Review articles

Evolution of the mammary gland from the innate immune system?

Claudia Vorbach,[1]* Mario R. Capecchi,[2] and Josef M. Penninger[1]

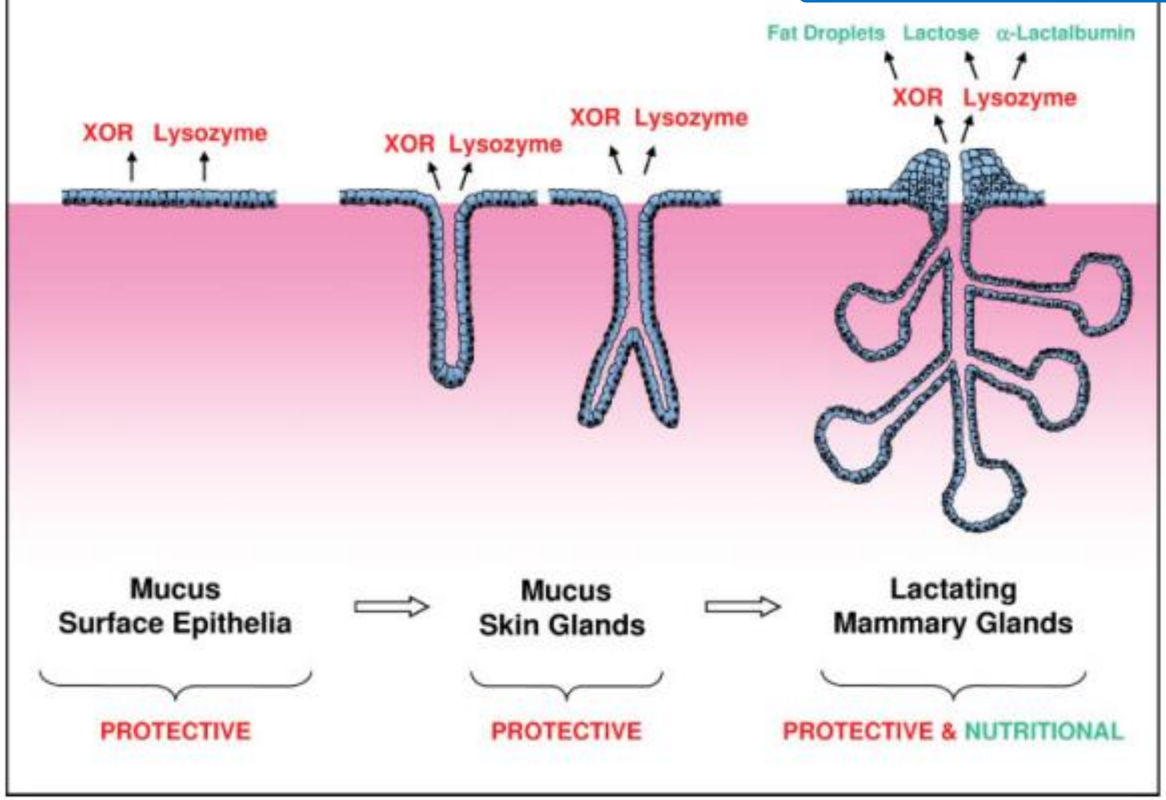
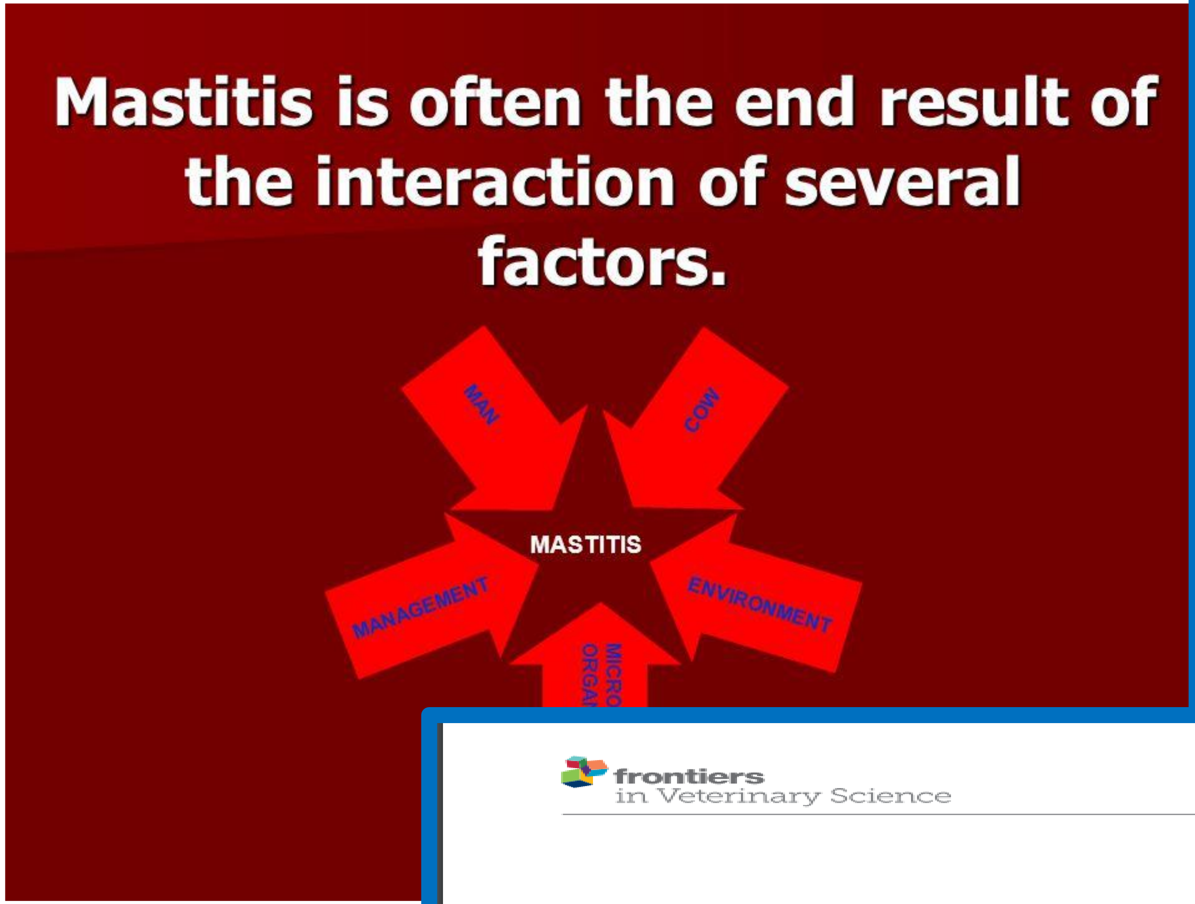


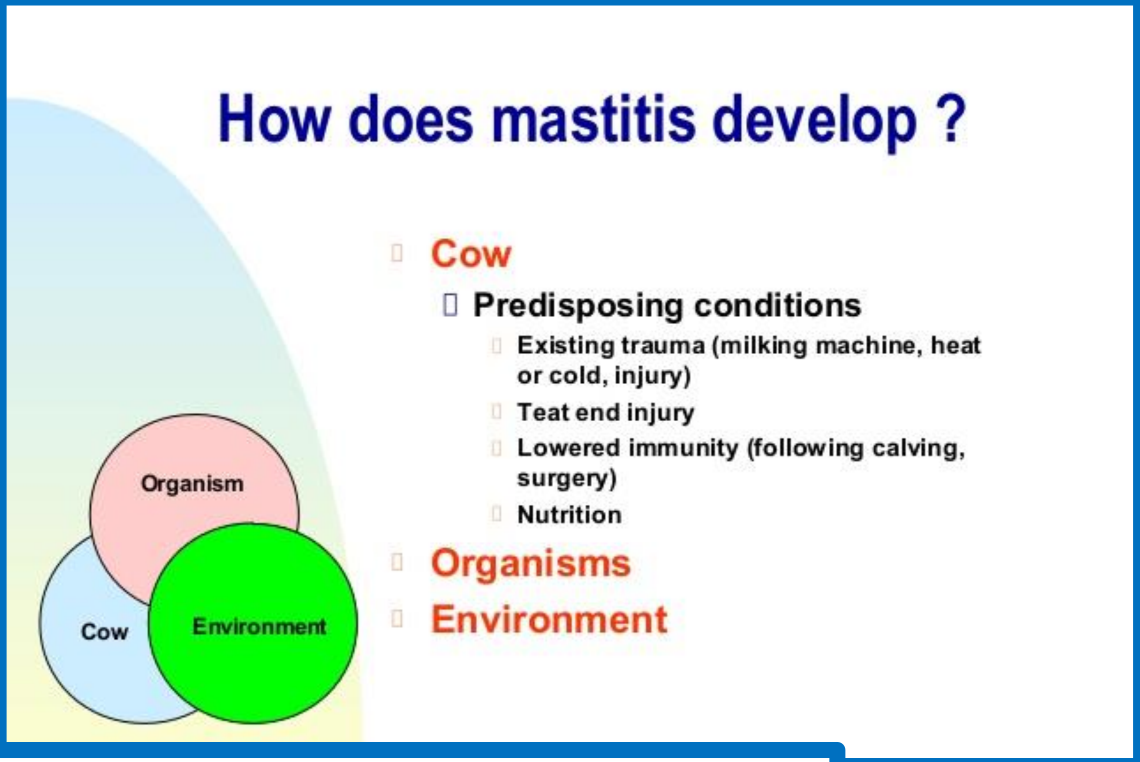
Figure 3. Proposed evolution of the mammary gland from a mucus-secreting epithelial gland. Mammary glands presumably evolved as mucus-secreting skin glands that similar to many mucus surface epithelia secreted antimicrobial enzymes such as XOR and lysozyme. The evolution of additional functions of XOR and lysozyme in the ancient mammary epithelium resulted in the secretion of fat droplets, α-lactalbumin and lactose. Consequently, the mammary gland evolved from a protective immune organ into a reproductive organ unique to the class mammalia.

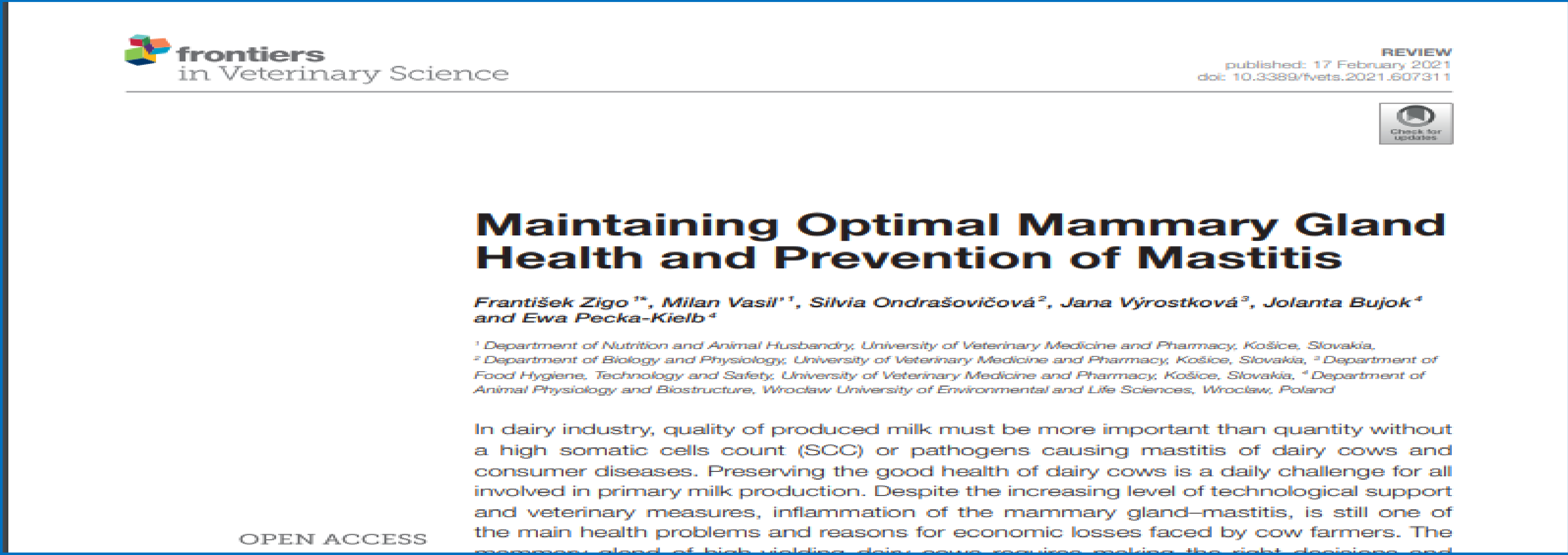
XOR / GENE SHARING
LYSOZYME / GENE DUPLICATION

МАСТИТИС

“Bovine mastitis is a disease of man with signs in the cow."
"Bad management will overwhelm the best immunology."







frontiers in Veterinary Science

REVIEW
published: 17 February 2021
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Maintaining Optimal Mammary Gland Health and Prevention of Mastitis

František Zigo[1*], Milan Vasiľ[1], Silvia Ondrašovičová[2], Jana Výrostková[3], Jolanta Bujok[4] and Ewa Pecka-Kiełb[4]

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OPEN ACCESS

In dairy industry, quality of produced milk must be more important than quantity without a high somatic cells count (SCC) or pathogens causing mastitis of dairy cows and consumer diseases. Preserving the good health of dairy cows is a daily challenge for all involved in primary milk production. Despite the increasing level of technological support and veterinary measures, inflammation of the mammary gland–mastitis, is still one of the main health problems and reasons for economic losses faced by cow farmers. The

