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Original Scientific Article

EFFECT OF TREATMENT WITH TRIS-EDTA / CHLORHEXIDINE TOPICAL SOLUTION ON CANINE *PSEUDOMONAS AERUGINOSA* OTITIS EXTERNA WITH OR WITHOUT CONCOMITANT TREATMENT WITH ORAL FLUOROQUINOLONES

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Summary: *Pseudomonas aeruginosa* (*P. aeruginosa*) infection in ear canals of dogs is associated with severe otitis externa (OE). Traditional use of antibiotics alone or in combination with topical antiseptic solutions to treat *Pseudomonas* OE is often associated with the development of antibiotic resistance. Tris-EDTA/chlorhexidine solution is very active against several species of gram-positive and gram-negative bacteria *in-vitro*. Therefore, *in-vivo* effect of topical antiseptic solution containing Tris-EDTA/chlorhexidine 0.15% on clinical and microbiological parameters in 18 dogs diagnosed with *Pseudomonas*- associated OE, with (n=12) or without (n=6) the concomitant use of oral fluoroquinolones, was evaluated over an eight week period.

Dogs were evaluated on days 0, 7, 14, 21, 28, 42 and 56. Clinical signs: pain, oedema, erythema and stenosis of the ear canal were evaluated and scored from 0 to 4. Overall combined score including all clinical signs was also calculated to show all clinical signs as an independent variable. Ear canal swabs and scrubs were used to assess for culture and fluoroqinolone sensitivity, parasitological examination and cytological evaluation. Linear mixed effects model and the logistic regression with the random effect were used to evaluate the effect of Tris-EDTA/chlorhexidine (time) and fluoroquinolones treatment on clinical, cytological and bacteriological variables.

Results indicated that the number of rod shaped bacteria and neutrophil granulocites decreased with time regardless of *fluoroqinolone* treatment ($P<0.05$). Similarly, all clinical signs were affected by Tris-EDTA/chlorhexidine treatment independently of fluoroqinolone treatment ($P<0.05$). Fluoroqinolone resistance was observed in *P. aeruginosa* in both, dogs that were, and those that were not treated with fluoroqinolones. Treatment with Tris-EDTA /chlorhexidine 0.15% combination solution seems to be beneficial for resolution of *Pseudomonas*- associated OE in dogs, which was independent of fluoroquinolone treatment.

Key words: otitis externa; dogs; *Pseudomonas aeruginosa*; Tris-EDTA; chlorhexidine; fluoroquinolones

Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) infection in an ear canal is usually associated with a severe otitis externa (OE) in dogs. Clinical features of *Pseudomonas* otitis include purulent discharge with severe erythema, ear canal erosions and ulcers with frequent bleeding, pain and discomfort. Tympanic membrane rupture can occur in cases assuming a chronic character, which is caused by proteolytic enzymes secreted by *P. aeruginosa,* and inflammatory cells' derived lizozymes. Such complications lead to otitis media (1,2).

Pathogenesis of canine otitis is complex and includes predisposing (narrow ear canals, high humidity, pendulous ear pinnae), primary (allergies, foreign bodies, ear mites and skin keratinisation defects as most common), secondary (bacteria, yeasts) and perpetuating factors (hyperplasia of the ear canal tissue, ear drum or middle ear mu-

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cosa due to chronic inflammation) (1). Primary factors only are capable of causing OE. Other factors in immunologically competent animals are required to combine their virulence with each other to be able to cause OE. Hypersensitivity reaction is the most common primary cause of canine OE (> 40%) (1). Predisposing, primary, secondary as well as perpetuating factors should be recognized and eliminated for successful treatment of canine OE. *Pseudomonas* infection in OE is considered to be of secondary nature.

Treatment of *Pseudomonas* otitis includes topical and systemic antibiotics, and regular ear irrigation with antiseptic solutions. Treatment with nonsteroidal anti-inflammatory drugs (NSAIDs) or glucocorticoids is recommended to control pain and inflammation (1). Treatments delivered topically are superior to systemic treatment regimens, because they achieve sufficient contact and drug therapeutic concentration (3, 4). Traditional use of antibiotics alone or in combination with antiseptic solutions to treat *Pseudomonas* OE is often associated with the development of antibiotic resistance, which increases the morbidity and the mortality of the disease, and predisposes the disease to assume chronic character (5, 6).

