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Efficacy of Enrofloxacin and Silver Sulfadiaznine Topical Otic Suspension for the Treatment of Canine Otitis Externa

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Abstract : The aim of this study was to evaluate the in vivo and in vitro efficacy of enrofloxacin-silver sulfadiazine (Baytril® otic, Bayer, USA) for the treatment of otitis externa in dogs. Twenty-four dogs with otitis externa were included in this double-blinded, randomized study. The experimental group was treated with the Baytril® otic and the distilled water was applied to the control group. Both groups were administered each solution twice daily for 7 days and next 7 days off treatment. On days 0, 7 and 14, clinical signs, bacteriological and fungal counts were graded using semi-quantitative scales, respectively. For the evaluation of in vitro efficacy of Baytril® otic, we also performed Minimal Inhibitory Concentration (MIC) test by agar dilution method against *Staphylococcus pseudintermedius*, *Pseudomonas aeruginosa* and *Malassezia pachydermatis*. In the experimental group, the sum of clinical scores was decreased 81.0% and microbial scores were significantly reduced 87.0% at days 14, compared with day 0. The results of MIC testing were showed the concentration of enrofloxacin and silver sulfadiazine in Baytril® otic is high enough to kill for 3 infectious agents. No adverse reactions were observed in any of the dogs during this study. These results suggest that Baytril® otic are efficient and safe treatment for canine otitis externa.

Key words **:** Otitis externa, dog, enrofloxacin, Baytril®otic.

Introduction

Otitis externa is an acute or chronic inflammatory disease of externa ear canal and common in small animal clinical practice (4,7). Otitis externa may result from numerous causes and they are divided three general categories : Predisposing conditions, primary causes, and perpetuating factors (15). Bacteria and yeasts are usually regarded as important perpetuating factors can maintain, worsen, or prevent resolution of otitis externa. Small number of gram-positive cocci and yeast such as Malassezia can inhabit in the normal exteranl ear canals, however, overgrowth of this organisms occurs following alteration of the microenvironment seconday to underlying primary disease and they stimulate an inflammatory response and worsen clinical disease (2,10,11,16). Consequently affected dogs present with otic pruritus, pain, otic discharge, erythematous and swollen (4,7). So cytological examination to detect increasing the number of infectious organisms and clinical evaluation of ear canal inflammation and change are useful method of identify otitis externa.

For treatment of otitis externa, veterinarians can try systemic and topical therapeutics. Although systemic therapeutic is useful treatment for eroded or ulcerated cases, systemic antibiotics may not achieve sufficient tissue concentrations to kill infectious organisms and to prevent antibiotic resistance. So topical therapy is a key to successful resolution of otitis externa which is essentially a surface infection (14). In addition, the choice of antibiotics based on culture and susceptibility testing is valuable to achieve a high enough concentration of the appropriate drugs and prevent infectious organisms obtain antimicrobial resistance.

The test product used in this study contains enrofloxacin and silver sulfadiazine. Enrofloxacin, one of the fluroquinolones class have good activity against a wide range of gram negative bacilli and gram positive cocci such as *Staphylococcus pseudintermedius*. Especially this drug has long been used for resistant Pseudomonas otitis (1,12,13,14,19,20). Silver sulfadiazine is a sulfonamide compound with antibacterial and antimycotic properties used topically in veterinary medicine. Silver sulfadiazine as well as enrofloxacine has shown excellent activity against *Pseudomonas aeruginosa.* It has also activity against *Malassezia pachydermatis* despite lower in vitro activity than other antimycotic agents (14,17,19).

The aim of this study was to evaluate the in vivo and in vitro efficacy of enrofloxacin-silver sulfadiazine (Baytril® otic, Bayer, USA) for the treatment of otitis externa in dogs by using clinical and cytological scoring and MICs test.

Materials and Methods

Animals

Twenty four dogs affected with otitis externa qualified for

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this study. It was conducted at one veterinary teaching hospital and 2 local veterinary clinics. They were diagnosed otitis externa based on clinical examination and cytological examination. Prior to first topical instillation, ear cleaning was performed by using warmed normal saline.

Treatment

As a general guide of Baytril otic, in the experimental group, dogs weighing less than 16 kg would receive 5-10 drops per treatment while those weighing greater than 16 kg would receive 10-15 drops per treatment. To control groups, 0.9% saline was applied. Both groups were administered each solution twice a day for 7 days and off of treatment for the following 7 days.

