J Vet Clin 25(3) : 159-164 (2008)

Journal of Veterinary Clinics
www.ksvc.or.kr

Therapeutic Effect of Injection-Acupuncture with Bee-Venom (Apitoxin) in Cases of Canine Otitis Externa

Sang-Hun Kim, Hyung-Kyou Jun, Suk Kim*, Myung-Jo You, Moo-Hyung Jun and Duck-Hwan Kim[1]**

College of Veterinary Medicine, Chungnam National University, Daejeon 305-764, Korea
**College of Veterinary Medicine, Gyeongsang National University, Jinju 660-701, Korea*
***College of Veterinary Medicine, Chonbuk National University, Jeonju 561-756, Korea*

(Accepted: June 5, 2008)

Abstract : This study was carried out to determine the therapeutic effect of injection-acupuncture (AP) with bee-venom (apitoxin) in cases of canine otitis externa (COE). Fifteen dogs with naturally-acquired otitis externa were used in this study. The dogs were divided into the following antibiotics group (control group), apitoxin group (experimental group A) and apitoxin combined with antibiotics group (experimental group B). All groups were treated by ear cleaning with normal saline once on day 1. The control group was treated with susceptible antibiotics, and experimental group A was given injection-AP with apitoxin (100 μg/head) at TH17 (Yi Feng), SI19 (Ting Gong), GB03 (Shang Guan) and TH03 (Zhong Zhu) bilaterally. Experimental group B was treated with susceptible antibiotics and injection-AP with apitoxin at the same acupoints as experimental group A. All the groups were treated 3 times/week for 2 weeks. The identity of the causative agents, the changes in the clinical signs, otoscopic findings, bacterial count in the auricular discharges, and total WBC counts and neutrophil/lymphocyte (N/L) ratio in the peripheral blood were investigated in all groups. In bacterial isolation, *Staphylococcus* spp. combined with *Streptococcus* spp. was detected higher than other agents. The bacterial cell count in experimental group A was significantly decreased at 2 weeks ($p < 0.01$), and those in experimental group B was significantly decreased at 1 week ($p < 0.01$) and 2 weeks ($p < 0.01$) compared by those of control group, respectively. The changes of clinical score in experimental group B were significantly decreased at 2 weeks ($p < 0.01$) compared by those of control group, but, those of experimental group A was similar to those of control group. The changes of total WBC counts and neutrophil/lymphocyte (N/L) ratio were no significant difference found. In conclusion, injection-AP with apitoxin is an effective treatment for COE and might be an alternative method for treating COE.

Key words **:** apitoxin, canine, injection-acupuncture, otitis externa.

Introduction

Canine otitis externa (COE) is a disease that is commonly encountered in small animal clinical practice (7). COE is an inflammation of the ear canal, which can result from a variety of causes. These causes include predisposing factors (conformation, excessive moisture, excessive cerumen production, treatment effects and obstructive ear disease, etc.), primary causes (microorganisms, parasites, hypersensitivity diseases, keratinization disorders, foreign bodies, glandular disorders, autoimmune diseases and viral disease, etc.) and perpetuating factors (bacteria, yeast, progressive pathological changes and otitis media, etc.) (8,31). The treatment of COE includes cleaning of the ear canal (ceruminolytic agents), topical therapy (various combinations of glucocorticoids, antibiotics, antifungals and parasiticides), systemic therapy (antibiotics, antifungals, parasiticides and glucocorticoids) and surgery (lateral ear canal resection, vertical canal ablation and total ear canal ablation) (24,30).

Bee-venom (apitoxin) has strong anti-bacterial, anti-fungal, and radioprotective effects (14,19). These properties are consistent with its strong anti-inflammatory effects (19,22). More than 30 different substances have been extracted from apitoxin (19). Peptides such as mellitin, apamin, peptide 401, adolapin and protease inhibitors are the main pharmacological components that reduce inflammation (11,32). In addition to its anti-inflammatory properties, apitoxin is a strong immunologic agent that stimulates the protective mechanisms of the body against disease (2,19,34).

Regarding research on the use of apitoxin therapy in the veterinary field, the therapeutic effects of apitoxin have been investigated in equine laminitis (15) as well as in calves with bacterial diarrhea (3), and in sows with mastitis, metritis and agalactia syndrome (4).

Due to the excessive use of antibiotics and the increase in antibiotics-resistant bacteria, there has been increasing interest in complementary or alternative medicine such as acupuncture and injection-acupuncture (AP) etc. However, there are no reports on therapeutic effect of injection-AP with

[1]Corresponding author.
E-mail : dhkim@cnu.ac.kr

apitoxin in COE. The aim of this study was to determine the therapeutic effect of injection-AP with apitoxin on COE.

Materials and Methods

Experimental animals

Fifteen dogs with naturally-acquired otitis externa (Maltese; 3 males and 6 females, Shitzu; 2 females, Poodle; 1 female, Yorkshire terrier; 1 female, Mongrel; 2 males) with weights ranging from 1.5 to 4.7 kg, and ages ranging from 3 to 48 months were used in this study. The dogs were divided into the antibiotics group (control group: five dogs) and apitoxin group (experimental group A: five dogs), apitoxin combined with antibiotics group (experimental group B: five dogs).

