Efficacy of a Topical Ear Formulation with a Pump Delivery System for the Treatment of Infectious Otitis Externa in Dogs: a Randomized Controlled Trial

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ABSTRACT

In a randomised, multicentric field clinical trial on dogs with otitis externa, the efficacy of an hydrocortisone aceponate-gentamicin-miconazole otic suspension (Easotic, Virbac, Carros, France) applied once daily for 5 days was compared to that of a betamethasone valerate-gentamicin-clotrimazole otic suspension (Otomax, Schering Plough Animal Health, Levallois-Perret, France) applied twice daily for 7 days. One-hundred and seventy-six dogs with bacterial and/or fungal otitis externa were included in the study and received one of the two ear medications. On days 0, 5, 7 and 14 clinical signs, as well as bacteriological and fungal counts at cytology from ear samples, were graded using semi-quantitative scales. Bacterial and fungal cultures were also performed on day 0. A control visit was performed on day 49 to check for relapses. The aggregate clinical scores were reduced by 83.2% with Easotic and 86.2% with Otomax on day 14, with no significant difference between groups. Clinical recovery (>75% reduction of the aggregate score) was recorded in 72.2% of cases with Easotic and 69.9% of cases with Otomax on day 14. Microbial scores were reduced in both groups over the study period, with a higher cytological recovery rate on day 14 in the Easotic group (61.3%) versus Otomax (37%) (odd ratio=2.7). None of the dogs with clinical recovery on day 14 presented a relapse up to day 49. Side effects were minor and transient. In this study Easotic applied once daily for 5 days proved efficient and safe for the treatment of canine otitis externa.

INTRODUCTION

Otitis externa is common in dogs with a reported frequency between 4% and 20% of patients consulting at veterinary clinics.[1,2] The condition refers to any inflammatory condition of the external ear canal commonly associated with pain and discomfort (pruritus). It is usually classified as erythemato-ceruminous or suppurative depending on the type of discharge material present.[1,3]

Otitis externa is a multifactorial syndrome involving primary, predisposing and secondary or perpetuating factors. Primary factors include hypersensitivities (atopic dermatitis, adverse food reactions, contact hypersensitivity), ectoparasites (most com-

monly *Otodectes cynotis*), foreign bodies, keratinisation defects and idiopathic inflammation. Predisposing factors are related to the anatomy of the ear (pendulous pinnae, stenotic canals, hypertrichosis) and environmental conditions such as temperature and humidity. Secondary bacterial and/or fungal infections are perpetuating factors that exacerbate the inflammatory condition.[1,3] The dominant pathogens found in canine otitis externa are the bacteria *Staphylococcus intermedius* (coccus) and *Pseudomonas aeruginosa* (rod), and the budding yeast *Malassezia pachydermatitis*.[4,5,6,7,8] The diagnosis of otitis externa is achieved through clinical history, clinical examination of the ear canal and cytology of ear exudates.[3,9,10,11]

Treatment of canine otitis externa is usually local although systemic therapy is required in some cases (stenotic ear canal, exacerbated expression of the disease). Long term management of the condition can be challenging in case of frequent relapses or chronicity associated with persistence of underlying causes, which should be identified and corrected.[1,3,12] Because of the central role of inflammation in the pathogenesis of the condition, and frequent association with mixed bacterial and yeast infection, topical treatment is usually achieved with a product containing an antibiotic, an antifungal agent and a corticosteroid.[1,3,13,14,15,16] Administration of the ear medication must be undertaken daily at home by the owner over several days until resolution of the problem. Correct application of the ear treatment in accordance with veterinarian's prescription may prove practically difficult however, especially when the auricular condition is painful for the dog and treatment must be repeated twice daily. Achieving good restraint of the dog while counting the exact number of drops required can be challenging, and discouragement may thus happen before the end of the treatment period, leading to poor compliance and reduced therapeutic efficacy.

To improve compliance and reduce dog handling, an antibiotic-antifungal-corticoid otic suspension with a single daily dose delivered through a pump delivery system was developed recently (Easotic, Virbac). The objective of product development was to simplify first line treatment of otitis externa in several ways: delivery of a preset volume (1 mL) of the suspension into the ear canal through a flexible canula by a single depression of the pump on the dispenser, reduced number of administrations (once daily dosing) and shorter duration of treatment application (5 days). The suspension includes gentamicin, miconazole and hydrocortisone aceponate as active ingredients. While gentamicin and miconazole are respectively well known antibiotic and antifungal agents documented for use in otitis externa,[4,6,17,18,19,20,21,22,23] the non-halogenated diester glucocorticoid hydrocortisone aceponate is only documented to date for its use in a spray formulation as an anti-inflammatory agent on the skin of other parts of the body in dogs.[24,25] Pharmacological data document an improved benefit/risk ratio of non-halogenated diester glucocorticoids as compared to corticosteroids previously used, thanks to increased entry into, and storage within, the epidermis (lipophilicity), and fewer local and systemic side effects (metabolism in skin structures).[26,27,28,29]

The objective of this study was to compare the efficacy and safety of the pump-dosed Easotic suspension administered per label (1 mL once daily for 5 days) to that of a reference drop-dosed treatment Otomax (Schering Plough Animal Health) administered per label (4 to 8 drops twice daily for 7 days) for the treatment of acute canine otitis externa. The control product was selected as it includes similar antibacterial (gentamicin) and antifungal (clotrimazole) components, as well as a potent glucocorticoid agent (betamethasone valerate), and it is also indicated as a first line treatment of canine otitis externa.

