

NON-ANTIBIOTIC APPROACHES TO CONTROL MASTITIS IN DAIRY COWS

4 November 2024 – 30 April 2026

Visegrad Grant No. 22420065

Visegrad Fund project team meeting
9. – 10.2.2026

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The mission of the fund is to advance ideas for sustainable regional cooperation in Central Europe.



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Implementation and participating countries

Project start: 11/2024

Project end: 04/2026

The general mission of the Visegrad Fund is to advance ideas for sustainable regional cooperation in Central Europe.

Slovakia

Czech Republic

Poland

Hungary

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Organizations

Main applicant: University of Veterinary Medicine and Pharmacy in Košice, Slovakia

Coordinator of the project: Zuzana Lacková, DVM., PhD.

Partner 1: Mendel University in Brno, Czech Republic

co-researcher: Petr Sláma, Assoc. prof., Ing., PhD.

Partner 2: Wroclaw University of Environmental and Life Sciences, Poland

co-researcher: Ewa Pecka-Kielb, Dr. hab. inż. PhD, prof. Uczelni

Partner 3: University of Veterinary Medicine Budapest, Hungary

co-researcher: David Sandor Kiss, Assoc. prof., Dr. PhD.

Website

The screenshot shows the website interface for the project. At the top, there is a navigation bar with links: AIS | Outlook Office 365 | Moodle | WebKredit | Informácie pre uchádzačov | Informácie pre študentov | Kontakty | Mapa univerzity. On the right of this bar are flags for Slovakia and the United Kingdom. Below the navigation bar is the university logo and name: UNIVERZITA VETERINÁRSKEHO LEKÁRSTVA A FARMÁCIE V KOŠICIACH. To the right of the logo are icons for Outlook Office365, Stravovanie WebKredit, Mapa univerzity, Portál AIS, and Intranet. A secondary navigation bar contains: UNIVERZITA | UCHÁDZAČI | ŠTUDENTI | VEDA A VÝSKUM | SLUŽBY | VEREJNOSŤ | KONTAKTY | PORTÁL KVALITY. Below this is a dark green banner with the title: NON-ANTIBIOTIC APPROACHES TO CONTROL MASTITIS IN DAIRY COWS and a home icon labeled DOMOV. Under the banner is a breadcrumb trail: MEDZINÁRODNÝ VYŠEHRADSKÝ FOND > NON-ANTIBIOTIC APPROACHES TO CONTROL MASTITIS IN DAIRY COWS. The main content area features the title: NON-ANTIBIOTIC APPROACHES TO CONTROL MASTITIS IN DAIRY COWS. At the bottom left of the content area is the Visegrad Fund logo.

Website

<https://v4dairycows2024.uvlf.sk/>

NON-ANTIBIOTIC APPROACHES TO CONTROL MASTITIS IN DAIRY COWS

• Visegrad Fund

4 November 2024 – 30 April 2026



• Mendelova
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• v Brně



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ACKNOWLEDGEMENT

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• Mendel
• University
• in Brno



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Objectives of the project

- The project objectives focused on addressing current issues arising from dairy farming conditions.
- Three main domains covering the following areas:

1. DIAGNOSIS OF MASTITIS CAUSED BY CONTAGIOUS AND ENVIRONMENTAL BACTERIA

2. INTRODUCTION OF SELECTIVE DRYING PROGRAMMES FOR COWS

3. DEVELOPMENT OF A NANOPARTICLE-BASED DISINFECTANT

1. DIAGNOSIS OF MASTITIS CAUSED BY CONTAGIOUS AND ENVIRONMENTAL BACTERIA

- Examination of 8 dairy farms
- 3 farms in Slovakia
- 2 farms in Poland
- 2 farms in the Czech Republic
- Total 1100 cows
- Tested 4400 quarters with CMT

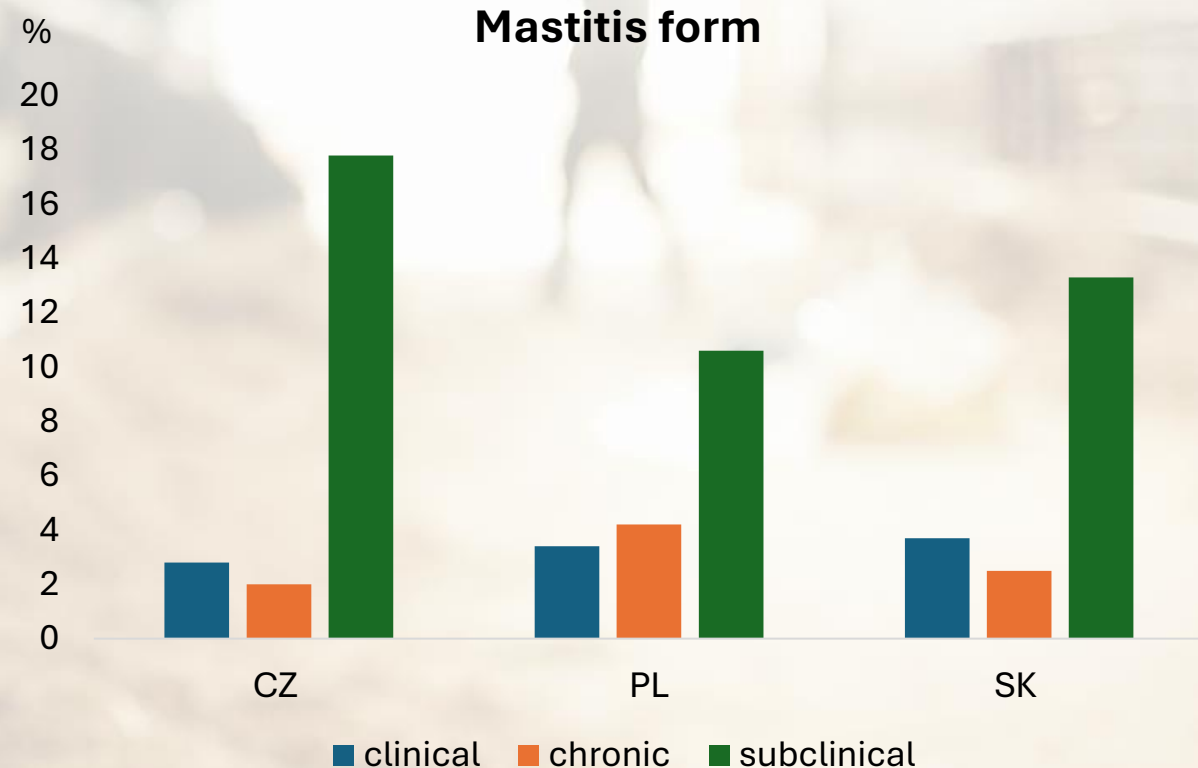


Figure: Clinical mastitis cases with positive bacteriological cultivation

Note: (A1 – A2) clinical IMI caused of *Str. uberis*, (B1 – B2) clinical IMI caused of *S. aureus*

1. DIAGNOSIS OF MASTITIS CAUSED BY CONTAGIOUS AND ENVIRONMENTAL BACTERIA

- 847 healthy cows (77%)
- 253 CMT positive result (23%)
- 230 infected samples (21%)
- highest incidence of subclinical forms of mastitis in all monitored farms (10,6% - 17,8%)
- incidence of clinical forms between 2-4,2%



1. DIAGNOSIS OF MASTITIS CAUSED BY CONTAGIOUS AND ENVIRONMENTAL BACTERIA

- 136 cases (59.1% of the infected samples) contained the most commonly isolated staphylococci.
- Non-aureus staphylococci (NAS) represented the most commonly detected bacteria (42.6% of positive findings)
- *S. aureus* (16.5 %) were the second most abundant pathogens
- *E. coli* (11.3%)
- streptococci (*Str. uberis*: 9.1%; *Str. agalactiae*: 3.4%)
- enterococci (6.1%).

