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Zuzana Lacková,

Ibrahim F. Rehan, František Zigo, Ewa Pecka-Kielb,

Petr Sláma, Dávid Sándor Kiss

**Advances in the Understanding and Management of Mastitis in
Dairy Cows**



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Editors:

Zuzana Lacková, DVM., Ph.D.

Department of Animal Nutrition and Husbandry,
University of Veterinary Medicine and Pharmacy,
Košice, Slovakia

Ibrahim F. Rehan, Assoc. prof., DVM., Ph.D.

Department of Husbandry and Development of
Animal Wealth, Faculty of Veterinary Medicine,
Menoufia University, Shebin Alkom, Egypt
Department of Biotechnology and Nutrigenomics,
Institute of Genetics and Animal Biotechnology,
Polish Academy of Sciences, Postepu, Jastrzebiec,
Poland

František Zigo, Assoc. prof., DVM., Ph.D.

Department of Animal Nutrition and Husbandry,
University of Veterinary Medicine and Pharmacy,
Košice, Slovakia

Ewa Pecka Kielb, Assoc. prof. inż. Ph.D.

Department of Animal Physiology and
Biostructure Wrocław University of
Environmental and Life Sciences, Wrocław,
Poland

Peter Sláma, Assoc. prof. Ing. Ph.D.

Department of Animal Morphology, Physiology and
Genetics, Mendel University in Brno, Czech
Republic

Dávid Sándor Kiss, Assoc. prof., MSc., Ph.D.

Department of Physiology and Biochemistry,
University of Veterinary Medicine, Budapest,
Hungary

Reviewers:

Alina Pikhtirova, Assoc. prof., DVM., Ph.D., Department of Public Health Sumy State University

Bariş Güner, Assoc. prof., DVM., Ph.D., Balıkesir University Faculty of Veterinary Medicine,
Department of Obstetrics and Gynecology, Balıkesir, Türkiye

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Preface

Despite the increasing demand for and consumption of milk, European Union regulations require that milk and dairy products originate from healthy animals, which significantly limits production. The continuous maintenance of animal health in dairy herds intended for market milk production is influenced by a variety of external and internal factors. An imbalance among these factors can lead to deteriorating animal health and welfare, ultimately reducing the quantity and quality of milk produced. This, in turn, results in higher costs and significant economic losses for farmers.

One of the primary contributors to disease development is inadequate housing conditions, poor milking hygiene, improper nutrition, mistreatment of animals, and ineffective herd management. Among these health challenges, mastitis stands out as one of the most prevalent, problematic, and economically demanding diseases in dairy farming worldwide. Its onset and consequences have detrimental effects on herd health and welfare, leading to increased treatment expenses, changes in herd management, and a decline in milk quality. Primary milk producers are directly connected to the broader food industry and dairy product manufacturers; therefore, the occurrence of mastitis also indirectly affects the economic viability and profitability of the entire dairy supply chain.

The primary goal of this book is to provide a comprehensive overview of the diverse factors contributing to mastitis and reduced milk quality. By fostering a clear understanding of the mechanisms underlying mastitis, farmers, veterinarians, and livestock handlers will be better equipped to implement effective control strategies. Although complete eradication of mastitis is unlikely - largely due to the constant presence of environmental pathogens such as coagulase-negative staphylococci, *Streptococcus uberis*, and *Escherichia coli* successful management is still achievable. Furthermore, the development of a universal vaccine remains improbable, given the wide variety of infectious agents involved. As a result, effective control of mastitis depends on adopting sound herd management practices grounded in a thorough understanding of the disease's etiology and pathogenesis. The central aim of this book is to deepen the reader's understanding of mastitis. If this knowledge helps reduce infection rates, thereby improving both dairy farm profitability and animal welfare, the authors will consider their objective successfully met. Accordingly, this book seeks to explore and synthesize current knowledge on the etiology, pathogenesis, risk factors, diagnostic methods, treatment strategies, and economic impact of mastitis, with a particular emphasis on its prevention and control in dairy cattle,

Editors.

Table of Contents

1	Chapter - Dairy herd management	10
1.1	NUTRITION	10
1.1.1	<i>Nutrition and feeding strategy</i>	11
1.1.1.1	Nutritional principles	12
1.1.1.2	Compliance with the principles of phase feeding in dairy cows and its monitoring	13
1.1.1.3	Feeding management of dairy cows using the TMR system	13
1.1.1.4	Farm grouping and animal transfers	15
1.2	BREEDING AND REPRODUCTION	15
1.2.1	<i>Goals and planning</i>	15
1.2.2	<i>Reproductive health problems</i>	17
1.2.3	<i>Heat detection – using the latest technologies</i>	21
1.2.4	<i>Calf health</i>	22
1.2.5	COW WELFARE	25
1.2.5.1	Technological housing systems	25
1.2.5.2	Principles of cow comfort	30
1.2.6	HEAT STRESS AND COMFORT	31
1.2.6.1	Causes of heat stress	32
1.2.6.2	Protecting animals against heat stress	32
1.2.6.3	Means to minimize the risk of heat stress	32
1.2.7	<i>Veterinary care</i>	34
2	Chapter – Milk Production	36
2.1	HORMONAL SYSTEM	36
2.2	NERVE IMPULSES	39
2.3	MILKING TECHNOLOGIES AND ADHERENCE TO HYGIENE PROCEDURES	40
3	Chapter - Mastitis – inflammation of the mammary gland	46
3.1	CONTAGIOUS MASTITIS	47
3.2	ENVIRONMENTAL MASTITIS	47
3.3	CLASSIFICATION BASED ON CLINICAL SYMPTOMS	48
3.3.1	<i>Clinical Mastitis</i>	48
3.3.2	<i>Subclinical Mastitis</i>	49
3.3.3	<i>Persistent Mastitis</i>	49
3.4	ETIOLOGIC AGENTS OF MASTITIS	49
3.4.1	<i>Major Pathogens</i>	49
3.4.2	<i>Staphylococcus aureus</i>	51
3.4.3	<i>Streptococci Species</i>	52
3.4.4	<i>Coliforms</i>	53
3.4.5	<i>Mycoplasma Species</i>	53
3.4.5.1	Minor Pathogens	54
3.5	PATHOGENESIS	54
3.6	ECONOMIC IMPACT OF MASTITIS	57
	Milk Production Losses	59
	Effects on Milk Composition and Quality	59
3.7	RISK FACTORS INFLUENCING THE DEVELOPMENT OF MASTITIS	60
3.7.1	<i>Host Factors</i>	61
3.7.1.1	Age	61
3.7.1.2	Breed	61
3.7.1.3	Parity	61
3.7.1.4	Stage of Lactation	61
3.7.1.5	Milk Yield	61
3.7.2	<i>Agent Factors</i>	62
3.7.3	<i>Managemental Factors</i>	62

3.7.3.1	Milking Hygiene.....	63
3.7.3.2	Bedding Material and Cleanliness	63
3.7.3.3	Unsuitable condition of dairy cows	63
3.7.3.4	Feeding Practices.....	63
3.7.3.5	Occurrence of mycotoxins in feed	65
3.7.3.6	Ventilation and Housing Environment	65
3.7.3.7	Heat Stress.....	65
4	Chapter - DIAGNOSIS OF DAIRY MASTITIS	66
4.1	CALIFORNIA MASTITIS TEST (CMT)	66
4.2	SOMATIC CELL COUNT (SCC).....	68
4.2.1	SCC as a Diagnostic Marker	69
4.2.2	SCC Composition dynamics during mastitis.....	71
	Table 14: Economic and Milk Quality Implications	71
4.2.2.1	Technological Advances in SCC Monitoring	72
4.3	ELECTRICAL CONDUCTIVITY.....	72
4.4	BIOCHEMICAL TESTS.....	74
4.5	DIRECT MICROSCOPY.....	74
4.6	MOLECULAR BIOLOGY TOOLS	74
4.7	MAMMARY ULTRASOUND.....	75
4.8	BLOOD GAS ANALYSIS.....	77
4.9	ON-FARM CULTURE	78
4.9.1	On-farm culture tests distribution.....	79
4.9.1.1	MicroMast rapid test.....	79
4.9.1.2	ClearMilk Test.....	81
4.9.1.3	Pure milk test.....	81
4.9.1.4	VetoRapid test	81
4.9.1.5	AccuMast test	82
4.10	INFRA-RED THERMOGRAPHY	82
5	Chapter - POSSIBLE TREATMENTS AND ANTIBIOTIC RESISTANCE OF BOVINE MASTITIS85	
5.1	TREATMENT	86
5.1.1	Antimicrobial treatment	86
5.1.1.1	Intramammary infusion	87
5.1.2	Systemic treatment	87
5.1.3	Anti-Inflammatory treatment	87
5.1.4	Antibiotic therapy in lactation and dry periods	88
5.1.5	Antibiotic resistance for mastitis in cattle.....	89
5.1.5.1	Mechanisms of antibiotic resistance	91
5.1.5.2	Common resistant pathogens in mastitis.....	92
5.1.5.3	Impact of antibiotic resistance on mastitis management.....	93
5.1.5.4	Strategies to combat antibiotic resistance in mastitis	93
5.1.5.4.1	Reducing antibiotic use	93
5.1.5.4.2	Implementing effective hygiene practices	94
5.1.5.4.3	Vaccination.....	94
5.1.5.4.4	Prognostic advances.....	94
6	Chapter - NON-ANTIBIOTIC APPROACHES TO BOVINE MASTITIS MANAGEMENT	95
6.1	BACTERIOPHAGE THERAPY	96
6.2	BACTERIOCINS	97
6.2.1	Mechanisms of action	98
6.2.2	Types of bacteriocins	98
6.2.3	Challenges and future directions	98
6.3	NANOPARTICLE-BASED THERAPY	99
6.4	PHYTOTHERAPY	101
6.5	ANTIMICROBIAL ANIMAL PROTEINS.....	102
6.6	PROBIOTICS: USE OF LACTIC ACID BACTERIA	103
7	Summary	111

8	Register	113
9	References	117
10	Biography of editors.....	134

Figures and Tables

Figure 1: Comparison of production with the absence of feed food refusals before the next feeding.....	14
Figure 2: Congenital morphological causes - freemartinismus	18
Figure 3: Severe incomplete fusion of the Müllerian ducts affects fertility.....	19
Figure 4: Cystic ovaries.....	19
Figure 5: Schematic figure depicting placenta collection after natural delivery. Black arrows indicate the placentomes, which were harvested and dissected for collection.....	20
Figure 6: Retained placenta.....	21
Figure 7: Heat detection	22
Figure 8: Factors affecting hazards and risks associated with bedding materials in dairy cow housing	26
Figure 9: Housing cows in cubicles	27
Figure 10: Soft cubicle mats, latex mats and waterbeds mat	29
Figure 11: Properly ventilated and shaded barn vs. poorly ventilated and shaded barn	33
Figure 12: Mobile milking parlour.....	41
Figure 13: Parallel, Tandem, Herringbone and Rotary type of parlor.....	42
Figure 14: Robotic milking system	44
Figure 15: The key elements of good milking hygiene.....	45
Figure 16: Factors influencing the development of mastitis in dairy cows	46
Figure 17: Classification of mastitis depending on the stage of lactation, clinical symptoms and etiological factors	50
Figure 18: Clinical mastitis cases with positive bacteriological cultivation	52
Figure 19: Process of udder infection	56
Figure 20: Breeding and economic impacts of mastitis	57
Figure 21: Incidence of production diseases depending on milk yield	62
Figure 22: Infected quarter wit positive CMT and sample cultivation	68
Figure 23: Portable SCC counter	72
Figure 24: A - Mammary ultrasound evaluation using longitudinal scanning (B-mode). Frequency: 6.5 MHz; Depth: 150 mm	76
Figure 25: Milk sample cultivation on MicroMast test.....	80
Figure 26: Micromast test interpretation	80
Figure 27: MicroMast test interpretation	80
Figure 28: ClearMilk test – interpretation.....	81
Figure 29: Infrared thermographic image of a mastitis-affected quarter	83
Figure 30: Determination of severity and treatment of mastitis according to clinical symptoms ..	86
Figure 31: Future prospectives of mastitis	95
Table 1: Farms by size.....	16
Table 2: Performance of different farm types with respect to economic characters	16

Table 3: Most common methods which are used during monitoring and early detection of disorders during transition period	17
Table 5: Comparison of the composition of milk and colostrum (%).....	23
Table 4: Identifying common problems in calves.....	24
Table 6: Parameters for free housing with cubicles (lying boxes) or pen housing for dairy cows	26
Table 7: The body's reaction to extreme temperatures in connection with the development of a stress reaction	31
Table 8: The role of various hormones on mammary function during lactation.....	38
Table 9: Classification of mastitis-causing pathogens	51
Table 10: Direct and indirect production losses.....	59
Table 11: Overview of Se and vitamin E deficiency syndromes in ruminants	64
Table 12: Intepretation of CMT	67
Table 13: Estimated milk losses due to increased somatic cell count.....	70
Table 14: Economic and Milk Quality Implications.....	71
Table 15: Known antibiotics used in mastitis prevention	89
Table 16: Activity of some plant extracts and essential oils against microorganisms of bovine mastitis in vitro and in vivo studies.....	106

1 Chapter - Dairy herd management

Dairy herd management is a critical component of production farms aiming to sustain high milk yields over the long term. To achieve this, farms must effectively manage several key areas: nutrition, reproduction, animal welfare, and milking practices. In addition to these core aspects, farms must also navigate external challenges such as weather conditions, transportation logistics, and rising operational costs. Coordinating all of these factors can be complex; however, with careful planning and the strategic application of current knowledge and modern technologies, production farms can maintain efficiency, ensure animal well-being, and achieve long-term productivity and profitability (Israeli Dairy School, 2022).

1.1 Nutrition

Cattle farmers can significantly enhance both the efficiency of milk production and the health of their herds by providing a properly formulated and thoroughly mixed feed ration (TMR). Such a ration must meet the specific nutritional requirements associated with the different phases of lactation - a concept referred to as phase feeding. In addition to optimizing productivity, a well-balanced TMR also strengthens the immune system of dairy cows, thereby increasing their resistance to mastitis-causing pathogens (Eastridge, 2006).

When formulating and mixing feed, it is essential to select ingredients that are microbiologically safe, particularly to prevent contamination by molds and mycotoxins. Feed contaminated with such agents has immunosuppressive effects, weakening the animal's defense mechanisms and increasing vulnerability to pathogens invading the mammary gland. Similarly, a nutritionally imbalanced diet-lacking sufficient energy, protein (nitrogenous compounds), and other essential nutrients can compromise immune function and reduce the cow's ability to fend off infections (Gombart et al., 2020).

Nutritional requirements vary significantly throughout the stages of lactation and can influence immune function both directly by modulating the cow's immune responses and indirectly by contributing to metabolic disorders. These disorders, most commonly occurring in the periparturient period, can increase the cow's susceptibility to mastitis. All essential nutrients have the potential to trigger metabolic disorders when present in excessive or insufficient amounts in the diet. For example, hypocalcemia (milk fever) has been associated with delayed teat sphincter closure, which increases the risk of bacterial entry into the mammary gland. Research has shown

that cows suffering from milk fever are 8.1 times more likely to develop mastitis and 9 times more likely to contract coliform mastitis. Additionally, mastitis has been linked to other conditions such as ketosis, retained placenta, and fatty liver syndrome. Cows with fatty liver exhibit a delayed clearance of *Escherichia coli* from the mammary gland, prolonging infection and inflammation.

Therefore, a diet enriched with appropriate levels of vitamins, minerals, and immunostimulatory nutrients is crucial. Such nutritional strategies enhance the immune defenses of dairy cows, improving their resilience against bacterial infections and reducing the incidence and severity of mastitis (Eastridge, 2006).

1.1.1 Nutrition and feeding strategy

The first essential step in planning a proper nutritional strategy for dairy cows is to determine the appropriate amount of feed required. It is crucial to recognize that dairy cows must consume more nutrients than they expend through milk production in order to maintain optimal health, productivity, and reproductive performance. When calculating feed intake according to current nutritional standards, the dry matter (DM) content of the feed ration - i.e., the weight of the feed after moisture has been removed is used as the basis. Dry matter intake (DMI) serves as a critical indicator of nutritional adequacy and is calculated as a percentage of the cow's body weight (Lardy et al., 2018).

The following values are commonly used as guidelines for different categories of dairy cattle:

- adult, non-pregnant cows: approximately 1.2% of body weight (in dry matter)
- pregnant, non-lactating cows: approximately 2.0% of body weight
- lactating dairy cows: 1.2% of body weight plus an additional 5 kg of DM per 10 L of milk produced.

These figures clearly highlight the importance of adjusting feed rations in accordance with both body weight and milk yield. Properly balanced feeding not only supports health and productivity but also ensures that the nutritional demands of lactation and pregnancy are adequately met. To achieve peak lactation, it is essential that cows are provided with adequate quantities of high-quality feed, particularly during early lactation when nutritional demands are at their highest. Without sufficient intake of energy, protein, and other nutrients, cows may lose body condition, become more susceptible to disease, and experience reduced milk yield. Ration

management requires continuous monitoring of feed intake, body weight, and milk production, enabling timely dietary adjustments and supporting long-term sustainability in dairy herd management (Brouček et al., 2014).

1.1.1.1 Nutritional principles

One of the fundamental principles of dairy cow nutrition is to ensure an adequate energy supply, primarily through the intake of starch and fat. Depending on regional forage availability, starch can be provided through cereal grains such as wheat, corn, barley, sorghum, or other concentrate feeds. The fermentation of these grains in the rumen contributes to lactose synthesis in milk, thereby directly influencing milk yield and composition. In addition to carbohydrates, cows require dietary fats, which should comprise up to 6% of the total feed ration. Common fat sources include oilseeds, spent malt, and vegetable oils. Generally, solid fats are preferred over liquid forms due to better digestibility and rumen stability. Another essential dietary component is vitamins. While most fresh forages naturally contain adequate amounts of vitamins and minerals, feeding dried or ensiled forages - especially during the off-season can significantly reduce their vitamin content. Therefore, during these periods, it is necessary to supplement the feed ration with additional vitamins to prevent deficiencies and maintain optimal cow health (Erickson and Kalscheur, 2020).

Feed management on a dairy farm typically consists of two main components:

- forage maintenance
- silage storage.

Grass remains one of the most cost-effective and nutritionally valuable forages for dairy herds. The goal of grassland management is to maintain pastures in a young, vegetative state, which corresponds to the highest nutrient density. Achieving this requires consistent efforts in fertilization, irrigation, rotational grazing, and timely harvesting. Farms with an abundance of grass can conserve the surplus as hay, ensuring a steady feed supply during periods of low forage growth. In regions where grass is less available, crops like maize, barley, or legumes serve as alternative staple feeds. The choice of forage depends heavily on local availability and quality, which can vary significantly by geographic region.

Based on the selected forages, it may be necessary to supplement the diet with concentrates and protein-rich additives, such as:

- soybean or rapeseed meal
- rice bran
- citrus pulp.

These supplements help compensate for nutritional deficiencies in the base forage, particularly when dry matter feeds lack essential nutrients (Lardy et al., 2018).

1.1.1.2 Compliance with the principles of phase feeding in dairy cows and its monitoring

The goal of phase feeding in dairy cows is to formulate rations according to their current maintenance and production needs, ensuring precise dosing and mixing of forages, concentrates, by-products, minerals, and vitamins into a unified total mixed ration (TMR). This approach allows for maintaining a stable nutritional composition of the feed available to the cows.

When monitoring phase feeding, it is important to track the nutrient content in analyzed TMR samples, actual dry matter intake (determined as the difference between the feed offered and the feed refusals), the lactation curve, milk fat and protein concentrations, as well as changes in body condition throughout lactation and pregnancy. Evaluating these parameters is essential for properly assessing the quality of the TMR and for the early identification of potential issues within the phase feeding system on the farm (Vajda and Maskařová, 2016).

1.1.1.3 Feeding management of dairy cows using the TMR system

Phased feeding of dairy cows in free-range housing requires providing the necessary amount and ratio of nutrients through a TMR. Free access to TMR helps stabilize the digestive system, nutrient metabolism, reproductive processes, and maintain animal body condition. The main advantages of TMR feeding include precise feed dosing, which allows for an optimal ratio forage to concentrate components, increases the effective intake and utilization of nutrients, and simultaneously reduces the risk of disease in the herd.

On-farm feeding monitoring includes tracking feed intake and its nutritional composition (protein, fiber, fat, starch, minerals such as Se, Zn, Cu, and vitamins E and A), as well as observing selective feeding behavior.

A particle separator is used to assess the structure of the TMR, which measures the physical effectiveness of the feed and supports proper rumen function and chewing activity. Analysis of

feed distribution in the trough allows for evaluating the extent of selective feeding. Uneaten, separated TMR components can affect rumen pH and total feed intake, which in turn impacts fermentation processes (Simoni et al., 2021). In parallel, fresh feed should be available at all times. Feed bunks (or feeding tables) should never be completely empty; a maximum of 3% feed food refusal is recommended to prevent shortages before the next feeding cycle (Fig. 1).



27.4 kg milk production vs 29 kg milk production
No feed food refusal vs 3% of feed food refusal

Figure 1: Comparison of production with the absence of feed food refusals before the next feeding

Source: Schaumann Agri International GmbH (2023)

Ensuring equal access to feed for all animals is critical. Dominant individuals may otherwise bully or displace subordinate cows, especially younger animals, resulting in nutritional imbalances and reduced feed intake. The feeding process should follow a structured sequence:

- forage should be offered first to promote rumination and saliva production
- only after forage intake should concentrated nutritional supplements be added.

A widely accepted guideline is to provide 1 kg of concentrate per 2 kg of milk produced. Additionally, feeding frequency, timing (day/night), duration, and dry matter intake significantly influence the digestive physiology of dairy cows. These factors affect rumen fermentation, intestinal health, rumination activity, and even the consistency and quality of feces. For example, studies have shown that distributing feed throughout the entire day can lead to a 12.5% increase in

digestive tract activity, underlining the importance of feeding time as a key management tool (Erickson and Kalscheur, 2020).

As well as controlling and dosing TMR, water intake is important at 60–70 liters of water per cow/day, with an additional 4–5 liters needed for every liter of milk produced. To meet these demands, cows must have unrestricted access to water troughs, particularly immediately after milking, as this is when a significant proportion of daily water intake occurs. Therefore, sufficient watering points must be available as cows exit the milking parlour.

1.1.1.4 Farm grouping and animal transfers

Cow groups on a farm are typically formed according to the stage of lactation, milk production level, and herd size. The most effective approach is to divide dairy cows into three production groups and two dry-off groups, with each group receiving a different feed ration. Such grouping significantly affects feed intake, milk yield, body condition, and overall animal health. When moving cows between groups, it is important to consider milk production, days in milk, and the body condition score of each cow. To prevent a marked decline in milk yield, it is recommended to move whole groups rather than individual animals, make transfers during feeding time, maintain the same feeding-space allotment and lying-area availability, and avoid overcrowding. (Barrientos-Blanco et al., 2022).

1.2 BREEDING AND REPRODUCTION

1.2.1 Goals and planning

Effective reproductive management requires careful planning, monitoring, and record-keeping. Interestingly, larger farms with a greater number of dairy cows often achieve better reproductive performance than smaller operations (Tab. 1 and Tab. 2).

This may be attributed to more structured management systems and better use of technology and labor resources. However, regardless of the farm's size, every production unit should implement a detailed routine for monitoring cow health and reproductive status. Maintaining regular and accurate farm records is essential for predicting estrus (heat), ensuring timely insemination, and identifying pregnant cows or those exhibiting signs of reproductive or systemic illness. In addition, farms should have well-defined protocols in place for monitoring the health and productivity of cows during the transition period (three weeks before and after calving) as well

as throughout gestation (Tab. 3). Early detection of health or reproductive issues allows for prompt intervention, thereby improving overall fertility rates, animal welfare, and farm profitability (Džermeikaitė et al., 2023).

Table 1: Farms by size

Farm by size (pcs)	Intercalving	Milk production
Up to 20	541	5044
21 - 50	431	5771
51 - 100	433	5871
101 - 220	419	7038
221 - 500	411	7947
over 500	405	10061

Source: Schaumann Agri International GmbH (2023)

Table 2: Performance of different farm types with respect to economic characters

Economic traits	Type of farm								
	Small			Medium			Large		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Lactation yield 1 (l)	1293	8620	3152	1078	7543	3189	1293	8620	4016
Lactation yield 2 (l)	1186	6012	2608	1270	6178	2901	1649	5723	3219
Service period (days)	88	1050	190	70	446	170	60	303	128
Inter calving period (days)	340	1330	470	366	726	455	340	583	408
AI per conception	1	10	2.29	1	5	1.96	1	3	1.8
First successful service (month)	12	39	21.7	15	46	23.9	12	42	19.2
First calving (month)	21	48	30.7	24	55	32.9	21	51	28.2

Source: George et al. (2020)

Table 3: Most common methods which are used during monitoring and early detection of disorders during transition period

Methods	Disorders
Metabolic profile test	Metabolic diseases and stress level
Body condition score (BCS)	Ketosis and fatty liver
Hi-tag rumination systems (neck collars)	Indigestion, ketosis, and displaced abomasum
Behavior Recorder systems (IGER)	Hypocalcemia, ketosis
Cow manager system (rumination and activity)	Hypocalcemia, ketosis
Urine pH	Subclinical hypocalcemia and subclinical keto
Metabolomics	Ketosis and subclinical mastitis
Liver activity index (LAI)	Metabolic health condition

Source: Emam et al. (2024)

1.2.2 Reproductive health problems

Infertility in dairy cows is defined as a condition where animals are neither fully fertile nor completely sterile. The causes of infertility are complex and multifactorial, involving disruptions at various stages of the reproductive process such as the development and maturation of the Graafian follicle, onset of estrus, ovulation, fertilization, implantation, fetal development, delivery, and expulsion of the placenta. Any disturbance in these physiological processes - whether due to disease, malnutrition, improper herd management, genetic and congenital abnormalities, hormonal imbalances, or environmental stressors can result in infertility.

Congenital morphological causes are often hereditary and involve developmental abnormalities of the reproductive organs. Common conditions include:

- ovarian hypoplasia and aplasia
- anomalies of the tubular parts of the reproductive tract
- hermaphroditism and freemartin (Fig. 2)
- arrested development of the Müllerian ducts (Fig. 3)
- double cervix.

Functional causes of infertility include:

- cystic or inactive ovaries leading to anestrus (Fig. 4)

- early embryonic mortality resulting in repeated inseminations
- prolonged gestation.

Anestrus often results from hormonal imbalances. Repeated insemination is typically defined as the need for three or more services to achieve conception (Kováč et al., 2023).

Infectious causes of infertility may result from infections of bacterial, protozoal, viral, or mycoplasmal origin. Systemic or nonspecific infections often lead to:

- endometritis
- metritis
- pyometra.

Endometritis is characterized by purulent vaginal discharge and significantly reduces fertility. Affected cows typically require more inseminations (2.0 vs. 1.6), exhibit longer intercalving intervals (394 vs. 383 days), and face higher culling rates for infertility (13.6% vs. 6.2%). These infections often occur at parturition, especially in cows experiencing:

- retained placenta
- assisted deliveries
- postpartum paresis



Figure 2: Congenital morphological causes - freemartinism

Note: Freemartinism is a distinct form of intersexuality which arises as a result of a vascular anastomosis of the adjacent chorioallantoic sacs of heterozygous fetuses in multiple pregnancies. Freemartin is an infertile female with a modified genital tract born cotwin, or in greater multiples, with a bull with which it has exchanged whole blood.

