

BEYOND ANTIBIOTICS: NATURAL ALTERNATIVES IN THE FIGHT AGAINST BOVINE MASTITIS

https://doi.org/10.56238/sevened2024.037-118

Leonardo Bindelli Verly¹, Ana Carla Rangel Rosa², Tamiris da Silva Gumiere³, João Victor Andrade⁴, Adriely Valerio de Macêdo⁵, Isabelle Lucas Braga Perin⁶, Maria Eduarda and Macedo⁷, Cecília Fernandes Patta Muller Marques⁸, Gilvana de Oliveira Costa⁹, Híllary Ozorio Gobeti Caprini¹⁰ and Mário Ferreira Conceição Santos¹¹.

ABSTRACT

Bovine mastitis is one of the main diseases that affect dairy farming, resulting in economic losses and impacts on animal health and milk quality. Conventional antibiotic treatment faces increasing challenges due to antimicrobial resistance, driving the search for natural and sustainable alternatives, such as the use of herbal medicines and essential oils from medicinal plants. Recent studies highlight the potential of several plant extracts with antimicrobial properties. Research has demonstrated the effectiveness of plants such as Psidium cattleianum, Tagetes minuta and Psidium guajava against Staphylococcus aureus and Corynebacterium bovis. Other studies have indicated that essential oils of species such as Origanum vulgare, Zataria multiflora and Thymus vulgaris are effective in controlling mastitis, reducing bacterial and somatic cell counts, in addition to improving the clinical

¹ Undergraduate in Pharmacy Department of Pharmacy and Nutrition Federal University of Espírito Santo ² Master's student in Genetics and Breeding Graduate Program in Genetics and Breeding Federal University of Espírito Santo ³ Undergraduate student in Biological Sciences Department of Biology Federal University of Espírito Santo ⁴ Undergraduate Degree in Chemistry Department of Chemistry and Physics Federal University of Espírito Santo ⁵ Undergraduate student in Biological Sciences Department of Biology Federal University of Espírito Santo ⁶ Undergraduate student in Chemistry Department of Chemistry and Physics Federal University of Espírito Santo ⁷ Undergraduate student in Biological Sciences Department of Biology Federal University of Espírito Santo ⁸ Undergraduate student in Pharmacy Department of Pharmacy and Nutrition Federal University of Espírito Santo ⁹ Master's student in Genetics and Breeding Graduate Program in Genetics and Breeding Federal University of Espírito Santo ¹⁰ Undergraduate student in Pharmacy Department of Pharmacy and Nutrition Federal University of Espírito Santo ¹¹ Professor Department of Chemistry and Physics Federal University of Espírito Santo



conditions of the animals. Experiments carried out with animal models and dairy cows have shown promising results. Essential oils, applied intramammary or topically, have demonstrated antimicrobial, immunomodulatory and anti-inflammatory effects, without compromising milk quality or causing significant adverse effects. The use of natural alternatives, such as herbal medicines and essential oils, can reduce dependence on antibiotics, minimize risks of chemical residues in milk, and provide a more sustainable approach to mastitis management. The combination of these strategies with hygienic management practices and early diagnosis is essential for effective and integrated disease control, benefiting animal health and milk production.

Keywords: Antimicrobial resistance, Veterinary phytotherapy, Essential oils, Sustainable management, Animal health.



INTRODUCTION

The entire milk production system, from small farms to the dairy industry, is extremely important to ensure the quality of the final product. Several factors influence the quality of milk, such as animal genetics, management, nutrition, herd health and the milking method applied. One of the great challenges of the global dairy sector is the presence of mastitis in the herd, a very common disease in Brazil and in the world and that significantly affects the performance of the entire dairy system until it reaches the consumer's table (SAAB *et al.*, 2014; BELOTI, 2015).

Mastitis is the inflammation of the parenchyma of the mammary gland, which can present itself in different forms, such as acute, superacute, subacute or chronic, characterized by a series of physical and chemical changes in the milk, as well as pathological changes in the glandular tissue. Mastitis can also be classified according to the clinical condition that the patient presents, into clinical and subclinical mastitis, and according to the mode of contagion and causative pathogens, into contagious and environmental (RADOSTITS, 2000; SEW; LEE; COSTA, 2012). The disease can be caused by stress, trauma to the mammary gland, infection by microorganisms such as fungi, yeasts, viruses and bacteria, these being the most frequent agents. The most important changes observed in milk are discoloration, the appearance of clots and the presence of a large number of defense cells (leukocytes) (LOPES; L; RONDA, 2013; COELHO *et al.*, 2016).

The presence of this disease in the herd ends up compromising the quality of the milk, putting at risk the safety of its handlers and the final consumer, which makes mastitis the most important disease among the pathological conditions that plague the dairy herd around the world, since this disease generates great losses in the economic sphere for both the producer and the industry, in addition to public health problems (MASSOTE *et al.*, 2019).

