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Study of Mastitis Incidence in Cows of Dairy Farms in East Kazakhstan: Impacts of Nutrition, Endometritis and Mycotoxin Contamination

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Abstract: This research aimed to investigate the prevalence, etiological factors, and diagnosis of mastitis in dairy farms located in East Kazakhstan. The study was conducted on two dairy farms, “Balke” and “Madi-R,” and examined a total of 490 cows. The findings indicate that the incidence of clinical and subclinical mastitis varied among the years studied. In 2016, the average incidence was 35.4%, which decreased to 19.6% in 2017, increased to 28.5% in 2018 and further decreased to 16.4% in 2019. Subclinical mastitis also exhibited variations in prevalence, with rates of 36.5% in 2016, 21.5% in 2017, 19.3% in 2018 and 22.6% in 2019. Feed analysis revealed the presence of mycotoxins synthesized by myogenic microorganisms. Additionally, milk samples from cows with mastitis identified bacteria such as *Streptococcus agalactiae*, *Staphylococcus aureus*, *Escherichia coli*, and *Micrococcus lysodeikticus* as the main microflora. The study also assessed the fertility rate in cows with mastitis and uterine pathology, finding a significantly lower rate of 1.4-1.9% compared to cows suffering only from mammary gland disease, which exhibited a fertility rate of 6.3%. Blood samples from cows with mastitis showed reduced levels of calcium, phosphorus, reserve alkalinity, and carotene compared to normal levels. In conclusion, this research provides valuable insights into the prevalence, etiological factors, and diagnosis of mastitis in East Kazakhstan's dairy farms. The findings emphasize the importance of implementing strategies to manage mastitis effectively and prevent its detrimental effects on cow health and productivity.

Keywords: Udder, Etiology, Diagnosis, Mastitis, Feed

Introduction

Dairy farming is one of the most important industries in modern livestock production worldwide. Inflammation of the mammary gland (mastitis) is a widespread disease among dairy cows, which is of significant economic importance (Rukmana *et al*., 2021; Cobirka *et al*., 2020). Mastitis in dairy farms can be caused by bacterial infection, poor milking hygiene, cow factors such as age and stress, environmental factors, and improper use of antibiotics. Prevention includes maintaining good milking hygiene practices and implementing a comprehensive herd health program (Haxhiaj *et al*., 2022).

The damage caused by this disease results from a decrease in the milk productivity of affected animals, a reduction in milk quality, premature culling of cows with chronic mastitis, expenses for treatment, and other factors. Mastitis can be caused by any factor from the external or internal environment that disrupts the integrity of the mammary gland. These factors may be physical, chemical, or biological in nature (Boboš *et al.*, 2013). Therefore, the study of specific causes that contribute to the development of mastitis and its diagnosis has great practical significance. The economic impact of mastitis on the dairy industry is estimated to be billions of dollars worldwide each year. Therefore, prevention and management of mastitis is crucial for dairy farmers to maintain the health and productivity of their herds. Fighting mastitis has been and remains one of the most serious problems in dairy farming (Freu *et al.*, 2023).

The type and quality of fodder can potentially contribute to the development of mastitis in dairy cows. The presence of molds, mycotoxins, and other contaminants in the fodder can lead to a weakened immune system, making cows more susceptible to bacterial infections that cause mastitis (Pachauri *et al.*, 2013; Jovaisiene *et al.*, 2016). It is known that more than 100 different microorganisms from the external environment, upon entering the cow's udder, are capable of provoking inflammation and regardless of the cause, mastitis primarily develops with active participation of the microflora. Pathogenic streptococci and staphylococci are considered the most common microbial cells present in the secretion of the affected quarter in 75-90% of cases (Riekerink *et al.*, 2008; Naranjo-Lucena and Slowey, 2022).

In dairy farms, the disease of mastitis significantly reduces milk production indicators. Consumption of cow's milk during mastitis is not allowed, as this milk contains numerous pathogenic microorganisms and various harmful impurities (Silva *et al.*, 2021; Bagri *et al.*, 2018). Inflammatory processes in mammary glands alter the physicochemical properties of milk. As is known, one of the etiological factors of mastitis is microorganisms, most often coliforms. However, another factor in the development of mastitis is fungi. According to some authors, their frequency of occurrence ranges from 2-13% of cases (Sağlam *et al.*, 2017; Breen *et al.*, 2021; Yao *et al.*, 2020). Staphylococcal mastitis is one of the most dangerous for dairy herds. This is evidenced by data on its prevalence in various countries (Grima *et al.*, 2021; Merl *et al.*, 2003).