Table: Udder pathogens isolated from milk samples of all monitored dairy herds

Pathogens	Number of isolates	% (n=230)
NAS	98	42.6
<i>S. aureus</i>	38	16.5
<i>Escherichia coli</i>	26	11.2
<i>Streptococcus uberis</i>	21	9.1
<i>Streptococcus agalactiae</i>	8	3.4
<i>Streptococcus</i> spp.	10	4.3
<i>Enterococcus</i> spp.	14	6.1
Mixed infection	15	6.5
Total	230	100

1. DIAGNOSIS OF MASTITIS CAUSED BY CONTAGIOUS AND ENVIRONMENTAL BACTERIA

- In 136 isolates of Staphylococci, *in vitro* resistance to 14 antimicrobials
- Of the tested staphylococci, **105 isolates** (77.2%) showed resistance to one or more antimicrobials:
 - To one antimicrobial, **51 isolates** (37.5%) were resistant.
 - To two or more antimicrobials, **54 isolates** (39.7%) of all tested staphylococci were resistant.
- Multi-drug resistance to three or more antimicrobial classes was recorded in **16 isolates** (11.7%).
- Tested staphylococci showed multi-resistance to a combination of antimicrobial classes, such as **aminoglycosides, β -lactams and macrolides**.

1. DIAGNOSIS OF MASTITIS CAUSED BY CONTAGIOUS AND ENVIRONMENTAL BACTERIA

- *S. aureus* still comes on top in the number of chronic or severe mastitis cases, as well as the number of virulence factors but some NAS species could have same aggressive potential based on their production of hemolysis, biofilm and multi-drug resistance.
- Regarding the virulence of both *S. aureus* and NAS species associated with bovine mastitis, especially in combination with resistance patterns, is important for designing efficient prophylaxis and treatment guidelines.

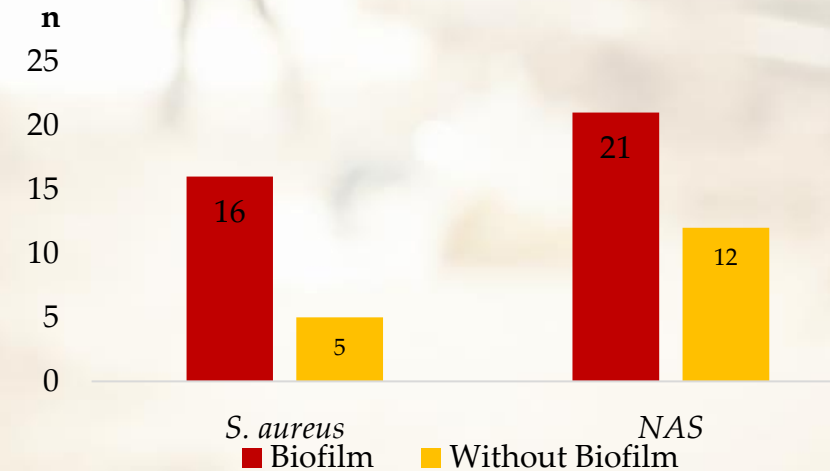


Figure: Comparison of resistance to more than one antimicrobials in isolates forming or non-forming biofilm

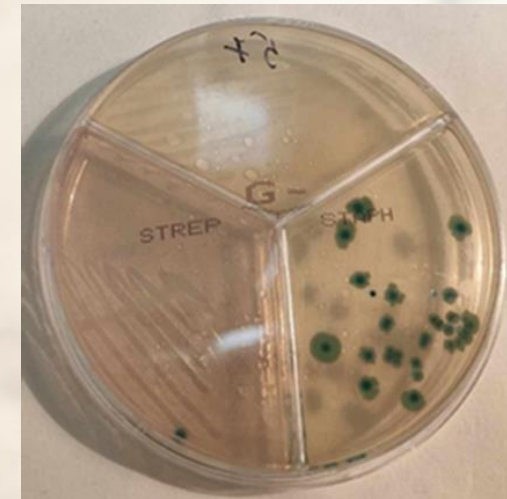
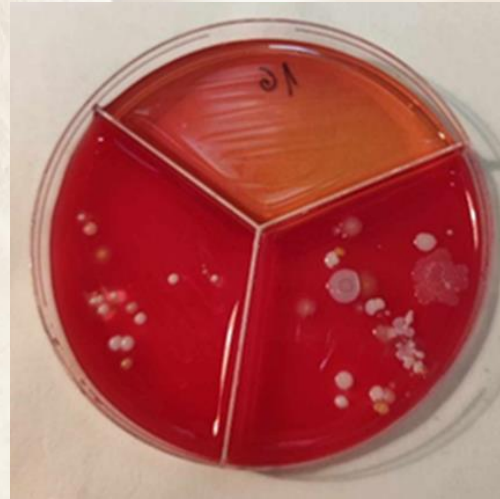
2. INTRODUCTION OF SELECTIVE DRYING PROGRAMMES -FOR DAIRY COWS

- main goal was to reduce the use of intramammary antibiotics during drying therapy and to introduce farm cultivation tests
- included 600 cows from two dairy farms
- clinical examination each cow and analysis based on the results of milk yield control
- introduction of rapid farm cultivation tests
- selection of the method of drying of dairy cows (without or with the use of intramammary ATB)

2. INTRODUCTION OF SELECTIVE PROGRAMMES OF DRYING FOR DAIRY

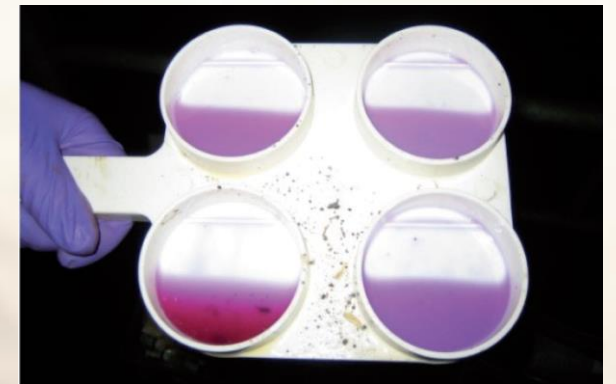
- Before drying therapy, quarter milk samples were examined and two methods for rapid detection of udder pathogens were compared: **MicroMast** rapid plates versus the **ClearMilk Test**

Figure: Samples cultivation on MicroMast and ClearMilk test



2. INTRODUCTION OF SELECTIVE PROGRAMMES OF DRYING FOR DAIRY

- results showed that although the CMT under on-farm conditions does not enable pathogen identification, it can indicate ongoing or early-stage udder inflammation through elevated SCC.
- comparing two rapid milk sample cultivation methods, the sensitivity of positive samples was 84.7% with MicroMast plates and 93.1% with the ClearMilk test.



