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 disease[2,3,4]. Still the complex interplay between infecting *Treponema* species and their relative contributions to pathogenesis is largely unknown for this disease and must be elucidated.

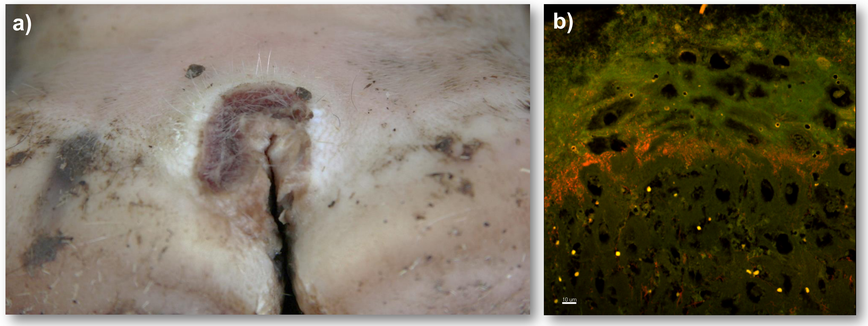

Figure 1. Digital dermatitis lesion (DD12) at a proliferative stage: a) macroscopic, b) microscopic depiction of the same lesion. Bacteria are visualized by Fluorescence *in situ* hybridization applying a *T. phagedenis* specific probe (red) and a general eubacterial probe (green).

Gene name	Function
Putative virulence genes	
Msp	Major outer sheath protein. Binding to fibronectin, laminin, collagen types I and IV, hyaluronic acid.
Hly	Hemolycin. Exhibits hemooxidative and hemolytic activities
FlgG	Flagellar basal body rod protein
FlgE	Flagellar hook protein
hbpB	Hemin-binding protein B Lipoprotein
PrtP	Serine protease, dentilisin Possibly involved in bacterial adhesion, tissue penetration and immune evasion
flaA	Flagellar filament outer layer protein
licCA	Cholinephosphate cytidylyltransferase
mglA	Galactose/methyl galactoside import ATP-binding protein
Reference genes	
hexo	Hexokinase family protein (glycolysis)
tpiA	Triosephosphate isomerase (Glycolysis)
lnt	Apolipoprotein N-acyltransferase (Lipid metabolism)
16S rRNA	Specific for *T. pedis*, *T. phagedenis*, *T. medium/vincentii* and *T. denticola*

Table 1. Design of 48.48 Dynamic array.

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 genes[1] and 3 reference genes (Table 1). 16S rRNA genes were applied for species identification.

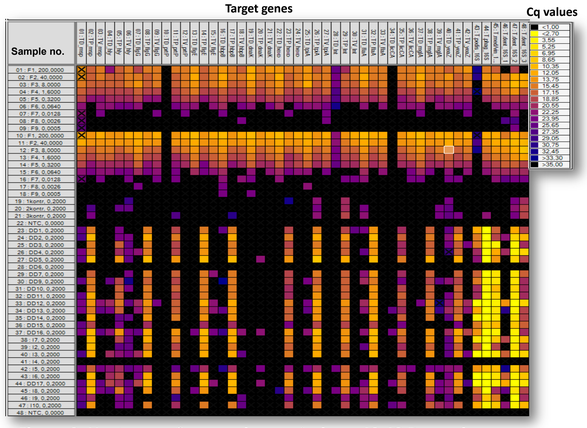

Figure 2. Heat map of expression analysis on 48.48 Dynamic array.

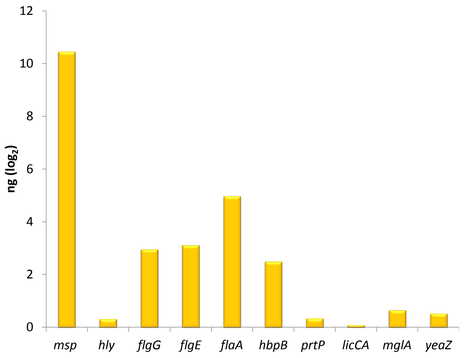

Figure 3. Mean expression (log_2) of *T. phagedenis*-like phylotypes in positive samples (n=23).

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

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