Mastitis causes significant economic damage to the country's farms and it is necessary to conduct timely diagnostic studies. Mastitis is a major economic concern for dairy farmers, as it can lead to reduced milk production and quality, increased veterinary costs, decreased reproductive efficiency, and increased time and labor costs. Effective prevention and control measures are necessary to minimize the economic impact of mastitis on dairy farming operations (Tomanić *et al.*, 2023; Suwito and Nugroho, 2019).

The treatment of mastitis in dairy cows involves veterinary care, supportive care, and management practices such as antibiotics, anti-inflammatory medications, supportive care measures, management practices, and culling if necessary (Nadăș *et al.*, 2021).

The aim of this study was to investigate the prevalence, etiological factors, and diagnosis of mastitis in the conditions of East Kazakhstan's dairy farms.

Materials and Methods

Study Area and Objects

The study was conducted at the "Balke" farm in Beskaragay district and the "Madi-R" farm in Znamensk rural district of Semey city, Abay region, Republic of Kazakhstan. Beskaragay district is located in the dry steppe subzone on the right bank of the Irtysh River. The climate of Beskaragay district is sharply continental, characterized by large daily and annual temperature fluctuations. Winters are severe and long, while summers are short, hot, and dry. Windy days are common throughout the year.

The winter season is prolonged and cold, with an average temperature reaching -17°C in January, occasionally dropping to -45°C. Summers are hot and dry, with an average temperature of 21°C in July. Winter is dominated by heavy snowstorms, while summer experiences dusty storms. The average annual precipitation is 275-300 mm. The growing and development period for plants is 140 days, which is favorable for the ripening of various cereal crops, vegetables, and melons. The main water source is the Irtysh River.

Znamensk rural district also has a sharply continental climate with significant annual, seasonal, and daily temperature fluctuations. Winter temperatures can reach as low as -42 to -43°C (January-February), while summer temperatures range from +35 to +45°C. North winds prevail from October to March, while southern winds dominate from July to September. The average annual precipitation ranges from 200-300 mm, with the majority falling during the spring and autumn periods. The first snowfall occurs in late October and a consistent snow cover forms in the second half of November, lasting until the first half of April. The soil freezes to a depth of 0.8-1.2 m during the winter period. Summers are hot and dry.

Mastitis in both its subclinical and clinical forms was studied in the farms over the course of December 2016 to November 2019, encompassing all four seasons. The research was conducted using 210 lactating Holstein-Friesian cows aged 5-6 years with a live weight of 450-500 kg from the cattle farm "Balke" in the Beskaragay district, as well as lactating crossbred 280 cows of dairy breeds with the local Kazakh Whiteheaded meat breed aged 5-6 years with a live weight of 350-470 kg from the cattle farm "Madi-R" in the Znamensky rural district of the city of Semey, Eastern Kazakhstan region.

Clinical studies were conducted and medical histories were created for each animal, which fully described each system: Body temperature, pulse, respiration, general condition, condition of lymph nodes, digestive, respiratory, nervous, and cardiovascular systems were all measured. The diagnosis was established based on the collection of medical history, and clinical and laboratory research results.

Feeding Conditions in Different Seasons

To explore potential predisposing factors contributing to mastitis, an examination was conducted on the dietary practices of cows during both the summer-autumn and winter-spring seasons.

Throughout the summer-autumn period, the animals were allowed to graze on pasture and were provided with a mixture of grass hay (from both steppe and meadow), along with supplementary components such as salt, minerals, and water. Additionally, during milking, each cow received 3.5 kg of compound feed.

In the winter-stable period, the diet of the cows comprised 25 kg of silage, 10 kg of a singular feed source, 5 kg of hay, 3.5 kg of compound feed, 1.5 kg of sunflower meal, 4 kg of a vitamin-mineral supplement, 150 g of molasses, 25 g of lime and 60 g of salt. The daily ratio for primiparous cows was established at 14.6 feed units.

For primiparous cows in the summer-autumn period, the feeding ration included 20 kg of silage, 10 kg of a single feed source, 8 kg of hay, 350 g of compound feed per liter of milk, 1.5 kg of sunflower meal, 3 kg of a vitamin-mineral supplement, 100 g of molasses, 25 g of lime and 50 g of salt. This resulted in a daily ration equivalent to 8.8 feed units.