HEALTH AND ECONOMIC IMPACT

Mastitis has a great prominence in the international context due to the high cost of its treatment, and is also considered the disease that most affects the dairy sector (SANTOS *et al.*, 2017). According to Peres Neto (2011), approximately 38% of the mortality of the dairy cattle herd is caused by mastitis. Every year, on average, 1 in 3 dairy cows shows signs of apparent inflammation in the udder. Among this number, 7% are discarded and 1% die as a result of the disease. Another important aspect is that more than 25% of the



economic losses resulting from diseases in the dairy herd are influenced by mastitis (PERES NETO, 2011).

In addition to the economic aspect of the activity, the dairy segment plays a very important social role for the country. The dairy sector is responsible for the manufacture of milk and dairy products, intended to meet the needs of thousands of rural families and for the generation of numerous direct and indirect jobs (DEMEU, 2009). According to Maliszewski (2020), Brazil is an important player on the world stage, reaching the fourth position among the countries with the highest milk production. Even with all the country's growth in this area, there is still a great concern about productivity, milk quality, and producers' income (MALISZEWSKI, 2020).

According to Lopes, Manzi and Langoni (2018), in Brazil the economic impact caused by clinical mastitis was estimated at around R\$0.1090 to R\$0.5985/kg of milk for average annual frequencies of 1 and 15% of mastitis (LOPES; MANZI; LANGONI, 2018). Bovine mastitis causes several losses in all sectors of milk and dairy production. The economic repercussions range from expenses with medicines and specialized services, to the disposal of the positive animal and the already contaminated milk. This is because, in addition to being a risk to public health as a result of the elimination of pathogens that cause zoonoses and toxins produced by milk microorganisms, milk has a drop in quality. Of all the losses mentioned, the disposal of milk, either because an animal is complying with a grace period for the use of antibiotics, or because of the poor quality related to its chemical compounds, is the largest in the production chain, second only to the disposal of animals (MAIOCHI; RODRIGUES; WOSIACKI, 2019).

Another aspect to be considered is the concern with public health, since several microorganisms present in the milk of contaminated animals are capable of causing foodborne infections or toxinfections, as a result of the production of toxins, which are not inactivated by pasteurization or boiling methods (OLIVEIRA *et al.*, 2016).

CAUSES AND SYMPTOMS OF MASTITIS CAUSATIVE AGENTS

Bovine mastitis is a medical condition of great importance in dairy farming, which affects not only the health of the animals, but also the quality and quantity of the milk produced. Characterized by inflammation of the mammary gland, mastitis can be triggered by a variety of pathogens, including bacteria, fungi, viruses, and, in rare cases, algae (TOZZETTI *et al.*, 2008; SILVA; MOTA, 2009). These causative agents differ in their modes of transmission, virulence, and impact on milk production, which makes a comprehensive



understanding of each essential for the development of effective prevention and control strategies (ELHADIDY; ZAHRAN, 2014).

Bacteria are by far the most common pathogens associated with bovine mastitis, accounting for the majority of clinical and subclinical cases of the disease. They can be classified into two major groups: contagious agents and environmental agents. Contagious agents are mainly spread during milking and through direct contact between cows. Pathogens such as Staphylococcus aureus, Streptococcus agalactiae and Mycoplasma spp. are significant examples of this group. Staphylococcus aureus is particularly problematic due to its ability to adhere to the mammary duct epithelium and form biofilms, which makes it difficult for the animal's immune system and antibiotic treatment to eliminate it (KHAZANDI et al., 2018). In addition, S. aureus often has resistance to multiple drugs, further complicating treatment (FERREIRA, 2020; RUEGG, 2021). S. agalactiae is another common contagious pathogen that is usually limited to the mammary gland, facilitating its spread during milking. This microorganism is one of the main causes of chronic subclinical mastitis, characterized by persistent inflammation that may go unnoticed until significant damage has occurred (ANDREIS; PERRETEN; SCHWENDENER, 2017; KHAZANDI et al., 2018). Mycoplasma spp., especially M. bovis, are less common pathogens but cause severe mastitis that is refractory to treatments. Due to the absence of a cell wall, Mycoplasma is resistant to many conventional antibiotics, making eradication of this pathogen a challenge (NICHOLAS; FOX; LYSNYANSKY, 2016).

Figure 1 - Characteristic staining of colonies of mastitis-causing microorganisms inoculated into chromogenic culture media triplate: 1 - Streptococcus uberes; 2- Streptococcus agalactiae/dysgalactiae; 3 - Enterococcus spp.; 4 - Lactococcus spp.; 5 - Staphylococcus aureus; 6 - Klebsiella spp./Enterobacter spp.; 7 - Prototheca spp./Leaven; 8 - Escherichia coli.



Source: GRANJA (2020).

In addition to contagious pathogens, there are environmental agents that are found in the environment around cows, such as soil, feces, and water. Infection usually occurs when these microorganisms enter the teat canal, especially during or shortly after milking when the teat sphincter is open. Among the environmental pathogens, *Escherichia coli* and *Klebsiella* spp. are particularly remarkable. *E. coli* is an opportunistic environmental pathogen that causes acute clinical mastitis, often resulting in severe inflammation and severe systemic symptoms. The adaptation of *E. coli* to the bovine mammary gland is a key factor in its pathogenicity (KLAAS; ZADOKS, 2018; MURINDA *et al.*, 2019). *Klebsiella* spp., in turn, are associated with humid and dirty environments and cause severe acute mastitis. These bacteria are often resistant to many commonly used antibiotics, complicating infection management (MURINDA, 2019). Another significant environmental pathogen is *Streptococcus uberis*, which can cause both clinical and subclinical mastitis. This microorganism is prevalent in humid environments rich in organic matter (ALVAREZ-URIA *et al.*, 2018; CHENG *et al.*, 2020).