Clinical evaluation

Clinical examination and scoring were consistently performed by the same investigator. The investigator examined otoscopically and scored at pre-treatment (Day 0), final treatment (Day 7) and post-treatment (Day 14). During each examination, ears were examined with 8 clinical signs (head shaking, pruritus, excoriation, pain, exudate, erythema, stenosis, suppuration) and was recorded each by clinical score based on severity (range: 0 to 3, except head shaking scored from 0(none) to 1(present)). Every adverse event had to be reported.

Cytological examination

For cytological examination, samples of ear exudate were collected from the external auditory canal using a cotton swab moistened with sterile normal saline. Each sample was smeared onto a clean slide, heat fixed and stained with Diff-Quik. Cytological examination was also performed by the same investigator. The investigator scanned the slide to detect and evaluate infectious organisms at high magnification field (× 1,000). The scoring was estimated by using a semi-quantitative scale for each organism count (Table 1).

Table 1. Semi-quantitative scale for bacterial and fungal counts recorded at cytology from ear swab

Organism	Count/hpf	Score
Malassezia yeasts	0	0
	1-2	1
	3-8	2
	> 8	3
Rods	0-2	0
	3-8	1
	9-40	2
	> 40	3
Cocci	0-2	0
	3-8	1
	9-40	2
	> 40	3

Minimum Inhibitory Concentrations (MICs) test

Samples of ear exudate were cultured aerobically on Sheep blood agar, MacConkey agar and Sabouraud dextrose agar. Bacteriological cultures were grown on blood agar supplemented with 5% sheep blood and MacConkey agar at 37ºC for 18-24 hours. Mycological cultures were grown on Sabouraud dextrose agar at 28ºC for 48-72 hours. Identification of orgarnisms was made using polymerase chain reaction band method. Pure PCR product of 16S gene was obtained and sequencing. Then use NCBI (National Center for Biotechnology Information) BLAST to search the bacterial DNA nucleotide sequencing and identify the species that most closely matches their sequence. All isolates were tested concerning their MICs against Enrofloxacin, Silver sulfadiazine (SSD), and Enrofloxacin/SSD by agar dilution method. For MICs, powder form of Enrofloxacin (Bayer, Korea) and SSD (Sigma, USA) were diluted with Ammonium hydroxide. After pure culture were grown, bacterial isolates were inoculated into Mueller Hinton Agar (Difco) at 37ºC for 18 hours and yeast were inoculated into Sabouraud Dextrose Agar (Difco) at 28ºC for 48 hours. All test agar plate were contained different concentrations of antimicorbial agent. Serial two-fold dilutions of enrofloxacin, SSD and Enrofloxacine/SSD in concentrations from 0.06-512 μg/ml, 0.06-512 μg/ml and 0.006/0.125-512/1024 μg/ml, respectively, were used to determine the in vitro susceptibility of the microbial organisms.

Statistical Analysis

All analysis was performed using commercial statistical software (SigmaPlot for Windows version 12.0, Systat Software, Inc.). The significance threshold was set to $P < 0.05$. Each clinical and cytological score was calculated the average and compared the changes between before treatment (Day 0) and after treatment (Day 7, Day14). Score reducing was expressed by percentage reduction method. One-way ANOVA was used to identify significant relationships of clinical and cytological scroe changes. In treatment group, scores of first visit (Day 0), second visit (Day 7) and final visit (Day 14) were compared.

Results

Twenty four dogs qualified for study enrollment (4 in the control group and 20 in the experiment group). Patient ages ranged from 1 year to 10 years. Eight different breeds were represented with Beagle (10/42%), Maltese (8/34%), Cocker Spaniel (1/4%), Poodle (1/4%), Chihuahua (1/4%), Pug (1/4%), Yorkshire Terrier (1/4%) and Shih Tzu (1/4%). No side effects by the eardrop were reported in control and treatment group.