Mastitis Detection

Various methods were employed to detect and diagnose mastitis in cows. Subclinical mastitis was identified using tests such as bromthymol blue, mastidine, dimastine, Whiteside, milk sedimentation, and the California Mastitis Test. Additionally, the Express Mastitis Diagnostic Device (EMDD) and Lactan were utilized for diagnosis (Mukhamadieva *et al.*, 2022). For the determination of dry matter content, density, water-to-fat ratio, and somatic cell count in milk, the "Lactan 4.1-mini" device was utilized and the Miltek device was used to identify mastitis.

Chuzhebaeva *et al*. (2021) research was conducted in two stages, comprising diagnostic studies in the first stage and the development and refinement of a blood and feed analysis scheme based on the results obtained during the experimental work in the second stage. Each animal had an individual medical history created and the diagnosis was based on A. P. Studenstov's classification of mastitis (Berdnyk *et al.*, 2021).

Biochemical Analysis

To define the levels of hemoglobin, red blood cells, and white blood cells in the blood, a modern hematological analyzer, PCE 90 vet (Erma, Japan), was employed for the research. The analysis of cow's blood samples using the hematological analyzer PCE 90 vet (Erma, Japan) involved the following method. Initially, blood samples were collected from the cows using appropriate aseptic techniques and transferred into suitable anticoagulant tubes to prevent clotting. Next, the blood samples were carefully mixed to ensure proper dispersion of the anticoagulant. The PCE 90 vet hematological analyzer was then prepared for analysis according to the manufacturer's instructions. To begin the analysis, a small volume of the well-mixed blood sample was aspirated into a specialized cuvette or cartridge designed for use with the PCE 90 vet analyzer. The cuvette was securely inserted into the instrument, which automatically initiated the analysis process. The parameters determined include Red Blood Cells (RBC), Hemoglobin (HGB), Hematocrit (HCT), White Blood Cells (WBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red blood cell Distribution Width (RDW %), Platelets (PLT), Mean Platelet Volume (MPV), Platelet Crit (PCT) and Platelet Distribution Width (PDW). The histogram outputs the volume of red blood cells, white blood cells, and platelets (Garba *et al.*, 2019).

Determination of the Chemical Composition of the Feed

The determination of moisture content followed the guidelines outlined in the reference method (GOST 13496, 2000a). To begin the process, opened beakers containing the feed samples were carefully positioned within a drying cabinet that had been preheated to a specific temperature of (130±2)°C. The drying duration commenced when the temperature within the cabinet reached 130°C and it lasted for a total of 40 min.

Determination of Carotene in Feeds

The determination of carotene in feeds involved a method based on the extraction of carotene using petroleum ether or gasoline, followed by the measurement of the color intensity of the extracted solution using photometric analysis. The color intensity is directly correlated with the carotene content present in the feed samples (GOST 13496, 2000b). This approach allowed for the accurate quantification of carotene, an essential component with nutritional significance, in the feeds under investigation.

Determination of the Reserve Alkalinity of Blood

The reserve alkalinity of blood was determined utilizing Kondrakhin's method, which involves a series of steps. Initially, blood plasma is subjected to sulfuric acid treatment in one segment of a flask, inducing the liberation of carbon dioxide from the bicarbonates present. The released carbon dioxide is subsequently absorbed by a sodium hydroxide solution located in the other segment of the flask. Subsequent to this, the surplus sodium hydroxide unreacted with carbon dioxide, and half of the sodium hydrocarbonate ($NaHCO_3$) generated during the absorption process is titrated using a sulfuric acid solution. The quantity of initially bound sodium hydroxide facilitates the calculation of the amount of carbon dioxide released from the plasma, which corresponds to the bicarbonate content (Kondrakhin *et al*., 1985).

Statistics

Each experiment was conducted in triplicate and the results were presented as the mean ± Standard Error of the Mean (SEM) from three independent observations. Statistical analysis was performed using Statistica 12.0 software (Statsoft Inc., Tulsa, OK, USA). The differences between samples were assessed using a one-way Analysis of Variance (ANOVA). To compare the means, the Tukey Honestly Significant Difference (HSD) test was employed. A p-value less than 0.05 was considered statistically significant, indicating a significant difference between the samples being compared.