Tris-EDTA/chlorhexidine solution is very active against several species of gram-positive and gram-negative bacteria, and performs better than chlorhexidine solution alone (7, 8, 9). It also increases susceptibility of *P. aeruginosa* to enrofloxacin "*in vitro*" (10). Therefore, Tris-EDTA/chlorhexidine solution may prove to be beneficial for canine *Pseudomonas* OE patients (8, 9) and is unlikely to select for bacterial resistance (9).

The *in-vivo* activity of topical antiseptic solution containing Tris-EDTA/chlorhexidine against *Pseudomonas* OE in dogs has not yet been reported. Preliminary results of the recent multicenter double blind placebo controlled study reported good efficacy against purulent canine otitis. Specific species of bacteria involved were not reported (11). Therefore, the purpose of this study was to assess *in-vivo* effect of topical antiseptic solution containing Tris-EDTA/chlorhexidine 0.15% on clinical and microbiological parameters in dogs diagnosed with *Pseudomonas*- associated OE, with or without concomitant use of oral fluoroquinolones.

Material and methods

Eighteen private-owned dogs diagnosed with OE associated with *P. aeruginosa* infection were included with the owners's consent. Dogs diagnosed with hypothyroidism or demodicosis were not included in the study, nor were patients included that were diagnosed with any of diseases that might require treatment unrelated to otitis. Dogs with hypersensitivity to products used to treat OE were also excluded. The study was approved by the Animal Ethical Committee, Ministry of Agriculture of Republic of Slovenia, No. 4.4.-27/2010.

All dogs were subjected to detailed clinical examination, including blood analysis, before being included into the study. A detailed case history was taken, with special emphasis on previous antibiotic treatment. Pain, oedema, erythema and stenosis of the ear canal were evaluated independently by at least two clinicians from the same clinical background and scored from 0 to 4 (11). The average score was then used for analysis.

Overall combined score including all clinical signs was also calculated to show all clinical signs as an independent variable.

After otoscopic evaluation of the ear canal, two sterile cotton swabs were inserted into the lumen and swabbed against the surface of the ear canal at the junction between the vertical and horizontal ear canals where the cartilage bends at about a 45º angle (12). The first cotton swab was used for the culture and sensitivity testing and the second was rolled onto one glass slide that was later heat-fixed and stained with a modified Wright's stain (Diff-Quik^{R}, Median Diagnostics, Dudingen, Switzerland).

Epidermal scrub was made for parasitological examination from the vertical ear canal using ear curette. Dogs have had then their ears thoroughly rinsed with water saline for removal of the inflammatory debris. Treatment with topical saline solution is beneficial even though it can cause some ear canal maceration (13). The ear canal was dried up using suction via the catheter.

All dogs included in the study were treated with Tris-EDTA /chlorhexidine 0.15% combination solution (OtodineR, Industria Chimica Fine ICF, Palazzo Pignano, Italy) once daily. Oral antibiotic (ciprofloxacin or enrofloxacin, 5 mg/kg SID) and/or oral methylprednisolone (1,0 mg/kg SID for 5-7

days, than 0,5 mg/kg every other day) treatments were instituted by the attending practitioners/ clinicians based on their personal clinical preferences. Similarly, the decision to discontinue such treatment was also made independently of those involved in the study; however, it could have been supported by laboratory reports provided by the study group.

Other ongoing treatment regimens for included dogs were standardized including regular rinsing of the ear canal (days 0, 7, 14, 21, 28, 42, 56) with saline solution for removal of the inflammatory debris (2). Owners of the dogs were instructed to fill the infected ear canals with Tris-EDTA /chlorhexidine 0.15% solution once daily accompanied by a gentle two minute massage of ears towards the head.

