



Sustainable Solutions for Small Ruminants

Genome-wide association analysis of resistance to paratuberculosis and mastitis in dairy sheep

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Agris

Agenzia regionale
per la ricerca in agricoltura



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Introduction

Mastitis

- Major health problem in dairy ruminants:
 - Lactation incidence of clinical Mastitis (5%)
 - Prevalence of subclinical mastitis [10 - 50%] } sheep
- Economic losses
 - Involuntary culling
 - Veterinary treatments
- Inflammation of the udder mainly caused by bacterial infection
 - (*S. aureus*; SCN)
- Dramatic increase in milk somatic cell count (SCC) due to the massive recruitment of neutrophils in the udder
- SCC has been widely considered an indicator of udder health (Rupp et al., 2003, Riggio, 2012)
 - used to detect sub-clinical mastitis in cattle and sheep
 - as selection criterion for resistance to mastitis in the French Lacaune breeding scheme (Rupp et al., 2002)

Introduction

Paratuberculosis (Johne's disease)



A sheep showing signs of OJD.

- Granulomatous enteritis of ruminants
 - Chronic weight loss, which may occur from 2 years of age, with most animals succumbing to disease at 3-5 years of age
 - Prevalence : 2-32% in different countries
- Causative agent: *Mycobacterium avium subsp. paratuberculosis* (**MAP**)
- Economic losses
 - Reduction on milk production
 - Weight losses
 - Costs for diagnostic test and control programs
- Possible influence on human health
 - Crohn's disease (multiple sclerosis,& Type-1 diabetes)

Introduction

Paratuberculosis Control strategies:

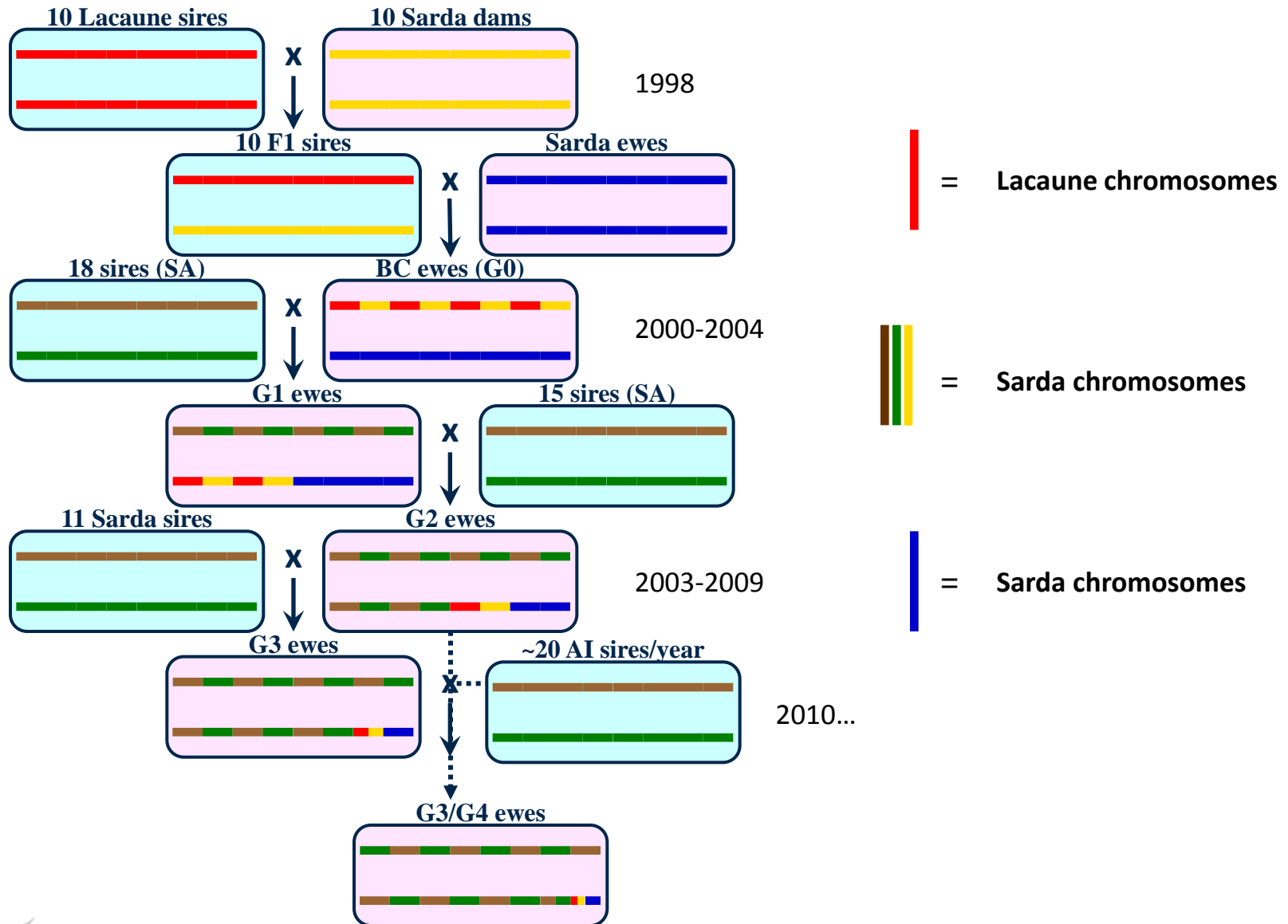
- No treatments
- Flock management & Removal of infected animals
- Vaccination
 - infected animals continue to shed Map in the environment
- Selection for resistance
 - Possible (h^2 in cattle E[0.06-0.10]; in sheep E[0.11-0.30] in this experimental population)
 - Difficult accurate diagnosis

