Strategies for Identifying and Preventing Fungal Mastitis in Dairy Cows

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Abstract
Mastitis is one of the most important diseases with a multifactorial aetiology that causes high economic losses in dairy industry, having a direct negative impact on the profitability of the farm and animal welfare. Fungal mastitis is widespread in dairy cows, and in recent years, fungal agents have been frequently reported as being responsible for mastitis. The aim of this study is to identify the species of fungi in various milk samples collected from cows diagnosed with clinical mastitis, in order to monitor the number of somatic cells. A total number of 30 samples of milk collected from cows diagnosed with mastitis were studied to determine if the disease was caused by the presence of fungal species or other etiological agents. In the analysed milk samples, a number of species of fungi belonging to the genera: Fusarium, Penicillium, Cladosporium and Aspergillus were identified. The presence of fungal contamination in the environment is almost ubiquitous, thereby strategies to prevent fungal mastitis in dairy farms, have become the main goal for most farmers, in order to increase the quality of raw milk.

Keywords: dairy cows, fungal species, somatic cells, prevention strategies.

Introduction

The most prevalent and costly disease in the dairy business is mastitis or the inflammation of the mammary gland. Fleischer was the first to describe a case of mycotic mastitis, according to Fernandes and collaborators [1]. Giesecke and collaborators did a thorough study of this disorder, listing the fungus species isolated in bovine mastitis milk samples [2]. Minami and collaborators described clinical mastitis caused by Cryptococcus laurentii in a cow in 1976, which was the first description of mycotic mastitis by Brazilian authors [3,4].
Mastitis signs include abnormalities in milk such as a watery appearance, flakes, clots, or pus [5-8]. The disease causes potassium, lactoferrin, and casein levels in milk to drop. Because calcium in milk is linked to casein synthesis, disruption of casein production results in reduced calcium levels in milk. Milk from mastitis-affected cows has a higher somatic cell count, lowering the quality of the milk.
Leucocytes are discharged into the mammary gland as a result of microbial invasion through the teat canal. External symptoms of this disease include swelling, heat, redness, hardness, and pain in the udder.
Mastitis is commonly transmitted through milking machines, milkers' hands, and other materials. Antibiotic medication is frequently the veterinarian and dairy farmer's initial option of treatment for infected cows without a prior identification of the mastitis-causing microbes. The incidence of fungal mastitis caused by yeast or yeast-like bacteria has increased dramatically in the past decade [5,9,10]. The use of infected syringes, cannulas, or antibiotic formulations to treat various conditions has been connected to the development of fungal mastitis. Moreover, injuries

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to the teats might lead to the development of a yeast infection [5].

The mycotic mastitis agents most frequently isolated from affected glands are species of Candida. They may cause clinical mastitis, which is characterized by pain, fever that lasts for a long time, tenderness, an inflammatory response in the mammary gland and related lymph nodes, and a decrease in the quantity and quality of milk produced by dairy cows [11].

Antimycotic drugs have been used to treat yeast mastitis in the past, but they are highly cytotoxic and not always efficient [5]. Mastitis is brought on by an inflammatory reaction that is triggered when germs invade the cow mammary gland through the teat canal [12].

Despite the fact that both moulds and yeasts are common in nature, only yeasts are typically considered the culprits behind fungal mastitis infections in ruminants. To ascertain whether an intramammary infection has occurred, tests are needed. These tests must either detect the harmful microorganism directly or covertly exhibit an inflammatory response, such as an increase in somatic cell count [12]. Given the importance of the disease, especially in terms of economic losses, this study presents strategies for the prevention of fungal mastitis and improvement of raw milk quality by monitoring the number of somatic cells, assessing the milk physical-chemical and the mycological examination.

Materials and methods

Identification of cows with mastitis

The dynamic activity of cows was monitored using the Afimilk program, which is the most advanced software in the world for the management of dairy farms. Based on the developed algorithm, the software sent alerts every time the milk quantity and conductivity suffers modifications.

Collection of milk samples

Before collecting the milk samples, the udder, the teat, and the milkers' hands were carefully cleansed with soap and water and disinfected with 70 percent ethyl alcohol. The first strips of milk were discarded and then about 20 ml of milk was collected in sterile containers. Samples were collected from 50 dairy cows in both cold and warm seasons, identified by Afimilk software as having high electrical conductivity. The following assay were performed for each collected sample: number of somatic cells, physical-chemical parameters and the mycological examination.