MATERIALS AND METHODS

This multicentered, controlled, randomised clinical field trial was conducted over 10 months. It was performed in accordance with GCP (Good Clinical Practice) guide-

lines.

Study centres and animal selection

Dogs with otitis externa were recruited in 32 veterinary clinics in France (10 sites), Germany (10 sites) and Spain (12 sites).

The investigators selected the animals according to the following inclusion criteria: dogs of various breeds over 3 months, in good general health, diagnosed with bacterial and/or fungal otitis externa based on clinical signs and cytological examination of ear swabs. The dog could be diagnosed at initial presentation or because of a relapse of a previous episode. Otitis externa was classified as erythemato-ceruminous (presence of cerumen and erythema with associated bacterial and yeast isolates observed microscopically) or purulent (presence of pus with bacteria detected at microscopy). In case of bilateral otitis, only the ear in the worst condition on day 0 was considered in the evaluation. Informed consent was obtained from the owners of all dogs prior to their participation in the study.

Non-inclusion criteria included: dogs that received any topical or systemic antifungal, antibiotic, corticoid or cyclosporin treatment in the 15 days before the trial, or any long-acting injectable glucocorticoid in the 3 months before the trial, dogs whose ears were cleaned with an antiseptic product on day 0, dogs with a negative microbial cytology, dogs with a rupture of the tympanic membrane, pregnant or lactating females, dogs with associated pyoderma, parasitic otitis, otitis due to foreign body, and dogs with advanced stages of proliferative or occlusive otitis.

Study design

Treatments

At each site on day 0, the dogs were allocated to one of the two treatment groups using treatment allocation envelopes based on pre-established randomisation. The investigators were not blinded to the type of treatment received by dogs. In the test group, 1 mL of Easotic (Virbac, Carros, France), a suspension containing 1.11mg of hydrocortisone aceponate, 15.1mg of miconazole nitrate and 1505 IU of gentamicin sulphate per mL, was administered once daily in each ear for 5 days. The daily dose of 1mL was delivered by one single depression of the pump on the head of the dispenser. In the control group, 4 drops (dogs<15kg) or 8 drops (dogs≥15kg) of Otomax (Schering Plough, Levallois-Perret, France), a suspension containing 0.88mg of betamethasone valerate, 8.80 mg of clotrimazole and 2640 UI of gentamicin sulphate per mL, were administered twice daily for 7 days. Both products were used according to label instructions.

Concomitant topical or systemic administration of glucocorticoid, antibiotic, antifungal, anti-histamine or non steroidal anti-inflammatory agents, as well as cyclosporine, were not allowed during the study period. The use of ear cleansers was forbidden as well.

Schedule

The animals were observed 4 times during a 2-week period: day 0, day 5, day 7 and day 14. An additional control visit was performed on day 49 to check for relapses of dogs in clinical remission on day 14. In case of aggravation or reappearance of clinical signs between planned visits, the owner had to bring his dog back to the veterinarian to perform clinical and cytological examinations. If the investigator decided to administer an additional treatment, the dog was withdrawn from the trial and treated appropriately by the investigator.

Clinical examination

At each visit, the ears were examined and 8 clinical signs were recorded: head shaking, excoriation-crusts, quantity of exudate, stenosis, pruritus, pain, erythema and suppuration. Each clinical sign was scored on a severity scale from 0 to 3 (0 = none, 1 = slight, 2 = moderate, 3 = severe), except head shaking scored from 0 (none) to 1 (presence). A total clinical score was calculated from the addition of individual scores at each examination time. Every adverse event had to be reported.

Cytological examination

A cytological examination from swab

sampling of the external ear canal was performed at each visit. The clinician wet the sterile cotton-tipped swab with isotonic saline before sampling. The investigator then rolled the tip of the swab onto a clean microscope slide. After drying, the slide was stained with a quick staining kit (Diff-Quik, Baxter Healthcare, Dade Division, Miami, FL). The investigator scanned the slide to detect bacteria and/or fungi. The swab was also smeared on an additional dry-cleaned slide and sent to the central laboratory (Vébiotel, Veterinary Analyses, Microbiology Unit, Arcueil, France). At the laboratory, the slides were scanned at low magnification (x40-100) after May-Grünwald-Giemsa staining, to select a representative area. Populations of yeast, coccoid and rod-shaped organisms were estimated from 10 consecutive microscope fields at higher magnification (x1,000, oil immersion) using a semi-quantitative scale (Table 1). The same person, blinded to treatment groups, performed microorganism counting on all slides of the study.