Source. Vetscraft (1) (2025)

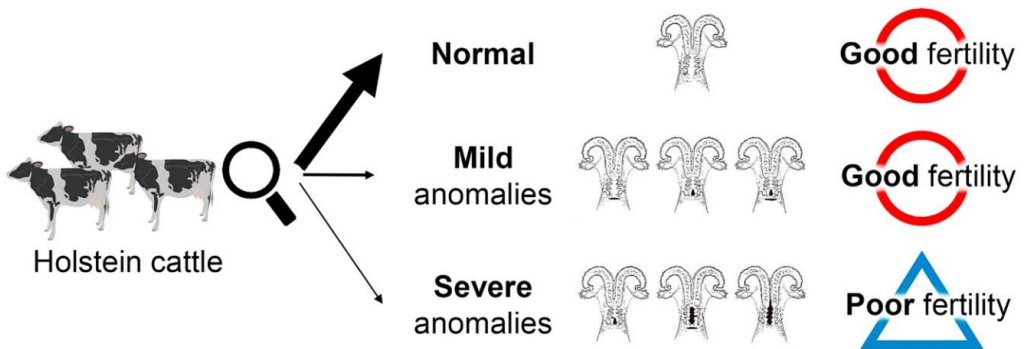


Figure 3: Severe incomplete fusion of the Müllerian ducts affects fertility

Source: Ishiyama (2019)

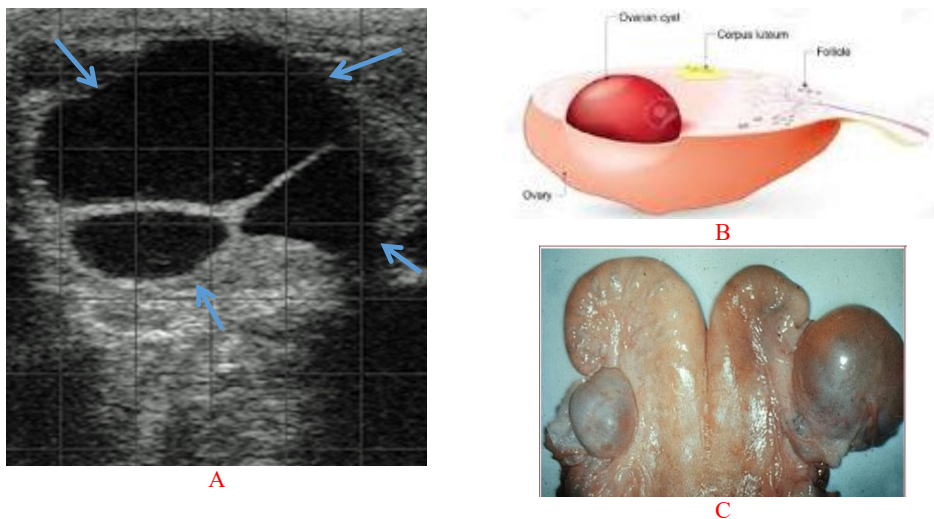


Figure 4: Cystic ovaries

Source: A - Mouncey (2015); B - Dinkissa (2022); C - Vetscraft (2) (2025)

Acute metritis presents with fever and depression and may progress to chronic metritis, characterized by persistent purulent discharge. Pyometra, the accumulation of pus in the uterus, is a common cause of anestrus. These disorders are more likely when multiple cows calve in the same area without proper hygiene, exposing each other to pathogenic microorganisms (Al-Hamedawi, 2011).

Retained placenta occurs when the placenta fails to separate from the uterine wall 12 to 24 hours after a normal delivery (Fig. 5). If retained for more than 2–3 days, the placenta undergoes decomposition within the uterus, increasing the risk of postpartum infections (Inaba et al., 2022).

Diagnosis of infertility is based on:

- reproductive performance indicators (e.g., insemination interval, service period, insemination index, intercalving interval)
- clinical signs of reproductive disorders (Fig. 6).

Effective prevention of reproductive disorders focuses on optimizing reproductive performance to minimize involuntary culling, which is most often due to conception failures. Achieving and maintaining optimal fertility is essential for improving both longevity and profitability in dairy herds (Bonneville-Hébert et al., 2011).

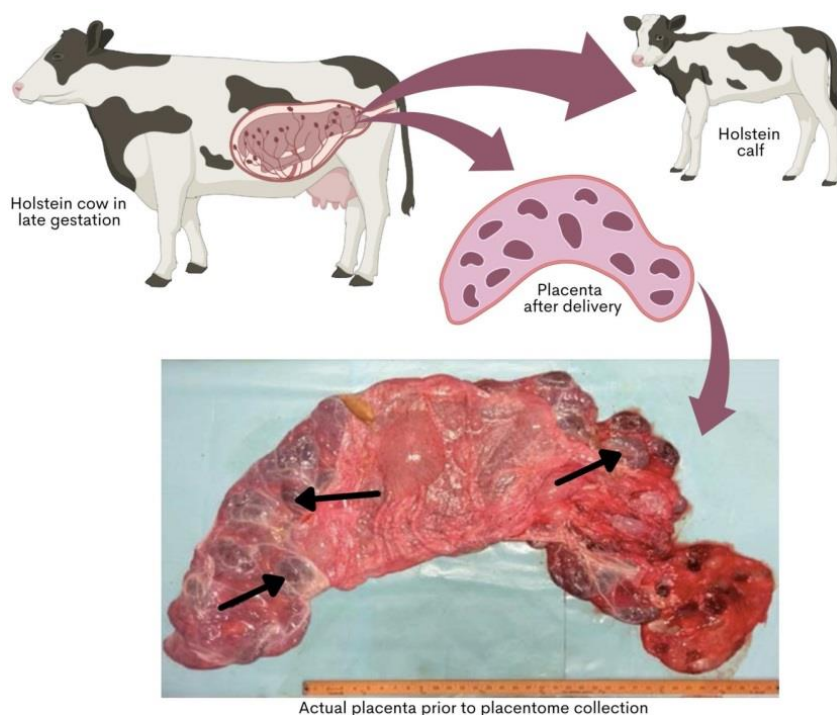


Figure 5: Schematic figure depicting placenta collection after natural delivery. Black arrows indicate the placentomes, which were harvested and dissected for collection

Source: Guadagnin (2023)



Figure 6: Retained placenta

Source: Skelly (2018)

1.2.3 Heat detection – using the latest technologies

The cornerstone of maximizing reproductive efficiency in dairy herds is the accurate detection of estrus (heat). Timely and correct identification of cows in heat significantly increases the likelihood of successful insemination and improves overall fertility rates. On many farms, personnel are trained to visually monitor cows for behavioral signs of estrus. One of the most prominent signs is mounting behavior, where a cow attempts to jump onto another. Additional signs include:

- mucous vaginal discharge
- restlessness
- vocalization
- reduced feed intake
- swollen vulva (Kováč et al., 2023) (Fig. 7).

This increase in activity and behavioral change is also detectable using automated activity monitoring systems, which function similarly to pedometers used in humans. These devices track parameters such as movement, gait, and steps taken. A sudden spike in activity levels is often indicative of estrus. Using pedometers or similar activity monitoring tools can significantly

improve estrus detection rates, especially in large-scale or extensive farming operations where visual observation of each individual animal may not be feasible. It is a comprehensive solution for improving herd reproductive indicators (e.g. shortening the intercalation period, reducing the number of inseminations, increasing the % of available cows) and monitoring the health of breeding cows (24/7 monitoring of food intake, rumination and activity). The system very reliably detects, determines the most suitable time for insemination and warns against long-term inactivity of breeding cows. These technologies enhance efficiency in herd management, reduce labor costs, and allow for more precise timing of artificial insemination, ultimately contributing to higher conception rates and better reproductive performance (Roelofs et al., 2005).

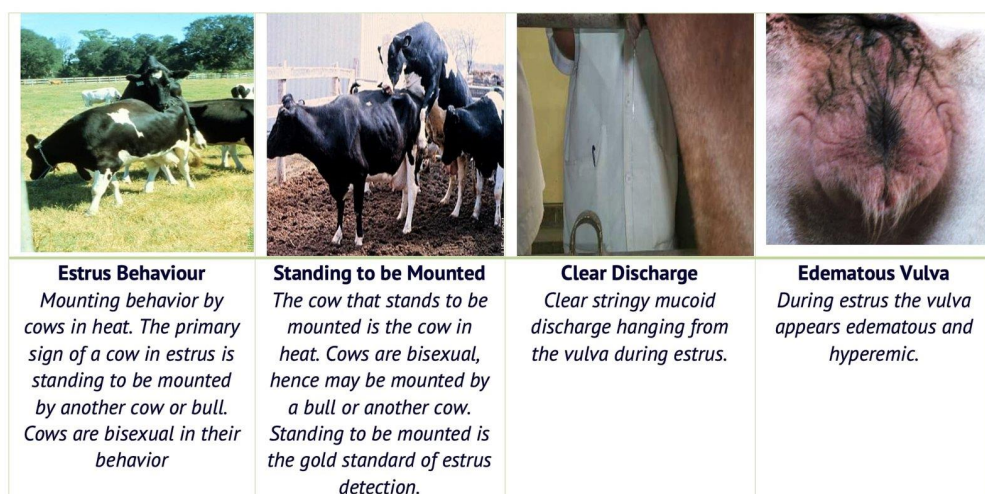


Figure 7: Heat detection

Source: Vetscraft (3) (2025)

1.2.4 Calf health

A key factor in maintaining the health of calves during the first weeks of life is providing a clean environment. Calves should be placed in a dry, clean pen immediately after birth. They may be housed individually or in small groups; however, when group housing is used, calves should be separated according to age and size. The main objective is to minimize their exposure to manure, which may contain pathogens responsible for calf diseases. Calf diseases are the result of a complex interplay of management practices, environmental conditions, infectious agents and risk factors at

calf. Various diseases have been considered as causes of calf morbidity and mortality; however, neonatal diarrhea and pneumonia in older calves are responsible for most of the morbidity and mortality in the calf hood (Tab. 4). In addition, several risk factors for calf morbidity and mortality have been identified, such as insufficient or no colostral immunity, overcrowding and poor hygiene, naive immune system in neonates and stress factors such as cold ambient temperature and frequent mixing of animals, and calf nutrition status. Therefore is essential to keep all feeding equipment clean and to ensure that staff consistently practice proper hand hygiene when moving between handling adult cows and calves (AHDB, 2025).

Colostrum intake is critical for immunity development. Each calf should receive about 4 liters of colostrum within the first 12 hours after birth, administered either with a feeding tube or a bottle (Tab. 5).

Table 4: Comparison of the composition of milk and colostrum (%)

Components	Milk	Colostrum*
Water	87.0 – 88.0	74.5 – 75.5
Fat	3.8 – 4.2	6.7 - 5.4
Protein	3.1 - 3.5	14.0 - 15.1
Lactose	4.6 - 4.8	2.7 – 2.9
ASH	0.7 – 0.8	1.0 – 1.2
Total solids	12.0 – 13.5	23.0 – 24.0

Note: *First postpartum milking. Optimal content of immunoglobulins 150-200 g in 1 l. The calf should be watered for 2-12 hours then reduced ability to absorb immunoglobulins through the intestinal wall.

Because the rumen is not fully developed during the first months of life, calves are unable to digest solid feed. During this period, they should consume milk equal to approximately 15% of their body weight daily. From the first week onward, concentrates and high-quality hay can be gradually introduced to stimulate rumen development and accustom calves to solid feeding. This practice helps support healthy growth and prepares calves for the weaning process (Baldwin et al., 2004).

Table 5: Identifying common problems in calves

Sings of good health and vigour	Look for early signs of disease	Late signs of disease
Bright, playful, curious, keen to drink milk.	Quiet, slow to stand, still drinking milk.	Dull, reluctant to stand unaided, off milk.
Respiratory		
		
Clear eyes and nose. No cough. Normal temperature 38-39°C	Discharge from eyes and nose. Coughed on movement. High temperature >39.5°C	Severe discharge with pus. Frequent coughing/wheezing. High temperature >39.5 °C
Scours		
		
Clean hindquarters. Well formed faeces. Normal temperature 38-39°C	Dirty hindquarters. Loose faeces. Variable temperature	Wet hindquarters. Hair loss. Dehydrated. Watery faeces. High temperature >39.5 °C

Source: AHDB (2025)

1.2.5 COW WELFARE

1.2.5.1 Technological housing systems

The technological system for cattle breeding consists of:

- housing,
- feeding,
- watering,
- milking of cows,
- removal of excrement,
- movement corridors and areas,
- ventilation,
- additional so-called comfort aids.

The risk of health problems and the transmission of infectious diseases in dairy cows can be reduced by improving overall cleanliness and hygiene, as well as through targeted management of housing, feeding, and watering practices (The Cattle Site, 2022). The main risk factors associated with housing and cow management are illustrated in Figure 8.

Housing systems for dairy cows are generally divided into two basic categories:

- free housing with cubicles (lying boxes) or pen housing (Tab. 6).
- tethered housing.

Housing forms the foundation of the technological system of dairy farming, and its design determines the choice and functionality of other components. Housing parameters should provide conditions that allow animals to fully realize their productive potential while ensuring adequate nutrition. At the same time, the spatial needs of the cows must be respected, particularly with regard to their natural behaviors. Proper housing design should ensure that animals remain clean, while minimizing the need for manual labor (Brouček et al., 2014).

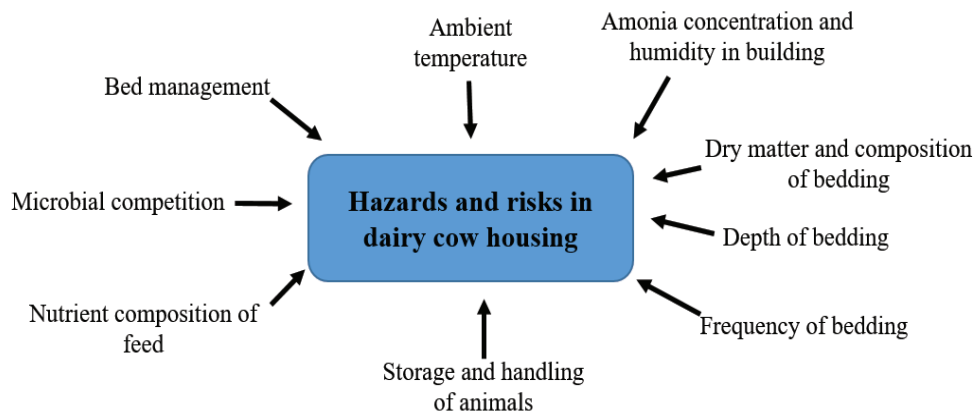


Figure 8: Factors affecting hazards and risks associated with bedding materials in dairy cow housing

Source: Zigo et al (2021).

Table 6: Parameters for free housing with cubicles (lying boxes) or pen housing for dairy cows

Weight (kg)	Lying boxes		Pen housing	
	Length (mm)	Width (mm)	Area (m ²)	Depth (mm)
600	2430	1233	5.8	8250
650	2478	1251	6.0	8450
700	2517	1269	6.2	8600
750	2551	1279	6.3	8750

Source: Brouček et al. (2014).

Both free and tethered housing systems have distinct advantages and disadvantages. According to Praks et al. (2007), the risk of foot-and-mouth disease is higher in free-housing systems; however, the prevalence of mastitis is significantly lower than in tethered-housing systems. Hoof diseases are more easily managed in tethered systems, but natural behavior is severely restricted. Permanent tethering may negatively affect cow welfare, leading to insufficient resting comfort, limited exercise, and restricted social interactions (Popescu et al., 2014).

Loose housing systems, in contrast, allow more natural behavior but reduce the level of direct human–animal contact. This may complicate the early detection of behavioral changes or disease symptoms, potentially delaying treatment and increasing the risk of premature culling (Beaver et

al., 2021). In addition, monitoring individual feed intake in free housing systems is difficult, which can increase the likelihood of nutritional imbalances, metabolic disorders, and infertility (Popescu et al., 2014).

Practical experience indicates that the most suitable housing system for dairy cows is free housing with cubicles (Fig. 9).



Clean bedding = cows spend more time lying down, limbs suffer less



When cows are overfed, they spend more time waiting in the corridors for a chance to lie down than to eat. Every hour of lying down under 12 hours is minus 1-1.5 L of milk

Figure 9: Housing cows in cubicles

Source: Schaumann Agri International GmbH (2023).

Properly designed lying boxes provide animals with sufficient comfort and welfare, help maintain cleanliness, and enable undisturbed rest due to reduced interaction with other animals. The benefits of cubicle housing can only be fully achieved if the dimensions are correctly adapted to the body size of the cattle. Cubicles must ensure maximum comfort when lying down and allow natural standing and lying movements. If the cubicles do not provide adequate comfort, cows may choose alternative resting places, such as the feed alley or manure passage, resulting in increased soiling. To accommodate the entire herd, cubicles should be dimensioned according to the largest animals in the group (Brestenský, 2020). Free-range housing is most effective when farmers minimize the introduction of pathogens into the barn and prevent their transmission between cows or from humans to cows. One of the most important ways to limit pathogen growth and survival is to maintain clean, dry bedding. Such bedding not only reduces microbial survival but also improves

cow hygiene by absorbing moisture from the body. In addition, comfortable bedding encourages longer lying periods. Increased lying time is associated with higher milk production, which ultimately leads to greater profitability for dairy producers (McLanahan, 2021).

Free housing systems have the greatest positive impact when farmers can effectively minimize the entry of pathogens into the barn and prevent their transmission between cows or from humans to cows. A key strategy to reduce pathogen survival and growth is maintaining clean and dry bedding. Clean, dry bedding not only decreases pathogen survival but also improves cow hygiene by absorbing excess moisture from their bodies

The welfare of dairy cows is closely linked to the type of bedding material used, as well as its impact on barn hygiene, cow comfort, and udder health. Commonly used bedding materials include straw, sawdust, sand, heat-treated manure and mattresses. Each has distinct advantages and limitations:

- Straw is beneficial as it increases lying time, contributing to comfort and overall welfare.
- Recycled manure solids (RMS) have been used as a substitute bedding material in recent years to create sufficient comfort for dairy cows due to the frequent lack of straw. RMS consists of dry matter and a nutrient-rich fraction obtained by mechanical or gravitational separation of slurry manure removed from dairy cow housings systems. To ensure its hygienic quality and optimum pH, RMS is often combined with straw and other components such as limestone or zeolite.
- Sand is highly valued for maintaining cow cleanliness and health; it supports faster healing of tarsal lesions and hoof diseases.
- Mattresses for dairy cows are specialized bedding made from materials like rubber, foam, and latex to provide comfort, support, and hygiene. Options include rubber mats for durability and insulation, high-density latex mattresses that conform to the cow's body to reduce pressure points, and gel-infused foam that helps dissipate heat (Fig. 10). The goal is to create a safer and more comfortable lying surface to prevent injuries and improve cow well-being.

Types of mattresses:

Rubber cow mats: A practical and durable option that provides good insulation, especially in cold weather. They are often used in stall flooring to keep cows safe and comfortable.

Latex cow mattresses: Made from 100% natural latex foam, these are known for their resilience and ability to mold to the cow's body. This distributes pressure evenly, reducing stress on joints and preventing injury.

Gel-infused foam mattresses: These mattresses are designed to conduct heat away from the cow, helping prevent heat buildup and reduce small milk leaks.

Waterbed mattresses: These provide a stable and consistent cushion for cows when they lie down. They have a separation between the front (knee) and the rear (belly/heel) water compartments. This gives the Dual waterbeds extra knee comfort. Dual waterbeds are suitable for all types of freestall divider. The mattresses are supplied on rolls and rolled into the barn, after which they are anchored and filled with water.

Foam mattresses: These can be a good option, though some studies suggest they can lead to higher hock scores and lameness compared to other bedding materials.



Figure 10: Soft cubicle mats, latex mats and waterbeds mat

Source: North Brook (2025)

Reduced lying time forces cows to spend longer periods standing on hard surfaces. This often leads to a posture where the forelegs rest on the bedding and the hind legs remain on the passage, which significantly increases the risk of lameness. Concrete is the most common flooring material in dairy barns due to its durability, cost-effectiveness, and ease of cleaning. However, concrete surfaces are closely associated with lameness problems. To mitigate these risks, floors should be flat and slightly rough to prevent slipping, but without sharp edges that could cause injury. Grooved concrete is considered suitable, while rubber mats or strips placed on the surface can reduce the

negative effects of hardness, roughness, and slipperiness. Poor bedding and flooring management, combined with inadequate barn hygiene, can lead to reduced lying comfort, increased incidence of lameness, and higher risks of mastitis. These factors contribute to premature culling and a shortened productive life of dairy cows (Brestenský, 2020).

1.2.5.2 Principles of cow comfort

Cow comfort is a key factor contributing to high milk production. A comfortable environment must provide:

- clean, dry bedding
- sufficient lying space
- shade in hot weather
- non-slip indoor flooring.

The design of the production farm should be adapted to the region, particularly the local climate. Where sufficient affordable bedding material is available, traditional barns can be a comfortable and cost-effective solution. Free housing barns are generally more expensive, but they offer several advantages. These barns are typically arranged with individual cubicles along the sides and a wider open space in the center. This layout allows cows to move freely, enter cubicles to rest or feed, and ensures better separation of manure from bedding (Molnár, 2022).

When designing housing systems for dairy cows, several critical aspects must be taken into account:

- adequate space in stalls, allowing cows to move both forward and sideways
- proper ventilation to ensure good air quality and temperature regulation
- non-slip flooring outside the stalls to reduce the risk of lameness
- sufficient feed space for all cows to access feed simultaneously
- efficient waste management to keep manure separate from both bedding and feed
- soft, comfortable bedding, aerated daily and replaced regularly
- easy access to clean water and feed at all times.

Although optimizing these housing details may require higher initial investment, it pays off through improved cow health, welfare, and productivity.

1.2.6 HEAT STRESS AND COMFORT

Heat stress has a profound negative impact on the health, welfare, and performance of dairy cows, resulting in substantial economic losses for the dairy industry. Cows in peak lactation are especially vulnerable, with the effects of heat stress becoming evident at temperatures as low as 21 °C. Global warming not only increases the number of hot and tropical days but also extends the duration of daily high temperatures. This prolonged exposure reduces feed intake, impairs nutrient digestibility, and decreases overall productivity (Tab. 7) (Brestenský, 2015).

Table 7: The body's reaction to extreme temperatures in connection with the development of a stress reaction

Body's reaction to extreme temperatures		
Stress	Low	High
Mild (low intensity and duration)	constriction of superficial blood vessels, bristled hair, increased muscle tension and tremors, lower respiratory rate, huddling of animals, increased locomotor activity, reduced body surface area.	dilation of superficial blood vessels, increased water intake, decreased muscle tension, polypnea, greater dispersion of animals, increased body surface area, less movement.
Medium	reduced overall activity, animals bristled and little movement.	lethargy and weakness, stretched body, labored breathing.
Heavy	labored breathing, decreased metabolism, animal dies.	weakness, stiffness, coordination disorders, convulsions, death.

Source: Tančin et al. (2024)

1.2.6.1 Causes of heat stress

Microbial activity in the rumen generates heat, contributing to the overall thermal load of dairy cows. Lactating cows produce more metabolic heat than dry cows, with high-yielding cows generating significantly more heat than those with lower milk production. Additional heat stress arises from solar radiation and from heat accumulation in poorly ventilated barns. As a result, cows divert part of the energy normally allocated to milk production toward thermoregulation, leading to reduced productivity (West, 2003).

1.2.6.2 Protecting animals against heat stress

Cows dissipate accumulated heat through three main mechanisms:

- convection – release of heat into the surrounding air
- conduction – transfer of heat to a cooler surface, such as the floor,
- evaporation – cooling through sweating and respiration.

When these mechanisms are insufficient, cows experience heat stress and rely on thermoregulation to reduce excess body heat. This leads to shorter feeding times, reduced digestibility and nutrient utilization, and altered behavior, such as seeking cooler, damp areas or drafts. Consequently, milk yield and overall productivity decline (Das et al., 2016).

1.2.6.3 Means to minimize the risk of heat stress

Proper barn ventilation prevents heat accumulation and supports convection, enabling heat dissipation through the body surface. Ventilation is achieved using air inlets and outlets located mainly along the perimeter walls, complemented by an open ridge slot in the roof. When indoor temperatures exceed 24 °C, airflow must be increased with fans, typically placed in lying and feeding areas or other locations where cows spend most of their time (Mondaca, 2019).

Shading also plays a critical role in protecting animals from direct solar radiation. On pastures, natural shade is provided by trees, while in barns it can be ensured by shelters or shading nets. Without adequate shading, cows crowd into shaded areas, reducing convection efficiency and increasing the risk of contamination in the milking parlour with environmental pathogens (Fig. 11).



Figure 11: Properly ventilated and shaded barn vs. poorly ventilated and shaded barn

Source: Schaumann Agri International GmbH (2023)

Another effective measure against heat stress is ensuring sufficient water intake. When cows lack access to adequate fresh water, their bodies prioritize vital functions over milk production. Reduced water consumption also leads to decreased feed intake, which further lowers productivity (Țogoe and Mincă, 2024).

Digestion of fiber in the rumen generates heat, so nutritional adjustments are necessary when cows are at risk of heat stress. Heat production can be reduced by decreasing the proportion of roughage and increasing the share of easily digestible concentrates. However, this raises the risk of rumen acidosis. For this reason, roughage must be of the highest quality, and the proportion of acid detergent fiber (ADF) should not drop below 19% of dry matter in the ration. The risk of acidosis can be further reduced by partially replacing concentrate energy with bypass fats, which bypass the forestomachs and are absorbed in the abomasum and small intestine (Mondaca, 2019).

During periods of high temperatures, supplementation with minerals such as potassium, sodium, and magnesium is advisable. In addition to ration composition, feeding management must also be adapted. Feed should not remain in troughs for extended periods in hot weather, as its sensory quality deteriorates. Larger feed portions should preferably be offered in the evening, when ambient temperatures are lower (Ben Salem and Smith, 2008).

Evaporation cooling can be achieved either by lowering the animal's body temperature or by cooling the surrounding air. Evaporative cooling systems generate a fine mist of water droplets that evaporate before reaching the ground, thereby reducing the ambient air temperature. On hot days with low relative humidity (around 30%), this method can decrease air temperature by up to 20%, although under some conditions the air temperature may initially rise slightly due to moisture

saturation. The system works by injecting water into the environment through high-pressure nozzles, which requires a pump and a high-pressure pipe network. However, potential drawbacks include the risk of animals inhaling water particles, which may lead to respiratory complications, and the formation of a water film on the coat. Such a film can interfere with heat transfer from the animal's body to the environment, reducing the effectiveness of cooling (Beigtan et al., 2024).

1.2.7 Veterinary care

Veterinarians are an integral part of dairy cow health management. While farm staff can manage many routine tasks, veterinarians are indispensable for administering medications, responding to health emergencies, performing diagnostic tests, and confirming cases that require culling. It is therefore important to budget for veterinary services and establish a strong relationship with a local veterinarian. This ensures that expert support is available whenever antibiotic treatment or other interventions are required (Raber et al., 2018).

Although veterinary care can be financially demanding, untreated health problems are often even more costly. Losses from mastitis, reduced productivity in cows affected by lameness, and the death of calves or adult animals can have a significant economic impact. Most production farms spend more on treating diseases than on preventing them. While some conditions cannot be fully prevented, strengthening preventive care helps reduce their incidence, making it the most cost-effective strategy in the long term. Preventive measures include routine treatments such as vaccinations, but veterinarians can also provide valuable advice, training, and consultation on herd health. When preventive veterinary care becomes a planned and consistent part of the farm budget, it not only improves cow health but also enhances long-term farm profitability (Raber et al., 2018).

To maintain high herd health and productivity, farmers and veterinarians should aim to achieve the following targets:

- ensure that at least 60% of cows have a somatic cell count (SCC) below 100,000/ml
- maintain bulk tank SCC below 200,000/ml
- keep mortality and culling within 60 days after calving under 3.5%
- limit the incidence of clinical mastitis to less than 2% per month
- limit the incidence of subclinical mastitis to less than 15% per month
- keep annual culling due to mastitis below 5%

- produce “plus-cows” (to have as many inseminated and confirmed pregnant cows as possible within 90 days after calving)
- improve the intercalving period to less than 411 days
- extend the productive lifespan of cows to more than three years (Kováč et al., 2023).

2 Chapter – Milk Production

Factors influencing the quantity and quality of milk production in dairy cows include breed, individual animal characteristics, dry period management, disease incidence, and nutrition. Nutritional factors play a key role and may differ depending on the production system. These include the overall nutritional level, the ratio of roughage to concentrates, feed particle size (feed processing), and nutrient concentration in the ration. All of these directly affect milk composition, including fat content and somatic cell count (Kováč et al., 2023).

The productive capacity of dairy cows depends on the coordination of multiple factors. In addition to nutrition and feeding, environmental conditions, animal care, and milking techniques must also be considered. Milk production itself is regulated by an integrated system that involves the hormonal system, nervous stimuli, environmental influences, and nutrition (Gross, 2021).

In a well-adjusted system, these factors act in harmony, allowing cows to reach their maximum production potential during each phase of lactation. Conversely, disruption of any single factor may cause a sharp decline in milk yield. Interestingly, milk as the final product of this interaction also exerts a feedback effect, influencing the intensity of these regulatory processes (Chreneková et al., 2019).

2.1 Hormonal system

The hormonal system is hereditary but undergoes changes, for example during pregnancy or illness, when its disruption may lead to a decline in milk production. The pituitary gland plays a central role in this process, as it secretes a complex of hormones necessary for maintaining milk secretion. In addition, it regulates the activity of the adrenal glands, thyroid gland, and gonads, all of which influence lactation to varying degrees (Miholová, 1999).

The adenohypophysis, also known as the anterior pituitary, is the front lobe of the pituitary gland that produces and secretes most of the pituitary's hormones in response to signals from the hypothalamus. These hormones regulate key physiological processes such as growth, reproduction, metabolism, and stress response, and they control other endocrine glands. The bovine adenohypophysis, or anterior pituitary, is a key endocrine gland in cows that produces hormones regulating growth, reproduction, and metabolism, including prolactin, growth hormone, luteinizing hormone, and follicle-stimulating hormone. Its function is highly responsive to nutritional status and reproductive cycles, and it is subject to regulation by the hypothalamus (Suga et al. 2011).

From a physiological perspective, the mammary gland depends on the synergistic action of two anterior pituitary hormones: prolactin (LTH, luteotropic hormone) and somatotropin (STH, growth hormone). These are the key factors inducing lactogenesis (the initiation of milk formation and secretion) and galactopoiesis (the maintenance of lactation) (Tab. 8; Červený, 2007).

