Session 29

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Bovine lactoferrin gene polymorphism and its association with prevalence of sub-clinical mastitis caused by *Staphylococcus* species

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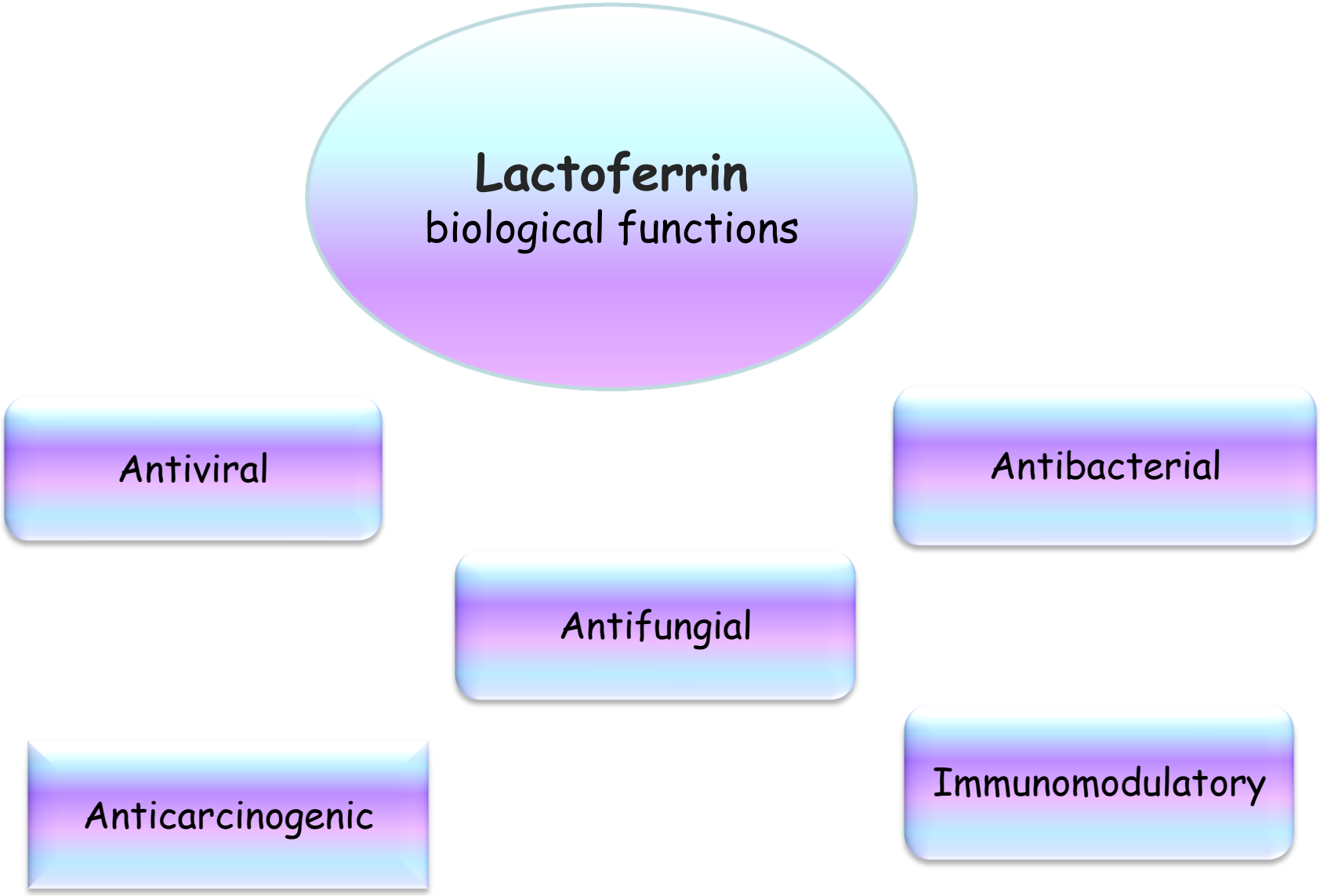
Presentation outline

In this presentation…

- Review on lactoferrin gene polymorphism and mastitis
- Methods (RFLP – PCR and bacteriological examination of milk)
- Results
- Conclusions


Lactoferrin
biological functions
Antiviral
Antibacterial
Antifungial
Anticarcinogenic
Immunomodulatory

Antibacterial activity

Iron dependent

Iron independent

Lactoferrin content in milk

Lactoferrin concentration in milk

0.1mg/ml

Dramatic increase:

- in colostrum
- mammary gland secretion occurring during involution
- in milk of animals suffering from mammary gland inflammation

Lactoferrin content in milk

Milk from quarters, in which mastitis pathogens
can be observed,
contains more lactoferrin than from uninfected ones.

