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# A 2-step strategy to infer genome-wide associations for endoparasite traits in local DSN cattle

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JLU



# Endoparasite infections in dairy cows

- Infections with endoparasites lead to reduced milk production and detrimental impacts on fertility (e.g., Charlier et al., 2005; Dank et al., 2015)  
→ high economic losses
- Increase of anthelmintic resistance (e.g., Gasbarre et al., 2014; Sutherland et al., 2011)
- Increasing interest in local breeds being best adapted to various environmental conditions



# Why genomic studies in endangered DSN cattle?

- „*Deutsches Schwarzbuntes Niederungsrind*“ /Black Pied cattle
- One of the founding breeds of Holstein Friesian

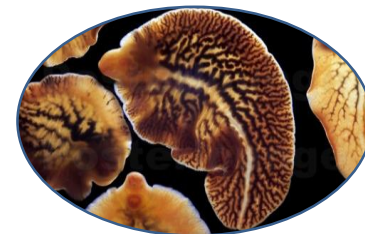


Better fertility  
and productive life  
(Biedermann et al., 2005)

More resistant to metabolic  
disease (Jaeger et al., 2016)

More robust under harsh  
environmental conditions  
(Al-Kanaan et al., 2016)




**Less  
susceptibility to  
endoparasite  
infections?**



# Background and preliminary work

- Faecal examination of 1166 dairy cows (2006 observations), 17 herds
- 4 different Black and White cattle selection lines
- HF milk, HF pasture, HF New Zealand, dual-purpose cows (DSN)

## 1. Quantitative-genetic parameters for endoparasite traits:

	Endoparasite trait	$h^2 \pm SE$	
	Gastrointestinal nematodes (GIN)	$0.05 - 0.06 \pm 0.04$	$r_g = 1.00$ $r_g = -0.10$ $r_g = 0.03$
	Lungworm larvae ( <i>Dictyocaulus viviparus</i> )	$0.05 \pm 0.04$	
	Liver fluke ( <i>Fasciola hepatica</i> )	$0.33 \pm 0.06$	

# Genomic scales for endoparasite traits

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## 2. Genome-wide association study (GWAS) for endoparasite traits in DSN

- a) Identification of SNP markers being associated with endoparasite resistance
- b) Analysis of potential candidate genes
- c) Pathway analyses

# Selective genotyping approach

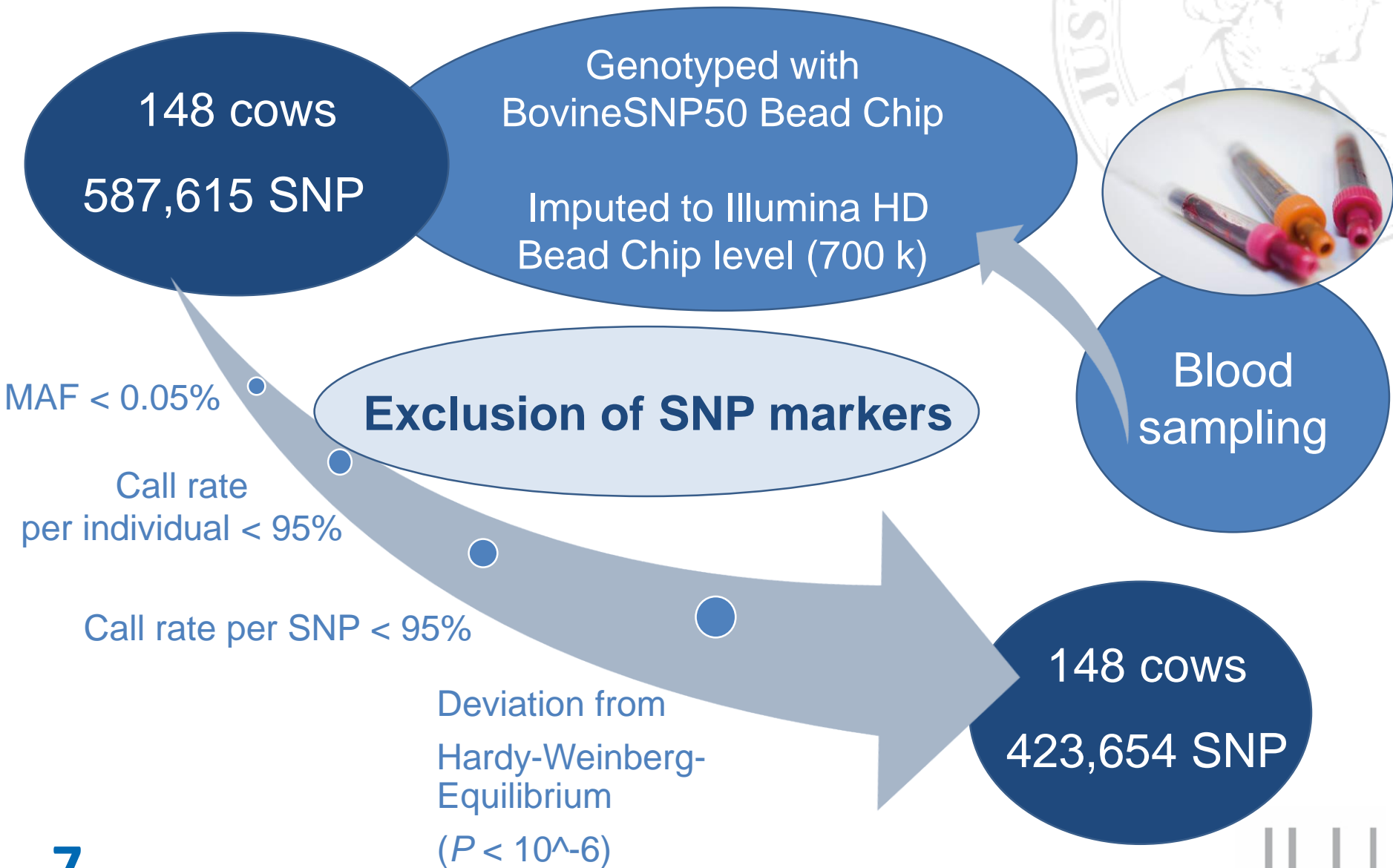
- Collection of blood samples from 148 **dual-purpose DSN cows**
- 3 herds