2. INTRODUCTION OF SELECTIVE PROGRAMMES OF DRYING FOR DAIRY

- After clinical examination, CMT evaluation and rapid farm culture tests, the cows were dried as follows:

The first category - dairy cows, in which intramammary infections not been recorded during the lactation.

- These dairy cows were dried without antibiotics, using keratin preparation (which definitely closes the teat canal and prevents pathogenic germs from entering it) or a combination of keratin preparation and teat immersion in a protective solution.



2. INTRODUCTION OF SELECTIVE PROGRAM DRYING FOR DAIRY



- **The second group** - consists of dairy cows with an SCC higher than 400×10^3 in 1 ml; mastitis has been recorded during lactation, but the current mammary gland does not show clinical inflammation.
- In this case, an intramammary antibiotic with long-term effect was applied to each quarter, in conjunction with a keratin plug or a teat protective solution.
- The selection of an antibiotic for drying was based on culturing quarter milk samples and mapping the overall sensitivity of bacteria to the antibiotics used (antibiogram) in each holding.

2. INTRODUCTION OF SELECTIVE PROGRAMMES OF DRYING FOR DAIRY

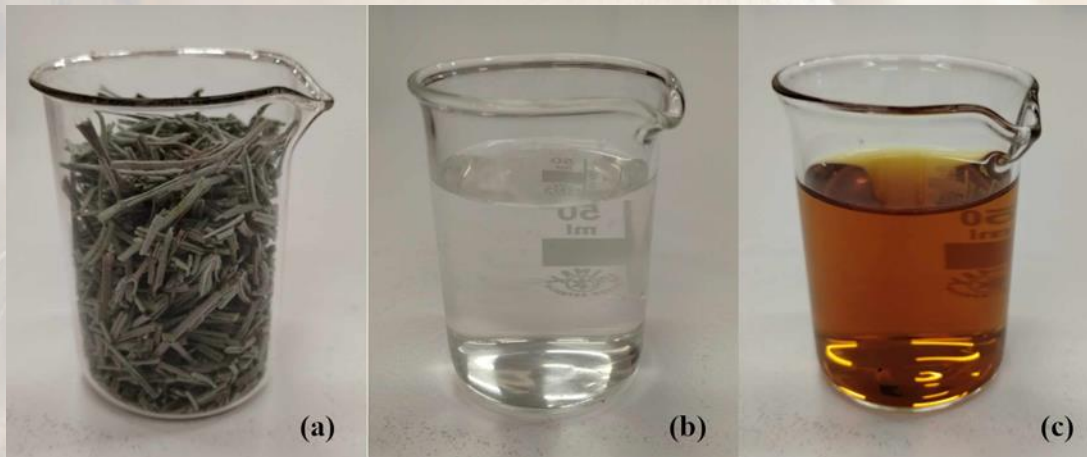
The third group - included dairy cows with current clinical or chronic mastitis, and dairy cows with dry quarters on which an antibiotic preparation was applied.

- In these dairy cows, it was necessary to treat the infected quarter with intramammary antibiotics during proper milking.
- Following this, the dairy cows in the second group were dried according to the above scheme using an intramammary antibiotic preparation, and the teat was closed with teat sealant or with a protective teat solution.

3. Development of a nanoparticle-based disinfectant

- goal focused on the development of a new disinfectant for mammary teats after milking
- novel process for synthesizing silver nanoparticles using dry lavender leaves (Lav-AgNPs) as both a reducing agent and stabilizer, and evaluates their inhibitory effect on udder pathogens of dairy cows

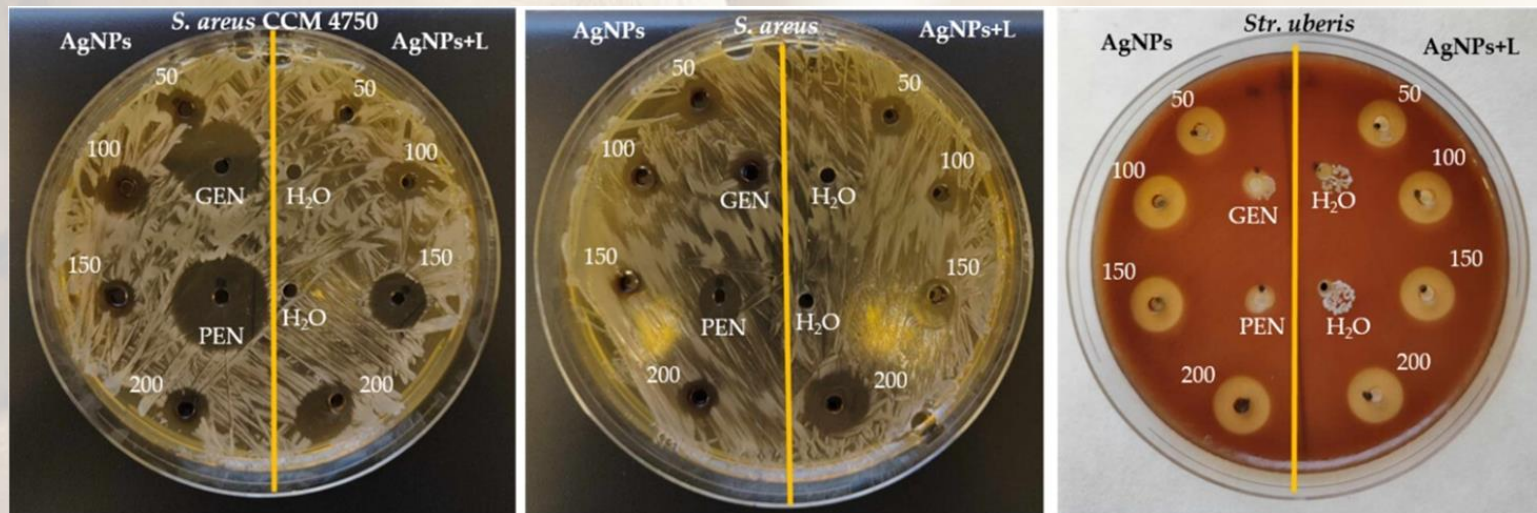
Figure: Dry lavender leaves (a), AgNO₃ solution (b) and extract of dried lavender leaves (c)



3. Development of a nanoparticle-based disinfectant

- In-vitro bactericidal testing of Lav-AgNP concentrations of 150 and 200 $\mu\text{g/ml}$ showed 100% or higher effect against resistant udder pathogens *S. aureus* and *Str. uberis*. For the other tested NAS, the relative efficacy was lower than 100%.

