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Evaluation of ozone therapy as an adjunct to the management of otitis externa in dogs#

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Abstract

A study was conducted in twelve dogs showing at least two signs of otitis externa, to evaluate the efficacy of ozone therapy in treatment of otitis externa in dogs. The selected dogs were randomly divided into two groups with six animals each. Group I underwent otic flushing with 0.9 per cent normal saline under general anaesthesia, while the other group was treated with 55 µg/mL ozonated distilled water in the same manner. All the animals received antibiotic therapy based on the results of antibiogram of otic discharge, along with topical antibiotic and antifungal therapy. Maximum reduction in otitis index score, pruritus severity scale and pain severity score were achieved by day 21 of treatment in all the animals. Radiographically ear canal appeared air filled and widened on fourth week of treatment, suggestive of absence of exudate and oedema in both groups. On clinical and otoscopic examination ulcerations, oedema, erythema, pain and otorrhea was found to be reduced by second week of treatment in Group I, compared to third week in Group I. Even though there was reduction in total viable bacterial count between day one and day seven of treatment in both groups, a marked reduction was observed by day 14 in Group II. Hence, ozone therapy may be considered as an adjunct to the management of otitis externa in dogs along with systemic and topical antibiotic therapy.

Keywords: *Otitis externa, ozone therapy, otic flushing*

Otitis externa is one of the most common ear diseases of dogs with multifactorial aetiology (Rosser, 2004). A comprehensive approach to the treatment of canine otitis externa, by excluding the possible causes and addressing the actual reasons, is warranted to resolve the disease permanently (Prakash, 2013). Ozone therapy is an emerging therapeutic modality in human and veterinary patients for the treatment of infected wounds, ulcers, skin diseases, endometritis, ear infections, autoimmune diseases and diabetes mellitus (Peteoaca *et al.,* 2020). It has been reported that, medical ozone can influence the immunomodulatory, antioxidant, oxidant, analgesic, antimicrobial and anti-inflammatory mechanisms of the body at certain concentrations (Bocci *et al.,* 2009 and Elvis and Ekta, 2011). Hence, the study was undertaken with the objective to evaluate the efficacy of ozone therapy in the treatment of otitis externa in dogs.

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Materials and methods

The study was conducted in twelve dogs of different age, breed and sex with unilateral or bilateral otitis externa presented to Teaching Veterinary Clinical Complex, Mannuthy and University Veterinary Hospital, Kokkalai during a period from October 2023 to June 2024.

The dogs were randomly divided into two groups with six animals in each group *i.e.* Group I and Group II. Group I consisted of six dogs serially numbered as I_1, I_2, I_3, I_4, I_5 and I_6 and Group II consisted of six dogs serially numbered as II_1, II_2, II_3, II_4, II_5 and II_6. The dogs were subjected to detailed physical and clinical examination at weekly intervals upto one month. Haematological parameters like total erythrocyte counts, haemoglobin concentration, total leukocyte counts, differential leucocyte count, volume of packed cells and platelet count were estimated on the first day and after two weeks from all the animals. Biochemical parameters like total thyroxine and random serum glucose were estimated on the first day presentation. Otic discharge was taken for cytological examination, culture and antibiotic sensitivity test (ABST) on the day of presentation. Detailed examination of the ear using otitis index scoring system, pruritus severity scale, pain severity score and aural temperature (Hill *et al*., 2010; Nuttall and Bensignor, 2014) were carried out in each group at weekly intervals upto one month. Total viable bacterial count of otic discharge was assessed on the day of presentation, 7th day and 14th day post treatment. Otoscopic examination was performed on the first day of presentation and 14th day treatment. Radiographic examination of the ear canal was performed on the first day of presentation and 30th day of treatment.

Group I animals underwent otic flushing (Fig. 2) with 0.9 percent normal saline under general anaesthesia, while the other group was treated with 55 µg/mL ozonated distilled water in the same manner. A medical ozone generator (Fig. 1) was used for the production of ozone gas (O_3) from oxygen. All the animals received antibiotic therapy based on the results of antibiotic sensitivity tests of otic discharge, along with topical antibiotic and antifungal therapy. Statistical significance of the study was analysed by repeated measures of ANOVA, independent t-test, Wilcoxon signed ranks test and Kaplan-Meier survival plots.

