

# The effectiveness of ozone as a therapeutic alternative for treating bovine mastitis

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## ABSTRACT

**Objective:** This narrative review aimed to evaluate the effectiveness of ozone as a therapeutic alternative for bovine mastitis, considering its antimicrobial and anti-inflammatory properties in comparison with conventional antibiotic treatments.

**Design/methodology/approach:** A review of the literature published between 2000 and 2025 was conducted, encompassing experimental assays, field studies, theses, reviews, and scientific articles. The purpose of this review was to analyze the doses used, the routes of administration, and the clinical and bacteriological outcomes observed in both clinical and subclinical mastitis.

**Limitations on study/implications:** The principal limitation identified was the inconsistency among the protocols employed, particularly with regard to doses, concentrations, frequency of application, and routes of administration.

**Findings/conclusions:** The local or intramammary use of ozonotherapy has been shown in several studies to be as effective as antibiotics. Its main advantages include the absence of milk residues, low cost, and a reduction in the use of antimicrobial agents. Nevertheless, further controlled studies are required to establish standardized protocols that can be effectively implemented in veterinary practice.

**Keywords:** Bovine mastitis, ozonotherapy, veterinary medicine.

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## INTRODUCTION

Bovine mastitis is one of the most prevalent and economically significant diseases in the dairy industry worldwide (Ruegg, 2017). It is defined as an inflammation of the mammary gland, generally caused by bacterial infections, which may occur in either clinical or subclinical form, thereby affecting both the quality and quantity of the milk produced (Bradley, 2002). In addition to compromising animal welfare, mastitis causes substantial economic losses due to decreased milk production, treatment costs, disposal of contaminated milk, and the premature culling of animals from the herd (Rainard *et al.*, 2018).



Traditionally, the treatment of this condition has relied on the use of intramammary and systemic antibiotics. However, the prolonged and, in some cases, inappropriate use of these drugs has led to problems such as the development of bacterial resistance, the presence of residues in milk, and the environmental impact derived from the use of pharmacological products (Kalmus *et al.*, 2011). In light of these limitations, it is necessary to explore more sustainable, effective, and safe therapeutic alternatives for both the animal and the consumer (Pereira *et al.*, 2016). Moreover, the intramammary application of ozone leaves no residues (Prabhakaran *et al.*, 2025), eliminating the need for withdrawal periods that result in economic losses, while also offering low cost and demonstrated effectiveness in the treatment of both clinical and subclinical mastitis (Sciorsci *et al.*, 2020).

In this context, the use of ozone has emerged as a promising option. Ozone, a gas with a high oxidative capacity, possesses antimicrobial, anti-inflammatory, and regenerative properties that have been exploited in human medicine and, more recently, in veterinary medicine (Filippi *et al.*, 2012). Several studies have shown that ozonotherapy applied topically, intramammarily, or in ozonated water may reduce bacterial load, accelerate tissue recovery, and decrease the need for antibiotics in the treatment of mastitis (Ogata and Nagahata, 2000), in both its clinical and subclinical forms (Fuentes *et al.*, 2023; Machado *et al.*, 2023).

The use of ozone constitutes a viable therapeutic alternative for the treatment of bovine mastitis, owing to its potential to improve clinical outcomes, reduce the adverse effects associated with conventional treatments, and contribute to safer and more sustainable dairy production.

## CURRENT CHALLENGES

Bovine mastitis has been extensively studied because of its high prevalence in dairy herds and its complex interaction with environmental, microbiological, and physiological factors. It is a multifactorial disease that, in most cases, results from a bacterial infection, although it may also be caused by yeasts, mycoplasmas, viruses, and even trauma (Ruegg, 2017). Its development is closely associated with the ability of microorganisms to colonize the mammary parenchyma and trigger a local inflammatory response characterized by leukocyte infiltration, release of proinflammatory cytokines, tissue damage, and alterations in milk composition, which may progress clinically or subclinically depending on the pathogen involved and the animal's immunological status (Bradley, 2002).

The most common etiological agents include contagious bacteria such as *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Mycoplasma* spp., as well as environmental bacteria such as *Escherichia coli*, *Klebsiella* spp., and *Streptococcus uberis* (Rainard *et al.*, 2018). The usual route of entry is the teat canal, through which microorganisms penetrate and colonize the mammary tissue. The ability of these pathogens to evade the immune system and adhere to the mammary epithelium is crucial for establishing infection, especially in chronic cases such as those caused by *S. aureus* (Kalmus *et al.*, 2011).

From a pathophysiological perspective, the inflammatory process begins when bacteria or their products are recognized by pattern recognition receptors (PRRs), mainly Toll-like

receptors (TLRs), present in macrophages and mammary epithelial cells. This interaction activates an inflammatory cascade mediated by cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF- $\alpha$ ), which induce fever, pain, and leukocyte recruitment to the site of infection (Rainard *et al.*, 2018). Local inflammation manifests as edema, hyperemia, and pain in the mammary gland, accompanied by alterations in the color, texture, and homogeneity of the milk.