The release of these hormones not only directs overall metabolism in favor of the mammary gland but also stimulates processes within secretory cells. This includes their regular turnover through proliferation and apoptosis, which ensures functional renewal and efficiency of the mammary tissue (Jelínek and Koudela, 2003). The first stage of lactogenesis is characterized by increased enzymatic activity in the mammary gland and the differentiation of cellular organelles, accompanied by limited milk secretion before parturition. The second stage is associated with the abundant secretion of all milk components in most animals. Colostrum is produced just before parturition, and this secretion continues for several days postpartum.

Hormonal regulation during the second stage of lactogenesis - the onset of abundant milk secretion at parturition involves increased secretion of prolactin (LTH), adrenocorticotrophic hormone (ACTH), and estrogens, accompanied by a decline in progesterone secretion. These hormones interact in several ways:

- **Prolactin** induces casein synthesis in mammary parenchymal tissue through gene expression, with **glucocorticoids** required for this process.
- **Progesterone** contributes to the formation of prolactin-binding receptors in mammary tissues but also competes with glucocorticoids for binding sites. Thus, a decline in progesterone levels is a prerequisite for the initiation of lactogenesis.
- An increase in **prostaglandin** production just before parturition causes regression of the corpus luteum, resulting in a subsequent decline in progesterone levels.
- In cattle, **estrogen concentrations** begin to rise about one month before parturition, peaking approximately two days before calving. Estrogens stimulate lactogenesis by promoting the release of prolactin and likely other anterior pituitary hormones.
- A sharp increase in **growth hormone (STH)** secretion from the adenohypophysis occurs just before parturition, directing nutrients from the bloodstream into the mammary gland to support milk synthesis (Chreneková et al., 2019).

After calving, the peak of milk production in dairy cows occurs between the 4th and 8th week of lactation. Once this peak is reached, milk yield gradually declines. To sustain lactation, both the

number of milk-producing cells and the activity of alveolar cells must be maintained. In addition to the secretion of hormones required for lactation, regular removal of milk from the mammary gland, either by milking or by suckling is essential. This process is closely linked to neurohumoral regulation at all stages: preparation for secretion, initiation of lactation immediately after calving, the period of sustained milk production, and milk ejection from the alveoli (Sopková et al., 2013).

Table 8: The role of various hormones on mammary function during lactation

Hormone	Function
Prolactin	This is the primary hormone for initiating lactation. High levels are seen around calving, and it promotes the development and function of the milk-producing cells.
Progesterone	Along with estrogen, it stimulates the growth of mammary secretory tissue. However, it also inhibits the full differentiation of these cells until after birth.
Estrogen	Stimulates the growth of the mammary ducts. It works with progesterone to promote the proliferation of secretory tissues.
Adrenocorticotropic hormone	Hormone made by the pituitary gland that stimulates the adrenal glands to produce cortisol, a key stress hormone. It plays a crucial role in the body's response to stress, regulating blood sugar, metabolism, and inflammation.
Glucocorticoides	These are essential for the biosynthesis of milk components like casein and lactose, working with prolactin and insulin to activate these processes.
Insulin	This hormone is also required for the biosynthesis of milk proteins and lactose, acting alongside glucocorticoids and prolactin
Bovine somatotropin	Also known as growth hormone, stimulates overall growth and metabolic processes, which are vital for supporting high milk production. Growth hormone (GH) is widely known for its galactopoietic effect in lactating dairy cattle.

Source: Wall and McFadde (2012)

According to Chreneková et al. (2019), the increase in prolactin (LTH) secretion during milking is primarily the result of udder and teat stimulation. Without this stimulation, LTH release does not occur. After lactation begins, basal prolactin levels may fall to a minimum during milking, but this does not negatively affect milk yield.

Milk contains a relatively high level of calcium, making functional adrenal glands essential for maintaining lactation. Both mineralocorticoids and glucocorticoids, which play key roles in metabolic regulation, are necessary in ruminants and non-ruminants alike. Glucocorticoids are steroid hormones produced from the cortex of adrenal glands (gluco-corti-coids: glucose-cortex-steroids). Glucocorticoids have a pivotal role in the glucose, protein, and fat metabolism of the body. They originate from steroid precursors and are synthesized primarily in the zona fasciculata of the adrenal cortex. The essential glucocorticoid in the body is cortisol. Cortisol acts on glucose metabolism to cause hyperglycemia. This effect is not only involved in maintaining normal glucose homeostasis but at times of stress, glucose happens to be the only substrate that provides energy to the critical organs of the body. Hyperglycemia is caused by increasing the synthesis of enzymes involved in glycogenolysis and gluconeogenesis (Suga et al. 2011). Plasma corticosteroid concentrations are higher in lactating animals compared to non-lactating ones, with high-producing cows having the greatest requirements. Parathyroid hormone (PTH), secreted by the parathyroid glands, also supports lactation by stimulating calcium resorption from bones and facilitating the conversion of vitamin D to its active form, which is required for calcium absorption in the intestine (Vajda and Maskal'ová, 2016).

2.2 Nerve impulses

Factors influencing milkability are often associated with nervous stimuli, the importance of which is sometimes underestimated by milkers both during preparation of the cow for milking and during the milking process itself. The stimuli generated during milking have both physiological and psychological justification, and they significantly influence milk secretion and yield. Modern milking equipment is designed to replicate the physiological conditions of manual milking; however, any deviation from the correct settings such as fluctuations in vacuum can have long-term negative effects on milkability (Tančín et al., 2001).

Improper handling also plays a role. Forcing cows into the milking parlor or rough and unprofessional treatment before or during milking negatively affects milk let-down and secretion.

In the long term, insufficient removal of milk leads to an accumulation of residual milk, which inhibits its own synthesis and limits total yield. Quality also deteriorates, as retained milk provides nutrients for bacterial growth and serves as an inoculum for potential pathogens, thereby increasing the risk of mastitis (Vasil' et al., 2016).

Disorders of the milk ejection reflex are closely linked to stress factors. These disorders can arise through two mechanisms:

- **central disorders**, originating in the central nervous system
- **peripheral disorders**, occurring at the level of the udder (Skřivánek, 2011).

Central milk ejection disorders are characterized by the cessation of oxytocin release from the neurohypophysis into the bloodstream. They are primarily caused by emotional stress. Such disorders are most commonly observed:

- during the first few milkings after calving,
- when milking in the presence of the cow's own calf or when combining suckling and milking,
- after weaning, especially following long-term contact between cow and calf,
- during suckling by another calf in systems using foster cows,
- after relocation to new milking conditions (e.g., following reconstruction of housing or milking facilities),
- after transfer from the maternity pen to the production barn, or when regrouping cows prior to milking,
- when changing milkers, in the presence of nervous or aggressive milkers, or with frequent organizational changes during milking.

Peripheral milk ejection disorders are mainly caused by human factors, such as inappropriate handling of animals before milking or incorrect use of technology. These factors stimulate the release of adrenaline into the bloodstream, which interferes with milk ejection (Tančin and Tančinová, 2008).

Both central and peripheral disorders can be prevented by improving conditions before and during milking and by ensuring calm, consistent, and professional handling of cows by milkers (Brestenský et al., 2015).

2.3 Milking technologies and adherence to hygiene procedures

The selected milking technology and work procedures, together with the dairy cow's response to external stimuli, significantly influence the milking process, milk quality, and udder health. The current level of milking equipment technology, the quality of materials used for structural components, and the degree of automation of the milking process reflect advancements

in the understanding of milking physiology, milk hygiene, and milk handling. Many work procedures have been automated, and the technical reliability of milking machines has improved. At the same time, working conditions for operators have become more comfortable, even with increased demands for adjustments in milking procedures. Paradoxically, when assessing milk quality, there remains ongoing discussion about factors, practices, and interrelationships that continue to negatively affect it and persist in primary production for various reasons (Tančin et al., 2006).

On most farms of Visegrad countries, milk is obtained using machine milking in separately located milking parlors. Depending on stall design, parlors can be either stationary or mobile. A mobile milking system is a movable milking solution, typically mounted on a platform or trailer, that brings the milking equipment to the animals instead of the animals to a fixed barn. This system provides flexibility by allowing farmers to milk cows in different locations, such as pastures, and can be used as a temporary or permanent setup. It combines the functionality of a traditional milking parlor with the ability to be easily relocated (Fig. 12).



Figure 12: Mobile milking parlour

Source: McDonald (2012)

Types of milking parlors:

Herringbone parlors: In these systems, cows stand at an angle to the milker, allowing multiple cows to be milked in a line or staggered pattern.

Parallel parlors: In parallel milking parlours all cows stand in parallel to each other and milking only begins when each cow is in its stall.

Rotary parlors: A circular platform rotates, bringing each cow to a stationary milker. This is efficient for milking large herds, as the cows move themselves into the correct position.

Side Open or Tandem: In side opening or tandem stalls, cows stand in an end-to-end configuration during milking, and units are attached from the side. This parlor type is less affected by variations in individual cow milking times than in herringbone or parallel parlors, as cows are moved one at a time rather than in groups (Fig. 13).

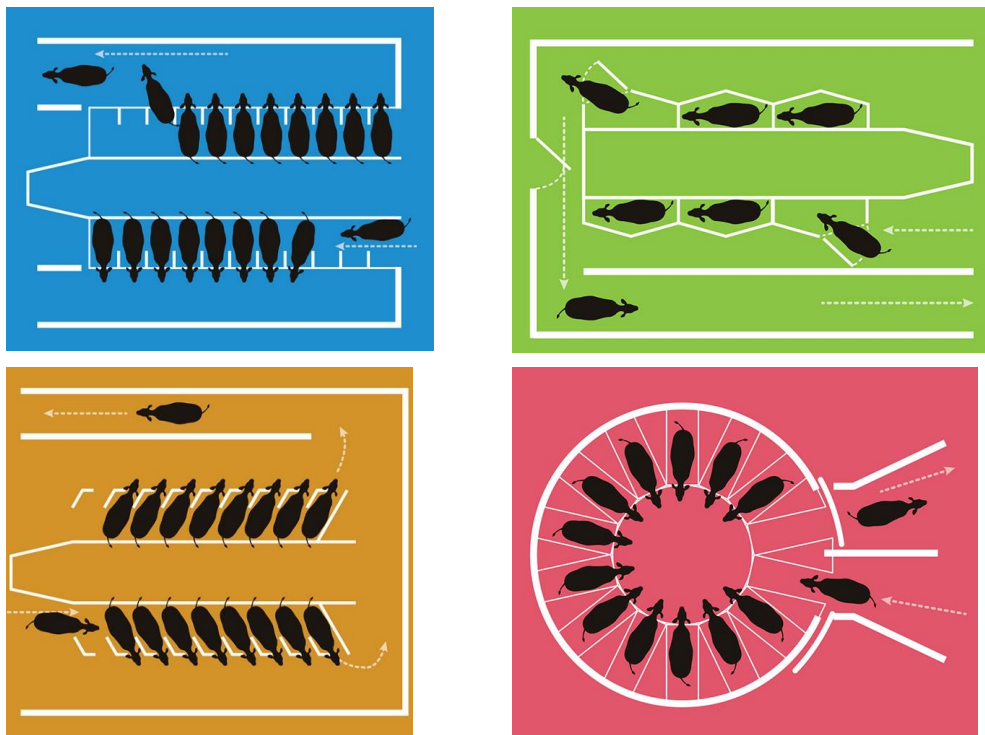


Figure 13: Parallel, Tandem, Herringbone and Rotary type of parlor

Source: Reinemann and Rasmussen (2016)

Flat barn parlors: Cows are milked in a straight line, often in a "flat barn" setting. In this parlors the milking area and the operator's area are at the same or similar elevation. Various stall designs are used, from simple stanchions in which the cow must back out of the stall after milking to more complicated gates that cows walk through after milking.

Robotic: This on-farm technology allows a cow to be milked when she chooses, in a self-service fashion. Each cow has a radio frequency identification (RFID). A cow enters a robotic milking unit when she wishes to be milked and is identified by her tag. The robot is triggered to prepare for the milking process. This means dropping her specially formulated ration into the feed bin, and automatically cleaning her udder. Then a laser beam or camera detects the exact position of the teat and the milking apparatus attaches. The milking stops once the robot senses the flow of milk has slowed. A gate then opens and the cow is free to return to the rest of her day.

Long-term breeding efforts have resulted in dairy cows with high productivity and the ability to release milk rapidly. Despite these advances, machine milking remains an unnatural method of milk extraction. To minimize its potential negative impact on the cow, it is essential even with modern machine milking technologies to respect the physiology of milk ejection and adhere to strict hygiene requirements (Brestenský et al., 2015).

On most farms, cows are milked twice daily, which yields approximately 40% more milk compared to once-daily milking. Increasing the frequency to three times per day results in an additional 20% increase in milk yield. This effect is most pronounced in cows during their first lactation and diminishes with age. The increased production associated with more frequent milking can be explained by:

- reduced intramammary pressure due to shorter intervals between milkings,
- increased stimulation of hormonal activity supporting milk production, and
- reduced negative effects on secretory cells caused by milk component accumulation (Bouška et al., 2006).

Specific in this regard are robotic milking parlors, which operate non-stop to manage the physiological needs of cows and their milking. The transition to an automatic milking system requires careful preparation, not only because the acquisition of robotic milking represents a significant investment, but also because the need for further training of farmers or employees cannot be underestimated. Robotic or automatic milking systems can milk cows without the need for human labor. The animals themselves decide when to be milked and the system, thanks to installed accessories, records various detailed information about each milking, as well as other data about the animal, such as its weight, temperature, and physical activity, which the farmer can check and analyze on his computer or mobile device. However, this does not mean that the importance of having quality milkers has decreased. Their work still ensures the smooth functioning of the

farm, contributes to high cow productivity, health and welfare. Common reasons for using milking robots are to improve the working conditions and lifestyle of the farmer or dairy farm workers, or to reduce the costs of operating the milking parlor and the problems of finding quality milkers, but also to benefit the health and welfare of the cows (Fig. 14).



Figure 14: Robotic milking system

Source: Mikšovský Marcinková (2023)

Proper milking hygiene plays a crucial role in maintaining udder health (Fig. 15). Preparation for milking should include cleaning the udder and providing sufficient stimulation to ensure an optimal milk let-down reflex. Correct attachment of teat cups, continuous monitoring of the milking process, and regular maintenance of machine functionality are essential to prevent teat contamination by environmental pathogens. After milking, teat tips must be disinfected within one minute of cluster removal, and thorough sanitation of the milking machine and pipeline system must be ensured (Brestenský et al., 2015).

An essential part of hygiene measures in milk production is the initial treatment of milk immediately after milking. This includes cleaning (mechanical filtration), rapid cooling, and maintaining the milk at low temperatures until it is transported to the dairy. These steps are critical for preserving the high hygienic quality of raw milk. To ensure its quality during handling and storage, milk must be cooled immediately after milking to 3–5 °C and kept at this temperature throughout the storage period (Viguier et al., 2009).

Adherence to strict hygiene principles during milking minimizes the risk of mastitis transmission between cows and reduces damage to teat tissue. The hygienic quality of milk is also

influenced by the disinfectants and cleaning agents used in the milking parlor, which ensure proper teat disinfection and sanitation of equipment, as well as by the overall organization of milking procedures. In addition, the motivation, professionalism, and competence of milkers play a crucial role. Achieving a high standard of milk hygiene is very difficult without the active participation and personal responsibility of farm staff (Tančin et al., 2005).

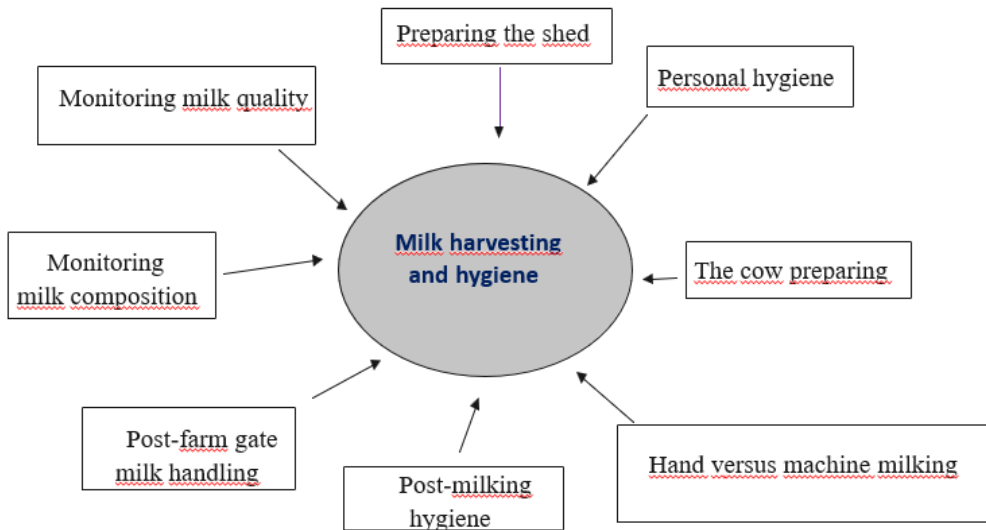


Figure 15: The key elements of good milking hygiene

Source: Zigo et al. (2021)

3 Chapter - Mastitis – inflammation of the mammary gland

Mastitis is an inflammatory condition of the mammary gland, most frequently caused by microbial infections, particularly bacteria. While physical and chemical irritants can also induce inflammation, the predominant etiology involves pathogenic bacteria that infiltrate the udder tissue, multiply, and release toxins detrimental to mammary tissue integrity (Radostits et al., 2007).

Globally, mastitis ranks among the most significant diseases affecting dairy livestock, with negative consequences on milk productivity and widespread distribution across dairy-producing regions. It is recognized as a multi-etiological disease (Fig. 16), with over 150 different microbial species implicated in its pathogenesis.

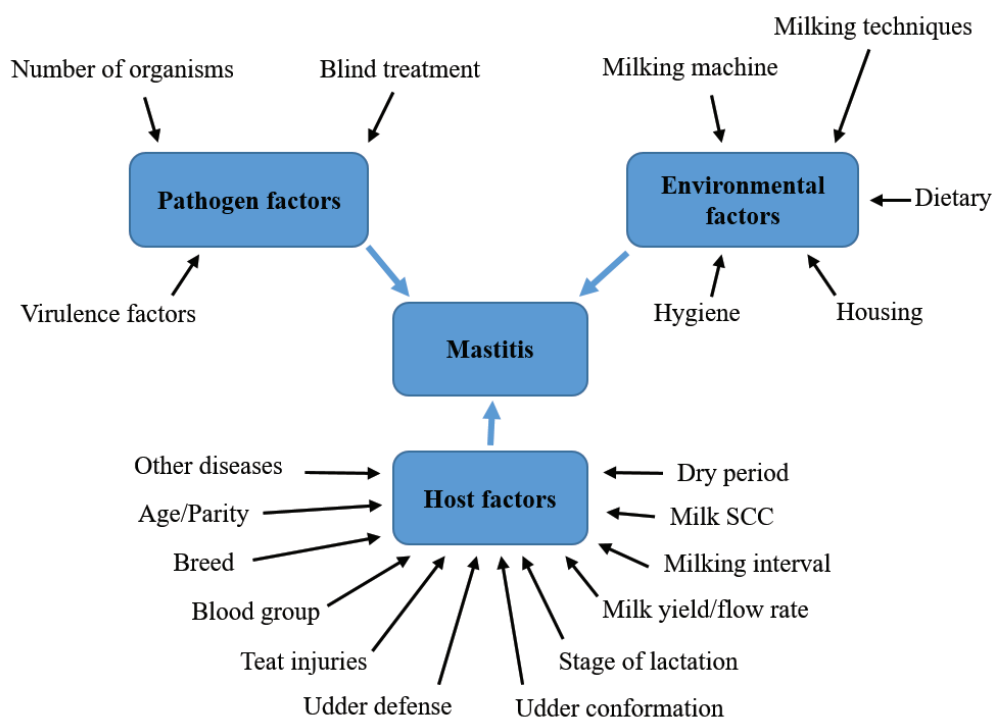


Figure 16: Factors influencing the development of mastitis in dairy cows

Source: Zigo et al. (2021)

A broad spectrum of microorganisms including bacteria (e.g., *Escherichia coli* (G-), *Staphylococcus aureus* (G+), *Klebsiella pneumoniae* (G-), *Streptococcus agalactiae* (G+),

Corynebacterium bovis (G+), *Mycobacterium bovis* (G+), actinomycetes (e.g., *Nocardia asteroides* (G+)), fungi (e.g., *Candida albicans*, *Aspergillus* spp., *Cryptococcus neoformans*), *Mycoplasma* spp. (G-), viruses, and even algae (*Prototheca* spp.) have been associated with the onset of mastitis in dairy herds. Based on the transmission route of the causative agents, mastitis is broadly classified into contagious and environmental types (Radostits et al., 2007).

3.1 Contagious Mastitis

Contagious mastitis is primarily caused by bacterial pathogens that reside in the udder and are transmitted from infected to healthy cows, predominantly during the milking process. Transmission occurs via contaminated milking equipment, the hands of milkers, or soiled cloths. Key contagious pathogens include *Streptococcus agalactiae* (G+), *Staphylococcus aureus* (G+), *Corynebacterium bovis* (G+), and *Mycoplasma* spp. (G-). Among these, *S. aureus* (G+) is the most isolated organism worldwide in both subclinical and chronic mastitis (Blowey and Edmondson, 2010).

The incidence of contagious mastitis depends on the microbial load, virulence, and the cow's physical and immunological defenses. Prevalence can vary significantly based on breed and geographical region, with *S. aureus* (G+) infection rates estimated between 7% and 40% of cows at any given time. *Streptococcus agalactiae* (G+) tend to localize in the ducts of the mammary gland, where antibiotics are effective. *Streptococcus agalactiae* (G+) is very sensitive to penicillin, so the therapeutic efficacy is relatively high. However, *Staphylococcus aureus* (G+) forms clusters of infection protected from antibiotics by scar tissue (Fox and Gay, 1993).

3.2 Environmental Mastitis

Unlike contagious mastitis, environmental mastitis is caused by pathogens that do not reside on the skin or within the udder but are introduced via contact with contaminated surroundings. Common pathogens include Coagulase-negative staphylococci (CNS), *Streptococcus uberis* (G+), *Str. dysagalactiae* (G+), and coliform bacteria such as *Escherichia coli* (G-) and *Klebsiella* spp (G-). These organisms originate from bedding, manure, feed, and general farmyard conditions (Radostits et al., 2007).

Environmental mastitis is more prevalent in cows housed indoors, especially in poorly managed environments with wet or soiled bedding. Infections with *E. coli* are most observed

immediately before and after parturition, highlighting the importance of clean, dry conditions during the dry and periparturient periods. Although previously less common, environmental mastitis now accounts for a growing proportion of mastitis cases globally. Most environmental infections are presented as acute clinical mastitis and are often short-lived. Notably, *Str. dysgalactiae* (G+) has shown both environmental and contagious transmission patterns (Blowey and Edmondson, 2010).

3.3 Classification Based on Clinical Symptoms

3.3.1 Clinical Mastitis

Clinical mastitis is characterized by visible signs of inflammation in the udder and abnormalities in the milk, such as clots, flakes, and changes in color or consistency. The affected quarter of the udder may appear swollen, warm, and painful to the touch, and clinical signs are often accompanied by systemic symptoms such as fever, anorexia, and lethargy. In well-managed herds, the incidence of clinical mastitis typically remains below 5% (Tomita and Hart, 2001).

This form of mastitis can develop suddenly and is often caused by both Gram-positive pathogens (e.g., *Staphylococcus aureus*) and Gram-negative bacteria (e.g., *Escherichia coli*), which trigger strong immune responses, including the acute-phase inflammatory reaction. Clinical mastitis leads to a significant reduction in milk yield and, in severe cases, may progress to gangrenous mastitis (Keane, 2019).

Forms of Clinical Mastitis:

- **severe clinical mastitis**

Cows exhibit extreme illness, often with marked depression and risk of death. The udder may become gangrenous ("black mastitis"). Initially, the milk may appear normal, despite the cow being clearly unwell. However, it soon becomes visibly abnormal, often discolored or containing clots.

- **acute clinical mastitis**

The cow may or may not show systemic illness. The affected quarter is typically hot, swollen, and painful. The milk is abnormal, possibly discolored, and may contain clots or blood. Effective detection and prompt treatment are essential to reduce long-term damage to the udder and minimize economic losses (Oliveira et al., 2013).

3.3.2 Subclinical Mastitis

Subclinical mastitis lacks visible signs but is associated with elevated somatic cell counts (SCC) and detectable pathogens in milk. This type affects more than 50% of animals in some herds and accounts for 15 - 40 times more cases than clinical mastitis. Despite the absence of overt symptoms, it reduces milk quality and quantity and acts as a reservoir for infection transmission within herds (Tomita and Hart, 2001).

Its economic implications are severe, being responsible for 60–70% of total mastitis-related losses in countries like the United States. Prolonged infections may hinder antibiotic efficacy due to fibrous encapsulation of pathogens. Pathogens such as *S. aureus* and environmental organisms like *Str. uberis* are commonly implicated (Atasever and Erdem, 2009).

3.3.3 Persistent Mastitis

Chronic mastitis represents a persistent inflammatory condition that can extend across lactation periods. Often originating as subclinical, it may occasionally flare into acute or subacute episodes. Chronic cases are marked by fibrous tissue development, leading to palpable nodules or indurations within the udder (Taverna and Dhong, 2007).

3.4 Etiologic Agents of Mastitis

3.4.1 Major Pathogens

Among bacterial pathogens, *Staphylococcus aureus* (G+) is a predominant cause of both clinical and subclinical mastitis in India (Fig. 17; Kováč et al., 2023), while *Candida* species represent the leading fungal pathogens in cases of mycotic mastitis. In European countries, the prevalence of fungal mastitis in dairy cow's ranges from 1% to 10%. Data from Lassa and Smulski (2013) report the involvement of *Arcanobacterium pyogenes* (G+) (31.0%), coliforms (16.4%), fungi (3.3%), algae (0.9%), and *Corynebacterium* spp. (G+) (0.8%) as significant contributors to mastitis. Approximately 90% of pathogens responsible for udder inflammation are environmental in origin. These pathogens are commonly found in cow-barn environments, where increased humidity and pollution lead to a higher microbial load on animals. Notably, except for *Actinomyces pyogenes* (G+), which is exclusively associated with clinical mastitis, most microorganisms can cause both clinical and subclinical mastitis (Malinowski et al., 2006).

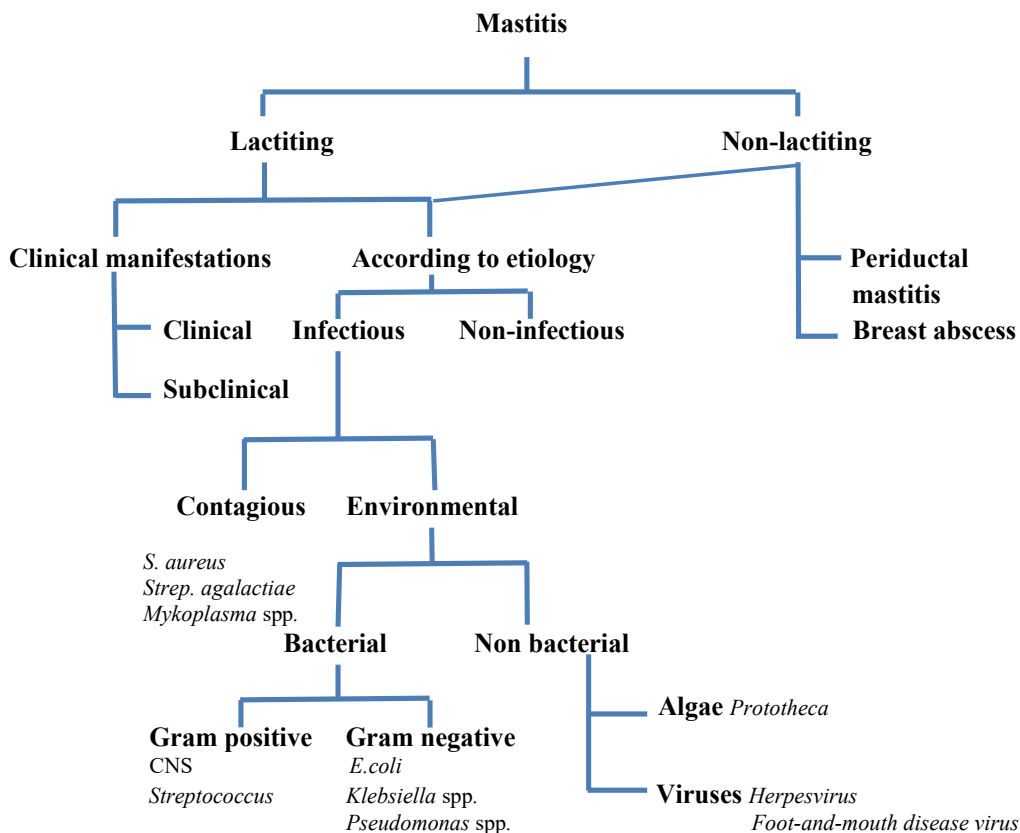


Figure 17: Classification of mastitis depending on the stage of lactation, clinical symptoms and etiological factors

Source: Kovač et al. (2023)

Staphylococcus aureus (G+), *Escherichia coli* (G-), and *Klebsiella* spp. (G-) cause the greatest milk production losses in primiparous cows. In older cows, *Streptococcus* spp. (G+), *S. aureus* (G+), *Actinomyces pyogenes* (G+), *E. coli* (G-), and *Klebsiella* spp. (G-) are the most detrimental (Tab. 9). Major pathogens are typically associated with clinical mastitis and lead to significant alterations in milk composition (Pal, 2018).