## Selection criteria:

1. Herd prevalence for gastrointestinal nematodes
2. Selection of most resistant and most susceptible cows within herds
  - 50% of cows being infected
  - 50% being not infected
3. Minimization of genetic relationships within and between groups

# Genotyping, Imputing and Quality control



# Pre-correction of endoparasite traits

1. Calculation of residuals for endoparasite traits using the whole dataset of 1166 cows

$$y_{ijklm} = \mu + \text{Farm}_i + \text{Parity}_j + \text{GenLin}_k + \text{SP}_l + \text{Lstage}_m + e_{ijklm}$$

$y_{ijklm}$  = observations for endoparasite traits (n = 2006)

$\mu$  = overall mean effect

$\text{Farm}_i$  = fixed effect of the  $i^{\text{th}}$  farm

$\text{Parity}_j$  = fixed effect of the  $j^{\text{th}}$  parity number

$\text{GenLin}_k$  = fixed effect of the  $k^{\text{th}}$  genetic line

$\text{SP}_l$  = fixed effect of the  $l^{\text{th}}$  sampling period

$\text{Lstage}_m$  = fixed effect of the  $m^{\text{th}}$  lactation stage

$e_{ijklm}$  = random residual effect



# Association analysis in GCTA (Yang et al., 2011)

## 2. Implementation of the GWAS in GCTA using residuals

$$y_i = a_i + \text{SNP}_i + e_i$$

$y_i$  = corrected phenotypic values  
(residuals of endoparasite traits)

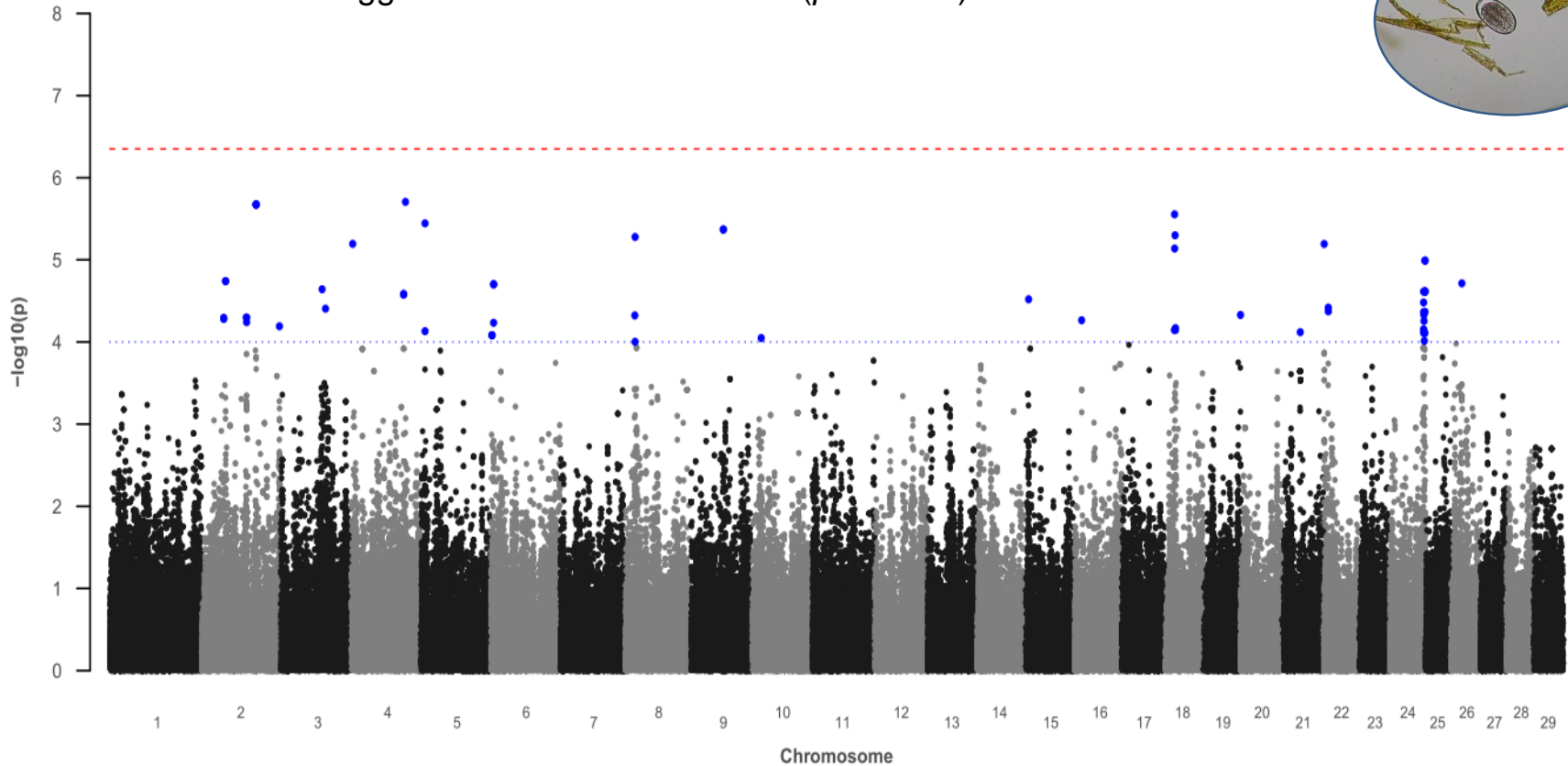
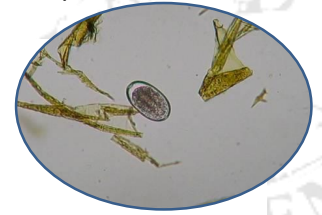
$a_i$  = random polygenetic additive-genetic effect on  
the basis of genomic relationship matrix

$\text{SNP}_i$  = fixed single-locus SNP effect

$e_i$  = random residual effect

# Manhattan-Plot for gastrointestinal nematodes

- Bonferroni-corrected genome-wide significance threshold ( $p = 4.47 \times 10^{-7}$ )
- Suggestive candidate threshold ( $p = 1e-04$ )