Materials: cow milk, 70% ethyl alcohol, sterile containers, plates, culture medium PDA, NaOH solution 4%, Decon solution, Triton solution, staining solution, CMT reagent.

Assessment of total somatic cell count

The California mastitis test (CMT) is most widely used screening test for mastitis diagnosis that assesses the somatic cell count in a milk sample. The samples are combined with the CMT reagent, which results in the lysis of somatic cells and the release of DNA that solidifies into a gel. Based on the intensity of the reaction, a qualitative evaluation of the CMT test result is made [13].

Assessment of the electrical conductivity (Draminski portable device)

About 15 ml of milk were added to the test cup, until the milk reached the indicated mark. Milk from each quarter of the gland was analysed individually with a prior sanitization of the device between determinations.

Assessment of sodium content

This assay was performed using a 4% solution of sodium hydroxide. Briefly, 3 ml of milk and 3 ml of 4% NaOH solution were added to a test tube. The sample was shaken and the results were read in 10-20 minutes. Gel formation is an indicative of a positive result [13, 14].

Assessment of the physical-chemical parameters of milk

The automated analyser - FTIR CombiScope 600/300 (LactoScope FTIR 600/300 and SomaScope LFC 600/300) was used to determine physical-chemical parameters (fat, dry matter, protein, lactose, casein) and somatic cell count (SCC), a technique that offers the advantages of reducing analysis time (200 milk samples / h), costs and reagents consumption. Additionally, the determinations are carried out automatically, in an objective manner.

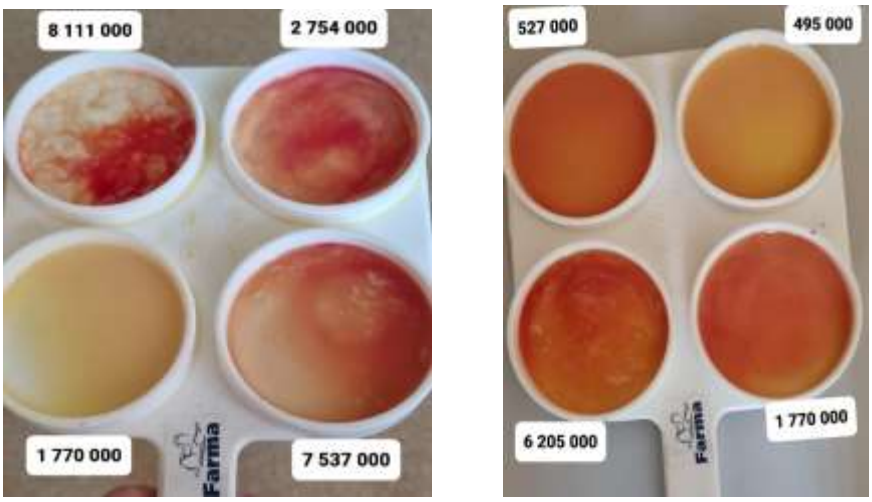
Mycological examination

The seeding in the plate was performed on a PDA type medium enriched with antibiotic (PenStrep). The plates were kept at thermostat, at 25ºC. Only

plates containing less than 150 colonies were considered. In order to identify the colonies of micro-mycetes that were to be taxonomically framed, preparations were made between the blade and the lamella and then they were examined under a microscope.
The peculiarities of the mycelial apparatus were analysed, thus identifying the genera and even the species of micro-mycetes from mastitis milk [15].

Results and discussion

Bovine mastitis is caused by physical, chemical, and biological agents. Early diagnosis of the subclinical form of mastitis is crucial for the infection's successful treatment and management. Mastitis often affects highly productive dairy cows more severely than low productive ones. Since some medications, including penicillin and tetracycline, operate as a source of nitrogen for various species of fungi, fungal mastitis is frequently missed by the expert during the first round of treatment. As a result, administration of antibiotics might worsen the condition. Since many of these fungi do not respond to antibiotic treatment, including tetracycline, treating fungal mastitis may be challenging. Because of this, the majority of mastitis cases continue to be incurable and a source of infection for nearby animals.
The aim of this study was to identify the species of fungi in various milk samples collected from cows diagnosed with clinical mastitis, in order to monitor the total number of germs and the number of somatic cells. Milk samples were collected from cows identified with a high somatic cell count both in the warm season and in the cold season. In mastitis, apart from the increased number of somatic cells, the milk suffers several other physical-chemical modifications. Due to the large number of somatic cells in the samples, when the CMT reagent is added, a gel of different consistency is formed depending on the number of somatic cells in analysed the sample (Figure 1). This assay is considered a “cow side” test, since it is quick, simple and may be performed easily by farm staff.