Treatment procedure

All groups were treated by ear cleaning with normal saline once on day 1. And the control group was medicated with one of the following: amoxicillin-clavulanic acid 12.5 mg/kg, ciprofloxacin 15 mg/kg, and doxycycline 5 mg/kg according to the results of an antibiotics sensitivity test. Experimental group A was treated with injection-AP with apitoxin only. The acupoints used in this study were TH17 (Yi Feng), SI19 (Ting Gong), GB03 (Shang Guan) and TH03 (Zhong Zhu) bilaterally. An apitoxin solution was made from commercial apitoxin (100 μg/head, Apitoxin®, 1mg/bottle, Guju Pharmacological Co., Korea) mixed with 0.1 ml of 2.5% lidocaine hydrochloride (1:1) and were diluted with saline (0.8 ml). The apitoxin solutions were injected into each acupoint (approximately 0.1-0.2 ml/acupoint). Experimental group B was given the susceptible antibiotics medication, as in the control group, as well as injection-AP with apitoxin. All groups were treated 3 times/week for 2 weeks.

Bacterial isolation

Samples were collected from the ear canal of all dogs prior to treatment. All bacterial strains obtained in this study were isolated and identified using the standard method and VITEC identification system (Biomeriux, France).

Total bacterial cell count

The bacteria isolated from COE were suspended in 1 ml PBS. 100 μl was then spread over brain heart infusion agar (BHI agar), incubated at 37°C for 48 h, and the total bacterial colony forming units (CFU) were counted before treatment, as well as 1 and 2 weeks after treatment.

Antibiotic sensitivity test

The bacteria isolated from the COE were cultured in BHI broth and 100 μl was spreaded over a BHI agar plate. Various antibiotics disks were tested and the level of bacterial antibiotic resistance was measured from diameter of the clear zone (> 2.0 cm; +++, 1.0-2.0 cm; ++, < 1.0; +, –; not sensitive).

Clinical scores

Pruritus, cerumen, redness, swelling, heat, pain and odor were chosen as the score calculation elements, and each element was divided into several phases, as listed in Table 1.

Blood analysis

The samples were collected from cephalic vein by venipuncture. The WBC count, and N/L count were determined using an automatic cell counter (MS9-5V, Melet Schloesing Laboratories, France). The blood analyses were determined before treatment, as well as 1 and 2 weeks after treatment, respectively.

Statistical analysis

The significant differences ($p < 0.01$) between the control and experimental groups were analyzed using a paired Student's t-test contained in the Microsoft Excel package (Microsoft Corp., USA).

Table 1. Criteria for evaluation of clinical score

Score	Score calculation elements						
	Pruritus	Cerumen	Redness	Swelling	Heat	Pain	Odor
0	No	Little	No	No	No	No	No
1	Mild	Slight	Mild	Slight	Mild	Mild	Slight
2	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
3	Severe	Severe	Severe	Severe	Severe	Severe	Severe

Table 2. Bacterial distribution detected in dogs with otitis externa

Agents	*Staphylococcus* spp. + *Streptococcus* spp.	*Staphylococcus* spp. + *Pseudomonas* spp.	*Staphylococcus* spp.	*Staphylococcus* spp. + *Malassezia* spp.	*Staphylococcus* spp. + *Bacillus* spp.
Percent	5/15 dogs (33.3%)	4/15 dogs (26.7%)	4/15 dogs (26.7%)	1/15 dogs (6.7%)	1/15 dogs (6.7%)

Results

Bacterial isolation

The causative agents of COE detected were *Staphylococcus* spp. + *Streptococcus* spp. (33.3%, 5/15 dogs), *Staphylococcus* spp. + *Pseudomonas* spp. (26.7%, 4/15 dogs), *Staphylococcus* spp. only (26.7%, 4/15 dogs), *Staphylococcus* spp. + *Malassezia* spp. (6.7%, 1/15 dogs) and *Staphylococcus* spp. + *Bacillus* spp. (6.7%. 1/15 dogs) (Table 2).