Clinical results

Mean total clinical score was not significantly changed in the control group (Table 2). In the Experimental group, mean clinical score was reduced 64.5% between pre-treatment (Day

Table 2. The total clinical score (group average) by treatment and day

	N	Pre-treatment	Final treatment	Post-treatment
Control group	4	13.25	14.75	13.5
Experimental group	20	14.50	5.15	2.75

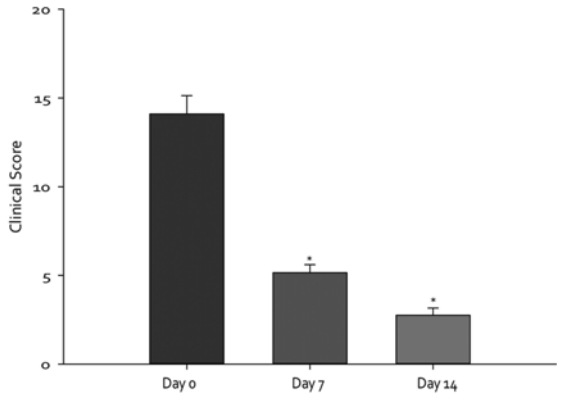

Fig 1. Clinical score change in Baytril Otic^{R} treatment. Mean global clinical score was reduced 64.5% between Day 0 (14.5) and Day 7 (5.15) (*p < 0.05). At Day 14 (2.75) mean clinical score was reduced 81.0% (*p < 0.05).

0) and final treatment (Day 7) ($p < 0.05$). At the post-treatment (Day 14) the mean clinical score was reduced 81% compared to pre-treatment ($p < 0.05$) (Fig 1).

Between Day 0 and Day 7, scores of stenosis (64.9%), head shaking (75.0%), suppuration (70.3%), pruritus (61.7%), pain (56.7%), erythema (58.59%), excoriation (60.6%), and exudates (73.9%) were decreased. Between Day 0 and Day 14, each clinical score were decreased range from 68.0% to 94.6%. P value between Day 0 and Day 14 was under 0.05 in all scores (*p < 0.05) (Fig 2,3).

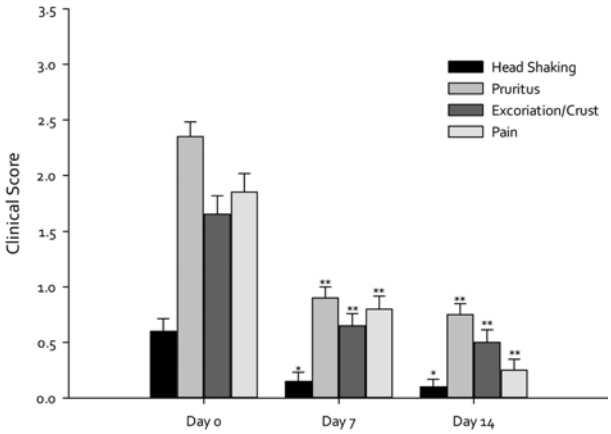

Fig 2. Clinical score change in Baytril Otic Treatment Group (*p < 0.05, **p < 0.01).

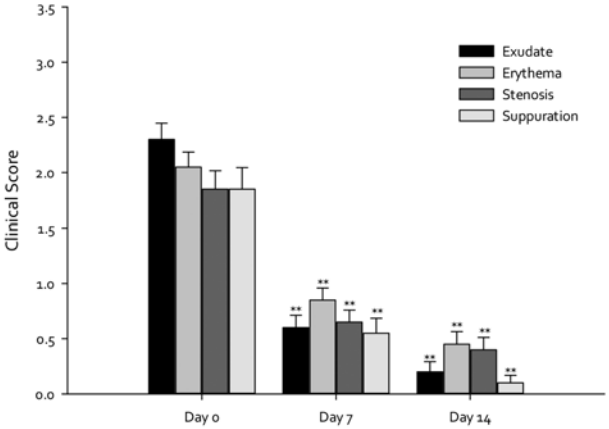

Fig 3. Clinical score change of ear canal in Baytril Otic Treatment Group (*p < 0.05, **p < 0.01).

Cytological results

In experimental group cytological recovery was achieved (Table 3). Between Day 0 and Day 7, Malassezia (76.2%), cocci (89.3%) and rod (57.19%) were improved. At Day 14, Malassezia (85.7%), rod (85.7%) and cocci (89.3%) infection were significantly reduced compare to Day 0 ($p < 0.005$) (Figs 4,5). In control group, there are no significant difference between Day 0 and Day 14.