Figure: *In-vitro* bactericidal potential of Lav-AgNPs and pure AgNPs against selected resistant udder pathogens ($\mu\text{g/ml}$)



3. Development of a nanoparticle-based disinfectant

- new green-synthesis process with plant extracts such as lavender has proven to be an effective and promising approach for eliminating udder pathogens.
- due to the relatively rapid preparation of disinfectant solutions and the cost-effective use of plant materials, green synthesis of nanoparticles appears to be a modern, forward-looking direction in hygiene and veterinary medicine.

3. Development of a nanoparticle-based disinfectant

- In the near future, we will test this product on cows during the milking process and compare it with other disinfectants.
- Proposed title: **Effects of Biosynthesized Silver Nanoparticles and Other Disinfectants on the Microbial Load of the Cow's Udder**

Authors: Zigo František, Vargová Mária, Balážová Ľudmila, Ondrašovičová Silvia, Lacková Zuzana, Farkašová Zuzana, Pecka-Kielb Ewa, Pavlík Aleš, Schilling-Tóth Boglárka Mária



Project events

- **1st meeting of project research teams (online), Košice, SK**

In 04.11.2024 Slovakia, UVMP Košice

- **1st research phase – Data collection**

From 19.1.2025 to 21.1.2025 Czechia, Brno

- **Participation in conference**

- **From 26.3.2025 to 28.3.2025 Slovakia, Piešťany**

- **2nd research phase – Data collection**

From 1.9.2025 to 3.9.2025 Hungary, Budapest

Project events

➤ **3rd research phase – Data collection**

From 2.10.2025 to 3.10.2025 Poland, Wroclaw

➤ **Participation in conference**

From 20.11.2025 to 21.11.2025 Hungary, Budapest

➤ **2nd meeting of project research teams, Košice, SK**

From 09.02.2026 to 10.02.2026 Slovakia, UVMP Košice

➤ **Participation in conference**

From 02.3.2026 to 04.3.2026 Poland, Zakopane

Outputs

1x Scientific book in review

2x Research article in review

3x Conference

(Slovakia, Piešťany; Poland, Wrocław; Hungary, Budapešť)

1x Planned Conference (Poland, Zakopane)

Scientific book (137 pp.)

Advances in the Understanding and Management of Mastitis in Dairy Cows

Category: professional monograph

Authors: Zuzana Lacková, Ibrahim F. Rehan, František Zigo, Ewa Pecka-Kielb, Petr Sláma, David Sandor Kiss

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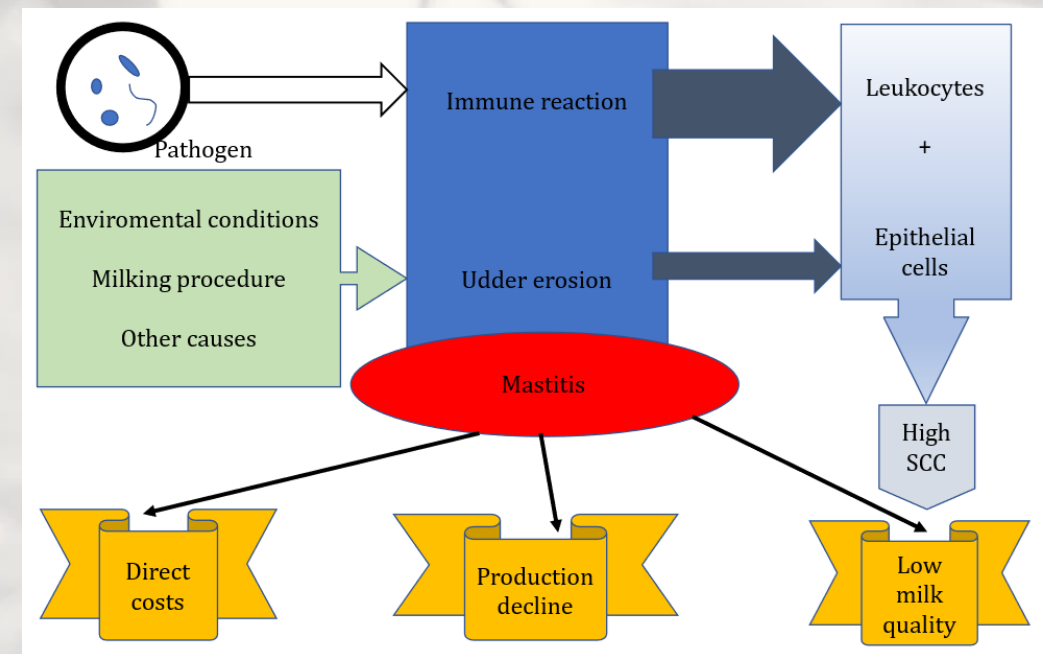
Scientific book

The central aim of this book is to deepen the reader's understanding of mastitis.

This knowledge helps reduce infection rates, thereby improving both dairy farm profitability and animal welfare, the authors will consider their objective met.

Accordingly, this book seeks to explore and synthesize current knowledge on the:

- etiology,
 - pathogenesis,
 - risk factors,
 - diagnostic methods,
 - treatment strategies,
 - economic impact of mastitis,
- with a particular emphasis on its prevention and control in dairy cattle.

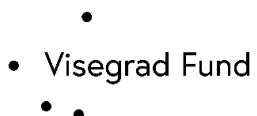


Research article

Prevalence and etiology of mastitis in dairy herds in eastern Slovakia

Zuzana Lacková, František Zigo, Zuzana Farkašová, Mária Vargová, Ewa Pecka-Kiełb, Boglárka Schilling-Tóth, Petr Sláma, Ibrahim F. Rehan

Journal of Dairy, Veterinary & Animal Research



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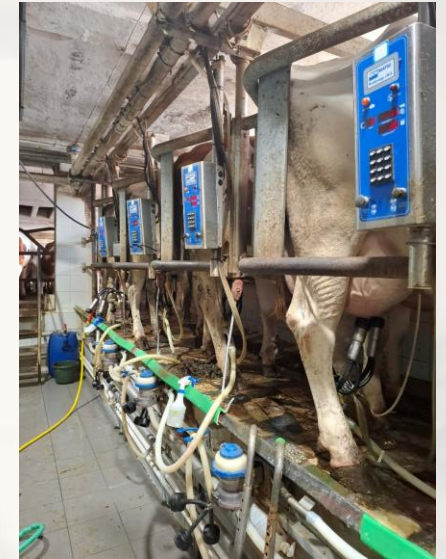


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Prevalence and etiology of mastitis in dairy herds in eastern Slovakia

- The primary objective was to evaluate the impact of environmental and physiological factors on mammary gland health through the identification of intramammary infections (IMI) and the associated pathogen spectrum.
- A study conducted in 10 herds in eastern Slovakia on a sample of 1,000 dairy cows (100 cows from each farm) during early lactation.
- The findings revealed a 20.7% overall incidence of mastitis within the monitored herds. Subclinical mastitis represented the most significant portion, affecting 14.2% of the examined cows, while clinical mastitis (CM) accounted for 6.5% of the positive cases. Microbiological analysis identified staphylococci as the dominant etiological agents, responsible for 55.8% of IMI cases. Other significant pathogens included Gram-negative bacteria (18.6%) and streptococci (17.8%).
- Due to their high pathogenicity and virulence factors including biofilm formation, hemolysin production, and DNA hydrolysis play a pivotal role in the pathogenesis of IMI.