Results and discussion

The current study evaluated the efficacy of ozone therapy along with systemic and topical antibiotic therapy in twelve dogs with otitis externa. The mean age of dogs affected were 3.29±1.40 years that ranged from one and half years to six years and these findings were in agreement with the findings of Shenoy (2004). The body weight of the dogs in the study ranged from 10 to 39 kg. The incidence of otitis externa was highest in German Shepherd, Labrador Retriever, Pug and Beagle (16.66 per cent each) followed by Husky, Pitbull, Akita and nondescript dogs (8.33 per cent each) in the present study. Mircean *et al.* (2008) and Kumar *et al.* (2014) also reported a higher rate of occurrence of otitis externa in German Shepherd and Labrador Retriever dogs. Out of the 12 animals under study, 66.7 per cent were female dogs and 33.3 per cent were male dogs. Prakash (2013) also reported that female dogs were more affected by otitis than male dogs. The duration of the illness varied from seven days to thirty days with a mean ± S.E of 12.41±6.54 days. Similar results were also reported by Hendricks *et al.* (2002). Haematological parameters like total erythrocyte counts, haemoglobin concentration, total leukocyte counts, differential leucocyte count, volume of packed cells and platelet count did not show any significant difference after the treatment in both groups and these findings were in accordance with the findings of Shenoy (2004), Reshmi (2010) and Prakash (2013). Biochemical parameters like total thyroxine and random serum glucose were in the normal range without any significant difference. Fernandez *et al.* (2006) also reported that endocrine diseases were less frequently associated with otitis externa. The dogs were subjected to

Fig 1. Medical ozone generator

Fig 2. Otic flushing

general anaesthesia to facilitate restraint while performing video otoscopy and otic flushing as per the suggestions of Shenoy (2004) and Cole (2011).

Clinical symptoms like shaking of head, scratching of ear (in all animals) and drooping of ear (one from Group I) were observed. These symptoms were also observed by Coatesworth (2011) in otitis affected dogs. Clinical symptoms subsided within two weeks of treatment in all the animals of Group II, compared to 50 percent dogs in Group I. Early reduction of clinical signs in Group II, might be due to the anti-inflammatory effect of ozone as reported by Maria (2020) in a study of treatment of ozonide for chronic eczematous external otitis. Erythema, oedema, malodourous discharge, presence of ulcers, pain on palpation of the external ear canal and stenosis of ear canal were noticed in animals (Fig. 4) selected for the study but free from any obvious trauma and foreign body in the ear canal which were reported as other causes of otitis externa by Rosser (2004) and Mircean *et al.* (2008). The colour of otic discharge observed were yellowish white, yellowish, yellowish brown, brownish, brownish black and greenish black and the findings were consistent with that of Reshmi (2010). The consistency of the otic discharge observed were creamy, waxy and watery as per Roshan *et al.* (2018). A reduction in inflammatory otic discharge, erythema and healing ulcers were noticed by second week of treatment in all the animals of Group II, compared to third week in Group I. Oedema and pain were reduced in all animals of both group by second week of treatment. Earlier resolution of pain in ozone treated animals might be due to the analgesic effect of ozone as reported by Teixeira *et al.* (2013) in a study of dogs comparing the analgesic effect of ozone and meloxicam following ovariohysterectomy. Otic flushing removed excess cerumen or discharge occluding the ear canal in both groups and this is in accordance with the findings of Scott *et al.* (2001). Early reduction of clinical signs in dogs that underwent ozone therapy may be due anti-inflammatory, antimicrobial, analgesic action of ozone as reported by Shimizu *et al.* (2013) and Varol *et al.* (2022) in various canine or feline systemic and local diseases and experimentally induced keratitis in rabbits respectively.