a **b**

Figure 1. Milk samples identified with an increased number of somatic cells

One of the modifications induced by mastitis is the increase of sodium ions which subsequently generates an increased electric conductivity [13]. Apart from the automatic determination of electric conductivity, at the milking parlour, using the Afimilk software, Draminski portable device may be used to assess the electric conductivity in farms. Values below 250 are indicative of possible infection in quarters, while values above 300 are indicative of healthy milk samples (Figure 2).

Figure 2. Draminski portable device for assessing milk conductivity

The increase in salt content, induced by mastitis, may be also confirmed by the sodium hydroxide test (4%). The formation of a indicated a positive result for milk samples from cows with mastitis (Figure 3). Although the method is less precise, it has the advantages of being affordable and easy to perform by farm staff [13,14].

Figure 3. Assessment of sodium content for confirming mastitis

The automated analyser - FTIR was used to determine physical-chemical parameters (fat, dry matter, protein, lactose, casein) and somatic cell count (SCC) (Figure 4).

Figure 4. Automated analyser - FTIR CombiScope

Samples with an increased number of somatic cells were subsequently examined mycologically to identify the probability of the occurrence of fungal mastitis. In the cold season, 5 cases of fungal mastitis were identified in the experimental group, while in the warm season, 6 cases were registered. Physical-chemical parameters, the total number of somatic cells and the results of the mycological examination for the samples collected in the warm season are presented in Table 1, respectively Figure 5. The same parameters were assessed for the samples collected in the cold season (Table 2, Figure 6).

Table 1. Results of the samples collected in the cold season and identified as suspicious with the fungal mastitis

Sample	SCC x 1000/ mL	Fat %	Protein %	Lactose %	Casein %	S.U. %	Mycological examination
1	495	3.45	3.40	4.71	2.99	11.04	numerous yeast colonies, 1 large colony of the genus Fusarium - F. moniliforme, 1 colony of the genus Fusarium - F. avenaceum, 1 colony of the genus Penicillium - P. purpurogenum (pathogenic; has exudate and red pigment diffused in the environment)
2	2,754	1.54	3.60	4.62	3.17	9.69	5 yeast colonies, 1 colony of the genus Fusarium, 2 colonies of the genus Aspergillus - A. fumigatus
3	527	3.40	3.36	4.61	3.23	10.98	2 yeast colonies
4	7,537	1.15	3.90	4.58	1.82	7.06	8 colonies of the genus Aspergillus - A. fumigatus, 2 colonies of the family Mucoraceae
5	2,564	2.07	6.02	1.85	5.69	7.13	1 yeast colony, 4 large colonies of the genus Penicillium - P. chrysogenum (2 colonies show exudate)

Somatic cell count (SCC), dry matter (S.U.)