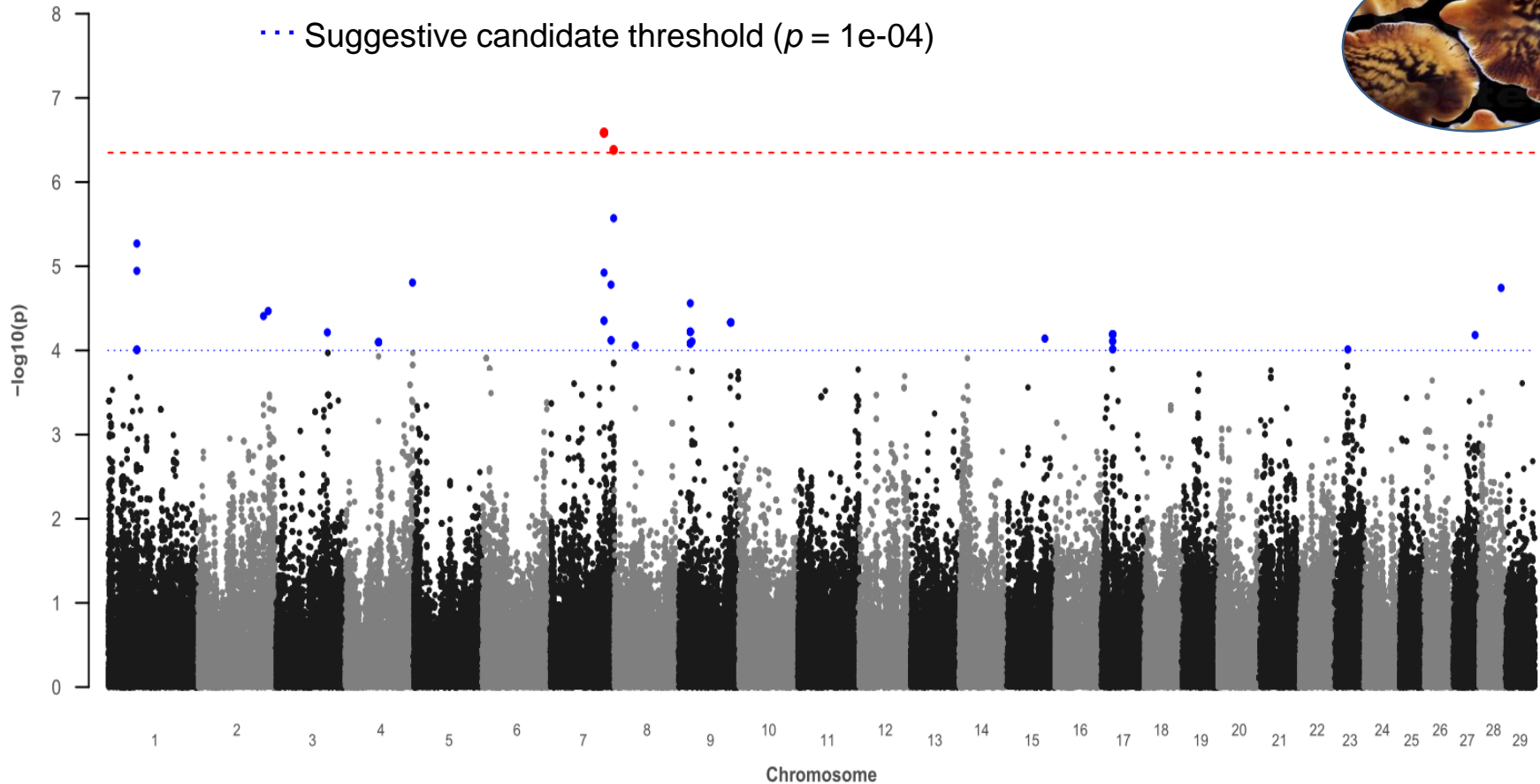
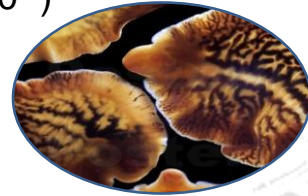


- 68 variants reached suggestive candidate threshold ( $p_{\text{Cand}} = 1 \times 10^{-4}$ )
- 11 candidate genes

# Manhattan-Plot for liver flukes (*F. hepatica*)

-- Bonferroni-corrected genome-wide significance threshold ( $p = 4.47 \times 10^{-7}$ )

••• Suggestive candidate threshold ( $p = 1e-04$ )