Research article

Effect of Humic Substances in the Diet of Dairy Cows on Rumen Fermentation, Blood and Immunological Parameters, Milk Composition and Mammary Gland Health

Halás Šimon, Zigo František, Récky Andrej, Lacková Zuzana, Farkašová Zuzana, Arvaiová Juliana, Timkovičová-Lacková Petra, Mudroňová Dagmar, Zahumenská Jana, Pecka-Kielb Ewa, Pavlík Aleš, Schilling-Tóth Boglárka Mária

Effect of Humic Substances in the Diet of Dairy Cows on Rumen Fermentation, Blood and Immunological Parameters, Milk Composition and Mammary Gland Health

- addition of Humic substances (HS) to the TMR
- supplementation during the entire drying period and in the first week after calving (68 days total)
- dose at 100 g/HS/head/day of HS
- evaluation of parameters of milk, blood, phagocytic activity, rumen content, occurrence and etiology of mastitis after calving





Effect of Humic Substances in the Diet of Dairy Cows on Rumen Fermentation, Blood and Immunological Parameters, Milk Composition and Mammary Gland Health

Results revealed that HS supplementation:

- stabilized ruminal pH
- increased acetate-to-propionate ratio (C2:C3; 3.46% vs 2.56%)
- decreased ammonia concentrations
- reduced serum and milk urea levels
- increased milk protein and non-fat dry matter content
- decreased SCC
- reduced number of udder quarters infected with mastitis pathogens

Effect of Humic Substances in the Diet of Dairy Cows on Rumen Fermentation, Blood and Immunological Parameters, Milk Composition and Mammary Gland Health

- the chosen dose of 100 g HS per cow per day has beneficial effects on stabilizing the ruminal environment and on key indicators of ruminal fermentation, milk production, and udder health.
- consequently, we recommend that farmers include HS in the TMR for dry and fresh cows as part of a comprehensive herd health program.



Conference – Piešťany, Slovakia

1. SLÁMA, Petr – PAVLÍK, Aleš – TANČIN, Vladimír – KOLESÁROVÁ, Adriana.

Fytonutrienty a jejich vliv na imunitní buňky. In *Bezpečnosť a kvalita potravín : Zborník vedeckých prác.* 1. vyd. - Nitra : Garmond, 2025. ISBN 978-80-8266-081-7, online, 153-154.

2. LACKOVÁ, Zuzana - ZIGO, František - FARKAŠOVÁ, Zuzana - ONDRAŠOVIČOVÁ, Silvia - VARGOVÁ, Mária - PECKA-KIEŁB, Ewa - SLÁMA, Petr

- PAVLÍK, Aleš - KISS, Dávid Sándor. Význam hygienického programu dojenja v prvovýrobe mlieka. In *Bezpečnosť a kvalita potravín : Zborník vedeckých prác.* 1. vyd. - Nitra : Garmond, 2025. ISBN 978-80-8266-081-7, online, 202-206.

VÝZNAM HYGIENICKÉHO PROGRAMU DOJENIA V PRVÝVÝROBE MIEKA
THE IMPORTANCE OF A HYGIENE MILKING PROGRAM IN PRIMARY MILK PRODUCTION

Zuzana Lacková, František Zigo, Zuzana Farkašová, Silvia Ondrašovičová, Mária Vargová, Petr Pecka-Kiełb, Aleš Pavlík, Dávid Sándor Kiss

Abstract: The aim of the study is to evaluate the impact of a hygiene milking program on the quality of milk produced in primary milk production. The study was conducted in a dairy farm in Slovakia. The results show that the hygiene milking program significantly reduces the number of somatic cells and bacteria in the milk, which improves its quality and safety. The program also helps to reduce the risk of mastitis and other diseases in the cows. The study concludes that a hygiene milking program is an essential part of primary milk production and should be implemented in all dairy farms.

Keywords: hygiene milking program, primary milk production, somatic cells, bacteria, mastitis, milk quality.

1. Úvod: Mlieko je základnou potravou pre ľudí a zvieratá. Jeho kvalita závisí od viacerých faktorov, vrátane hygieny dojenja. Cieľom štúdie je posúdiť vplyv hygienického programu dojenja na kvalitu mlieka v prvovýrobe. Výskum bol realizovaný na mliekarskej farme v Slovenskej republike. Výsledky ukázali, že hygienický program dojenja významne znižuje počet somatických buniek a baktérií v mlieku, čo zlepšuje jeho kvalitu a bezpečnosť. Program tiež pomáha znížiť riziko mastitidy a iných ochorení u kráv. Záverom štúdie je, že hygienický program dojenja je nevyhnutnou súčasťou prvovýroby mlieka a mal by byť implementovaný vo všetkých mliekarských farmách.

2. Materiál a metódy: Výskum bol realizovaný na mliekarskej farme v Slovenskej republike. Dojnice boli rozdelené do dvoch skupín: kontrolnej a skúsenej. Skúsenej skupine bolo aplikované hygienické program dojenja. Vzorky mlieka boli zberané pravidelne a analyzované na počet somatických buniek a baktérií. Štatistická analýza bola vykonaná pomocou softvéru SPSS.

3. Výsledky a diskusia: Výsledky štúdie ukázali, že hygienický program dojenja významne znižuje počet somatických buniek a baktérií v mlieku. Táto zmena je významná pre kvalitu a bezpečnosť mlieka. Navyše, program dojenja pomáha znížiť riziko mastitidy a iných ochorení u kráv. Záverom štúdie je, že hygienický program dojenja je nevyhnutnou súčasťou prvovýroby mlieka a mal by byť implementovaný vo všetkých mliekarských farmách.

4. Záver: Hygienický program dojenja je nevyhnutnou súčasťou prvovýroby mlieka a mal by byť implementovaný vo všetkých mliekarských farmách. Tento program pomáha zlepšiť kvalitu a bezpečnosť mlieka a znížiť riziko ochorení u kráv. Ďalšie štúdie by mali zistiť, či táto metóda môže byť aplikovaná aj v iných druhoch zvierat.

5. Referencie: [List of references]

6. Kontaktné údaje: [Contact information]

PHYTONUTRIENTS AND THEIR EFFECT ON IMMUNE CELLS

Petr Sláma¹, Aleš Pavlík², Vladimír Tančín¹, Adriana Kolesárová²
¹Mendel University in Brno, Czech Republic
²Slovak University of Agriculture in Nitra, Slovakia

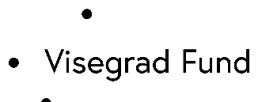
INTRODUCTION: Phytonutrients are increasingly being studied for their beneficial effects on both human and animal health. These compounds, including flavonoids, alkaloids, polyphenols, and terpenes, can influence various biological processes, such as immune system modulation, anti-inflammatory response, and cellular antioxidant protection. Research suggests that certain phytonutrients may support immune response, protect cells from oxidative stress, and contribute to overall homeostasis in the body. Special attention is given to their ability to modulate the function of blood immune cells, which play a critical role in immune defense. In the context of both animal and human health, these natural compounds represent a promising tool for the prevention and treatment of various diseases, including cattle diseases such as mastitis, an inflammation of the mammary gland.

