



NAS:
PREVALENTIE EN IMPACT OP UIERGEZONDHEID EN
MELKPRODUCTIE

Uiergezondheidspanel 9/10/2019
Leuvenum

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M-team^{UGent} and Mastitis and Milk Quality Research Unit
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MISSIE

“GEDREVEN DOOR PASSIE, GEÏNSPIREERD DOOR WETENSCHAP
EN GESTERKT DOOR ERVARING MASTITIS MANAGEMENT
GEMAKKELIJK MAKEN VOOR RUNDVEEDIERENARTSEN EN
MELKVEE-ADVISEURS MET EEN INTERESSE IN
UIERGEZONDHEID.”

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Driven by **passion**, inspired by **science** and
strengthened by **experience**

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NON-AUREUS STAPHYLOCOCCI

General information

- Heterogenous group
 - >50 species and subspecies¹
 - >10 species isolated from bovine milk²
- As a group still considered as harmless teat skin flora opportunists to mildly pathogenic organisms

¹ Plessens et al., 2011
² Vanderhaeghen et al., 2014

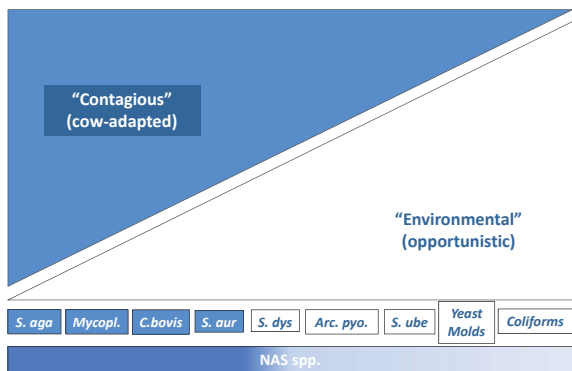


DIFFERENT CLASSIFICATIONS

- Based on the **structure of their cell wall**:
 - Gram-positive *versus*
 - Gram-negative *versus*
 - Pathogens without cell wall.
- Based on their **epidemiology**:
 - Contagious ("cow/udder adapted") *versus*
 - Environmental ("opportunistic") pathogens
- Based on their **virulence characteristics**:
 - Major *versus*
 - Minor pathogens



CLASSIFICATION | EPIDEMIOLOGY



NON-AUREUS STAPHYLOCOCCI

Source

- Some NAS species: part of the normal teat skin/apex flora -> adapted to the cow/udder – *S. chromogenes* and *S. epidermidis*
- Other NAS: environment but seems to be adapted to the cow/udder -> can cause intramammary infections – *S. simulans* and *S. haemolyticus*. As a group: harmless teat skin flora opportunists to mildly pathogenic organisms
- Other NAS: environment of the cow -> only rarely cause intramammary infections – *S. saprophyticus*, *S. fleuretti*, *S. cohnii*,...



NON-AUREUS STAPHYLOCOCCI

Means of spread

- Some from cow to cow similar to *Staphylococcus aureus*
- Other infections probably originate from the environment



NON-AUREUS STAPHYLOCOCCI

Basic prevention and control measures



NON-AUREUS STAPHYLOCOCCI

Miscellaneous information

- NAS: most common bacteria isolated from milk samples obtained from well-managed dairy herds all over the world. A prevalence of 10 to 15% of the quarters is common
- Unusually high prevalence of CNS infections



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13

NON-AUREUS STAPHYLOCOCCI

Miscellaneous information

- Infections caused by NAS
 - Typically subclinical mastitis
 - SCC: x2 to x3.
 - ±10% of infections: SCC >500.000 cells/ml.

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14

J. Dairy Sci. 94:2329-2340
doi:10.3168/jds.2010-3741
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Some coagulase-negative *Staphylococcus* species affect udder health more than others

- K. Supré,^{1*} F. Haesebrouck,¹ R. N. Zadoks,² M. Vaneechoutte,³ S. Piepers,⁴ and S. De Vliegher⁵

genomic spacer PCR. The distribution of CNS causing IMI was highly herd-dependent, but overall, *Staphylococcus chromogenes*, *Staphylococcus xylosum*, *Staphylococcus colmi*, and *Staphylococcus simulans* were the most prevalent. No CNS species were found to cause clinical mastitis. The effect of the most prevalent species on the quarter milk somatic cell count (SCC) was analyzed using a linear mixed model, showing that *Staph. chromogenes*, *Staph. simulans*, and *Staph. xylosum* induced an increase in the SCC that is comparable with that of *Staphylococcus aureus*. Almost all CNS species were able to cause persistent IMI, with *Staph. chromogenes* causing the most persistent infections. In conclusion, accurate species identification cannot be ignored when studying the effect of CNS on udder health, as the effect on SCC differs between species and species distribution is herd-specific. *Staphylococcus chromogenes*, *Staph. simulans*, and *Staph. xylosum* seem to be the more important species and deserve special attention in further studies. Reasons for herd dependency and possible