Parasitological and cytological examination

Native smears of the epidermal scrubs were examined microscopically under low magnification (x40) for the presence of parasites. Cytological evaluation of the smears was performed in a semi-quantitatively manner (14), adjusted to an oil-immersion (x1000) magnification. Microscopic slides were screened at low (x100) magnification to locate most significant areas with cell monolayer. Inflammatory cells, rod shaped bacteria, cocci and yeasts were quantified in ten different oil-immersion fields (OIF, 1000x magnification). The average number was then calculated across all examined fields. Reference ranges were set and modified by the appropriate factor to adjust for 1000x magnification based on the study from 2002 by Ginel and coworkers (14). Therefore, equal or more than 2 yeasts, ≥ 10 cocci, > 0 rods and > 0 inflammatory cells per OIF were considered abnormal.

Bacteriological examination

Ear swabs were inoculated onto blood agar plates (Columbia agar supplemented with 5% sheep blood) and incubated aerobically at 37°C for 48+/-2 hours. After 24-h and 48-h incubation the plates were examined for the growth of *Pseudomonas spp.* or other pathogenic bacteria. Colonies morphologically consistent with *P. aeruginosa* were subcultured on fresh blood agar plates for subsequent identification.

Bacterial isolates were identified using methods described by Quinn and coworkers (15). The biochemical characteristics of isolates were determined using the commercial kit Api 20NE (BioMerieux, France).

Anti-microbial susceptibility testing

The cultured bacteria were tested for the enrofloxacine and ciprofloxacine susceptibility using the disk-diffusion method by Kirby-Bauer according to accepted guidelines (Clinical and Laboratory Standards Institute M100-S20).

Statistical analysis

The effect of treatment with antibiotic and time (Tris-EDTA /chlorhexidine 0.15% treatment) on the continuous outcomes of observed variables was verified with linear mixed effects model, where dogs were considered as a random effect. Interaction between treatment and time was also considered in the model. Variable methylprednisolone was included in the model as a controlling covariate in order to remove the possible confounding effect of this variable. The pre planed between-times and between-treatment comparisons were carried out with the contrast analysis.

The effect of time and treatment on binary outcomes was estimated with the logistic regression with the random effect. Like in the case of the continuous outcomes variable methylprednisolone was included as a controlling covariate to remove the confounding effect. The pre planed between-time and between-treatment differences were estimated with the contrast analysis. A p-value equal or less than 0.05 was considered as statistically significant. All analyses were performed with R language for statistical computing (version 2.8.1: R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.)

Results

Eighteen dogs (mean age: 8.3 years, mean weight 21.77 kg) were included in this study. Breeds included were German Shepherd (5/18), Shar Pei (4/18), Cocker Spaniel (3/18), Highland

Terrier (2/18), English Cocker Spaniel (1/18) and mixed-breed (3/18). Twelve dogs (12/18) were treated orally with antibiotics. Eleven dogs (11/18) were treated with methylprednisolone. All dogs had *P. aeruginosa* associated otitis externa at the beginning of the study. Atopic syndrome was determined to be the primary cause for the disease in seventeen dogs (17/18). The primary cause for the disease could not be determined in one dog (1/18).

Parasitology and cytology

No parasites were detected from ear canal epidermal scrubs from any of the patients.

All dogs (18/18) had rod shaped bacteria in their ear canal. Treatment with antibiotics had no effect (P=0.3), whereas time had a significant effect on the reduction of rod shaped bacteria in the ear canal (P<0.0001) (Table 1). Thirteen (13/18) dogs had rod shaped bacteria in their ear canals on day 56; from those eight (8/12) were and five (5/6) were not treated with oral fluoroqinolone.

Fourteen dogs (14/18) had cocci present in their ear canal at the beginning of the study. Treatment with antibiotics had no effect on the number of cocci in the ear canal (P=0.06). The number of cocci did not change over time (P=0.08) (Table 1). Six (6/18) dogs had cocci in their ear canals on day 56; from those four (4/12) were and one (1/6) was not treated with oral fluoroqinolone.

Seven dogs (7/18) had yeasts present in their ear canal. Neither, treatment with antibiotics (P=0.4) or time (P=0.4) had an effect on the presence of yeasts in the ear canal (Table 1). Only one dog (1/18) had yeasts in the ear canal on day 56; the dog was not treated with fluoroqinolones. Treatment with methylprednisolone had no effect on the number of rod shaped bacteria, cocci or yeasts in the ear canal (P□0.3).