Table 9: Classification of mastitis-causing pathogens

Source	Mammary Gland	Environment
Major pathogens	<i>S. aureus</i> (G+) <i>Str. agalactiae</i> (G+) <i>(Str. dysgalactiae</i> (G+)) <i>Mycoplasma bovis</i> (G-)	Environmental streptococci: <i>Str. uberis</i> (G+), <i>Str. equinus</i> (G+), <i>(Str. bovis</i> (G+)), <i>(Str. dysgalactiae</i> (G+)) <i>Enterococcus</i> spp. (G+): <i>E. faecalis</i> , <i>E. faecium</i> , <i>E. durans</i> Coliforms: <i>E. coli</i> (G-), <i>K. pneumoniae</i> (G-), <i>K. oxytoca</i> (G-), <i>Enterobacter aerogenes</i> (G-) Non-coliforms: <i>Proteus</i> spp. (G-), <i>Serratia</i> spp. (G-), <i>Yersinia</i> spp. (G-) <i>Others:</i> <i>Pseudomonas aeruginosa</i> (G-), <i>Arcanobacterium pyogenes</i> (G+)
Minor pathogens	CNS: <i>S. chromogenes</i> (G+), <i>S. haemolyticus</i> (G+), <i>S. epidermidis</i> (G+), <i>S. simulans</i> (G+), <i>S. sciuri</i> (G+) <i>Corynebacterium bovis</i> (G+)	<i>Yeasts</i> <i>S. Fungi</i>

Note: Table modified according to Heikkilä et al. (2018).

3.4.2 Staphylococcus aureus

Staphylococcus aureus, a Gram-positive bacterium (G+), is a leading cause of bovine mastitis. It colonizes the teat skin and migrates through the teat canal into the mammary gland. The organism possesses numerous virulence factors, including the ability to adhere to milk fat globules, facilitating rapid entry into mammary tissue. Intramammary infection (IMI) with *S. aureus* (G+) often results in chronic clinical mastitis (Fig. 18) that may persist throughout the cow's lifetime (Yang and Bäckhed, 2008).

S. aureus (G+) is also zoonotic; chronically infected cows can serve as reservoirs, transmitting the bacterium to humans via direct contact (e.g., during hand milking) or through the consumption of unpasteurized milk. Certain strains produce toxins that pose health risks to both

cows and humans; these toxins are not destroyed by pasteurization. Thus, *S. aureus* (G+) infections are of concern for both animal and public health. This pathogen is among the most frequently isolated from mastitis cases globally (Zigo et al., 2022, 2024).

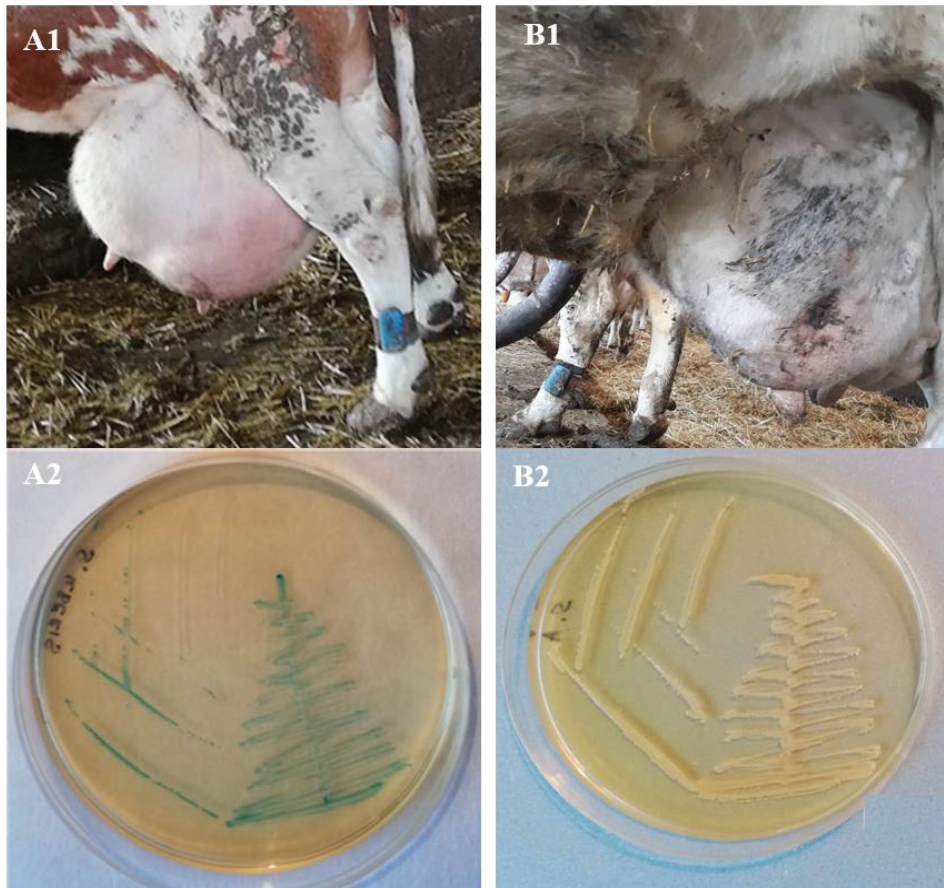


Figure 18: Clinical mastitis cases with positive bacteriological cultivation

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

Photo: Zigo (2021)

3.4.3 Streptococci Species

Three primary *Streptococcus* spp. (G+) cause bovine mastitis: *Str. agalactiae* (G+), *Str. dysgalactiae* (G+), and *Str. uberis* (G+). Their prevalence and pathogenic impact vary by region.

Streptococcus agalactiae (G+) is an obligate udder pathogen and is highly contagious. It possesses multiple virulence factors and can cause both clinical and subclinical mastitis. It primarily infects the gland cistern and ducts, causing swelling and irritation (National Mastitis Council - NMC, 1999).

Streptococcus dysgalactiae (G+) is generally considered an environmental pathogen but shows characteristics of both environmental and contagious organisms. It is less contagious than *Str. agalactiae*, and thus, herd prevalence is typically lower. Although often associated with "summer mastitis," it can cause mastitis year-round. It is found in the cow's environment, on teat skin, and even in the rumen (Schukken et al., 2011).

Streptococcus uberis (G+) is primarily environmental, with the cow's surroundings serving as its reservoir. It resists control methods like teat dipping due to its environmental origin. Although it is not traditionally considered contagious, recent evidence suggests it may be transmitted between cows (Fig. 18).

3.4.4 Coliforms

Coliform bacteria such as *Escherichia coli* (G-) and *Klebsiella spp.* (G-) are common mastitis pathogens. They are part of the normal gut flora of cattle and contaminate bedding and other environmental materials through feces. Cows are especially vulnerable to coliform mastitis during the puerperal period, when their immune defenses are suppressed. All Gram-negative bacteria, including coliforms, produce endotoxins that trigger a strong inflammatory response. *Klebsiella spp.* (G-) infections often affect multiple cows within a herd. These infections are typically severe but develop more gradually than those caused by *E. coli*, lasting longer and often resulting in subclinical mastitis.

Escherichia coli (G-) commonly causes acute clinical mastitis, characterized by rapid progression and, in some cases, fatal outcomes. Although *E. coli* (G-) may also cause subclinical mastitis, these strains differ from those that induce clinical disease. The prevalence of *E. coli* (G-) and *Klebsiella spp.* (G-) in mastitis cases varies by herd and region (Dogan et al., 2005).

3.4.5 Mycoplasma Species

Mycoplasma spp. (G-) is highly contagious but less prevalent than *S. agalactiae* (G+) and *S. aureus* (G+). These bacteria damage secretory tissues and induce fibrosis of both glandular tissue and lymph nodes. Cows of all ages are susceptible, particularly during early lactation. High-

yielding cows may harbor the pathogen without showing clinical signs. Mycoplasmosis is often associated with mastitis outbreaks following the introduction of new animals to a herd or a history of respiratory disease. When mastitis recurs, presents without obvious signs, or fails to respond to antibiotic treatment, *Mycoplasma* infection should be suspected (Fox et al., 2005).

3.4.5.1 Minor Pathogens

Minor pathogens, including *Corynebacterium bovis* (G+), *Pseudomonas aeruginosa* (G-), and coagulase-negative staphylococci (CNS), typically cause mild infections and are predominantly associated with subclinical mastitis. Interestingly, these organisms may exert a protective effect against major pathogens, potentially through mechanisms such as competitive inhibition, antagonism, leukocyte induction, or enhancement of the cow's immune response (Lam et al., 1997).

CNS are opportunistic pathogens and natural colonizers of teat skin. They rarely cause clinical mastitis and are often excluded from routine mastitis studies. However, their prevalence has increased in recent decades, and they are now recognized as important mastitis agents globally, although still not classified as major pathogens. This group includes over 50 species and subspecies, such as *S. chromogenes* (G+), *S. epidermidis* (G+), *S. hyicus* (G+), *S. simulans* (G+), *S. haemolyticus* (G+), and *S. xylosus* (G+), which are frequently isolated from mastitis cases (Tab. 9). CNS are strongly linked to subclinical mastitis and show higher prevalence in primiparous animals. They vary in antimicrobial susceptibility, virulence factors, and the host's immune response (Taponen et al., 2009).

Other agents implicated in bovine mastitis include fungi (*Candida albicans*, *C. tropicalis*, *Cryptococcus neoformans*, *Geotrichum candidum*), algae (*Prototheca spp.*), *Mycoplasma spp.* (G-), *Chlamydia spp.* (G-) and viruses such as bovine herpesviruses, cowpox virus, pseudocowpox virus, vesicular stomatitis virus, foot-and-mouth disease virus, and bovine papillomaviruses. These viruses can cause teat lesions - particularly in the ductus papillaris - compromising the udder's natural defense mechanisms and predisposing it to bacterial infections (Wellenberg et al., 2002).

3.5 Pathogenesis

A thorough understanding of mastitis pathogenesis is essential for developing effective detection methods. Although a wide variety of bacterial strains are the primary causes of mastitis, viral, algal, and fungal infections have also been reported. Normally, the teat canal is tightly closed

by sphincter muscles, which prevent pathogen entry. The canal is lined with keratin, a waxy substance that inhibits bacterial migration. However, keratin's protective effect is limited. As parturition approaches, fluid accumulates in the mammary gland, increasing internal pressure and causing the teat canal to dilate and leak secretions, making the gland more susceptible to infection. During milking, keratin is flushed out and the teat canal stretches, requiring approximately two hours to close again. Once bacteria enter the teat, they must evade the udder's cellular and humoral defenses; if successful, they multiply within the mammary gland (Fig. 19; Rainard et al., 2006).

Once inside the mammary gland, pathogens release toxins that stimulate leukocytes and mammary epithelial cells to secrete chemoattractants. These include cytokines such as tumor necrosis factor- α (TNF α), interleukins (IL-1, IL-8), eicosanoids like prostaglandin F 2α (PGF 2α), reactive oxygen species, and acute phase proteins (APPs) such as haptoglobin (Hp) and serum amyloid A (SAA). These signals recruit polymorphonuclear neutrophils (PMNs), the primary immune cells, to the infection site (Zhao, 2021).

PMNs combat infection by engulfing and destroying bacteria through both oxygen-dependent and oxygen-independent mechanisms. Their intracellular granules contain bactericidal peptides, enzymes (e.g., myeloperoxidase), and both neutral and acidic proteases, such as elastase and cathepsins B, D, and G. While these components are essential for killing bacteria, they also damage some mammary epithelial cells. This contributes to reduced milk production and increased levels of enzymes like *N*-acetyl-*D*-glucosaminidase (NAGase) and lactate dehydrogenase (LDH) in milk. After completing their role, most PMNs undergo apoptosis, and the remaining cells are cleared by macrophages. The accumulation of dead PMNs and sloughed epithelial cells in milk elevates the somatic cell count (SCC), a key marker of mastiti (Paape, 2003).

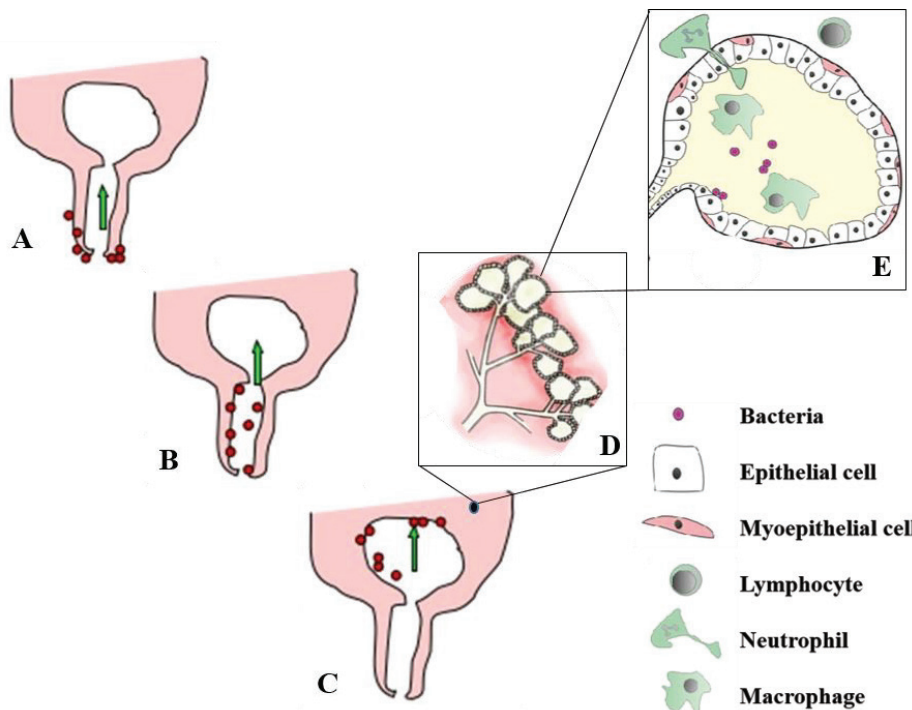


Figure 19: Process of udder infection

Note: A – Organisms invade the udder through teat canal, B, C - Migrate up the teat canal and colonize all parts where the milk gets and finally setup the infection in mammary gland, D - alveoli and secretory mammary epithelial cells. After getting bacterial infection, cellular defence mechanism become active and phagocytic cells (from blood) effort to engulf and kill the bacteria, phagocytosis by products and release of bacterial toxins damage to the secretory mammary epithelial cells (E).

Source: Zigo et al. (2021)

If the infection becomes chronic, internal swelling of the mammary epithelium and structural damage to alveoli occur. This disrupts the blood-milk barrier, allowing ions such as sodium, chloride, hydrogen, potassium, and hydroxide to leak into the milk. In severe cases, blood may appear in the milk due to extensive barrier damage. Clinically, this results in visible udder inflammation-swelling and reddening as well as changes in milk characteristics, including increased conductivity, pH, water content, and the presence of clots or flakes (Zhao, 2021).

3.6 Economic Impact of Mastitis

Mastitis leads to significant economic losses for dairy farmers through both direct and indirect costs (Fig. 20), impacting milk production, treatment, and herd management. Mastitis is a globally prevalent health issue in dairy animals, manifesting in both sporadic and epidemic forms, and causing substantial economic burdens on the dairy sector. Estimates suggest that the disease is responsible for annual financial losses of approximately 33 billion USD. As a complex and multifactorial disorder, the development of mastitis is influenced by various factors associated with the host, infectious agents, and environmental conditions. It is primarily characterized by alterations in the milk's physical, chemical, and microbiological properties, alongside pathological changes in the mammary tissue (Radostits et al., 2007).

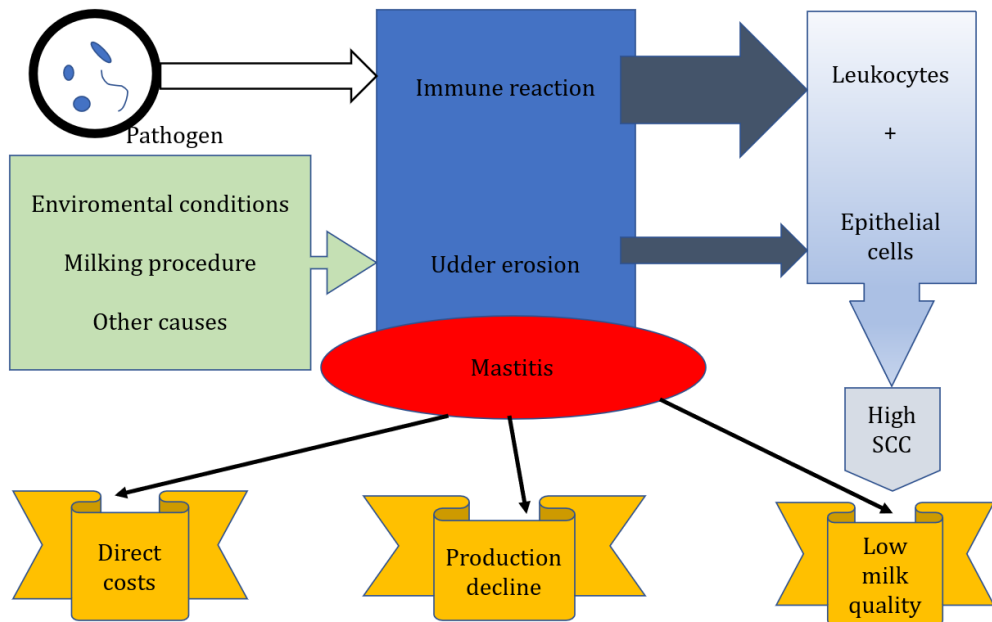


Figure 20: Breeding and economic impacts of mastitis

Source: Arvaiova et al. (2023)

Economically, subclinical mastitis has a more profound impact due to its insidious nature and prolonged effects on milk yield. Moreover, several mastitis-causing pathogens are of zoonotic significance, posing risks of transmission to humans and potentially causing illnesses such as brucellosis, campylobacteriosis, listeriosis, tuberculosis, and Q fever.

The total economic cost includes:

- **production losses** (milk yield and quality reductions)
- **control expenditures** (treatment and prevention costs, Tab. 10)

Therefore, mastitis incurs both **visible** costs (like treatment and discarded milk) and **hidden** costs (like reduced fertility and increased culling), resulting in a substantial economic burden on dairy operations. Effective mastitis management require understanding and mitigating both direct and indirect losses to improve herd health and farm profitability (Osterås, 2005).

Indirect costs from mastitis can include SCC penalties and increased culling rates. In Britain, mastitis has been documented to be the leading cause of premature culling in dairy cattle. In addition, an association between clinical mastitis and reduced reproductive performance in lactating dairy cattle has been reported; cows with clinical mastitis prior to being confirmed pregnant showed increased days to first service, days open, and services per conception. In summary, clinical and subclinical intramammary infection is a major issue for the dairy industry, with broad-ranging impacts and consequences (Radostits et al., 2007).

Table 10: Direct and indirect production losses

Direct Losses	
Milk Production Losses	<ul style="list-style-type: none"> • both clinical and subclinical mastitis reduce milk yield • yield losses persist even after recovery • severity depends on pathogen type, cow parity, and lactation stage • multiparous cows experience more severe losses than primiparous. pathogens causing major losses include <i>s. aureus</i>, <i>e. coli</i>, <i>klebsiella spp.</i>, <i>streptococcus spp.</i>, and <i>a. pyogenes</i>
Effects on Milk Composition and Quality	<ul style="list-style-type: none"> • mastitis lowers fat and casein content • clinical and subclinical forms cause fat loss ranging from 3 to 22 kg per lactation (1.5 - 7.5%) • milk quality deterioration affects processing, cheese yield, shelf life, and flavor
Treatment Costs	<ul style="list-style-type: none"> • includes expenses for drugs and medications • costs vary by country and local regulations
Discarded Milk	<ul style="list-style-type: none"> • milk from cows showing clinical signs or under antibiotic treatment must be discarded • discarded milk loss includes both the lost product and associated feeding costs, making it more expensive than unrecovered yield loss
Mortality	<ul style="list-style-type: none"> • delayed recognition and treatment increase risk of death, especially in cases caused by gram-negative bacteria
Veterinary Services	<ul style="list-style-type: none"> • costs for veterinary diagnosis and treatment visits • can be mandatory or optional depending on legislation
Diagnostics	<ul style="list-style-type: none"> • costs for lab tests, cultures, equipment, reagents, and advanced diagnostics like pcr
Materials and Investment	<ul style="list-style-type: none"> • includes costs of consumables like disinfectants and drugs (renewable materials) • non-renewable investments include infrastructure like milking parlors and their maintenance

Indirect Losses	indirect costs are often hidden and less obvious but affect long-term herd productivity
Culling	<ul style="list-style-type: none"> • clinical mastitis increases the risk of culling and mortality • risk influenced by lactation stage and reproductive status (open vs. pregnant cows) • subclinical mastitis (high somatic cell count >300,000 cells/ml) is a key driver for culling decisions • economic costs include replacement heifer costs and lost future production
Subsequent Disorders	<ul style="list-style-type: none"> • mastitis predisposes cows to additional mastitis cases and other disorders such as lameness, ketosis, displaced abomasum, and paresis • it negatively affects reproductive performance, causing extra inseminations and longer calving intervals
Extra Labor	<ul style="list-style-type: none"> • increased labor for veterinary care, medication, and altered milking routines • clinical cases may require about 2 extra hours of labor per case. • opportunity cost of labor must be considered when evaluating economic impact

Source: modified table according to Arvaiova et al. (2023)

3.7 Risk factors influencing the development of mastitis

The mammary gland of dairy cows is continuously exposed to microorganisms present in the rearing environment. Under unfavorable conditions, these pathogens have ideal opportunities to penetrate the udder and establish intramammary infections, ultimately leading to impaired udder health. According to Foltys and Kirchnerová (2003), the causes of mastitis can be broadly divided into two categories:

- diseases of the mammary gland and excretory ducts caused by microbial pathogens
- diseases of the mammary gland and excretory ducts resulting primarily from incorrect milking procedures.

Of critical importance in understanding mastitis is the complex interaction between the infectious agent and the host. A cow's susceptibility to mastitis is influenced by multiple factors, including age and parity, stage of lactation, milk yield, anatomical predispositions, and especially the immunological condition and reactivity of the mammary gland. The variety and interplay of these factors make mastitis a polyfactorial disease.

Several host-related characteristics, pathogen-specific traits, and environmental or management factors can significantly increase the risk of mastitis development in dairy cows (Peeler et al., 2000).

3.7.1 Host Factors

3.7.1.1 Age

Adult cows are more frequently affected by mastitis compared to younger ones, with the incidence peaking around 5 years of age.

3.7.1.2 Breed

Certain breeds are more susceptible to mastitis. For example, among Swedish breeds, Swedish Holstein cows show a higher incidence compared to Swedish Red cows. Crossbred cows are generally more prone to mastitis than local breeds, possibly due to anatomical differences in teat structure and lower genetic resistance to infections.

3.7.1.3 Parity

The risk of mastitis tends to increase with the number of lactations. Older cows, particularly those beyond four lactations, are more susceptible. Multiparous cows are at higher risk of clinical mastitis, although this trend reverses during the early stages of lactation (Lakew et al., 2009).

3.7.1.4 Stage of Lactation

Clinical mastitis is more common during the early stages of lactation, while the likelihood of subclinical mastitis increases as the number of days in milk rises (Persson et al., 2009).

3.7.1.5 Milk Yield

Mastitis is more prevalent in high-yielding cows, followed by medium and low-yielding animals. This is possibly due to factors such as udder and teat anatomy, increased susceptibility linked to specific genes, and reduced phagocytic cell efficacy due to dilution effects in high-

producing cows. In dairy farming, mastitis, laminitis, and metritis are distinguished as the main three production diseases. From fig. 21 shows that the incidence of the most common production diseases increases with high milk yield. In dairy cows with milk yield over 10,000 liters per lactation, the risk of mastitis is up to 35% (Radostits et al., 2007).

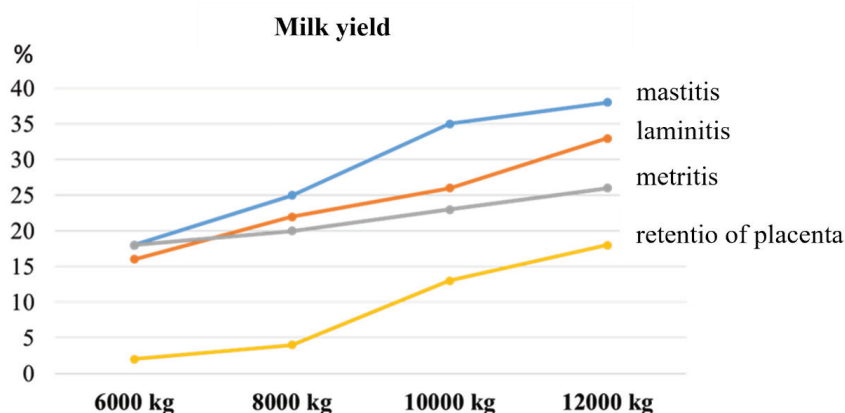


Figure 21: Incidence of production diseases depending on milk yield

Source: Zigo et al. (2021)

3.7.2 Agent Factors

Pathogenic bacteria responsible for mastitis possess various virulence factors, including adhesion molecules for colonization, capsule formation, virulence genes carried on plasmids, and toxin production. For example, *Escherichia coli* (G-) produces lipopolysaccharide endotoxins that contribute to inflammation during coliform mastitis. *Staphylococcus aureus* (G+) isolated from infected udders produce multiple virulent factors, such as enterotoxins, hemolysins, hyaluronidase, and leukocidins. Additionally, antibiotic resistance is common in some strains for instance, certain *Staphylococci* produce β -lactamase, which confers resistance to penicillin (Olsen et al., 2006).

3.7.3 Managemental Factors

Environmental and managemental conditions significantly influence mastitis risk. Cows housed in unsanitary conditions or subjected to poor milking hygiene are more likely to develop mastitis. Tick infestations around the udder also elevate the risk. Environmental mastitis is further associated with conditions such as wet or dirty bedding, milking wet udders, inadequate pre-milking teat preparation, and poor fly control. It means environmental contamination and

suboptimal husbandry practices are critical contributors to the incidence of mastitis in dairy cows. The environment in which cows are housed, milked, and fed provides abundant opportunities for the introduction of pathogens to the teat canal (Almaw et al., 2008).

3.7.3.1 Milking Hygiene

Improper milking hygiene, including inadequate cleaning and disinfection of teats and milking equipment, can lead to the transmission of both environmental and contagious mastitis pathogens. Practices such as failing to use individual towels for drying teats and improper hand hygiene of milkers significantly elevate mastitis risk. The National Mastitis Council (NMC) (1999) highlights that pre- and post-milking teat disinfection are critical steps in reducing pathogen load and subsequent intramammary infections.

3.7.3.2 Bedding Material and Cleanliness

The choice and management of bedding material directly affects mastitis prevalence. Organic bedding materials, such as straw or sawdust, can harbor environmental mastitis pathogens like *Streptococcus uberis* and coliforms if not regularly replaced or kept dry. Frequent bedding replacement and dry, clean stalls reduce the microbial burden and exposure of teats to pathogens (EFSA, 2009).

3.7.3.3 Unsuitable condition of dairy cows

Poor condition of dairy cows is a common cause of production and metabolic problems in the herd. Condition assessment serves as an important tool in nutritional management and overall herd management and is used in a 5-point assessment system. Overweight cows are more susceptible to metabolic diseases and may have complications during childbirth, while emaciated individuals suffer from reduced milk production due to lack of energy reserves. The combination of condition assessment, milk production, reproductive indicators and nutrition according to the lactation phase provides valuable information for analyzing the herd condition and proposing appropriate measures in the herd (Vajda and Maskařová, 2016).

3.7.3.4 Feeding Practices

Insufficient nutrient composition of the feed ration and an inappropriate ratio of rumen-degradable and non-degradable proteins as well as the amino acid profile of proteins can negatively affect the immune system of cows with a higher risk of inflammation. The unbalanced content of

starch, minerals and vitamins in TMR reduces the body's resistance to infections (Khan et al., 2024). Poor feeding practices that result in nutritional deficiencies, especially of trace elements like selenium and zinc or vitamin E (Tab. 11), impair the cow's immune defenses, increasing susceptibility to mastitis. Additionally, feeding methods that encourage cows to lie down immediately after eating - before the teat canal has closed - can raise infection risk (EFSA, 2009).

