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EFFECTIVENESS OF OZONE TREATMENT IN CURING UTERINE INFECTIONS AND ASSOCIATED MASTITIS IN DAIRY ANIMALS

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ABSTRACT

To find the association between uterine infection and mastitis and to compare the effectiveness of ozone treatment and antibiotics in curing these infections, 200 dairy cows were screened by culturing uterine and milk samples on blood agar containing 5% sheep blood. A total of 52 animals either suffering from mastitis or uterine infections were isolated and divided into four groups to receive antibiotic or ozone treatments i.e., MA (mastitis animals treated with antibiotics), MO (mastitis animals treated with ozone), UA (uterine infected animals treated with antibiotics) and UO (uterine infected animals treated with ozone). The UA and UO groups were further treated with ovsynch estrus synchronization protocol (GnRH-PGF2α-GnRH) on 14th day after the start of ozone or antibiotic treatment. The estrus response and pregnancy rate were determined to evaluate the efficiency of ozone or antibiotic treatment in uterine infected animals. The results showed a significant (P<0.05) association between uterine infection and mastitis based on the presence of common pathogen. The cure rates in MO (53.8%) was higher (P<0.05) than MA (23.1%). Similarly, estrus response and pregnancy rate were significantly higher (P<0.05) in UO group than UA group. In conclusion, ozone therapy is more effective in curing mastitis and uterine infections.

Keywords: antibiotic therapy, mastitis, ozone therapy, uterine infection

INTRODUCTION

The profitability of a dairy farm is determined by the productive and reproductive efficiency of animals which are mainly affected by mastitis and postpartum uterine infections. The most common cause of uterine infection is pathogenic microorganisms depending on their virulence and presence of predisposing factors. The postpartum uterine infections occur due to the contamination of uterus with bacteria during parturition (Azawi, 2008) and main predisposing factors include abortion, twin birth, still birth, dystocia and retained placenta (Bell and Roberts, 2007). These bacteria cause inflammation of endometrium that results in delayed involution of uterus. This inflammation also suppresses the secretion of luteinizing hormone and disturbs the follicular growth and ovulation in dairy animals (Sheldon *et al.,* 2002). The metritis and endometritis are amongst the most common uterine infections in dairy animals (Sheldon *et al.,* 2006). The cows with metritis or endometritis have prolonged service period, decreased conception rate and a greater number of services per conception that eventually lead to economic losses (Kim and Kang, 2003). On the other hand, the mastitis which is mostly caused by *Staphylococcus* and *streptococcus* species results in decreased milk production and severe economic losses due to milk discard and treatment cost (DeGraves and Fetrow, 1993). In addition, the mastitis results in abortion, embryonic loss, decreased conception rate, and increased number of services per conception (Kumar *et al.,* 2017). The association between mastitis and uterine infections is not well known, however, it could be speculated that the translocation of bacteria and toxins from uterus to the udder or vice versa may occur (Bacha and Regassa, 2010).

To date enormous efforts continue to control uterine infection and mastitis through a wide variety of antibiotics. However, the antibiotic residues in milk and meat is considered a major

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problem and may produce various consequences for consumers. Instead of using antibiotics in most countries, ozone has been used for inactivation of microorganisms in dairy industry and has also been successfully used to treat bovine mastitis (Ogata and Nagahata, 2000). Ozone therapy is a safe, effective and inexpensive method of curing mastitis without leaving antibiotic residues in raw milk (Ogata and Nagahata, 2000). Ozone induces oxidation that damages the bacterial capsule and subsequently plasma membrane by stimulating the peroxidases and may inhibit DNA replication. Ozone is viricidal because it inhibits viral replication and damage their lipid molecule in capsids (Đuričić *et al.,* 2015). Although ozone therapy has not been sufficiently used for the treatment of various diseases in medical and veterinary practice, it has exhibited very promising results as an alternative to the use of antibiotics (Samardžija *et al.,* 2017). As milk, meat and other dairy products are common foods for humans, therefore, to avoid the potential risks of antibiotic resistance due to indiscriminate use of antibiotics in animal production, there is great need to use naturally occurring bioactive substances, such as ozone, for the treatment of various diseases in dairy animals. Therefore, the objectives of the present study were to determine the possible association between postpartum uterine infection and mastitis and to compare the effectiveness of ozone treatment with antibiotics in curing these infections.