Protein concentration is to some extent pathogen specific

Bovine lactoferrin gene

Bovine chromosome 22
17 exons
34.5 kbp genomic DNA

Lactoferrin gene polymorphism occurs in coding and regulatory regions as well as in introns

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Bovine lactoferrin gene

- Several studies have investigated connection between lactoferrin gene polymorphism and mastitis susceptibility (SEYFERT et al (1994, 1996), LI et al (2004) KAMIŃSKI et al (2006), (WOJDAK – MAKSYMIEC et al. (2006), SENDER et al.(2006).
- Association between lactoferrin gene polymorphism occurring in intron 6 and somatic cell count has been found (WOJDAK – MAKSYMIEC et al. (2006), SENDER et al.(2006)
- These studies focused only on the phenotypic value of the somatic cell count as the indicator of mastitis and did not consider pathogens which cause inflammation

Objective of the study

The objective this study was to verify hypothesis that polymorphism, occurring in intron 6 of bovine lactoferrin gene is associated with prevalence of sub-clinical mastitis caused by Staphylococcus species (Staphylococcus aureus and CNS) in Polish dairy cattle.

Material and methods

479

Polish Black – and – White Holstein cows

in two experimental herds

(303 and 176 cows)

two years

(2004 and 2005)

Material and methods

Bacteriological status of the mammary gland

Collecting and culturing duplicate composite milk samples.

680 milk samples from 216 cows in two herds

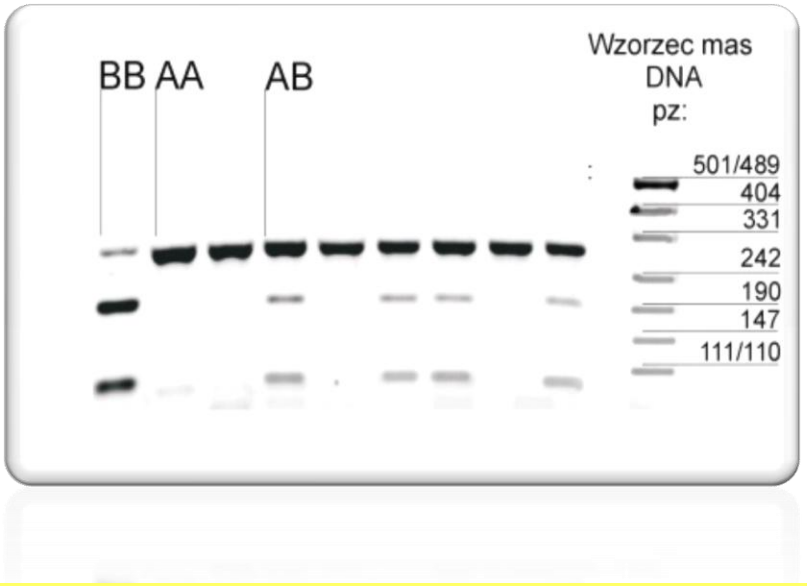
338 samples from 109 cows in first heard

342 samples from 107 cows in second herd

The bacteriological examination of composite milk samples and the diagnosis of sub-clinical *mastitis* were performed according to the methods recommended by IDF

PCR – RLFP method

- Reaction was performed with the use of touch down protocol from 70°C to 60°C (for 20 cycles)
- A PCR product was digested with restriction enzyme *EcoRI*
- Allele frequency in the studied population was verified with χ2 test in order to observe whether it remained in accordance with the Hardy – Weinberg Equilibrium



BB –201 and 100 bp
AA – 301
AB – 301, 201 and 100 bp

Statistical methods

Analysis of variance (GLM procedure of SAS) was used to evaluate the prevalence of subclinical mastitis caused by different bacterial species. (pooled, *S. aureus*, CNS, *Str. dysgalactiae)*.