MATERIALS AND METHODS: Macrophage cells were isolated from blood using a separation method based on Histoopaque density gradient centrifugation. Blood samples were first diluted with a physiological solution and then carefully layered onto Histoopaque 1077 separation medium. After centrifugation under appropriate parameters, different cellular fractions were separated, with macrophages being collected from the interface between the plasma and the separation medium. These cells were then washed multiple times with phosphate-buffered saline (PBS) and resuspended in a culture medium for further analysis.

To assess cell viability and identify apoptotic and necrotic cells, flow cytometry with fluorescent staining was used. The cells were first incubated with YO-PRO-1 and propidium iodide (PI) dyes, which allow differentiation of cell death types. YO-PRO-1 penetrates early apoptotic cells, while propidium iodide labels necrotic and late apoptotic cells with severely damaged membranes. After staining, the samples were analyzed using a Bio-Rad FC500 flow cytometer (Brno, Czechia), where fluorescence intensity was measured for individual cells. The obtained data were evaluated based on the separation of cell populations according to fluorescence intensity, enabling the quantification of live, apoptotic, and necrotic cells under different experimental conditions.

RESULTS AND DISCUSSION: Isolated macrophage cells were exposed to various concentrations (5-100 µg/ml) of berberine, and cell viability was evaluated after 3 and 18 hours of incubation. The results indicate that at the concentration of the tested compound increases, the percentage of necrotic cells rises in both time intervals (3 and 18 hours). This trend suggests a dose-dependent cytotoxic effect of the compound. A comparison between the 3-hour and 18-hour incubation times reveals that prolonged exposure leads to a higher percentage of necrotic cells across all tested concentrations. This suggests that the cytotoxic effect of the substance is not only dose-dependent but also influenced by the duration of exposure. These observed effects align with literature reports stating that increased doses of cytotoxic compounds lead to heightened toxic effects on sensitive cells. Specifically, berberine, an alkaloid found in plants of the *Berberis* genus, is known for its hepatocellular and hepatocellular properties. Although its primary mode of action is considered to be related to its potential cytotoxic effects on various cell lines, which could explain the observed increase in necrotic cells depending on concentration and exposure duration. It is important to note that cytotoxic effects may be influenced not only by concentration and exposure time but also by the specific properties of the tested cells and their sensitivity to the compound.

CONCLUSIONS: The study was conducted as part of the Visegrad Fund project (202402) - Immunohistochemistry in control of mastitis in dairy cows. The project is co-financed by the governments of Czechia, Hungary, Poland and Slovakia through Visegrad Grants from the International Visegrad Fund. The mission of the fund is to advance ideas for sustainable regional cooperation in Central Europe.



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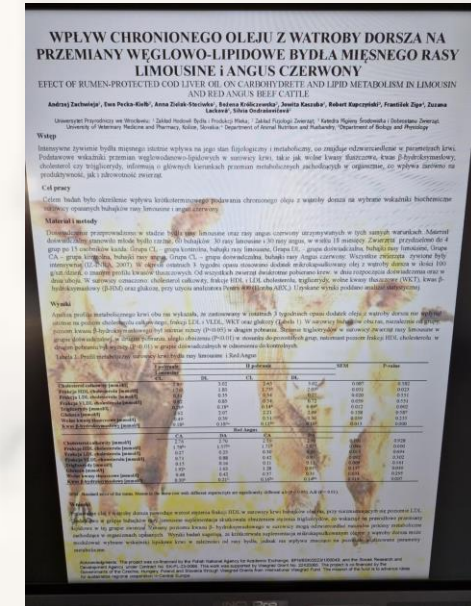
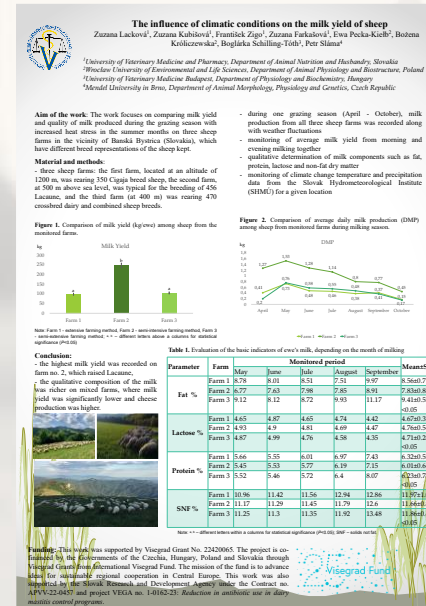
WROCLAW UNIVERSITY OF ENVIRONMENTAL AND LIFE SCIENCES



Conference – Wroclaw, Poland

1. LACKOVÁ, Zuzana - KUBIŠOVÁ, Zuzana - ZIGO, František - FARKAŠOVÁ, Zuzana - PECKA-KIEŁB, Ewa - KRÓLICZEWSKA, Bożena - SCHILLING-TÓTH, Boglárka Mária - SLÁMA, Petr. The influence of climatic conditions on the milk yield of sheep. In One Health: ludzie, zwierzęta i środowisko - nasza wspólna przyszłość. Konferencja naukowa. *One Health: ludzie, zwierzęta i środowisko – nasza wspólna przyszłość.* - 2025, s. 26-28.

2. ZACHWIEJA, Andrzej - PECKA-KIEŁB, Ewa - ZIELAK-STECIWKO, Anna - KRÓLICZEWSKA, Bożena - KASZUBA, Jowita - KUPCZYŃSKI, Robert - ZIGO, František - LACKOVÁ, Zuzana - ONDRAŠOVIČOVÁ, Silvia. Wpływ chronionego oleju z wątroby dorsza na przemiany węglowo-lipidowe bydła mięsnego rasy limousine i angus czerwony. In One Health: ludzie, zwierzęta i środowisko - nasza wspólna przyszłość. Konferencja naukowa. *One Health: ludzie, zwierzęta i środowisko – nasza wspólna przyszłość.* - 2025, s. 36-38.



Conference – Budapest, Hungary

1. **KISS, Dávid Sándor - ZIGO, František - LACKOVÁ, Zuzana - ONDRAŠOVIČOVÁ, Silvia - PECKA-KIEĽB, Ewa - TÓTH, István - SCHILLING-TÓTH, Boglárka Mária - SLÁMA, Petr.** Green synthesis of silver nanoparticles and their antibacterial activity against udder pathogens. In FoodConf - Conference on food science, technology and innovation. *FOODCONF - Science, technology and innovation : Book of abstracts.* - Budapest : Élelmiszertudományi Alapítvány, 2025, p. 104.

2. **LACKOVÁ, Zuzana - ZIGO, František - ONDRAŠOVIČOVÁ, Silvia - PECKA-KIEĽB, Ewa - SLÁMA, Petr - KISS, Dávid Sándor.** Mastitis in dairy cows and its impact on reproductive parameters. In FoodConf - Conference on food science, technology and innovation. *FOODCONF - Science, technology and innovation : Book of abstracts.* 1. vyd. - Budapest : Élelmiszertudományi Alapítvány, 2025, p. 105.