Aim

To Detect QTN affecting resistance to paratuberculosis and mastitis in dairy sheep by using the Ovine SNP50K BeadChip



Experimental population



Somatic Cell Count (SCC) measurements

- Milk samples bimonthly collected at a.m. and p.m. milking
- **SCC** (cells/ml) measured by a Fossomatic Cell Counter
- Log-transformation of a.m and p.m. SCC to obtain Somatic Cell Score:
 - **SCS** = $\text{Log}_2(\text{SCC}[\text{cells/ml}]/100)+3$
- Daily Somatic Cell Score (**SCSd**) = Arithmetic mean of a.m & p.m SCS
- Only lactations with more than 3 SCSd
- Lactation Somatic Cell Score (**LSCS**) = arithmetic mean of SCSd

Generation	# animals with measurements	SCCd		
		n	Mean	std
G0	928	36,527	643.8	1751.3
G1	762	19,419	670.7	1890.9
G2	769	23,461	772.1	1896.3
tot	2,459	79,407	688.3	1830.5

Available LSCS records

GEN	n	Mean	std	n for QTN detection
G0	3,277	4.06	1.34	903
G1	2,488	4.07	1.27	749
G2	2,611	4.29	1.25	702
Tot	8,376	4.09	1.29	2354

$$\text{Phenotypes for QTN detection} = \underline{a_i} + \underline{\sum e_{ij}} / n$$

Phenotypes adjusted for generation-specific environmental effects

- **Repeatability model** : $a \sim N(0, I * \sigma_a^2)$; $e \sim N(0, I * \sigma_e^2)$
- **Environmental effects** : n. lambs born; production year; **group of management**; **age at lambing**; lambing month; parity age class;

Paratuberculosis resistance measurements



Endemically infected flock

All ewes slaughtered 5 years of age

Since 2000:

- DEAD: Clinical signs and deaths of PTB (n=273)
- HISTO: Histopathological exam of specimen from mesenteric lymph nodes and ileum and Ziehl-Nielsen staining (n=1,861)
 - Available at slaughtering or death
- SERO: ELISA test to measure the **optical density values (OD)** of the MAP antibodies in serum (12,033)
 - once/year for the G0, twice/year for the G1 and G2

PTB resistance: which phenotype?