Aural temperature was measured with non-contact digital infrared thermometer (Yanmaz *et al*., 2015) and the mean ± S.E. values of affected and normal ears were within the normal range in all animals. Yoshida *et al*. (2002) also suggested that aural temperature had minor role in the development of otitis externa. A gradual reduction in otitis index score (Fig. 8) and pruritus severity score (Fig. 9) were observed in all the animals of Group II. Significant reduction in otitis index score and pruritus severity scale were appreciated by seventh day and second day of treatment in Group I and Group II respectively and this earlier reduction in ozone treated animals may be due to the reduction of bacterial infection and anti-inflammatory effect of ozone, as observed in a clinical study of ozonide treatment for chronic eczematous external otitis (Maria, 2020). A gradual reduction in pain severity score (Fig. 10) was observed throughout the observation period in both groups with significant reduction appreciated at seventh day of treatment. The pain severity scores dropped to zero in animals that underwent ozone therapy by 21st day which may be due to the analgesic effect of ozone as reported by Teixeira *et al.* (2013) in a study of ozone as an analgesic after ovariohysterectomy in dogs.

Among the total number of isolates, five cases (41.67 percent) were gram-positive cocci and seven (58.3 percent) were gram-negative rods. Reshmi (2010) and Prakash (2013) also reported a higher incidence of gram-negative bacterial infections in canine otitis externa. Yellowish purulent and greenish otic discharges were observed in gram negative bacterial infection and brownish waxy to watery discharge was noticed in gram positive bacterial infection or yeast infection during the study and which was similar to the findings of Shaw (2017). Antibiotic sensitivity test revealed that the most sensitive antibacterials were Cephalexin, Enrofloxacin, Amikacin, Amoxicillin and Gentamicin while the most resistant were Ceftriaxone, Ciprofloxacin, Amoxicillin clavulanic acid, Azithromycin and Tetracycline. Cytological examination of otic discharge (Fig. 5) revealed the presence of more than 30 bacteria, 3-6 epithelial cells, more than 20 neutrophils, more than 30 budding yeast cells and RBCs,

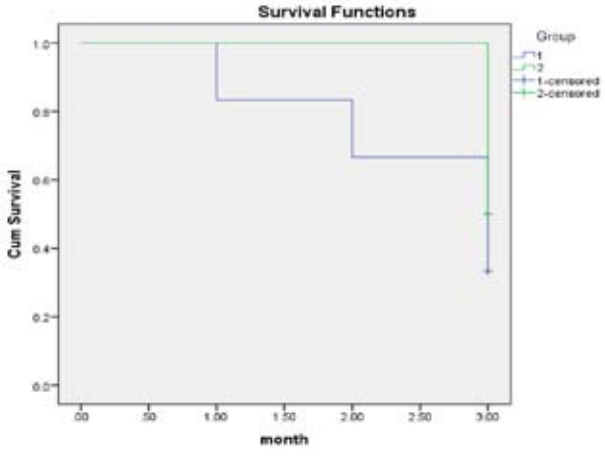


Fig 3. Kaplan-Meier survival plots for probability of cure stratified by two treatment groups (p – 0.33)

Table 1. Mean ± S.E of total viable count (CFU/mL) between days in dog with otitis externa (n=12)

Group	Day 1	Day 7	Day 14
I	36.90±14.33^{a}	10.11±4.8^{ab}	21.46±10.23^{a}
II	34.71±14.33^{a}	9.31±4.81^{ab}	2.61±10.23^{a}

Superscript with different alphabet differ significantly within a row (p=0.03)

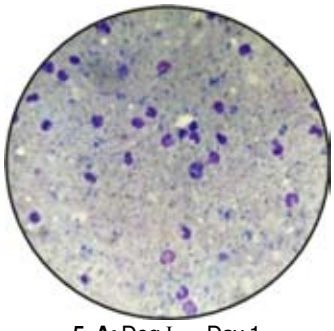
4. A: Dog I_3 – Day 1

4. B: Dog I_6 – Day 1

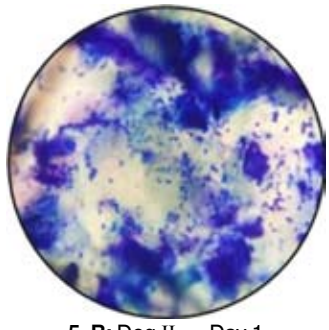
4. C: Dog I_3 – Day 30

4. D: Dog II_6 – Day 30

Fig 4. Findings of clinical examination of the ear: 4.A and 4.B: Presence of exudates, oedema, erythema, ulcerations on 1st day of presentation in normal saline group and ozone therapy group respectively; 4.C and 4.D: Absence of clinical findings on 30th day of treatment



5. A: Dog I_6 – Day 1



5. B: Dog II_6 – Day 1

5. C: Dog I_6 – Day 14

5. D: Dog II_6 – Day 14

Fig 5. Cytological examination findings of otic discharge: 5.A and 5.B: Presence of bacterial organisms, neutrophils, yeast organisms and epithelial cells on 1st day of presentation in normal saline group and ozone therapy group respectively; 5.C and 5.D: Reduction of cytological examination findings on 30th day of treatment.