- ±10% of inf

Supré et al., 2011

15

NON-AUREUS STAPHYLOCOCCI

Miscellaneous information

- Infections caused by NAS
 - Typically subclinical mastitis
 - SCC: x2 to x3.
 - ±10% of infections: SCC >500.000 cells/ml.
 - ±10% of clinical samples. Still, the possibility exists that a proportion of these clinical cases is actually caused by a different pathogen that could not be isolated.

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16

Table 1. Culture results, severity, and pathogen-specific incidence rates of clinical mastitis (IRCM) from a 1-yr survey conducted on 50 randomly selected Flemish dairy herds

Culture result	n	Cases ¹ (%)	Severity ²			IRCM ³
			Mild ⁴	Moderate	Severe	
<i>Streptococcus uberis</i>	154	18.2	62.3	33.8	3.9	1.3
<i>Escherichia coli</i>	131	15.5	45.8	36.6	17.6	1.1
<i>Staphylococcus aureus</i>	62	7.3	64.5	29.6	4.8	0.5
<i>Streptococcus dysgalactiae</i>	61	7.2	63.9	34.4	1.6	0.5
Non-aureus staphylococci	42	5.0	64.3	26.2	9.5	0.4
<i>Corynebacterium bovis</i>	25	3.0	72.0	28.0	0.0	0.2
Other coagulase-negative cocci ⁵	18	2.1	83.3	16.7	0.0	0.2
Yeast	17	2.0	58.8	23.5	17.6	0.1
Other pathogen	46	5.4	60.9	32.6	6.5	0.4
Mixed culture ⁶	35	4.1	68.6	29.0	11.4	0.3
Contaminated sample ⁶	87	10.3	76.7	19.8	3.5	0.7
Total culture positive	677	80.1	62.5	30.1	7.4	5.7
No growth	168	19.9	65.5	29.2	5.4	1.4
Total	845	100.0	63.1	29.9	7.0	7.1

¹Number of cases with the specific culture result/total number of cases.

²Mild = only clots in milk; moderate = hard quarter without general signs; severe = systemic illness.

³Quarter cases per 10,000 cow-days at risk.

⁴Besides *Streptococcus uberis*.

⁵Isolation of 2 different pathogens.

⁶Isolation of 3 or more different pathogens.

NON-AUREUS STAPHYLOCOCCI

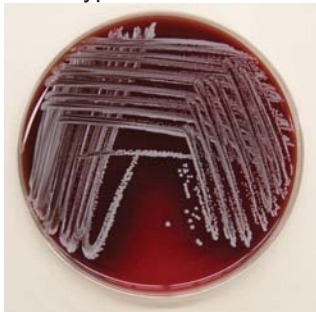
Miscellaneous information

- Infections caused by NAS
 - Typically subclinical mastitis
 - SCC: x2 to x3.
 - ±10% of infections: SCC >500.000 cells/ml.
 - ±10% of clinical samples. Still, the possibility exists that a proportion of these clinical cases is actually caused by a different pathogen that could not be isolated.
- NAS: predominant cause of intramammary infections in pregnant heifers and heifers in early lactation



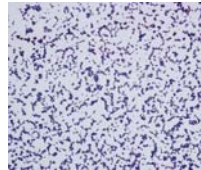
NON-AUREUS STAPHYLOCOCCI

Phenotypic characteristics



NAS on esculin-blood agar. Mostly no hemolysis.