Healthy/Sick

- Problems in defining the MAP infectious status
 - SERO: multiple measures, not always concordant :
 - ~ 10 % animals* SERO⁻ at the last 2 samplings had at least 1 SERO⁺ result during the career
 - No full agreement among different diagnostic tests:
 - ~ **13%** always SERO⁻ animals* showed lesions or clinical sings

* at least 4 Elisa tests available

PTB resistance: analyzed variables

Sample-to-positive ratio (S/P) :

$$\text{S/P} = \frac{(\text{OD of sample} - \text{mean of OD of negative controls})}{(\text{mean of OD of positive controls} - \text{mean of OD of negative controls})}$$

SERO

$$\text{S/P}_{\text{mean}}: \sum(\text{S/P})/n, n > 1$$

Histology

$$\text{HS:} \begin{cases} = 1, \text{ if HISTO}^+ \text{ or DEAD}^+ \\ = 0, \text{ if HISTO}^- \text{ and DEAD}^- \end{cases}$$

Combined

$$\text{C:} \begin{cases} = 100\%, \text{ if HISTO}^+ \text{ or DEAD}^+ \text{ or last 2 Elisa Tests}^+ \\ \quad (\text{S/P} > 70) \\ = \% \text{ Elisa tests } ^+, \text{ otherwise} \end{cases}$$

PTB resistance variables

GEN	HS						S/Pmean	
	n	-	<i>HISTO+</i>	<i>DEAD+</i>	+	Prev	n	Mean
G0	779	523	118	138	256	0.33	727	28.6
G1	593	453	73	67	140	0.24	667	38.2
G2	670	510	92	68	160	0.24	780	31.7
Tot	2042	1486	283	273	556	0.27	2174	32.7

Phenotypes for QTN detection = **EBV+residual**

Fixed effect: year of birth

h^2 :