6. A: Dog I_1 – Day 1

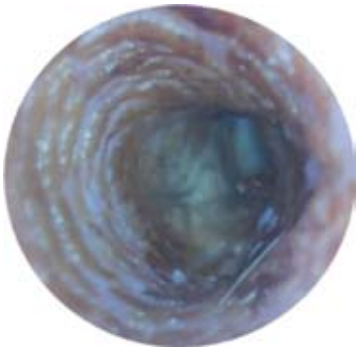
6. B: Dog II_4 – Day 1

6. C: Dog I_1 – Day 30

6. D: Dog II_4 – Day 30

Fig 6. Radiographic examination of ear; 6.A and 6.B: uneven walls with narrowing of ear canal lumen noticed on 1st day of presentation; 6.C and 6.D: increased ear canal lumen diameter in both animals with more pronounced change noticed in ozone treated animal on day 30 of treatment

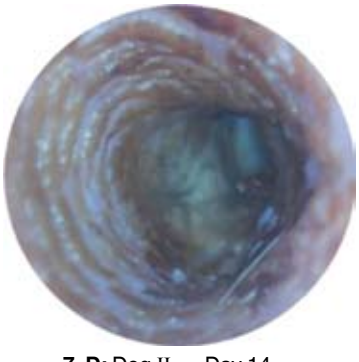
7. A: Dog I_5 – Day 1



7. A: Dog II_3 – Day 1

Fig 7. Otoscopic examination of ear canal; 7.A and 7.B: ulcerations, oedema, erythema, otorrhoea and stenosis could be noticed in 1st day of presentation in both animals

7. C: Dog I_5 – Day 14



7. D: Dog II_3 – Day 14

Fig 7. Otoscopic examination of ear canal; 7.C and 7.D: reduction of clinical findings in both animals with intact glistening tympanic membrane on day 14 of treatment in ozone treated animals.