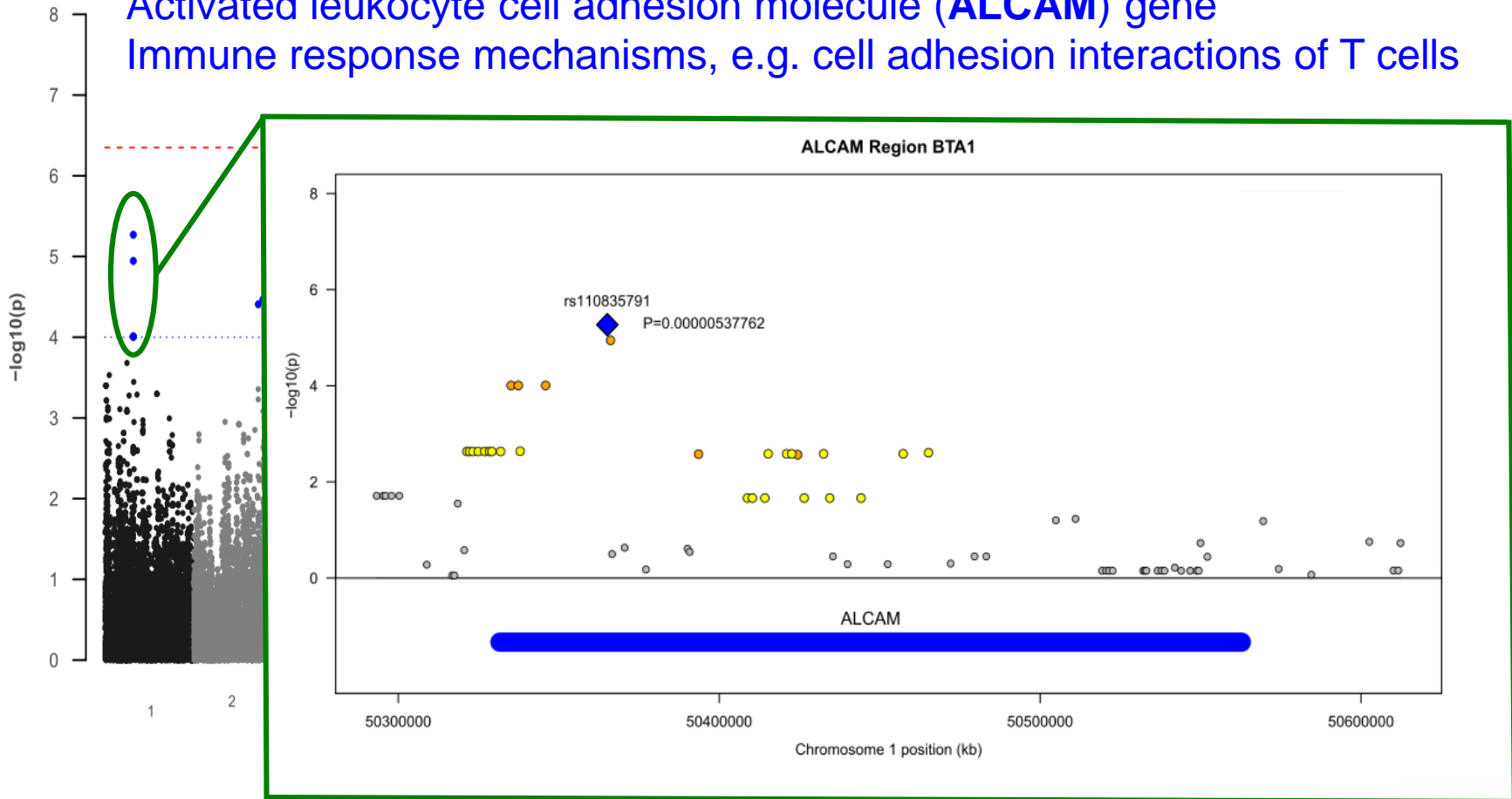


- 3 variants reached  $p_{\text{Bonf}}$  and 53 variants reached  $p_{\text{Cand}}$
- 7 candidate genes

# Gene annotation: Liver fluke (*F. hepatica*)

••• Candidate threshold ( $p = 1e-04$ )    - - - Bonferroni corrected genome-wide threshold ( $p = 4.47 \times 10^{-7}$ )

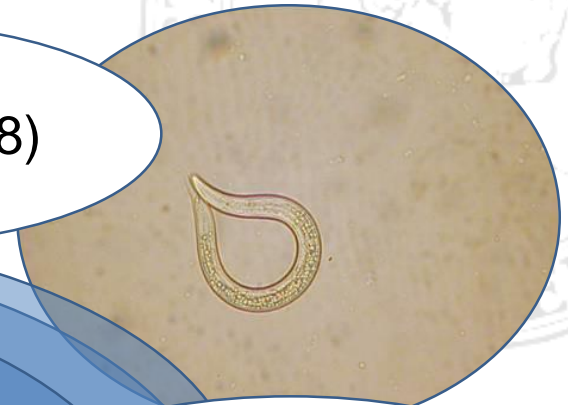
Activated leukocyte cell adhesion molecule (**ALCAM**) gene  
Immune response mechanisms, e.g. cell adhesion interactions of T cells



# Pathway-analyses (DAVID, Huang et al., 2008)

Cell adhesion interactions of T cells

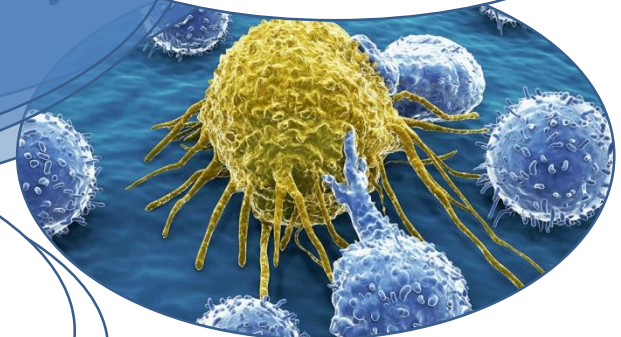
(BTA 28)



KCNJ3 (BTA2)  
EGFR (BTA 22)

Cell adhesion molecules  
cytokine signaling pathway  
immunological pathways

CDH2 (BTA 24)



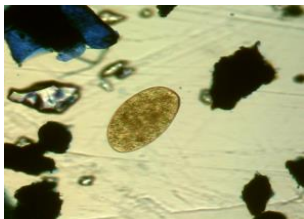
Important functions in cellular immune response, involved in host defense mechanisms during infections with *Neospora caninum*