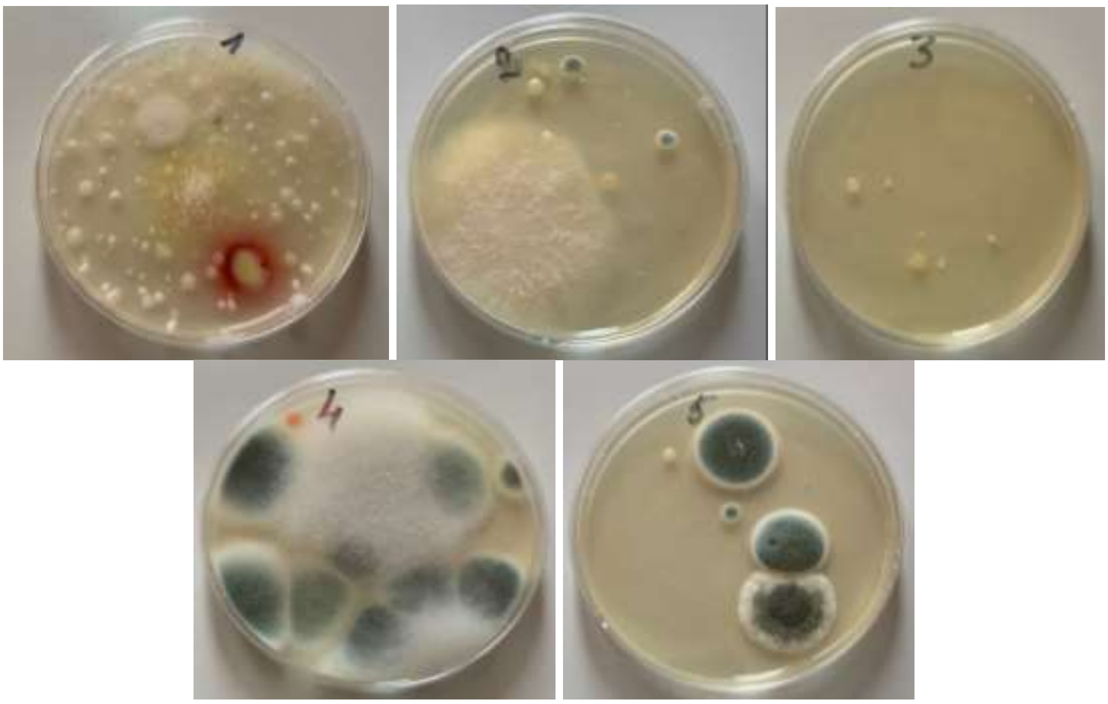


Figure 5. The mycological examination of the samples identified with the fungal mastitis harvested in the cold season

Table 2. Results of the samples collected in the warm season and identified as suspicious with the fungal mastitis

Sample	SCC x 1000 / mL	Fat %	Protein %	Lactose %	Casein %	S.U. %	Mycological examination
1	505	3.46	3.49	4.76	2.95	11.56	25 yellow yeast colonies, 4 Fusarium colonies (2 small, 2 large)
2	330	3.85	3.36	4.94	2.87	11.97	numerous yeast colonies (> 150)
3	24	3.55	3.46	4.66	3.01	11.77	3 small colonies of the genus Fusarium, numerous yeast colonies (> 50)
4	8,111	1.05	3.08	3.15	6.42	9.65	15 yeast colonies of different sizes
5	6,205	1.77	5.26	0.47	3.98	7.65	1 colony of the genus Penicillium - P. crysogenum, 5 small colonies of the genus Penicillium, 2 colonies of the genus Fusarium (1 colony F. moniliforme, 1 colony F. avenaceum), 1 yeast
6	1,770	1.80	3.80	4.66	3.12	9.25	9 yeasts, 1 colony of genus Cladosporium, 6 colonies of genus Fusarium, 1 colony of genus Apergillus – *A. fumigatus*

Somatic cell count (SCC), dry matter (S.U.)