Results

The study conducted on the dairy farms "Balke" and "Madi-R" has provided insights into the occurrence of mastitis in cows over different years. A total of 490 cows were examined across both farms. The findings indicate that the incidence of clinical mastitis varied among the years studied. In 2016, clinical mastitis was observed in an average of 35.4% of cases, whereas in 2017, the incidence dropped to 19.6%. In 2018, it increased to 28.5%, and in 2019, it further decreased to 16.4%. Similarly, the prevalence of subclinical mastitis also exhibited differences in incidence rates across the years (Fig. 1).

According to clinical forms of mastitis, serous mastitis accounted for 14.4% in 2016, 15.6% in 2017, 20.6% in 2018 and 10.6% in 2019. Fibrous mastitis was 17.7% in 2016 and 4.2% in 2019. Catarrhal mastitis was 32% in 2016 and 21.3% in 2019. Hemorrhagic mastitis was 1.1% in 2016 and 7.9% in 2018. Purulent mastitis was 2.2% in 2016 and 2.1% in 2019. Additionally, there were certain differences in the prevalence of subclinical mastitis by year. Specifically, it was 36.5% in 2016, 21.5% in 2017, -19.3% in 2018 and 22.6% in 2019.

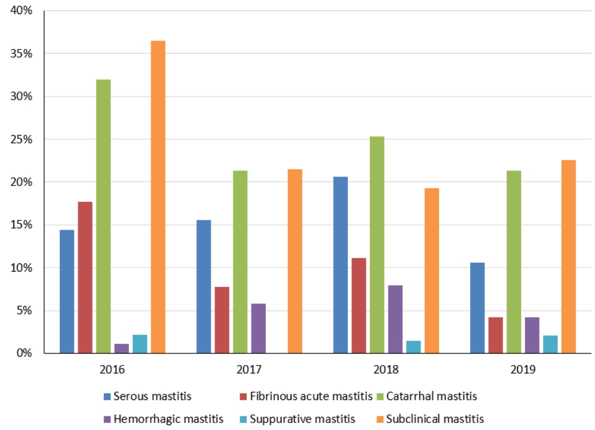

Fig. 1: Prevalence of mastitis in cows from Balke and Madi-R farms

The research results showed that in cows with mastitis, endometritis was diagnosed. In 2016, endometritis was detected in 36.6% of cases, in 2017-56.6%, in 2018-31.1% and in 2019-23.3% of cases (Table 1).

During the examination of the herd of dairy cows of the Holstein breed, it was observed that the animals were defecating semi-liquid feces and were more interested in sorting their feed rather than chewing it. A selective clinical examination of the lactating cows revealed signs of mineral metabolism disorder, as almost all cows had thin tails, which indicates a disturbance in the phosphorus-calcium balance.

Additionally, blood samples from 26 cows with mastitis were sent to the veterinary laboratory for analysis of carotene, calcium, phosphorus, and reserve alkalinity. The results of the analysis are presented in Tables 2-3.

If the normal blood levels of dairy cows should contain in mg% calcium -11.3-13.1, phosphorus -4.0-4.6, reserve alkalinity -460-540 and carotene -0.5-2.28, then according to the conducted study, in the blood of cows with mastitis, these indicators are reduced or are at the lower limit. In cows with mastitis, the serum levels in mg% were found to be from 8.0-10.8 for calcium, from 3.0-4.3 for phosphorus, from 320-440 for reserve alkalinity, and from 0.220-0.988 mg% for carotene.

Insufficient exercise was also one of the reasons for mastitis.

Our research analysis has shown that during the summer season when there is an adequate amount of protein, carbohydrates, vitamins, and minerals in green feed, cases of mastitis in cows occur less frequently. To identify the causes of mastitis, an analysis of the contamination of feed with mold fungi was carried out. For this purpose, feed samples were collected for analysis of mycogenic sensitization, which is used in preparing feed mixtures. Ready-made feed mixtures were also collected to detect the level of contamination by mold fungi, yeasts, and microorganisms.