# SNP effect correlations within endoparasite traits

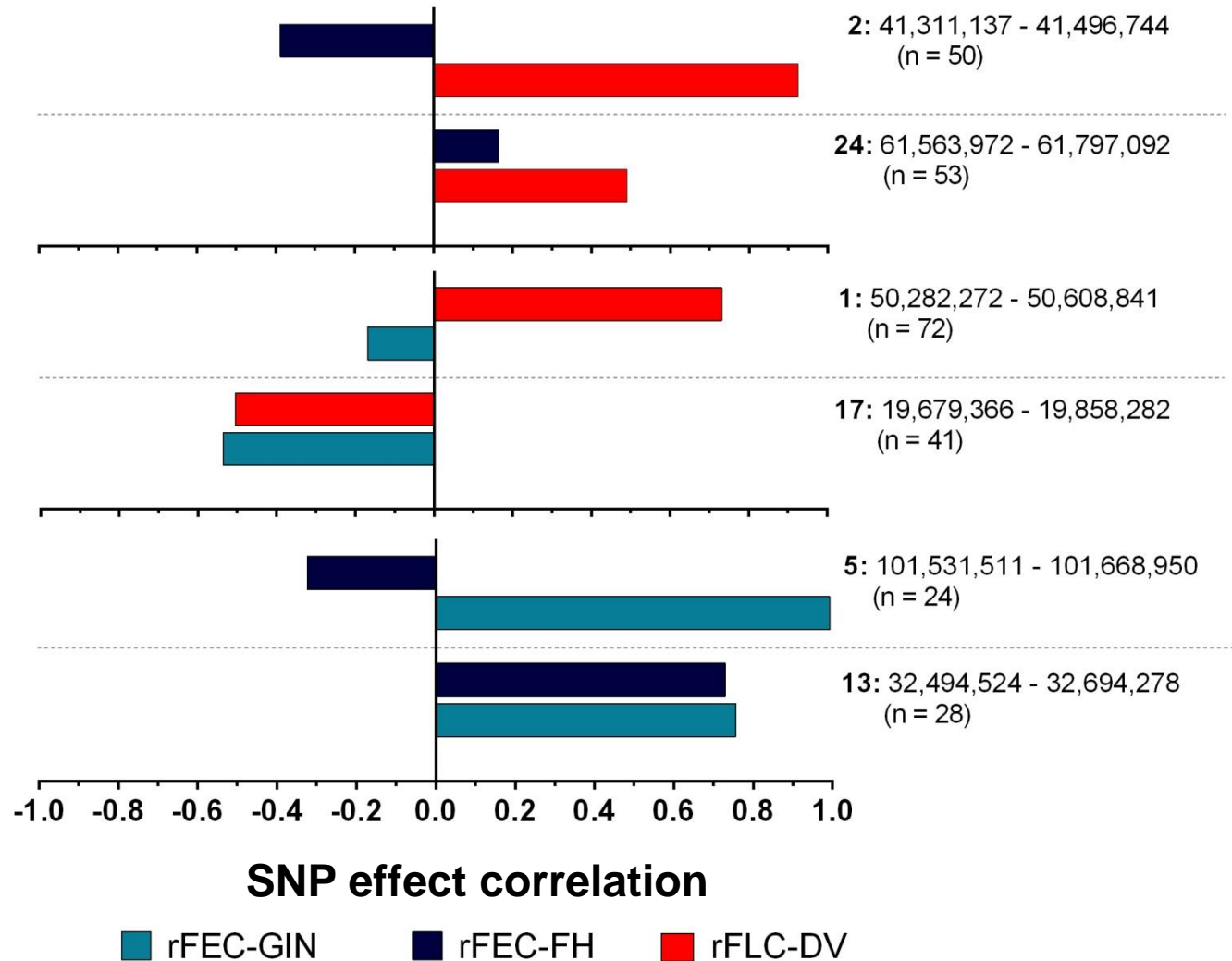
ROI für GIN



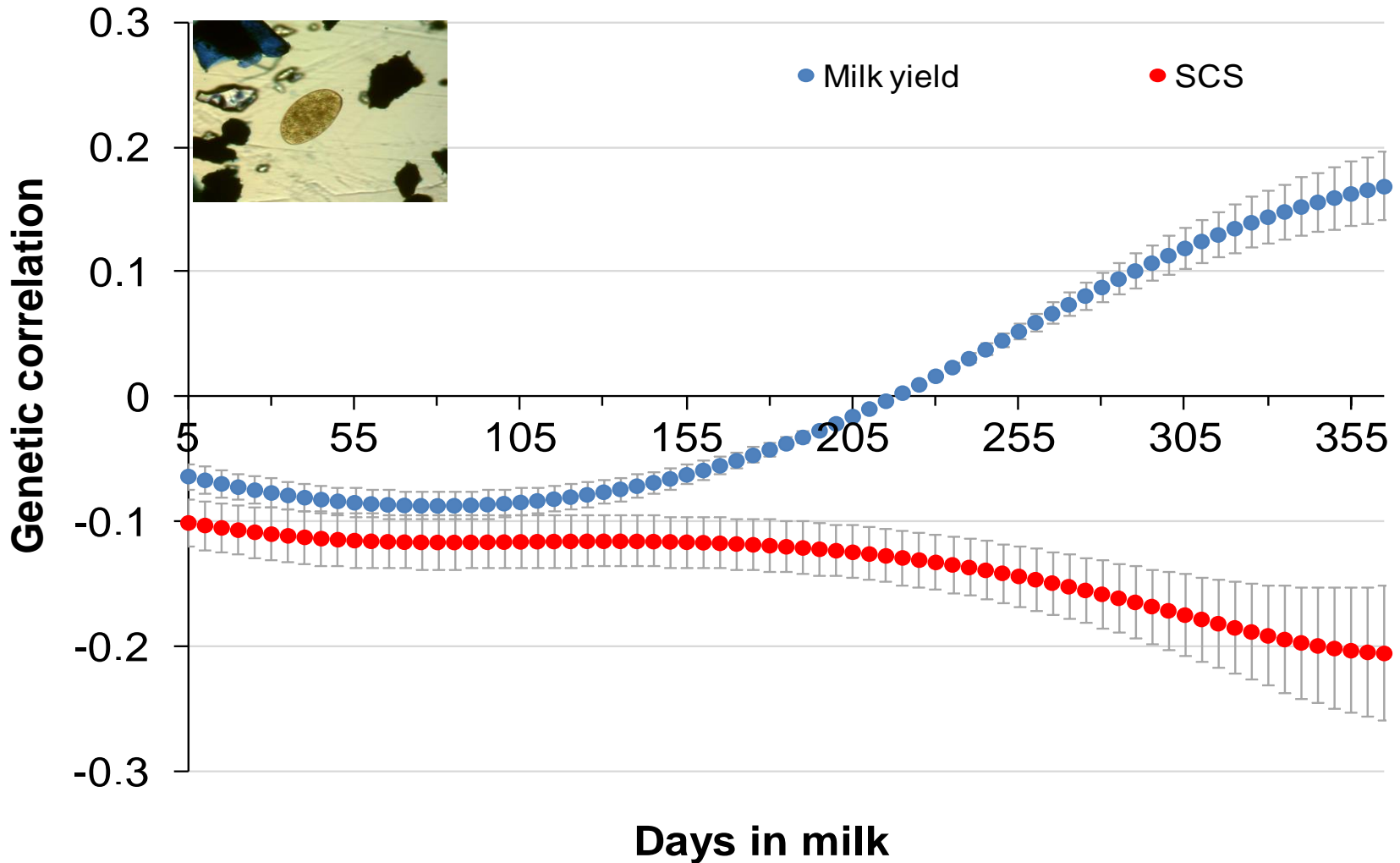
ROI for liver flukes



ROI for lungworms



# ... and between endoparasite and test-day traits



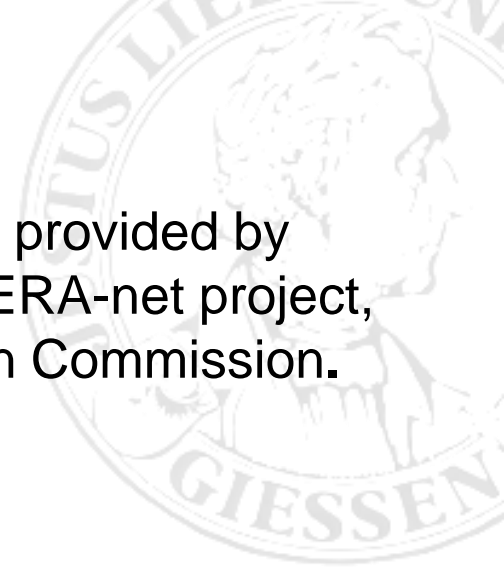
# Conclusions

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- The **2-step approach** using pre-corrected phenotype data based on a larger dataset is a **valid strategy for** a small number of phenotypes from **small-sized cattle populations**
- **53 potential candidate genes** were related to **endoparasite resistance**
- **7 pathways** associated with **immune response mechanisms** to endoparasite infections or involved in host-pathogen interactions for candidate genes located on BTA 1, 2, 6, 13, 22, 24 and 28
- **Joint genetic basis** for the two nematodes **GIN** and ***D. viviparus*** (high correlations on genetic and genomic scales, same genes (**NAV3**) and pathways)