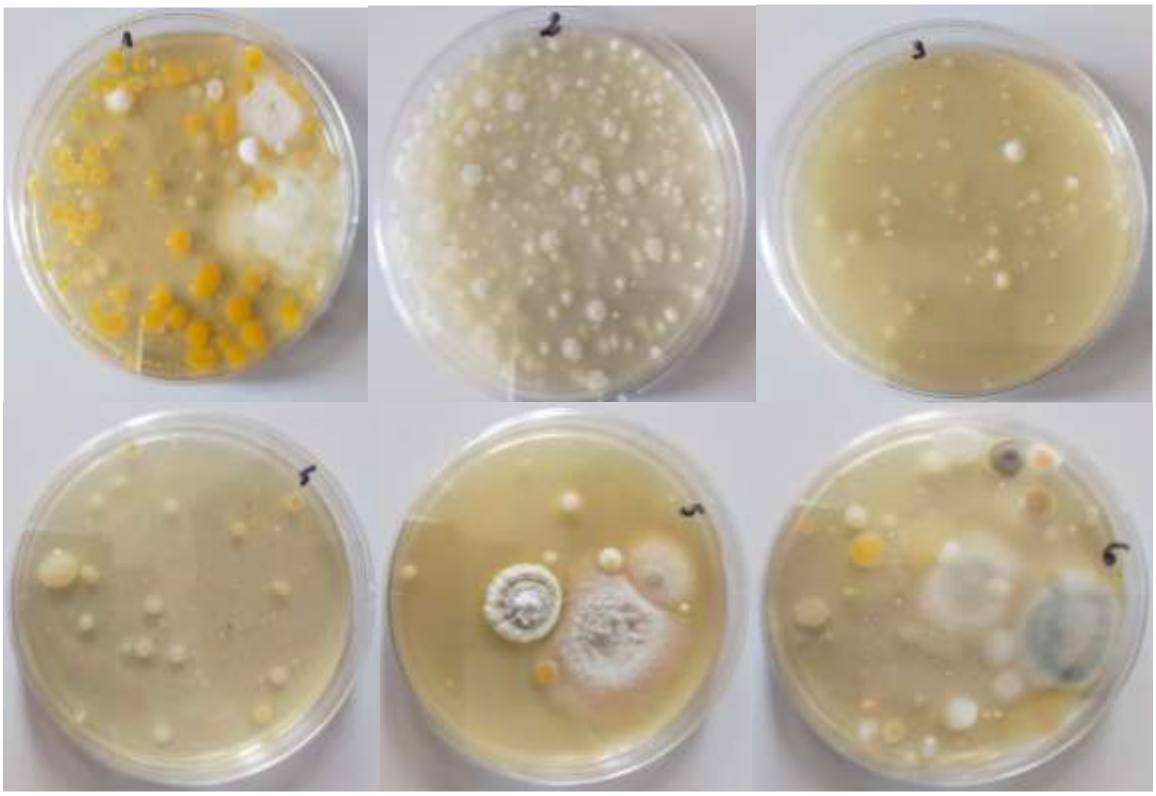


Figure 6. The mycological examination of the samples identified with the fungal mastitis harvested in the warm season

Colonies from the genera Fusarium, Penicillium, Cladosporium and Aspergillus were predominantly identified, but yeast colonies were also present in a relatively large number. Yeasts are often the cause of mammary gland infections in dairy cows. Most cases appear in the form of localized foci and/or after treatment with antimicrobials. Yeasts are generally considered

saprobic and have been isolated from milk from healthy animals. However, in some cases, they are also present in milk samples from animals with mastitis.
Currently, the study of mycoses in animals and humans is becoming increasingly important due to the fact that many species of yeasts, yeast-like and filamentous fungi, previously considered non-pathogenic, have acted as opportunistic agents, causing disease in their hosts [15].

Strategies to prevent and control mastitis in dairy farms

The appearance of mastitis in cows includes is influenced by many factors such as the stage of lactation, breed, history of mastitis and parity. In spite of the udders' defence systems, the microbial infection may occasionally overwhelm and can lead to lead to mastitis. Inappropriate livestock management and husbandry practices, like unsanitary livestock environment, insufficient floor space given to the animals, inappropriate ventilation, and incorrect milking procedures are also factors that may determine the occurrence of mastitis in a dairy farm.
Several management strategies and control measures for bovine mastitis prevention and control may be implemented in farms with the aim of improving milk quality, dairy cattle health and diminish the economic burden of mastitis (Figure 7).