Neutrophil granulocytes were detected in all dogs with *Pseudomonas* otitis (18/18). Their number was not affected by the treatment with antibiotic (P=0.6); however, the number of *neutrophil* granulocytes reduced over time (P=0.0005) (Table 2). Fourteen (14/18) dogs had *neutrophil* granulocytes in their ear canals on day 56; of those nine (9/12) were treated with oral fluoroqinolone and five (5/6) were not treated with oral fluoroqinolone.

Table 1: Ear cytology - microorganisms (No. (number) / OIF - 1000x magnification)

	Ear cytology (bacteria & yeast)						
	Day						
	0	**7**	**14**	**21**	**28**	**42**	**56**
Rods*	(No./OIF)						
NoAtb	38.3±10.0	21.8±6.2	15.5±9.0	15.3±9.3	12.8±8.2	28.2±13.6	26.5±15.5
Atb	68.2±18.9	35.8±13.7	28.1±7.2	17.9±5.5	11.3±4.0	15.2±4.8	3.1±1.2
Cocci	(No./OIF)						
NoAtb	4.9±3.0	1.8±1.8	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.1	1.1±0.7
Atb	11.6±5.0	5.2±2.0	8.8±4.0	9.6±3.1	6.7±2.6	3.6±1.0	2.2±0.9
Yeast	(No./OIF)						
NoAtb	2.3±2.2	0.4±0.3	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.1
Atb	0.2±0.1	0.1±0.1	0.1±0.1	0.5±0.5	0.1±0.1	0.0±0.0	0.0±0.0

Legend: Rods: rod shaped bacteria. Cocci: sphericaly shaped bacteria. NoAtb: not treated with antibiotics (n=6). Atb: treated with antibiotics (n=12). * Significant effect of time -treatment with Tris-EDTA /chlorhexidine.

Table 2: Ear cytology – inflammatory cells (No. (number) / OIF - 1000x magnification)

	Ear cytology (neutrophil granulocytes & macrophages)						
	Day						
	0	**7**	**14**	**21**	**28**	**42**	**56**
Neutrophil*	(No./OIF)						
NoAtb	1.4±0.7	0.7±0.1	0.3±0.1	0.6±0.2	0.4±0.1	0.6±0.1	0.8±0.4
Atb	1.2±0.3	0.9±0.2	0.6±0.1	0.4±0.1	0.4±0.1	0.6±0.1	0.2±0.1
Macrophage	(No./OIF)						
NoAtb	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Atb	0.0±0.0	0.1±0.1	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

Legend: NoAtb: not treated with antibiotics (n=6). Atb: treated with antibiotics (n=12). * Significant effect of time -treatment with Tris-EDTA /chlorhexidine

Table 3: Ear and ear canal clinical signs score (0 (the variable (clinical sign) absent) – 4 (the variable (clinical sign) very evident)

	Clinical signs						
	Day						
	0	**7**	**14**	**21**	**28**	**42**	**56**
Erythema*	(Score)						
NoAtb	1.7±0.2	1.7±0.2	1.3±0.2	1.0±0.0	0.8±0.2	0.3±0.2	0.3±0.2
Atb	2.3±0.3	1.4±0.1	1.4±0.1	1.1±0.1	0.7±0.2	0.5±0.2	0.4±0.1
Oedema*	(Score)						
NoAtb	0.8±0.3	1.0±0.3	0.5±0.2	0.5±0.2	0.4±0.2	0.2±0.2	0.0±0.0
Atb	1.2±0.4	0.8±0.2	0.7±0.3	0.4±0.2	0.2±0.2	0.2±0.1	0.3±0.1
Pain* @	(Score)						
NoAtb	0.5±0.3	0.3±0.2	0.8±0.4	0.0±0.0	0.0±0.0	0.2±0.2	0.0±0.0
Atb	1.2±0.5	0.4±0.2	0.4±0.2	0.3±0.1	0.3±0.3	0.0±0.0	0.0±0.0
Stenosis*	(Score)						
NoAtb	1.2±0.4	0.8±0.4	0.8±0.4	0.3±0.2	0.4±0.2	0.2±0.2	0.0±0.0
Atb	1.8±0.4	1.2±0.3	0.8±0.3	0.4±0.2	0.3±0.2	0.2±0.1	0.2±0.1
ClinScore*	(Score)						
NoAtb	4.2±0.9	3.8±0.8	3.3±0.8	1.8±0.4	1.6±0.5	0.8±0.5	0.3±0.2
Atb	6.3±1.3	3.7±0.8	3.3±0.8	2.3±0.6	1.3±0.6	0.8±0.3	0.8±0.4

Legend: ClinScore: Overall clinical score combining Erythema, Oedema, Pain and Stenosis. NoAtb: not treated with antibiotics. Atb: treated with antibiotics. * Significant effect of time -treatment with Tris-EDTA /chlorhexidine. @: significant effect of methylprednisolone on variable.