- **Negative correlations** between **GIN** or ***F. hepatica*** and **SCS** within most genomic regions



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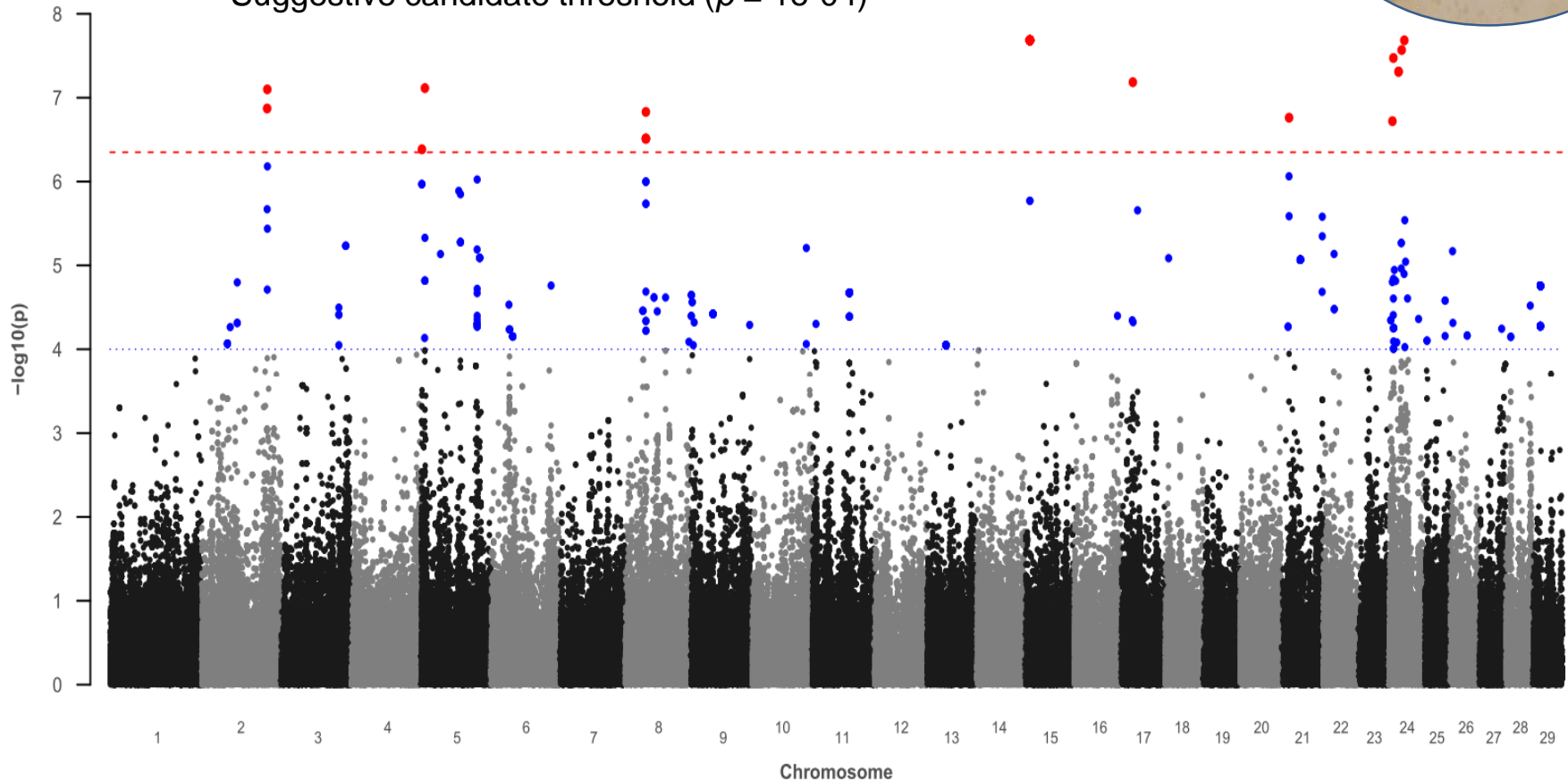


We gratefully thank the H. Wilhelm Schaumann Stiftung for the provision of a PhD grant

# Manhattan-Plot for bovine lungworms

- - Bonferroni-corrected genome-wide significance threshold ( $p = 4.47 \times 10^{-7}$ )

••• Suggestive candidate threshold ( $p = 1e-04$ )



- 41 variants reached  $p_{\text{Bonf}}$  and 311 variants reached  $p_{\text{Cand}}$

- 35 candidate genes

# Genotyping and Imputing

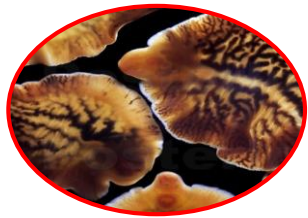
- Genotyped with BovineSNP50 Bead Chip (50k SNP chip)
- Imputing to Illumina HD Bead Chip level (i.e. 700,000 SNPs) using a multi-breed reference panel of 2188 animals

- The reference panel comprised:

- 48 DSN animals genotyped with the Illumina HD Bead Chip array (Illumina Inc., San Diego, CA, USA)
- 2140 sequenced animals from the 1000 bull genome project database (Daetwyler et al., 2014) downscaled to Illumina HD Bead Chip density including 30 sequenced DSN animals

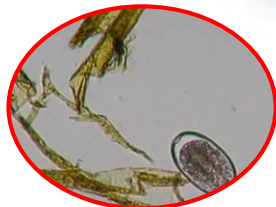
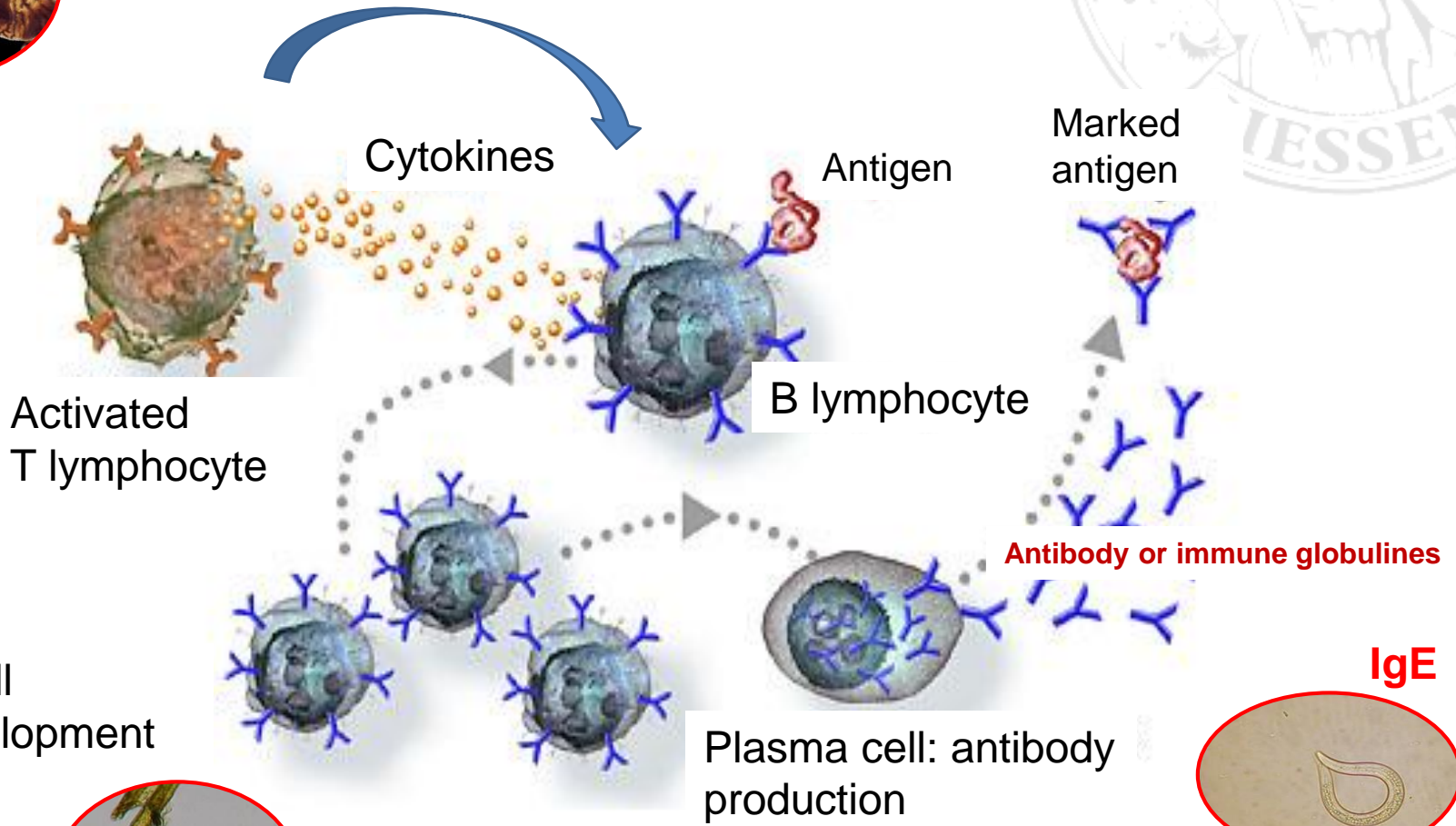