Table 11: Overview of Se and vitamin E deficiency syndromes in ruminants

Species	Syndrome	Affected system, resp. organ
Cattle	Nutritional myodystrophy of calves	Skeletal muscle, myocardium
	Retained placenta	Placental connection with the uterus
	Ovarian cysts	Ovaries
	Decreased production, mastitis	Udder, mammary gland
	Immune system disorders	Decrease Th lymphocyte production and phagocytic activity
	Anemia	Erythrocytes
Sheep/goat	Nutritional myodystrophy	Skeletal muscle, myocardium
	Infertility	Loss of uterine tone
	Decreased production, mastitis	Udder, mammary gland
	Immune system disorders	Decrease Th lymphocyte production and phagocytic activity

Source: Zigo et al. (2021)

Similarly, increased lipomobilization and the occurrence of metabolic diseases after calving are often the result of negative energy balance and inappropriate phase feeding, when dairy cows are not adequately differentiated according to production level before calving. Overfeeding low-producing cows can lead to excessive condition and fattening, which increases the risk of intense lipomobilization, fatty liver infiltration and ketosis. These disorders negatively affect the

reproduction of dairy cows and are associated with immunosuppression, which increases susceptibility to diseases such as metritis, mastitis and pneumonia (Esposito et al., 2014).

3.7.3.5 Occurrence of mycotoxins in feed

Mycotoxins can significantly increase the risk of mastitis in dairy cows because they weaken the immune system, damage the intestinal barrier and cell membranes, disrupt liver function and promote inflammatory processes in the body. Monitoring the content of mycotoxins in feeds and using quality detoxification products is an important preventive strategy to protect the health of dairy cows. The most important mycotoxins associated with an increased incidence of mastitis include deoxynivalenol (DON), aflatoxins, fumonisins and zearalenone (Corassin and Oliveira, 2023).

3.7.3.6 Ventilation and Housing Environment

Inadequate barn ventilation promotes high humidity and ammonia accumulation, which can irritate teat skin and predispose it to infection. Well-designed housing should provide ample airflow, minimize the growth of environmental pathogens and maintain udder health (Blowey and Edmonson, 2010).

3.7.3.7 Heat Stress

Heat stress has emerged as a significant risk factor for mastitis, as it negatively affects immune competence and alters cow behavior. During periods of heat stress, cows are more likely to congregate near water troughs or in shaded, often contaminated areas, increasing exposure to pathogens. Heat stress impairs neutrophil function, decreasing the cow's ability to clear intramammary infections. Effective cooling systems, such as fans and sprinklers, and providing adequate shade, are essential for mitigating heat stress-related mastitis risks (Cheng and Han, 2020).

4 Chapter - DIAGNOSIS OF DAIRY MASTITIS

Mastitis occurs in two primary clinical forms: clinical and subclinical. Clinical mastitis presents with overt symptoms and is often manageable with timely therapeutic intervention. In contrast, subclinical mastitis lacks visible signs and is frequently undetected without diagnostic testing. The most used diagnostic tools include the California Mastitis Test (CMT), somatic cell count (SCC), and bacteriological culture of milk samples. Advanced techniques such as measurement of N-acetyl-D-glucosaminidase (NAGase), lactate dehydrogenase (LDH), and electrical conductivity (EC), as well as molecular diagnostics like PCR, are also available, albeit less commonly employed in routine screening. Reports on the prevalence of mastitis have shown significant variability, influenced by factors including geographic location, cattle breed, management practices, and diagnostic techniques. As a result, the overall prevalence of mastitis—regardless of its classification into clinical or subclinical forms has been reported to range widely from 0.4% to 81.1%. In Ethiopia, investigations into the occurrence of bovine mastitis have primarily relied on clinical examination of the udder for detecting clinical cases, while subclinical infections were typically diagnosed using cow-side tests. Among these, the CMT emerged as the most frequently utilized method, followed by the indicator paper test (Umer et al., 2015).

Clinical examination of the udder should begin with visual inspection, followed by palpation to detect signs such as swelling, pain, asymmetry, or teat abnormalities (e.g., blind teats). Milk should also be examined for discoloration and the presence of clots, which may indicate mastitis (Quinn et al., 2006).

4.1 California Mastitis Test (CMT)

The CMT is a simple, low-cost, and rapid on-farm diagnostic tool commonly used to detect subclinical mastitis during milking. The test involves collecting milk into a CMT paddle, followed by the addition of an equal amount of reagent-sodium alkyl aryl sulfonate using a gentle horizontal swirling motion for approximately 10 to 15 seconds. Sodium alkyl aryl sulfonate is an anionic surfactant that reduces surface tension and interacts with the cell membranes and nuclei of somatic cells in milk. It disrupts osmotic balance, blocks oxidation, stimulates proteolytic enzymes, and increases milk viscosity (Tanni et al., 2021). These reactions cause the formation of a gel, which corresponds to leukocyte counts ranging from 200,000 to 5,000,000 cells per milliliter—indicative of intramammary infection (IMI). Additionally, infected milk samples often exhibit a more intense

purple coloration due to their basic or alkaline pH. The deep purple color signals an abnormal milk composition caused by mammary gland infection (Marshall et al., 2023).

Several studies have demonstrated the correlation between CMT scores, and somatic cell counts (SCC). For instance, Hoque et al. (2015) found a significant positive relationship where higher CMT scores corresponded with elevated SCC levels. This finding was supported by Kaşıkçı et al. (2012), who categorized CMT reactions into three groups (CMT+, CMT++, CMT+++ or T, 1-3) and confirmed a direct correlation with SCC values (Tab. 12).

Table 12: Interpretation of CMT

CMT score	Somatic cell range	Interpretation
N (negative)	0 – 200,000	Healthy quarter
T (trace)	200,000 – 400,000 (±50,000)	Latent mastitis or subclinical mastitis
1	400,000 – 1,200,000 (±150,000)	Subclinical mastitis
2	1,200,000 – 5,000,000 (±800,000)	Subclinical or Serious mastitis
3	Over 5,000,000 (±300,000)	Serious mastitis

Note: CMT - The Californian Mastitis Test, SCC- somatic cell count, Latent mastitis* - milk appears normal, but infection is still present in samples of raw milk without changing of SCC with negative CMT score, Subclinical mastitis* - no signs are observed, the udder and milk appears normal, but infection is still present with positive CMT score and increased SCC. Serious* or clinical mastitis – signs range from mild to severe with positive CMT score, bacteriological cultivation, high level of SCC, changing the consistency of the milk with the presence of flakes, clots or pus and reduction or loss of milk production with clinical signs.

Source: Zigo et al. (2021)

The CMT is extensively validated for identifying infected quarters in cows outside the early lactation period (Fig. 22). Its ability to detect both clinical and subclinical mastitis toward the end of lactation is particularly valuable for implementing selective dry cow therapy (SDCT). Poutrel and Rainard (1981) reported that positive CMT scores several weeks before drying off accurately

predicted IMI in over 80% of cases. Similarly, Sanford et al. (2006) found a high negative predictive value for IMI caused by major pathogens when performing CMT on the day of dry-off. Bhutto et al. (2012) also highlighted CMT's good sensitivity and negative predictive value, although its positive predictive value and specificity were lower.



Figure 22: Infected quarter with positive CMT and sample cultivation

Source: Zigo et al. (2021)

The accuracy of the CMT can improve by testing duplicate or multiple milk samples. Fundamentally, the test works by disrupting the cell membranes of somatic cells in the milk sample, allowing DNA to react with the reagent (alkyl aryl sulfonate) and form a gel. This makes CMT a practical cow-side indicator of somatic cell count and an effective technique for detecting subclinical mastitis (David et al., 2005).

4.2 Somatic Cell Count (SCC)

Somatic cells, which include lymphocytes, macrophages, polymorphonuclear cells, and some epithelial cells, are integral components of the udder's natural defense mechanisms. The somatic cell count (SCC) serves as an indicator of the inflammatory response to intramammary infection (IMI). To differentiate between infected and uninfected quarters, a threshold SCC of 200,000 to 250,000 cells/mL is generally accepted. Milk with an SCC below 200,000 cells/mL is considered normal, whereas elevated SCC values are indicative of inflammation within the udder (Pillai et al., 2001).

4.2.1 SCC as a Diagnostic Marker

Somatic cell counts (SCC) is widely regarded as the gold-standard indirect marker for detecting intramammary infections (IMI) and monitoring udder health. A normal, healthy mammary gland typically has an SCC below 100,000 to 200,000 cells/mL, representing the baseline activity of resident immune cells. When an infection occurs, somatic cells, particularly neutrophils, are rapidly recruited to the site of infection as part of the mammary gland's inflammatory defense (Paape, 2003).

The threshold SCC level for distinguishing infected from uninfected quarters is generally set at 200,000 to 250,000 cells/mL. Milk from quarters with SCC above this range is considered to originate from inflamed tissue and is highly likely to harbor pathogens associated with mastitis. Quarters with SCC exceeding 1,000,000 cells/mL often correspond to clinical mastitis cases, where inflammation is visible and may be accompanied by abnormal milk and udder swelling (Schukken et al., 2003).

Under practical farming conditions, a farmer may lose several hundred euros (€130–250) per dairy cow per lactation in market profits from milk sales when SCC reach 350,000–400,000/ml. Applied to a herd of 100 dairy cows, using interpretative values (Table 13), and assuming a milk price of €0.40/l with a marginal SCC purchase threshold of 400,000/ml, this equates to an annual financial loss of approximately €20,000 (Tongel, 2009).

A mastitis control system within a herd can be implemented through regular SCC monitoring in pooled milk samples, based on monthly performance reports. If somatic cell counts in pooled milk exceed 400,000/ml for two consecutive months, the farm faces milk classification problems and must address the underlying causes of the unfavorable condition. The quickest corrective measure is to identify and separate cows with elevated SCC. These cows should be milked last into separate containers to ensure that milk with high SCC (often contaminated with microorganisms) does not mix with the bulk milk in the cooling tank. However, this approach represents only a short-term solution to reducing SCC levels in pooled milk samples (Zajac et al., 2012).

Table 13: Estimated milk losses due to increased somatic cell count

(SCC/ml)	Milk loss (%)	Production losses 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 (2005)

Farmers often classify chronically ill or incurable dairy cows into so-called “millionaire cows” in which multiple treatments fail to reduce SCC or eliminate the mastitis-causing pathogen. These cows represent not only a persistent source of high SCC but also a serious risk for transmitting resistant strains to other cows within the group and across the herd. Based on clear evidence and the frequency of chronic, untreatable mastitis caused by contagious pathogens in individual cows, culling should be considered. Removing cows with repeated mastitis and ineffective treatment outcomes is one of the most effective measures to lower herd SCC levels and reduce bacterial pressure, particularly in the case of contagious infections (Tab. 14; Tančin et al., 2005).

Another approach is prevention through strict monitoring of mastitic cows and their timely treatment. Differentiating between new (mainly subacute or acute) and chronic infections requires daily diagnostics and record-keeping. Treatment must begin immediately after detection, ideally following thorough milking of the affected quarter as first aid. Early diagnosis of mastitis, combined with bacteriological cultivation and the targeted selection of antibiotics based on sensitivity testing, significantly improves treatment effectiveness. This approach reduces SCC, restores milk yield, and proves to be both cost-effective and sustainable in the long term. For

example, reducing herd SCC from 600,000 to 300,000 in 100 dairy cows, at a milk price of € 0.40 per kg, can yield an annual financial gain of approximately € 6,700 through decreased production losses under the same feeding and management conditions (Harmon, 2006).

4.2.2 SCC Composition dynamics during mastitis

During IMI, the relative proportions of somatic cell types change dramatically. In healthy milk, macrophages predominate (~60%), followed by lymphocytes (~30%) and PMNs (~10%). However, during mastitis, the proportion of neutrophils can exceed 90% of total somatic cells, reflecting their key role in phagocytosis and pathogen clearance. This cellular shift is a hallmark of the mammary gland's acute response to infection (Rainard et al., 2006). It is important to interpret SCC with awareness of physiological and environmental modifiers:

- **Parity:** Older cows tend to have higher baseline SCC compared to heifers.
- **Stage of Lactation:** SCC often increases late lactation due to epithelial cell shedding and lower milk yield.
- **Milking Management:** Poor hygiene, machine malfunction, and over-milking contribute to elevated SCC.
- **Stress and Seasonality:** Heat stress and seasonal variations can transiently elevate SCC.

Table 14: Economic and Milk Quality Implications

Milk Quality Implications through SCC	
Reduced milk yield	Production losses of 0.4 kg/day per 100,000 cell/mL SCC increase have been reported
Altered milk composition	High SCC lowers lactose and casein content while increasing sodium and chloride, negatively impacting processing properties
Shortened shelf life	Elevated SCC accelerates spoilage due to higher enzyme activity

Source: Modified table according to Tančin et al. (2005)

4.2.2.1 Technological Advances in SCC Monitoring

New technologies for SCC monitoring include:

- **Automated milking systems:** Integrated SCC sensors provide continuous monitoring for early mastitis detection.
- **Portable SCC counters:** Handheld devices enable on-farm testing for rapid decision-making (Fig. 23).
- **Infrared thermography:** Can complement SCC by identifying localized inflammation in the udder.
- **SCC in mastitis control strategies**
SCC data is foundational to mastitis control programs:
- **Selective dry cow therapy:** High-SCC cows can be targeted for antimicrobial treatment during the dry period, reducing unnecessary antibiotic use.
- **Herd-level management:** Bulk tank SCC serves as a proxy for overall udder health and milk quality compliance (Olde Riekerink et al., 2012).



Figure 23: Portable SCC counter

Source: DeLaval (2025)

4.3 Electrical Conductivity

Electrical conductivity (EC) measures the ability of a material to conduct an electric current, defined as the reciprocal of electrical resistance. Electrical resistance (or impedance) is measured in ohms (Ω) and calculated as voltage divided by current (amperes), whereas conductivity is measured in Siemens (S) and calculated as current divided by voltage. Specifically, the resistance

of an electrolyte solution is defined by the resistance of a 1 cm³ cube of the solution (Nielen et al., 1992).

In milk, EC depends primarily on the concentration of ions, especially sodium (Na), potassium (K), and chloride (Cl). During intramammary infection (IMI), the integrity of tight junctions and the active ion-pumping systems in the mammary gland are compromised, leading to leakage of these ions into the milk and thereby increasing EC (Norberg, 2005).

Several factors can influence the EC beyond mastitis. For instance, a 2009 study found that metabolic disorders such as milk fever caused the greatest increase in EC, followed by pneumonia, ketosis, off-feed status, left displaced abomasum, retained placenta, and lameness—all conditions associated with elevated EC levels. Interestingly, increases in EC were detectable up to three days before clinical diagnosis of mild mastitis using other methods.

Methods to evaluate EC vary, but recent studies propose cut-off values of individual quarter conductivity > 6.5 mS/cm and a difference between the highest and lowest quarter conductivity > 1.0 mS/cm for infection diagnosis. EC accuracy has been found to compare favorably with other indirect diagnostic methods, particularly when measuring strip milk rather than foremilk obtained after milk ejection (Janzekovic et al., 2009).

Research comparing EC in mastitis caused by primary versus secondary pathogens observed significant differences in EC between infected and uninfected quarters. Both differential conductivity (difference between quarters) and absolute conductivity (individual quarter values) were useful, with absolute conductivity sometimes providing superior diagnostic performance, especially when measured in stripping milk.

The ability of EC to detect infections may also vary by bacterial species. For example, *Staphylococcus aureus* (G+) and *Streptococcus uberis* (G+) infections are more readily detected via EC than coagulase-negative staphylococci (CNS), possibly due to the less severe epithelial damage and localized nature of CNS infections in teat canal or cistern regions. Despite these findings, the EC's utility in detecting subclinical mastitis remains somewhat controversial, and additional research is required. However, combining EC with other parameters has been shown to significantly improve predictive accuracy.

In summary, mastitic milk typically exhibits higher electrical conductivity than normal milk, mainly due to tissue damage and the resultant increased concentrations of sodium and chloride ions (Ebrahimie et al., 2018).

4.4 Biochemical Tests

Biochemical tests are essential tools for the identification of bacterial species, relying on the unique biochemical activities exhibited by different organisms. Since bacterial physiology varies across species, differences in metabolic pathways (e.g., carbohydrate, protein, and lipid metabolism), the production of specific enzymes (e.g., coagulase, N-acetyl- β -D-glucosaminidase [NAG-ase], urease, and catalase), and the ability to utilize specific compounds serve as diagnostic markers. Many mastitis-causing bacteria can be identified using these tests. For example, *Staphylococcus* species can be characterized using the coagulase, catalase, Voges-Proskauer (VP), urease, and thermonuclease tests. *Streptococcus* species are commonly identified by sugar fermentation profiles, the catalase test, and esculin hydrolysis. Additionally, the IMViC series of tests (Indole, Methyl Red, Voges-Proskauer, and Citrate utilization) is widely used to identify *Escherichia coli* (G-) (Quinn et al., 2006).

4.5 Direct Microscopy

Milk samples can be centrifuged, and smears prepared from the sediment for microscopic examination. Gram staining is routinely used to identify Gram-positive pathogens such as *Staphylococcus* and *Streptococcus*, and can also detect yeasts like *Candida albicans*, which when *Mycobacterium fortuitum* or *M. bovis* are involved, a standard Ziehl–Neelsen stain is appropriate (Kalorey et al., 2007).

4.6 Molecular Biology Tools

Polymerase chain reaction (PCR) is employed to enhance the diagnosis of mastitis pathogens, particularly when conventional biochemical methods are unreliable for accurate species identification. PCR operates on the principle that bacterial cells possess nuclear DNA composed of nucleotide sequences that are unique to each species. This molecular technique is especially valuable for detecting obligate udder pathogens such as *Streptococcus agalactiae* (G+) in bulk milk samples, even in herds with a low prevalence of infection, where traditional culture methods may fail to identify the pathogen (Katholm et al., 2012).

4.7 Mammary Ultrasound

Ultrasonography is a non-invasive diagnostic technique widely employed to evaluate both physiological and pathological conditions of the reproductive organs in ruminants. In recent years, its applications have broadened to include assessing subcutaneous fat and diagnosing respiratory disorders. Increasing evidence supports the value of mammary ultrasonography in detecting lesions and structural alterations in the mammary parenchyma and teat. Furthermore, it provides useful prognostic information on treatment outcomes. Notably, the ability to pre-identify animals that are unlikely to respond to intramammary antibiotic therapy is particularly significant, both for mitigating the development of antimicrobial resistance and for minimizing the economic losses associated with mastitis. The echographic assessment of the mammary gland in ruminants is most commonly performed using the transcutaneous (direct-contact) technique. This procedure typically employs linear, convex, or sector transducers, selected based on imaging depth and anatomical region (Flöck and Winter, 2006).

Ultrasonographic imaging of healthy udder parenchyma typically reveals a homogeneous hypoechogenic texture with interspersed anechoic structures representing blood vessels, milk alveoli, and lactiferous ducts (Fig. 24). The gland cisterns appear as large, homogeneous anechoic areas punctuated by a few hypoechoic dots, corresponding to milk presence (Mourya et al., 2020). In a study involving 52 dairy cattle, mammary parenchymal alterations were categorized into seven distinct sonographic patterns: (1) physiological sonographic pattern characterized by medium homogeneous echogenicity with isolated anechoic lactiferous ducts and vessels; (2) non-homogeneous hyperechoic areas with few lactiferous ducts; (3) abscesses, defined as round, well-circumscribed structures of variable size with a distinct capsule and hypoechoic content; (4) non-homogeneous regions with hyperechoic zones and gas formation; (5) hematomas, identified as large septal spaces containing anechoic to hypoechoic fluid; (6) non-homogeneous hypoechoic areas; and (7) spotted hypoechoic areas. Based on these patterns, a grading system for sonographic changes was proposed: physiological sonographic pattern, mild changes (affecting only small portions of udder tissue with subtle sonographic differences), moderate changes (involving larger areas with more marked alterations), and severe changes (where the normal udder architecture is largely obliterated) (Flöck and Winter, 2006).

Another study investigated the potential of ultrasonography to predict the clinical outcome of mastitis. Udder examinations focused on identifying one or more of the following

ultrasonographic signs: hyperechoic spots within the parenchyma, structural alterations in the milk ducts, and non-homogeneous parenchyma. Statistical analysis revealed that among these indicators, only the presence of non-homogeneous parenchyma was a significant predictor of whether the milk would remain marketable.

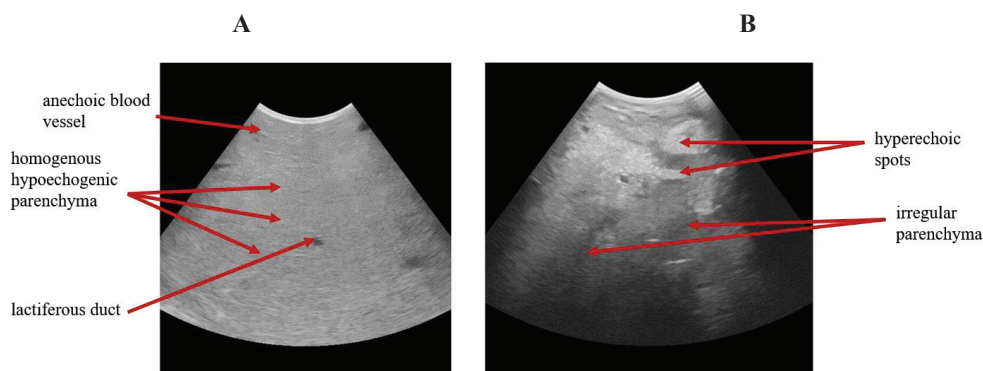


Figure 24: A - Mammary ultrasound evaluation using longitudinal scanning (B-mode). Frequency: 6.5 MHz; Depth: 150 mm

Note: Figure B - An example ultrasound evaluation using longitudinal scanning (B-mode) of a quarter infected with *E. coli*. Frequency: 6.5 MHz; Depth: 150 mm; Focus: 50 mm. The image shows multiple hyperechoic spots and irregular parenchymal structure.

Source: Tommasoni et al. (2023)

Concerning B-mode echography of the teat in ruminants, this is most performed using the water bath technique combined with vertical scanning. Under normal physiological conditions, the teat wall presents three distinct layers: the skin appears as a thin, hyperechoic line; beneath it lies the muscular and connective tissue layer containing blood vessels, which appears as a thicker, homogeneous, hypoechoic layer; and the innermost mucous membrane is seen as a hyperechoic layer. A study evaluating teat structure alterations in cases of subclinical mastitis reported that the teat cistern appeared as an anechoic area with few hypoechoic dots corresponding to milk content. Additionally, the affected teat showed irregular outlines of the teat canal and cistern, homogeneous hypoechoic contents, and loss of the normal three-layered appearance of the teat wall (Mourya et al., 2020).

4.8 Blood gas analysis

The animal's clinical status plays a crucial role in assessing the success of mastitis treatment. Hematobiochemical testing is a useful and reliable indicator of the severity of syndromic conditions and serves as a valuable tool for diagnosis and prognosis in many diseases. Several studies have evaluated the main alterations during mastitis, aiming to extend the use of blood parameters for this disease. The application of a blood gas analyzer (Emogas analyzer) enables the acquisition of prognostic indices in real-time under field conditions. Blood gas analysis is already widely used in the evaluation and management of various pathological conditions. For instance, in calves, it is valuable for assessing acid–base status and establishing appropriate treatment protocols for respiratory disease and diarrhea (Ider et al., 2021).

Recently, researchers investigated variations in blood parameters in cows with different milk production levels. They found a direct correlation between the partial pressure of oxygen and milk production, possibly due to increased oxygen-transport capacity in higher-producing cows. Additionally, bicarbonate (HCO_3^-) levels were higher in high-producing cows. Conversely, elevated blood glucose concentrations were associated with low milk production, likely reflecting increased glucose utilization in cows producing more milk to meet the mammary gland's energy demands (Pawliński et al., 2023).

Several studies have also explored ultrasound evaluation of the mammary gland in mastitis cases. A 2022 study used blood gas analysis in cows with high somatic cell counts (SCC) to assess mammary gland permeability, focusing on sodium (Na) and potassium (K) levels as important indicators. Results showed higher K levels and an increased Na:K ratio in animals with high SCC, supporting the use of blood gas parameters for rapid assessment of mammary gland health (Feng et al., 2022). Furthermore, Qayyum et al. (2016) reported significantly lower serum calcium, phosphorus, and zinc levels in infected cows compared with healthy cows.

Another study highlighted decreased blood glucose levels in clinical mastitis cases, often attributed to negative energy balance (NEB), which is common during the transition period. NEB leads to reduced serum glucose, impairing the immune system's energy supply and predisposed animals to mastitis. Alternative hypotheses include reduced blood sugar supply to infected udder due to altered blood flow, increased glucose transport from milk to the extracellular space to maintain osmotic balance in response to elevated Na and chloride (Cl) levels during mastitis, and local glucose depletion caused by immune cell accumulation at infection sites (Zhang et al., 2020).

4.9 On-farm culture

On-farm culture (OFC) is a diagnostic system that enables veterinarians to guide producers in making timely and strategic treatment decisions for clinical mastitis cases without the delays associated with laboratory culture processing. Early OFC methods used blood and MacConkey agar plates, allowing differentiation of microorganisms into Gram-positive, Gram-negative, or no-growth categories within 24 to 32 hours (Ganda et al., 2016).

Numerous studies have demonstrated the effectiveness of OFC in reducing intramammary antibiotic use both in the short and long term. For example, Lago et al. (2011) reported that implementing OFC reduced intramammary antimicrobial use by half and tended to shorten the withholding period by one day. Importantly, this reduction did not significantly affect key clinical outcomes, including days to clinical cure, bacteriological cure risk, risk of new infections, or treatment failure within 21 days after the mastitis event. A companion study assessing long-term effects showed that selective treatment based on OFC did not alter the recurrence of clinical mastitis in the same quarter, somatic cell count (SCC), milk production, or cow survival throughout the remainder of the lactation.

Chromogenic culture media have emerged as valuable alternatives for rapid microbial identification by differentiating bacterial species or groups based on colony color. These media contain chromogenic substrates that release dyes upon enzymatic hydrolysis by specific microorganisms, thereby imparting distinctive colors to colonies. Studies have demonstrated the high diagnostic accuracy of chromogenic media for instance, Borchardt and Heuwieser (2022) reported 100% sensitivity and 99.8% specificity for *Staphylococcus aureus* (G+) detection in clinical mastitis milk samples.

While chromogenic media show satisfactory performance for identifying main pathogens in subclinical mastitis caused by Gram-positive bacteria, limitations exist, such as challenges in identifying *S. aureus* (G+) in some cases. Additionally, comparisons of bi- and tri-plate culture methods demonstrated adequate accuracy for identifying *S. agalactiae* (G+), *S. dysgalactiae* (G+), *S. uberis* (G+), *E. coli* (G-), *Klebsiella* spp. (G-), and *Enterobacter* spp. (G-) in clinical and subclinical mastitis. The accuracy of OFC depends on the pathogen type and mastitis presentation. Moreover, the expertise of the observer in milk microbiology significantly improves interpretation of OFC results, which is crucial for making appropriate management decisions, especially when adopting selective treatment protocols (Garcia et al., 2021).

Despite the advantages of OFC, laboratory culture remains essential for confirming bacterial species and *Mycoplasma* (G-) presence, as well as determining antimicrobial susceptibility—particularly the detection of beta-lactamase production by *S. aureus* (G+). This information is critical for optimizing treatment protocols for clinical and subclinical mastitis and implementing effective control measures.

Milk from bulk tanks can be cultured to assess herd-level pathogen prevalence, while individual cow or quarter milk samples are cultured as part of mastitis investigations. Various selective culture plates such as biplates (MacConkey agar and blood agar with 1% esculin) and triplates (MacConkey agar, blood agar, and TKT agar-containing thallium, crystal violet, and staphylococcal toxin in 5% blood agar with 1% esculin) are used to differentiate Gram-positive, Gram-negative, and no-growth results. These tools aid rational and targeted antimicrobial use. Additionally, mycological media such as Sabouraud dextrose agar and Pal sunflower seed medium are used to isolate fungi and algae like *Prototheca* from mastitic milk (Pal, 2018).

Chromogenic culture media and OFC represent promising approaches for rapid, on-farm identification of mastitis pathogens, facilitating timely and targeted treatments that could improve antimicrobial stewardship on dairy farms.

4.9.1 On-farm culture tests distribution

4.9.1.1 MicroMast rapid test

The MicroMast rapid cultivation test is designed for on farm cultivation and identification of bacteria causing mastitis (Fig. 25). It comes in a kit for aseptic milk sampling, plates for cultivation and an incubator. The MicroMast is divided into three zones. The first zone is used to exclude any contamination of the test kit. The Second zone is used for the identification of gram-positive bacteria including *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus uberis*, *Enterococcus* spp., *Corynebacterium* spp., *Trueperella pyogenes* and *Bacillus* spp.

Finally, in the third zone we can identify gram negative bacteria including *Escherichia coli*, *Klebsiella* spp., *Proteus vulgaris*, *Pseudomonas* spp., *Serratia marcescens*, *Pasteurella* spp. and other from the family *Enterobacteriaceae* (Fig. 26 and Fig. 27).