Although less common, fungal infections are becoming increasingly relevant, especially in herds where antibiotics are frequently used, which can alter the natural microbial flora of the mammary gland and allow fungi to proliferate. *Candida* spp. and *Aspergillus* spp. are the main fungi involved (DUBIE *et al.*, 2015). Yeasts of the genus *Candida* can cause both subclinical and clinical mastitis, often associated with immunosuppression or long-term use of antibiotics (DUBIE *et al.*, 2015; MOUSA; ELMONIR; ABDEEN, 2016). Fungi of the genus *Aspergillus* are responsible for rarer infections, which usually occur in cases of contamination of milk or milking equipment (ABD EL-RAZIK *et al.*, 2011).

Viruses, although less often associated with bovine mastitis, can play a significant role in certain conditions. *Bovine herpesvirus* (BHV) and Bovine Viral Diarrhea Virus (BVDV) are notable examples. *Bovine herpesvirus* can cause mastitis as part of a systemic infection, although it is best known for its association with respiratory and reproductive diseases (ALTUN; ÖZDEMIR; SAĞLAM, 2019). The Bovine Viral Diarrhea Virus can also affect the mammary gland, leading to inflammation and changes in milk quality, even if its main clinical presentation is systemic (BRODERSEN, 2014).

Algal infections are extremely rare, but they can occur in regions with contaminated water problems. *Prototheca* algae is the main cause, leading to chronic mastitis that is often refractory to treatment. This condition is especially difficult to treat due to algae's resistance to conventional mastitis control methods (JAGIELSKI *et al.*, 2019; KANO, 2020).



CLINICAL SIGNS AND DIAGNOSIS

Bovine mastitis can be identified by changes in the udder, such as swelling, warmth, redness, hardness, or pain when it is clinical mastitis. Other indicators include abnormalities in the milk, such as watery appearance, presence of flakes or clots (ACOSTA *et al.*, 2016). In cases of subclinical mastitis, no signs of udder infection or changes in milk are visible.

The most evident symptoms of clinical mastitis include moderate to severe swelling of the udder, which also presents excessive heat to the touch and reddish color (AMORIM; SANTANA, 2021). The udder can also cause discomfort to the cow when handled (FONSECA, 2021). In severe situations, the cow's body temperature may increase and the milk produced may have a watery appearance, as well as flakes, clots, pus or even blood (LANGONI *et al.*, 2017).



Figure 2 - Detection of hardened, swollen and painful parts of the udder during palpation.

Fonte: OLIVEIRA et al. (2015).

Other symptoms associated with mastitis may include reduced milk production, lack of appetite, sunken eyes, decreased mobility (due to udder pain or general malaise), and signs of diarrhea and dehydration (AMORIM; SANTANA, 2021). In acute cases of clinical mastitis, the cow may appear extremely debilitated. In contrast, subclinical mastitis may not present with immediately obvious symptoms, other than an elevated somatic cell count in the milk (ACOSTA *et al.*, 2016).

An early diagnosis of clinical mastitis is crucial to prevent losses in milk production and enable effective treatment from the beginning of the disease, in addition to avoiding



dissemination to other animals and alteration of milk properties (BORGES *et al.*, 2020). Common diagnostic methods include visual inspection of the animal's udder and analysis of milk characteristics, such as the presence of lumps during milking (SANTOS *et al.*, 2017).



Figure 3 - Milk with changes in consistency and color.

Fonte: Oliveira et al. (2015).

Animals with a history of significant recurrence of infections should be isolated to avoid transmission to other healthy animals. Those who are being treated for the first time must strictly follow the waiting periods of the medications used (ACOSTA *et al.*, 2016). Effective strategies for mastitis control should include a preventive program that identifies the pathogens involved and prevents the development of antimicrobial resistance, a serious threat to human and animal health (SILVA; MOTA, 2019).

Identifying the causative agent of the infection is essential to determine the appropriate treatment of mastitis, as different pathogens require different management approaches. This allows dairy farmers to work together with veterinarians to develop personalized disease control strategies that adapt to the specific needs of their dairy operation (FONSECA, 2021).

Treatment of mastitis usually involves the use of antibiotics, although in resistant cases, other therapeutic approaches may be necessary to combat chronic infections (RODRIGUES *et al.*, 2018). Effective mastitis management is not only one of the biggest



costs for the dairy industry, but also a crucial factor for the well-being of dairy cows (AMORIM; SANTANA, 2021).

