

EADGENE European Animal Disease Genomics Network of Excellence for Animal Health and Food Safety

Genomics for Animal Health: Outlook for the Future
13- 14th October 2009, Muséum National d'Histoire Naturelle, Paris, France

Gene expression profiling in divergent lines of sheep for enhanced insight into genetic resistance to mastitis

Rachel Rupp

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Background

- **Mastitis** = infection of the mammary gland caused by bacteria
- Health problem of **major economic importance** in dairy ruminants
- Evidence of **genetic control** of mastitis
Direct measure (clinical mastitis) or indirect predictor (milk somatic cell counts) are heritable
Numerous QTL for SCC have been reported
- Breeding programs based on SCC implemented worldwide
- Genes and mechanisms of genetic control still unknown

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Divergent selection of sheep

Study genes and mechanisms underlying genetic resistance to mastitis using a divergent selection experiment in sheep based on SCC.

Apply multidisciplinary research, including transcriptomic profiling, on a genetic animal model developed in an INRA experimental unit

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Divergent selection of sheep

Population (Lacaune breed)

Progeny-tested rams (SCS EBV) ~ 2*6/year

INRA Experimental unit (La Fage, Roquefort)

since 2003, First lactations since 2005

High SCS line (susceptible) n = 2*200
Low SCS line (resistant) 3σs

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Resistance to natural infection is enhanced in Low SCC lines, compared to High SCC lines

Trait	High SCS line		Low SCS line	
	N	mean	N	mean
SCS (se) , Lsmears (236 L1; 2005-2008)	750	4.02(0.3)	755	2.42 (0.2)
Clinical mastitis (384 lactations; 2005-2008)		18 cases (9.8%)		5 cases (2.4%)
Mammary abscesses (% affected ewes; 2005-2008)	283	38%		7%
Milk Bacteriological Examination (% positives)	586	45%	536	24%

OR=3.1[1.9;5.2]

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Shorter duration of infection in Low SCS line, compared to high SCS line

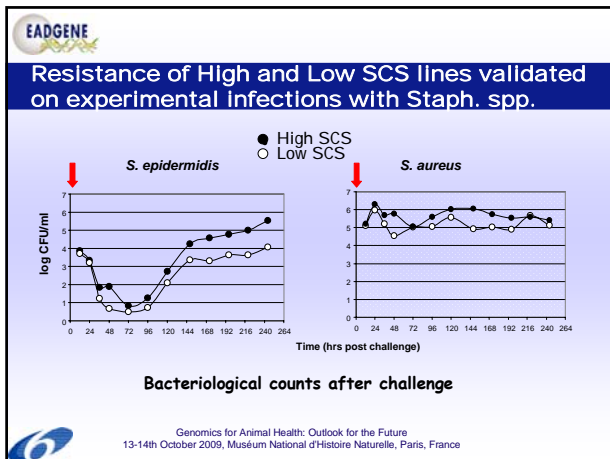
Estimated probability

Sampling time point

Bacteriological negativation rate

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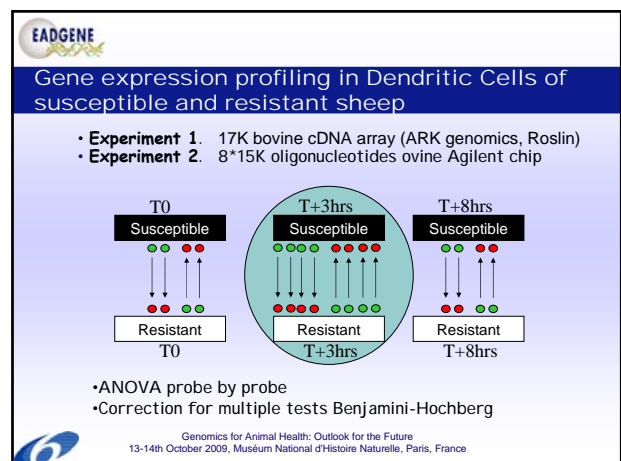
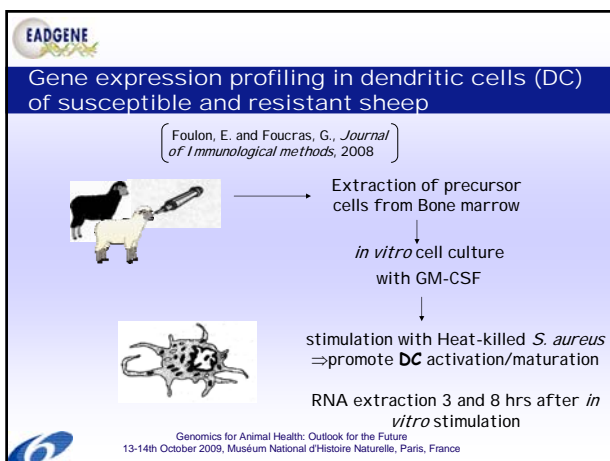
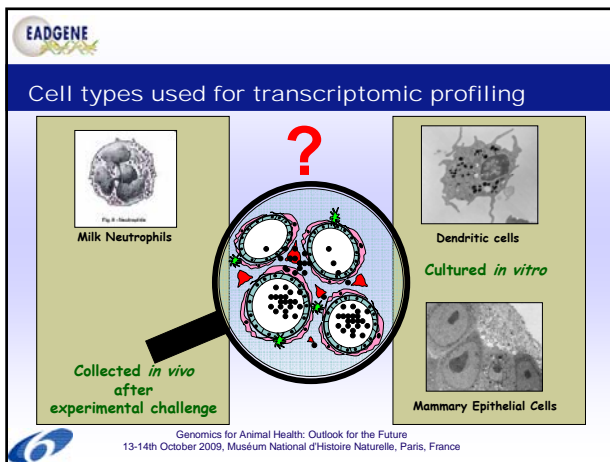