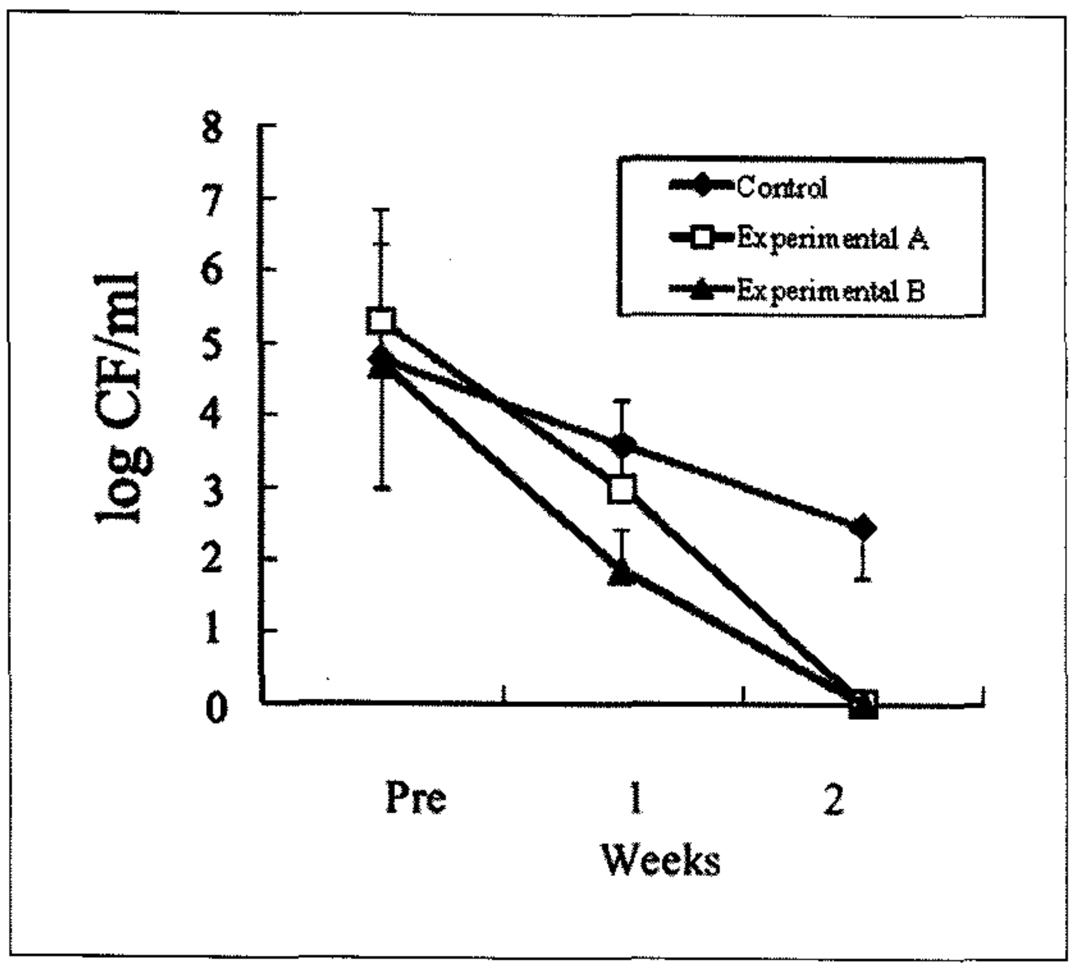

Fig 1. The change of bacterial cell counts in cerumen of otitic dogs with each treatment (*significant differences between control group and experimental group A ($p < 0.01$), **significantly difference between control group and experimental group B ($p < 0.01$)).

The change of bacterial cell count

The control group showed a slightly decreasing bacterial replication rate after treatment with the susceptible antibiotics (before: 4.8 ± 1.80, 1 week: 3.6 ± 0.45 and 2 weeks: 2.5 ± 0.72 log CFU/ml). However, experimental group A showed a dramatically decreasing bacterial replication rate (before: 5.3 ± 1.51, 1 wk: 3.0 ± 1.21 and 2 wks: 0.0 ± 0.00 log CFU/ml). Experimental group B showed similar results to experimental group A (before: 4.7 ± 1.67, 1 wk: 1.9 ± 0.55, 2 wks: 0.0 ± 0.00 log CFU/ml). The bacterial cell count in experimental group A was significantly decreased at 2 weeks ($p < 0.01$), and experimental group B was significantly decreased at 1 week ($p < 0.01$) and 2 weeks ($p < 0.01$), compared by those of control group, respectively (Fig 1).

Clinical score

The clinical signs including pruritus, cerumen, redness, swelling and odor were greatly improved after the 2-week treatment in experimental groups, and the control (Fig 2). In particular, there was a significant decrease in the clinical