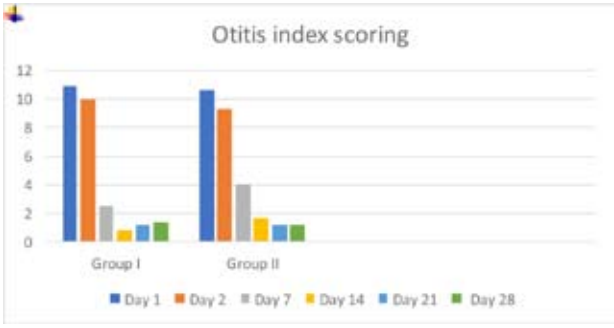


Fig 8. Otitis index scoring in Group I and Group II animals

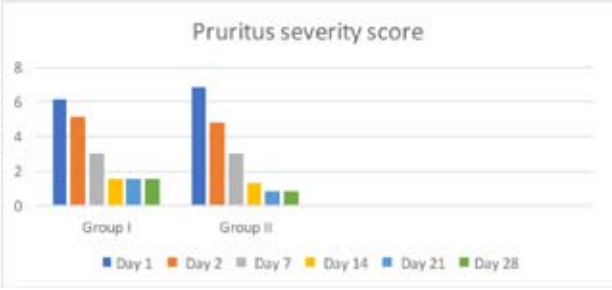


Fig 9. Pruritus severity score in Group I and Group II animals

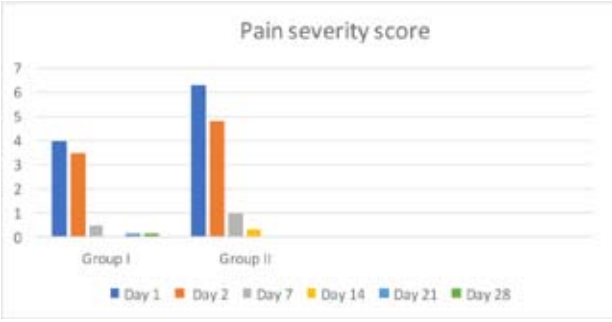


Fig 10. Pain severity score in Group I and Group II animals

per field on the first day of presentation. Coatesworth (2011) and Prakash (2013) also observed similar findings on cytological examination of ear discharge. A reduction in number of bacteria, yeast cells, neutrophils and red blood cells were observed by fourteenth day of treatment in both the groups. Reduction of bacterial, yeast cell and neutrophil count in dogs of Group II may be due to the antibiotic, antifungal and anti-inflammatory effect of ozone as reported by Nithish (2022) in ozone therapy for subclinical endometritis in dairy cow and experimentally induced keratitis in rabbits (Varol *et al.*, 2022) respectively. Reduction of epithelial cells was noticed by day 14 in all the animals of Group II, which may be attributed to the effect of ozone on wound healing (Zeng and Lu, 2018). The mean total viable count (CFU/mL) of otic discharge (Table 1) significantly reduced between day one and day seven in both the groups. Despite this, Group II animals exhibited marked reduction in mean total viable count, compared to the rise in Group I by day 14 and the results were comparable with that of Nithish (2022).

Plain radiographs of the skull were taken in ventro-dorsal view (Fig. 6) for evaluating changes in the external ear canal (Gotthelf, 2004). Uneven walls with narrowing of the ear canal lumen and uneven walls with slight opacity and air-filled ear canal lumen were observed on the first day of presentation in selected animals. Similar observations of narrowing and irregularity of the ear canal lumen due to oedema was reported by Cole *et al.* (2002), Gortel (2004) and Rosser (2004) in canine otitis externa. Prakash (2013) reported that the opacity of the ear canal lumen might be due to exudates or any growth projecting in the external ear canal. There was an increase in the lumen diameter and air-filled ear canal lumen in all the animals by 30th day of treatment. Increased lumen diameter in the current study was suggestive of the absence of exudate and oedema (Prakash, 2013) in all animals with more pronounced change observed in ozone treated animals. Otoscopic evaluation of the ear canal (Fig. 7) was performed on the day of presentation in all the dogs and two weeks after in dogs selected randomly. Video otoscopic findings like status of integument of the ear canal, ulcerations, oedema, erythema, otorrhoea, degree of stenosis and alterations in the morphology of the tympanic membrane were recorded in selected dogs. Better resolution of ulcerations, erythema and oedema was noticed in animals which underwent ozone therapy and it may be attributed to the reduction in bacterial infection in response to ozone treatment and anti-inflammatory effect of ozone (Melanie *et al.,* 2024). The topical and systemic antimicrobial therapy along with otic flushing reduced exudation and oedema and the tympanic membrane appeared intact, smooth and glistening by day 14 of treatment. Gortel (2004) also reported that otic flushing and antimicrobial therapy reduced exudates in dogs with otitis externa. However, better visualisation of tympanic membrane could be noticed in ozone treated animals, which might be due to anti-inflammatory and antimicrobial properties of ozone. The post-therapy recurrence was assessed monthly in all animals for up to three months (Fig. 3). Recurrence of otitis externa was detected in both groups by third month with more recurrence rate in animals of Group I. There was 70 percent recurrence in Group I, compared to 50 percent in Group II. The higher cure rate in Group II might reflect a direct effect of ozone on the etiological agents (Bocci *et al.*, 2009; Elvis and Ekta, 2011). Sustained antimicrobial and anti-inflammatory effect of ozone could be due to the effect of modulation of immune system by ozone (Menendez *et al.*, 2012).

Conclusion

The present study was undertaken with the objective to evaluate the efficacy of ozone therapy in treatment of otitis externa in dogs. Ozone therapy at a concentration of 55 µg/mL along with systemic and topical antibiotic therapy achieved remission of clinical signs by second week of treatment and there was a significant reduction in otitis index score, pruritus severity scale and

pain severity score noticed on day two of ozone therapy, compared to day seven in animals treated with normal saline. A progressive reduction in total viable bacterial count of otic discharge was also observed in animals that underwent ozone therapy, which proved it to be an effective antibacterial agent.

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Conflict of interest

The authors declare that they have no conflict of interest.

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