MATERIALS AND METHODS

This study was conducted in urban and peri urban areas of Multan (latitude, 30.2^{o}N and longitude, 71.4^{o}E).

Experimental design

The study was conducted in two successive experiments. The experiment 1 was aimed to determine possible association between mastitis and uterine infection in dairy cows. For this purpose, a total of 200 dairy cows during the puerperium period (≤40 days postpartum) were initially screened for mastitis through California Mastitis Test (CMT). The animals positive for mastitis upon CMT were further screened for the presence of intrauterine infections by culturing uterine mucus on blood agar. Then the association between uterine infection and mastitis was determined based on the presence of common pathogen in both infections. The experiment 2 was conducted to compare the effectiveness of antibiotics and ozone therapy on curing mastitis and uterine infections. In this experiment, the animals (n=52) either suffering from mastitis or uterine infections were randomly divided into two equal groups (M and U). Each group then further divided into two subgroups (MA; n=13 or MO; n=13 and UA; n=13 or UO; n=13) to receive antibiotics or ozone therapy. The mastitis affected animals in MA groups were treated with commercially available intramammary antibiotic “infusion (Cloxam-LC; selmor phamaceuticals Pvt. Ltd. Pak.) containing ampicillin and doxacillin, twice a day for five successive days, whereas MO group was treated with intramammary infusion of ozone for single time” as per manufacturer’s instructions. Milk samples were collected at 7th, 14th and 21th day post-treatment for CMT to check the efficiency of each treatment regimen according to the already described procedure (Schalm *et al.,* 1971). Similarly, animals in UA group were treated with intrauterine antibiotic infusion “(OXY-5; salmor phamaceuticals Pvt. Ltd. Pak.) containing oxytetracycline,” antibiotics infusion twice a week for the period of two weeks, whereas, the animals in UO group were treated with intrauterine ozone for 10 second on alternative days for a week. On day 14th after the start of ozone or antibiotic treatment, all the animals in UA and UO groups were treated with the standard ovsynch estrus synchronization protocol (GnH; day 0, PGF2α; day 7th and GnRH; day 9th). All the animals that showed behavioral signs of estrus were mated with a breeding bull. The efficiency of each treatment regimen was determined based on estrus response and pregnancy rate.

Milk and uterine sample collection

Milk samples were collected from those animals which were CMT positive according to (Harmon *et al.,* 1990). Before starting sample collection each teat was scrubbed with (70% alcohol) cotton swab and disposable (5ml) syringes were used for sample collection. Syringe was placed horizontally under the teat and plunger was pulled out without touching its inner surface. First two streaks from each teat were discarded. The uterine mucus samples were also collected from CMT positive animals. The uterine mucus samples were collected through artificial Insemination (AI) plastic rod connected with a syringe. Briefly the mucus samples were collected by passing AI rod through cervix and by applying negative pressure on syringe connected end of AI rod.

After sample collection, both ends of rods were sealed by melting with heat then sample were placed in ice box and transported to the laboratory at Department of Clinical Sciences, FVS-BZU, Multan analysis.

Culturing of uterine and milk samples

Uterine and milk samples were cultured on blood agar containing 5% sheep blood. Microbiological examination of both milk and uterine samples was done within 8 hours after sample collection. Both milk and uterine samples were inoculated on separate blood agar plates with the help of platinum loop and incubated for 48 hours at 37^{o}C. Different colors, appearance and type of colonies were observed and characterized, and presence of different bacteria in both uterine and milk samples were further confirmed by Gram staining and Catalyze test. Association between uterine infection and mastitis was determined based on common pathogen cultured on blood agar.