HS=0.116

S/Pmean=0.30

C=0.133



Genotypes

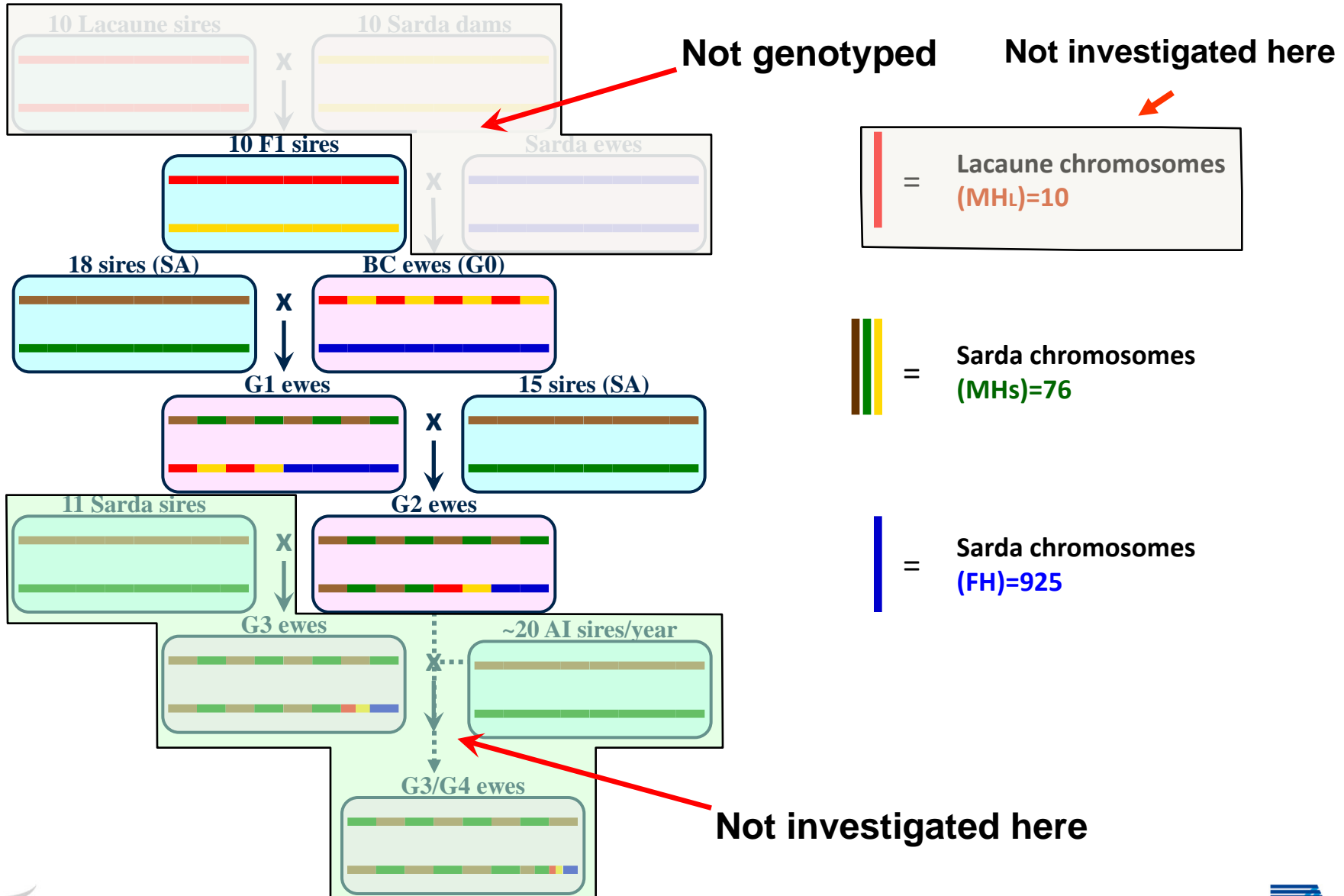
Ovine SNP50 BeadChip → 54,241 SNPs

- SNPs not located on the 26 autosomes
- SNPs with call rate < 0.95
- SNPs with MAF < 0.05

= 44,859 SNPs



Experimental population



LD-LA MODEL

Multiple regression of the offspring performances on probabilities of inheriting linear combinations of male founder haplotypes (PCA from IBD matrix)

IBDp between Sarda founder (76) haplotypes (hap=41 SNP)

IBDp Sarda vs Lacaune =0

PCA' IBD matrix (Pc > 0.90 of variance & eigenvalue>1)

Model: $y = 1\mu + As + XV\beta + e$

y = vector of phenotypes; μ = overall mean; s = vector of the fixed sire effects;

β = vector of the fixed effects of selected linear combinations of MH $\beta = \begin{bmatrix} \beta_L \\ \beta_S \end{bmatrix}$

e = vector of random residuals;

A = incidence matrix relating phenotypes with sires (relationship coeff.);

X = incidence matrix allocating transmission probabilities MH -> OH ;

V = eigenvectors relating MH with selected linear combinations $V = \begin{bmatrix} V_L & 0 \\ 0 & V_S \end{bmatrix}$



Test and Significance threshold

F Test (at each SNP position):

Genomic $H_0 \rightarrow \sigma^2 = 0$

Sarda $H_0 \rightarrow \sigma_s^2 = 0$

Lacaune $H_0 \rightarrow \sigma_L^2 = 0$

Significance

- H_0 chromosome wise (CW) and genome wise (GW) maximum test distribution
- 10,000 permutations within sire family
- Random deviates from family effect