Macrophages were detected in eight (8/18) cytology samples. Their number was not affected by the treatment with antibiotics (P=0.5), nor it changed over time (P=0.4) (Table 2). None of the animals had macrophages in their ear canal on day 56.

Ten (8/18) dogs had rod shaped bacteria present in their ear canals at the conclusion of the study (day 56), which were not determined as *P. aeruginosa* (*Proteus sp., E. coli* and *Corynebacterium sp.*) (2). Seven (7/12) were present in the fluoroquinolone treated patients and one (1/6) was present in the patient not treated with fluoroquinolone. Treatment with antibiotics did not influence the presence of other rod shaped bacteria on day 56 of the study (OR=4.7; 95% CI: 0.68-4.0; P=0.1), nor was the likelihood of the presence of other rods at the end of the study influenced by the treatment with methylprednisolone (OR=0.78; 95% CI: -2.1-1.7; P=0.9).

Clinical signs

Treatment with antibiotics had no effect on ear canal erythema (P=0.6), oedema (P=0.9), stenosis (P=0.6) or pain (P=0.5). Similarly, treatment with methylprednisolone had no effect on erythema (P=0.3), oedema (P=0.6) and stenosis (P=0.3); however, it had a significant effect on pain (P=0.048). Time had a significant effect on all clinical signs individually (P<0.001) (Table 3).

Clinical signs estimated together as one overall variable were also not affected by treatment with antibiotics (P=0.6), nor were they affected by the treatment with methylprednisolone (P=0.2). The severity of overall clinical signs diminished over time (P<0.0001) (Table 3).

Bacterial culture

All dogs included in the study had their initial bacterial culture positive for *P. aeruginosa*. Six dogs that were treated with antibiotics (6/12) had their bacterial culture still positive for *P. aeruginosa* on day 56. One dog (1/6) that was not treated with antibiotics had its bacterial culture still positive for *P. aeruginosa.* on day 56. Treatment with antibiotics had no influence on the bacterial cultures to test positive for *P. aeruginosa* at the conclusion of the study (OR=1.75; 95% CI: 0.2-14.4; P=0.6). Similarly, treatment with methylprednisolone had no influence on the bacterial cultures to test positive for *P. aeruginosa* at the conclusion of the study (OR=2.8; 95% CI: 0.3-21.7; P=0.3).

Antibiotic resistance of Pseudomonas sp.

Six dogs (6/18) had resistant *Pseudomonas sp.* present in their ear canal on the initial bacteriology, which increased to twelve (12/18) on further evaluations. Treatment with antibiotics was not shown to increase the likelihood for *P. aeruginosa* to develop antibiotic resistance against fluoroquinolones (OR=1.2; 95% CI: 0.016-4.7; P=0.3). Treatment with methylprednisolone also was not influencing *P. aeruginosa* to develop antibiotic resistance against fluoroquinolones (OD=2.3; 95% CI: 0.5-1.7; P=0.1). Similarly, the development of specific antibiotic resistance against enrofloxacin or ciprofloxacin was not affected by treatment with fluoroqinolones or methylprednisolone (P>0.05).

Discussion

This paper evaluated clinical, cytological and bacteriological characteristics of canine OE after topical treatment with Tris-EDTA /chlorhexidine 0.15% solution in cases where the disease was complicated with *P. aeruginosa* infection. Parameters were compared between cases that were and those that were not treated with fluoroquinolones. Significant observations were: 1) When dogs were treated with Tris-EDTA /chlorhexidine 0.15% combination solution, oral antibiotic treatment did not contribute to the resolution of *Pseudomonas* OE and 2) *P. aeruginosa* may be selected for antibiotic resistance regardless of the presence of the antibiotic treatment.

