A genome wide association study for Bovine Digital Dermatitis using M-stages


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67th Annual Meeting of the European Federation of Animal Science
Belfast UK, 29 Aug 2016, Session 12
Bovine Digital Dermatitis (BDD)
(=Pappillomatous Digital Dermatitis/ Hairy Heel Warts)

- infectious claw disease with global distribution
- multifactorial etiology -> also host genetic factors could play important role (Scholey et al., 2012)
- different Cow Types regarding the susceptibility to BDD (Döpfer et al., 2004)
- cows seem to differ in their immune response against Treponema spp. (Gomez et al., 2014)
- $h^2$ based on M-stages: 0.13 – 0.52 (Schöpke et al., 2015)

➢ identification of chromosomal region(s) contributing to a cow’s resistance against BDD, or affecting the course of disease
M-scale system

M0 M1 M2 M3 M4 M4.1

signs of chronicity:
0: none
1: hyperkeratosis
2: proliferation

BDD-cycle

Zinpro corporation 2014
Döpfer et al., 1997, 2012
M-scale system

- M0
- M2 – 0
- M2 – 1
- M2 – 2
- M4 – 1
- M4 – 2

\(\leftrightarrow\) scored M-stages and signs of chronicity

signs of chronicity:
0: none
1: hyperkeratosis
2: proliferation

Döpfer et al., 1997, 2012

Zinpro corporation 2014
Main features

- 7 farms in northeast Germany between October 2015 and April 2016
- > 8,000 cows scored for stages of BDD, 3 times at intervals of 3 weeks

scoring:
- with DD Check App (Zinpro 2015)
- rotary milking parlour (external rotary, herringbone, side-by-side)
- 1 score per cow (hind legs)
- both legs affected → more severe lesion documented
Data edition (1)

scoring data (BDD project)

8,148  • cows scored

7,378  • remove cows with missing values, scored twice without identification/pedigree

7,215  • merge with calving data (DIM < 450)
Data edition (1)

number of observations per cow (N=7,215)

<table>
<thead>
<tr>
<th>number of obs.</th>
<th>frequency</th>
<th>percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>985</td>
<td>13.65%</td>
</tr>
<tr>
<td>2</td>
<td>1353</td>
<td>18.75%</td>
</tr>
<tr>
<td>3</td>
<td>4877</td>
<td>67.60%</td>
</tr>
</tbody>
</table>

distribution on farms (N=6,230)

<table>
<thead>
<tr>
<th>farm</th>
<th>N cows</th>
<th>frequency M2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1161</td>
<td>4.18%</td>
</tr>
<tr>
<td>2</td>
<td>635</td>
<td>25.11%</td>
</tr>
<tr>
<td>3</td>
<td>1845</td>
<td>2.01%</td>
</tr>
<tr>
<td>4</td>
<td>1147</td>
<td>1.80%</td>
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<tr>
<td>5</td>
<td>528</td>
<td>5.35%</td>
</tr>
<tr>
<td>6</td>
<td>348</td>
<td>4.51%</td>
</tr>
<tr>
<td>7</td>
<td>566</td>
<td>6.28%</td>
</tr>
</tbody>
</table>

scoring data (BDD project)

8,148 • cows scored

7,378 • remove cows with missing values, scored twice without identification/pedigree

7,215 • merge with calving data (DIM < 450)
Data edition (2)

**genotype data (Kuh-L project)**

- **19,166**
  - 4,960 50K
  - 14,206 10K imputed to 50K

**scoring data (BDD project)**

- **8,148**
  - cows scored
  - 7,378
    - remove cows with missing values, scored twice without identification/pedigree
  - 7,215
    - merge with calving data (DIM < 450)