The immune system responds through the rapid migration of neutrophils to the infected tissue, where they phagocytose bacteria and release enzymes and free radicals. Although this response is essential for controlling infection, it may also damage secretory epithelial cells, leading to reduced milk production and, in prolonged cases, to fibrosis and mammary atrophy (Ruegg, 2017).

Clinically, mastitis is classified into two main forms: clinical and subclinical. Clinical mastitis is characterized by visible signs in the milk (clots, blood, pus) and in the udder (edema, redness, heat, and pain). Systemic signs such as fever, anorexia, and decreased productive performance may also be observed. Clinical mastitis may be mild, moderate, or severe, depending on the intensity of the inflammation and the type of etiological agent involved (Bradley, 2002). In contrast, subclinical mastitis presents no evident clinical signs, but involves persistent inflammation that causes alterations in milk composition, including an increase in somatic cell count (SCC), a decrease in lactose and protein content, and the presence of pathogens. It is more difficult to detect, yet it has a significant economic impact because of its silent and persistent effect on dairy production (Ruegg, 2017).

For diagnosis, several tools are available to identify the presence and type of mastitis. Among the tests most commonly used in the field is the California Mastitis Test (CMT), which detects an increase in somatic cell count (SCC) through the formation of a viscous gel. SCC values above 200,000 cells/mL generally indicate subclinical inflammation (Ruegg, 2017). In addition, microbiological culture of milk is essential to identify the etiological agent and determine its antimicrobial susceptibility profile. At present, molecular techniques such as polymerase chain reaction (PCR) have been incorporated as more sensitive and specific methods, particularly for detecting chronic infections caused by *Mycoplasma* spp. or strains of *S. aureus* (Rainard *et al.*, 2018).

The pathophysiology of bovine mastitis represents a major challenge for dairy herd health. Subclinical forms, owing to their silent nature, may go unnoticed and generate sustained production losses, whereas clinical forms may severely compromise animal health. Understanding these processes is fundamental for the development of more effective therapeutic strategies, including alternatives to conventional treatment such as ozonotherapy.

## JUSTIFICATION

Ozone (O<sub>3</sub>) is a molecule composed of three oxygen atoms, characterized by its high reactivity and oxidative capacity. Its therapeutic use is based on the controlled application of specific concentrations that induce moderate oxidative stress, capable of activating antioxidant defense mechanisms without causing cellular damage (Bocci, 2011). This

property makes it a therapeutic alternative of growing interest, especially in infectious and inflammatory contexts in which conventional treatments, such as antibiotics, have lost efficacy or promoted microbial resistance (Franzini *et al.*, 2016). Ozone exhibits a potent broad-spectrum antimicrobial action, effective against Gram-positive and Gram-negative bacteria, fungi, and viruses. Its mechanism of action is based on the oxidation of membrane lipids, proteins, and nucleic acids of microorganisms, leading to loss of cellular integrity and bacterial death (Sechi *et al.*, 2001; Zielinska *et al.*, 2020). This property is particularly relevant against persistent pathogens such as *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Escherichia coli*, as well as against mycotic agents such as *Candida* spp. Recent studies have demonstrated that the intramammary application of ozone is effective in cases of mycotic mastitis, achieving clinical and microbiological resolution without the use of conventional antifungals, thereby broadening its therapeutic spectrum beyond bacterial infections (Köker, 2024). Furthermore, ozone promotes modulation of the cellular antioxidant system. Upon reacting with unsaturated fatty acids and other biological compounds, ozone generates reactive oxygen species (ROS) and lipid oxidation products (LOPs), including hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). These molecules act as second messengers that activate intracellular pathways such as the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, which stimulates the production of endogenous antioxidant systems, including superoxide dismutase, catalase, and glutathione peroxidase, thereby strengthening the organism's capacity to limit tissue damage, preserve mammary epithelial cells, and favor recovery of milk production (Bocci, 2011; Filippi *et al.*, 2012). Another important aspect of ozone's mechanism of action is its ability to modulate the immune response. At the cellular level, ozone has been shown to stimulate the activation of macrophages, T lymphocytes, and natural killer (NK) cells, promoting the production of T helper type 1 (Th1) cytokines such as interferon gamma (IFN- $\gamma$ ) and tumor necrosis factor alpha (TNF- $\alpha$ ), while reducing immunosuppressive cytokines such as interleukin-10 (IL-10) and transforming growth factor beta (TGF- $\beta$ ). This modulation contributes to a more effective immune response against infections and to the regulation of chronic inflammatory processes. In addition, an increase in phagocytosis and the destructive capacity of neutrophils following ozone exposure has been documented (Zielinska *et al.*, 2020). In veterinary medicine, ozone may be administered in various forms, including ozonated gas, ozonated water, carrier oils such as ozonated sunflower oil, or by means of ozonated autohemotherapy. These routes allow for the effective treatment of infectious diseases such as mastitis, as well as inflammatory conditions, wounds, dermal infections, and metritis (Fuentes *et al.*, 2023). In the specific case of bovine mastitis, recent studies suggest that ozone not only reduces bacterial load, but also promotes recovery of mammary tissue by stimulating angiogenesis, improving microcirculation, and reducing local inflammation (Franzini *et al.*, 2016). The use of ozone represents a safe, effective, and environmentally friendly alternative in the treatment of infectious diseases, particularly in the context of increasing antimicrobial resistance. Unlike antibiotics, it does not generate residues in milk or meat, and its controlled application has been shown to be well tolerated by animals, with no relevant adverse effects when established protocols are followed (Filippi *et al.*, 2012).