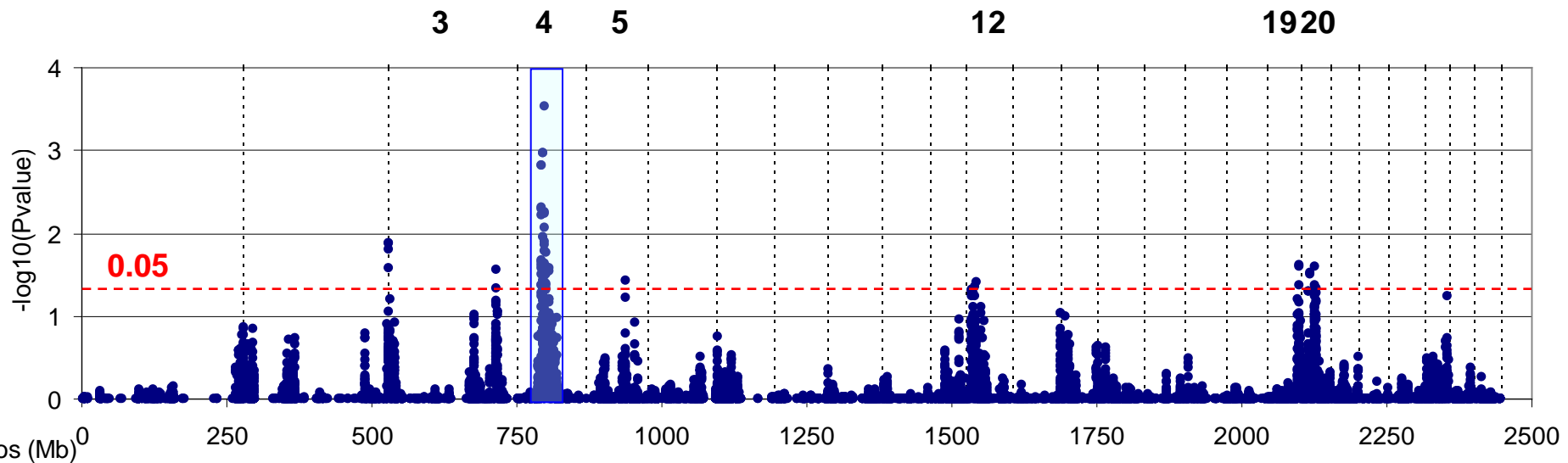
Results



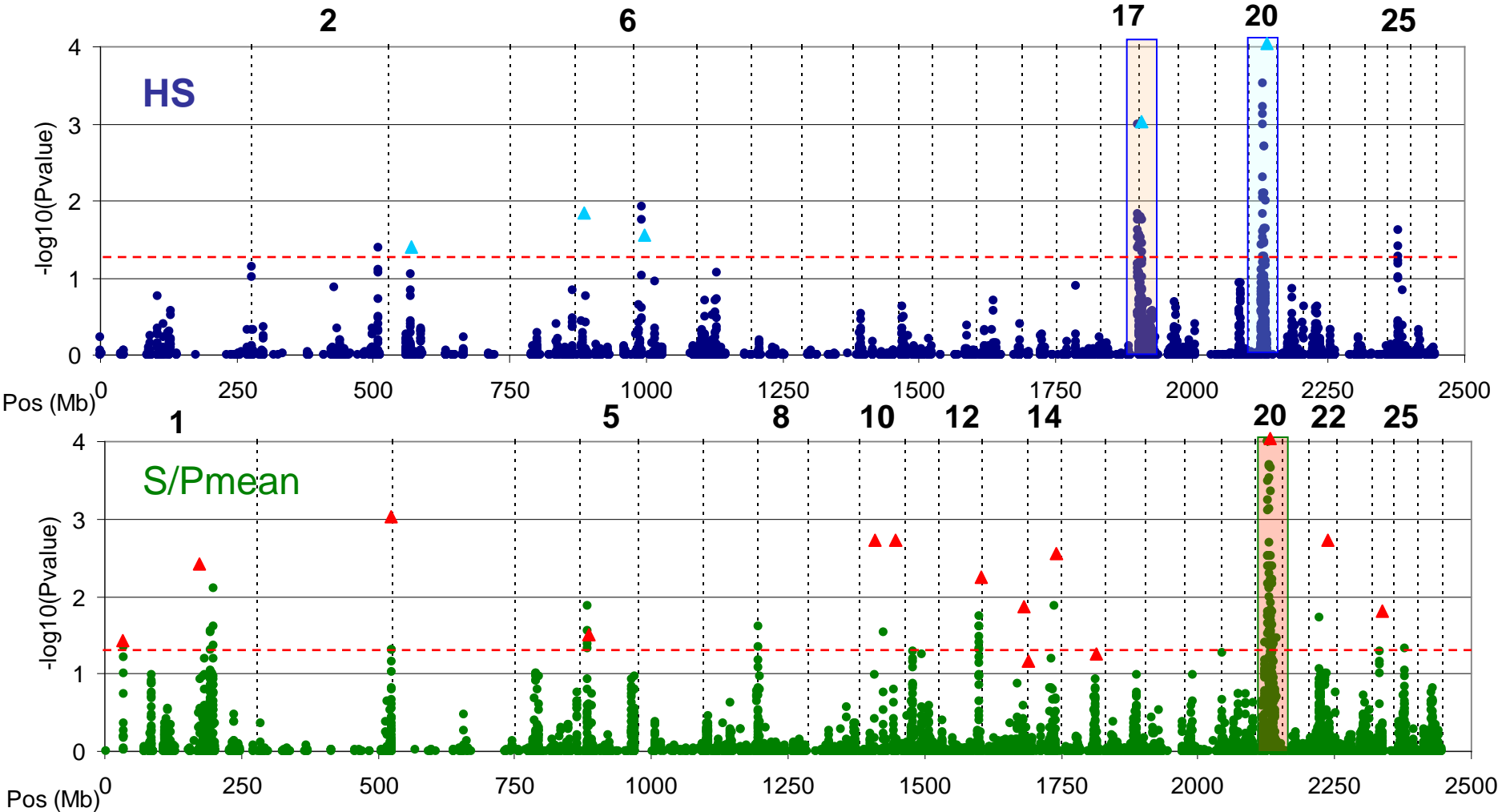
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Genomic regions associated with LSCS