Gram-positive cocci in pairs or irregular clusters



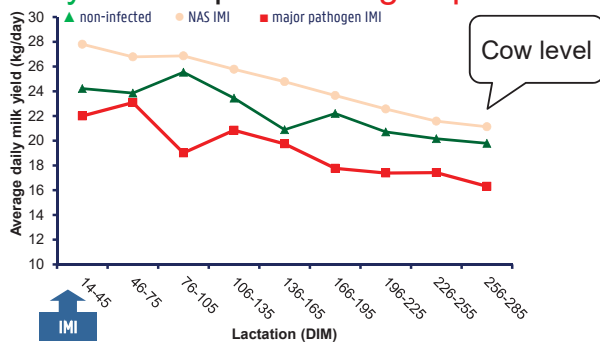
INTRODUCTION

- A high proportion of heifers freshens with an intramammary infection (IMI)¹
- Non-*aureus* staphylococci (NAS): most common mastitis pathogens in fresh heifers¹

¹ De Vliegher et al., 2012



Milk yield - Impact NAS group IMI



Piepers et al., 2010



INTRODUCTION

- NAS in early lactation yield: ↑ milk yield^{1,2}
- No data available at the quarter level



¹ Piepers et al., 2010
² Compton et al., 2007



PART 1

Effect of intramammary infection with non-aureus staphylococci in early lactation in dairy heifers on quarter somatic cell count and quarter milk yield during the first 4 months of lactation



MATERIALS & METHODS

Herds and heifers



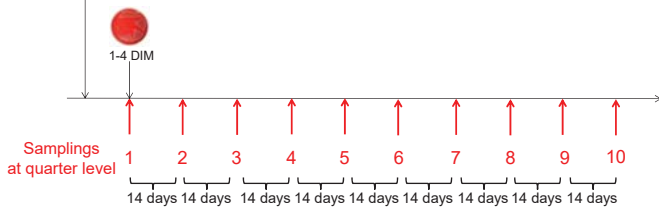


MATERIALS & METHODS

Study design

SCC

Calving



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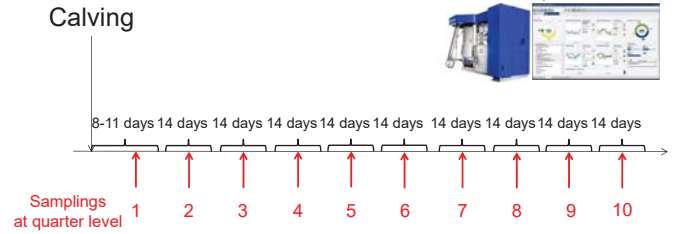


MATERIALS & METHODS

Study design

Milk yield

Calving



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26



MATERIALS & METHODS

Quarter IMI status

- ~ standard bacteriological culturing

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27



MATERIALS & METHODS

Statistical analyses

Linear mixed regression models (SAS 9.4)

- Continuous outcome variables
 - qMY
 - LnqSCC
- Random effects
 - Heifer
 - Quarter within heifer
- Herd forced in the model

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28



MATERIALS & METHODS

Statistical analysis

Linear mixed regression models (SAS 9.4)

- Infection status in early lactation as categorical independent variable (*predictor of main interest*)
- Quarter position (2 levels) as categorical independent variable
- Continuous independent variables
 - DIM
 - DIM*DIM

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29



RESULTS

Descriptive analysis:

- 324 quarters from 82 heifers at 1-4 DIM (BC + SCC)
- 3161 samples throughout lactation (SCC)

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RESULTS | IMI status in early lactation (1 – 4 DIM)

Quarter infection status	Herd			Overall	% of quarters	% of pos. samples
	1	2	3			
Negative	113	43	64	220	67.9	
NAS	30	16	22	68	21.0	76.4
<i>Coryne. spp</i>	2	1	3	6	1.9	6.7
<i>Bacillus spp.</i>	1	3	2	6	1.9	6.7
<i>S. aureus</i>	0	0	2	2	0.6	2.3
<i>Esc. pos. cocci</i>	0	1	1	2	0.6	2.3
<i>E. coli</i>	5	0	0	5	1.5	5.6
Contaminated	8	3	4	15	4.6	
Overall	159	67	98	324		

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31



RESULTS | Evolution of qMY and qSCC

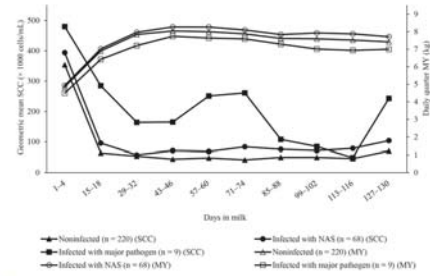


Figure 1. Evolution of average quarter daily milk yield (MY) and geometric mean quarter milk SCC during the first 4 mo of lactation in quarters that were noninfected, infected with NAS, or infected with a major pathogen in early lactation (1-4 DIM).