The earliest studies in this field include the work of Ogata and Nagahata (2000), who administered intramammary gaseous ozone in cases of acute clinical mastitis using concentrations of 20-30  $\mu\text{g}/\text{mL}$  (10 mL per mammary quarter for three consecutive days). They obtained a recovery rate of 60% in quarters treated exclusively with ozone and detected no residues in milk. Complementarily, Enginler (2015) evaluated different intramammary doses and concluded that moderate concentrations (20-30  $\mu\text{g}/\text{mL}$ ) achieved a more favorable balance between therapeutic efficacy and tissue tolerance. In the field of subclinical mastitis, Fuentes *et al.* (2023) demonstrated that the application of ozone (20-40  $\mu\text{g}/\text{mL}$ , 10 mL/quarter for three days) significantly reduced somatic cell count (SCC) and improved milk quality parameters, although its efficacy was lower than that of conventional antibiotic treatments. Likewise, Argudo and Soria (2017) compared the efficacy of gaseous ozone (50 mL at 35  $\mu\text{g}/\text{mL}$ ), ozonized saline solution (50 mL at 35  $\mu\text{g}/\text{mL}$ ), and intramuscular ceftiofur (1.6 mg/kg) in mild and moderate clinical mastitis, obtaining recovery rates of 77.8%, 33.3%, and 83.3%, respectively. The authors concluded that gaseous ozone showed efficacy comparable to that of the antibiotic, whereas ozonized saline solution was less effective.

Other studies, such as that of Wakade *et al.* (2025), evaluated the combined use of gaseous ozone (25  $\mu\text{g}/\text{mL}$ , 10 mL/quarter) and antibiotics, reporting cure rates of 60% with ozone alone, 70% with antibiotics, and 100% with the combination, thus suggesting a potential synergistic effect. Sertkol *et al.* (2018) reported the clinical recovery of cows treated with intramammary ozone in Turkey, further supporting its utility under production conditions. Research has also explored alternative formulations. Raimundo *et al.* (2024) used intramammary ozonated oil (10 mL,  $\sim 1,200$  mg/L of  $\text{O}_3$ ) in cows with mild clinical mastitis, achieving clinical improvement in four of six animals and negative cultures in half of the cases. *In vitro* studies by Sechi *et al.* (2001) and Orlandin *et al.* (2021) demonstrated the potent bactericidal activity of ozonated oils against mammary pathogens such as *Staphylococcus aureus* and *Streptococcus agalactiae*. In field studies, Torrico *et al.* (2018) applied intramammary gaseous ozone (30  $\mu\text{g}/\text{mL}$ , 10 mL/quarter for three days) in 165 affected quarters, observing a 39% reduction in microbial load and absence of clinical signs in 23% of cases. Kwon *et al.* (2005) also reported positive results in the treatment of bovine mastitis with gaseous ozone, reinforcing its therapeutic action against this disease. Taken together, the literature indicates that ozonotherapy has broad potential as an alternative or adjunct in the treatment of bovine mastitis (Table 1). Its benefits include reduction of bacterial load, improvement of clinical and productive parameters, and absence of residues in milk, thereby contributing to a reduction in antibiotic use (Travagli *et al.*, 2020). However, the heterogeneity of protocols, concentrations, and routes of administration, together with the limited sample size in some trials, underscores the need to establish standardized guidelines and to conduct larger controlled clinical trials capable of confirming its efficacy and safety (Sciorsci *et al.*, 2020). Moreover, although several studies mention lower costs associated with the use of ozone instead of antibiotics for the treatment of mastitis, no studies were found in which a direct cost comparison between both treatments was performed.

