples: CDNA from biopsies taken from cows just after slaughter: Controls (n=3), different stages of DD lesions (n=25). High-throughput reverse transcription qPCR analysis was performed on a 48.48 Dynamic Array (Fluidigm) (Figure 2), targeting 9 putative virulence genes1 and 3 reference genes (Table 1). 16S rRNA genes were applied for species identification.

Results
Although all three major groups of DD-related treponemes appeared to be present in most of the lesions (16S rRNA positive results), the expression results indicated that only T. phagedenis-like species were metabolically active (Figure 2). T. denticola/T. pedis-like and T. medium/T. vincentii-like species may either be present in low numbers and/or they may be less active than T. phagedenis. An example of the distribution of T. phagedenis-like species in DD lesions is shown in Figure 1b. From this picture it can be seen that T. phagedenis-like bacteria are highly active in the deeper part of the lesions, which is in good accordance with the expression results. The most active genes were related to functions such as host adhesion and motility (Figure 3).

Conclusion
The method of parallel gene expression analysis presented here needs further optimization but seems promising and may be broadly applicable for high throughput investigation of the infection dynamics of Treponema in DD lesions.

References

Figure 1. Digital dermatitis lesion (DD12) at a proliferative stage: a) macroscopic, b) microscopic depiction of the same lesion. Bacteria are visualized by fluorescence in situ hybridization applying a T. phagedenis specific probe (red) and a general eu-bacterial probe (green).

Figure 2. Heat map of expression analysis on 48.48 Dynamic array.

Figure 3. Mean expression (log2) of T. phagedenis-like phylotypes in positive samples (n=23).

Table 1. Design of 48.48 Dynamic array.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Function</th>
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<tbody>
<tr>
<td>Hly</td>
<td>Hemolycin. Exhibits hemoxidative and hemolytic activities</td>
</tr>
<tr>
<td>FlgG</td>
<td>Flagellar basal body rod protein</td>
</tr>
<tr>
<td>FlgE</td>
<td>Flagellar hook protein</td>
</tr>
<tr>
<td>PrP</td>
<td>Hemin-binding protein D-Lipoprotein</td>
</tr>
<tr>
<td>flaA</td>
<td>Flagellar filament outer layer protein</td>
</tr>
<tr>
<td>licCA</td>
<td>Cholinephosphate cytidylyltransferase</td>
</tr>
<tr>
<td>mglA</td>
<td>Galactose/methyl galactoside ATP-binding protein</td>
</tr>
<tr>
<td>yeaZ</td>
<td>Hexokinase family protein (glycolysis)</td>
</tr>
<tr>
<td>msp hly flgG flgE flaA hbpB prtP licCA mglA yeaZ</td>
<td>Putative virulence genes</td>
</tr>
</tbody>
</table>

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