Microbiology results

In this investigation, 56 microbiological specimens obtained

Table 3. The cytological score (group average) by treatment and day

	N	Pre-treatment	Final treatment	Post-treatment
Control group	4	4.25	4.00	4.25
Experimental group	20	3.85	0.80	0.50

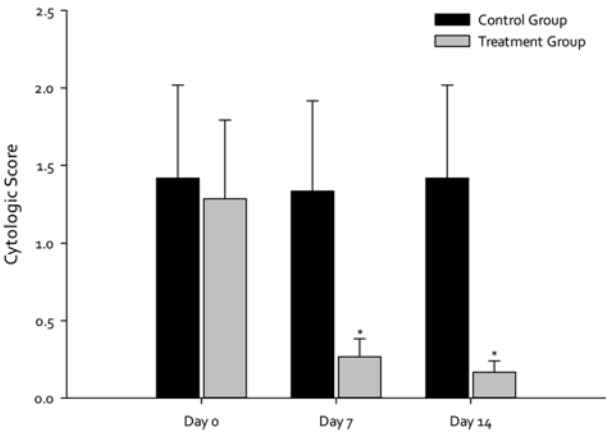

Fig 4. Cytological score change in Baytril otic treatment group and control group (*p < 0.05).

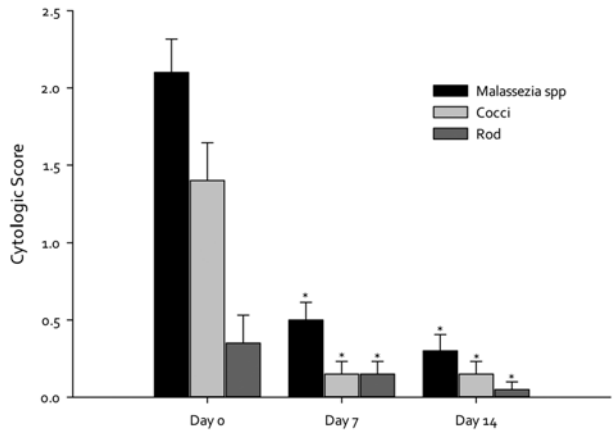

Fig 5. Cytological score change in Baytril Otic treatmentaccording to the orgnisms (*$p < 0.05$).

from 24 case of unilateral and bilateral otitis externa. Two of the samples produced 'no growth'. The remaining 54 samples yielded 29 bacteria and 25 yeast for a total of 54 microbial isolates.

A total of 24 *S. pseudintermedius*, 4 *P. aeruginosa,* 1 *Proteus spp*, and 25 *M. parchydermatis* isolates were identified. The breakpoints were easily determined in all strains.

The results are shown in Table 4. Enrofloxacin did not inhibit in vitro growth of Malassezia spp at concentration up to 2 μg/ml. Enrofloxacin exhibited in vitro activity against the bacteria *Psedomonas aeruginosa* under 16 μg/ml. SSD exhibited its lowest in vitro activity against Malassezia spp at the 100 μg/ml.

Discussion

The test solution, Baytril otic is combination of Enrofloxacine and Silver sulfadiazine in the ratio of 1:2. Each compound of this solution were used empirically for treating of canine otitis externa (15). This study was designed to demonstrate the efficacy of Enrofloxacin-Silver sulfadiazine topical otic combination for treatment otitis externa in dogs.

Acute or chronic inflammation of externa ear canal is common in small animal veterinary practice (4,8). The dogs with otitis externa was shown variable change of behaviors and histology. The most common clinical sign is head shaking and scratching (7). The histological features of affected ear by otitis externa are hyperplasia and inflammation of epidermis, dermis, and hair follicle. These changes lead to stenosis, erythema, exudate and suppuration (3,9,16). In this study, all the dogs had erythema and exudate. This was expected result since these are the most frequent signs in otitis externa of dogs (16). Compared to control group, in experimental group, significant reduction of the clinical score were detected. At Day 7, all scores were decreased range from 56.8% to 75%. Between Pre-treatment (Day 0) and Post-treatment (Day 14), exudate (91.3%) and suppuration (94.6%) were reduced dramatically. Three cases got the no score at Day 14.