МУЛТИФАКТОРИЈАЛНЕ И ПОЛИЕТИОЛОШКЕ ПРИРОДЕ

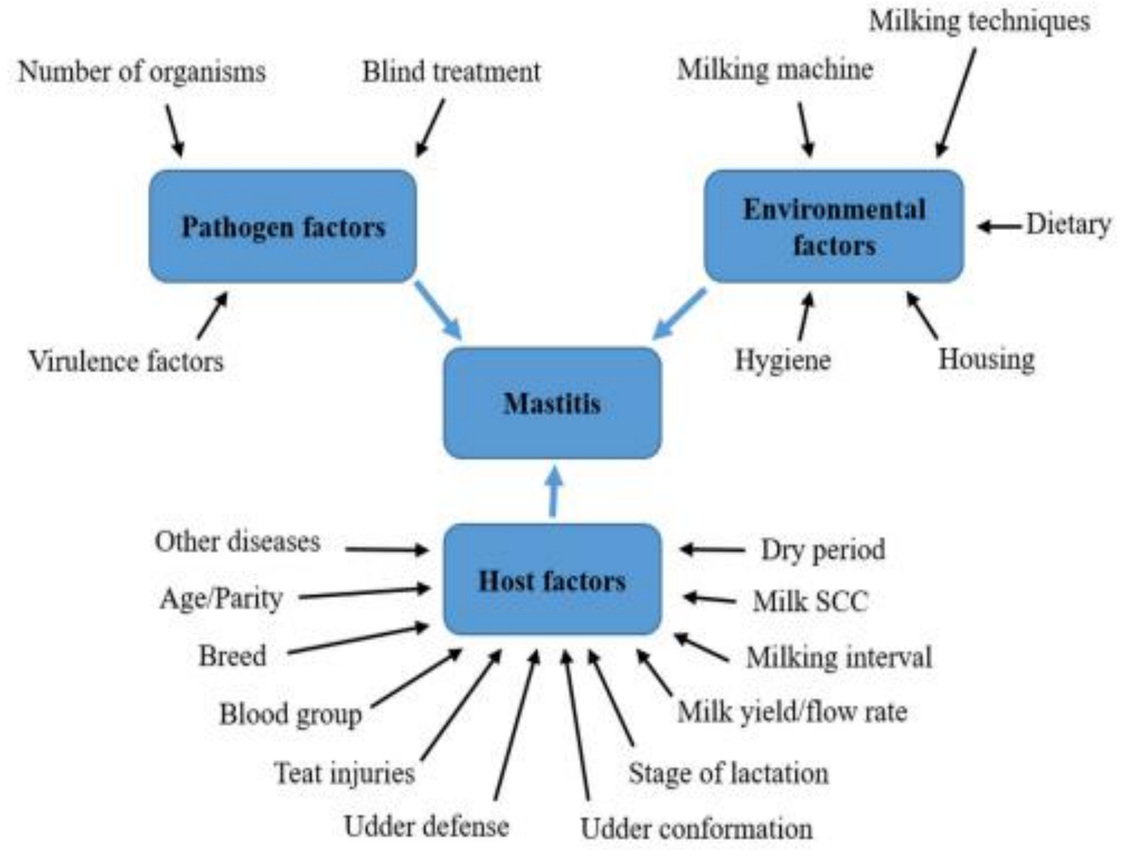

FIGURE 1 | Factors influencing the development of mastitis in dairy cows. Soure: Adapted from Abebe et al. (17).

J. Dairy Sci. 102:4452–4463
https://doi.org/10.3168/jds.2018-15657

Relationship between the probability of veterinary-diagnosed bovine mastitis occurring and farm management risk factors on small dairy farms in Austria

Clair L. Firth,[1]* Christian Laubichler,[2] Corina Schleicher,[2] Klemens Fuchs,[2] Annemarie Käsbohrer,[1] Christa Egger-Danner,[3] Josef Köfer,[1] and Walter Obritzhauser[1,4]
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ABSTRACT

Bovine mastitis is the most frequently reported disease among dairy cows worldwide. Treatment of udder disease often involves the use of antimicrobial substances, which is difficult to justify with respect to their possible effect on the development and spread of antimicrobial resistance. Prevention of udder disease is therefore always preferable to treatment. The study presented here statistically analyzed the probability of mastitis occurring during 3,049 lactation periods on 208 farms and attempted to ascertain which on-farm

were likely to increase the probability of mastitis occurring, included lactation number (OR = 1.18), farming part time (OR = 1.55), and udders on the farm being classed by herd veterinarians as medium to severely soiled (OR = 1.47). The study presented here was able to confirm several management factors recommended to reduce the probability of mastitis occurring during a cow's lactation period, with particular relevance for the small dairy herds common to Austria.
Key words: mastitis, farm management, generalized linear mixed model, udder disease

Table 5. Estimated model parameters from the generalized linear mixed model based on 3,049 lactation periods on 208 farms

Fixed effect	Data form[1]	Data source	Coefficient	95% CI	Odds ratio (OR)	95% CI OR
Intercept			−1.65	−2.40, −1.09	0.19	0.09, 0.34
Lactation no.	Numerical	Livestock data	0.17	0.12, 0.21	1.18	1.13, 1.24
Part-time farmer	Binary Y/N	Farmer survey	0.44	0.06, 0.81	1.55	1.06, 2.25
Regular access to pasture	Binary Y/N	Farmer survey	−0.32	−0.66, −0.01	0.73	0.52, 0.99
Automatic shut-off	Binary Y/N	NMR[2] survey	−0.41	−0.71, −0.06	0.67	0.49, 0.94
Access to feed immediately after milking	Binary Y/N	Farmer survey	−0.85	−1.36, −0.17	0.43	0.26, 0.84
Regular bacteriological milk culture	Binary Y/N	Farmer survey	0.43	0.10, 0.75	1.53	1.10, 2.11
Udder hygiene score: medium to severely soiled	Binary Y/N	Veterinarian survey	0.38	−0.02, 0.72	1.47	0.98, 2.06
$\hat{\sigma}^2_{farm}$ [3]			0.45	0.46, 0.80		

[1]Y/N = yes/no.

[2]NMR = National Milk Performance Recorders.

[3]Estimated variance component of the random farm effect.

Table 1. Subject areas covered by the questionnaires

Subject area	Maximum no. of questions
(1) Farmer questionnaire	
Education	2
Farm type	3
Housing systems and hygiene	13
Mastitis management/prevention/treatment	13
Milking/parlor equipment	8
Dry cow management	14
Animal health	6
Calf management	7
Feed and water	13
Foot health	4
Access to pasture or outdoor yards	7
Pest control and biosecurity	6
(2) National milk performance recorder questionnaire	
Hand hygiene at milking	3
Premilking preparation	7
Milking routine	12
Postmilking routine	4
Milking machine	10
Maintenance of parlor machinery	4
Cleanliness of parlor	2
Housing systems and hygiene	10
(3) Herd veterinarian questionnaire	
Most common mastitis pathogens	4
Most commonly used dry cow tubes	3
Use of teat sealants	2
Use of mastitis vaccines	5
Udder hygiene (using photographic scale)	1
Housing systems and hygiene	4

Cow Environment

CLEAN

" Hygiene is a dominant factor in mastitis control, influenced primarily by cow comfort, ventilation and bedding"

Fetrow et al (2002)

efsa
European Food Safety Authority
Annex to the EFSA Journal (2009) 1143, 1-284

SCIENTIFIC REPORT OF EFSA

Effects of farming systems on dairy cow welfare and disease[1]

Report of the Panel on Animal Health and Welfare

(Question No EFSA-Q-2006-113)

“Iceberg” КОНЦЕПТ

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- ПРИСУСТВО РЕЗИДУА АНТИБИОТИКА У МЛЕКУ
- ПРИСУСТВО ПАТОГЕНИХ МИКРООРГАНИЗАМА

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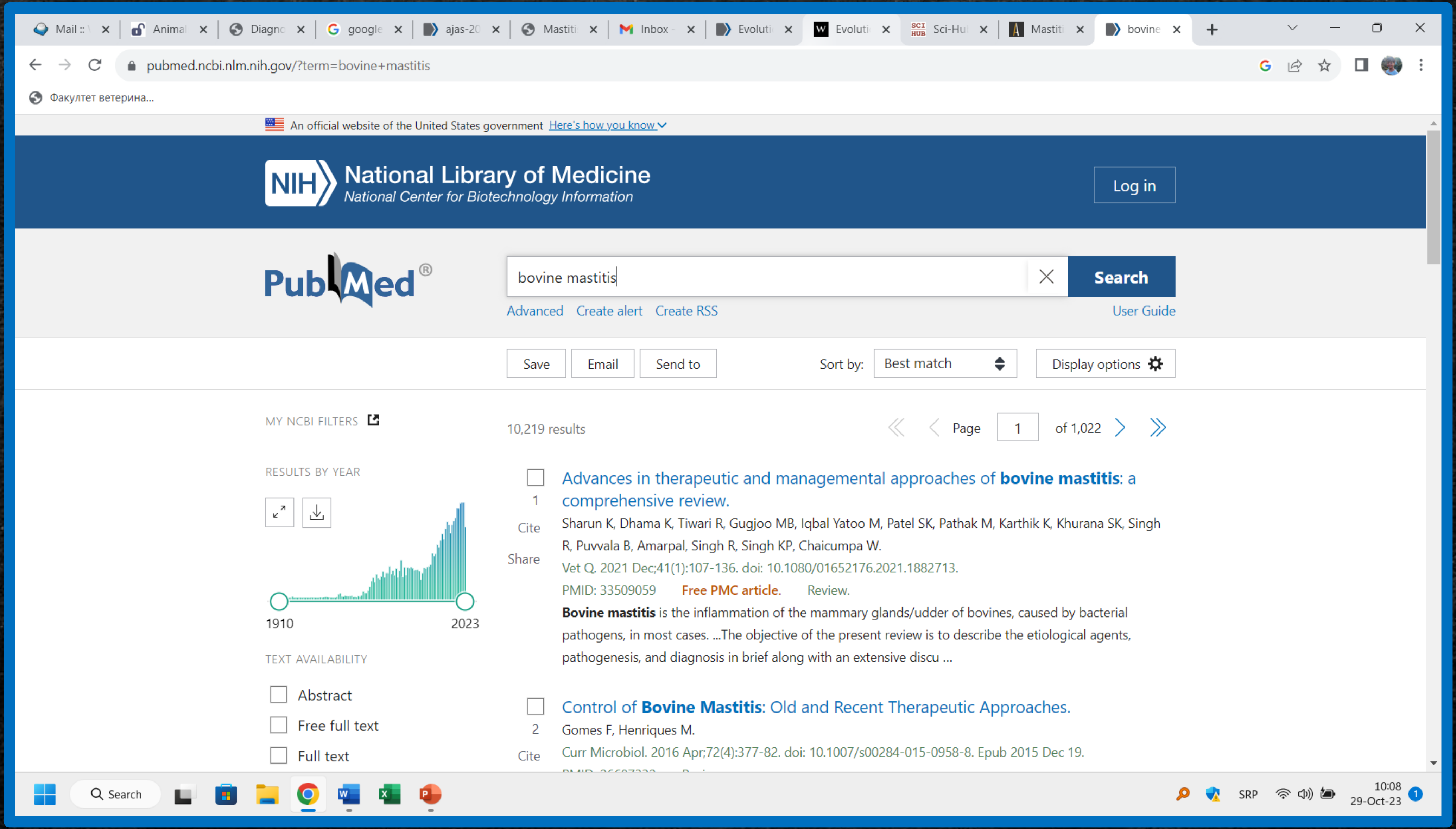

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provide an overview of some conventional and emerging ...

3 Cite Share

Causes, types, etiological agents, prevalence, diagnosis, treatment, prevention, effects on human health and future aspects of **bovine mastitis**.

Ashraf A, Imran M.

Anim Health Res Rev. 2020 Jun;21(1):36-49. doi: 10.1017/S1466252319000094. Epub 2020 Feb 13.

PMID: 32051050 Review.

Mastitis is among the most common and challenging diseases of dairy animals. It is an inflammation of udder tissues due to physical damage, chemical irritation, or infection caused by certain pathogens. **Bovine mastitis** has been known for ages, but its complex ...

4 Cite Share

A 100-Year Review: **Mastitis** detection, management, and prevention.

Ruegg PL.

J Dairy Sci. 2017 Dec;100(12):10381-10397. doi: 10.3168/jds.2017-13023.

PMID: 29153171 Free article. Review.

Mastitis is the most frequent disease of dairy cows and has well-recognized detrimental effects on animal wellbeing and dairy farm profitability. ...During the last century, tremendous significant advances in **mastitis** control have been made but changing herd structu ...

5 Cite Share

Importance of **bovine mastitis** in Africa.

Motaung TE, Petrovski KR, Petzer IM, Thekisoe O, Tsilo TJ.

Anim Health Res Rev. 2017 Jun;18(1):58-69. doi: 10.1017/S1466252317000032. Epub 2017 Jun 13.

PMID: 28606203 Review.

Bovine mastitis is an important animal production disease that affects the dairy industry globally. ...Here, published cases supporting the occurrence and importance of **bovine mastitis** in certain regions of Africa are outlined....

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SRP 10:11 29-Oct-23

Matrix Assisted Laser Desorption Ionization-Time Of Flight Mass Spectrometry (MALDI-TOF MS)

MATRICOM POTPOMOGNUTA IONIZACIJA LASERSKE DESORPCIJE POVEZANE SA MS TEMELJENOM NA VREMENU LETA IONA

animals MDPI

Review

Diagnosing Intramammary Infection: Meta-Analysis and Mapping Review on Frequency and Udder Health Relevance of Microorganism Species Isolated from Bovine Milk Samples

Daryna Kurban [1,2,3,*], Jean-Philippe Roy [1,2,3], Fidèle Kabera [1,2,3], Annie Fréchette [1,2,3], Maryse Michèle Um [1,2,3], Ahmad Albaaj [1,2,3], Sam Rowe [4], Sandra Godden [5], Pamela R. F. Adkins [6], John R. Middleton [6], Marie-Lou Gauthier [7], Greg P. Keefe [2,8], Trevor J. DeVries [2,9], David F. Kelton [2,10], Paolo Moroni [11,12], Marcos Veiga dos Santos [13], Herman W. Barkema [2,14] and Simon Dufour [1,2,3,*]

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14 Faculty of Veterinary Medicine, University of Calgary, Calgary, AB T2N 4N1, Canada
* Correspondence: daryna.kurban@umontreal.ca (D.K.); simon.dufour@umontreal.ca (S.D.)