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32



RESULTS | Effect of IMI in early lactation (1-4 DIM)

qIMI status at calving	N	SCC(x1000)	P-value	qMY	P-value
Noninfected	220	66	...	7.35	...
Infected with NAS	68	89	<0.001	7.39	0.83
Infected with major pathogen	9	140	<0.001	6.84	0.31

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33



CONCLUSION

- NAS (group) IMI in early lactation
 - a slight but significant increase in qSCC during the first 4 mo in lactation
 - no significant difference in daily qMY
 - no difference in milk prolactin level in early lactation
- IMI with NAS (as a group) shortly after calving -> no adverse effect on later production

¹ Valckenier et al., 2019

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34



PART 2

The effect of intramammary infection in early lactation with non-aureus staphylococci in general and *Staphylococcus chromogenes* specifically on quarter milk somatic cell count and quarter milk yield

Valckenier et al., 2019b

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35

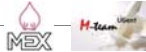


AIMS

- To determine
 - the infection dynamics of the most prevalent NAS species in the first 2 weeks after calving
 - the (species-specific) effect of transient and persistent IMI in the first 2 weeks after calving on
 - the quarter milk somatic cell count (SCC)
 - the daily quarter milk yield (MY)

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36

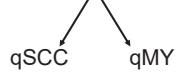


AIMS

- To determine
 - Quantification of the NAS species-specific impact



- The (species-specific) effect of transient and persistent IMI in the first 2 weeks after calving on

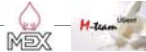
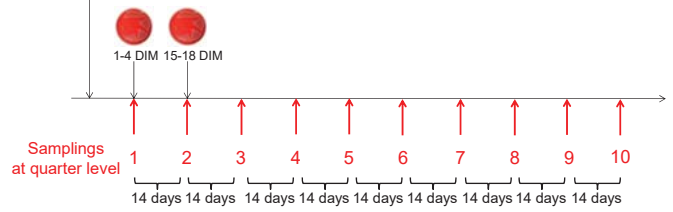


MATERIALS & METHODS

SCC

Study design

Calving

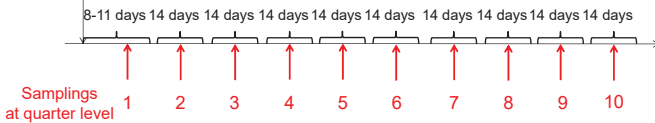


MATERIALS & METHODS

Study design

Calving

Milk yield



MATERIALS & METHODS

Quarter IMI status

- ~ standard bacteriological culturing
- NAS isolates: further identification to species level
 - Transfer RNA intergenic spacer PCR (tDNA-PCR)
 - Sequencing (16S rRNA gene – rpoB gene)



RESULTS | IMI status at 1 – 4 and 15 – 18 DIM

Table 1. Overview of bacteriological culture results from samples (n = 324) collected at the first (1-4 DIM) and second (15-18 DIM) sampling day

Bacteriological culture result	First sampling day		Second sampling day	
	N ¹	% of samples (n = 324)	N	% of culture-positive samples (n = 64)
Culture-negative ²	220	67.9	245	76.6
Culture-positive				
<i>Corynebacterium</i> spp.	6	1.9	6	1.9
<i>Bacillus</i> spp.	6	1.9	16	4.9
<i>Staphylococcus aureus</i> ³	2	0.6	1	0.3
Esculin-positive cocci ⁴	2	0.6	7	2.2
<i>Escherichia coli</i> ⁵	9	2.8	5	1.6
NAS ⁶	68	21.0	34	10.5
Contaminated ⁷	13	4.0	13	4.0

¹Number of samples.

²Resulting in the IMI status "noninfected."

³Resulting in the IMI status "infected with major pathogen."

⁴Resulting in the IMI status "infected with NAS."

⁵More than 2 bacterial colony types.



RESULTS | IMI status at 1 – 4 and 15 – 18 DIM

Table 2. Overview of the quarter-level IMI (qIMI) status at the first sampling day (1-4 DIM) and the associated qIMI at the second sampling day (15-18 DIM) in 297 quarters from 82 dairy heifers in 3 herds^a

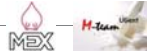
qIMI status at first sampling day	qIMI status at second sampling day					
	Noninfected	As a group	Same NAS spp. as on first sampling day	Different NAS spp. as on first sampling day	No species identification possible	NAS ^b
Noninfected	179	15	—	—	—	3
NAS	45	16	9	4	3	1
<i>Staphylococcus aureus</i>	0	1	—	—	—	0
Esculin-positive cocci (EPC)	1	0	—	—	—	0
<i>Escherichia coli</i>	2	1	—	—	—	0
Undefined ^c	—	—	—	—	—	5

^aOnly quarters that were noninfected (n = 220), NAS-infected (n = 68), and major pathogen-infected (*Staph. aureus*-infected, n = 2; EPC-infected, n = 2; *E. coli*-infected, n = 5) at the first sampling day, and thus relevant for further statistical analysis in the scope of this study, are shown.