CONVENTIONAL TREATMENT WITH ANTIMICROBIALS COMMON ANTIMICROBIALS

Antimicrobial therapy is widely used to prevent and control mastitis. However, even with the best antimicrobial treatments available, bacterial cure failures are frequent, especially in the case of mastitis caused by *S. aureus* and antimicrobial resistance. In addition, antimicrobial resistance in bacteria constitutes a danger to public health, and the long-term use of antimicrobials is seen as an important contributing factor to this resistance. Several strains isolated from mastitis cases have demonstrated resistance to multiple antimicrobials, such as penicillin-G, gentamicin, streptomycin, ampicillin, ciprofloxacin, and oxytetracycline (CHANDRASEKARAN *et al.*, 2014). To achieve effective antibacterial therapy against mastitis, it is necessary for the active drug to achieve and maintain concentrations above the minimum necessary to inhibit infection at the affected site for the time required to suppress the production and release of toxins by the pathogen responsible. Several elements can interfere with this process, including pathological changes in the glandular tissue of the mammary gland, bacterial factors associated with mastitis, inadequate selection and overuse of antibiotics, and also the way medications are administered (HOSSAIN *et al.*, 2017).

Antibiotic therapy for mastitis should be targeted to achieve therapeutic efficacy and economic advantages, both in terms of increasing productivity and reducing sources of infection. Acute and hyperacute cases of mastitis can be managed with antibiotics and always require additional therapeutic support (including fluid administration, electrolyte replacement, and nonsteroidal anti-inflammatory agents). In the case of cows in the dry period, the most effective treatment for subclinical mastitis involves the intramammary application of long-acting antibiotics, combined with drying of the mammary glands (BENEDETTE *et al.*, 2008). However, due to the type of microorganism involved, location of the infected sites, degree of mammary gland hardening, duration of infection, and other undefined factors, it is difficult to quantify the clinical efficacy of an antimicrobial (TOZETTI *et al.*, 2008).

An analysis of the antimicrobial activity of several antibiotics was conducted against S. *aureus* samples obtained from 63 cows. Drugs tested included: ampicillin, cephalexin, ciprofloxacin, streptomycin, gentamicin, norfloxacin, and tetracycline. The results revealed that more than half of the agents tested demonstrated sensitivity, with efficacy exceeding



50%. Among the antibiotics evaluated, those that stood out for their efficacy were gentamicin, cephalexin, and ciprofloxacin, with a success rate of 100%. Then, norfloxacin showed an efficacy of 94.6%. On the other hand, the least effective antimicrobials were tetracycline and ampicillin, with resistance rates of 10.82% and 13.51%, respectively (SAEKI *et al.*, 2011). Another analysis was conducted using the Disk Diffusion procedure with the commercial strains of *S. aureus*. A total of 16 antimicrobial agents were examined: Florfenicol 30 µg, Enrofloxacin 5 µg, Gentamicin 10 µg, Amikacin 30 µg, Tetracycline 30 µg, Norfloxacin 10 µg, Cephalexin 30 µg, Cephalexin 30 µg, Gentamicin 30 µg, Neomycin 30 µg, Amoxicillin 30 µg, Ampicillin + Sulbactam 10/10 µg, Cefoxitin 30 µg, Bacitracin 10 µg, Ampicillin 10 µg. Total resistance to the drugs Ampicillin and Penicillin was observed. Cefoxitin and Bacitracin showed reduced efficacy, while the others proved to be effective in combating the bacteria responsible for mastitis (SILVA *et al.*, 2022).

With the emergence of evidence of drug-resistant strains, it becomes evident that antibiotics will no longer be effective in combating mastitis. This is a consequence of decades of antimicrobial use and abuse in both human and veterinary medical practice. As a result, there is international attention focused on the search for alternative options for the treatment of bacterial diseases (LI *et al.*, 2023).

CHALLENGES AND LIMITATIONS

The overuse of antibiotics in dairy herds has increased, leading to microbial resistance and making it difficult to treat diseases (ALVES *et al.*, 2020). In relation to *S. aureus*, durability varies among herds due to its ability to develop immunity, being one of the pathogens that cause mastitis with a significant economic impact (COSTA *et al.*, 2013).

Streptococcus agalactiae is a bacterium commonly found in milk and has become a major obstacle for farms by causing persistent and chronic infections. The presence of antibiotic resistance genes in this bacterium increases concern about the effective treatment of infections (FONSECA, 2021).

Therefore, from a hygienic point of view, milk should be pleasant, clean, fresh and safe (LEITE JÚNIOR *et al.*, 2011). Antibiotic residues in milk pose health risks to consumers and cause technological problems in the dairy industry, negatively affecting fermentation processes during the production of derived products (FERREIRA *et al.*, 2014). Milk may contain residues of substances such as antibiotics, disinfectants, and pesticides that are administered to animals or used on the farm. Antibiotics can be detected in milk after being administered intramammary, intramuscularly, intrauterinely, orally or subcutaneously (LEITE JÚNIOR *et al.*, 2011).



NATURAL ALTERNATIVES

PHYTOTHERAPY AND MEDICINAL PLANTS

The use of herbal medicines can be a more accessible and natural alternative in the fight against bovine mastitis, since the plant source for this treatment is often easy to access and low cost, providing the producer with an alternative for the treatment of this disease (DANTAS *et al.*, 2010). Because this disease is commonly caused by bacteria, there is the use of natural products that have antimicrobial activity, making the need for scientific research in this area increasingly greater (SPERANDIO *et al.*, 2019).