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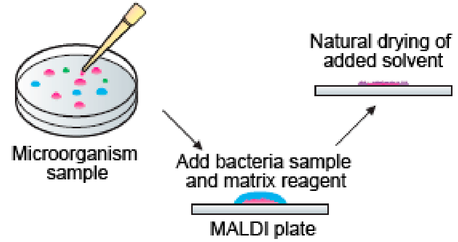
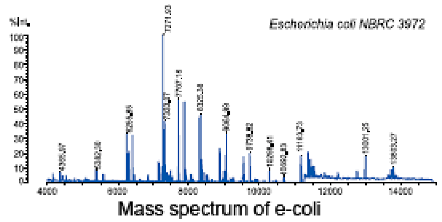
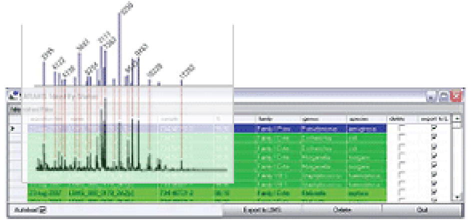
Citation: Kurban, D.; Roy, J.-P.; Kabera, F.; Fréchette, A.; Um, M.M.; Albaaj, A.; Rowe, S.; Godden, S.; Adkins, P.R.F.; Middleton, J.R.; et al. Diagnosing Intramammary Infection:







Simple Summary: Innovations in veterinary medicine diagnostic methods can help to better identify the microorganisms causing bovine mastitis. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry is such an innovation. This tool can identify many different microorganisms cultured from bovine milk samples to species-level. However, not all these microorganisms are necessarily pathogenic to the cow udder. Using 93,353 milk samples from different countries, we aimed to determine the diversity and proportion of different microorganisms cultured from bovine milk of apparently healthy cow mammary glands and of clinical mastitis cases, and identified by MALDI-TOF. Moreover, we highlighted the current knowledge gaps on the effect of these microorganisms on udder health. We revealed a great diversity of microorganisms in both types of samples, but, for most species (206 of 294), little literature regarding their udder health relevance was available. However, some microorganisms with little or no scientific literature were frequently isolated from clinical mastitis samples; thus, suggesting that they may be relevant in terms of udder health. For other species, more research is needed to clarify their role.

Abstract: Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry provides accurate species-level identification of many, microorganisms retrieved from bovine milk samples. However, not all those microorganisms are pathogenic. Our study aimed to: (1) determine the species-specific prevalence of microorganisms identified in bovine milk of apparently healthy lactating quarters vs. quarters with clinical mastitis (CM); and (2) map current information and knowledge gaps on udder health relevance of microorganisms retrieved from bovine milk samples. A mixed study design (meta-analysis and mapping review) was chosen. We gathered several large Canadian, US and Brazilian data sets of MALDI-TOF results for organisms cultured from quarter milk samples. For meta-analysis, two datasets (apparently healthy quarters vs. CM samples) were

РЕЗУЛТАТИ

- Among the quarter-milk samples from apparently healthy quarters, only 26 groups of microorganisms had an estimated prevalence 0.1% with 11 of them being related to the Staphylococcus genus, though one of the microorganisms, Staphylococcus sciuri, was recently reassigned to the novel genus Mammaliicoccus with Mammaliicoccus sciuri as the type species. The five most prevalent species were Staphylococcus chromogenes (6.7%, 95% CI 4.5–9.2%), Aerococcus viridans (1.6%, 95% CI 0.4–3.5%), Staphylococcus aureus (1.5%, 95% CI 0.5–2.8%), Staphylococcus haemolyticus (0.9%, 95% CI 0.4–1.5%), and Staphylococcus epidermidis (0.7%, 95% CI 0.2–1.6%). For most species, the estimated prevalence did not differ between countries.

РЕЗУЛТАТИ

- Among the 43,924 quarter-milk CM samples, 43 groups of microorganisms had an estimated prevalence 0.1%. The most frequent genus was, again, the Staphylococcus genus (n = 11, when including Staphylococcus sciuri), followed by Streptococcus genus (n = 7). The 5 most frequent species in CM samples were Escherichia coli (11%, 95% CI 8.1–14.3%), Streptococcus uberis (8.5%, 95% CI 5.3–12.2%), Streptococcus dysgalactiae (7.8%, 95% CI 4.9–11.5%), Staphylococcus aureus (7.8%, 95% CI 4.4–11.9%), and Klebsiella pneumoniae (5.6%, 95% CI 3.4–8.2%). Again, for most microorganisms, the estimated prevalence was not significantly affected by the country of origin.

Table 4. Prevalence of the different microorganisms isolated from the milk of mammary quarters of dairy cows with clinical mastitis and identified using MALDI-TOF estimated using random meta-analyses conducted on the raw data from 43,924 samples using 8 datasets (Canada, USA and Brazil). Only microorganisms with prevalence ≥0.1% are reported.

Microorganism	Prevalence (95% CI)	95% Prediction Interval [a]
Escherichia coli	11.0 (8.1–14.3)	4.5–19.9
Streptococcus uberis	8.5 (5.3–12.2)	1.4–20.4
Streptococcus dysgalactiae	7.8 (4.9–11.5)	1.1–19.8
Staphylococcus aureus	7.8 (4.4–11.9)	0.4–22.6
Klebsiella pneumoniae	5.6 (3.4–8.2)	1.0–13.2
Staphylococcus chromogenes	2.3 (0.5–5.3)	0.0–14.0
Serratia marcescens	1.0 (0.5–1.7)	0.0–3.4
Trueperella pyogenes	0.9 (0.1–2.2)	0.0–6.0
Corynebacterium bovis	0.9 (0.1–2.1)	0.0–5.9
Staphylococcus haemolyticus	0.7 (0.1–1.8)	0.0–4.2
Unspeciated [b] *Staphylococcus*	0.7 (0.0–2.8)	0.0–10.3
Staphylococcus simulans	0.6 (0.4–0.8) *	0.1–1.2
Staphylococcus sciuri [c]	0.6 (0.1–1.6)	0.0–4.4
Unspeciated [b] *Corynebacterium*	0.6 (0.1–1.4)	0.0–3.8
Lactococcus garvieae	0.6 (0.1–1.3)	0.0–2.9
Unspeciated [b] *Bacillus*	0.6 (0.0–2.5)	0.0–9.4
Staphylococcus xylosus	0.5 (0.0–1.6)	0.0–5.4
Lactococcus lactis	0.5 (0.0–1.4)	0.0–4.1
Streptococcus agalactiae	0.4 (0.0–2.0)	0.0–7.6
Unspeciated [b] *Streptococcus*	0.4 (0.0–1.1)	0.0–3.5
Enterobacter cloacae	0.3 (0.2–0.4)	0.2–0.4
Streptococcus equinus	0.3 (0.1–0.6)	0.0–1.2
Staphylococcus epidermidis	0.3 (0.0–1.2)	0.0–3.8
Aerococcus viridans	0.3 (0.0–1.2)	0.0–4.1
Enterococcus saccharolyticus	0.3 (0.0–1.0)	0.0–2.4
Candida rugosa [d]	0.3 (0.0–0.9)	0.0–2.6
Klebsiella oxytoca	0.3 (0.0–0.8)	0.0–1.8
Enterococcus faecium	0.3 (0.0–0.8)	0.0–2.0
Pasteurella multocida	0.3 (0.0–0.7) *	0.0–1.5
Candida krusei [d]	0.2 (0.0–0.7) *	0.0–2.1

УЗРОЧНИЦИ МАСТИТИСА

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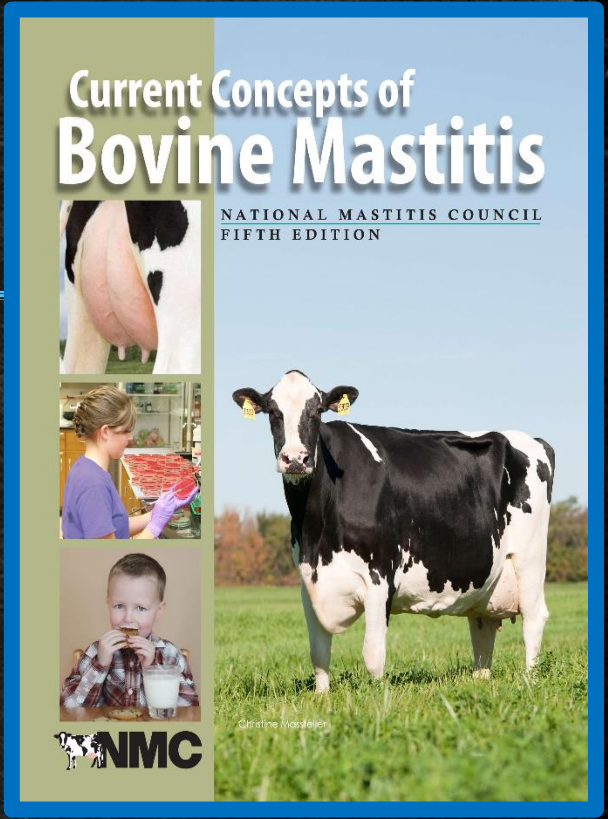
- Zadoks, R.N.; Middleton, J.R.; McDougall, S.; Katholm, J.; Schukken, Y.H. Molecular Epidemiology of Mastitis Pathogens of Dairy Cattle and Comparative Relevance to Humans. J. Mammary Gland. Biol. Neoplasia 2011, 16, 357–372. - 200 MO

- Current Concepts of Bovine Mastitis listed the following microorganisms as important mammary gland pathogens: Staphylococcus aureus, Streptococcus agalactiae, Streptococcus uberis, Streptococcus dysgalactiae and other Streptococcus-like microorganisms, Mycoplasma bovis and other Mycoplasma spp., Corynebacterium bovis, Escherichia coli, Klebsiella spp., Enterobacter spp., Citrobacter spp., Enterococcus faecalis, Enterococcus faecium, non-aureus staphylococci (NAS) (Staphylococcus chromogenes, Staphylococcus hyicus, Staphylococcus warneri, Staphylococcus epidermidis, Staphylococcus cohnii, Staphylococcus simulans, Staphylococcus xylosus, Staphylococcus sciuri, Staphylococcus saprophyticus), Pseudomonas aeruginosa, Trueperella pyogenes, Nocardia spp., mycobacteria, Serratia spp., Bacillus cereus, yeasts (Candida), molds, and algae (Prototheca).



УЗРОЧНИЦИ МАСТИТИСА

- **УЗРОЧНИЦИ КОНТАГИОЗНОГ МАСТИТИСА**
  - *Streptococcus agalactiae*
  - *Staphylococcus aureus*
  - *Mycoplasma spp.*

УЗРОЧНИЦИ МАСТИТИСА

- **УЗРОЧНИЦИ МАСТИТИСА ИЗ ОКОЛИНЕ/"*environmental pathogens*"**
  - *Escherichia coli*
  - *Klebsiella* spp.
  - *Enterobacter* spp.
  - *Citrobacter*
  - *Streptococcus dysgalactiae*
  - *Streptococcus uberis*
  - *Trueperella pyogenes* (*Actinomyces pyogenes*, *Arcanobacterium pyogenes*)
  - Enterokoke
  - Kvasci
  - Jednoćelijske alge (*Prototheca zopfii*)

Table 1. Classification of mastitis pathogens Adapted from Constable and colleagues (2017) [12].

Contagious	Environmental	Opportunistic
Staphylococcus aureus *Streptococcus agalactiae* *Corynebacterium bovis* *Mycoplasma spp*	*Escherichia coli* *Streptococcus uberis* *Streptococcus dysgalactiae* *Klebsiella spp* *Corynebacterium pyogenes*	Non-aureus Staphylococci: *Staphylococcus simulans* *Staphylococcus chromogens*

Environmental

Streptococcus uberis

E. coli

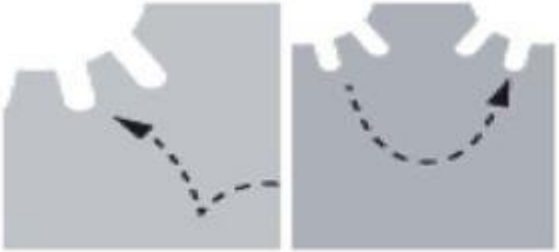
Coliforms
(Klebsiella, Serratia, Enterobacter)