Genomic regions associated with PTB resistance



Combined variable

Bin3 = positive/negative ELISA result at 3 years of age



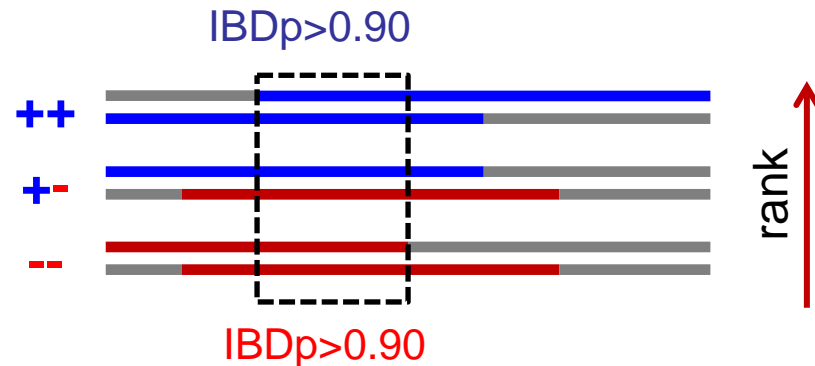
Resequencing



The most significant regions were further investigated by whole genome resequencing of **trios of animals** in which the QTNs are expected to be segregating

$$\text{Max} (\alpha_{H+} - \alpha_{H-})$$

$$\text{IBD}_p (H+, H-) = 0.00$$



Trait	OAR	Loc. (Mb)	P-value	Contrast (s.d.u)	Segment length (bp)	SNP ++; +-; --
Bin3	20	24.93	<0.0001	0.834	1,176,499	2,561
S/Pmean	20	24.93	<0.0001	0.968	1,176,499	2,561
LSCS	4	47.74	0.0003	0.498	584,716	1,351



Conclusions

- The performed analysis allowed to detect:
 - 1 region close to 5%GW significance associated with LSCS on OAR4
 - 1 region on OAR20 significantly associated (5%GW) with different phenotypes related to Paratuberculosis resistance
- Functional role of identified SNPs is under investigation
- Estimation of haplotype frequency of regions of interest is ongoing in the whole registered population

Differences in classifying the infectious status of animals for traits that lack definitive diagnostic criteria, particularly in subclinical stages, as PTBC as well as subclinical mastitis, can affect GWA analysis results



THANKS FOR YOUR ATTENTION!