Table 1: Prevalence of mastitis and pathology of reproductive organs in cows

	2016		2017		2018		2019	
Diseases of cows by year	Total	%	Total	%	Total	%	Total	%
Total clinical mastitis	56	35,4	31	19,6	45	28,5	26	16,4
Subclinical mastitis	34	36,5	20	21,5	18	19,3	21	22,6
Total mastitis	90	100	51	100	63	100	47	100
Pathologies of reproductive organs in cows								
Endometritis / mastitis	33	36,6	23	56,6	28	31,1	21	23,3

Table 2: Content of carotene, calcium, phosphorus, and reserve alkali in blood serum of cows of the farm "Balke"

	Inventory number	Number of calves	Date of calving	Results, mg % Ca	P	Reserve alkali	Carotene
1	16608	4	16.02	9,3	3,2	320	0,931
2	14162	6	10.01	9,6	3,3	360	0,514
3	16612	4	04.02	8,0	3,6	360	0,436
4	18237	2	01.02	9,5	3,5	350	0,425
5	14365	6	07.03	9,6	3,3	360	0,484
6	17215	3	02.03	9,9	4,0	400	0,480
7	14011	6	08.03	9,0	3,0	420	0,410
8	2113	9	11.03	8,0	3,0	320	0,484
9	17679	4	20.03	10,0	3,0	380	0,438
10	14398	7	10.04	9,1	3,4	350	0,416
11	7109	8	17.03	9,9	3,9	440	0,511
12	150152	5	16.01	9,2	3,7	400	0,492
13	17219	4	14.02	10,8	4,3	440	0,518
14	18144	2	26.02	9,3	3,7	380	0,661
15	17649	4	28.03	8,4	3,5	340	0,497

Table 3: Content of carotene, calcium, phosphorus, and reserve alkali in blood serum of cows of the farm "Madi-R"

#	Inventory number	Number of calves	Date of calving	Results, mg % Ca	P	Reserve alkali	Carotene
1	18056	3	02.02	10,6	4,1	360	0,501
2	18153	3	30.03	9,2	3,3	330	0,488
3	183652	3	07.02	9,1	3,4	350	0,467
4	16039	4	22.01	9,6	3,9	360	0,477
5	19394	7	27.02	9,0	3,8	320	0,362
6	10651	5	21.02	10,8	3,6	350	0,410
7	14384	6	10.04	9,7	4,0	370	0,988
8	17248	5	19.13	8,1	4,0	360	0,491
9	14086	6	21.02	9,4	3,5	340	0,344
10	18305	4	11.04	9,9	3,4	330	0,309
11	10588	7	18.04	8,5	3,8	360	0,220

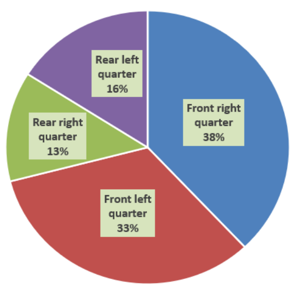

Fig. 2: Mastitis prevalence by udder fractions, %

Table 4: Results of microbiological analysis of mammary gland secretions

Bacteria	2016	2017	2018	2019
Streptococcus agalactiae	+	+	+	+
Staphylococcus aureus	+	+	+	+
E. Coli	+	+	+	-
Micrococcus lysodeikticus	-	+	+	-

Table 5: Results of microflora sensitivity determination of mammary gland secretion to antibiotics

	Bacteria			
Name of antibiotics	*Streptococcus agalactiae*	*Staphylococcus aureus*	*E. Coli*	*Micrococcus lysodeikticus*
Oxytetracycline	+++	+++	+++	+++
Levomycetin	+++	++-	+++	+-+
Gentamicin	++-	---	++-	---
Benzylpenicillin	---	+++	---	---
Erythromycin	+++	+++	++-	+-+

Note: +++ sensitive++- low-sensitivity--- sustainable

Penicillium spp. and *Mucor ramosissimus* were discovered in the moldy grain, with the colonies of *Penicillium* spp. exhibiting various sizes ranging from 5-20 mm in diameter and an exudate of a pale-yellow color.

Colonies of *Mucor ramosissimus* were 25 mm in diameter with uniform growth, having denser mycelial growth in the center compared to the periphery. *Mucor circinelloides* were detected in the chopped feed, with surface colonies being gray and fluffy and rapid growth covering the entire petri dish within three days. All the identified micromycetes are mycotoxic and synthesize mycotoxins, which contribute to chronic intoxication and disturbances in metabolism.

The presence of black mold *Alternaria alternata* and a large amount of *Bacillus subtilis* or hay bacillus in all samples of feed raises particular concern. In the next stage, laboratory analysis of milk gland secretions was performed. Bacteriological examination of 15 milk samples from cows with mastitis revealed the microflora. As a result, it was found that the main microflora of milk from cows with subclinical mastitis consists of bacteria such as *Streptococcus agalactiae*, *Staphylococcus aureus*, *E. coli*, and *Micrococcus lysodeikticus* (Table 4).

