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Comparison of Intramammary Ozone Administration Doses in Dairy Cows with Clinical Mastitis

Sinem Özlem Enginler¹, Ahmet Sabuncu¹, Beren Başaran Kahraman², Ömür Koçak³, Esmâ Yıldar⁴ & Özlem Güzel⁴

ABSTRACT

Background: Mastitis is an economically important disease leading cost associated problems. The most accurate relationship between intramammary infection and somatic cell count (SCC) can be evaluated at quarter level when SCC exceeds 100.000 cells/mL. In subclinical mastitis, pathogens do not cause enough disruption in the alveolar tissue to be seen in the milk but SCC increase indicates the infected milk. Ozone (O₃) is an unstable polymerized oxygen created by the passage of air or oxygen over high energy electrodes within an ozone generator system or by ultraviolet light. The aim of this study was to compare the treatment efficacy of ozone (O₃) insufflation daily via latex free syringe in different doses (30-60-70 µg/mL) and high dose O₃ insufflation (70 µg/mL) with combination of antibiotic and only antibiotic treatment in dairy cows with clinical mastitis.

Materials, Methods & Results: A total of 32 lactating cows with clinical signs of mastitis whom had got 79 infected mammary quarters were used for the study. Mastitis was diagnosed by typical clinical symptoms of the mammary gland and general clinical symptoms of systemic infection. California mastitis test (CMT) was performed to all quarters and recorded before the treatment. Somatic cell count (SCC) of each milk sample of the infected quarters was recorded before the treatment. Microbiological examination was conducted on aseptically collected milk samples from infected quarters before the treatment. Ozone insufflation was applied daily in different doses (30, 60 and 70 µg/mL) intramammary via latex free 100 mL syringe every day for one week and only antibiotic was administered intramuscularly to the mastitic cows for 5 days and high dose ozone insufflation (70 µg/mL) with combination of antibiotic therapy after milking of the animals. On day 8; CMT and SCC were repeated to evaluate the efficacy of the treatments on the milk samples. The statistical analysis of CMT and SCC values between groups was conducted according to Paired Sample t-test and *P* value of < 0.05 was considered significant.

Discussion: In the current study, CMT scores were found to best decreased in 70 µg/mL O₃+AB combination group in post-treatment (1.63 ± 0.10) scores when compared to pre-treatment (1.21 ± 0.08) scores and the difference was found statistically significant (*P* < 0.01) between pre- and post-treatment of CMT scores. In another study, the initial CMT score was reported as 10 ± 0.97 and found to be decreased to 4.55 ± 0.83 on the 3rd day after ozone treatment. SCC values were reported as decreased gradually towards to normal range after 3 weeks ozone and antibiotic treatment in a study. SCC results are tended to be decreased in this study in line with their study after one week therapy but in this study SCC values did not reach to normal reference ranges. It may be due to the shorter treatment duration of this study when compared to their study. The most isolated bacteria was detected as *E. coli* and *Staphylococcus aureus* (n=12 for each) in the present study, *S. aureus* was also the most isolated bacteria (n = 7) in a previously published study. In conclusion, high doses ozone (60-70%) and high dose ozone (70%) in combination with antibiotic can be effectively used to treat clinical mastitis cases in dairy cattle. The best result was achieved in high dose ozone (70 µg/mL) with combination of antibiotic therapy in the cases of clinical mastitis in dairy cattle in current study.

Keywords: antibiotic, clinical mastitis, intramammary insufflation, ozone therapy.