A study conducted by Krummenauer, Ponzilacqua, and Zani (2019) investigated the antimicrobial activity of Brazilian medicinal plant extracts against bacterial strains of *S. aureus* and *Corynebacterium bovis* isolated from cases of bovine mastitis. Among the extracts tested, decocts of *Psidium cattleianum*, *Tagetes minuta* and *Psidium guajava* were observed to demonstrate effective activity against both bacterial species. In addition, the extracts of *Polygonum hydropiperoides*, *Casearia sylvestris*, *Achyrocline satureioides*, and *Bidens pilosa* exhibited specific antimicrobial activity against *S. aureus*, but not against *C. bovis*. On the other hand, the extracts of *Allium sativum* and *Matricaria chamomilla* did not show antimicrobial activity against the strains analyzed. The results of this study highlight the potential of medicinal plants from the Brazilian flora in the treatment of bovine mastitis, offering new perspectives for the development of effective and selective active ingredients against specific pathogenic bacteria, especially *S. aureus* (KRUMMENAUER; PONZILACQUA; ZANI, 2019).

The use of natural products in the treatment of mastitis has been an interesting alternative, since most bacteria have a resistance against the drugs already used, making treatment difficult (AUSTREGESILO-FILHO *et al.*, 2023). Based on the positive results, and the great potential of several plant species, the field of veterinary phytotherapy can benefit greatly from these studies, offering a viable alternative to the antibiotics that are generally used (BEZERRA *et al.*, 2009).

CASE STUDIES AND RESULTS

PRACTICAL EXAMPLES: IN VIVO STUDIES WITH PROMISING RESULTS

The study conducted by Montironi *et al.* (2019) tested the efficacy of *Minthostachys verticillata essential oil* in the treatment of mastitis caused by the pathogenic agent *Enterococcus faecium* through a murine model. The immunomodulatory and protective effects of the EO of *M. verticillata,* at different concentrations, were evaluated against macrophage phagocytosis analysis, reactive oxygen species (ROS) production,

polymorphonuclear neutrophil (PMNs) infiltration, cytokine mRNA expression (IL-1 β , TNF- α and IL-10) and bacterial count in the mammary glands of mice that received intramammary injections of *E. faecium* to induce mastitis. It was observed that the intramammary application of EO at a concentration of 3.6mg/ml activated the phagocytosis mechanisms of macrophages, inducing the production of ROS, in addition to reducing the infiltration of PMNs and the expression of proinflammatory cytokines (IL-1 β and TNF- α). Increased production of IL-10, an anti-inflammatory cytokine, has also been reported. In addition, a decrease in bacterial load was observed compared to the control groups, which suggests that the immunomodulatory effect of M. *verticillata* EO contributes efficiently against the pathogen that causes mastitis (MONTIRONI *et al.*, 2019).

A study conducted in South Korea evaluated the therapeutic effect of oregano essential oil (Origanum vulgare) in a group of 18 dairy cows diagnosed with subclinical mastitis caused by S. aureus and E. coli. The cows were separated into 4 groups -Negative Control Group (CON), which was treated only with saline solution; Positive Control Group (GEN), treated with gentamicin ointment (10g/tube); OEO-1 group, treated with a dose of 0.9ml of oregano OE ointment, twice a day for three days, and finally, the OEO-2 group, treated with twice the dose of OEO-1 group EO for the same period of time. Before and after the stipulated period of time, the physical conditions of the udder, the Somatic Cell Count (SCC) and the leukocyte count (WBC) and the presence of bacteria (S. aureus and E. colli) were evaluated. As a result, a significant improvement in udder physical conditions was observed in the groups treated with gentamicin and EO. A considerable reduction in SCC and leukocyte count was also reported compared to the control group. The presence of S. aureus and E. colli was not detected in the milk of the groups treated with OEO. The results of the research indicate that oregano essential oil is effective in the treatment of bovine subclinical mastitis, as it not only reduced somatic cell count and leukocyte count, but also eliminated the pathogens that cause the infection. In addition, treatment with EO improved the physical conditions of the udder without causing significant damage, making it a promising and natural alternative to antibiotics (CHO et al., 2015).

Harati *et al.* (2022) evaluated in Iran the efficacy of *Zataria multiflora essential oil* in the treatment of subclinical bovine mastitis caused by *S. aureus*. The study was carried out with 40 dairy cows that were separated into two groups – Control Group, which was treated with gentamicin and OEZM Group, which was treated with an intramammary ointment containing the essential oil of *Z. multiflora* at a concentration of 10% (v/v). Both groups were treated twice daily for the three-day period. Milk and blood samples were collected before treatment (T0) and at regular intervals after the start of treatment (T24, T48 and T72 hours)



for the analyses of Somatic Cell Count CCS, bacterial count, milk composition parameters and hematological and biochemical parameters. The results demonstrated that *Z. multiflora* EO was effective in reducing bacterial count and SCC, without altering milk composition parameters or causing systemic adverse effects, making it a promising alternative for the treatment of subclinical mastitis (HARATI *et al.*, 2022).