In the laboratory tests conducted in 2016, the following strains of microorganisms were isolated: *Streptococcus agalactiae*, *Staphylococcus aureus*, and *E. coli* in sample #1. In the bacteriological examination conducted in 2017, *Streptococcus agalactiae*, *Staphylococcus aureus*, *E. coli*, and *Micrococcus lysodeikticus* were detected in the milk sample.

In 2018, during the examination of a sample of mammary gland secretion from cattle, the causative agents of cow mastitis, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Staphylococcus aureus* and *E. coli*, *Micrococcus lysodeikticus*, were isolated from the samples. In 2019, during the examination of another sample of mammary gland secretion from cattle, the causative agents of cow mastitis, *Streptococcus agalactiae* and *Staphylococcus aureus*, were isolated from the samples. Diagnostic examination of the mammary gland of cows involved analyzing mastitis by quarters of the udder (Fig. 2).

According to our observations, inflammation of the mammary gland by quarters was as follows: 38% for the front right quarter, 33% for the front left quarter, 13% for the rear right quarter, and 16% for the rear left quarter. Subsequently, the sensitivity of the microflora to antibiotics was determined (Table 5).

Staphylococci were sensitive to oxytetracycline, chloramphenicol, benzylpenicillin, and erythromycin. Streptococci were sensitive to oxytetracycline, chloramphenicol, and erythromycin. *Escherichia coli* were sensitive to oxytetracycline, chloramphenicol, and erythromycin. *Micrococci* were sensitive to oxytetracycline, chloramphenicol, and erythromycin.

Discussion

Mastitis is a disease of the mammary gland that leads to a decrease in milk quality and quantity. Endometritis of various etiologies can cause this disease, leading to infertility, sterility, and prolonged estrus in the uterus herd (Čobanović *et al.*, 2021). Predisposing factors for the development of mastitis and endometritis are the weakening of the body's resistance as a result of improper, inadequate, and incomplete nutrition, hypodynamia, overwork, avitaminosis, and trauma of the birth canal (Mohammad *et al.*, 2023).

There were some differences in the prevalence of subclinical mastitis by year. For example, in 2016 it was 36.5%, while in 2017 it was 21.5%, in 2018 it was 19.3% and in 2019 it was 22.6%. In contrast, clinical mastitis cases were registered in 35.4% of cows on average in 2016, 19.6% in 2017, 28.5% in 2018 and 16.4% in 2019. According to some scientists, clinical mastitis is registered in 2.0-4.7% of cases in dairy herds, while subclinical mastitis is registered in 12.9-30.0% of cases (Khasapane *et al.*, 2023; Mironchik *et al.*, 2020).

According to the results of clinical examination of cows and laboratory diagnosis of milk gland secretions, it has been established that one of the etiological factors in the development of mastitis is the Candida albicans fungus. Additionally, the study results on the effectiveness of various rapid mastitis tests for detecting subclinical mastitis have been obtained. As a result of the research, it has been found that the mastidin and California tests show the highest results in diagnosing subclinical mastitis (Kivaria and Noordhuizen, 2007; Eldesouky *et al*., 2016).

The results of the study showed that in cows with mastitis, endometritis was also diagnosed. In 2016, endometritis was 36.6%, in 2017 it was 56.6%, in 2018 it was 31.1% and in 2019 it was 23.3%. Analyzing the data of the conducted study, it can be concluded that both subclinical and clinical mastitis have a high level of prevalence. Thus, mastitis in cows in the conditions of dairy complexes and farms has a wide distribution. When milking cows with traditional milking installations, the incidence of clinical mastitis on average was 5.2-5.6% and the subclinical form was 13.7-14.1% (Semenov *et al*., 2021).

Research articles and reviews, as well as the results of our own observations, suggest that mastitis and endometritis are widespread and that pathogenic and opportunistic microflora play an important role in their etiology (Paiano *et al*., 2020). It has been established that cows with gynecological diseases (retained placenta, endometritis) develop various forms of mastitis in 32.4% of cases. The presence of prepartum edema in cows, due to impaired blood and lymph circulation, contributes to a decrease in the resistance of the mammary gland parenchyma to the effects of opportunistic microflora (Bacha and Regassa, 2010).

In the group of animals affected by both mastitis and uterine pathology, a significantly lower fertility rate of 1.4-1.9% was observed when compared to cows solely experiencing mammary gland disease, which exhibited a fertility rate of 6.3%. This trend was particularly evident in animals with subclinical mastitis (Girma and Tamir, 2022).