3. **ZIGO, František - LACKOVÁ, Zuzana - FARKAŠOVÁ, Zuzana - KISS, Dávid Sándor - ONDRAŠOVIČOVÁ, Silvia - PECKA-KIEĽB, Ewa - SCHILLING-TÓTH, Boglárka Mária - PAVLÍK, Aleš.** Comparison of rapid cultivation tests to detection mastitis in dairy cows. In FoodConf - Conference on food science, technology and innovation. *FOODCONF - Science, technology and innovation : Book of abstracts.* 1. vyd. - Budapest : Élelmiszertudományi Alapítvány, 2025. ISBN 978-615-02-4608-6, p. 107.

Green synthesis of silver nanoparticles and their antibacterial activity against udder pathogens

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The study presents a novel process for synthesizing silver nanoparticles using dry lavender leaves (Lav-AGNs) as both a reducing agent and stabilizer, and evaluates their inhibitory effect on udder pathogens of dairy cows.

Material and methods: For the practical purposes of the study, two dairy farms were monitored, with a total of 480 cows milked twice daily. Before milk sampling, cows were subjected to clinical examination and udder palpation. After sensory evaluation, the anterior milk strips from each quarter were evaluated using CMT-10 for catalase activity, hemolysis, pigment formation, coagulable, and green staining were performed using the methods published by Malinovskii et al. (2006). Species identification was performed using biochemical tests, including STAPHINEX 24, STRIPTOX 24, ID 100 (BioMérieux, IA, USA) and evaluated with the TMS 2100 (BioMérieux, IA, USA) and evaluated with the TMS 2100 (BioMérieux, IA, USA). Silver nanoparticles were obtained from the mixing of silver nitrate solution with reducing substances extracted from lavender leaves in the different concentrations: 50 µg/l, 100 µg/l, 150 µg/l and 200 µg/l. Pure silver nanoparticles (Ag) were used as a control group at the same concentration as Lav-AGNs and penicillin (PEN) at a concentration of 10 IU/ml.

Pathogens resistant to tetracycline antibiotics that were isolated from mastitic cows (*Streptococcus agalactiae* and *Streptococcus uberis*) were tested for inhibitory activity by the disk diffusion method.

Results: Figure 1: In vitro bactericidal potential of Lav-AGNs and pure AgNPs against selected resistant udder pathogens (µg/ml).

As the concentration of Lav-AGNs increased, the bactericidal ability also increased, resulting in a larger diameter of the antibacterial ring.

Analysis of the obtained results shows that Lav-AGNs at concentrations of 150 µg/ml and 200 µg/ml had an effect on the inhibition zone for resistant *Streptococcus agalactiae* of 13 mm as well as for *Streptococcus uberis* (12.4 mm). For penicillin, the inhibition zone was 2 mm for both pathogens. In conclusion, green synthesis provides an antibacterial product that represents an alternative to antibiotic substances.

Figure 2: Relative efficiency of AgNPs and Lav-AGNs with respect to penicillin (%).

Figure 2 shows the calculation of the relative antibacterial activity of AgNP and Lav-AGNP with respect to the reference antimicrobial agents. The inhibition zones were calculated for penicillin and their percentages affected of reference concentrations of AgNP and Lav-AGNP against all tested pathogens. The calculation showed that the relative efficiency of 150 and 200 µg/ml showed 100% or higher effect against *S. aureus* and *S. uberis*. For the other pathogens, the relative efficiency was lower than 100%.

Table 1: Inhibition zone of AgNPs and Lav-AGNs against udder pathogens causing bovine mastitis (mm) at different concentrations.

Pathogen	AgNP 50 µg/ml	AgNP 100 µg/ml	AgNP 150 µg/ml	AgNP 200 µg/ml	Lav-AGN 50 µg/ml	Lav-AGN 100 µg/ml	Lav-AGN 150 µg/ml	Lav-AGN 200 µg/ml
<i>S. agalactiae</i>	13	13	13	13	13	13	13	13
<i>S. uberis</i>	12.4	12.4	12.4	12.4	12.4	12.4	12.4	12.4
<i>S. aureus</i>	2	2	2	2	2	2	2	2
<i>E. coli</i>	2	2	2	2	2	2	2	2
<i>K. pneumoniae</i>	2	2	2	2	2	2	2	2

Table 2: Inhibition zone of AgNPs and Lav-AGNs against udder pathogens causing bovine mastitis (mm) at different concentrations.

Pathogen	AgNP 50 µg/ml	AgNP 100 µg/ml	AgNP 150 µg/ml	AgNP 200 µg/ml	Lav-AGN 50 µg/ml	Lav-AGN 100 µg/ml	Lav-AGN 150 µg/ml	Lav-AGN 200 µg/ml
<i>S. agalactiae</i>	13	13	13	13	13	13	13	13
<i>S. uberis</i>	12.4	12.4	12.4	12.4	12.4	12.4	12.4	12.4
<i>S. aureus</i>	2	2	2	2	2	2	2	2
<i>E. coli</i>	2	2	2	2	2	2	2	2
<i>K. pneumoniae</i>	2	2	2	2	2	2	2	2

Table 3 shows that pure colloid particles of AgNPs did not have such inhibitory effect as the colloidal particles of AgNPs prepared using an extract from dried lavender leaves in the same concentrations. As the concentration of AgNPs increased, the bactericidal ability also increased, resulting in a larger diameter of the antibacterial ring. The maximum inhibition zone of Lav-AGNs was 13 x 0.45mm for *S. aureus* and 14x0.55 mm for *S. uberis*.

Table 1 shows that pure colloid particles of AgNPs did not have such inhibitory effect as the colloidal particles of AgNPs prepared using an extract from dried lavender leaves in the same concentrations. As the concentration of AgNPs increased, the bactericidal ability also increased, resulting in a larger diameter of the antibacterial ring. The maximum inhibition zone of Lav-AGNs was 13 x 0.45mm for *S. aureus* and 14x0.55 mm for *S. uberis*.

Conclusions: This work was supported by Visegrad Grant No. 22420055: Non-antibiotic approaches to control mastitis in dairy cows. The project is co-financed by the governments of Czechia, Hungary, Poland and Slovakia through Visegrad Grants from the International Visegrad Fund. The mission of the fund is to advance ideas for sustainable regional cooperation in Central Europe. This work was also supported by the Research and Development Support Agency based on Contract no. APVV-22-0447 and project VEGA-1-0162-23.

supported by Visegrad Fund

Mastitis in dairy cows and its impact on reproductive parameters

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The aim of the work was to evaluate the incidence and etiology of mastitis and its impact on reproductive parameters of dairy cows.

Material and methodology: Cattle breeding (mainly Holstein type) 172 dairy cows that had 10 days post-calving with a history of intramammary infection in the previous lactation.