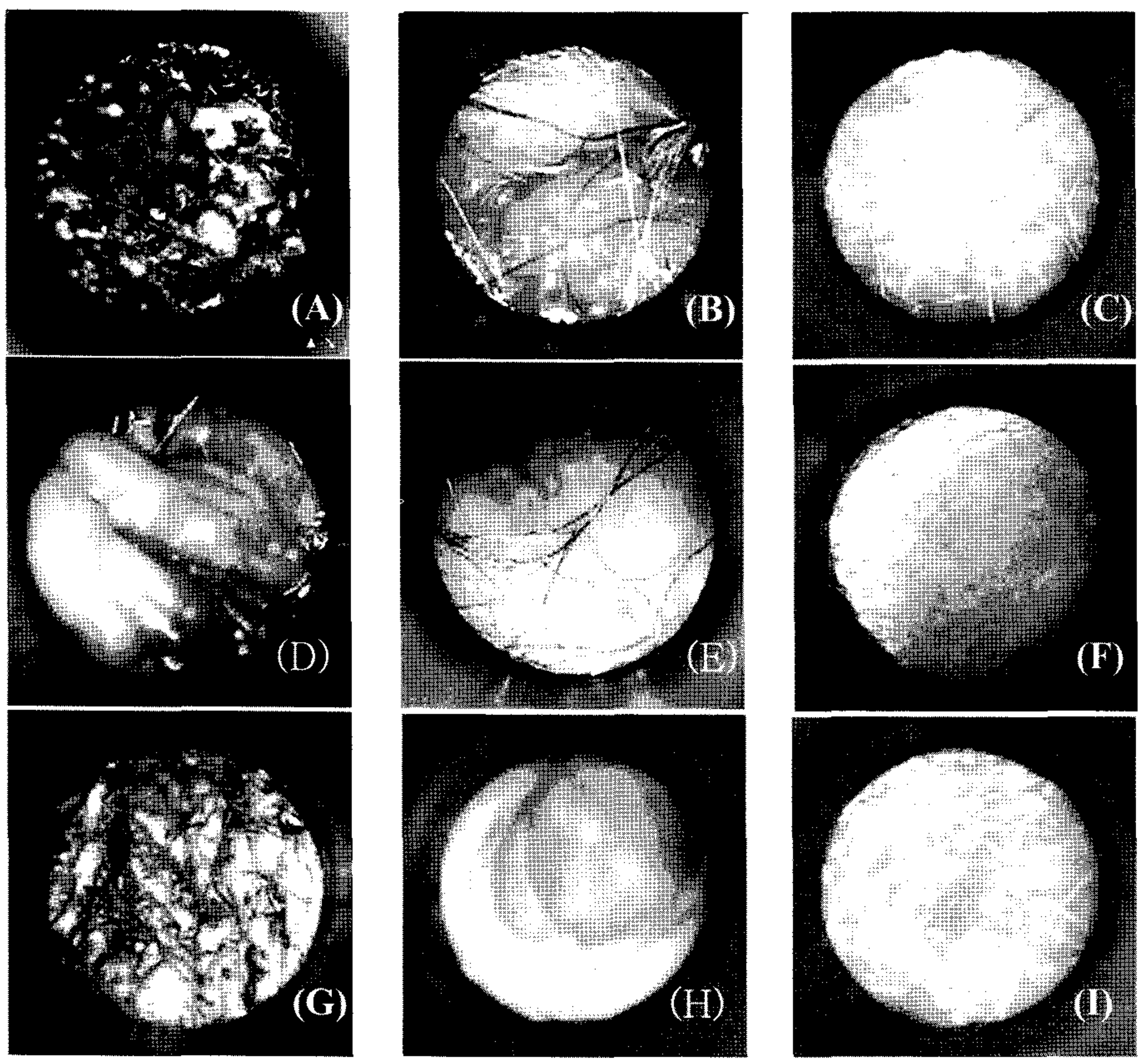

Fig 2. Otoscopic features after injection-acupuncture with apitoxin. (the control group; pre-treatment (A), 1 week after treatment (B), 2 weeks after treatment (C), the experimental group A; pre-treatment (D), 1 week after treatment (E), 2 weeks after treatment (F), the experimental group B; pre-treatment (G), 1 week after treatment (H), 2 weeks after treatment (I)).