According to research conducted in 28 dairy herds in New Zealand, the mean annual cumulative incidence of clinical mastitis in one or more quarters was 12.7 cases per 100 cows. The incidence was higher in young 2-year-old and old (>or = 9 years) cows compared to 3 and 4-year-old cows. Frisian breed cows had a higher incidence than Jersey or crossbred cows. As milk production increased, the frequency of mastitis also increased, resulting in a significant economic loss (McDougall *et al*., 2007a-b; Sharma *et al*., 2012).

Over the course of 50 years in India, economic losses from the disease, along with the increasing frequency of cases, have increased by 115 times (Sharma *et al*., 2012). Certain cases of poisoning by staphylococcal enterotoxin can lead to a fatal outcome in humans. Toxins produced by pathogenic *Streptococcus* in milk can cause meningitis in newborns and angina. The *E. coli* toxin can cause severe forms of enteritis in the small intestine. *Pyogenic bacteria* can lead to pyogenic and necrotic diseases in human tissues and organs (Saed and Ibrahim, 2020; Alkasir *et al*., 2016).

Blood samples from 26 cows with mastitis were also analyzed for levels of carotene, calcium, phosphorus, and reserve alkalinity. Cows with mastitis had serum levels of 8.0-10.8 mg% for calcium, 3.0-4.3 mg% for phosphorus, 320-440 for reserve alkalinity, and 0.220-0.988 mg% for carotene.

Such a disturbance in mineral metabolism, especially in combination with protein and vitamin deficiencies in the diet, led to their disruption. Improper calcium and phosphorus ratio in the diets of dairy cows during the dry period leads to hypocalcemia in animals immediately after calving, which is directly related to the development of mastitis (Tenhagen *et al*., 2009). The quality of feed also plays an important role. Feeds should not contain mechanical impurities, substances that are toxic and harmful to the health and life of animals. In accordance with veterinary-sanitary rules, each batch of silage, haylage, mixed feed, etc. must undergo various laboratory tests (toxicological, microbiological, biochemical, etc.) both before being laid and before being fed to animals (Reinhardt *et al*., 2011).

Microscopic fungi were found in all samples during the investigation. *Alternaria alternate* and *Mucor circinelloides* were found in alfalfa. *Penicillium chrysogenum* and *Mucor ramosissimus* were found in barley. *Mucor circinelloides* was found in feed mash. The presence of black mold *Alternaria alternate* and a large number of *Bacillus subtilis* raises particular concern. Only the meal was found to be uninfected.

When developing measures for preventing mastitis in farms, it is necessary to take into account the balanced and high-quality nature of feed included in the cow's diet. According to Batrakov *et al*. (2009); Zecconi *et al*. (2018), cows are subjected to metabolic stress during the early lactation period, which in turn reduces the immune response of the body to infectious agents. The decrease in the immune status of the cow's body leads to outbreaks of subclinical diseases, including mastitis. Feeds containing residual pesticides, elevated levels of nitrates and nitrites, and those affected by mold and fungi pose a particularly high risk.

During the investigation, the microflora of milk from cows with subclinical mastitis was identified. The following bacteria were detected: *Streptococcus agalactiae*, *Staphylococcus aureus*, *Escherichia coli*, and *Micrococcus lysodeikticus*.

The most important role in the etiology of mastitis (up to 90% of cases) is played by *streptococci*, *staphylococci*, and less frequently, bacteria from the group of *Escherichia coli*. The main causative agent of inflammation (28-85% of cases) is *Str. agalactiae*, which belongs to serological group B and has adapted better to the conditions of existence in the mammary gland compared to other streptococci. The second place after *Str. agalactiae* in terms of the number of mastitis cases is occupied by *Str. uberis* (serological group E), which is isolated from the surface of the cow's body, udder and teats. Studies show that *Staph. aureus* (30.5-29.3%), *Staph. epidermidis*

(32.5-24.9%), *Str. agalactiae* (22.0-17.7%), *Str. dysgalactiae* (16.6-15.9%), *Str. uberis* (4.6-3.5%) and other types of microorganisms (*E. coli, Str. pneumoniae*) constitute less than 2% of the etiological structure of infectious mastitis (Algharib *et al.*, 2020; Butler *et al*., 2000; Duarte *et al*., 2015; Shigidi and Mamoun, 1981; Iraguha *et al.*, 2017).