**1,885 cows with genotypes (scored at least two times)**
### Traits

<table>
<thead>
<tr>
<th>Binary Traits</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBIN*</td>
<td>1 = at least one obs. with $DD(=M2/M4)$, else = 0</td>
</tr>
<tr>
<td>TBINA*</td>
<td>1 = ... with $M2$, else = 0</td>
</tr>
<tr>
<td>TCHRONA</td>
<td>1 = ... with proliferation of skin, else = 0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type Traits</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCTM2*</td>
<td>counting $M2/ M4/$ proliferative(MP) events</td>
</tr>
<tr>
<td>TCTM4</td>
<td>1 = never, 2 = one time, 3 = repeatedly</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Categorical Traits</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSEVCAT*</td>
<td>1 = $M0$, 2 = at least once $M4$, 3 = at least once $M2$</td>
</tr>
<tr>
<td>TCHRCAT</td>
<td>1 = 0, 2 = ... hyperkeratosis, 3 = ... proliferation</td>
</tr>
<tr>
<td>TMCSEV</td>
<td>0 = $M0$, 1 = M4-1, 2 = M4-2, 3 = M2-0, 4 = M2-1, 5 = M2-2</td>
</tr>
<tr>
<td>TTRANS*</td>
<td>score for classified transitions between $M$-stages</td>
</tr>
</tbody>
</table>

*analogous to Schöpke et al., 2015*
fixed effects: farm (1 to 7), parity (1 to 5), DIM (50d steps, 0 to 350+), number of observations per cow (2 or 3)

software:

PLINK v.1.07 (Purcell et al., 2007)
- genome wide + chromosomewise
- --hardy --maf 0.05
- “qqman” for Manhatten plots R version 3.0.2 (R Core Team, 2013)
- no fixed effects for binary traits

- genome wide
- “Proc glimmix” for binary traits
- “Proc mixed” for type and categorical traits
Results PLINK - overview

**binary traits**

- TBIN
- TBINA
- TCHRONA

**type traits**

- TCTM2
- TCTM4
- TCTMP

**categorical traits**

- TSEVCAT
- TCHRCAT
- TMCEV
- TTRANS
Results - TCTM4 in detail

- Interesting region on BTA 11 in PLINK and SAS
- BONFERRONI adjusted p-value significant (p < 0.05) in PLINK and SAS for 5 different traits (TCHRONA, TCTM4, TCTMP, TCHRCAT, TTRANS)
Further analysis

GWAS in 6 different variants/data edits:

A all 1,885 animals
B without farm 2
C just 50K typed animals
D healthy animals from „poor farms“ and affected from „good farms“
E only 2 categories (best-worst) for type and categorical traits
F combination of D and E

- interesting region on BTA11 substantiated in 5 of 6 variants with BONF< 0.05
- traits representing chronicity/ course of disease (TCHRONA, TCTM4, TCTMP, TCHRCAT, TTRANS)
- clear trend for the significant traits in genotype frequencies
Conclusion and outlook

- promising literature research for BTA11:
  - link to alteration of gene expression in immune cells upon spirochetal infection
  - theory: variation in the intensity of expression level alteration affect the individual immune response and thus the course of disease (healing/chronic-proliferation)?

- analysis of 2 to 10 SNPs haplotypes revealed „good“ and „poor“ haplotypes for TCHRONA and helped to identify 12 animals for sequencing

- Research for other interesting regions on BTA 6, 13, 19, 26 and 29 is in progress...
Thank you for your attention!

we gratefully acknowledge:

• BMBF, FBF and DHV for sponsoring Kuh-L project
• project partners: University Kassel, vit, RBB and RinderAllianz

special thanks to Dörte Döpfer, the BDD-team Halle and the participating farms
Data overview (1)

M-stages:

M0

M2

M4

visit 1  visit 2  visit 3

visit 1  visit 2  visit 3

visit 1  visit 2  visit 3
Data overview (2)

signs of chronicity:

- **none (0)**
- **hyperkeratosis (1)**
- **proliferation (2)**
## Fixed effects

<table>
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<th>farm</th>
<th>frequency</th>
<th>percent</th>
<th>cumulative frequency</th>
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<td>18.64</td>
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<th>frequency</th>
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