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Gene expression profiling studies

- To compare transcriptomic profiles in resistant and susceptible sheep
- To characterize transcriptomic profiles during pathogen-specific responses
- Using
 - Bovine cDNA microarray (20K), ARK genomics
 - Ovine oligo array (15k), Agilent

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Gene expression profiling in Dendritic Cells of susceptible and resistant sheep

Differentially expressed genes between resistant and susceptible sheep:

- EXp 1 (bovine cDNA array), BH5%
 - IL1 α ; S1003A : unknown protein
- EXp 2 (ovine oligo array), BH5%
 - 14 gènes
- 6 genes in common including TLR2 and IL6

« Pattern recognition receptors in recognition of bacteria and viruses (IPA canonical pathway) »

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Gene expression profiling in Dendritic Cells of susceptible and resistant sheep

■ Susceptible
 □ Resistant

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Collection of milk Neutrophils after challenge of susceptible and resistant sheep

Milk neutrophils

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Collection of milk Neutrophils after challenge of susceptible and resistant sheep

6 resistant (SCS-) / 30g / 6 susceptible (SCS+)

Lambing 1: *Staph. epidermidis* 10³ CFU
 Antibiotic
 +12 hrs
 Collection of milk cells (neutrophils)

Lambing 2: *Staph. aureus* 10³ CFU
 +12 hrs
 Collection of milk cells (neutrophils)

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Gene expression profiling in milk Neutrophils of susceptible and resistant sheep

- 8*15K oligonucleotides ovine Agilent chip
- 22 microarrays / 2-colour dye switch design (Cy3-Cy5)

•Editing and Normalization \Rightarrow 9327 probes
 •ANOVA probe by probe
 •Correction for multiple tests Benjamini-Hochberg

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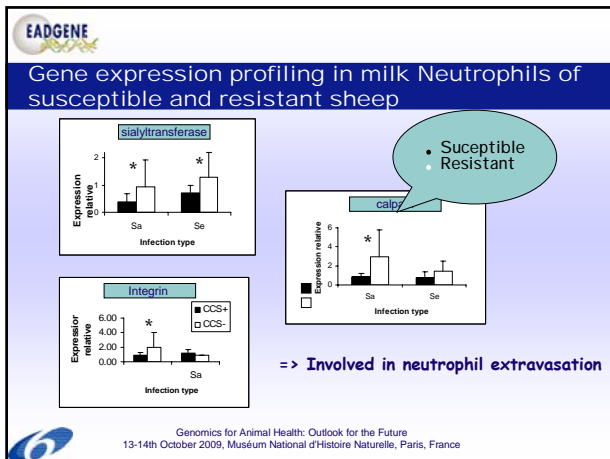
Gene expression profiling in milk Neutrophils of susceptible and resistant sheep

- 15 differentially expressed genes in neutrophils
- 12 grouped in a common Network
- Positional candidates (QTL)

Over-expressed
 ■ resistant
 ■ susceptible

Ingenuity Pathway Analysis, version 7.5

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Prospects

- 1- Deciphering response to mastitis : « EADGENE »
 - Various Cell types/tissue
 - Divergent lines in sheep and goat
 - Species: Cattle, Sheep, Goat
- 2 - QTL detection using high-throughput SNP chips
 - Sheep : « SheepSNPQTL »
 - Cattle : « CARTOFINE »
- 3 - Fine mapping and trait dissection: EU project «3SR»

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