According to our observations, inflammation of the mammary gland was observed in quarters as follows: Right front quarter -38%, left front -33%, right rear 13%, and left rear -16%. The affected front quarters (29%) produce less milk and are therefore milked faster than the rear quarters, resulting in a non-productive milking process that leads to harmful vacuum effects on tissues and the development of mastitis. Mastitis was observed in 53.0% of animals affecting the rear quarters, which is associated with their high productivity and longer milking times due to the lack of additional milking, milk is constantly retained in them, causing irritation of the mammary tissues leading to the development of mastitis. In most cases (89.0%), 1-2 quarters of the udder are involved in the inflammatory process, while 3-4 quarters are affected less frequently (7.0%) (Zhang *et al.*, 2016; De Souza *et al.*, 2012).

One of the commonly used treatment regimens for cows with staphylococcal mastitis is injections of penicillin at a dose of 1 million units and streptomycin at a dose of 1 g, diluted in 10 mL of 0.5% novocaine solution. These antibiotics are administered intramuscularly twice a day for 3-5 days.

According to our study, the in vitro sensitivity to penicillin was 20% and to streptomycin was 40%. However, the therapeutic effect reached 70% due to the synergistic effect of these two antibiotics (Bengtsson *et al.*, 2009; Russi *et al.*, 2008).

One of the common factors leading to low effectiveness of antibiotic treatment in cows with mastitis is therapy without consideration of the sensitivity of the microflora to antimicrobial agents. In addition, the therapeutic efficacy depends on the route of administration of antimicrobial agents, including intramuscular, intra-arterial, and intradermal at the base of the udder (intralymphatic) (Ali *et al.*, 2021; Moroni *et al.*, 2006). To increase the clinical and economic efficiency of the treatment of cows with mastitis, it is necessary to conduct treatment taking into account the sensitivity of the causative agents of the disease to antimicrobial agents.

Calving of Cows

Clinically healthy animals in the gestation period were monitored. The influence of the form of mastitis previously experienced on the subsequent occurrence of reproductive system pathologies, the number of days of infertility, and the conception index were determined. In cases of subclinical mastitis, uterine subinvolution, endometritis, and retained placenta occurred 1.9-2.3 times more frequently compared to non-affected animals. In contrast, in cases of clinically pronounced mastitis, these conditions were detected 27.5% more frequently than in subclinical mastitis. Both subclinical and more significantly, clinically pronounced mastitis experienced during the gestation period increased the number of days required for the restoration of reproductive function and decreased the conception index.

During calving, cases of postpartum pathologies such as retained placenta, uterine subinvolution, and endometritis were recorded for cows. Subsequently, the incidence of mastitis in the postpartum period was determined.

However, milk from cows displaying symptoms of mastitis is not suitable for calves. Such milk often exhibits visual changes such as a yellowish color, clumps, a slimy texture, or an unpleasant odor. It may contain a high number of bacteria that can cause digestive tract problems in calves and increase their risk of developing diarrhea.

Conclusion

In conclusion, this study conducted on two dairy farms in East Kazakhstan found that the incidence of clinical and subclinical mastitis varied among the years studied. Cows with mastitis were found to have end metritis and signs of mineral metabolism disorder. Blood analysis showed reduced levels of carotene, calcium, phosphorus, and reserve alkalinity in cows with mastitis. Insufficient exercise was also identified as a contributing factor. Analysis of feed for mycogenic sensitization revealed the presence of microscopic fungi in almost all samples. These findings provide valuable insights into the prevalence, etiological factors, and diagnosis of mastitis in dairy farms in East Kazakhstan. Further research is needed to explore the effectiveness of various management practices, genetic factors, alternative treatments, and environmental influences on mastitis in dairy cows. It is recommended to implement comprehensive mastitis prevention programs, promote biosecurity measures, maintain regular monitoring and early detection, keep accurate records, and encourage collaboration to enhance mastitis prevention and control in dairy farming.

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Author's Contributions

Nurzhamal Mukhamadieva: Wrote a manuscript.

Dinara Zainettinova: Performed the calculations and analysis.

Mardan Julanov, Vasyl Stefanik and Nursulu Julanova: Developed a model and computational structure and analyzed the data.

Zhanat Nurzhumanova and Meruert Alimbekova: Performed analysis.

Nurmagambet Akzhigitov: Collected samples, and performed analysis.

Ethics

The animal study protocol (protocol code P042-2.11, dated 20.04.2021) received approval from the institutional review board of Shakarim University of Semey.

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