Determination of prevalence and cure rate of sub clinical mastitis

Milk samples of mastitis affected animals were collected on day 7th, 14th and 21st post treatment and tested for the presence of subclinical mastitis through California Mastitis Test (CMT). Prevalence of mastitis was calculated by dividing the animals with positive CMT by total number of animals which were screened. The cure rate of subclinical mastitis was calculated by dividing the CMT negative animals by total number of mastitis affected animals.

Determination of estrus expression and pregnancy rate

All the animals in UA and UO groups were observed for estrus twice a day (morning and evening) for 30 minutes. The estrus intensity was confirmed according to the signs as described previously (Van Eerdenburg *et al.,* 1996). Animals were also palpated per rectum for presence of uterine tone and vaginal mucus discharge. Estrus response was determined by dividing the animal observed in estrus over total animals in each group (Yousuf *et al.,* 2015)**.** All the animals that showed behavioral signs of estrus were mated with a breeding bull. The pregnancy diagnosis was done on day 40 post breeding through ultrasonography. Pregnancy rate was calculated by dividing the number of pregnant animals by total number of animals which showed estrous response.

Statistical analysis

The data was analyzed by using Statistical Software (SPSS; version 20.0, IBM Corp. Armonk, NY). The association between uterine infection and mastitis was determined by applying Z-test. Data for estrus response, pregnancy rate and sub clinical mastitis cure rate were analyzed by using Chi square test.

RESULTS

Association between uterine infection and mastitis

Out of 200 animals, 57 were CMT positive, so, the prevalence of mastitis was 28.5%. Out of 57 mastitis affected animals, 11 were positive for uterine infection, thus 19.29% of the mastitis affected animals have both mastitis and uterine infection**.** In this study *Staphylococcus* spp., *streptococcus* spp., *E. coli* and *Corynebacterium* were isolated and identified on basis of characteristics shown in (Table 1). The bacteria isolated from the milk samples were *Staphylococcus* spp., *Streptococcus* spp., and *E. coli*. *Staphylococcus* was the most prominent isolate as compared to *Streptococcus* and *E. coli* (Table 2). The bacteria isolated from the uterine mucus samples were *Staphylococcus* spp., *Streptococcus* spp., *E. coli,* and *Corynebacterium*. Some samples had bacterial isolates as mixed isolates. *Staphylococcus* and *Corynebacterium* were the main isolates followed by *Streptococcus* spp., and *E. coli* (Table 3). The *Staphylococcus* and *Streptococcus* species were common in animals having both mastitis and uterine infection (Table 4). This result showed that there was significant (P<0.05) association between uterine infection and mastitis based on the presence of common pathogen.

Table 1. Colony and biochemical characteristics of isolated bacteria

Characteristics	***Staphylococcus spp.***	***Streptococcus spp.***	***E. coli***	***Corynebacterium***
Hemolytic pattern on blood agar	α and β	β	β	β
Gram Staining	Gram positive	Gram positive	Gram negative	Gram positive
Microscopic appearance of organism	Cocci	Cocci in chain	Rod	Rod/club
Catalyze test	+v	-v	+v	+v

Table 2. Bacterial species (%) isolated from milk of sub clinical mastitis affected animals

Bacteria	Single Isolates No. %	Mixed Isolates No. %	Total No. %
Staphylococcus spp.	39 (68.5)	-	39 (68.5)
Streptococcus spp.	14 (24.5)	-	14 (24.5)
Staphylococcus + Streptococcus	-	02 (3.5)	02 (3.5)
Streptococcus spp. E. coli	-	02 (3.5)	02 (3.5)
Total	53 (93)	04 (07)	57 (100)