INTRODUCTION

Mastitis can lead to cause infection independently in each mammary quarter, mostly in single quarter [11]. The most accurate relationship between intramammary infection and somatic cell count (SCC) can be evaluated at quarter level when SCC exceeds 100.000 cells/mL [13]. Besides healthy quarter's SCC is consistently quite low and usually remains below 100.000 cells/mL [7]. In subclinical mastitis, pathogens do not cause enough disruption in the alveolar tissue to be seen in the milk but SCC increase indicates the infected milk [2]. In clinical mastitis, clinical signs can be recorded as mild, moderate or severe [12]. The intramammary antibiotic administration is the most common method to treat mastitis in dairy cows [8]. However, antibiotic treatment during lactation has lower recovery rate for many mastitis pathogens [4,9,15]. Ozone (O_3) is an unstable polymerized oxygen which is created by the passage of air or oxygen over high energy electrodes within an ozone generator system or by ultraviolet light [18]. After a short period of exposure, bacteria, spores and viruses may be inactivated by ozone therapy [1]. Ozone shows its efficacy with different mechanisms including the activation of erythrocytes and immune cells and it is a disinfectant against the anaerobic bacteria [19].

The objective of this study was to compare the treatment efficacy of O_3 insufflation via latex free syringe in different doses (30-60-70 $\mu\text{g/mL}$) and high dose O_3 insufflation (70 $\mu\text{g/mL}$) with combination of antibiotic (intramuscularly) and only antibiotic treatment (intramuscularly) in dairy cows with clinical mastitis.

MATERIALS AND METHODS

Examination of the udder and selection of cows for treatment

Thirty-two lactating cows with clinical signs of mastitis whom had got 79 infected mammary quarters were used for this study. Udders of the cows were checked for redness, pain, heat, hardness and swelling by inspection. Samples from each quarter were collected and controlled for any variation in milk colour and consistency. Mastitis was diagnosed by typical clinical symptoms of the mammary gland (hardness, pain, redness, heat and swelling) and general clinical symptoms of systemic infection (watery milk, anorexia, decreased milk yield).

California Mastitis Test (CMT)

Milk samples were collected while the cows were strained in standing position after all the quarters were cleaned, they were washed with tap water. The teat end was dried and cleaned with alcohol. California mastitis test solution consisted of 3% sodium lauryl sulfate and bromocresol¹ was mixed at the same amount of milk, CMTs were scored due to no reaction; as 0, for a weak positive (+), for a distinct positive (++) , for a strong positive (+++) on collected milk samples. CMT was performed to all quarters of the cows, the infected quarters were diagnosed and recorded before the treatment. At the end of the treatment CMT was repeated on day 8 to evaluate the curative effect of the treatment regimens.

Somatic Cell Count (SCC)

Hundered mL of milk samples were collected into the sterile bottles aseptically from infected quarters and transported for analysis of SCC within 4 h to the laboratory of Faculty of Veterinary Medicine, Istanbul University. In one of the 100 mL milk samples somatic cell counts were measured using a Fossomatic 90 instrument² after 40°C heat treatment at duration of 15 min [15]. SCC of each infected quarters were recorded before and after the treatment on day 8.

Microbiological examination

For microbiological examination approximately 100 mL of milk samples were collected into the sterile bottles aseptically from infected quarters before the treatment. Milk samples were inoculated into Nutrient Broth with horse sera, onto Nutrient Agar with 7% sheep blood and MacConkey agar plates. Inoculations onto blood agar plates were duplicated for each sample and incubated both aerobic and microaerobic conditions. MacConkey agar plates were incubated aerobically, at 37°C for 24-48 h. Nutrient broths were also incubated microaerobically, at 37°C for 24-48 h. Gram staining was performed from the cultures and bacteriological methods were used for the identification [10]. The isolates were investigated for their *in vitro* susceptibility to 11 antimicrobial agents. The antibiotic susceptibility tests according to the guidelines from the Clinical and Laboratory Standards Institute [3] were performed to select the convenient antimicrobial agent. Resistance was determined by measurement of inhibition of growth around the antimicrobial disk according to the zone diameter interpretative standards of [3].