In the study conducted in Lebanon by Abboud *et al.* (2015), the efficacy of thyme (Thymus vulgaris) and lavender (Lavandula angustifolia) essential oils against pathogens that cause bovine mastitis (S. aureus and Streptococcus spp.) was evaluated. The experiment was conducted on four farms, where dairy cows were examined weekly for five months for the presence of clinical mastitis using the California Mastitis Test (CMT). Farm 1 had 5 positive cows for mastitis, while Farm 2, 3 and 4 had 6, 4 and 7 diagnosed cows, respectively. The treatment differed in each farm, with Farm 1 treated with intramammary (IM) injection of 10% thyme EO in methanol, Farm 2 with IM of 10% lavender EO in methanol, Farm 3 IM with the combination of the two essential oils at 10% methanol and Farm 4, in which 4 cows received external application of thyme EO in petroleum jelly and 3 cows received external application of the OE of lavender in petroleum jelly. All treatments were performed for 4 consecutive days and there was clinical evaluation and milk collection to evaluate the parameters of its composition and bacterial count during all days. The results showed that both application methods (intramammary and external) showed a significant reduction in bacterial count and an improvement in the clinical conditions of the affected cows, without significant adverse effects. Farm 1 obtained the best result, demonstrating the highest efficiency of thyme EO compared to lavender in combating bovine mastitis (ABBOUD et al., 2015).

FINAL CONSIDERATIONS

Therefore, bovine mastitis represents a major challenge for dairy farming, impacting both the health of the animals and the quality and quantity of milk produced. The diversity of causative agents requires a comprehensive approach to diagnosis, treatment, and prevention. While antibiotics are widely used, growing antimicrobial resistance and the adverse effects associated with their long-term use highlight the need for more sustainable and effective alternatives.

Recent studies suggest that treatments based on plant essential oils may offer a promising solution, with several extracts showing significant antimicrobial efficacy against common mastitis pathogens. In addition, the use of herbal medicines can reduce



dependence on antibiotics and reduce the risks of chemical residues in milk, benefiting both animal and human health.

Thus, the implementation of integrated strategies, combining hygienic management practices, early diagnosis, thoughtful use of antibiotics and adoption of natural treatments, is essential to control bovine mastitis in an effective and sustainable way.



REFERENCES

- 1. Abbood, M., et al. (2015). In vitro and in vivo antimicrobial activity of two essential oils Thymus vulgaris and Lavandula angustifolia against bovine Staphylococcus and Streptococcus mastitis pathogen. Middle East Journal of Agriculture Research, 4(4), 975-983.
- 2. Abd El-Razik, K. A., et al. (2011). New approach in diagnosis and treatment of Bovine Mycotic Mastitis in Egypt. African Journal of Microbiology Research, 5(31), 5725-5732.
- 3. Acosta, A. C., et al. (2016). Mastites em ruminantes no Brasil. Pesquisa Veterinária Brasileira, 36(7), 565-573.
- 4. Altun, S., Özdemir, S., & Sağlam, Y. S. (2019). The presence and prevalence of Bovine Parainfluenza 3 (BPIV-3), Bovine Papillomaviruses (BPV), Bovine Herpesvirus 1 (BHV-1) in subclinical mastitis in cattle. Kocatepe Veterinary Journal, 12(2), 135-143.
- 5. Álvarez-Uria, G., et al. (2018). Global forecast of antimicrobial resistance in invasive isolates of Escherichia coli and Klebsiella pneumoniae. International Journal of Infectious Diseases, 68, 50-53.
- 6. Alves, B., et al. (2020). Sensibilidade de Staphylococcus aureus aos antimicrobianos usados no tratamento da mastite bovina: Revisão. Pubvet, 14(4), 141.
- 7. Amorim, A. V., & Santana, D. A. (2021). Mastite bovina: Reflexões sobre controle e prevenção. Scientia Generalis, 2(2).
- 8. Andreis, S. N., Perreten, V., & Schwendener, S. (2017). Novel β-Lactamase blaARL in Staphylococcus arlettae. MSphere, 2(3).
- 9. Austregesilo-Filho, P., et al. (2023). Atividade antimicrobiana do extrato de Melaleuca leucadendra (Myrtaceae) em bactérias causadoras da mastite bovina. Peer Review, 5(16), 115-128.
- 10. Beloti, V. (2015). Leite: Obtenção, inspeção e qualidade. Londrina: Editora Planta.
- 11. Benedette, M. F., et al. (2008). Mastite bovina. Revista Científica Eletrônica de Medicina Veterinária, 11.
- 12. Bezerra, D. A. C., et al. (2009). Atividade biológica da jurema-preta (Mimosa tenuiflora (Wild) Poir.) sobre Staphylococcus aureus isolado de casos de mastite bovina. Revista Brasileira de Farmacognosia, 19(4), 814-817.
- 13. Borges, J. L., et al. (2020). Uso do núcleo homeopático anti mastite no controle de mastite em vacas leiteiras: Relato de caso. Revista Thêma et Scientia, 10(1).
- 14. Brodersen, B. W. (2014). Bovine viral diarrhea virus infections: Manifestations of infection and recent advances in understanding pathogenesis and control. Veterinary Pathology, 51(2), 453-464.
- 15. Chandrasekaran, D., et al. (2014). Pattern of antibiotic resistant mastitis in dairy cows. Veterinary World, 7(6).