Figure 25: Milk sample cultivation on MicroMast test

Source: Zigo (2024)

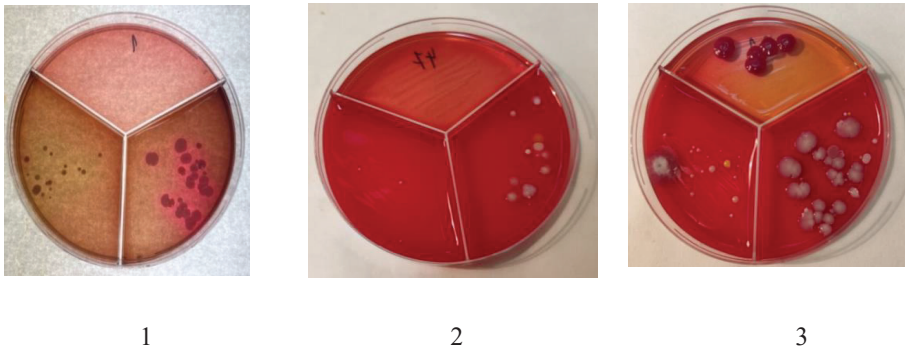


Figure 26: Micromast test interpretation

Note: Figure 1: Zone A - identification of Gram-positive and -negative pathogens, Figure 2: Zone A - identification of Gram-positive and -negative pathogens, Figure 3: Contaminated sample.

Source: Zigo (2024)

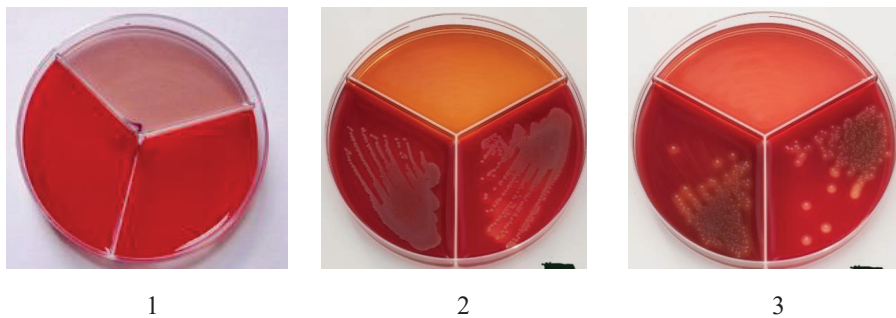


Figure 27: MicroMast test interpretation

Note: Figure 1 – no grown, Figure 2: *S. aureus* (G+), Figure 3: *Str. agalactiae* (G+)

Source: Zigo (2024)

4.9.1.2 ClearMilk Test

The ClearMilk test is another rapid cultivation test designed to identify bacteria causing mastitis directly on the farm. It consists of a petri dish containing three different chromogenic agars. One for the identification of Staphylococci (G+), one for Streptococci (G+), and one for the identification of other gram-negative bacteria. The milk sample is inoculated on the agar and incubated for 22-26 hours at 37.5 degrees. After incubation, the samples are analyzed, and the bacteria are identified based on color (Fig. 28).

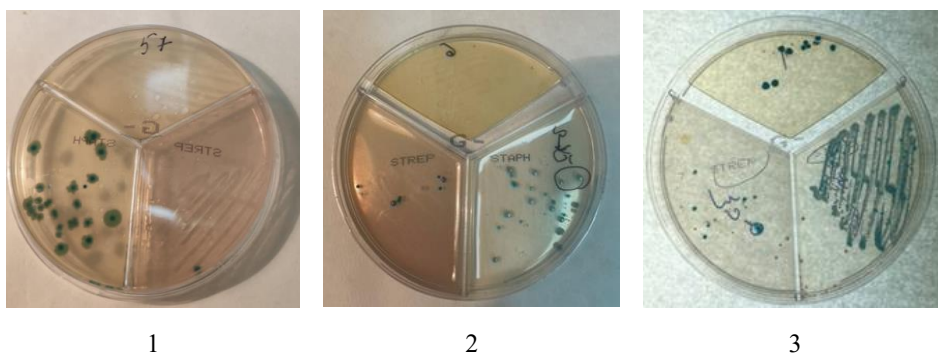


Figure 28: ClearMilk test – interpretation

Note: Figure 1: Positive zone for *Staphylococci* (G+), Figure 2: positive zones for *Staphylococci* (G+) and *Streptococci* (G+), Figure 3: Contaminated sample.

Source: Zigo (2024)

4.9.1.3 Pure milk test

Pure milk test is an on-farm cultivation test, developed for the quick and easy detection of mastitis causing pathogens. The test comes with a three-sector petri dish containing different chromogenic agars for the easy identification of bacteria. Color changes in different sectors shows the pathogen present. This allows for the preparation of targeted treatment.

4.9.1.4 VetoRapid test

VetoRapid is a rapid mastitis test which allows differentiation of mastitis causing pathogen within 24 hours. It gives an accurate result which allows for planning of the suitable treatment of the individuals. Test is easy to use and by comparing color of the incubated sample and booklet from the kit, the main bacteria causing mastitis can be detected (Vetoquinol 2022).

4.9.1.5 AccuMast test

AccuMast is a cultivation kit designed to detect specific pathogens that cause mastitis. It can accurately identify pathogens in just 16 hours. The kit contains a petri dish divided into three main areas containing selective growth media. One-third allows for the growth of Gram-negative bacteria; the second, for *Streptococcus* spp. (G+), and the last one for *Staphylococcus* spp. Within these sections, chromogenic media allow different bacteria to grow in distinct colors, enabling easy identification. Data from this test can be used to prepare effective ATB treatment (Calibre Control 2020).

4.10 Infra-red thermography

Infrared Thermography (IRT) is based on the physical principle that any object with a temperature above absolute zero emits electromagnetic radiation in the infrared spectrum. The amount and wavelength of this emitted radiation are directly related to the temperature of the object's surface and are governed by well-established physical laws. IRT devices detect this radiation using a sensor array, converting it into thermographic images. In these images, each pixel represents a specific surface temperature, typically color-coded: warmer areas appear red or white, while cooler regions appear blue or black. In veterinary medicine, and particularly in bovine practice, IRT has found broad applications. It is widely used for heat detection and ovulation prediction, lameness assessment, evaluation of animal welfare, and monitoring of feed utilization efficiency (Sathiyabarathi et al., 2016).

In the context of mastitis, IRT offers a non-invasive and rapid tool for early detection. Mastitis induces an inflammatory response in the mammary gland, leading to the release of prostaglandins and an increase in both systemic and localized udder-surface temperatures. This thermal change is detectable via IRT, often before clinical signs become visually apparent, making it a valuable adjunct in mastitis surveillance (Fig. 29; Machado et al., 2021).

Recently, several studies have established a significant positive correlation between somatic cell count (SCC) and udder surface temperature. Infrared thermography (IRT) has shown promising potential in detecting early-stage mastitis due to its ability to detect localized inflammation through elevated temperatures. However, several variables must be considered when performing thermal imaging, as IRT results can be influenced by external and physiological factors. These include mechanical brushing of the udder, direct solar radiation, wind speed, parity, stage of lactation, and pregnancy. Research has shown that the thermographic imaging of the forequarters

of the udder yields a stronger correlation with SCC compared to the rear quarters. This is likely due to the rear udder region being more exposed to environmental variables and prone to physical trauma during milking, which may artificially elevate temperature readings and lead to inaccurate interpretations. Therefore, it is essential to calibrate the thermal imager properly and to adhere to a standardized imaging protocol. A commonly adopted emissivity value for cow udder surface imaging is 0.98, as supported by several studies (Zaninelli et al., 2018).

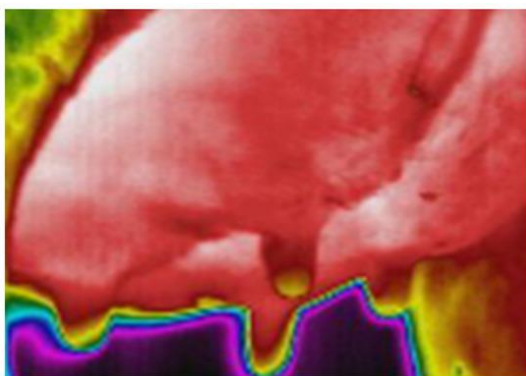


Figure 29: Infrared thermographic image of a mastitis-affected quarter

Note: The red coloration observed around the teat and teat orifices indicates elevated surface temperature, consistent with inflammatory changes. Notably, the absence of a temperature gradient between the udder and the teat contrasts with the typical pattern seen in healthy quarters, where teats are usually warmer than the udder base.

Source: Machado et al. (2021)

For optimal thermogram acquisition, a 2022 study recommended photographing the udder from three distinct angles to capture all quarters and repeating each shot at least three times to eliminate poor-quality images. The imaging distance typically ranges from 30 to 100 cm, with the thermal imager tilted at an angle of -15° to $+15^{\circ}$, adjusted based on udder conformation and the cow's position in the stall. This ensures all teats are included in the field of view. Images should be captured both before and after milking. Interestingly, no significant temperature differences were found between the left and right udder quarters in either condition (Khakimov et al., 2022).

As shown in Figure 15, the presence of red coloration at the teat and teat orifice indicates an elevated surface temperature due to inflammation. Unlike healthy quarters, which typically exhibit

a temperature gradient between the warmer teat and cooler udder base, the affected quarter shows a uniform temperature distribution, highlighting the inflammatory state.

Mastitis remains one of the most prevalent and economically damaging diseases in dairy cows, significantly impacting animal welfare and representing one of the leading causes of antibiotic use on farms. Considering increasing antimicrobial resistance, national and international regulations are promoting strategies to reduce unnecessary antibiotic use. Not all intramammary infections (IMIs) require antimicrobial treatment; therapeutic decisions should be based on bacterial culture and sensitivity testing rather than empirical treatment. The success of mastitis therapy depends on various factors, including the etiological agent, stage of lactation, parity, concurrent systemic diseases, and structural changes in the mammary parenchyma. Therefore, mastitis management must be guided by continuous veterinary oversight, with a focus on identifying cases with favorable prognostic indicators to optimize treatment outcomes and reduce antibiotic use (Zaninelli et al., 2018).

On-farm diagnostic tools are already being employed to support selective dry cow therapy (SDCT), such as the California Mastitis Test (CMT) and on-farm culture (OFC). IRT offers a noninvasive, rapid diagnostic approach that could enhance early detection of mastitis, allowing for more timely and effective treatment decisions. While it is an indirect diagnostic method, IRT has demonstrated relatively good sensitivity, specificity, and predictive values, and it can detect surface temperature changes that reflect varying severities of mammary gland infection.

Other emerging diagnostic techniques, such as ultrasound evaluation of the mammary gland and teat structures, have proven useful for identifying lesions or parenchymal alterations that may influence prognosis. Blood gas analysis, using portable analyzers, offers additional on-farm, real-time clinical data that may support prognosis and treatment decision-making.

5 Chapter - POSSIBLE TREATMENTS AND ANTIBIOTIC RESISTANCE OF BOVINE MASTITIS

As mastitis is a complex disease with multiple etiologies, complete eradication is not possible. However, good hygiene practices and regular screening of cows for subclinical mastitis must be maintained at the dairy farm to reduce the risk of mastitis. Most cases of mastitis are due to injury to the udder followed by microbial infection, and these can be avoided, and even if they occur accidentally, treatment should be rapid and regular. These can be handled by good management and cleanliness in sheds. Use of post-milking antiseptics and at teat opening also reduces the risk of microbial entry, thus considered an effective management practice for the prevention of contagious mastitis (Olde Riekerink et al., 2012).

Measures aimed at preventing new cases of mastitis include breeding, fly control, optimal nutrition, improved milking hygiene, avoidance of inter-sucking among young ones, post-milking teat disinfection, regular control of milking equipment, implementation of a milking order, and improved bedding material. Some of the vaccines already in field trials for the control of bovine mastitis include inactivated, highly encapsulated *S. aureus* cells; a crude extract of *S. aureus* exopolysaccharides; *S. aureus* CP5 whole-cell vaccine; inactivated and unencapsulated *S. aureus*; as well as *Streptococcus* spp. A cell-based vaccine has been developed for controlling bovine mastitis. Recombinant *Streptococcus uberis* GapC or a chimeric Christie Atkins Munch-Petersen (CAMP) antigen; pauA; live *Str. uberis* 0140J strain and bacterial surface extract, DNA vaccine containing clumping factor A of *S. aureus* and bovine IL18, DNA-Protein vaccine may be used against *S. aureus*, etc. (Yin et al., 2009).

Mastitis is regarded as the most widespread and economically devastating disease of dairy animals, presenting considerable challenges for disease control. Pal (2018) emphasized that the cost associated with its treatment and prevention remains one of the most significant financial burdens on the global dairy industry. Several predisposing factors, such as breed, age, lactation stage, milking hygiene, husbandry practices, environmental sanitation, milker hand hygiene, water quality, and farmer awareness, contribute to the incidence and transmission of the disease. Best practices recommend that animals undergoing antibiotic treatment be milked last, and their milk be discarded to prevent contamination. All equipment used must be sanitized thoroughly. Additionally, milk obtained during treatment and within 96 hours following the final antibiotic dose should not be utilized for human consumption.

5.1 Treatment

Despite significant advancements in preventive strategies and herd management practices, effective therapeutic measures remain essential for the control of clinical and subclinical mastitis. Current treatments involve the use of antimicrobials, anti-inflammatory agents, and supportive therapies (Figure 30; Zigo et al., 2021).

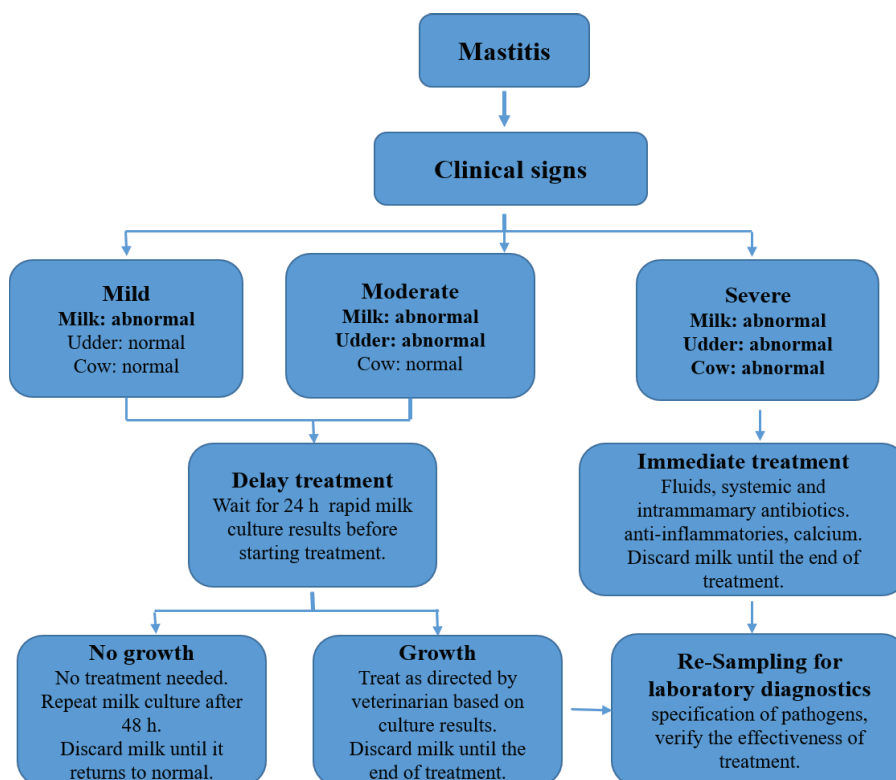


Figure 30: Determination of severity and treatment of mastitis according to clinical symptoms

Source: Zigo et al. (2021)

5.1.1 Antimicrobial treatment

Antimicrobials are fundamental to mastitis treatment due to their bactericidal or bacteriostatic effects. Beyond direct antibacterial activity, some agents influence pathophysiological processes. For instance, polymyxins can neutralize bacterial endotoxins, while others may inadvertently exacerbate infections by inducing massive endotoxin release or impairing phagocytic activity within the udder. Cefquinome, a fourth-generation cephalosporin, demonstrates

enhanced activity against Gram-negative bacteria and resistance to β -lactamases produced by many clinically significant pathogens. Sulfonamide-trimethoprim (TMS) combinations also remain widely used in treating susceptible infections (Schmid et al., 1994).

5.1.1.1 Intramammary infusion

The choice of treatment depends on the causative pathogen. Intramammary infusion (IMM) involves the direct delivery of antibiotics into the mammary gland via the teat canal. Prior to infusion, the affected quarter should be thoroughly stripped to eliminate bacteria-laden milk, clots, and debris. It can be facilitated with oxytocin (2–3 mL, IM) to promote milk letdown. Common intramammary antibiotics (Schering, 2008) include:

- **Benthatin**
- **Cloxacillin**
- **Erythromycin**
- **Streptomycin with Penicillin.**

5.1.2 Systemic treatment

Systemic antibiotic therapy, pioneered in Sweden and Israel, allows drugs to reach deeper udder tissues more effectively than IMM alone. Systemically administered antibiotics such as procaine penicillin G, amoxicillin, ampicillin, and oxytetracycline are effective in treating mastitis. To optimize efficacy and avoid resistance, *in vitro* sensitivity testing is recommended before administering treatment (Pal, 2018).

5.1.3 Anti-Inflammatory treatment

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in mastitis therapy due to their anti-inflammatory, analgesic, and antipyretic properties. These effects are primarily mediated through inhibition of the cyclooxygenase (COX) enzyme complex. COX has two isoforms:

- **COX-1:** Maintains physiological functions (constitutive)
- **COX-2:** Induced during inflammation

Most veterinary NSAIDs inhibit both isoforms. These drugs differ in pharmacokinetics, potency, and side effects. Common NSAIDs used in mastitis treatment:

- **Flunixin meglumine**
- **Tolfenamic acid**

- **Ketoprofen (high potency)**
- **Phenylbutazone (moderate potency)**

Ketoprofen is often reported as superior in field cases of clinical mastitis (Lohuis, 1991).

5.1.4 Antibiotic therapy in lactation and dry periods

Antibiotics are used both during lactation and the dry period for curative and preventive purposes, respectively. During lactation, efficacy depends on pathogen type, drug sensitivity, and infection duration. During the dry period, dry cow therapy (DCT) creates a protective window before the next lactation, preventing new infections and clearing existing ones.

However, growing resistance has reduced antibiotic efficacy, contributing to treatment failures, prolonged infections, and early culling resulting in significant economic losses.

Resistance is often linked to:

- overuse and misuse of antibiotics
- emergence of MRSA and ESBL-producing bacteria
- residual antibiotic presence in milk, which can:
 - Affect fermented product manufacturing.
 - Pose health risks to consumers
 - Contribute to antibiotic resistance in human flora (Gomes and Henriques, 2016).

Although milk from treated cows must be discarded during the withdrawal period, these challenges highlight the urgent need for alternative therapies and responsible antibiotic use in mastitis management. Typical antibiotics used in the treatment of mastitis are shown in Table 15.

Table 15: Known antibiotics used in mastitis prevention

Antibiotic	Examples	Mechanism of Action	Target Pathogens	Resistance Concerns	Reference
Beta-Lactams	Penicillin, Cephalosporins	Inhibit bacterial cell wall synthesis	<i>Staphylococcus aureus</i> (G+), <i>Escherichia coli</i> (G-)	Overuse has led to resistant strains	McDougall et al., 2020
Macrolides	Erythromycin	Inhibit protein synthesis by binding to bacterial ribosomes	<i>Streptococcus agalactiae</i> (G+)	Resistance documented in some Gram-positive strains	Siibak et al., 2009
Tetracyclines	Oxytetracycline, Doxycycline	Inhibit bacterial protein synthesis	Broad-spectrum, including environmental and contagious pathogens	Resistance seen in diverse mastitis pathogen	Becker et al., 2020
Fluoroquinolones	Enrofloxacin	Inhibit DNA gyrase and topoisomerase IV	Primarily <i>E. coli</i> (G-) and other Gram-negative bacteria	Use limited due to resistance and human health concerns	Fitzgerald et al., 2019

5.1.5 Antibiotic resistance for mastitis in cattle

Mastitis, characterized by inflammation of the mammary gland, typically due to infection, is one of the most prevalent and economically significant diseases affecting dairy cattle worldwide. It impacts not only milk production and composition but also animal health and welfare, with cascading effects on the global dairy industry. The economic burden of mastitis includes both direct and indirect losses, such as treatment costs, veterinary services, discarded milk, decreased yield and milk quality, premature culling, and compromised animal welfare (Pettersson-Wolfe et al., 2018).

The etiology of bovine mastitis is highly diverse. It is most commonly caused by bacterial pathogens, though fungi and algae have also been implicated. Over 140 microorganisms have been identified in mastitis cases worldwide, with the most frequent bacterial species including *Staphylococcus aureus* (G+), coagulase-negative staphylococci (CNS), *Streptococcus uberis* (G+), *Streptococcus agalactiae* (G+), and *Escherichia coli* (G-). These pathogens vary in their sources

and routes of transmission and are generally classified as contagious, environmental, or opportunistic, depending on their reservoirs and modes of spread (Kalińska et al., 2018).

Effective prevention is central to mastitis control and relies on strict adherence to biosecurity and management protocols. Preventive measures include optimal milking hygiene, post-milking teat disinfection, proper milking machine maintenance, early detection and treatment of clinical cases, selective dry cow therapy, and culling of chronically infected animals. Despite these efforts, challenges in consistent implementation have made mastitis a persistent issue on dairy farms, with antibiotic therapy continuing to be the mainstay of control (Pascu et al., 2022).

However, growing concerns over antibiotic resistance (AMR) and drug residues in milk and meat products are prompting a shift in treatment paradigms (Doehring and Sundrum, 2019). Overuse and misuse of antibiotics in both human and veterinary medicine have significantly contributed to the emergence of resistant pathogens, posing a serious threat to public health. According to the Antimicrobial Resistance Collaborators (2022), an estimated 4.95 million deaths were associated with bacterial AMR globally in 2019, with 1.27 million directly attributable to resistant infections.

Given this, substantial efforts are being directed toward alternative preventive and therapeutic strategies to reduce antibiotic dependence. Recent innovations include:

- **Bacteriophage therapy:** Leveraging bacteriophages to target and lyse specific bacterial pathogens
- **Vaccination:** Enhancing host immunity to reduce susceptibility to key mastitis pathogens
- **Natural therapeutics:** Utilizing plant extracts and essential oils for their antimicrobial properties
- **Animal-derived antimicrobials:** Investigating natural compounds such as lactoferrin and lysozyme
- **Probiotics and bacteriocins:** Employing beneficial microbes and their antimicrobial peptides to modulate the udder microbiome and inhibit pathogen growth (Alawneh et al., 2020).

Antibiotic resistance represents a growing challenge in mastitis management. While antibiotics have traditionally been effective in treating infections caused by *S. aureus* (G+), *E. coli* (G-), and *S. agalactiae* (G+), the rise in resistant strains is reducing treatment efficacy. This leads to prolonged disease duration, increased treatment costs, and, in some cases, the need for alternative

or more expensive therapies. Moreover, resistant bacteria pose significant risks to public health due to their potential transfer from animals to humans via the food chain (Schwarz et al., 2020).

Therefore, the fight against mastitis in dairy cattle must now balance effective disease control with the responsible use of antimicrobials. Exploring and validating alternative therapies is essential to safeguard animal health, maintain production efficiency, and mitigate the global AMR crisis.

5.1.5.1 Mechanisms of antibiotic resistance

Understanding the mechanisms by which bacteria develop antibiotic resistance is fundamental to addressing the challenges of mastitis treatment in dairy cattle. Resistance can arise via several molecular and physiological pathways, including:

- **Genetic mutation:** Spontaneous mutations in bacterial DNA can alter antibiotic target sites, such as enzymes or ribosomal components, thereby reducing antibiotic binding and efficacy. A notable example is mutations in penicillin-binding proteins (PBPs) in *Staphylococcus aureus* (G+), which confer resistance to beta-lactam antibiotics commonly employed in mastitis treatment (Jones et al., 2019).
- **Horizontal gene transfer (HGT):** Resistance genes can be rapidly disseminated among bacterial populations via mobile genetic elements such as plasmids, transposons, and bacteriophages. This mechanism has been particularly significant in the spread of beta-lactamase genes among mastitis-causing bacteria, enabling the enzymatic degradation of penicillins and cephalosporins, which are typically first-line therapies (Lima et al., 2020).
- **Efflux pumps:** Certain bacteria express efflux pumps that actively transport antibiotics out of the cell, lowering intracellular concentrations to sub-lethal levels. This is a common mechanism of resistance in *Escherichia coli* (G-), a major mastitis pathogen, contributing to multidrug resistance (Zong et al., 2020).
- **Biofilm formation:** Biofilms are structured communities of bacteria enclosed within a self-produced extracellular matrix that adheres to surfaces such as mammary tissue. Pathogens like *S. aureus* utilize biofilm formation to evade both host immune responses and antibiotic penetration, significantly complicating treatment and contributing to chronic or recurrent infections (Fitzgerald et al., 2019).

5.1.5.2 Common resistant pathogens in mastitis

A wide range of mastitis pathogens have developed resistance to conventional antibiotic therapies. The most clinically significant include:

- ***Staphylococcus aureus* (G+)**: A primary agent of both clinical and subclinical mastitis, *S. aureus* is particularly problematic due to the emergence of methicillin-resistant strains (MRSA). These strains are resistant to multiple beta-lactam antibiotics, including penicillins and cephalosporins, severely limiting treatment options.
- ***Escherichia coli* (G-)**: Often associated with acute clinical mastitis, *E. coli* has increasingly exhibited resistance to third-generation cephalosporins and fluoroquinolones. These resistant strains are difficult to manage and pose a significant therapeutic challenge in both herd-level and individual-animal care.
- ***Streptococcus agalactiae* (G+)**: Responsible for numerous subclinical mastitis cases, particularly in early lactation, *S. agalactiae* has developed resistance to penicillin and other beta-lactam antibiotics. This trend emphasizes the growing need for alternative prevention and treatment strategies.
- ***Klebsiella* spp. (G-)**: An environmental pathogen often associated with severe clinical mastitis, *Klebsiella* has demonstrated resistance to multiple antibiotic classes, including beta-lactams, aminoglycosides, and macrolides. This multidrug resistance profile complicates both treatment and farm-level control efforts (Becker et al., 2020).

5.1.5.3 Impact of antibiotic resistance on mastitis management

The emergence of antibiotic-resistant pathogens in bovine mastitis presents significant challenges for disease control, animal welfare, and farm productivity. The implications of resistance include:

- **Prolonged infection durations:** Infections caused by resistant organisms are harder to resolve, resulting in extended treatment durations and increased animal discomfort. This also raises the risk of chronic infection and recurrence.
- **Increased treatment costs:** Managing resistant infections often necessitates the use of second- or third-line antibiotics, which are more expensive. Additional interventions, such as immunomodulatory agents, further inflate treatment costs.
- **Milk quality deterioration:** Mastitis associated with resistant pathogens commonly results in elevated somatic cell counts (SCC), impairing milk quality. High SCC levels may lead to milk being rejected by processors, incurring direct economic losses.
- **Public health risks:** Resistant bacteria can be transmitted to humans through the consumption of contaminated milk or contact with infected animals. This zoonotic risk highlights the broader implications of resistance beyond the farm level (Fitzgerald et al., 2019).

5.1.5.4 Strategies to combat antibiotic resistance in mastitis

To address antibiotic resistance in mastitis management, several integrated strategies have been proposed and are being progressively adopted:

5.1.5.4.1 Reducing antibiotic use

A key component in managing resistance is minimizing unnecessary antibiotic use through:

- **Judicious Antibiotic Application:** Antibiotics should be used only when clinically indicated, with the selection guided by culture and sensitivity testing.
- **Selective Dry Cow Therapy (SDCT):** Unlike blanket therapy, SDCT involves treating only cows identified as infected or at high risk, thereby reducing overall antibiotic usage without compromising udder health.
- **Strategic Culling:** Removal of chronically infected or non-responsive cows helps prevent the persistence and dissemination of resistant strains within the herd (Krömker and Leimbach, 2017).

5.1.5.4.2 Implementing effective hygiene practices

Improving hygiene is fundamental to reducing infection pressure and minimizing treatment needs:

- **Milking Hygiene:** Adhering to rigorous milking protocols, including equipment sanitation and proper udder preparation, helps prevent both contagious and environmental mastitis.
- **Optimal Housing Conditions:** Providing clean, dry, and well-ventilated environments reduces bacterial loads and the incidence of environmental mastitis (Schwarz et al., 2020).

5.1.5.4.3 Vaccination

Vaccination offers a proactive approach by enhancing the animal's immune response to specific pathogens such as *E. coli* (G-) and *S. aureus* (G+). Effective vaccination can reduce infection severity, frequency, and the subsequent need for antibiotic interventions (McDougall et al., 2020).

5.1.5.4.4 Prognostic advances

Rapid and precise prognostic methods facilitate targeted therapy, reducing unnecessary antibiotic use:

- **Molecular and Culture-Based Techniques:** Methods such as PCR, on-farm culture, and antimicrobial susceptibility testing help identify causative agents and inform appropriate treatment plans (Fitzgerald et al., 2019).