Table 3. Bacterial species (%) isolated from uterine mucus of dairy cows

Bacteria	Single Isolates No. %	Mixed Isolates No, %	Total No. %
Staphylococcus spp.	13 (22.8)	-	13 (22.8)
Streptococcus spp.	09 (15.8)	-	09 (15.8)
E. coli	08 (14.0)	-	08 (14.0)
Staphylococcus spp. +E. coli	-	06 (10.5)	06 (10.5)
Streptococcus spp. + E. coli	-	11 (19.3)	11 (19.3)
Corynebacterium	10 (17.5)	-	10 (17.5)
Total	40 (70.1)	17 (29.9)	57 (100)

Table 4. Percentages of common bacteria in uterine infection and sub clinical mastitis cases in dairy cows

Bacteria	Mastitis	Uterine infection	Common bacteria (%)
Staphylococcus spp.	39	13	07 (12.3)
Streptococcus spp.	14	09	04 (7.0)
E. coli	-	08	-
Staphylococcus spp. + Streptococcus spp.	02	-	-
Staphylococcus spp. +E. coli	-	06	-
Streptococcus spp. + E. coli	02	11	-
Corynebacterium	-	10	-
Total	57	57	11 (19.3)

Cure rate of sub clinical mastitis

The cure rates of sub clinical mastitis on day 7th, 14th and 21st in both antibiotic and ozone treatment (MA and MO group). The cure rate was 23.1% and 53.8% in MA and MO group respectively (Figure 1). These results revealed that there was a significant difference (P<0.05) between antibiotic treatment and ozone treatment. The ozone therapy has better efficacy in curing mastitis.

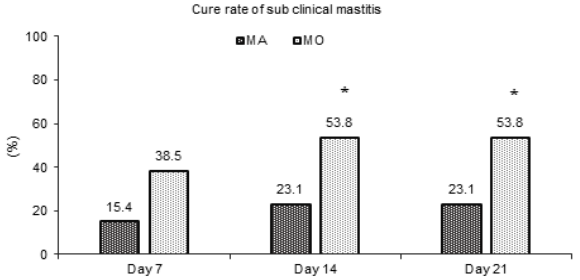

Figure 1. Cure rate of sub clinical mastitis on day 7th, 14th and 21st from the start of treatment in MA (mastitis affected animals treated with commercially available intramammary antibiotic) and MO (mastitis affected animals treated ozone)

Estrus response

The estrus response in UA and UO group was 46.2% and 61.5% respectively (Figure 2). This result revealed that there was higher estrus response in ozone treatment group as compared with antibiotic treated animals.

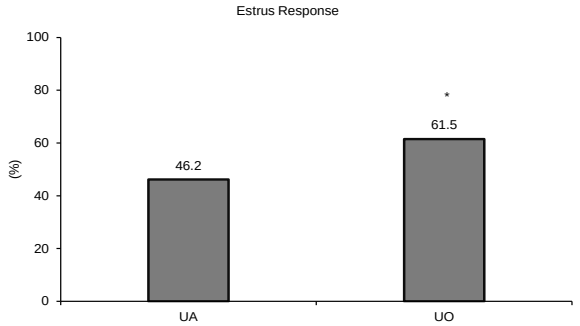

Figure 2. Estrus response (%) in UA (animals having uterine infection and treated with antibiotic) and UO (animals having uterine infection and treated with ozone) groups. (*****P<0.05).

Pregnancy rate

Pregnancy rate was 33.3% and 62.5% in UA and UO group respectively. This result showed higher there (P<0.05) pregnancy rate in ozone treated animals as compared with antibiotic treated animals (Figure 3).