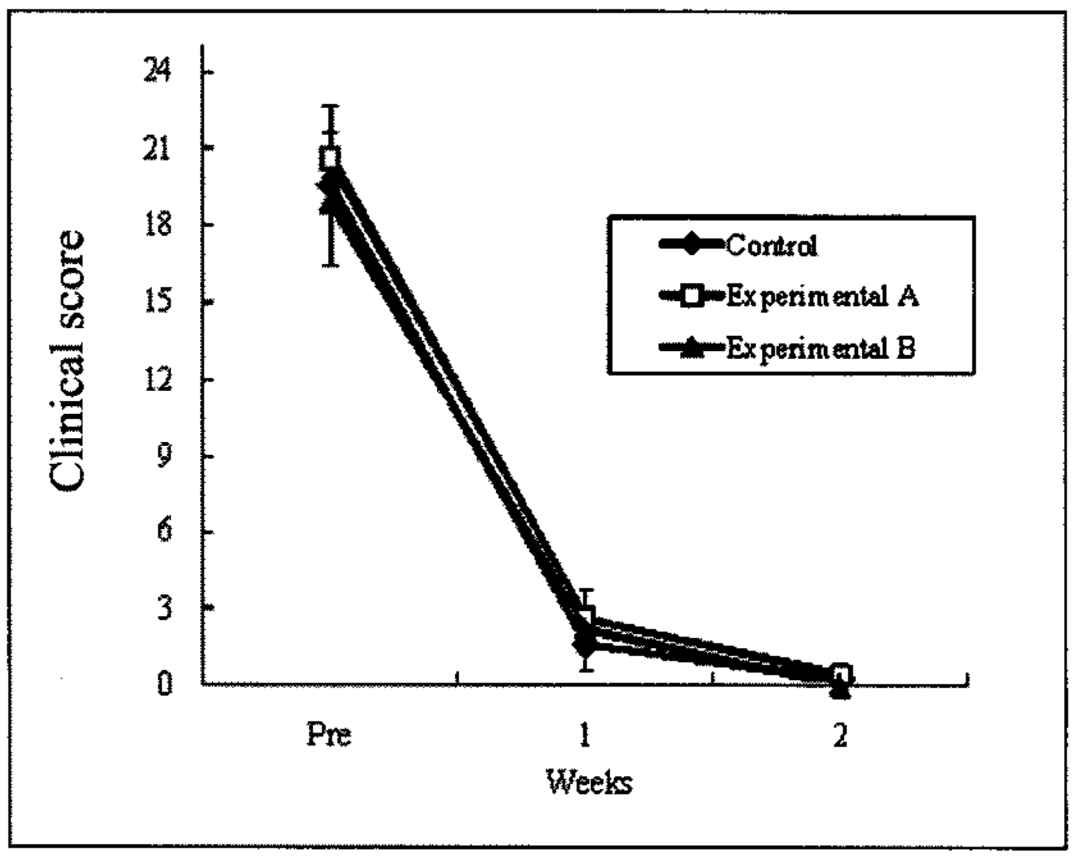

Fig 3. The change of clinical signs in control and experimental groups (*significant difference between control group and experimental group B ($p < 0.01$)).

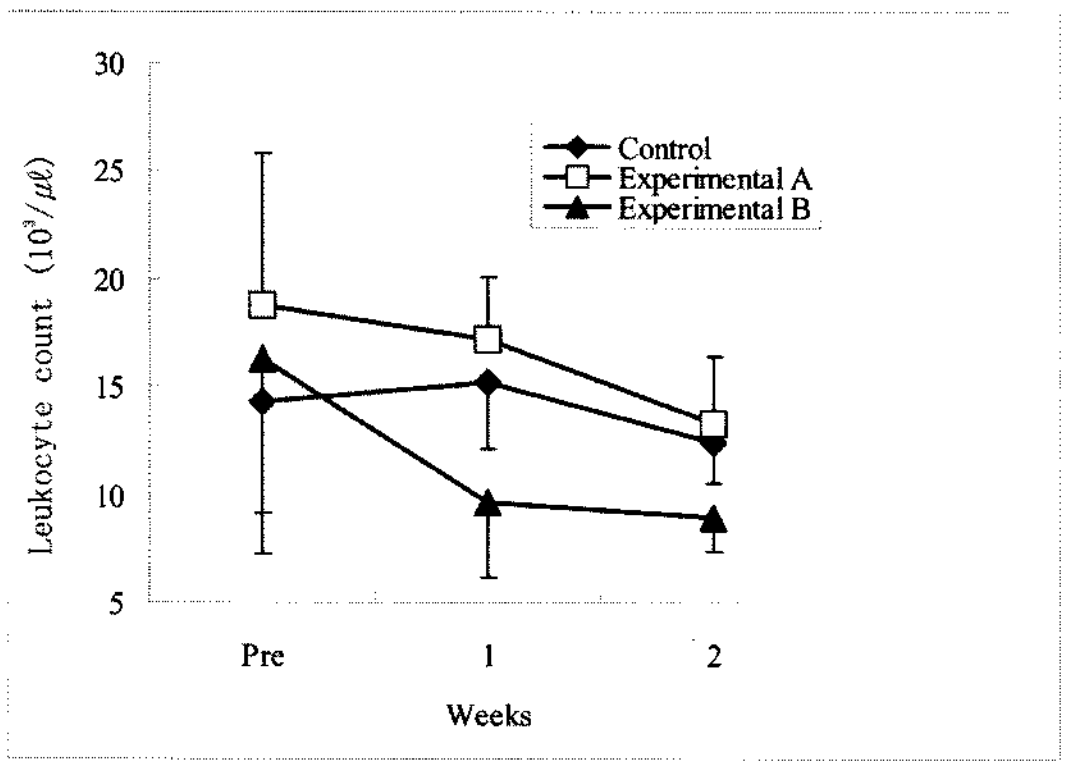

Fig 4. The change of total leukocyte counts with treatment in control and experimental groups.