Treatment regime

Mastitic cows were randomly divided into five groups. Group I ($70 \mu\text{g/L O}_3$): 7 cows with 12 infected quarters; Group II (only antibiotic (AB), im): 9 cows with 24 infected quarters. Group III ($70 \mu\text{g/mL O}_3$ + Antibiotic im): 9 cows with 24 infected quarters. Group IV ($30 \mu\text{g/mL O}_3$): 4 cows with 10 infected quarters; Group V ($60 \mu\text{g/mL O}_3$): 3 cows with 9 infected quarters. According to the antimicrobial agent selection, antibiotic (Sefakim®) was administered daily at 2.2 mg/kg dose 24-h intervals for 5 days intramuscularly (im) to groups II and III's animals and ozone gas was prepared with

ozone therapy device (Humazona)³ (Figure 1). Ozone insufflation was applied in different doses daily intramammary via latex free 100 mL syringe every day for one week after milking of the animals. On day 8; CMT and SCC were repeated to evaluate the efficacy of the treatment regimens on the collected milk samples (Figure 2).

Statistical analysis

The statistical analysis of CMT and SCC values between groups was conducted according to Paired Sample *t*-test and *P* value of < 0.05 was considered significant.



Figure 1. Ozone therapy device; ozone gas preparation for intramammary therapy.



Figure 2. Ozone insufflation via latex free syringe to a mastitic quarter in lactating cow with mastitis.

RESULTS

In this study, the general conditions of the cows and the inflamed quarters were improved in few days after 60, 70 µg/mL and AB+70 µg/mL ozone treatment, but no clinically improvement was detected in the animals treated with in 30 µg/mL and only antibiotic. The best improvement was detected in group AB+70 µg/mL ozone according to the clinical signs and in the consistency of milk samples in this group of animals. CMT results before and after treatment regimens are shown in Table 1. The results of mean SCC before and after treatment within groups are indicated in Table 2. The isolated pathogens from milk samples of 79

infected mammary quarters from thirty two lactating cows before treatments are shown in Table 3. Antibiotic susceptibility test results are shown for 11 antimicrobial agents in Table 4 for the isolates.

According to the statistical analysis in the current study, the most efficient treatment regime was found in group III when compared to other groups (I, II, IV and V) and was found statistically significant ($P < 0.001$). The efficacy of group I and V was found significant ($P < 0.05$) when compared to group II and IV in dairy cows with clinical mastitis. None of the *S. aureus* isolates were found to be resistant to methicillin.

Table 1. California mastitis test results of groups I (70 µg/mL O₃), II (AB, im), III (70 µg/mL O₃+AB, im), IV (30 µg/mL O₃), V (60 µg/mL O₃) before and after treatment. (Turkey, March 2014).

Group	CMT pre-treatment		CMT post-treatment		P
	Mean	Std Error	Mean	Std Error	
I (70 µg/mL O ₃)	1.50	0.23	1.25	0.18	NS
II (AB, im)	2.21	0.18	1.96	0.16	NS
III (70 µg/mL O ₃ +AB, im)	1.63	0.10	1.21	0.08	$P < 0.01$
IV (30 µg/mL O ₃)	1.70	0.30	1.60	0.22	NS
V (60 µg/mL O ₃)	1.50	0.22	1.20	0.13	NS

NS: Not significant.

Table 2. Mean SCC results before and after treatment regimens within groups I (70 µg/mL O₃), II (AB, im), III (70 µg/mL O₃+AB, im), IV (30 µg/mL O₃), V (60 µg/mL O₃) and significance are indicated. (Turkey, March 2014).

Treatment Groups	Number of infected quarter	Pre-treatment SCC		Post-treatment SCC		Significance
		Mean	Std Error	Mean	Std Error	
Group	n					
I (70 µg/mL O ₃)	12	1314.917	447.5818	766.1667	273.4989	$P < 0.05$
II (AB, im)	24	2240.708	292.9491	1822.125	215.6256	NS
III (70 µg/mL O ₃ +AB, im)	24	1216.125	142.2029	563.5417	60.41637	$P < 0.001$
IV (30 µg/mL O ₃)	9	1476.444	386.5198	1074.444	240.2342	NS
V (60 µg/mL O ₃)	10	989.6	250.545	452.6	102.3837	$P < 0.05$

NS: Not significant.