# Immune response mechanisms during endoparasite infections

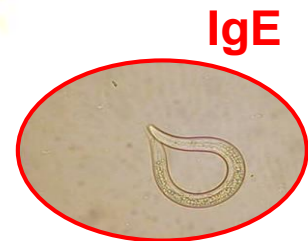


**IgG1, IL4, IL10**

Interleukin

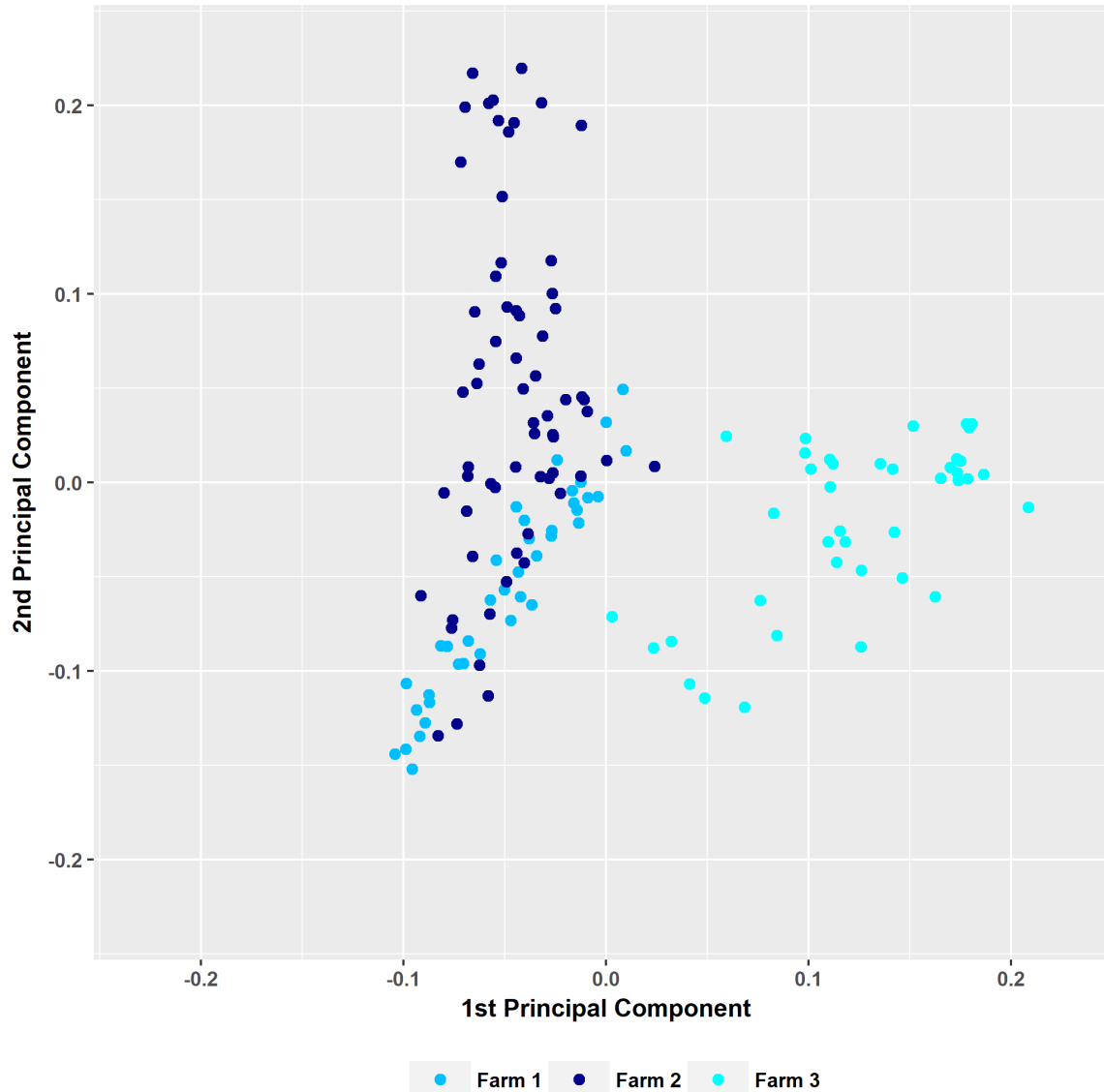


**IgA, IgG, IgM**

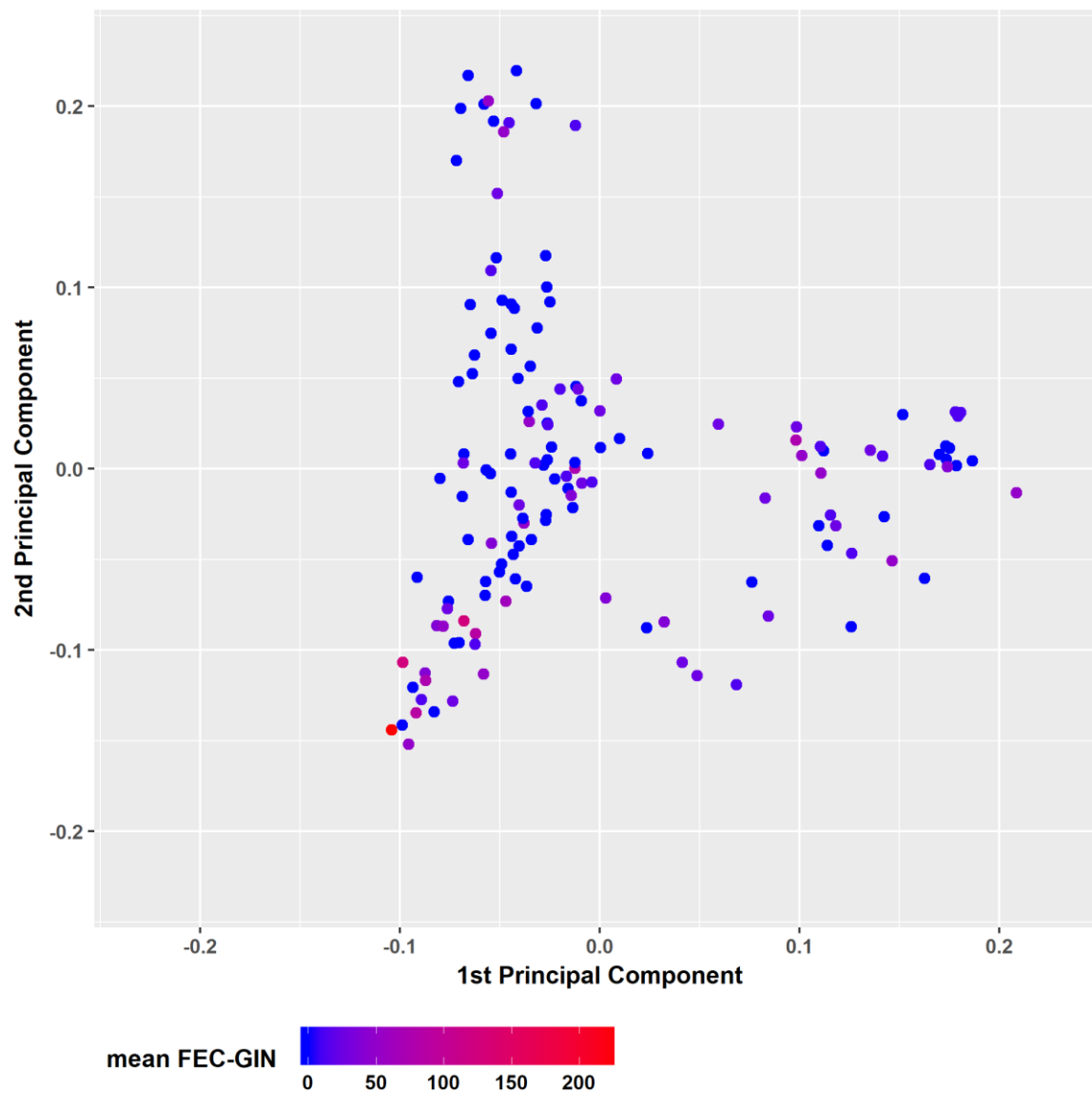


**IgE**

# PCA for the three herds



# PCA plotted by GIN



# PCA plotted by liver flukes (*F. hepatica*)