Staphylococcus spp. and *M. pachydermatis* were most frequent isolated bacteria and yeast from normal canine ear canals. Rods such as Proteus are rarely found in normal ear swab but can be existed (2,6,18). According to previously study, *S. pseudintermedius* and *M. pachydermatis* are most common pathogens from dogs with otitis externa (21). In this present investigation, *M. pachydermatis* was observed in 95.8% (23/24) cases and *S. pseudintermedius* was existed in 87.5% (21/24) cases. The majority of case were infected more than one pathogen (83.3%, 20/24). The most frequent multiple infection case was *M. pachydermatis* + *S. pseudintermedius*. These findings agreed with the previous studies (16,21) In this study the cytological score in experimental group decreased significantly after treatment. Between Day 0 and Day 14, Malassezia (85.7%), cocci (89.3%), and rods (85.7%) were improved. Especially, 10 cases got the score 0 point against *Malasessia* and in 17 cases cocci and rods were 100% reduction on Day 7 or Day 14.

In topical therapy, selection of appropriate antibiotics and

Table 4. Minimum Inhibitory Concentration (μg/ml) for Enrofloxacin, Silver sulfadiazine and Enrofloxacin/Silver sulfadiazine

Organisms	n	Antimicrobial	MIC range (μg/ml)	MIC50	MIC90
Staphylococcus pseudintermedius	24	Enrofloxacin	0.06-512	16	32
		SSD	16-32	32	32
		Enrofloxacin/SSD	< 0.06/ < 0.0125-16/32	N	N
P. aeruginosa	4	Enrofloxacin	4-16	N	N
		SSD	32	N	N
		Enrofloxacin/SSD	8/16	N	N
Proteus spp.	1	Enrofloxacin	< 0.06	N	N
		SSD	8	N	N
		Enrofloxacin/SSD	0.25/0.5	N	N
Malassezia parchydermatis	25	Enrofloxacin	1-4	2	2
		SSD	64	64	64
		Enrofloxacin/SSD	1/4-4/8	N	N

using sufficient volume are key to the successful resolution of otitis externa (14). If patients receive insufficient concentration of drug, organisms can get the resistance against antimicrobials. Previous studies indicate that an increasing resistance to antimicrobials presents problems in the treatment of otitis externa (7,10,11,12). Drug resistant veterinary pathogens make otitis externa get worsen and develop to chronic stage. Therefore, in order to apply adequate volume, the MICs test can be meaningful in the treatment of otitis externa. In the present study, 54 strains were isolated and identified from ear swab. All isolates were tested concerning their MICs against Enrofloxacin, SSD, and Enrofloxacin/SSD by agar dilution method. Compare to previous studies (5,11,17), Silver sulfadiazine had a similar in vitro activity against all isolates strains. Enrofloxacine show good efficacy against most of isolates, especially *Malassezia parchydermatis*. For all isolates, the combination of Enrofloxacin and SSD show marked efficacy compared to either of its components individually. This clear synergistic effect allows to reduce antibiotic concentrations and minimize the possibility of resistances to these drugs.

The result of clinical and cytological examination demonstrate that Baytril otic is effective for the treatment of otic externa complicated by the presence of *M. parchydermatis*, *S. pseudintermedius*, *P. aeruginosa*, and *P. mirabilis*. The data of MIC test show that the concentrations of two drugs in Baytril otic exceed MIC value against all isolated organisms.

Therefore, the combination of Enrofloxacine and Silver sulfadiazine is a good choice for treatment canine otitis external.