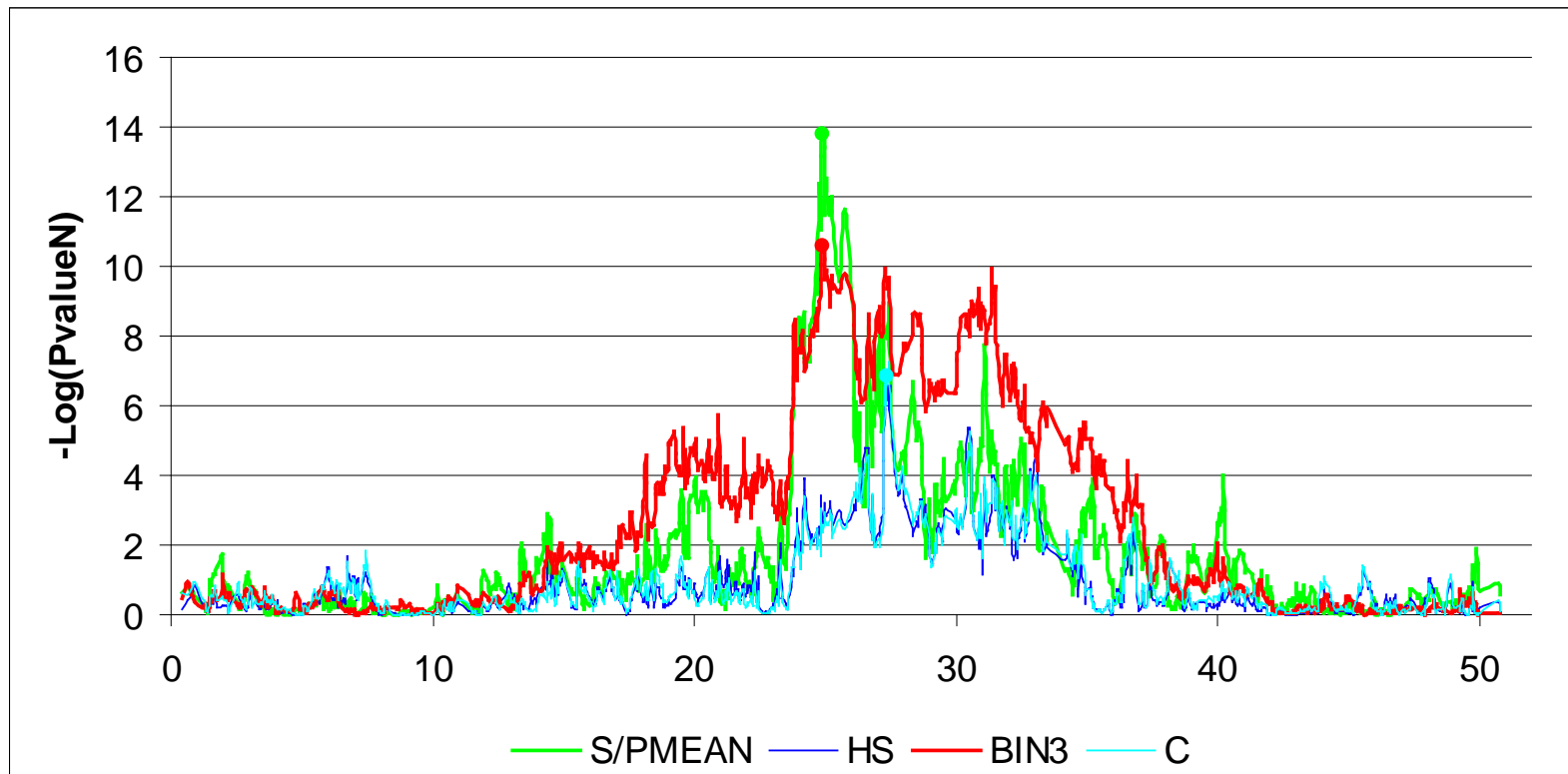




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OAR20 regions associated with PTB resistance



Ovine (Texel) version 3.1 Genome Assembly: Mappa di 1.176 Mbp da OAR20, posizione 24,389,449 - 25,565,948

Browser [Select Tracks](#) [Snapshots](#) [Custom Tracks](#) [Preferences](#)

Cerca

Elemento Genomico o Regione:

OAR20:24,389,449..25,565,948

Cerca

Save Snapshot

Load Snapshot

Esempi: [OAR1:50000000..50900000](#), [OAR10_3723681](#).

Origine dei dati

Ovine (Texel) version 3.1 Genome Assembly

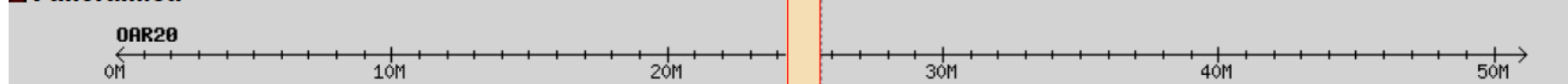
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Mostra 1.177 Mbp