Results: CMT test had a positive CMT test and an elevated SCC value in the first three months before drying off CMT test (indirect diagnostic test). Bacteriological examination was performed according to the methodology of Malinovskii and Platonova (2006) with identification of *Streptococcus agalactiae*, *Streptococcus uberis* and *Enterococcus faecalis* using STRIP-TEST test or ENTERO-test and identified using TMS Pro 7.0 software (Eiken Chemical, CZ).

Statistical analysis: Interval, insemination date, insemination period, service period and lactation length were obtained from the databases of the given breeding.

Table 1: Overview of CMT test evaluation and bacterial findings.

CMT test	Positive	Negative
112	381 (85%)	41 (15%)
112	62 (55%)	50 (45%)

Table 2: Overview of isolated pathogens from milk samples.

Isolated pathogen	Number of isolates
<i>Streptococcus agalactiae</i>	14 (37%)
<i>Streptococcus uberis</i>	2 (4%)
<i>Enterococcus faecalis</i>	12 (34%)
<i>Streptococcus agalactiae</i> (pathogenic)	20 (48%)

Table 3: Evaluation of reproductive parameters of dairy cows with isolated and without mastitis.

Reproductive parameter	Dairy cows with isolated and without mastitis		Dairy cows with unsatisfactory reproductive parameter	
	V [no./%]	N [no./%]	SKM [no./%]	KM [no./%]
Insemination interval (55-58 days)	86 (77%)	7 (23%)	4 (8%)	20 (13%)
Insemination interval (61-62)	86 (77%)	7 (23%)	4 (8%)	20 (13%)
Insemination interval (63-110 days)	86 (77%)	7 (23%)	4 (8%)	20 (13%)
Insemination interval (145-160 days)	86 (77%)	7 (23%)	4 (8%)	20 (13%)
Insemination interval (161-185 days)	86 (77%)	7 (23%)	4 (8%)	20 (13%)

Table 4: Overview of isolated pathogens from milk samples.

Isolated pathogen	Number of isolates
<i>Streptococcus agalactiae</i>	14 (37%)
<i>Streptococcus uberis</i>	2 (4%)
<i>Enterococcus faecalis</i>	12 (34%)
<i>Streptococcus agalactiae</i> (pathogenic)	20 (48%)

Conclusions: In dried milk samples, *Streptococcus agalactiae*, *Streptococcus uberis*, *Streptococcus agalactiae*, *Streptococcus uberis* and *Enterococcus faecalis* were detected in both forms of mastitis. Compared to optimal reproductive indicators, the results showed unsatisfactory reproductive values, especially in dairy cows with a clinical form of fatty udder.

Conclusions: This work was supported by Visegrad Grant No. 22420055: Non-antibiotic approaches to control mastitis in dairy cows. The project is co-financed by the governments of Czechia, Hungary, Poland and Slovakia through Visegrad Grants from the International Visegrad Fund. The mission of the fund is to advance ideas for sustainable regional cooperation in Central Europe. This work was also supported by the Research and Development Support Agency based on Contract no. APVV-22-0447 and project VEGA-1-0162-23.

supported by Visegrad Fund

Comparison of rapid cultivation tests to detection mastitis in dairy cows

František Zigo¹, Zuzana Lacková², Zuzana Farkašová³, Dávid Sándor Kiss⁴, Silvia Ondrašovičová⁵, Ewa Pecka-Kieľb⁶, István Tóth⁷, Boglárka Schilling-Tóth⁸, Aleš Pavlík⁹

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Abstract: The objective of this study was to evaluate and compare two methods for rapid detection of udder pathogens: MicroMast rapid plates versus the Chelex test. Both methods belong to cultivation-based laboratory methods for the detection of Gram-negative bacteria (*Streptococcus agalactiae* and *Streptococcus uberis*) and Gram-positive bacteria (*Streptococcus agalactiae* and *Streptococcus uberis*) in dairy cows. Subsequently, 144 quarter milk samples from the positive cows with California mastitis test scores 1-3 were subjected to laboratory cultivation using both rapid tests. Visegrad grants from these tests show the MicroMast test sensitivity for positive samples 94.1% and the Chelex test sensitivity at 82.1%. After biochemical and protein identification of cultured isolates, on both rapid tests MicroMast test and Chelex test were identified the same number of *Streptococcus agalactiae* spp. (*S. agalactiae* and *S. uberis*), *Streptococcus agalactiae* spp. (20 isolates). Based on the results, both tests are comparable and can therefore be used to practice for the rapid detection of udder pathogens.

Keywords: dairy cows, mastitis, MicroMast test, Chelex test, udder pathogens

MicroMast Rapid Test
This test is designed for rapid 24-hour culture of samples directly under vacuum conditions. The results were evaluated after 24 hours of incubation at 37 °C in each plate, which is detailed in these pages (Fig. 2). Growth in the Blue zone "A" is used to indicate contamination and to confirm bacterial growth (non-selective agar). This second zone "B" is intended for the identification of Gram-negative pathogens such as *Streptococcus agalactiae*, *Streptococcus uberis*, *Streptococcus agalactiae* (20S), *Streptococcus agalactiae*, *Streptococcus uberis*, *Enterococcus* spp., *Corynebacterium* spp. or *Bacillus* spp. The third zone "C" is intended for the identification of Gram-negative pathogens including *Enterobacteriaceae* and *Bacteroides* spp.

ChelexMilk Test
This test is used to test the rapid 24-hour cultivation of milk samples under on-farm conditions. Milk samples were applied to the surface of a plate containing three different selective chromogenic agars. The results were analyzed after inoculation of each plate, which is divided into three zones (Fig. 2). **Zone A:** This sector supports the growth of *Streptococcus agalactiae*. **Streptococcus agalactiae** sector: This sector is designed for the growth of *Streptococcus agalactiae*.

Conclusion: The results obtained from this study allowed us to demonstrate that although the use of the California Mastitis Test (CMT) under on-farm conditions does not enable pathogen identification, it can indicate an ongoing or early-stage inflammation of the udder. When comparing two rapid milk culture methods, the sensitivity of positive samples was observed to be 84.7% using MicroMast plates and 82.1% using ChelexMilk plates. The ChelexMilk test is a simple and fast method for the identification of Gram-negative pathogens. The MicroMast test is a simple and fast method for the identification of Gram-negative pathogens. The MicroMast test is a simple and fast method for the identification of Gram-negative pathogens. The MicroMast test is a simple and fast method for the identification of Gram-negative pathogens.

Conclusions: This work was supported by Visegrad Grant No. 22420055: Non-antibiotic approaches to control mastitis in dairy cows. The project is co-financed by the governments of Czechia, Hungary, Poland and Slovakia through Visegrad Grants from the International Visegrad Fund. The mission of the fund is to advance ideas for sustainable regional cooperation in Central Europe. This work was also supported by the Research and Development Support Agency based on Contract no. APVV-22-0447 and project VEGA-1-0162-23.

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Planned future cooperation

- Planning a new **Visegrad fund** focused on the development of a new nanoparticle-based disinfectant.
- Submission of bilateral projects within the framework of the **APVV scheme SK-PL, SK-HU or SK-CZ.**
 - **Horizon Europe – Cluster**
 - **ERA-Net One Health**
 - **COST Action**



Thank you for your attention