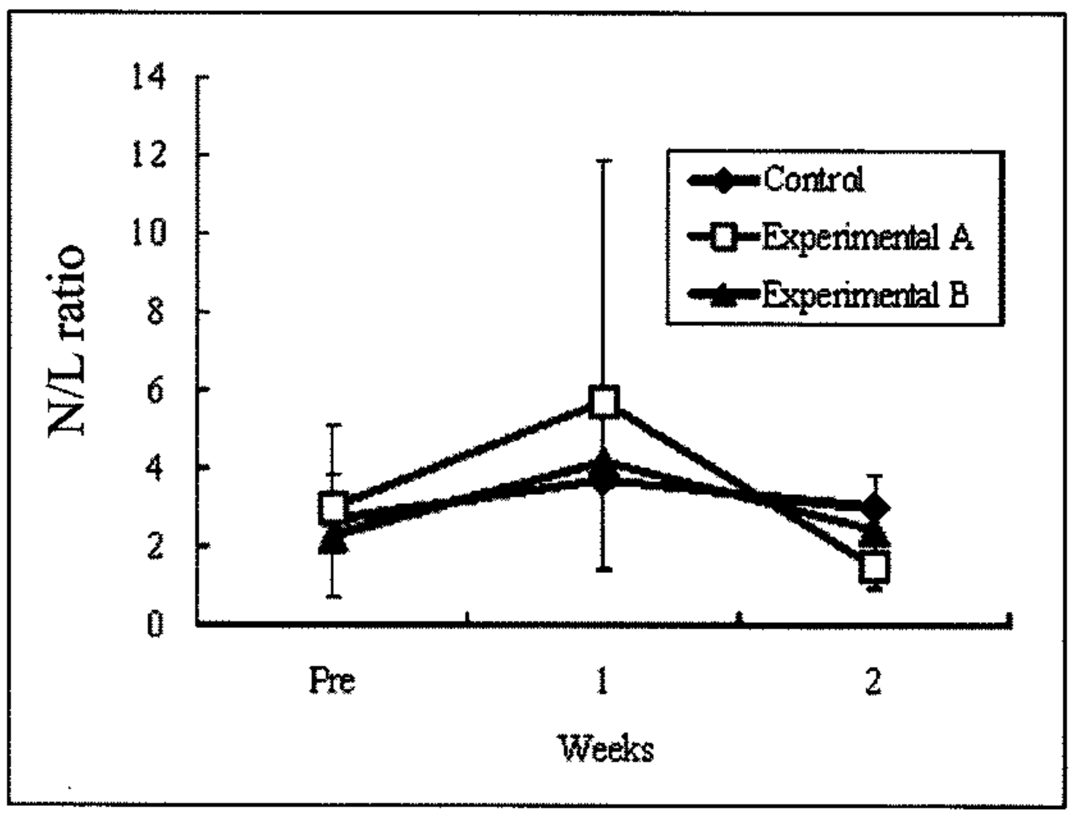

Fig 5. The change of neutrophil/lymphocyte (N/L) ratios with treatment in control and experimental groups.

score found 2 weeks after treatment in experimental group B ($p < 0.01$) (Fig 3).

Blood WBC counts and N/L ratio

The blood WBC counts and N/L ratios were measured using a blood cell counter analyzer. There was no significant difference found in the changes in the blood WBC counts (Fig 4) and N/L ratios (Fig 5) of the experimental groups, compared with those of the control.

Discussion

COE is a common disease occurring in 15% to 20% of dogs in veterinary clinical practice (7,8). There are various treatments for COE, which include antibiotics, antifungals, parasiticides and glucorcoticoid, administered topically or systemically (9,30,31). Surgery is essential in the case of chronic otitis externa (24).

Muller and Heusinger (27) reported that the most common bacteria species were coagulase-positive *Staphylococci, Pseudomonas* spp., beta-hemolytic *Streptococci* and *Proteus* spp. with *Staphylococci* being the most common isolate out of 413 swabs from dog and cat ears with otitis externa. In addition, the yeast *Malassezia pachydermatis* was isolated from the ears of 76% of the dogs with COE, often in combination with *Staphylococcus intermedius* bacteria (20). Chon *et al.* (5) also examined the prevalence of *Malassezia pachydermatis* (*M. pachydermatis*) from 589 dogs with COE at Chonbuk area in Korea, and reported a 36.7% (243/589 dogs) rate of infection with *M. pachydermatis* as well as a significantly higher prevalence of *M. pachydermatis* in 1 to 2 year old, pendulous ear dogs in the summer. Although the detection rate of the causative agent was slightly different, the agents identified in this study were similar to those reported previously.

Morris (26) reported that the medical regimens for the successful treatment of infectious otitis might vary widely according to the degree of pathologic changes in the external ear canals, the status of the tympanic membranes and the specific microorganisms involved, and that chronic otitis media is problematic, because an impaction of the bullae might involve antibiotic-resistant bacterial organisms.

On the other hand, Hariharan *et al.* (12) examined the *in vitro* susceptibility of a total of 1,819 bacterial isolates from canine and 103 isolates from feline otitis externa cases over 5 years. They reported that 90% of isolates were susceptible to gentamicin. The susceptibility of *Pseudomonas aeruginosa* to gentamicin and polymyxin B was 85% and 100%, respectively. Lilenbaum *et al.* (25) reported that resistance to antibiotics was frequently observed, with 90.9% of the isolates showing resistance to at least one drug, and resistance to three or more different drugs was a common finding, which has been observed with 16 strains (36.4%) of coagulase-positive and coagulase-negative *Staphylococci.* In addition, Guedeja-Marron *et al.* (10) reported an increased resistance to antibiotics, based on the results from the susceptibility of the bacterial isolates from chronic COE to twenty antibiotics.