Acknowledgement

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References

1. Anjum F, Mir A. Susceptibility pattern of pseudomonas aeruginosa against various antibiotics. Afr J Microbiol Res 2010; 4: 1005-1012.
2. Campbell JJ, Coyner KS, Rankin SC, Lewis TP, Schick AE, Shumaker AK. Evaluation of fungal flora in normal and diseased canine ears. Vet Dermatol 2010; 21: 619-625.
3. Cole LK. Anatomy and physiology of the canine ear. Vet Dermatol 2010; 21: 221-231.
4. Ettinger SJ, Feldman EC. Textbook of veterinary internal medicine: Diseases of the dog and the cat. St. Louis, Mo: Elsevier Saunders; 2010.
5. Freedom of information summary original new animal drug application NADA 141-176 Baytril Otic 2006.
6. Ginel PJ, Lucena R, Rodriguez JC, Ortega J. A semiquantitative cytological evaluation of normal and pathological samples from the external ear canal of dogs and cats. Vet Dermatol. 2002; 13: 151-156.
7. Harvey RG, Delauche AJ, Harari J. Ear diseases of the dog & cat. London: Manson Publishing; 2005:
8. Hill PB, Lo A, Eden C, Huntley S, Morey V, Ramsey S, Richardson C, Smith DJ, Sutton C, Taylor MD, Thorpe E, Tidmarsh R, William. Survey of the prevalence, diagnosis and treatment of dermatological conditions in small animals in general practice. Vet Rec 2006; 158: 533-539.
9. Huang H, Little CJL, McNeil PE. Histological changes in the external ear canal of dogs with otitis externa. Vet Dermatol 2009; 20: 422-428.
10. Keskin O, Tel O, Kaya NA. Aerobic bacteria and fungi isolated from external ear canal of healthy dogs and the antibiotic susceptibility of staphylococci. J Anim Vet Adv 2010; 9: 496-500.
11. Lyskova P, Vydrzalova M, Mazurova J. Identification and antimicrobial susceptibility of bacteria and yeasts isolated from healthy dogs and dogs with otitis externa. J Vet Med A Physiol Pathol Clin Med 2007; 54: 559-563.
12. Martín Barrasa JL, Lupiola Gómez P, González Lama Z, Tejedor Junco MT. Antibacterial susceptibility patterns of pseudomonas strains isolated from chronic canine otitis externa. J Vet Med B Infect Dis Vet Public Health 2000; 47: 191-196.
13. Metry CA, Maddox CW, Dirikolu L, Johnson YJ, Campbell KL. Determination of enrofloxacin stability and in vitro efficacy against staphylococcus pseudintermedius and pseudomonas aeruginosa in four ear cleaner solutions over a 28 day period. Vet Dermatol 2012; 23: 23-28.
14. Morris DO. Medical therapy of otitis externa and otitis media. Vet Clin North Am Small Anim Pract 2004; 34: 541-555.
15. Oliveira LC, Leite CA, Brilhante RS, Carvalho CB. Comparative study of the microbial profile from bilateral canine otitis externa. Can Vet J 2008; 49: 785-788.
16. Saridomichelakis MN, Farmaki R, Leontides LS, Koutinas AF. Aetiology of canine otitis externa: A retrospective study of 100 cases. Vet Dermatol 2007; 18: 341-347.
17. Schmidt A. In vitro activity of climbazole, clotrimazole and silver-sulphadiazine against isolates of malassezia pachydermatis. Zentralbl Veterinamed B 1997; 44 :193-197.
18. Tater KC, Scott D, Miller W, Erb H. The cytology of the external ear canal in the normal dog and cat. J Vet Med A 2003; 50: 370-374.
19. Trott D, Moss S, See A, Rees R. Evaluation of disc diffusion and MIC testing for determining susceptibility of pseudomonas aeruginosa isolates to topical enrofloxacin/silver sulfadiazine. Aust Vet J 2007; 85: 464-466.
20. Turkyilmaz S. Antibiotic susceptibility patterns of pseudomonas aeruginosa strains isolated from dogs with otitis externa. Turk J Vet Anim Sci 2008; 32: 37-42.
21. Zur G, Lifshitz B, Bdolah-Abram T. The association between the signalment, common causes of canine otitis externa and pathogens. J Small Anim Pract 2011; 52: 254-258.

개 외이염에 대한 Enrofloxacin과 Silver Sulfadiazine 국소제제의 치료효과

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요 약 : 본 연구는 개 외이염에 대한 enrofloxacin-silver sulfadiazine (Baytril® otic, Bayer, USA)의 치료효과를 평가하는 것이다. 24두의 외이염 이환견을 대상으로 처치군에는 바이트릴 오틱을, 대조군에는 증류수를 이도내 7일간 국소투약한 후 7일후에 임상증상, 미생물 및 효모균수를 통해 효과를 비교하였다. 또한 외이염에서 분리된 세균 및 효모균에 대한 바이트릴 오틱의 최소억제농도를 산출하였다. 주요 분리균으로는 *Staphylococcus pseudintermedius*, *Pseudomonas aeruginosa, Proteus spp* and *Malassezia pachydermatis*.이었다. 임상증상의 경감은 81%, 미생물수는 87% 감소하였다. 바이트릴 오틱은 분리된 균에 대한 살균효과가 충분하였고 국소투약에 대한 부작용은 관찰되지 않았다. 따라서 바이트릴 오틱은 개의 외이염 감염에 대한 효과적이며 안전한 제제로 판단된다.

주요어 : 외이염,,개, enrofloxacin, Baytril®otic