Contagious

Staphylococcus aureus

Mixed bacteria

Coagulase-negative staphylococci



ТРАНСМИСИЈА

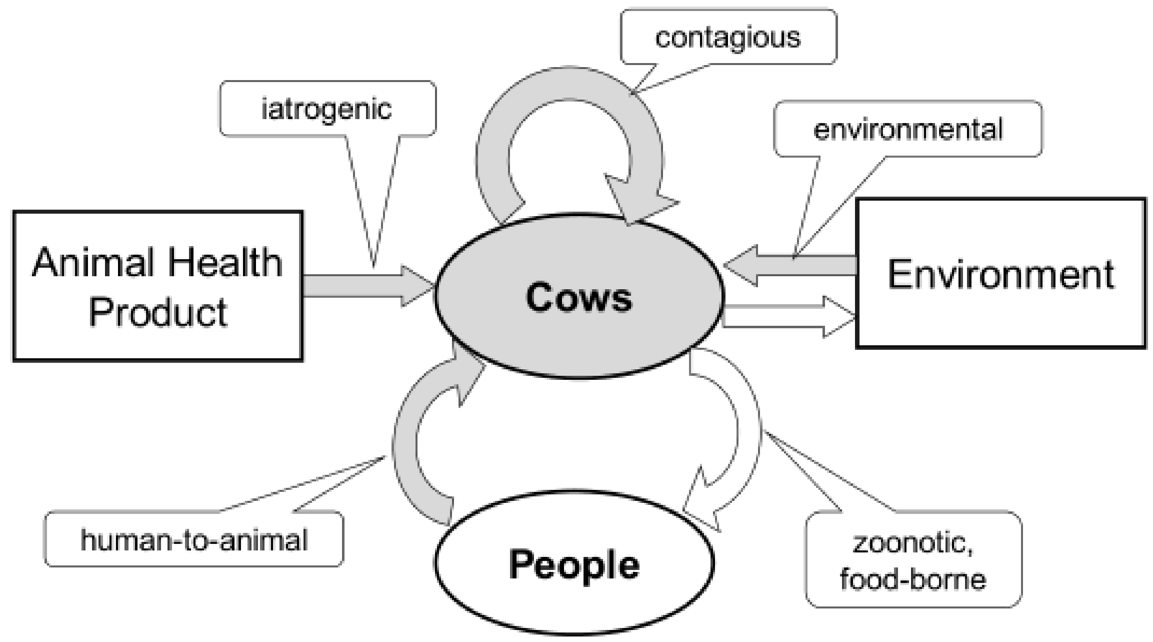
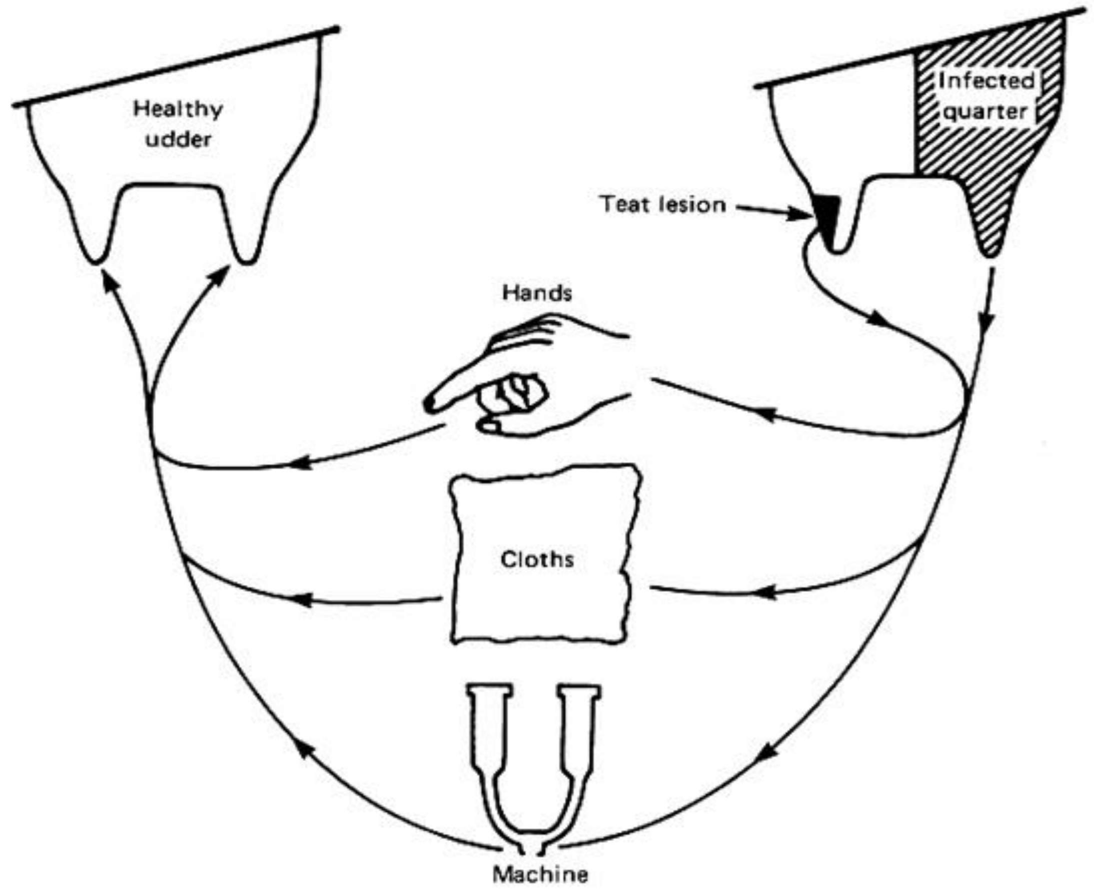


Figure 1. Possible sources and transmission routes for mastitis pathogens: from other cows, the environment, people or products. Mastitis control efforts must focus on the most important sources and routes.



Persistent infection
Dominant strain

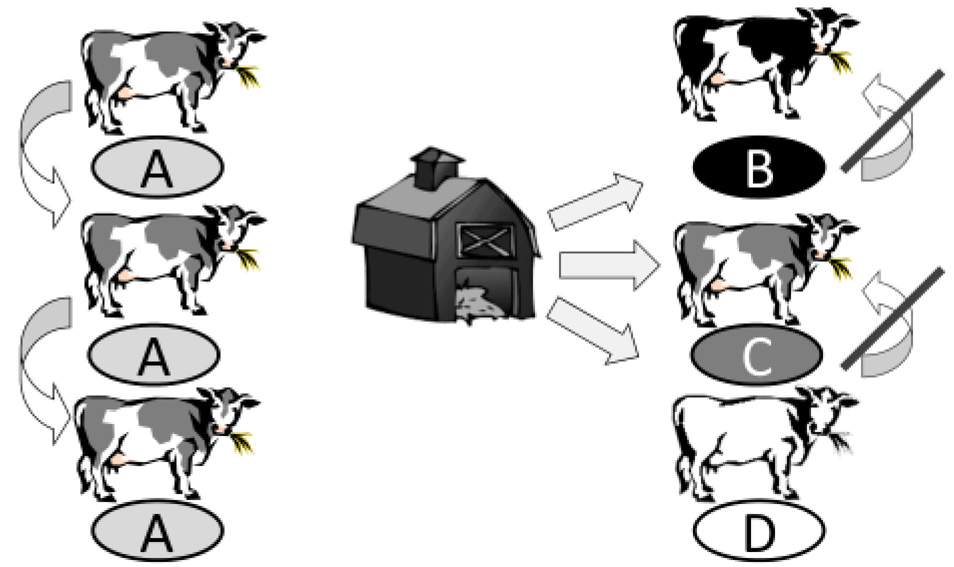


Figure 3. Contagious and environmental transmission as seen through strain typing: If cow-to-cow transmission occurs, all cows will be infected with the same strain (left). If cows are infected with different strains (right), they did not infect each other. Rather, they probably were infected from the environment, which contains many different strains of mastitis pathogens.

Transient infection
Multiple strain

bacteria that *“didn’t follow the textbook”*

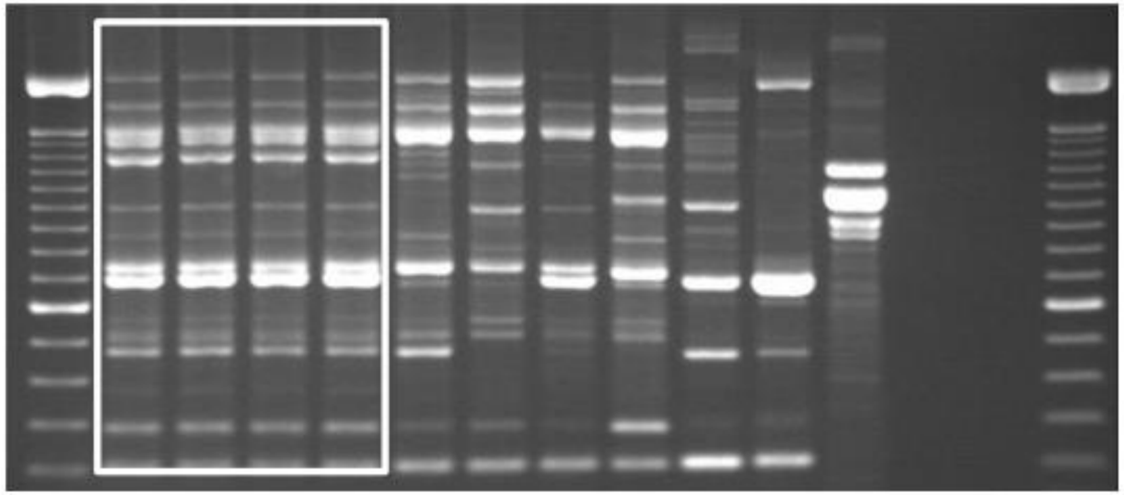


Figure 4. Example of strain typing results for mastitis-causing bacteria. Each “DNA fingerprint” or barcode-like typing result runs from top to bottom and represents a single cow with mastitis. The box encloses 4 identical results showing that 4 cows shared the same strain of *Klebsiella*. Other cows from this herd were infected with different strains of *Klebsiella*. The regular patterns on the left and right are DNA-ladders, which are included for quality control reasons, as are the empty lanes.

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DISCONTOOLS SUPPLEMENT

WILEY

An update on environmental mastitis: Challenging perceptions

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Summary
Environmental mastitis is the most common and costly form of mastitis in modern dairy herds where contagious transmission of intramammary pathogens is controlled through implementation of standard mastitis prevention programmes. Environmental mastitis can be caused by a wide range of bacterial species, and binary classification of species as contagious or environmental is misleading, particularly for *Staphylococcus aureus*, *Streptococcus uberis* and other streptococcal species, including *Streptococcus agalactiae*. Bovine faeces, the indoor environment and used pasture are major sources of mastitis pathogens, including *Escherichia coli* and *S. uberis*. A faeco-oral transmission cycle may perpetuate and amplify the presence of such pathogens, including *Klebsiella pneumoniae* and *S. agalactiae*. Because of societal pressure to reduce reliance on antimicrobials as tools for mastitis control, management of environmental mastitis will increasingly need to be based on prevention. This requires a reduction in environmental exposure through bedding, pasture and pre-milking management and enhancement of the host response to bacterial challenge. Efficacious vaccines are available to reduce the impact of coliform mastitis, but vaccine development for gram-positive mastitis has not progressed beyond the "promising" stage for decades. Improved diagnostic tools to identify causative agents and transmission patterns may contribute to targeted use of antimicrobials and intervention measures. The most important tool for improved uptake of known mastitis prevention measures is communication. Development of better technical or biological tools for management of environmental mastitis must be accompanied by development of appropriate incentives and communication strategies for farmers and veterinarians, who may be confronted with government-mandated antimicrobial use targets if voluntary reduction is not implemented.

KEYWORDS
antimicrobial use, bedding, coliforms, environmental mastitis, molecular epidemiology, streptococci

...DUCTION

The world population is growing and needs increasing amounts of

to provide every Chinese, especially children, sufficient milk each day.[1] There are an estimated 1.4 billion people in China—more milk will be needed to satisfy Wen Jiabao's dream. At the same time, the

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Molecular Epidemiology of Mastitis Pathogens of Dairy Cattle and Comparative Relevance to Humans

Ruth N. Zadoks · John R. Middleton · Scott McDougall · Jorgen Katholm · Ynte H. Schukken

Understanding the Sources, Transmission Routes, and Prognoses for Mastitis Pathogens

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Take Home Messages

- Within animal species, we recognize different breeds. Within bacterial species, we recognize different strains. Strain typing may help to identify sources and transmission routes of mastitis-causing bacteria so that we can target interventions and choose appropriate treatment options.
- Many of the most common mastitis-causing bacteria, including *Staphylococcus aureus* and *Streptococcus uberis*, can originate from the environment and spread from cow to cow. It is wrong to consider a bacterial species as "contagious" or "environmental". Transmission mechanisms are herd- and strain specific. Inspection of the farm, animals and records may be enough to identify the transmission route. Strain typing can help.

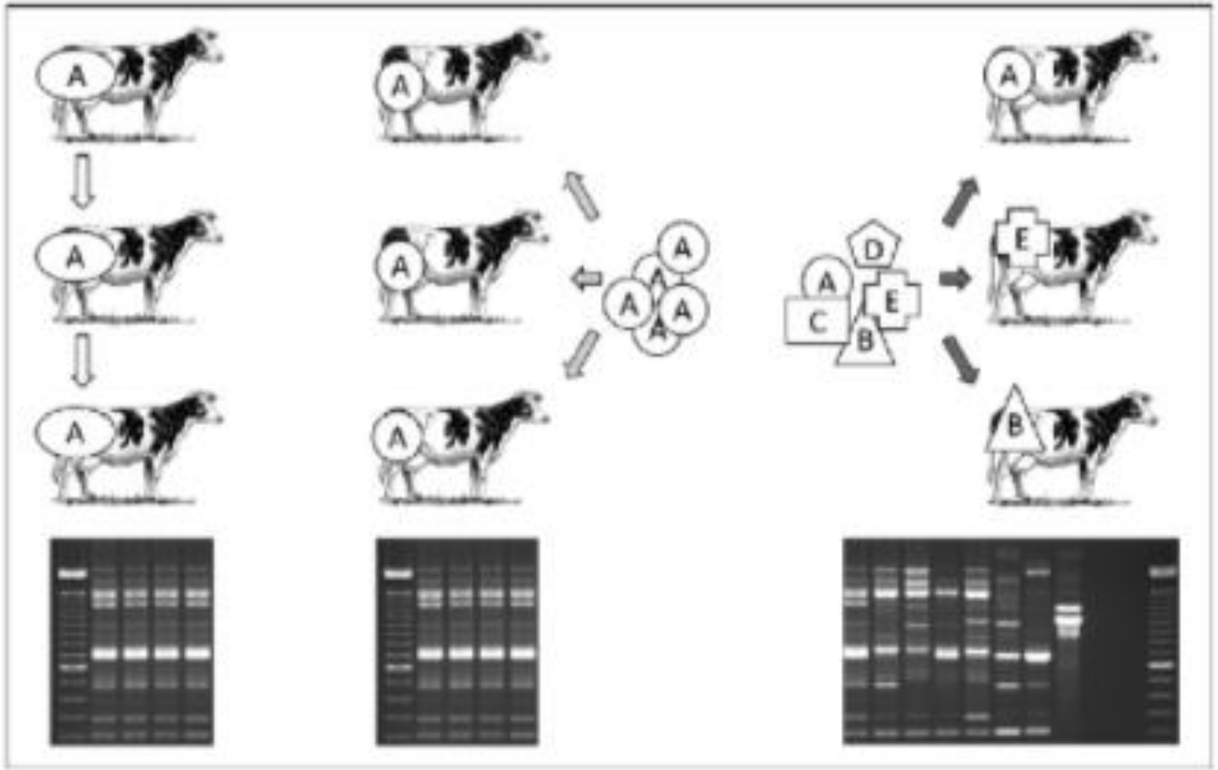

FIGURE 3 Modes of transmission (left: contagious; centre: environmental point source; right: heterogeneous environmental source) and resultant patterns of strain distribution (left, centre: homogeneous; right: heterogeneous), demonstrating that strain heterogeneity is proof of environmental origin of mastitis pathogens, but homogeneity is not proof of contagious transmission