**Table 1.**

Author / Year	Design / Sample	Treatment and dose	Main results	Clinical observations
Ogata & Nagahata (2000)	19 cows with acute clinical mastitis	O <sub>3</sub> gas, 10 mL/quarter, 20-30 µg/mL for 3 days (intramammary administration)	60% recovery; no milk residues detected	Better outcomes in cases caused by Streptococcus
Sechi <i>et al.</i> (2001)	In vitro study with mastitis-associated pathogens	Ozonized oil (Oleozone), dose not specified	High <i>in vitro</i> bactericidal activity	Supports the use of topical formulations
Kwon <i>et al.</i> (2005)	Clinical trial	O <sub>3</sub> gas, dose not specified (intramammary administration)	Clinical recovery observed	Protocol not described in detail
Enginler (2015)	Dose-comparison study	O <sub>3</sub> gas, 20-70 µg/mL; optimal range: 20-30 µg/mL (intramammary administration)	Moderate doses showed the best balance between efficacy and safety	High doses may irritate mammary tissue
Ablondi & Pogliacomì (2004)	1,323 cases of various conditions	Autohemotherapy + O <sub>3</sub> + local treatment	Good response in mastitis caused by <i>S. aureus</i> , <i>S. uberis</i> , among others	Not effective against coliforms or <i>S. agalactiae</i>
Maldonado <i>et al.</i> (2017)	Cows with subclinical endometritis	Ozonized water, 45 µg/mL, via intrauterine administration	Reduced inflammation; increased conception rate	Potentially extrapolable application
Argudo & Soria (2017)	54 cows with mild mastitis	O <sub>3</sub> gas, 35 µg/mL (50 mL) for 3 days <i>vs.</i> saline solution <i>vs.</i> antibiotic (intramammary administration)	O <sub>3</sub> : 77.8%; antibiotic: 83.3%; saline: 33.3%	Gaseous ozone showed efficacy comparable to that of the antibiotic
Sertkol <i>et al.</i> (2018)	Cows with clinical mastitis	O <sub>3</sub> gas, 20 mL, 60 µg/mL for 3 days (intramammary administration)	85% clinical recovery; 70% bacteriological recovery	No local irritation observed
Torrico <i>et al.</i> (2018)	165 quarters from 73 cows	O <sub>3</sub> gas, 10 mL/quarter, 30 µg/mL for 3 days (intramammary administration)	39% reduction in bacterial load; 23% of cases showed no clinical signs	Evaluated under real production conditions
Soto <i>et al.</i> (2022)	52 animals with mastitis and endometritis	Ozone <i>vs.</i> antibiotic, dose not specified; administered according to the pathology, either intramammarily or intrauterinely	Mastitis: 53.8% with ozone <i>vs.</i> 23.1% with antibiotic	O <sub>3</sub> was more effective and was also associated with a better reproductive response
Fuentes <i>et al.</i> (2023)	12 cows with subclinical mastitis (randomized study)	O <sub>3</sub> gas, 20-40 µg/mL, 10 mL/quarter for 3 days (intramammary administration)	Reduced somatic cell count (SCC); improved milk parameters; lower efficacy than antibiotics	A viable adjunct therapy that may reduce antimicrobial use
Machado <i>et al.</i> (2023)	5 cows with clinical and subclinical mastitis	Ozonized saline solution, 500 mL/quarter, plus intramammary O <sub>3</sub> at 40 µg/mL for 5 min; repeated every 24 h	Improvement within 24 h; reduced inflammation; clinical mastitis shifted to subclinical status, and subclinical cases became negative	No pain, edema, or erythema; reduction in clots
Raimundo <i>et al.</i> (2024)	6 cows (pilot study)	Ozonized oil (~1,200 mg/L) for 5 days (intramammary administration)	Clinical improvement in 4/6 cases; negative cultures in 3/6	No control group
Wakade <i>et al.</i> (2025)	30 cows with clinical mastitis	O <sub>3</sub> gas, 25 µg/mL <i>vs.</i> antibiotic <i>vs.</i> combination therapy (intramammary administration)	60% with ozone; 70% with antibiotic; 100% with the combination	Potential therapeutic synergy

## CONCLUSIONS

Bovine mastitis remains a disease of considerable economic and sanitary importance. Although antibiotics have proven effective, their use is constrained by important limitations, particularly bacterial resistance and the presence of residues in milk. The literature reviewed indicates that ozonotherapy, in its various forms, including gaseous ozone, ozonized solutions, and ozonized oils, represents a promising therapeutic alternative owing to its antimicrobial and anti-inflammatory properties, its lack of residue generation, and its lower cost. Although some findings remain variable, recent reviews consistently suggest that ozone may reduce the use of antimicrobials in cattle and contribute to food safety. Accordingly, ozonotherapy may be regarded as a viable therapeutic alternative for the treatment of both clinical and subclinical bovine mastitis. Nevertheless, greater standardization of doses, routes of administration, and treatment protocols is still required in order to consolidate its application in veterinary practice.

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