Table 3. Dispersions of the isolates that were collected from milk samples of infected quarters. (Turkey, March 2014).

Isolate/s	Number of isolates
<i>S. aureus</i>	12
<i>S. agalactiae</i>	8
<i>S. aureus</i> + <i>S. agalactiae</i>	2
<i>S. aureus</i> + <i>E. coli</i>	4
<i>E. coli</i>	12
<i>Gram Bacillus</i>	1
Total	39

Table 4. Percentages of *in vitro* susceptibility to selected antimicrobial agents according to microbiological evaluation. (Turkey, March 2014).

Antibiotic	Susceptibility rate (%)
Cefoperazone	70.8
Gentamicin	41.6
Ampicillin	79.1
Amoxicillin/Clavulanic acid	79.1
Trimethoprim/Sulfamethoxazole	50
Penicillin G	70.8
Streptomycin	62.5
Oxytetracycline	50
Ceftiofur	79.1
Kanamycin	54.1
Lincomycin	41.6

DISCUSSION

In the current study, we aimed to compare the treatment efficacy of ozone insufflation via latex free 100 mL syringe in different doses (30-60-70 µg/mL) and high dose ozone insufflation (70 µg/mL) with combination of antibiotic (intramuscularly) and only antibiotic treatment (intramuscularly) in dairy cows with clinical mastitis. In a study about the clinical mastitis cases caused by coliform organisms, ceftiofur therapy found to be reduced the proportion of cases that resulted in cow death or culling [5]. But long term usage of antibiotics can cause residues in milk that can be serious for public health [6]. The application of ozone

infusion into the inflamed quarter in clinical mastitis of dairy cattle can lead to sterilization of causative agents and detoxification of the inflamed quarter [8]. Shinozuka *et al.* [16] reported mammary irrigation regimen with ozone water as more effective to cure mastitis in dairy cows when compared to systemic antibiotic administration. They reported ozone water can be effective as first line treatment for mammary irrigation regimen for coliform mastitis and ozone was detected to have possibility to reduce the mortality [16].

Ogata & Nagahata [8] reported the initial CMT score as 10 ± 0.97 and found to be decreased to 4.55 ± 0.83 on the 3rd day after ozone treatment. In the current study, CMT scores were found to best reduce in 70 µg/

mL O₃+AB combination group in post-treatment (1.63 ± 0.10) scores when compared to pre-treatment (1.21 ± 0.08) scores and the difference was found statistically significant ($P < 0.01$) between pre- and post-treatment of CMT scores [8]. Ogata & Nagahata [8] reported SCC values as decreased gradually towards to normal range after 3 weeks ozone and antibiotic treatment. In this study, our SCC results are tended to be decreased in line with Ogata & Nagahata [8] after one week therapy but in this study SCC values did not reach to normal reference ranges. It may be due to the shorter treatment duration of this study when compared to their study. *Streptococcus uberis* have been found to show a strong resistance to ozone treatment in mastitis [8]. In the present study, the most isolated bacteria was detected as *E.coli* and *Staphylococcus aureus* (n = 12 for each), *S. aureus* was also the most isolated bacteria (n = 7) in their study [8]. Shinozuka et al. [17] have advocated ozone treatment which can cause lower endotoxin release from *E. coli* to the milk other than the treatment with antibiotics. They reported that the mechanism of ozone sterilization includes the destruction of the

microbial cell membrane. In the current study we have no information to support their findings.

CONCLUSION

High doses ozone (60-70%) and high dose ozone (70%) in combination with antibiotic can be effectively used to treat clinical mastitis cases in dairy cattle. Although this study's results demonstrate that ozone insufflation is an effective treatment regime to cure clinical mastitis, the best result was achieved in the group of high dose ozone (70 µg/mL) with combination of antibiotic therapy. So it was concluded that ozone therapy can be useful as an adjunctive therapy method in the cases of clinical mastitis in dairy cattle.

MANUFACTURERS

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Declaration of interest. The authors report no conflicts of interest. The authors are responsible for this manuscript type-writing and content.

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