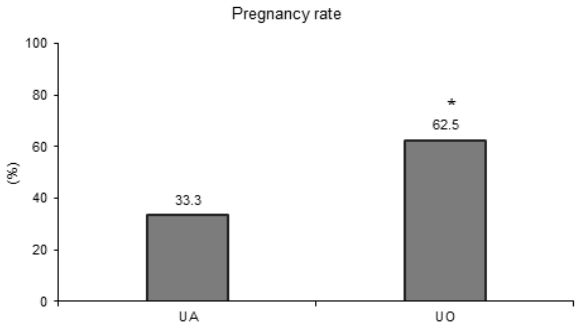

Figure 3. Pregnancy rate (%) in UA (animals having uterine infection and treated with antibiotic) and UO (animals having uterine infection and treated with ozone) groups. (*****P<0.05).

DISCUSSION

This study was conducted to determine the association between uterine infection and mastitis and to standardize the effectiveness of ozone treatment in uterine infection and mastitis in dairy animals. In this study the association between uterine infection and mastitis was observed. Bacteria isolated from uterine samples were mainly Staphylococcus spp, Streptococcus spp. *E. coli* and Corynebacterium. These findings were consistent with previous studies (Hussain *et al.,* 1990; Javed, 1991) where *Staphylococcus* spp. *Streptococcus* spp., *E. coli*, *Corynebacterium*, *Pasteurella* spp., and *Clostridium* spp. were reported as bacterial isolates from uterine samples of postpartum dairy animal. In the present study, *Staphylococcus* spp., *Streptococcus* spp. and *E. coli* were also isolated from milk samples of animals suffering from mastitis. The previous studies also documented that mastitis is mostly caused by *Staphylococcus* spp., *Streptococcus* spp. and gram-negative bacteria like *E. coli* (Fox and Gay, 1993; Lakew *et al.,* 2009). In our study association between uterine infection and mastitis was determined on the basis of common pathogen in uterine samples and milk samples of the same animal. The exact mechanism of association is unknown. This association may be due to the possibility of translocation of bacteria and bacterial products (toxins) from the uterus to the udder or vice versa (Bacha and Regassa, 2010).

In the present study mastitis was treated with antibiotic and ozone therapy and cure rates were 23.1% and 53.8% respectively. In previous study the cure rate of subclinical mastitis with intramammary antibiotic (amoxicillin) therapy was 30.4% (Owens *et al.,* 1988). Ozone has also been reported to treat acute clinical mastitis in Holstein cow with cure rate of 60% (Ogata and Nagahata, 2000).

In current study the estrus response in both antibiotic and ozone treatment group was 46.2% and 61.5% respectively. Similarly, the pregnancy rate in antibiotic and ozone group was 33.3% and 62.5% respectively. These results are not consistent with the previous reports (Goshen and Shpigel, 2006) where estrus response and pregnancy rates were 75.5% and 42.5 in animals treated with intrauterine infusion of oxytetracycline for metritis.

CONCLUSION

In conclusion, there is significantly better efficacy of ozone treatment in curing uterine infection and mastitis. Therefore, ozone therapy could replace antibiotics treatment of uterine infection and mastitis as ozone is safer than antibiotics and has no residual effects. It is recommended that this study may be replicated at different public and private dairy farms with large number of animals to evaluate the effectiveness of ozone therapy at large scale.

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CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

AUTHOR'S CONTRIBUTION

A. Ahmad: Methodology, data curation, and writing original draft

T. Ahmad: Funding acquisition, project administration, supervision, validation, writing-review and editing

E. Ahmad: Conceptualization, validation, writing - review and editing

M. Nadeem: validation, writing- review and editing

A. Sattar: writing- review and editing, validation, supervision

REFERENCES

Azawi, O. 2008. Postpartum uterine infection in cattle. Animal Reproduction Science, 105 (3-4): 187-208.

Bacha, B. and F. G. Regassa. 2010. Subclinical endometritis in Zebu x Friesian crossbred dairy cows: its risk factors, association with subclinical mastitis and effect on reproductive performance. Tropical Animal Health and Production, 42 (3): 397-403.

Bell, M. and D. Roberts. 2007. The impact of uterine infection on a dairy cow's performance. Theriogenology, 68 (7): 1074-1079.