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Genotypes of *Staphylococcus aureus*: On-farm epidemiology and the consequences for prevention of intramammary infections

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ABSTRACT

Staphylococcus aureus is a highly contagious mastitis-causing pathogen infecting dairy cattle worldwide. Previous studies have shown the presence of different genotypes (GT) on farms. In Switzerland, *Staph. aureus* genotype B (GTB) is contagious, whereas GTC and other genotypes cause sporadic, noncontagious mastitis. In this study, we evaluated the epidemiological properties of *Staph. aureus*, together with its genotypes and *spa* types, on Swiss dairy farms. A total of 21 dairy farms were sampled throughout Switzerland; 10 farms were positive for the contagious *Staph. aureus* GTB and 11 farms were negative for GTB. Samples were taken from

prevent IMI caused by contagious pathogens still hold for GTB but not for *Staph. aureus* genotypes that are opportunistic colonizers of bovine skin (e.g., GTC and GTA). For those genotypes, protection of the skin from minor lesions and wounds, particularly on the hocks, is essential.
Key words: *Staphylococcus aureus*, subtypes, milk, environment

INTRODUCTION

Staphylococcus aureus causes contagious and mostly chronic mastitis in cattle worldwide (Barkema et al., 2006), as well as large economic losses due to reduced

- ✓ ГЕНОТИП Б (ГТБ) (централна Европа),ГТЦ, ГТР
- ✓ ГТФ, ГТИ и остали (41)

ГТБ

VERY HIGH PRESSURE OF INFECTION

ГТЦ

ГТР

MULTI HOST PATHOGEN AND HOST SPECIES JUMPER

MICROBIAL GENOMICS

RESEARCH ARTICLE
Crestani *et al.*, *Microbial Genomics* 2021;7:000648
DOI 10.1099/mgen.0.000648

MICROBIOLOGY SOCIETY

OPEN DATA OPEN ACCESS

The fall and rise of group B *Streptococcus* in dairy cattle: reintroduction due to human-to-cattle host jumps?

Chiara Crestani[1,*], Taya L. Forde[1], Samantha J. Lycett[2], Mark A. Holmes[3], Charlotta Fasth[4], Karin Persson-Waller[4] and Ruth N. Zadoks[1,5,6]

Abstract

Group B *Streptococcus* (GBS; *Streptococcus agalactiae*) is a major neonatal and opportunistic bacterial pathogen of humans and an important cause of mastitis in dairy cattle with significant impacts on food security. Following the introduction of mastitis control programmes in the 1950s, GBS was nearly eradicated from the dairy industry in northern Europe, followed by re-emergence in the 21st century. Here, we sought to explain this re-emergence based on short and long read sequencing of historical (1953–1978; n=44) and contemporary (1997–2012; n=76) bovine GBS isolates. Our data show that a globally distributed bovine-associated lineage of GBS was commonly detected among historical isolates but never among contemporary isolates. By contrast, tetracycline resistance, which is present in all major GBS clones adapted to humans, was commonly and uniquely detected in contemporary bovine isolates. These observations provide evidence for strain replacement and suggest a human origin of newly emerged strains. Three novel GBS plasmids were identified, including two showing >98% sequence similarity with plasmids from *Streptococcus pyogenes* and *Streptococcus dysgalactiae* subsp. *equisimilis*, which co-exist with GBS in the human oropharynx. Our findings support introduction of GBS into the dairy population due to human-to-cattle jumps on multiple occasions and demonstrate that reverse zoonotic transmission can erase successes of animal disease control campaigns.

ПАТОГЕНЕЗА МАСТИТИСА

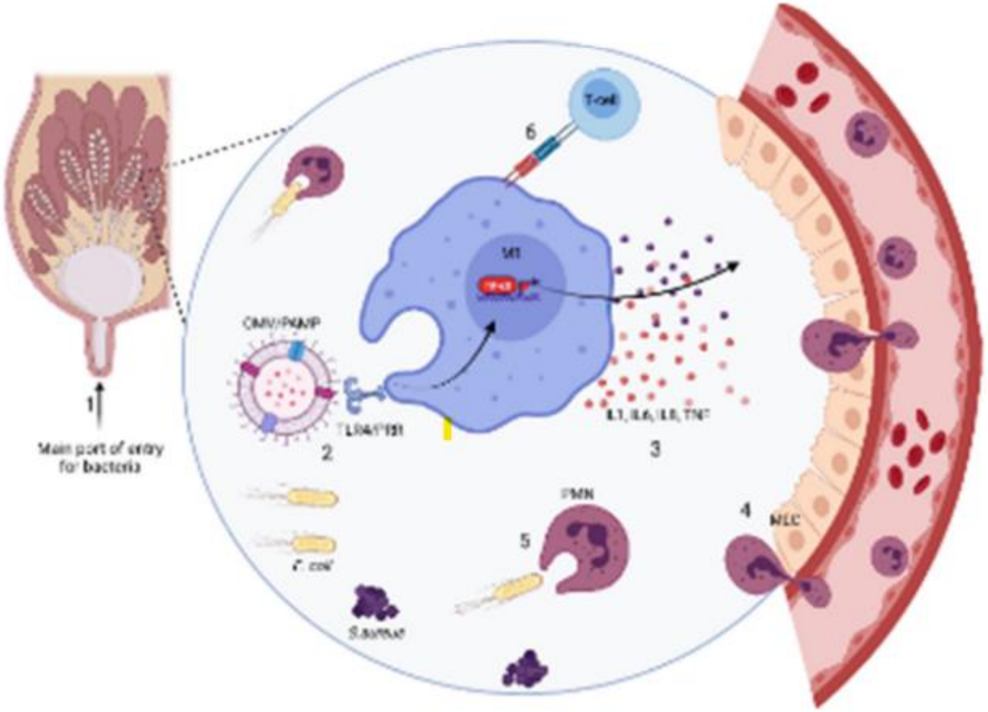
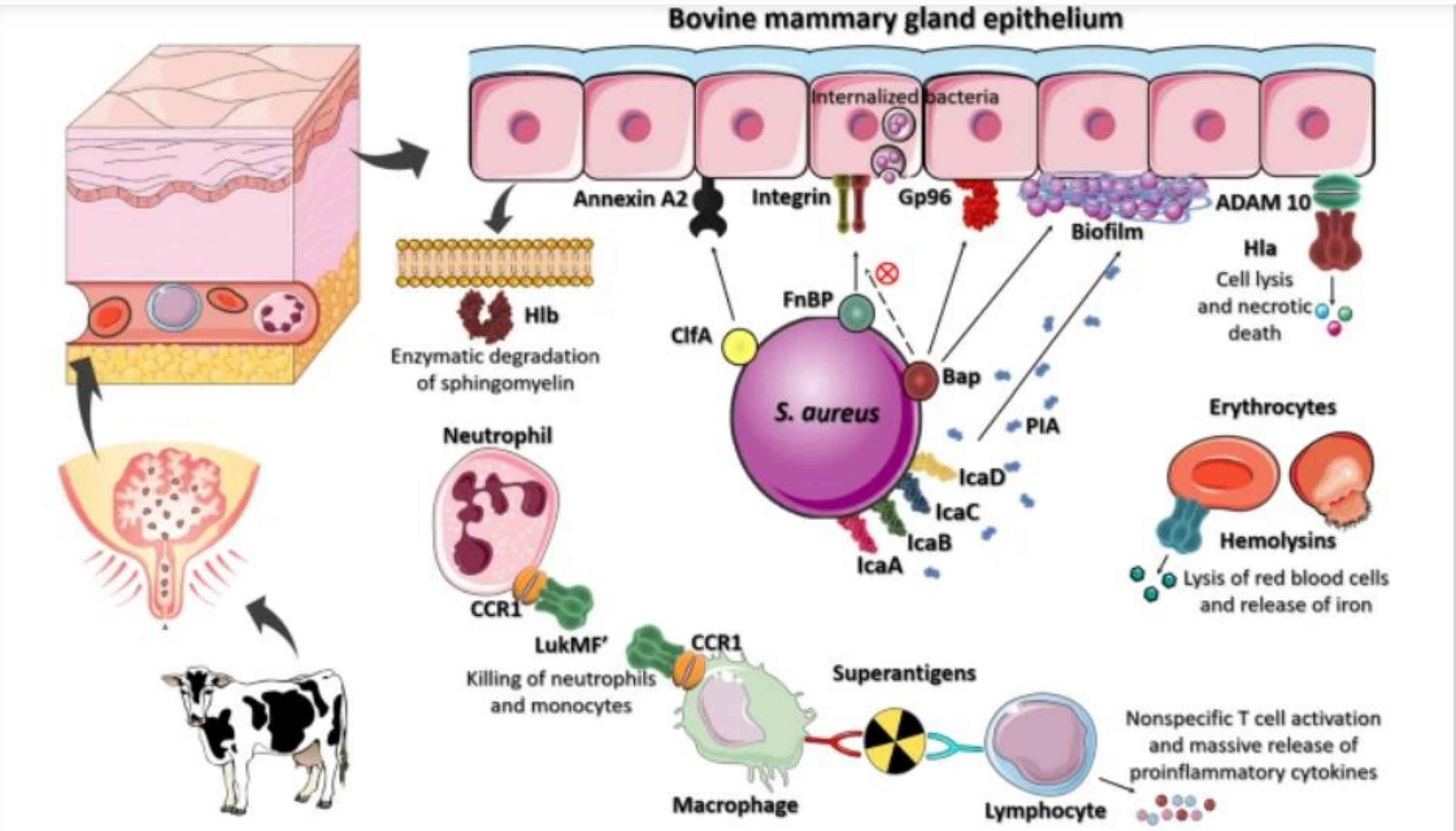


Figure 1. Schematic presentation of mastitis pathogenesis. Typically, (1) once bacteria invade the teat end and ascend into the teat canal and alveoli, a local immune reaction starts; (2) bacterial by products, such as lipopolysaccharide (LPS) or outer membrane vesicle (OMV) of Gram-negative pathogens act as pathogen-associated molecular pattern (PAMP), which is recognized by pathogen recognition receptors (PRR), specifically Toll-like receptor 4 (TLR4), on macrophage type 1 (M1). After this contact, (3) humoral elements, such as cytokines (IL1, IL6, and TNF) and chemokines [interleukin(IL)-8] are released that alert other white blood cells, mainly polymorphonuclear (PMN) leuckocytes in the systemic circulation and trigger the release of acute phase proteins; (4) Once PMN have entered in the infected area, through mammary epithelial cells (MEC), (5) they engulf and kill bacteria through phagoctysis. If this inflammation is persistent, (6) then adaptive immunity is activated via the interaction of macrophages with lymphocytes, like T-regulatory cells.

ФАКТОРИ ВИРУЛЕНЦИЈЕ *Staphylococcus aureus*



Proteins that have been implicated in the pathogenesis of *Staphylococcus aureus* of bovine origin. Surface proteins interact with host proteins to promote bacterial adhesion and invasion. Biofilm-associated protein (Bap) interferes in the internalization pathway mediated by FnBP and contributes to biofilm formation, a process that also depends on the *ica* locus. Toxins such as alpha and beta hemolysins, promote necrosis of the mammary gland tissue and lyse bovine erythrocytes to use hemoglobin as a source of iron. Leukotoxin LukMF´ binds to neutrophils present in the bovine milk. Staphylococcal superantigens activate T-cells resulting in the release of various pro-inflammatory cytokines. Figure created by authors using resources from Smart Servier Medical Art and BioRender

ПОВРШИНСКИ ПРОТЕИНИ /АДХЕЗИНИ: fibronectin-binding proteins A and B (fnbA and fnbB), fibrinogen-binding proteins also called clumping factors A and B (clfA and clfB), cell wall components (type 5 and 8 Capsules), collagen-binding proteins (can), and fibrinogen-binding proteins (fib), ica operon (PS/A, PIA), stafilokokni protein S (spA), koagulaza (coa), biofilm/associated proteins (bap)

ИНВАЗИНИ - у форми ензима и цитотоксина: леукоцидини, леукотоксини, хијалуронидаза, хемолизини

ЕГЗОТОКСИНИ – ентеротоксини (SEA до SEQ), токсин токсичног шок синдрома (TSST-1), и ексфолијативни токсини

РЕЗИСТЕНЦИЈА НА АНТИБИОТИКЕ (формирање биофилма и хоризонтални трансфер гена резистенције)

НЕСПЕЦИФИЧНА/УРОЂЕНА ОДБРАНА МЛЕЧНЕ ЖЛЕЗДЕ

1) Физичка баријера врха папиле
2) КОМПОНЕНТЕ ћелијске одбране: макрофаги, неутрофили
3) КОМПОНЕНТЕ хуморалног одговора - комплемент систем, имуно-модулирајући фактори, цитокини, лизозим, лактоферин, лактопероксидаза, трансферин, олигосахариди, реактивне врсте кисеоника, протеини акутне фазе

- Активирају се брзо на месту инфекције
- Нема функције памћења

ПРВА ЛИНИЈА ОДБРАНЕ - *Ductus papillaris*

- Примарна баријера за патогене МО - физичка баријера/извор антимикробних супстанци

- Кератински слој састављен од различитих масних киселина
  - Естерификоване и неестерификоване МК (миристинска, палмитоолеинска линоленска) имају бактериостатско деловање према: *Staph. aureus*, *Staph. hyicus*, *C. bovis* и *Str. agalactiae*