New approaches including topical application of bacitracin or chloramphenicol and a systemic therapy with cephalosporins by Guedeja-Marron *et al.* (10), beta-thujaplicin ear drops for *Malassezia*-related COE by Nakano *et al.* (28), the new pH-balanced, propylene glycol-free test ear cleanser (Epiotic Advanced, Virbac) by Reme *et al.* (29) and the ear cleanser containing 2.5% lactic acid and 0.1% salicylic acid by Cole *et al.* (6) have been made in an attempt to solve the problem of antibiotics-resistance and to increase therapeutic success for treatment of COE.

It is known that AP treatments including needle-AP, injection-AP, laser-AP and moxibustion are effective in treating various human and animal diseases (13,15-18,35). In particular, it was demonstrated that the therapeutic effects by injection-AP were superior to those by a conventional injection with the same drugs (13,16-18).

In this study, the authors examined the therapeutic effect of injection-AP with apitoxin for treatment of COE. The clinical symptoms of the dogs with COE were improved considerably by injection-AP with apitoxin, with superior results to those of a treatment with susceptible antibiotics.

The main component of apitoxin, mellitin, stimulates the hypophyseal-adrenal system and releases 100 times more cortisone than hydrocortisone (19,33). Mellitin also stabilizes the lysosomal cell membrane against inflammation (19). Like mellitin, apamin releases cortisol and inhibits the complement (C3) system that is involved in the inflammatory process (19,32). Peptide 401 blocks arachidonic acid and inhibits prostaglandin synthesis (1,11). Adolapin inhibits the microsomal cyclo-oxygenase system and has been shown to be 70 times stronger than indomethacin in animal models (19). It also inhibits platelet lipoxygenase, which is involved in the production of hydroperoxy-eicotetranonic acid and leukotrienes. Furthermore, adolapin inhibits thromboxane and prostacycline, which are activated during the inflammation process (21). Protease inhibitors inhibit carrageenin, prostaglandin E1, bradykinin and histamine-induced inflammation as well as chymotrypsin and leucineaminopeptidase (19).

Antibiotic-resistance is a problemic issue to solve in public heath and animal welfare. Therefore, identifying alternative treatment methods such as apipuncture is very important in veterinary clinical practice. However, adverse effects such as anaphylactic shock through the application of apipuncture can occur (19). Therefore, broad investigations including the effective dosage of apitoxin and the use of main component of apitoxin, mellitin, are needed.

In conclusion, apipuncture is an effective treatment for COE and might be an alternative method for treating COE.

References

1. Billingham ME, Morley J, Hanson JM, Shipolini RA, Vernon CA. An anti-inflammatory peptide from bee venom. Nature 1973; 245: 163-164.
2. Chang YH, Bliven ML. Anti-arthritic effect of bee venom. Agents Actions 1979; 9: 205-211.
3. Choi SH, Cho SK, Cui XH, Kang SS, Pak SC. Therapeutic effect of bee venom in calves with bacterial diarrhea. J Vet Clin 2000; 17: 57-61.
4. Choi SH, Kang SS, Cui XH, Pak SC, Joeng SI, Cho SK. Therapeutic effects of natural bee venom in sows with mastitis, metritis and agalactia syndrome. J Vet Clin 1999; 16: 397-403.
5. Chon SK, Park YJ, Kim HE, Kim NS. Prevalence of *Malassezia pachydermatis* in canine otitis externa in Chonbuk. J Vet Clin 2006; 23: 1-5.
6. Cole LK, Kwochka KW, Kowalski JJ, Hillier A, Hoshaw-Woodard SL. Evaluation of an ear cleanser for the treatment of infectious otitis externa in dogs. Vet Ther 2003; 4: 12-23.
7. Craig EG. Infectious disease of the dog and cat. In: Otitis Externa, WB Saunders Corp, Philadelphia 1998: 549-554.
8. Danny W, William H, Craig EG. Disease of otitis externa. In: Muller and Kirk's Small Animal Dermatology, WB Saunders Corp, Philadelphia 1995: 927-943.
9. Gotthelf LN. Small animal ear diseases: an illustrated guide. WB Saunders Corp, Philadelphia 2000: 45-112.
10. Guedeja-Marron J, Blanco JL, Ruperez C, Garcia ME. Susceptibility of bacterial isolates from chronic canine otitis externa to twenty antibiotics. Zentralbl Veterinarmed B 1998; 45: 507-512.
11. Hanson JM, Morley J, Soria-Herrera C. Anti-inflammatory property of 401 (MCD-peptide), a peptide from the venom of the bee. Apis Mellifera Br J Phamacol 1974; 50: 383-392.
12. Hariharan H, Coles M, Poole D, Lund L, Page R. Update on antimicrobial susceptibilities of bacterial isolates from canine and feline otitis externa. Can Vet J 2006; 47: 253-255.
13. Hong MS, Lee JY, Lee B, Lee SE, Seo JM, Song KH, Kim DH, Cho KW, Kim MC. The effect of laserpuncture and aquapuncture with methionine on the recovery in artificially induced hepatic damaged rats. J Vet Clin 2002; 19: 125-131.
14. Kanno I, Ito Y, Okuyama S. Radioprotection by bee venom. Nippon Igaku Hoshasen Gakkai Zasshi 1970; 29: 1494-1500.
15. Kim DH, Liu J, Choi SH, Macmanus P, Jennings P, Darcy K, Burke F, Leorald N, Rogers P. Acupuncture treatment in a case with equine laminitis. Vet Clin 2006; 23: 6-8.
16. Kim DH, Liu J, Lee YW, Song KH, Kang SK, Choi HJ, Seo KM, Choi SH, Nam TC, Rogers P. Treatment of canine cervical and lumbar disc disease by injection-acupuncture. J Vet Clin 2006; 23: 65-68.
17. Kim DH, Liu J, MacManus P, Jennings P, Darcy K, Burke F, Slattery G, Rogers P. Injection-acupuncture with dexamethasone and modified moxibustion treatment of a downer cow. J Vet Clin 2006; 23: 69-71.
18. Kim DS, Liu J, Lee JY, Kim MC, Shin ST, Kim DH. The anti-emetic effect acupuncture and aqua-acupuncture at PC-6 and BL-21 on the emesis by xylazine in dogs. Kor J Vet Res 2005; 45: 399-403.
19. Kim MH. The main component of bee venom. In: Bee Venom Therapy and Bee Acupuncture Therapy, Korean Education and Planning Co, Seoul 1992: 67-132.
20. Kiss G, Radvanyi S, Szigeti G. New combination for the