The statistical model was accounted for:

- lactoferrin genotype
- herd
- interaction between animal genotypes and herd
- repetition
- season of examination

Results

Allele	Frequency	X^2	df	Probability observed	Probability expected
A	0.7923	0.4190	1	0.5175	0.4931
B	0.2077				

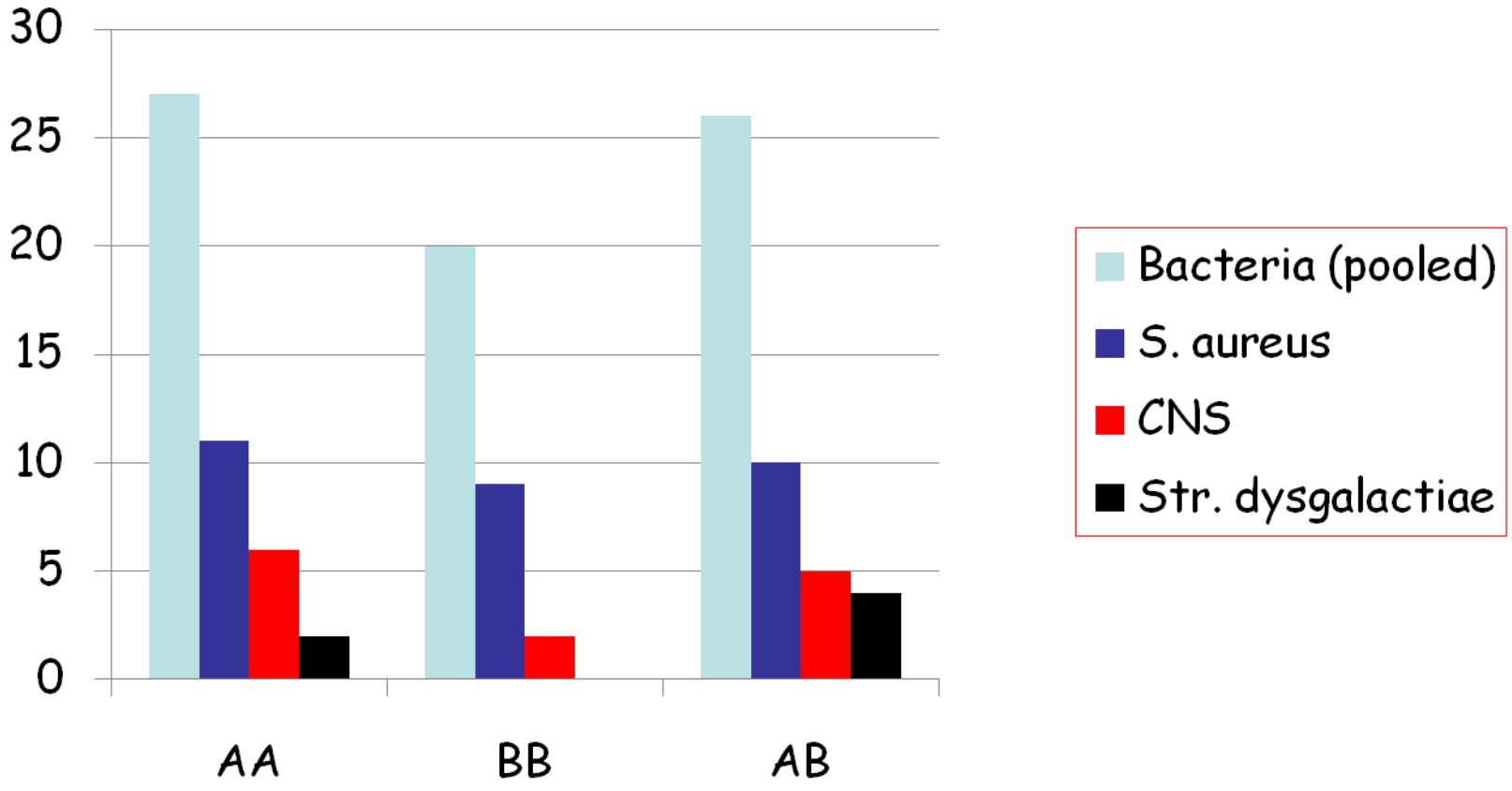
Allele frequency and testing for Hardy– Weinberg Equilibrium in studied population

Genotype	No. of cows	Frequency
AA	303	0.6326
BB	23	0.0480
AB	153	0.3194

Frequency of lactoferrin genotypes in studied population

Results

Lactoferrin genotypes effect on the prevalence of sub-clinical mastitis caused by different bacterial species



Conclusions

A significant association between lactoferrin genotype and mastitis caused by Staphylococcus species was not found mostly because a low number of animals, especially BB homozygote.
The results of this study with regard to bacteriology are presented as preliminary.

Conclusions

Probably observed polymorphism in intron 6 was not causal but linked to other polymorphism in lactoferrin loci involved in mastitis resistance.

Looking for a candidate gene for mastitis resistance lactoferrin gene polymorphism occurring in coding and regulatory regions of this gene should also be examined in relation to mastitis.