>> Gira

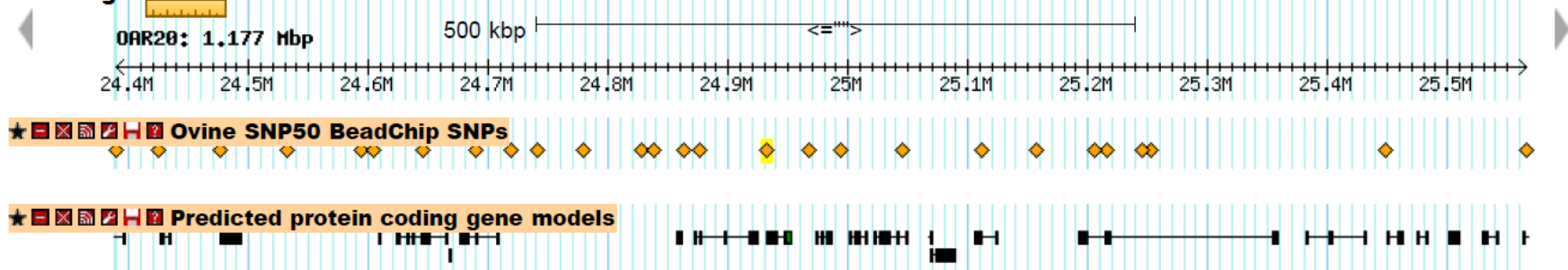
Panoramica



Region



Dettagli



Ovine (Texel) version 3.1 Genome Assembly: Mappa di 584.7 kbp da OAR4, posizione 47,204,156 - 47,788,872

Browser [Select Tracks](#) [Snapshots](#) [Custom Tracks](#) [Preferences](#)

Cerca

Elemento Genomico o Regione:

OAR4:47,204,156..47,788,872

Cerca

Save Snapshot

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Esempi: [OAR1:50000000..50900000](#), [OAR10_3723681](#).

Origine dei dati

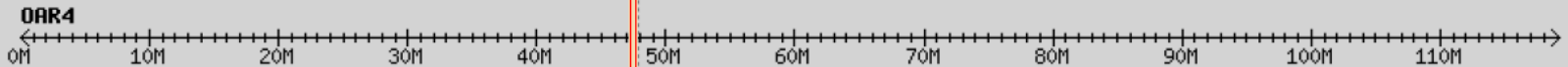
Ovine (Texel) version 3.1 Genome Assembly

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Mostra 584.7 kbp

Gira

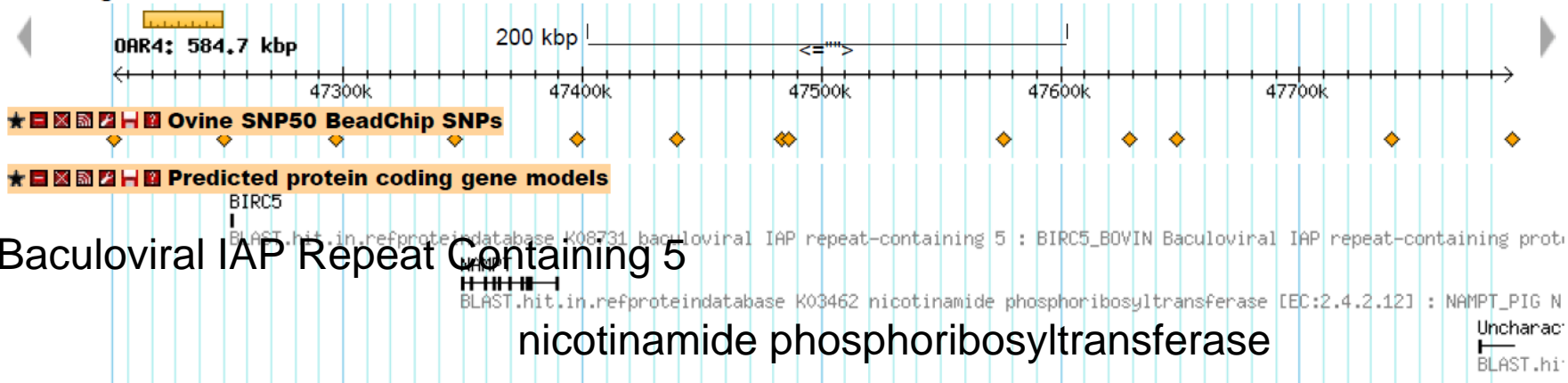
Panoramica



Region



Dettagli



Baculoviral IAP Repeat Containing 5