  - Катионски протеини (убиквитини) – електростатички на МО; алтерација ћелијског зида – осетљивост на промене осмотског притиска; лиза и смрт

Ћелијски елементи у секрету здраве/захваћене маститисом млечне жлезде

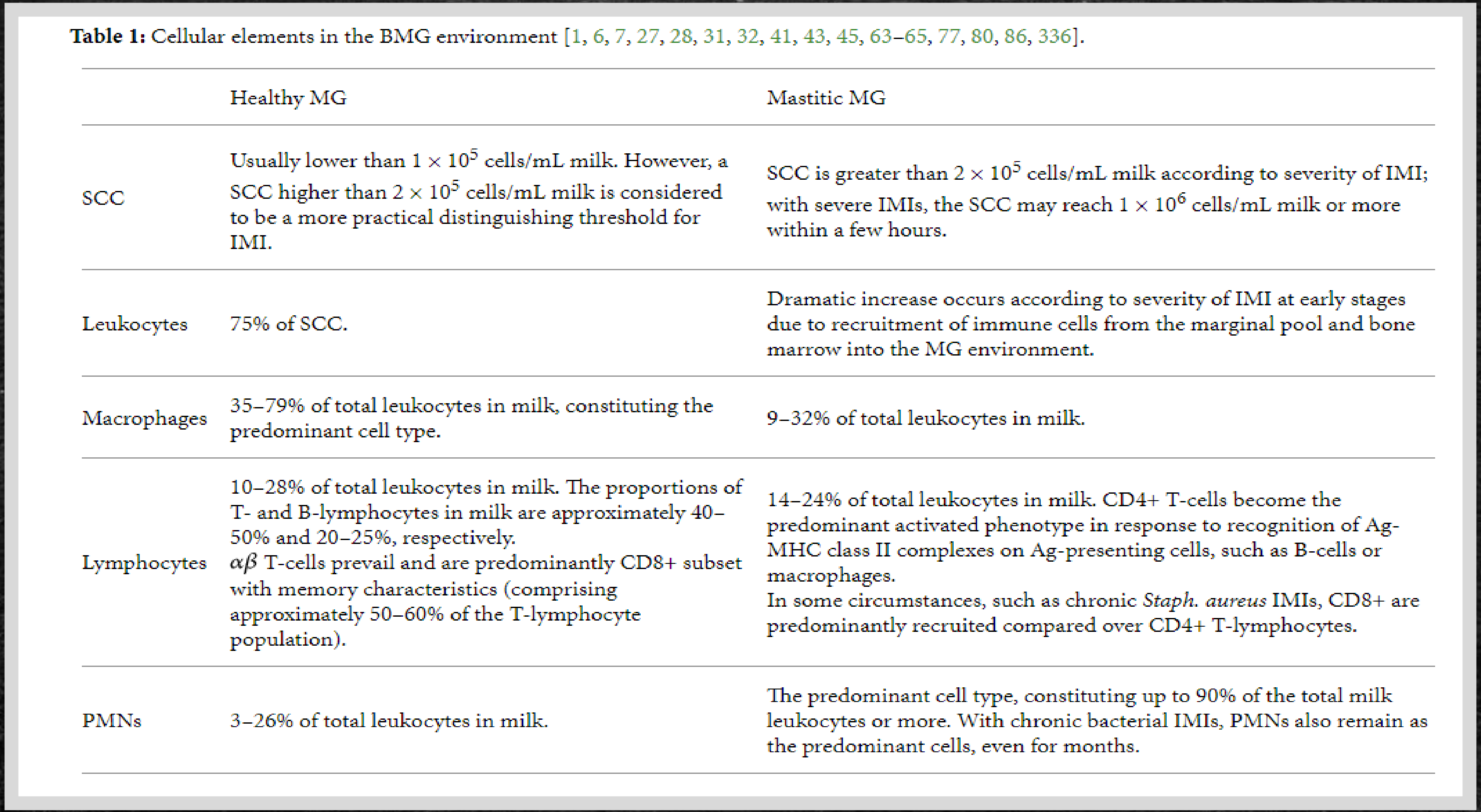


Table 1: Cellular elements in the BMG environment [1, 6, 7, 27, 28, 31, 32, 41, 43, 45, 63–65, 77, 80, 86, 336].

	Healthy MG	Mastitic MG
SCC	Usually lower than 1×10^5 cells/mL milk. However, a SCC higher than 2×10^5 cells/mL milk is considered to be a more practical distinguishing threshold for IMI.	SCC is greater than 2×10^5 cells/mL milk according to severity of IMI; with severe IMIs, the SCC may reach 1×10^6 cells/mL milk or more within a few hours.
Leukocytes	75% of SCC.	Dramatic increase occurs according to severity of IMI at early stages due to recruitment of immune cells from the marginal pool and bone marrow into the MG environment.
Macrophages	35–79% of total leukocytes in milk, constituting the predominant cell type.	9–32% of total leukocytes in milk.
Lymphocytes	10–28% of total leukocytes in milk. The proportions of T- and B-lymphocytes in milk are approximately 40–50% and 20–25%, respectively. $\alpha\beta$ T-cells prevail and are predominantly CD8+ subset with memory characteristics (comprising approximately 50–60% of the T-lymphocyte population).	14–24% of total leukocytes in milk. CD4+ T-cells become the predominant activated phenotype in response to recognition of Ag-MHC class II complexes on Ag-presenting cells, such as B-cells or macrophages. In some circumstances, such as chronic *Staph. aureus* IMIs, CD8+ are predominantly recruited compared over CD4+ T-lymphocytes.
PMNs	3–26% of total leukocytes in milk.	The predominant cell type, constituting up to 90% of the total milk leukocytes or more. With chronic bacterial IMIs, PMNs also remain as the predominant cells, even for months.

Број соматских ћелија у млеку из здраве млечне жлезде < 100.000 у мililитру. Токсини бактерија, компоненте ћелијског зида и производи метаболизма бактерија делују као хемотактични агенси за леукоците.

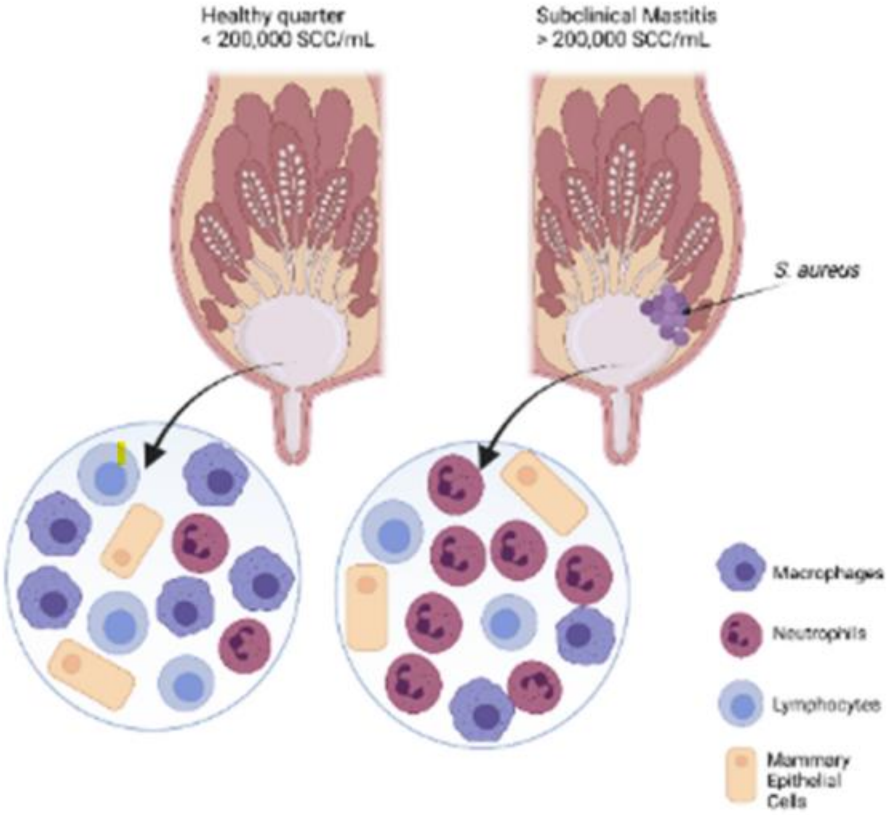


Figure 2. Presence of somatic cells in healthy and infected quarters of a cow. In the milk of a healthy quarter there are present more macrophages, followed by a small percentage of lymphocytes, neutrophils, and epithelial cells, whereas an infected quarter, with clinical mastitis or subclinical mastitis, is overpopulated with neutrophils and few macrophages, lymphocytes, and epithilail cells.

ЋЕЛИЈСКА ОДБРАНА

- Моноједарни фагоцитни систем (макрофаги)
- Полиморфонуклеарни фагоцитни систем (микрофаги) – ПМНЛ – НЕУТРОФИЛНИ ГРАНУЛОЦИТИ

ИНВАЗИЈА - ИНФЕКЦИЈА - ИНФЛАМАЦИЈА

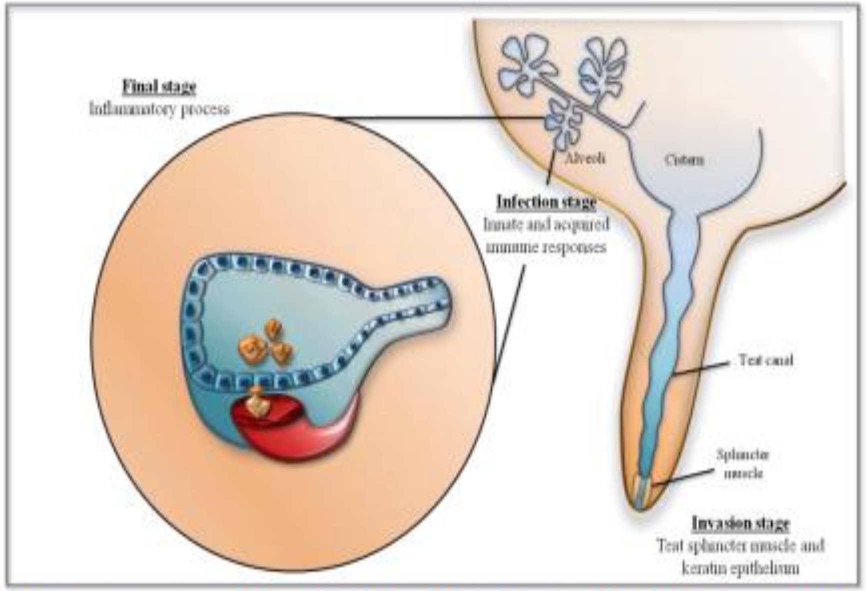


Figure 1. Schematic structure of the mammary gland and its defence barriers. During the invasion stage, bacteria must pass through the keratinised epithelium of the teat canal. These physical barriers are components of innate immunity. In the infection stage, bacteria become established within the mammary tissue. Mediators of the innate and acquired immune responses (macrophages, neutrophils, natural killer cells, lymphocytes, antibodies and cytokines) defend against this process, and the efficiency of these responses determines mastitis susceptibility. In the final stage, once the infection has been established, the presence of inflammation increases in the somatic cell count and clinical signs of infection become apparent.

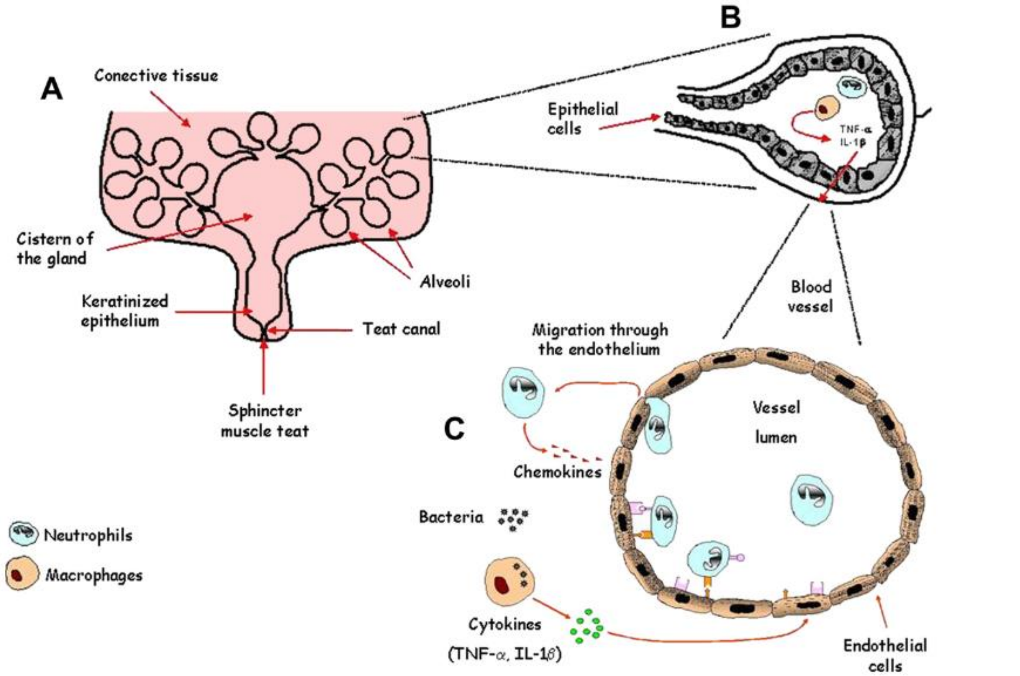


Figure 1 A. Schematic diagram of the bovine mammary gland showing the most important anatomic factors that act as defense barriers. The teat sphincter muscle represents the first line of defense, whereas the keratinized epithelium of the teat cistern is considered the second line. B. Cellular and soluble factors that participate in the innate immune response of the mammary gland. Macrophages located in the alveoli phagocytize bacteria that enter the mammary gland cistern. Activated macrophages release cytokines such as TNF-α and IL-1β. C. Endothelial cells from blood vessels adjacent to alveoli express adhesion molecules in response to pro-inflammatory cytokines; this, in turn, facilitates neutrophil recruitment from the bloodstream to the site of infection in order to eliminate the invading bacteria.