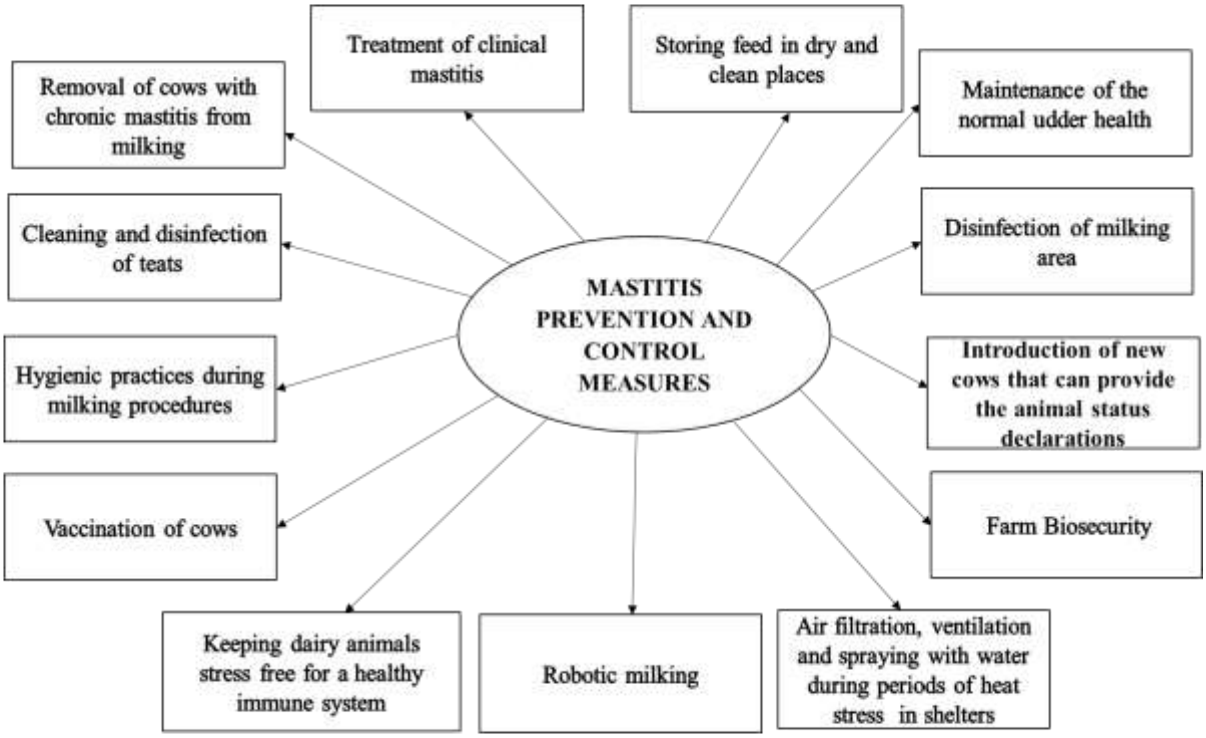


Figure 7. Management strategies for bovine mastitis prevention and control

Regular floor cleaning, proper milking techniques, udder washing before milking, and pre- and post-milking teat dipping in antiseptic solutions are the most popular ways to prevent mastitis in dairy cow farms. Other significant risk factors for the introduction of microbial infections that cause mastitis in the udder include physical injury to the teat skin, teat canal, and mammary cistern. However, despite decades of extensive research and prevention strategies at the herd level, bovine mastitis remains the illness inflicting the most economic losses to the dairy sector. Antibiotics are commonly used to treat mastitis. Yet, the focus should be switched away from this type of therapy and toward more cost-effective prevention measures. An alternative to antibiotics was investigated by several researchers in order to find an effective approach for management of bovine mastitis. Nanoparticles, vaccines, bacteriophages and cytokines are some examples of valid substitutes to antibiotics to control bovine mastitis. Modern molecular biological technologies provide excellent opportunities for studying the epidemiological and pathogenicity characteristics of bacteria, which may aid in the development of particular mastitis management measures for dairy herds. Studies on the host response and the link between somatic cell count and mastitis susceptibility provide opportunities for dairy cow genetic improvement.

Biotechnological approaches to mastitis prevention are still in the early stages of development, however there are a slew of issues. In experimental trials, many immunomodulation approaches for the prevention of mastitis have showed promised results, but the evidence is insufficient to justify commercial applications. Improvements in dairy cattle diet, housing, and environment are still critical in preventing mastitis, especially during the most vulnerable period after parturition.

New milking methods, such as robotic milking, may improve milking hygiene and udder health [16]. Fungi are typical pollutants in dairy products, and they thrive in this environment. They cause apparent and non-visual flaws including off-odour and -flavour, as well as major food waste and losses, as well as severe economic losses. Good manufacturing and hygiene procedures, air filtering, and decontamination systems are examples of prevention measures, while inactivation treatments, temperature control, and other control methods are examples of control methods.

Conclusions

In this study, colonies from the genera Fusarium, Penicillium, Cladosporium and Aspergillus were identified in the cows of the experimental group, but yeast colonies were also present in a relatively large number. During the hot season, due to thermal stress, the incidence of fungal mastitis was higher.

The treatment of fungal mastitis is challenging, as many of these fungi do not respond to antibiotics, using some of the antibiotics such as tetracycline as an energy source. For this reason, most cases of mastitis remain incurable and a source of infection for other animals in the herd.

The control of mycotic and bacterial mastitis should be based mainly on preventive methods, effective implementation of control measures such as correct handling and adequate hygiene during milking being essential.

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