DeGraves, F. J. and J. Fetrow. 1993. Economics of mastitis and mastitis control. The Veterinary Clinics of North America. Food Animal Practice, 9 (3): 421-434.

Đuričić, D., H. Valpotić and M. Samardžija. 2015. Prophylaxis and therapeutic potential of ozone in buiatrics: Current knowledge. Animal Reproduction Science, 159: 1-7.

Fox, L. K. and J. M. Gay. 1993. Contagious mastitis. The Veterinary Clinics of North America. Food Animal Practice, 9 (3): 475-487.

Goshen, T. and N. Y. Shpigel. 2006. Evaluation of intrauterine antibiotic treatment of clinical metritis and retained fetal membranes in dairy cows. Theriogenology, 66 (9): 2210-2218.

Harmon, R., R. Eberhart, D. Jasper, B. Langlois and R. Wilson. 1990. Microbiological procedures for the diagnosis of bovine udder infection. Arlington: National Mastitis Council, pp. 34.

Hussain, A., R. Daniel and D. O'Boyle. 1990. Postpartum uterine flora following normal and abnormal puerperium in cows. Theriogenology, 34 (2): 291-302.

Javed, M. 1991. MZ KHAN. Journal of Islamic Academy of Sciences, 4 (3): 242-244.

Kim, I. H. and H. G. Kang. 2003. Risk factors for postpartum endometritis and the effect of endometritis on reproductive performance in dairy cows in Korea. Journal of Reproduction and Development, 49 (6): 485-491.

Kumar, N., A. Manimaran, A. Kumaresan, S. Jeyakumar, L. Sreela, P. Mooventhan and M. Sivaram. 2017. Mastitis effects on reproductive performance

Lakew, M., T. Tolosa, W. Tigre. 2009. Prevalence and major bacterial causes of bovine mastitis in Asella, South Eastern Ethiopia. Tropical Animal Health and Production, 41 (7): 1525.

Ogata, A. and H. Nagahata. 2000. Intramammary application of ozone therapy to acute clinical mastitis in dairy cows. Journal of Veterinary Medical Science, 62 (7): 681-686.

Owens, W., J. Watts, R. Boddie and S. Nickerson. 1988. Antibiotic treatment of mastitis: comparison of intramammary and intramammary plus intramuscular therapies. Journal of Dairy Science, 71 (11): 3143-3147.

Samardžija, M., R. Turk, P. Sobiech, H. Valpotić, I. Harapin, D. Gračnerand D. Đuričić. 2017. Intrauterine ozone treatment of puerperal disorders in domestic ruminants: A Review. Veterinarski arhiv, 87 (3): 363-375.

Schalm, O. W., E. J. Carroll and N. C. Jain. 1971. Bovine mastitis. Philadelphia, Pa: Lea and Febiger. pp. 72.

Sheldon, I., D. Noakes, A. Rycroft, D. Pfeiffer and H. Dobson. 2002. Influence of uterine bacterial contamination after parturition on ovarian dominant follicle selection and follicle growth and function in cattle. Reproduction-Cambridge, 123 (6): 837-845.

Sheldon, I. M., G. S. Lewis, S. LeBlanc and R. O. Gilbert. 2006. Defining postpartum uterine disease in cattle. Theriogenology, 65 (8): 1516-1530.

Van Eerdenburg, F., H. Loeffler and J. Van Vliet. 1996. Detection of oestrus in dairy cows: A new approach to an old problem. Veterinary Quarterly, 18 (2): 52-54.

Yousuf, M. R., J. Martins, A. Husnain, U. Riaz, H. Riaz, A. Sattar, K. Javed and N. Ahmad. 2015. Effect of oestradiol benzoate on oestrus intensity and pregnancy rate in CIDR treated anoestrus nulliparous and multiparous buffalo. Animal Reproduction Science, 159: 104-108.

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