therapy of canine otitis externa. I. Microbiology of otitis externa. J Small Anim Pract 1997; 38: 51-56.
21. Kovurova KL, Michailova SG, Shkenderov SV. Further investigation on the anti-inflammatory properties of adolapin-bee venom polypeptide. Acta Physiol Pharmacol Bulg 1985: 11: 50-55.
22. Kwon YB, Lee HJ, Han HJ, Mar WC, Kang SK, Yoon OB, Beitz JH. The water-soluble fraction of bee venom produces antinoceptive and anti-inflammatory effects on rheumatoid arthritis in rats. Life Sci 2002; 71: 191-204.
23. Lee JD, Kim SY, Kim TW, Lee SH, Yang HI, Lee DI, Lee YH. Anti-inflammatory effects of bee venom on type ÉÉ collagen induced arthritis. Am J Chin Med 2004; 32: 361-367.
24. Lee SG. The result of surgical treatment of chronic otitis externa in dog. J Vet Clin 1997; 14: 99-102.
25. Lilenbaum W, Veras M, Blum E, Souza GN. Antimicrobial susceptibility of staphylococci isolated from otitis externa in dogs. Lett Appl Microbiol 2000; 31: 42-45.
26. Morris DO. Medical therapy of otitis externa and otitis media. Vet Clin North Am Small Anim Prac 2004; 34: 541-555.
27. Muller E, Heusinger A. Microbiological results of ear swabs from dogs and cats. Tierarztl Prax 1994; 22: 80-84.
28. Nakano Y, Matsuo S, Tani H, Sasai K, Baba E. Therapeutic effects of beta-thujaplicin eardrops on canine *Malassezia*-related otitis externa. J Vet Med Sci 2006; 68: 373-374.
29. Reme CA, Pin D, Collinot C, Cadiergues MC, Joyce JA, Fontaine J. The efficacy of an antiseptic and microbial anti-adhesive ear cleanser in dogs with otitis externa. Vet Ther 2006; 7: 15-26.
30. Rick L, Ronald D, James H. The external Ear Canal. In: Diagnostic Cytology and Hematology of the Dog and Cat, Mosby Inc, St Louis 1999: 83-87.
31. Rosser EJ. Causes of otitis externa. Vet Clin North Am Small Anim Pract 2004; 34: 459-468.
32. Shkenderov S, Kovurova K. Adolapin-a newly isolated analgesic and anti-inflammatory polypeptide from bee venom. Toxicon 1982; 20: 317-321.
33. Vick JA, Shipmann WH. Effects of whole bee venom and its fractions (apamin and melittin) on plasma cortisol levels in the dog. Toxicon 1972; 10: 377-380.
34. Yeo SW, Seo JC, Choi YH, Jang KJ. Induction of the growth inhibition and apoptosis by bee-venom in human breast carcinoma MCF-7 cells. J Kor Acu Mox Soc 2003; 20: 45-62.
35. Yoon YH, Liu J, Kim KW, Kim YS, Kim JY, Jang SH, Nam HJ, Song KH, Kim MC, Kim DH. The anti-emetic effect of needling acupuncture, aquapuncture and moxibustion at BL-20 and LIV-13 on the xylazine induced emesis in dogs. Kor J Vet Res 2005; 45: 297-302.