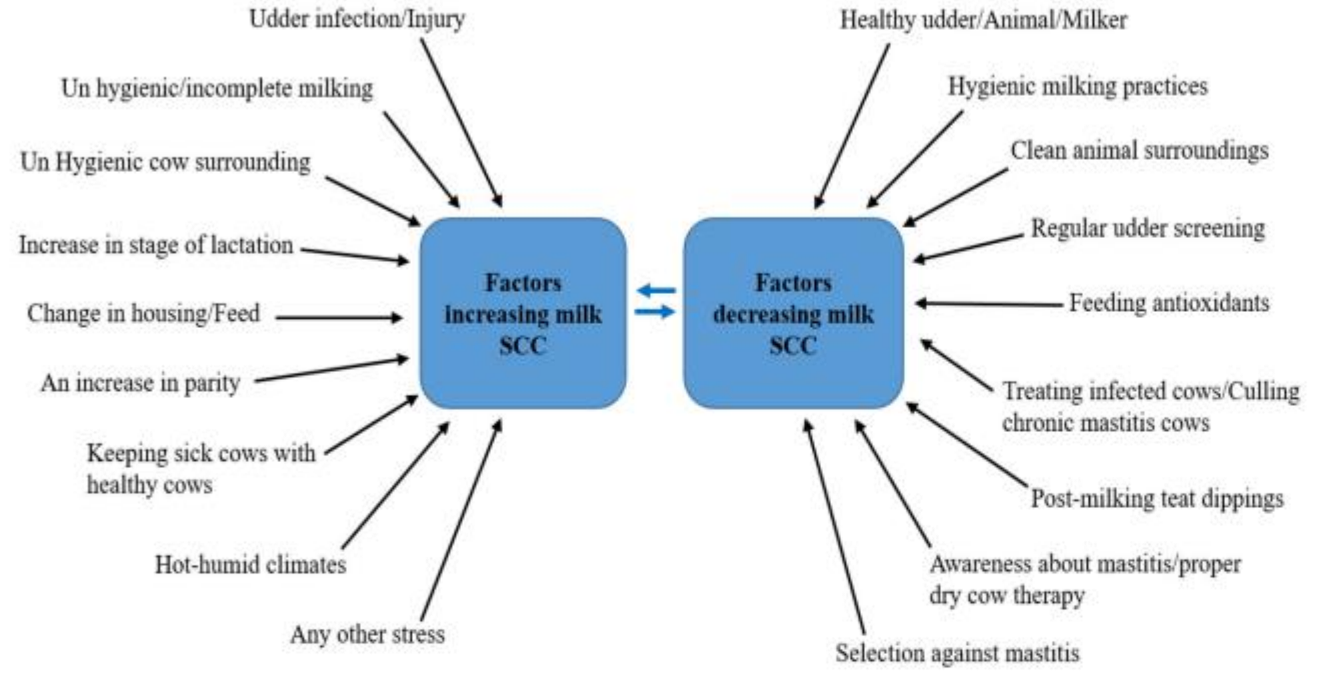


FIGURE 3 | Factors and management practices affecting the release of milk SCC. Source: Adapted from Alhussien and Dang (49).

TABLE 1 | Estimated milk losses due to increased SCC.

SCC/ml	Loss of milk (%)	Losses of milk production per dairy cow/year (kg)
100 000	3	180
200 000	6	360
300 000	7	450
400 000	8	550
500 000	9	590
600 000	10	635
700 000	10.5	680
800 000	11	725
900 000	11.5	750
1 000 000	12	770
1 600 000	12	770

Source: Tongel and Mihina (52).

СМАЊЕЊЕ БРОЈА СОМАТСКИХ ЋЕЛИЈА У ЗБИРНОМ МЛЕКУ КРАВА

- КРАВЕ СА ПОВЕЋАНИМ БРОЈЕМ СОМАТСКИХ ЋЕЛИЈА СЕ ИЗДВАЈАЈУ И МУЗУ ОДВОЈЕНО
- МЛЕКО ТИХ КРАВА СЕ НЕ МЕША СА ОСТАЛИМ МЛЕКОМ СА ФАРМЕ
- У СТАДУ ПРОСЕЧНЕ ВЕЛИЧИНЕ, ЈЕДНА КРАВА СА ПОВЕЋАНИМ БРОЈЕМ СОМАТСКИХ ЋЕЛИЈА МОЖЕ ДА ЗА 5-50% ПОВЕЋА БРОЈ СОМАТСКИХ ЋЕЛИЈА У ЗБИРНОМ МЛЕКУ

БРОЈ СОМАТСКИХ ЋЕЛИЈА У ПРОГРАМИМА ЗА СУЗБИЈАЊЕ МАСТИТИСА

- КРАВЕ СА ПОВЕЋАНИМ БРОЈЕМ СОМАТСКИХ ЋЕЛИЈА ТОКОМ ЛАКТАЦИЈЕ, И ИЗ ЛАКТАЦИЈЕ У ЛАКТАЦИЈУ, ИСКЉУЧУЈУ СЕ ИЗ ПРОИЗВОДЊЕ

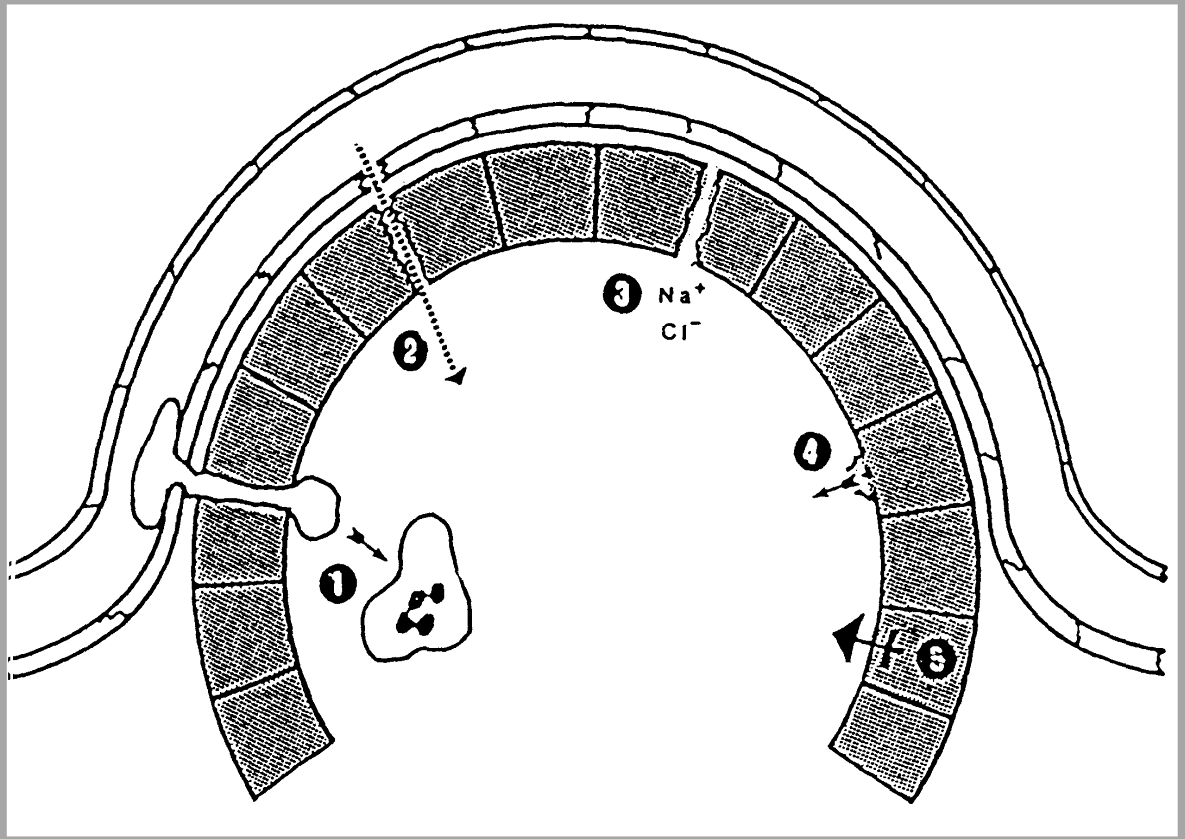
- КРАВЕ СА УТВРЂЕНОМ ИНТРАМАМАРНОМ ИНФЕКЦИЈОМ У ТРИ ИЛИ СВЕ ЧЕТИРИ ЧЕТВРТИ, ИСКЉУЧУЈУ СЕ ИЗ ПРОИЗВОДЊЕ

- КРАВЕ КОЈЕ СУ ЛЕЧЕНЕ ОД МАСТИТИСА ТРИ И ВИШЕ ПУТА, ИСКЉУЧУЈУ СЕ ИЗ ПРОИЗВОДЊЕ

ПРОМЕНА САСТАВА МЛЕКА ПРИ МАСТИТИСИМА

1. Прелаз ћелија из крви у млеко
2. Прелаз BSA из крви у млеко,

3. Јонске промене у млеку,
4. Дисрупција ћелија,
5. Смањење садржаја лактозе, казеина и масти



УТИЦАЈ МАСТИТИСА НА САСТАВ И ОСОБИНЕ МЛЕКА

- СМАЊЕЊЕ КОЛИЧИНЕ ЛАКТОЗЕ У ЦИТОСОЛУ – ИЗОТОНИЈА – ПРЕЛАЗАК ЈОНА
- СМАЊЕЊЕ САДРЖАЈА МАСТИ
  - Већи проценат естерификованих МК саC_4- C_{12}, а мањи проценат засићених МК/C16:0-C18:0/
  - Промене на мембрани масне капљице
  - Већи садржај слободних масних киселина
- Укупан садржај протеина се не мења
  - Смањење садржаја α -казеина, β- казеина, α-лакталбумина и β-лактоглобулина
  - Повећање БСА и имуноглобулина
  - Повећање садржаја паракапа казеина
  - Мањи садржај мицеларног казеина (46%)

УТИЦАЈ МАСТИТИСА НА ПРЕРАДУ МЛЕКА

- ПРОИЗВОДЊА ПАСТЕРИЗОВАНОГ МЛЕКА
- ПРОИЗВОДЊА ФЕРМЕНТИСАНИХ ПРОИЗВОДА ОД МЛЕКА
- ПРОИЗВОДЊА СИРА

ЗНАЧАЈ СУБКЛИНИЧКИХ МАСТИТИСА ЗА БЕЗБЕДНОСТ МЛЕКА И ПРОИЗВОДА ОД МЛЕКА

- УЗРОЧНИЦИ МАСТИТИСА ПАТОГЕНИ ЗА ЉУДЕ
  - *Streptococcus agalactiae* и *Staphylococcus aureus*,
  - ређе *Listeria monocytogenes*, *Brucella abortus*

- *Staphylococcus aureus*- ПРОДУКЦИЈА ЕНТЕРОТОКСИНА; MRSA

- AMR – ЛАТЕРАЛНИ ТРАНСФЕР ГЕНА

ДИЈАГНОСТИКА СУБКЛИНИЧКИХ МАСТИТИСА - IDF

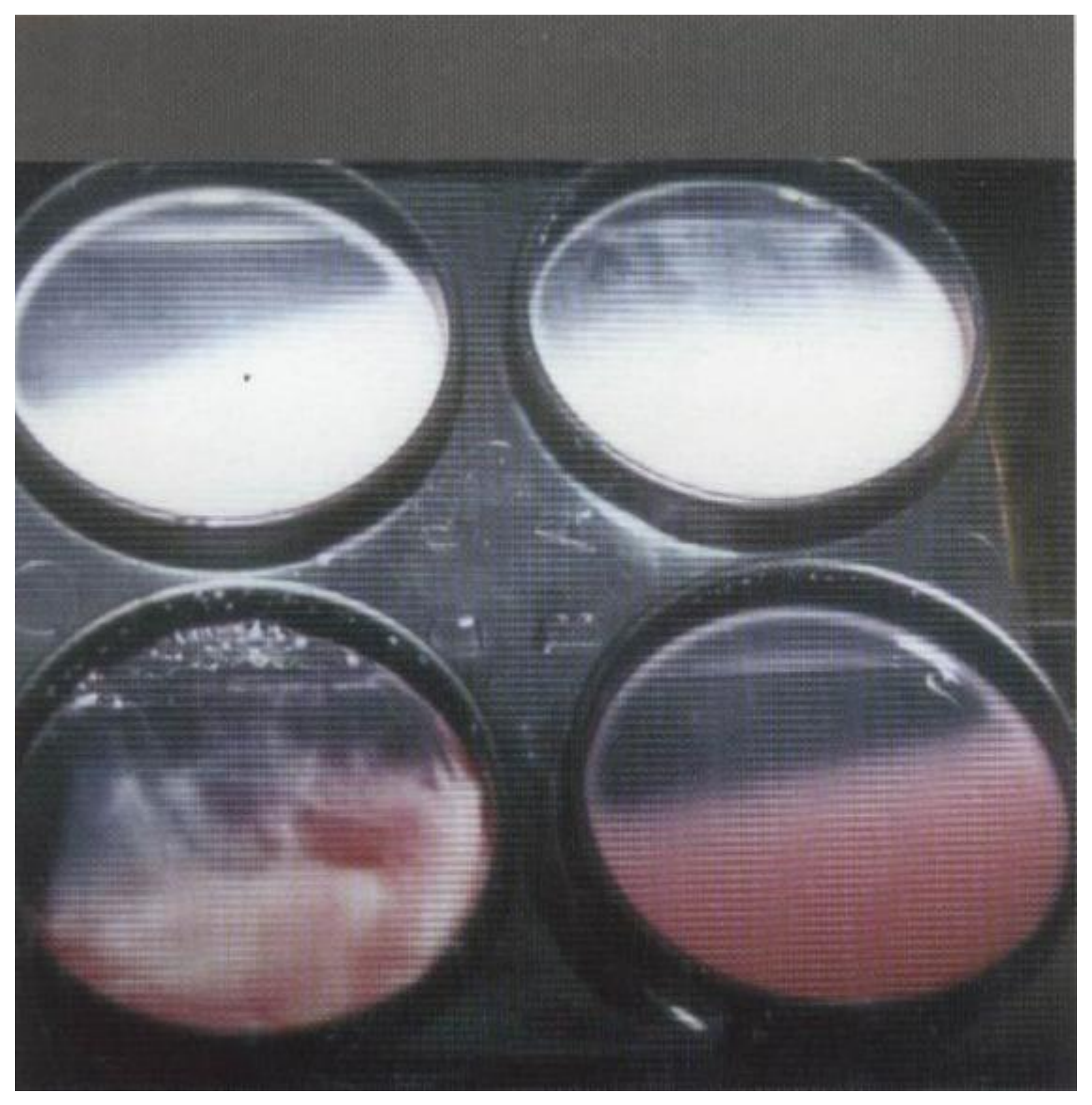
БСЋ/мл	**ПАТОГЕНИ МО**	
	НИСУ ИЗОЛОВАНИ	ИЗОЛОВАНИ
<500 000	ЗДРАВО ВИМЕ	ЛАТЕНТНА ИНФЕКЦИЈА
>500 000	НЕСПЕЦИФИЧНИ МАСТИТИС (ПОРЕМЕЋАЈ У СЕКРЕЦИЈИ)	МАСТИТИС