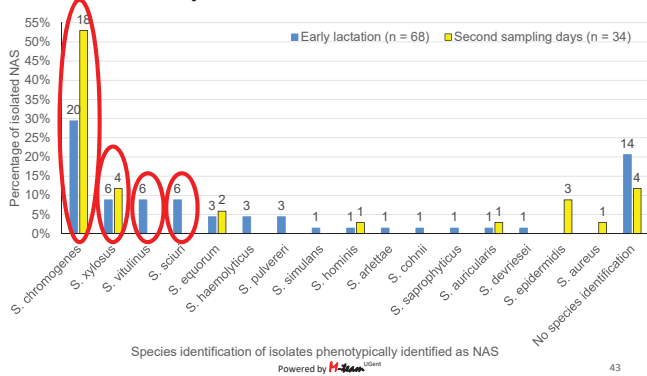
^bPhenotypically identified as NAS.

^c*Corynebacterium* spp.

^dContaminated samples (more than 2 phenotypically different colony types).



RESULTS | Species identification of NAS

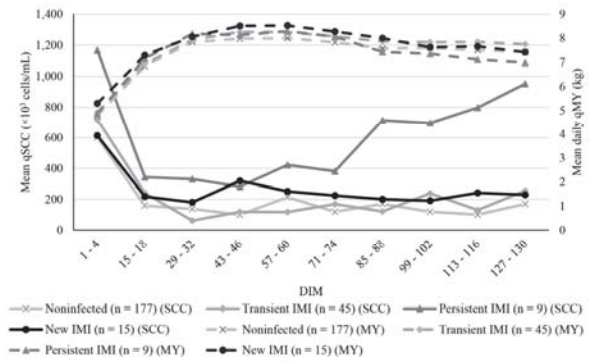


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43

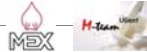


RESULTS | Evolution of qMY and qSCC



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46



RESULTS | Effect of IMI with *S. chromogenes* or other NAS

qIMI status at calving	N	SCC(x1000)	P-value	qMY	P-value
Noninfected	220	67	...	7.37	...
Infected with <i>S. chromogenes</i>	20	144	<0.001	7.31	0.85
Infected with other NAS	34	67	0.93	7.35	0.94
Infected with major pathogen	9	162	<0.001	6.95	0.47

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45

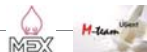


RESULTS | Effect of transient, persistent and new IMI with any *S. species*

qIMI status at 1st & 2nd sampling day	N	SCC(x1000)	P-value	qMY	P-value
Noninfected	177	63	...	7.37	...
Transient infection with a <i>S. sp.</i>	45	73	0.10	7.37	0.99
Persistent infection with a <i>S. sp.</i>	9	226	<0.001	7.33	0.93
New infection with a <i>S. sp.</i>	15	119	<0.001	7.72	0.35

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46



RESULTS | Effect of transient, persistent and new IMI with *S. chromogenes*

qIMI status at 1st & 2nd sampling day	N	SCC(x1000)	P-value	qMY	P-value
Noninfected	177	63	...	7.38	...
Transient infection with <i>S. chrom.</i>	12	93	0.01	7.46	0.86
Persistent infection with <i>S. chrom.</i>	8	340	<0.001	7.11	0.60
New infection with <i>S. chrom.</i>	5	302	<0.001	7.92	0.41

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47



CONCLUSION

- NAS: most prevalent pathogen in early lactation and in first 4 months of lactation
 - *Staph. chromogenes*: most prevalent species
 - *Staph. xylosum*: second most prevalent
 - *Staph. epidermidis*: not found in early lactation
 - *Staph. vitulinus* & *sciuri*: only in early lactation
- High spontaneous cure rate

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48



CONCLUSION

- *Staph. chromogenes*:
 - increased SCC
 - no effect on daily MY
 - IMI in early lactation: 40% persistent
- Persistent *Staph. chromogenes* IMI:
 - higher SCC compared to transient IMI
 - numerically lower daily MY compared to noninfected quarters



THANK YOU



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