- 16. Cheng, J., et al. (2020). Klebsiella pneumoniae isolated from bovine mastitis is cytopathogenic for bovine mammary epithelial cells. Journal of Dairy Science, 103(4), 3493-3504.
- 17. Cho, B., et al. (2015). Therapeutic effect of oregano essential oil on subclinical bovine mastitis caused by Staphylococcus aureus and Escherichia coli. Korean Journal of Veterinary Research, 55(4), 253-257.
- 18. Coelho, K.O., et al. (2016). Níveis de células somáticas sobre o perfil físico-químico do leite em pó integral. Ciência Animal Brasileira, 17(4), 534-539.
- 19. Coser, S. M., Lopes, M. A., & Costa, G. M. da. (2012). Mastite bovina: Controle e prevenção. Boletim Técnico, UFLA, Lavras MG, 93, 1-30.
- 20. Costa, G. M. da, et al. (2013). Resistência a antimicrobianos em Staphylococcus aureus isolados de mastite em bovinos leiteiros de Minas Gerais, Brasil. Arquivo do Instituto Biológico, 80(3), 297-302.
- 21. Dantas, S. A. F., et al. (2010). Avaliação de plantas medicinais no combate a mastite bovina. HOLOS, 4, 96-101.
- 22. Deméu, F. A. (2009). Simulação do impacto econômico da mastite em rebanhos bovinos leiteiros (Dissertação de Mestrado em Ciências Veterinárias). Universidade Federal de Lavras, Lavras.
- 23. Dubie, T., et al. (2015). An insight review on the role of fungi in mastitis of dairy animals and its economical importance. The Journal of Veterinary Science, 116, 440-445.
- 24. Elhadidy, M., & Zahran, E. (2014). Biofilm mediates Enterococcus faecalis adhesion, invasion and survival into bovine mammary epithelial cells. Letters in Applied Microbiology, 58(3), 248-254.
- 25. Ferreira, A. R. P., et al. (2014). Resíduos de antibióticos em leite in natura utilizado para processamento em laticínio localizado no município de Teresina–Piauí. Acta Tecnológica, 9(1), 9-12.
- 26. Ferreira, E. M. (2020). Fatores de patogenicidade de Staphylococcus spp. em leite de vacas com tratamento não convencional da mastite (Tese de Doutorado). Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, SP, Brasil.
- 27. Fonseca, M. E. da. (2021). Mastite bovina: Revisão. Pubvet, 15(2).
- 28. Granja, B. de M. (2020). Avaliação de meios de cultura cromogênicos para identificação rápida de microrganismos causadores de mastite bovina (Tese de Doutorado). Universidade de São Paulo, São Paulo, SP, Brasil.
- 29. Harati, H., et al. (2022). Efficacy of Zataria multiflora essential oil for treatment of Staphylococcus aureus detected by polymerase chain reaction in lactating dairy cows with subclinical mastitis. Iranian Veterinary Journal, 18(1), 34-45.



- 30. Hossain, M. K., et al. (2017). Bovine mastitis and its therapeutic strategy doing antibiotic sensitivity test. Austin Journal of Veterinary Science & Animal Husbandry, 4(1), 1030.
- 31. Jagielski, T., et al. (2019). Prevalence of Prototheca spp. on dairy farms in Poland a cross-country study. Microbial Biotechnology, 12(3), 556-566.
- 32. Kano, R. (2020). Emergence of fungal-like organisms: Prototheca. Mycopathologia, 185(5), 747-754.
- 33. Khazandi, M., et al. (2018). Genomic characterization of coagulase-negative staphylococci including methicillin-resistant Staphylococcus sciuri causing bovine mastitis. Veterinary Microbiology, 219, 17-22.
- 34. Klaas, I. C., & Zadoks, R. N. (2018). An update on environmental mastitis: Challenging perceptions. Transboundary and Emerging Diseases, 65, 166-185.
- 35. Krummenauer, A., Ponzilacqua, B., & Zani, J. L. (2019). Atividade antibacteriana de extratos naturais sobre agentes causadores de mastite bovina. BIOFARM Journal of Biology & Pharmacy and Agricultural Management, 15(4), 436-449.
- 36. Langoni, H., et al. (2017). Considerações sobre o tratamento das mastites. Pesquisa Veterinária Brasileira, 37(11).
- 37. Leite Júnior, B. R. de C., et al. (2011). Aplicação das boas práticas agropecuárias no processo de ordenha em uma propriedade rural do município de Rio Pomba, Minas Gerais. Revista do Instituto de Laticínios Cândido Tostes, 66(380), 31-39.
- 38. Li, X., et al. (2023). Alternatives to antibiotics for treatment of mastitis in dairy cows. Frontiers in Veterinary Science, 10.
- Lopes, L. O., Lacerda, M. S. de, & Ronda, J. B. (2013). Uso de antibióticos na cura e controle de mastite clínica e subclínica causada por principais microrganismos contagiosos em bovinos leiteiros: revisão de literatura. Revista Científica Eletrônica de Medicina Veterinária, 21, 1-15.
- 40. Lopes, B. C., Manzi, M. de P., & Langoni, H. (2018). Etiologia das mastites: Pesquisa de micro-organismos da classe Mollicutes. Veterinária e Zootecnia, 25(2), 173-179.
- 41. Maiochi, R. R., Rodrigues, R. G. A., & Wosiacki, S. R. (2019). Principais métodos de detecção de mastites clínicas e subclínicas de bovinos. Enciclopédia Biosfera, Centro Científico Conhecer Goiânia, 16(29), 1237–1251.
- 42. Maliszewski, E. (2020). Dia Mundial do Leite: Os desafios da cadeia. Agrolink. Available at: https://www.agrolink.com.br/noticias/dia-mundial-do-leite--os-desafios-da-cadeia_434701.html. Accessed on: June 2, 2024.
- 43. Massote, V. P., et al. (2019). Diagnóstico e controle de mastite bovina: Uma revisão de literatura. Revista Agroveterinária do Sul de Minas, 1(1), 41–54.