ЧИЊЕНИЦЕ

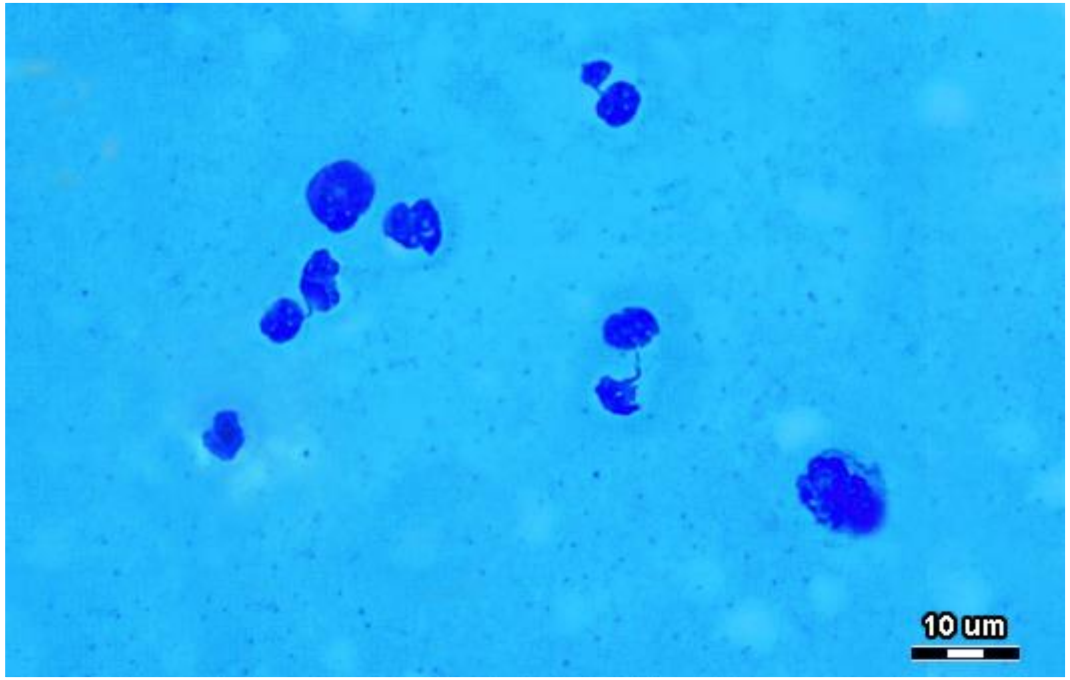
- РЕПРЕЗЕНТАТИВНЕ ФАРМЕ – 2-5% клиничких маститиса
- СУБКЛИНИЧКИ МАСТИТИСИ – 20-50 пута чешћи од клиничких
- 70% НОВИХ ИНФЕКЦИЈА – У ПРВОМ НЕДЕЉИ ЗАСУШЕЊА
- Субклиничка форма прелази у клиничку – у првих 14 дана по телењу
- ОКО 30% НОВИХ ИНФЕКЦИЈА НАСТАЈЕ У ЛАКТАЦИЈИ – ПРВЕ ТРИ НЕДЕЉЕ ЛАКТАЦИЈЕ (половина инфекција насталих у лактацији, преноси се у нову лактацију)

МЕТОДЕ ЗА ОДРЕЂИВАЊЕ БРОЈА СОМАТСКИХ ЋЕЛИЈА У МЛЕКУ

- ДИРЕКТНО МИКРОСКОПСКО ОДРЕЂИВАЊЕ БРОЈА СОМАТСКИХ ЋЕЛИЈА
- МАСТИТИС ТЕСТ, Whiteside ТЕСТ
- ПОМОЋУ СПЕЦИЈАЛНИХ АПАРАТА



МАСТИТИС ТЕСТ


10 um


FOSS

ПРОГРАМ ЗА СУЗБИЈАЊЕ МАСТИТИСА

- ЕКОНОМСКИ ОПРАВДАН
- ПОКАЗУЈЕ ВИДЉИВЕ РЕЗУЛТАТЕ
- ЛАКО ПРИМЕЊИВ
- ОХРАБРУЈУЋИ ЗА ФАРМЕРЕ

ОСНОВЕ ПРОГРАМА ЗА СУЗБИЈАЊЕ МАСТИТИСА

А. СКРАЋЕЊЕ ТРАЈАЊА ИНФЕКЦИЈЕ

B. РЕДУКОВАЊЕ ПОЈАВЕ НОВИХ ИНФЕКЦИЈА

C. ПРАЋЕЊЕ СТОПЕ ПОРАСТА ИНФЕКЦИЈА

СКРАЋЕЊЕ ТРАЈАЊА ИНФЕКЦИЈА

- ЛЕЧЕЊЕ У ЛАКТАЦИЈИ
- ТЕРАПИЈА У ЗАСУШЕЊУ
- ИСКЉУЧИВАЊЕ ИЗ ПРОИЗВОДЊЕ КРАВА СА ХРОНИЧНИМ КЛИНИЧКИМ МАСТИТИСИМА

ИЗБОР ПРОГРАМА ЗА СУЗБИЈАЊЕ МАСТИТИСА

- ТЕРАПИЈА СВИХ КРАВА У ЗАСУШЕЊУ ПРИМЕЊУЈЕ СЕ САМО АКО:
  - ПРОЦЕНАТ ИНФИЦИРАНИХ ЧЕТВРТИ ВЕЋИ ОД 40-50-%,
  - БРОЈ СОМАТСКИХ ЋЕЛИЈА У МЛЕКУ ВЕЋИ ОД 500 000/ml
  - СРЕДЊА ВРЕДНОСТ БРОЈА СОМАТСКИХ ЋЕЛИЈА У МЛЕКУ ВЕЋА ОД 250 000/ml
  - АКО ИМА 4 и ВИШЕ КЛИНИЧКИХ МАСТИТИСА НА 100 КРАВА

РЕДУКЦИЈА ПОЈАВЕ НОВИХ ИНФЕКЦИЈА

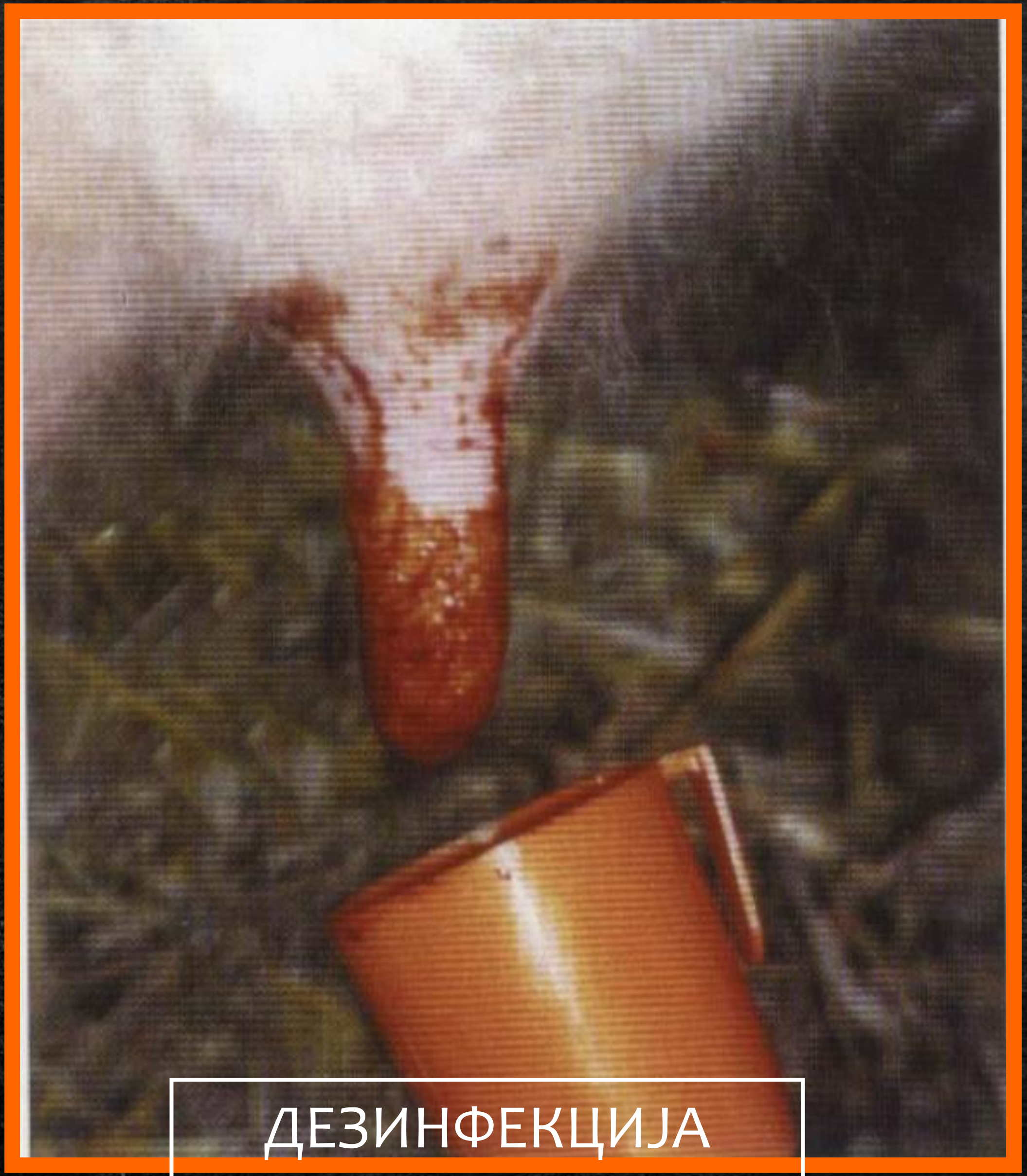
- ХИГИЈЕНА ПРИ МУЖИ
  - ДЕЗИНФЕКЦИЈА ПАПИЛА ПРЕ МУЖЕ (маститиси узроковани МО из околине)
  - ДЕЗИНФЕКЦИЈА СИСНИХ ЧАША ПОСЛЕ МУЖЕ СВАКЕ КРАВЕ – МЕЂУФАЗНА ДЕЗИНФЕКЦИЈА
  - ДЕЗИНФЕКЦИЈА ПАПИЛА ПОСЛЕ МУЖЕ

ДЕЗИНФЕКЦИЈА ПАПИЛА ПОСЛЕ МУЖЕ

- ДЕЗИНФИЦИЈЕНС ЗА ПОТАПАЊЕ ПАПИЛА ПОСЛЕ МУЖЕ:

  - не сме да оштеђује кожу папила
  - потпомаже санирање лезија на папилама
  - уништава све узрочнике маститиса на папили после муже
  - има продужено деловање и спречава контаминацију вимена између две муже
  - не утиче на хигијенску исправност млека

ПРИБОР ЗА ДЕЗИНФЕКЦИЈУ ПАПИЛА



ДЕЗИНФЕКЦИЈА ПАПИЛА ПОСЛЕ МУЖЕ

The 5 Point Plan

1. Teat disinfection - after every milking
2. Antibiotic drying off - dry cow therapy
3. Prompt antibiotic therapy -clinical cases
4. Culling cows - with repeated cases
5. Milking machine maintenance

Figure 6. New Zealand leaflet (MAF, 1974)

ИЗБОР ПРОГРАМА ЗА СУЗБИЈАЊЕ МАСТИТИСА

I. ТЕРАПИЈА У ЗАСУШЕЊУ → ИНФЕКЦИЈА У СТАДУ 15-18%

II. ТЕРАПИЈА У ЗАСУШЕЊУ, ДЕЗИНФЕКЦИЈА ПАПИЛА ПОСЛЕ МУЖЕ, КОНТРОЛА МАШИНА ЗА МУЖУ → НИВО ИНФЕКЦИЈЕ У СТАДУ ДО 10%

III ТЕРАПИЈА У ЗАСУШЕЊУ, ДЕЗИНФЕКЦИЈА ПАПИЛА ПРЕ И ПОСЛЕ МУЖЕ; КОНТРОЛА МАШИНА ЗА МУЖУ, ЛЕЧЕЊЕ СУБКЛИНИЧКИХ МАСТИТИСА У ЗАСУШЕЊУ → НИВО ИНФЕКЦИЈЕ У СТАДУ ДО 7%

- У СВА ТРИ ПРОГРАМА КЛИНИЧКИ МАСТИТИСИ СЕ ЛЕЧЕ У ЛАКТАЦИЈИ, А КРАВЕ СА ХРОНИЧНИМ ИНФЕКЦИЈАМА ВИМЕНА СЕ ИСКЉУЧУЈУ ИЗ ПРОИЗВОДЊЕ

ВЕНТИЛАЦИЈА

УДОБНО

ЧИСТО

СУВО

ОДРЖАВАЊЕ Т подлоге штала - 10°C ниже од телесне температуре