Therefore, the growing challenge of antibiotic resistance in mastitis pathogens necessitates a multifaceted approach that includes improved antibiotic stewardship, enhanced biosecurity, vaccination programs, and advanced diagnostics. Sustainable mastitis management depends not only on reducing antibiotic dependence but also on continuous investment in research to develop alternative therapeutic and preventive solutions.

6 Chapter - NON-ANTIBIOTIC APPROACHES TO BOVINE MASTITIS MANAGEMENT

As shown in Fig. 31 bovine mastitis remains one of the most significant production diseases affecting dairy herds worldwide, with profound economic implications due to both direct and indirect costs. The losses from reduced milk yield, poorer milk quality, treatment expenses, discarded milk, increased culling, and subsequent health complications place a heavy financial burden on milk producers.

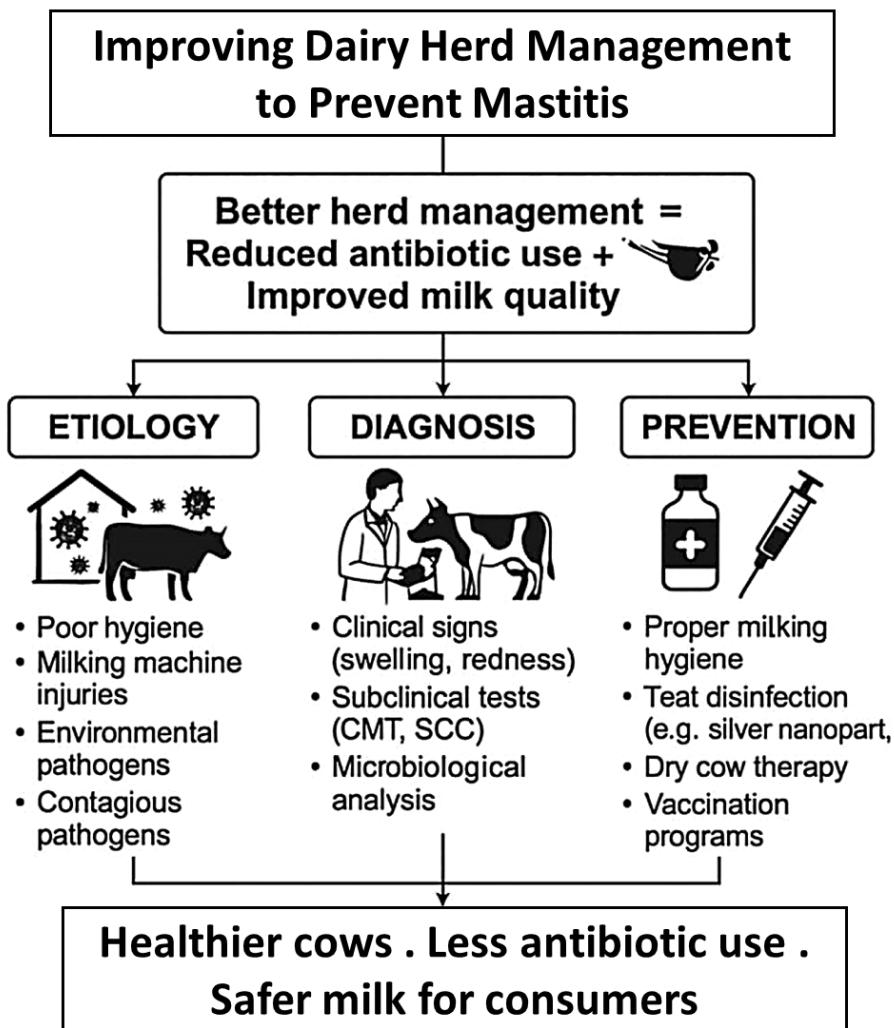


Figure 31: Future perspectives of mastitis

Source: Makumi et al. (2021)

Subclinical mastitis often goes unnoticed but contributes substantially to economic losses and health deterioration. To mitigate these impacts, regular screening for early detection, prompt and effective treatment, and rigorous follow-up of chronic and subclinical cases are essential. Raising farmers' awareness of the economic importance of subclinical mastitis and promoting best practices, such as proper milking procedures, hygiene, and environmental cleanliness, can greatly reduce infection risks and cross-contamination (Zigo et al., 2021).

Preventive measures, including the consistent use of disinfectants and vaccination in endemic regions, should be integrated into herd health management plans. Additionally, continued comprehensive research into mastitis prevalence and risk factors is critical to developing more effective control strategies. Finally, collaborative efforts involving public health agencies, academic institutions, and research organizations are crucial to minimizing mastitis' adverse effects on animal welfare, farm economics, and public health, thereby ensuring safer and more sustainable dairy production globally.

6.1 Bacteriophage Therapy

Bacteriophages, viruses that naturally occur in various environments such as fresh and saline water, sewage, soil, and living organisms, possess the unique ability to infect and lyse specific bacteria while remaining harmless to humans, animals, and plants. Upon infecting a bacterial cell, bacteriophages induce lysis, releasing progeny phages into the environment and enabling the targeted destruction of additional bacterial cells (Radzikowski et al., 2020).

Beyond their specificity and self-amplifying replication at infection sites, bacteriophages offer environmentally friendly properties, favorable biosafety profiles, thermostability, and potent lytic activity. Their use as therapeutic agents offers a viable alternative to antibiotics, especially given their biodegradability after infection resolution and reduced potential for resistance development (Makumi et al., 2021).

Numerous studies have demonstrated the efficacy of phages against mastitis-causing pathogens. Specifically, bacteriophage therapy has shown promising results against *Staphylococcus aureus*-induced mastitis. Varela-Ortiz et al. (2018) demonstrated that 36 strains of *S. aureus* (G+) from subclinical mastitis were susceptible to four phage lysates derived from a non-pathogenic *S. aureus* (G+) strain. Furthermore, the recombinant endolysin Trx-SA1 (a phage-derived protein) significantly reduced pathogen load and inflammation levels in clinical *S. aureus* (G+) mastitis cases (Fan et al., 2016). Endolysins act by hydrolyzing bacterial peptidoglycan, leading to cell lysis. In one study, bacteriophage perfusion achieved a 16.7% cure rate in *S. aureus*-induced mastitis (Gill et al., 2006).

Bacteriophage therapy has also shown effectiveness against other pathogens such as *Escherichia coli* (G-), *Streptococcus uberis* (G+), *Streptococcus agalactiae* (G+), *Klebsiella pneumoniae* (G-) and *Klebsiella oxytoca* (G-) (Amiri Fahliyani et al., 2018). To enhance efficacy and broaden the host range, phage cocktails have been developed. Geng et al. (2020) showed that a cocktail was more effective than single phages in treating *S. aureus* (G+) mastitis in a murine model. Similarly, a three-phage mixture reduced *S. aureus* (G+) isolates from bovine mastitis after 12 hours of incubation and a four-phage cocktail inhibited *E. coli* (G-) growth while reducing adhesion to mammary epithelial cells (Giraffa et al., 2017).

Thus, bacteriophage therapy holds promises for reducing antibiotic usage and enhancing dairy productivity. However, limitations exist, including potential dysbiosis that could increase susceptibility to future infections. Resistance to phages can also develop through mechanisms such as receptor modification, production of inhibitory substances, or prevention of phage replication (Drulis-Kawa et al., 2012).

Moreover, phage presence in dairy environments poses challenges, especially due to their ability to target lactic acid bacteria critically in dairy fermentation. This can result in undesirable fermentations, low-quality dairy products, and economic losses (Ortiz Charneco et al., 2023). Therefore, further research is necessary to optimize phage use in the udder and minimize adverse impacts on beneficial microbial flora.

6.2 Bacteriocins

Bacteriocins are ribosomally synthesized antimicrobial peptides produced by bacteria that inhibit closely related or similar bacterial strains. They have gained attention as promising alternatives to antibiotics for mastitis prevention and treatment, due to their potent activity and low toxicity.

Bacillus megaterium (G+) is a scientifically and practically significant, non-pathogenic organism with high potential for industrial biotechnology and the field of probiotics. This species has been shown to stimulate the immune system, improve intestinal health and positively affect the growth performance of animals. The key mechanism of its action is the production of megacin, a highly specific antimicrobial protein (bacteriocin). Supplementation with *B. megaterium* shows a clear immunomodulatory effect, as evidenced by increased serum levels of immunoglobulins (IgG, IgM, IgA) and a concomitant decrease in pro-inflammatory cytokine concentrations. In clinical practice, *B. megaterium* appears to be a promising tool in the prevention and supportive treatment of subclinical mastitis in dairy cows. Studies (Shkromada et al. 2022a; 2022b) confirm that this microorganism acts as a safe alternative or adjuvant to antibiotic treatment. Specifically, the *B.*

megaterium strain NCH 55 demonstrated a significant ability to inhibit the growth of mastitis-causing pathogens under experimental conditions.

6.2.1 Mechanisms of action

Bacteriocins primarily exert their effects by disrupting the integrity of bacterial cell membranes, leading to cell death. They have demonstrated strong activity against key mastitis pathogens, including *Staphylococcus aureus* (G+) and *Escherichia coli* (G-). Importantly, unlike traditional antibiotics, bacteriocins typically spare the normal microbiota, helping preserve the udder's natural defense mechanisms (Darbandi et al., 2022).

6.2.2 Types of bacteriocins

- Nisin: One of the most extensively studied bacteriocins, nisin is effective against a broad range of Gram-positive pathogens, including mastitis-associated bacteria.
- Lacticin: Produced by *Lactococcus lactis* (G+), lacticin has shown effectiveness in inhibiting mastitis-causing bacteria.

Emerging evidence suggests that bacteriocins not only prevent mastitis but may also reduce the need for antibiotics, thereby helping limit resistance development (Simons et al., 2020).

6.2.3 Challenges and future directions

Despite their potential, the widespread application of bacteriocins faces obstacles including limited stability, high production costs, and regulatory barriers. Nonetheless, advances in biotechnology and nanotechnology may help overcome these issues and promote bacteriocins as viable alternatives in mastitis control programs.

5.3. Vaccination

Vaccination remains a proactive and cost-effective approach to controlling infectious diseases. In dairy herds, vaccination against mastitis pathogens aims to prevent new infections, reduce disease severity, and lower somatic cell counts, thus contributing to reduced antibiotic usage (Fanelli et al., 2022).

Numerous studies support the effectiveness of mastitis vaccines. Wilson et al. (2007) observed reduced local and systemic symptoms following vaccination against enterobacterial mastitis. Chang et al. (2008) demonstrated that a recombinant staphylococcal enterotoxin C mutant vaccine (MastaVac) effectively prevented *S. aureus* (G+) infections during lactation, with vaccinated cows showing significantly lower SCCs and no *S. aureus* (G+) detection compared to 75% in unvaccinated cows.

Similarly, Collado et al. (2018) reported decreased clinical signs, bacterial loads, rectal temperatures, and milk yield loss after *S. uberis* (G+) infection in cows vaccinated with a *S. uberis*

(G+) subunit vaccine. For *E. coli* (G-), Gurjar et al. (2013) found that J-5 bacterin vaccination in late gestation led to increased serum immunoglobulin levels and reduced mastitis severity, though it did not prevent new infections. J5 vaccines have been reported to reduce coliform mastitis severity by 70–80%.

Commercial vaccines such as Startvac, targeting *S. aureus* (G+) and *E. coli* (G-), have shown mixed efficacy. While Schukken et al. (2014) observed only modest reductions in *S. aureus* (G+) infection rates. Conversely, Tashakkori et al. (2020) found no benefit, highlighting the variability in vaccine performance due to factors such as age, herd management, and environmental conditions.

Given the polymicrobial and variable nature of mastitis, vaccines offering broad protection across multiple strains are urgently needed. Nonetheless, vaccination should be integrated into comprehensive herd health management strategies that include hygiene, biosecurity, antibiotic use, and removal of chronically infected cows (Ismail, 2017).

6.3 Nanoparticle-Based Therapy

Nanoparticles have garnered substantial interest across diverse sectors, including industry, environmental science, agriculture, food technology, biomedicine, medicine, and consumer products, due to their distinctive chemical and physical characteristics. These characteristics encompass parameters such as density, optical behavior, electrical conductivity, chemical stability, bioavailability, bioabsorption, and anti-inflammatory properties, which collectively contribute to their efficacy in applications like targeted drug delivery, modulation of biological activity, and antimicrobial effects. Veterinary medicine has recently made significant strides in employing nanoparticles for disease prevention, diagnostic purposes, and therapeutic interventions. Nanoparticle-based therapies hold promise in addressing critical issues such as antimicrobial resistance and drug residues prevalent in dairy farming. Accordingly, nanoparticle-based approaches are emerging as innovative, safe, and promising options for both the prevention and treatment of bovine mastitis (Jafary et al. 2023).

A wide array of nanoparticles, including silver, gold, copper, zinc oxide, nanogels, and chitosan, have demonstrated considerable potential against pathogens responsible for mastitis, utilizing diverse mechanisms to inhibit or eradicate these microorganisms (Neculai-Valeanu et al. 2021). These nanoparticles primarily exert their antimicrobial action by interacting with bacterial cell membranes, resulting in membrane disruption, enzymatic inactivation, protein denaturation, and alterations in gene expression profiles. For instance, silver nanoparticles at a concentration of 10 µg/ml exhibited strong antibacterial activity against 90% of *Staphylococcus aureus* (G+) strains isolated from cows with subclinical mastitis after just seven minutes of *in vitro* exposure. Similarly, commercially

available silver and copper nanoparticles reduced the viability of *S. aureus* (G+) and *Escherichia coli* (G-) *in vitro* without inducing toxicity in mammary gland tissues. Moreover, when combined with the plant-derived compound quercetin, silver nanoparticles demonstrated enhanced antibacterial and anti-biofilm effects against multidrug-resistant *E. coli* (G-) strains isolated from mastitic cows (Ondrašovičová et al., 2025). The antimicrobial mechanisms attributed to silver nanoparticles include disruption of cell membranes, protein denaturation, inhibition of DNA replication, generation of reactive oxygen species (ROS), and interference with respiratory enzymes and proteins (Xu et al. 2020).

Gold nanoparticles have also shown potential to reduce biofilm formation by *S. aureus* (G+) by modulating membrane potential and suppressing adenosine triphosphate (ATP) synthesis, thereby impairing bacterial metabolism. Their relatively low cytotoxicity compared to other nanoparticles makes them particularly attractive for treating mastitis pathogens. Copper nanoparticles, due to their potent antibacterial and antifungal properties, especially against Gram-positive bacteria with thick cell walls, including methicillin-resistant *S. aureus* (G+) and *Candida* species, are also being considered as therapeutic agents for bovine mastitis (Kruk et al. 2015). The bactericidal effect of copper nanoparticles is largely attributed to their ability to generate reactive oxygen species and peroxides, which cause extensive damage to DNA, proteins, and lipids within bacterial cells (Angelé-Martínez et al. 2017).

Chitosan nanoparticles have demonstrated promising antimicrobial and antibiofilm activities against *S. aureus* (G+), coagulase-negative staphylococci isolated from the milk of cows with subclinical mastitis, and *Pseudomonas* (G-) strains isolated from clinical mastitis cases, all without adversely affecting bovine cell viability. Furthermore, the combination of nanoparticles with antibiotics has been shown to enhance antimicrobial efficacy against mastitis pathogens, including *S. aureus* (G+) (Zhu et al. 2018). For example, solid lipid nanoparticles loaded with antibiotics like tilmicosin have demonstrated prolonged and enhanced antibacterial activity against *S. aureus* (G+) *in vitro*, underscoring the promise of nanoparticle-based drug delivery systems. Collectively, nanoparticle technologies, whether used alone or in combination with antibiotics, offer an innovative and promising strategy for managing bovine mastitis. Nevertheless, comprehensive *in vivo* studies are essential to validate these preliminary findings and to assess the long-term safety and therapeutic efficacy of nanoparticle-based treatments under clinical conditions (Zhu et al. 2018).

6.4 Phytotherapy

Phytotherapy, representing one of the most ancient and widely embraced therapeutic systems in veterinary medicine, has experienced renewed interest in recent years as natural plant-based agents gain traction for controlling microbial infections. Numerous plants are regarded as valuable therapeutic sources owing to their bioactive chemical constituents, which confer health benefits to both humans and animals. These natural compounds are also favored due to their minimal drug residues in animal-derived products, reduced environmental impact, and cost-effective production processes. Several studies have highlighted the anti-inflammatory capabilities of phytotherapeutic compounds, which modulate pro-inflammatory mediators by inhibiting cytokines such as tumor necrosis factor-alpha, interleukin-1 beta, and interleukin-6 (Saeed et al. 2019).

Investigations into alternative mastitis therapies have revealed the effectiveness of plant extracts, which typically present fewer side effects and a lower risk of resistance development compared to conventional antibiotics. Medicinal plants are also recognized for their antimicrobial, anti-inflammatory, and immunomodulatory properties, contributing to their therapeutic value in mastitis management. The beneficial effects of these plants are often linked to their secondary metabolites, including phenolic acids, alkaloids, flavonoids, terpenoids, and volatile oils (Lopes et al. 2020).

Both *in vivo* and *in vitro* studies have confirmed the efficacy of various plant extracts and essential oils (EOs) in combating bovine mastitis (Table 16). The antimicrobial actions of essential oils are mainly ascribed to components such as geranyl acetate, eugenyl acetate, trans-cinnamaldehyde, menthol, carvacrol, thymol, geraniol, eugenol, p-cymene, limonene, terpinene, and carvone. The lipophilic nature of these compounds facilitates their penetration through bacterial lipid membranes, causing disruption of membrane integrity and structural organization. The susceptibility of microorganisms to these compounds can vary depending on their intrinsic characteristics. Plant extracts and essential oils exert their antibacterial effects by degrading the bacterial cell wall, compromising the cytoplasmic membrane, altering membrane proteins, inducing leakage of cellular contents, promoting cytoplasmic coagulation, and destabilizing the proton motive force essential for bacterial survival (Burt 2004).

Consequently, phytotherapy presents a viable alternative or adjunctive treatment approach for bovine mastitis. However, the use of plant extracts and essential oils is limited by extraction methodologies, formulation processes, and optimal administration practices. Furthermore, potential toxicities associated with plant derivatives require thorough investigation. Past research has identified toxic effects of certain essential oils and medicinal plants in animals. Additionally, essential oils have been implicated in hepatotoxicity, nephrotoxicity, vascular alterations, and oxidative stress,

underscoring the need for careful application in farm animals (Horky et al. 2019). Therefore, rigorous scientific validation of therapeutic benefits and comprehensive assessment of species-specific side effects and administration routes are imperative before widespread adoption of phytotherapeutic agents for mastitis.

To mitigate the adverse effects while augmenting antibacterial efficacy, recent studies have focused on encapsulating essential oils within nanoparticles. This technique enhances the chemical stability and solubility of active constituents, minimizes evaporation and degradation due to environmental factors such as oxygen, humidity, and pH, and facilitates controlled, sustained release that improves bioavailability and reduces toxicity. Encapsulation within nanocarriers protects the bioactive molecules from enzymatic degradation, oxidation, and adverse chemical interactions, an important consideration given that inflammatory changes in the mammary gland can alter treatment effectiveness (Troncarelli et al. 2013).

Encapsulation thus represents a highly effective strategy for essential oil formulation, with numerous studies corroborating its potential. For example, Salvia-Trujillo et al. (2015) demonstrated that nanoemulsions containing essential oils from lemongrass, clove, thyme, or palmarosa exhibited superior bactericidal activity against *Escherichia coli* (G-) *in vitro*. Similarly, nanoparticles encapsulating summer savory (*Satureja hortensis* L.) essential oil showed marked antibacterial activity against *Staphylococcus aureus* (G+), *Listeria monocytogenes* (G+), and *E. coli* (G-), with efficacy dependent on the concentration of the encapsulated oil (Feyzioglu and Tornuk 2016).

6.5 Antimicrobial animal proteins

Animal-derived proteins have demonstrated promising antimicrobial potential for the prevention and treatment of bovine mastitis. Among these, lactoferrin stands out as a multifunctional glycoprotein naturally present in milk and other exocrine secretions. Its primary mode of action is the sequestration of iron, an essential nutrient for bacterial growth. Additionally, lactoferrin increases bacterial cell membrane permeability and disrupts the outer membrane of Gram-negative bacteria by binding to lipopolysaccharides (LPS), thereby inhibiting bacterial biofilm formation. In addition to its direct antimicrobial effects, lactoferrin acts as an immunomodulator, playing a crucial role in the innate immune response (Shaheen et al., 2016).

Low lactoferrin levels in milk, especially during the non-lactating or drying-off period, correlate with increased infection rates. Intramammary administration of lactoferrin during this dry period has demonstrated significant protective effects against bacterial infections. Lactoferrin also exhibits antibacterial, antifungal, and antiviral properties, with curative effects documented in the treatment of acute bovine mastitis. Importantly, lactoferrin inhibits the growth of β -lactam-resistant

Staphylococcus aureus (G⁺) strains both *in vitro* and *in vivo*. It is also effective against other pathogens such as *Escherichia coli*, *Streptococcus agalactiae* (G⁺), and *Pseudomonas aeruginosa* (G⁻), and even certain microalgae implicated in mastitis like *Prototheca zopfii*, where it completely inhibits growth at low concentrations. However, some bacterial strains, including certain *E. coli* (G⁻), enterococci, and staphylococci, have demonstrated resistance to lactoferrin (Lacasse et al., 2008).

Studies have highlighted the synergistic potential of lactoferrin when combined with antibiotics. Petitclerc et al. (2007) reported that a combination of lactoferrin and penicillin G resulted in a higher cure rate (33.3%) for chronic mastitis caused by β -lactam-resistant *S. aureus* (G⁺), compared to penicillin G alone (12.5%). Furthermore, Diarra et al. (2003) found that this combination enhanced the sensitivity of *S. aureus* (G⁺) to phagocytosis.

Another important milk protein with antimicrobial properties is β -lactoglobulin (β -LG), a major whey protein. Some authors demonstrated *in vitro* that β -LG inhibits the growth of *S. aureus* and *Streptococcus uberis* (G⁺) in a dose-dependent manner but shows no activity against *E. coli* (G⁻). Among its genetic variants, β -LG A exhibits greater inhibitory effects than β -LG B. Notably, β -LG A combined with lactoferrin showed enhanced antibacterial activity against *S. aureus* (G⁺).

Lysozyme, found in milk, saliva, and serum, is another antimicrobial protein effective against bovine mastitis pathogens. It functions as a hydrolytic enzyme that cleaves the β -1,4-glycosidic bonds in the peptidoglycan of bacterial cell walls, primarily targeting Gram-positive bacteria. Its limited efficacy against Gram-negative bacteria is attributed to the protective LPS layer that restricts enzyme access. However, lysozyme's antibacterial activity is significantly enhanced when combined with antibiotics. Promisingly, the therapeutic injection of a human lysozyme-expressing vector into infected udder quarters has led to clinical symptom resolution, improved California mastitis test scores, and a relatively high microbiological cure rate (Ferraboschi et al., 2021).

6.6 Probiotics: Use of lactic acid bacteria

Probiotics are live microorganisms that confer health benefits to the host, with lactic acid bacteria (LAB) being the primary probiotic group. LAB are Gram-positive, non-motile, non-spore-forming cocci or rods, typically exhibiting facultative aerobic metabolism and lacking catalase production. Their hallmark is the fermentation of sugars into lactic acid which lowers pH and redox potential, inhibiting pathogenic growth (Dhama et al., 2017).

LAB naturally occur in various foods such as meat, milk, cereals, plants, and vegetables, and are classified based on optimal growth temperature into mesophilic (20°C-37°C) and thermophilic (40°C-45°C) groups. Major LAB genera include *Lactobacillus* (G⁺), *Lactococcus* (G⁺), *Aerococcus* (G⁺), *Bifidobacterium* (G⁺), *Carnobacterium* (G⁺), *Enterococcus* (G⁺), *Leuconostoc* (G⁺),

Advances in the Understanding and Management of Mastitis in Dairy Cows

Melissococcus (G+), *Oenococcus* (G+), *Pediococcus* (G+), *Streptococcus* (G+), *Tetragenococcus* (G+), *Vagococcus* (G+), and *Weissella* (G+) (Dortu and Thonart, 2009).

LAB are recognized as important members of natural ecosystems such as the intestinal and urogenital tracts. Within the mammary gland ecosystem, certain probiotic strains contribute to teat defense by antagonizing pathogens. As a result, probiotics have gained interest as alternative or complementary strategies for the prevention and treatment of bovine mastitis, aiming to reduce antibiotic use (Li et al., 2021).

Mechanisms of Protection: Probiotics protect against pathogens by directly inhibiting their growth or virulence factors and indirectly by competing for nutrients and colonization sites or by stimulating host immunity. Many LAB strains inhibit mastitis pathogens *in vitro* (Pellegrino et al., 2019). For example, *Lactobacillus brevis* (G+) 1595 and 1597 and *Lactobacillus plantarum* (G+) 1610 exhibit strong colonization of bovine mammary epithelial cells, preventing pathogen invasion. Additionally, *Lactobacillus casei* (G+) strains reduce adhesion and internalization of *S. aureus* (G+) in mammary epithelial cells. Intramammary administration of LAB elicits immunomodulatory effects that boost host defenses (Pellegrino et al., 2017). Notably, *Lactococcus lactis* strains have demonstrated cure rates comparable to antibiotic treatments for bovine mastitis. Bio-therapeutic formulations containing live *L. lactis* (G+) DPC3147, which produces the bacteriocin lacticin 3147, have shown efficacy equivalent to commercial antibiotics in treating clinical and subclinical mastitis. Similarly, teat dips containing live lacticin-producing *L. lactis* (G+) significantly reduced colonization by *Staphylococcus* (G+) (80%), *Streptococcus uberis* (G+) (90%), and *Streptococcus agalactiae* (G+) (97%) compared to controls (Klostermann et al., 2010).

Regular prepartum external application of a combination of four LAB strains (*Lactobacillus rhamnosus* (G+), *Lactococcus lactis* subsp. *Lactis* (G+), *Lactobacillus paracasei* (G+), and *Lactobacillus plantarum* (G+)) effectively prevented intramammary infections in dairy heifers caused by major mastitis pathogens. These same four strains also significantly inhibited growth of several mastitis-associated bacteria *in vitro*, including *S. aureus* (G+), *S. epidermidis* (G+), *S. xylosus* (G+), *Str. uberis* (G+), *Str. agalactiae* (G+), and *E. coli* (G+). Furthermore, a mixture of 13 LAB species from *Lactobacillus* (G+) and *Bifidobacterium* (G+) genera isolated from honey displayed antibacterial activity against bovine mastitis pathogens (Piccart et al., 2016).

Antimicrobial Factors: The antimicrobial effects of LAB derive from organic acids, hydrogen peroxide, diacetyl, and the production of bacteriocins and specific compounds such as reuterin. Bacteriocins also known as lantibiotics are post-translationally modified antimicrobial peptides with unique amino acids like lanthionine and β -methyl lanthionine. These modifications form intramolecular bridges that stabilize their structure. Several bacteriocins inhibit Gram-positive mastitis pathogens such as *Str. uberis* and *Str. agalactiae* (G+) (Vidal Amaral et al., 2022).

Among these, nisin is the most studied. It has Generally Recognized As Safe (GRAS) status and is widely used as a food preservative. Nisin shows strong efficacy in mastitis treatment and, importantly, milk from treated cows need not be discarded. Reported bacteriological cure rates are high: 90.1% for *Str. agalactiae* (G+), 50%–54.5% for *S. aureus* (G+), and 58.8% for coagulase-negative staphylococci (CNS), outperforming antibiotics (33.3%). Nisin works by inhibiting peptidoglycan synthesis and forming membrane pores. It also combats some drug-resistant mastitis pathogens, including methicillin-resistant *S. aureus* (G+) (MRSA) When combined with other antibiotics like rampolamine or antibiotics such as chloramphenicol, nisin's efficacy is enhanced, helping overcome resistance (Bennett et al., 2021).

Economically, nisin is cost-effective, with better outcomes reported in early lactation and multiparous cows than in primiparous cows. Its affordability and cure rates make it an attractive option for farmers treating subclinical mastitis. Lacticin, another bacteriocin produced by *L. lactis* (G+), shows broad antimicrobial activity, including against mastitis pathogens, highlighting its therapeutic potential. Lacticin, combined with teat sealants, inhibits bacterial growth during the dry period and may be more effective than nisin under similar conditions (Rodriguez et al., 2023).

The encouraging results from LAB and their bacteriocins suggest they are promising alternatives to conventional antibiotics for mastitis prevention and treatment. However, bacterial resistance to bacteriocins can develop and strategies such as combining different bacteriocins or bacteriocins with antibiotics may help overcome resistance. The multifaceted approach to mastitis management including antibiotics, vaccination, animal proteins, phytotherapy, bacteriophages, nanotechnology, probiotics, and bacteriocins reflects the complexity of this disease. LAB and their bacteriocins, through direct antimicrobial effects, immune modulation, and pathogen competition, offer valuable additional tools (Ceotto Vigoder et al., 2016).