Pseudomonas aeruginosa infections are challenging to manage due to its potential multidrug resistance and the ability to produce several extracellular factors, which are involved in the expression of virulence (16). The unpredictable behaviour of *P. aeruginosa* with regards to antibiotic resistance was also observed in this study. Antibiotic resistance developed at some point of the study in most cases that were treated with antibiotics, as well as in cases that were not treated with antibiotics. Regardless of that effect, 11/18 dogs in our study were negative for *P. aeruginosa* on bacterial culture on day 56, which was not affected by the antibiotic treatment.

The dose of enrofloxacin at 5 mg/kg SID used in this study is lover than recently suggested (17). However, the dose of 20 mg/kg SID is often avoided due to high incidence of side effects and overwhelming costs (17).

Treatment with Tris-EDTA /chlorhexidine 0.15% combination solution was proven safe in dogs with OE (8). Similarly, no side effects were observed in this study. Tris-EDTA /chlorhexidine 0.15% solution was found "in-vitro" to be active against all the pathogens most commonly involved in canine otitis, including *P. aeruginosa*, which showed sensitivity at dilutions between 1:8 and 1:32 (9). Tris-EDTA also has the ability to potentiate the penetration of enrofloxacin into the bacterial cell by mechanisms involving magnesium cations, which effectively reduces the bacterias' minimum inhibitory concentrations (5, 18). Such beneficial effects were not appreciated in this study's population.

Clinical signs (pain, oedema, erythema and stenosis) were quantitatively scored and analysed separately, as well as unified under the cumulative score and analysed as an overall clinical variable. This was done to minimize the effect of individual clinician's clinical estimates and to minimize possible deviation in the animal presentation (stoic animals vs. easily excitable animals). The resolution of clinical signs was similar between dogs that were or were not treated with fluoroquinolones. Treatment with methylprednisolone was included in the statistical analysis to evaluate its potential effect on the outcome of all investigated parameters and should not be interpreted as a function that would show the specific effect on any particular clinical signs. Methylprednisolone has not been found to have any influence on the disease outcome; however, it reduced pain in treated animals, as expected.

Cytological analysis of ear swab preparations is an important diagnostic tool to diagnose, estimate the severity of, and the success of implemented treatments for OE. It is more sensitive than bacterial culture at detecting organisms in the external ear canal (19). The presence of rod shaped bacteria, cocci, yeasts, *neutrophil* granulocytes and macrophages was quantitatively assesed. Epithelial cells were not included in the analysis, because of unpredictable presence in the clinical sample (14). Yeast and cocci infections resolved by the end of the study in all cases.

Rods are not part of the normal canine external ear canal flora; therefore, any presence of rods in the ear canal is considered pathological (20). Thirteen dogs (13/18) had rod shaped bacteria present in their ear canal on day 56 (Table 1). In cases where bacterial culture was negative to *P. aeruginosa*, other species of rod shaped bacteria (most frequently *Corynebacterium sp.*) were colonizing the ear canal. In cases where rod shaped bacteria were detected on the cytological smear, but no growth was present on the bacterial culture, the positive cytology result was attributed to the presence of dead bacteria in the cytological smear, or bacteria deteriorated during the process of sampling and/or culture procedures.

Inflammatory cells or phagocytosis should not be seen in cured ears' cytology smears (14), although some exceptions were reported (20). *Neutrophil* granulocytes were present on day 56 in 7/18 ear swabs. Nevertheless, their number was low and no phagocytosis was seen. The presence of neutrophil granulocytes in these cases could be attributed to unresolved underlying cause of OE. Our results indicate that the resolution of cytological indicators was not influenced by the treatment with fluoroquinolones, nor were they influenced by the treatment with methylprednisolone.

In conclusion, in this study the use of systemic fluoroquinolones had no beneficial effect on the outcome of *Pseudomonas* OE. Treatment with Tris-EDTA /chlorhexidine 0.15% combination solution seems to be efficient independently of antibiotic treatment, not disregarding the potential differences between topical and systemic antibiotic treatment. Treatment modalities without the use of antibiotics should be investigated to treat canine *Pseudomonas* OE.

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