Science and Connections: The Interdependence of Disciplines

Acetylsalicylic acid treatment in wastewater by electrocoagulation using aluminum electrodes: Electrical voltage effect



- 44. Montironi, I. D., et al. (2019). Minthostachys verticillata essential oil activates macrophage phagocytosis and modulates the innate immune response in a murine model of Enterococcus faecium mastitis. Research in Veterinary Science, 125, 333-344.
- 45. Mousa, W., Elmonir, W., & Abdeen, E. (2016). Molecular typing, virulence genes and potential public health implications of Candida albicans isolated from bovine milk. Japanese Journal of Veterinary Research, 64(Supplement 2), S211-S215.
- 46. Murinda, S. E., et al. (2019). Shiga toxin–producing Escherichia coli in mastitis: An international perspective. Foodborne Pathogens and Disease, 16(4), 229-243.
- 47. Nicholas, R. A. J., Fox, L. K., & Lysnansky, I. (2016). Mycoplasma mastitis in cattle: To cull or not to cull. Veterinary Journal, 216, 142-147.
- 48. Oliveira, G. C., et al. (2016). Perfil microbiológico de Streptococcus spp. como agentes causadores de mastite clínica em diversas regiões do Brasil. Revista de Educação Continuada em Medicina Veterinária e Zootecnia do CRMV-SP, 14(3), 74-74.
- 49. Oliveira, V. M., et al. (2015). Como identificar a vaca com mastite em sua propriedade: Cartilhas elaboradas conforme a metodologia e-Rural. Embrapa, Brasília, DF, Brazil.
- 50. Peres Neto, F. (2011). Mastite em vacas leiteiras revisão de literatura. Revista Científica Eletrônica de Medicina Veterinária, 16, 1-28.
- 51. Radostits, O. M., et al. (2000). Clínica Veterinária Um tratado de Doenças dos Bovinos, Suínos, Caprinos e Equinos. Guanabara Koogan, Rio de Janeiro, Brazil.
- 52. Rodrigues, T. P., et al. (2018). Mastite bovina Influência na produção, composição e rendimento industrial do leite e derivados. Arquivos de Pesquisa Animal, 1(1), 14-36.
- 53. Ruegg, P. L. (2021). What is success? A narrative review of research evaluating outcomes of antibiotics used for treatment of clinical mastitis. Frontiers in Veterinary Science, 8.
- 54. Saab, A. B., et al. (2014). Prevalência e etiologia da mastite bovina na região de Nova Tebas, Paraná. Semina: Ciências Agrárias, 35(2), 835–843.
- 55. Saeki, E. K., et al. (2011). Mastite bovina por Staphylococcus aureus: Sensibilidade às drogas antimicrobianas e ao extrato alcoólico de própolis. Acta Veterinaria Brasilica, 5(3), 284-290.
- 56. Santos, W. B. R. dos, et al. (2017). Mastite bovina: Uma revisão. Colloquium Agrariae, 13(Especial), 301-314.
- 57. Silva, A. T. F., & Mota, R. A. (2019). Mastite: Perguntas e respostas (1st ed.). EDUFRPE, Recife, Brazil.
- 58. Silva, S. G. M. da, et al. (2022). Resistance of Staphylococcus aureus and Escherichia coli to antibiotics. Research, Society and Development, 11(2).



- 59. Sperandio, J., et al. (2019). Atividade antimicrobiana e citotoxicidade in vitro do óleo essencial de Tagetes minuta L. visando à aplicação no controle da mastite bovina. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 71(4), 1251–1259.
- 60. Tozzetti, D. S., et al. (2008). Prevenção, controle e tratamento das mastites bovinas revisão de literatura. Revista Científica Eletrônica de Medicina Veterinária, 10, 1-7.