Table 16: Activity of some plant extracts and essential oils against microorganisms of bovine mastitis *in vitro* and *in vivo* studies

Plant	Main components	Tested Microorganisms	Study	Mode of administration	Action	References
<i>Mentha pulegium</i> L., <i>Melissa officinalis</i> L., <i>Nepeta cataria</i> L.,	Essential oil ethanol extract	<i>S. aureus</i> (G+) <i>E. coli</i> (G-)	<i>In vitro</i> (disk diffusion method)	-	All tested plant species demonstrated antibacterial properties, with particularly strong effects observed in squaw mint (<i>Mentha pulegium</i> L.), lemon balm (<i>Melissa officinalis</i> L.), catnip (<i>Nepeta cataria</i> L.), and their essential oils. Among these, lemon balm extract and its essential oil exhibited the highest levels of bacterial inhibition, producing zones of inhibition measuring 21–23 mm against <i>Staphylococcus aureus</i> (G+) and 19–20 mm against <i>Escherichia coli</i> (G-).	Arbab et al., 2022
<i>Cinnamomum cassia</i> <i>Origanum vulgare</i>	Essential oil	<i>S. aureus</i> (G+) <i>E. coli</i> (G-) <i>Candida albicans</i>	<i>In vitro</i> (agar well diffusion method)	-	Xanthan gum-based film-forming suspensions enriched with essential oils from <i>Cinnamomum cassia</i> and <i>Origanum vulgare</i> demonstrated significant antimicrobial activity against <i>Staphylococcus aureus</i> (G+), <i>Escherichia coli</i> (G-), and <i>Candida albicans</i> —pathogens commonly linked to bovine mastitis. The extent of microbial inhibition increased proportionally with	Barreiros et al., 2022

Advances in the Understanding and Management of Mastitis in Dairy Cows

<i>Terminalia chebula</i>	Ethyl acetate	<i>S. aureus</i> (G+) <i>E. coli</i> (G-) <i>Pseudomonas aeruginosa</i> (G-), <i>Bacillus megaterium</i> (G+)	<i>In vitro</i> (agar well diffusion method)	-	The ethyl acetate extract demonstrated broad-spectrum antibacterial effectiveness against all bacterial strains isolated from milk samples of cows affected by subclinical mastitis, showing comparable efficacy to that of amoxicillin.	Kher et al., 2018
<i>Allium sativum</i> <i>Bunium persicum</i> <i>Oryza sativa</i> <i>Triticum aestivum</i>	Methanolic extracts, Alkaloids, Flavonoids Saponins	<i>S. aureus</i> (G+) <i>E. coli</i> (G-) <i>K. pneumoniae</i> (G-)	<i>In vitro</i> (agar well diffusion method)	-	<i>Allium sativum</i> exhibited strong antibacterial effects against all tested bacterial strains, with <i>Bunium persicum</i> , <i>Triticum aestivum</i> , and <i>Oryza sativa</i> following in effectiveness. Among the tested compounds, alkaloids produced the largest inhibition zone (22 mm) against <i>Klebsiella pneumoniae</i> (G-), while the crude methanolic extract showed the highest inhibition zone (21.3 mm) against <i>Escherichia coli</i> (G-).	Amber et al., 2018
<i>Mintostachys verticillata</i> (Griseb.) Epling	Essential oil Limonene	<i>E. coli</i> (G-) <i>Bacillus pumilus</i> (G+) <i>Enterococcus faecium</i> (G+)	<i>In vitro</i> (Time Kill Assay, Biofilm Assays)	-	The essential oil effectively suppressed the growth of all tested microbial isolates even at low concentrations, whereas limonene displayed inhibitory activity exclusively against <i>Bacillus pumilus</i> (G+). Both the essential oil and limonene also demonstrated the ability to reduce biofilm formation in the isolated strains	Cerioli et al., 2018

Advances in the Understanding and Management of Mastitis in Dairy Cows

<i>Fumaria indica</i> <i>Adiantum capillus</i> <i>Nepeta cataria</i>	Aqueous extracts	<i>S. aureus</i> (G+) <i>E. coli</i> (G-), <i>Str. Agalactiae</i> (G+) <i>K. pneumoniae</i> (G-)	<i>In vitro</i> (disc diffusion method) <i>In vivo</i>	Intramammary infusions	<i>Fumaria indica</i> exhibited the strongest antibacterial activity against <i>Staphylococcus aureus</i> (G+), <i>Escherichia coli</i> (G-), <i>Streptococcus agalactiae</i> (G-), and <i>Klebsiella pneumoniae</i> (G-), followed by <i>Nepeta cataria</i> and <i>Adiantum capillus</i> . <i>In vivo</i> studies revealed that <i>Fumaria indica</i> led to the highest clinical recovery, with <i>Adiantum capillus</i> showing moderate results, while <i>Nepeta cataria</i> was associated with the lowest recovery rate.	Reshi et al., 2017
<i>Ocimum sanctum</i>	Eugenol	<i>Staphylococci</i> (G+) <i>Streptococci</i> (G+) <i>Corynebacterium</i> (G+)	<i>In vitro</i>	Oral (administration of <i>O. sanctum</i> leaf powder at 600 mg/kg body weight daily)	The treatment successfully eliminated 69.23% of intramammary infections and led to a noticeable reduction in somatic cell count (SCC), California Mastitis Test (CMT) scores, and levels of the acute-phase protein ceruloplasmin. <i>Ocimum sanctum</i> demonstrated significant immunotherapeutic potential in the management of bovine subclinical mastitis.	Shafi et al., 2016
<i>Minthostachys verticillata</i>	Essential oil Limonene	<i>Str. Uberis</i> (G+)	<i>In vitro</i> (microdilution assays)	-	<i>Minthostachys verticillata</i> essential oil and its major constituent, limonene has shown antibacterial activity and affected the biofilm formation of most of the <i>S. uberis</i> (G+) strains tested.	Montiromi et al., 2016
<i>Syzygium aromaticum</i>	Essential oils	<i>S. aureus</i> (G+)	<i>In vitro</i>		Essential oil from <i>Minthostachys verticillata</i> and its primary component, limonene,	Budri et al., 2015

Advances in the Understanding and Management of Mastitis in Dairy Cows

<i>Cinnamomum zeylanicum</i>	Eugenol Cinnamaldehyde	(agar well diffusion method, Biofilm Assays)	-	exhibited antibacterial properties and significantly influenced biofilm formation in the majority of tested <i>Streptococcus uberis</i> (G+) strains.	
<i>Origanum vulgare</i>	Essential oil	<i>In vivo</i>	Intramammary treatment	Administering essential oil twice daily for three days into inflamed udder quarters resulted in a reduction of white blood cells and somatic cell counts (SCC), with <i>Staphylococcus aureus</i> (G+) and <i>Escherichia coli</i> (G-) becoming undetectable in milk by the fourth day after treatment.	Cho et al., 2015
<i>Thymus vulgaris</i> <i>Lavandula angustifolia</i>	Essential oils	<i>In vivo</i>	Intramammary application External use in vaseline	Intramammary administration of these essential oils, prepared as a 10% methanolic solution, along with their combined mixture, led to a significant reduction in bacterial counts across various milk samples. Additionally, topical application of these oils in a Vaseline base demonstrated enhanced antibacterial effects against <i>Staphylococcus</i> (G+) and <i>Streptococcus</i> spp. (G+), achieving a 100% recovery rate when using thyme essential oil.	Abbound et al., 2015
<i>Cinnamomum zeylanicum</i> L., <i>Citrus bergamia</i> Risso, <i>Eucalyptus globulus</i> Labill.	Essential oil Thymol Carvacrol p-Cymene	<i>In vitro</i> (the paper disc diffusion method)	-	Out of the ten essential oils evaluated, <i>Satureja montana</i> , <i>Thymus vulgaris</i> chemotype thymol, and <i>Origanum vulgare</i> exhibited notable antibacterial activity against mastitis-causing bacteria in animals. A blend	Fratini et al., 2014

Advances in the Understanding and Management of Mastitis in Dairy Cows

<p><i>Foeniculum vulgare</i> Mill. <i>Origanum majorana</i> L. <i>Origanum vulgare</i> L. <i>Rosmarinus officinalis</i> L. <i>Satureja montana</i> L., <i>Thymus vulgaris</i> L. ct. <i>carvacrol</i> <i>Thymus vulgaris</i> L. ct. <i>thymol</i></p>		<p><i>S. xyloso</i> (G+), <i>E. coli</i>(G-).</p>			<p>of <i>Satureja montana</i> and <i>Thymus vulgaris</i> chemotype thymol essential oils demonstrated enhanced inhibitory effects against all tested bacterial strains.</p>	
<p><i>Thymus vulgaris</i> <i>Origanum vulgare</i> <i>Mexican oregano</i></p>	<p>Essential oils Thymol Carvacrol</p>	<p><i>Staphylococcus</i> spp. (G+)</p>	<p><i>In vitro</i> (agar diffusion method)</p>	-	<p>Essential oils derived from oregano, thyme, and Mexican oregano, along with their primary components' thymol and carvacrol, exhibited antimicrobial effects against <i>Staphylococcus</i> spp. (G+)</p>	<p>Dal Pozzo et al., 2011</p>
<p><i>Cinnamomum cassia</i></p>	<p>Essential oils Cinnamaldehy</p>	<p><i>S. aureus</i> (G+), <i>S. epidermidis</i> (G+), <i>S. xyloso</i> (G+) <i>S. hyicus</i> (G+) <i>E. coli</i> (G-)</p>	<p><i>In vitro</i> (disk diffusion assay)</p>	-	<p><i>Cinnamomum cassia</i> oil demonstrated inhibitory effects against all pathogen isolates tested from bovine mastitis. At elevated concentrations, it exhibited bactericidal activity against <i>Staphylococcus aureus</i> (G+) and <i>Escherichia coli</i> (G-), while at sub-inhibitory levels, it was able to suppress AL-2 signaling.</p>	<p>Zhu et al., 2016</p>

7 Summary

Maintaining the health of dairy cows and the quality of milk produced is a highly complex daily task that should be approached with care. The scientific papers presented in the book provide insights into risk factors for dairy mastitis, as well as innovative scientific methods and practical experience in mastitis diagnosis and control. Priority should be given to studying the impact of mastitis on small-holder production systems, as it can undermine household nutrition, especially among poor rural populations and adversely affect the socioeconomic conditions of farmers. The following are strategic recommendations for comprehensive mastitis control:

- A comprehensive roadmap for mastitis management should be developed by considering the perspectives of the three key stakeholders: the dairy sector, farmers, and consumers.
- Nationwide temporal and spatial mapping of mastitis prevalence should be conducted. Research should focus on identifying genome-based biomarkers linked with subclinical mastitis. Additionally, species and breed variations in susceptibility or resistance to mastitis require detailed assessment.
- Without a clear profile of causative organisms, the role of many microbes involved in mastitis may be overlooked, hindering effective treatment. Therefore, a metagenomic approach is essential to study the intra-mammary microbial community in both healthy and mastitis-affected animals to improve understanding of pathogenesis.
- Given the growing issue of antibiotic resistance and drug residues in milk, the indiscriminate use of antibiotics for mastitis treatment must be strictly controlled. Monitoring adherence to recommended milk withdrawal periods following antibiotic therapy is crucial to protect consumer health.
- Research should shift towards alternative disease control methods, such as immune modulation and nutritional support, to enhance udder immunity. Governments should allocate sufficient funds and infrastructure to support such research. A collaborative network program on mastitis control involving entities like the European Council of Agricultural Research, State Agricultural Universities, and related agencies should be established.
- A comprehensive education package on mastitis awareness, management, and control must be developed for farmers. Emphasis should be placed on udder health, hygiene, and nutrition, as these are critical in mastitis prevention. Best practices such as teat dipping after milking and avoiding letting cows sit immediately post-milking should be widely promoted through mass media and extension services.
- Veterinary services must be effectively organized, with regular training of personnel to ensure high-quality performance. Clear roles and responsibilities should be defined among all

stakeholders to facilitate the successful implementation of mastitis control programs. Awareness campaigns are also necessary to foster understanding and sustained participation among farmers, veterinarians, and other stakeholders.

8 Register

A

abnormal milk, 67, 69
AccuMast Test, 82
Actinomyces pyogenes, 49
actinomycetes, 47
acute phase proteins, 55
algae, 47, 49, 54, 79
alkaloids, 107
animal-derived antimicrobials, 90
antibacterial activity, 86, 99, 102, 103, 104, 108, 109, 132
antibiotic resistance, 62, 88, 91, 93, 94, 111
antibiotics, 47, 87, 88, 90, 92, 93, 98, 101, 103, 104, 105, 111, 123, 127
Antimicrobial Animal Proteins, 102
antimicrobial susceptibility, 54, 79, 94
Antimicrobial Treatment, 86
apoptosis, 55, 124
aqueous extracts, 108
Arcanobacterium pyogenes, 51
Aspergillus spp., 47
Automated Milking Systems, 72

B

bacteria, 46, 47, 53, 55, 56, 59, 62, 78, 79, 81, 87, 88, 89, 91, 93, 97, 98, 103, 104, 109, 119, 121, 126, 127, 130
bactericidal, 55, 86, 102, 110
bacteriocin, 104, 105, 123
bacteriophage therapy, 90, 97
bacteriostatic effects, 86
bedding materials, 26, 63
Beta-Lactams, 89
biofilm, 107, 108, 121
bovine herpesviruses, 54
bovine mastitis, 51, 52, 54, 93, 95, 97, 101, 103, 104, 106, 110, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 128, 130, 131, 132
bovine papillomaviruses, 54
breed, 47, 111, 126

C

C. tropicalis, 54
calf health, 22
California Mastitis Test, 66, 84, 108, 119
calves, 77, 122
Candida albicans, 47, 54, 74, 106
cattle, 53, 75, 91, 117, 118, 120, 121, 125, 126, 127, 128, 131, 132
ClearMilk test, 81
clinical symptoms, 50, 86
CMT score, 67
coagulase-negative staphylococci, 47
colostrum, 135
concentration, 73, 99, 102, 106, 126
contagious, 47, 48, 53, 63, 89, 94, 122
control, 3, 53, 62, 72, 79, 86, 91, 92, 93, 98, 111, 112, 122, 124, 125, 128, 131
Corynebacterium bovis, 47, 51, 54
Corynebacterium spp., 79
cow comfort, 30
cowpox virus, 54
cows, 53, 62
Cryptococcus neoformans, 47, 54
culling, 60, 93
cultivation test, 79, 81
cytokines, 55, 101

D

dairy cows, 1, 2, 130
Diagnostic Marker, 69
disinfection, 63
displaced abomasum, 60, 73
ducts, 47, 53, 75, 76

E

E. durans, 51
E. faecalis, 51
E. faecium, 51
electrical conductivity, 72, 125
electrical resistance, 72
Enterobacter aerogenes, 51
Enterobacteriaceae, 79

- Enterococcus* spp., 51, 79
environment, 53, 63, 127
environmental pathogens, 65
Escherichia coli, 46, 47, 50, 53, 62, 79, 89,
92, 97, 100, 102, 103, 106, 107, 108, 109,
110, 118, 119, 122, 124, 132
essential oils, 90, 101, 102, 106, 109, 116,
117, 118, 119, 121, 124, 125, 128
ethanol extract, 106
etiological factors, 50
etiology, 46
evaporation, 102
- F
flavonoids, 107
flora, 53, 88, 97
foot-and-mouth disease, 54
fungi, 47, 49, 54, 79, 124
- G
Geotrichum candidum, 54
gland cistern, 53
glucose levels, 77
gram-positive bacterium, 51
- H
hand hygiene, 63
haptoglobin, 55
heat, 32, 65, 119, 131
heat stress, 65, 71
herd, 10, 53, 54, 57, 58, 60, 79, 86, 92, 93, 99,
116, 122, 123, 131
housing systems, 25
- CH
chemoattractants, 55
chronic mastitis, 47, 49, 103
- I
immune response, 54, 94
immunity, 90, 104, 111, 127
immunological defenses, 47
inflammation, 46, 49, 56, 62, 68, 69, 72, 82,
83, 87, 96
Infra-Red Thermography, 82
inhibitory effects, 103, 109, 110, 126
interleukins, 55
intramammary infection, 51
intramammary infusion, 87
- K
K. oxytoca, 51
K. pneumoniae, 51, 107, 108
ketosis, 60, 73
Klebsiella oxytoca, 97, 116
Klebsiella pneumoniae, 46, 97, 107, 108, 132
- L
lactation, 49, 50, 53, 59, 60, 61, 67, 71, 82,
84, 88, 92, 98, 105, 117, 126, 128
lactoferrin, 90, 102, 103, 124, 127
lameness, 60, 73
latent mastitis, 67
leukocytes, 55
- M
macrolides, 89
macrophages, 55, 68, 71
major pathogens, 50, 51
management, 1, 2, 71, 72, 93, 127
mastitis, 1, 2, 46, 47, 48, 49, 53, 57, 59, 60,
61, 63, 66, 67, 71, 72, 84, 89, 92, 93, 108,
117, 119, 122, 123, 125, 126, 130, 131
metabolism, 103, 117
MicroMast Rapid Test, 79
microorganisms, 46, 49, 78, 99, 101, 103,
106, 117
mild infections, 54
milk composition, 50, 67, 120
milk ejection, 73
milk fever, 73
milk quality, 59
milking, 47, 51, 55, 59, 60, 62, 63, 66, 71, 83,
94, 111, 129, 132
milking hygiene, 62, 63
minor pathogens, 51, 54
monitoring, 69, 72, 132
mortality, 59
Mycobacterium bovis, 47
Mycoplasma bovis, 51

Mycoplasma spp., 47, 53, 54

N

natural therapeutics, 90
neutrophils, 55, 69, 71
Nocardia asteroides, 47
non-coliforms, 51
non-steroidal anti-inflammatory drugs, 87
nutrition, 111, 120, 121

O

obligate udder pathogen, 53

P

pariesis, 60
parity, 61, 71
penicillin, 47, 62, 87, 92, 103, 119, 127
phagocytic cells, 56, 61
phagocytosis, 56, 71, 103
phytotherapy, 101
plant extracts, 90, 101, 106, 124
plants, 101, 103, 116
pregnancy, 82
prevalence, 49, 52, 53, 54, 63, 74, 79, 111, 122
prevention, 58, 92, 97, 104, 105, 111, 126, 128, 132, 134
primary immune cells, 55
probiotics, 90, 103, 104, 119, 126
production, 14, 16, 50, 55, 57, 59, 60, 62, 67, 79, 91, 95, 97, 98, 101, 103, 104, 111, 117, 118, 119, 121, 122, 126, 127, 131, 134
production losses, 58, 70, 71
prostaglandin, 55
Proteus spp., 51
Prototheca spp., 47, 54
Prototheca zopfii, 103
Pseudocowpox virus, 54
Pseudomonas aeruginosa, 51, 54, 103, 107
public health, 52, 91
Pure Milk Test, 81

R

reproductive health problems, 17
reproductive status, 60

resistance, 88, 89, 91, 93, 97, 116
risk factors, 116, 128, 130
rumen, 53, 135

S

S. epidermidis, 51, 54, 104, 110
S. haemolyticus, 51, 54
S. chromogenes, 51, 54, 109
S. sciuri, 51
saponins, 107
screening, 122, 130
secretory mammary epithelial cells, 56
serious mastitis, 67
Serratia marcescens, 79
Serratia spp., 51
serum amyloid A, 55
somatic cell, 49, 55, 60, 67, 68, 70, 71, 82, 93, 98, 108, 109, 122, 126, 127, 129
somatic cell count, 55, 60, 67, 68, 70, 82, 108, 122, 129
Somatic Cell Count, 68
Stage of Lactation, 61, 71
Staphylococcus aureus, 46, 47, 49, 50, 51, 62, 73, 78, 79, 89, 92, 96, 99, 102, 103, 106, 108, 109, 110, 117, 118, 119, 120, 121, 126, 127, 128, 130, 131, 132
Str. bovis, 51
Str. dysgalactiae, 47, 48
Str. equinus, 51
Streptococcus agalactiae, 46, 47, 53, 74, 79, 89, 92, 97, 103, 104, 108, 131, 132
Streptococcus uberis, 47, 53, 63, 73, 79, 97, 103, 104, 108, 117, 119, 125
stress, 32, 65, 101, 119, 131
subclinical, 47, 49, 53, 54, 58, 59, 61, 66, 67, 68, 73, 76, 78, 79, 86, 92, 96, 99, 104, 105, 107, 108, 111, 116, 117, 118, 121, 122, 123, 124, 125, 126, 128, 131
subclinical mastitis, 49, 60, 67
symptoms, 49, 98
systemic antibiotic therapy, 87

T

teat disinfection, 63
therapeutic strategies, 118

- transition period, 77
- treatment, 54, 57, 58, 59, 72, 77, 78, 79, 81, 84, 86, 87, 88, 90, 91, 92, 93, 94, 95, 101, 102, 104, 105, 108, 109, 111, 117, 119, 120, 121, 123, 124, 125, 127, 128
- U
- unpasteurized milk, 51
- V
- vaccination, 90, 94, 98
- ventilation, 65
- vesicular stomatitis virus, 54
- veterinary care, 34
- veterinary diagnosis, 59
- VetoRapid Test, 81
- virulence factors, 51, 53, 54, 62, 104, 132, 134
- viruses, 47, 54
- W
- welfare, 84, 93, 117, 120, 127
- Y
- Yersinia* spp., 51
- Z
- zoonotic, 51, 58, 93

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10 Biography of editors

Zuzana Lacková, DVM., Ph.D.

Department of Nutrition and Animal Husbandry, University of Veterinary Medicine and Pharmacy, Košice, Slovakia.

Research and Academic Experience: Diagnostics and treatment of diseases of ruminants, poultry, companion animals and small fur animals. Skills in isolating and identifying bacteria from milk, blood and animal products. Teaching breeds and breeding of companion and farm animals and animal production technologies.

Research Area: Research activities aimed at solving problems in the field of ruminant breeding, as well as the breeding and diseases of companion animals. She has collaborated extensively on projects that bridge veterinary medicine with ruminants, particularly in the field of detection mastitis and milk quality.

Number of Published Papers and Citations: 293 scientific publications; 10 of them in SCI/WoS and Scopus, 11 textbooks, 66 registered citations in databases WoS.

Research Projects: The main researcher of one project Visegrad Fund. Co-author 2 of projects focused on the detection and prevention of mastitis. Co-solver of 4 Slovak projects dealing with issues of livestock breeding and diagnostics of diseases.

Ibrahim F. Rehan, Assoc. prof., Dr., BVM, Ph.D.

Institute of Genetics and Animal Biotechnology, Polish Academy of Sciences, Postepu, Jastrzebiec, Poland.

Department of Pathobiochemistry, Faculty of Pharmacy, Meijo University, Yagotoyama 150, Tempaku-Ku, Nagoya-Shi, Japan.

Department of Husbandry and Development of Animal Wealth, Faculty of Veterinary Medicine, Menoufia University, Shebin Alkom, Egypt.

Research and Academic Experience: He is a dedicated academic and accomplished researcher specializing in animal behavior, veterinary biotechnology, and glycomics. He currently serves as an associate professor at animal behavior and husbandry in the department of husbandry and animal wealth development, Faculty of Veterinary Medicine, Menoufia University, Egypt. Dr. Rehan has since developed a robust interdisciplinary research profile in glycomics, chemical biology, animal biotechnology, and stress physiology. He has collaborated extensively on projects that bridge veterinary medicine with molecular biology, particularly in the field of biomarker discovery. He is a co-author of several high-impact publications (2016–2022). His current scientific interests include mastitis-pathogen, stress biomarkers and alternative therapeutics in dairy animals.

Advances in the Understanding and Management of Mastitis in Dairy Cows

Research Area: With 20 years of international experience across Asia (Japan), the UK, Europe (Slovak Republic), and Africa (Egypt), I specialize in Animal Nutrition and Biotechnology and Veterinary Sciences. Also, I have expertise in MALDI-TOF/MS, Animal Production, Chemical Biology, and Drug Discovery.

Number of Published Papers and Citations: scientific publications; 50 registered citations in databases WoS and 1 textbook. Citation reached +500.

Research Projects: Co-author of 4 projects focused on the detection and prevention of mastitis. The main researcher of one project NSP Fund.

František Zigo, Assoc. prof., DVM., Ph.D.

Department of Nutrition and Animal Husbandry, University of Veterinary Medicine and Pharmacy, Košice, Slovakia.

Research and Academic Experience: Knowledge and methodological skills in the field in detecting of udder pathogens, their virulence factors and health safety of milk produced. Teaching activities on animal husbandry and technology of animal production.

Research Area: Research activities aimed at solving problems in the field of ruminant breeding and primary milk production, as well as poultry, pigeon and rabbits breeding and diseases. His current scientific interests include mastitis-pathogen, stress biomarkers and alternative therapeutics in dairy animals.

Number of Published Papers and Citations: He is a author of academic book titled "Occurrence of Mastitis in Dairy Herds and the Detection of Virulence Factors in Staphylococci", to be published by IntechOpen, UK. His work is widely cited, reflecting his growing influence in the fields of veterinary science and chemical biology. The scientific achievement consists from 250 peer-reviewed scientific publications, and 87 of them in SCI/WoS and Scopus, 2 monograph and 6 text books. In databases WoS and Scopus, 883 citations are registered, and the Hirsch index is 15.

Research Projects: The main researcher of 2 foreign and 4 Slovak projects focused on the detection and prevention of mastitis. Co-solver of 2 foreign NAWA project (Poland) and 4 Slovak projects.

Special Award: Inclusion and publication of a CV in the Encyclopedia of Personalities of the Czech and Slovak Republics, 2018, British Publishing House based on the BP index. Member of the editorial board of the International Journal of Avian & Wildlife Biology.

Pecka-Kielb Ewa, Assoc. prof., Inż. Ph.D.

Wroclaw University of Environmental and Life Sciences, Department of Biostructure and Animal Physiology, Norwida 31, Wroclaw 50-375, Poland

Advances in the Understanding and Management of Mastitis in Dairy Cows

Research and Academic Experience: Methodological skills in the field in physico-chemical analysis of colostrum and milk.

Research Area: Changes in physical and chemical properties of milk in cows, ewe with mastitis. Research on the digestive system of ruminants and monogastric animals. In vitro fermentation of rumen, caecum and colon digesta, testing different feeds and feed additives.

Number of Published Papers and Citations: The body of scientific achievement consists of 87 original publications, including: 51 papers published in JCR-listed journals, 3 monographs and 19 book chapters, 14 publications in non-JCR journals. Presented 125 papers and short communications at meetings and national and international conferences IF (impact factor) is 77.46 and the Hirsh index is 12. In database WoS are registered 534 citations.

Research Projects: The main researcher of 5 foreign. Co-solver of 2 foreign APPV project (Slovak) and 4 Poland projects.

Petr Sláma, Assoc. Prof., Ing., Ph.D.

Department of Animal Morphology, Physiology and Genetics, Mendel University in Brno, Czech Republic

Research and Academic Experience: Expertise in immunology, cell biology, and cell culture techniques, with a focus on detecting inflammatory cytokines related to mastitis. Teaching responsibilities include courses in animal anatomy, physiology, and immunology.

Research Areas: Research primarily focused on addressing issues related to bovine mastitis. Additional interests include cell apoptosis and the effects of phytonutrients on immune cells.

Publications and Citations: Author of 125 peer-reviewed publications indexed in the Web of Science Core Collection. These works have received 1,774 citations, with an H-index of 22.

Research Projects: Principal investigator of two Czech and two international projects focused on mastitis and phytonutrients. Co-investigator in an additional two Czech and two international projects.

Editorial Activities: Member of the editorial boards of the *Slovak Journal of Animal Science, Acta Fytotechnica et Zootechnica*, and *Archives of Ecotoxicology*.

Dávid Sándor Kiss, Assoc. prof., MSc., Ph.D.

Department of Physiology and Biochemistry, University of Veterinary Medicine, Budapest, Hungary

Research and Academic Experience: head of the Neurophysiology, Neuroendocrinology Research Group at the Dept. of Physiology and Biochemistry (University of Veterinary Medicine, Budapest, Hungary); visiting researcher at the MRC Anatomical Neuropharmacology Unit, University of Oxford (Oxford, UK); visiting researcher at the School of Medicine, Section of Comparative

Advances in the Understanding and Management of Mastitis in Dairy Cows

Medicine, Dept. Neurobiology and Ob./Gyn., Yale University (New Haven, USA); teaching activities on veterinary physiology and comparative physiology, physiology of extinct animals, in English, German and Hungarian

Research Area: cellular mechanisms of hypothalamic controlling of homeostatic processes; regulation of neuronal energy levels in the hypothalamus; effects of endocrine disruptors on the neuroendocrine regulation; hypothalamic functional asymmetry; role of astrocytes in the development of hepatic encephalopathy

Number of Published Papers and Citations: 81 scientific publications, 26 of them peer-reviewed original research articles or reviews; 113 citations, 67 of them registered in SCI/WoS & Scopus; h-index: 6

Research Projects: Involved in 3 international and 2 national projects