Microbiological culture

On day 0, a sample for bacteriological and fungal culture was collected from the external ear canal with a sterile polyurethane swab (Culturette EZ Collection and Transport Systems, BD Diagnostics, Franklin Lakes, NJ) and sent rapidly to the central laboratory (as above). The swab sample was plated on nonselective medium and incubated in both microaerophilic and aerobic atmospheres at 35°C for 18 to 24 hours. The bacterial strains were identified by standard identification tests and biochemical testing (API kits, Biomerieux, Marcy-l'Etoile, France). The antimicrobial sensitivity of bacterial strains to gentamicin, as well as the antifungal sensitivity of *Malassezia* strains to miconazole and clotrimazole, were determined by the agar disk diffusion method according to the standards of the French Society of Microbiology.

Efficacy criteria

Dogs were considered in the efficacy analysis if follow-up data were available beyond the first visit, if there were no major deviations to test or control treatment regimens and planned visits, and if no forbidden treatments were administered concurrently for an unrelated disease condition. Dogs withdrawn

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

Organism	Count per oil immersion field (x1,000)	Score
Malassezia yeasts	0	0
	1-2	1
	3-8	2
	>8	3
Cocci	0-2	0
	3-8	1
	9-40	2
	>40	3
Rods	0-2	0
	3-8	1
	9-40	2
	>40	3

from the study for treatment failure, or administered with any rescue therapy for the ear condition were included in the efficacy analysis. All animals with follow-up data available after the first visit were considered in the safety analysis.

The main efficacy criterion was the percentage reduction of the total clinical score on day 14 as compared to baseline (day 0). Other efficacy parameters included: reduction of the total clinical score from baseline on days 5 and 7, clinical and cytological recovery rates on day 14, and reduction of microbial semi-quantitative scores from cytology over the study period. Clinical recovery was defined as more than 75% reduction of the total score from baseline. Cytological recovery was achieved if mean *Malassezia* and cocci scores was 1 or lower and mean rod score was nil on 10 high-power fields with no neutrophils seen on microscope slides.

When clinical recovery was achieved on day 14, clinical relapse between day 14 and day 49 was defined as any increase in the total clinical score or need for additional treatment (whether the dog was brought back in that period by the owner for worsening of signs or only seen at the final check-up visit).

Safety of treatments was evaluated from the frequency and severity of adverse events.

Statistical analysis

All analyses were performed using commercial statistical software (S-PLUS 6.2, Insightful Corp, Seattle, WA). The significance threshold was set to $P<0.05$.

To check group comparability before treatment, qualitative parameters (animal gender, ear type, living habits, otitis type and history, antibiotic/antifungal susceptibility rate of bacterial and yeast species isolated from bacterial culture) as well as quantitative parameters (animal age and weight, total clinical score, microbial scores at cytology, number of bacterial and fungal strains isolated from bacterial culture) were compared between groups on day 0.

Qualitative parameters were compared using the Chi-square test or Fisher's exact test for low numbers. Quantitative parameters were analysed using Wilcoxon rank sum test.

The last observed value was used for parameter calculations in dogs that left the trial before day 14 because of treatment failure.

The reduction of the clinical score on days 5, 7 and 14 was compared between groups using an equivalence approach. The 95% confidence interval for the difference in clinical score reduction between the test and control treatment was calculated. The lower limit of the interval was then compared to -10%, the lower pre-set clinically acceptable difference.

Clinical and cytological recovery rates on day 14 were compared between groups using the 95% confidence interval of the odds ratio (significant difference if the interval of OR does not include 1). The Wilcoxon rank sum test was used to compare microbial scores between groups at each examination time.

The number of adverse events over the study period was compared between groups using Fisher's exact test.

RESULTS

Study population

One hundred and seventy-six dogs from 5 months to 13 years were recruited for the study (88 in the test product group and 88 in the positive control group). Various breeds were represented, including most frequently mixed breed dogs (22.2%), English cocker spaniels (6.8%), Labrador retrievers (6.3%), poodles (4%), West Highland white terriers (4%) and Yorkshire terriers (4%). The dogs ranged in size from 2.5 to 60kg. Twenty-four dogs were excluded from the analysis of treatment efficacy (Table 2). The main reason for exclusion of dogs in the control group (10 cases) was serious deviation from prescribed treatment regimen, as owners overtly reported missed doses or lack of proper drop counting. Only one owner in the test product group could not ensure that correct administration was performed. Other reasons for exclusion in both groups includ-

Table 2. *Animals excluded from the analysis of treatment efficacy and reasons*

Reason for exclusion	Number of dogs withdrawn (N=24/176)	
	Easotic (n=9/88)	**Otomax (n=15/88)**
Lack of compliance with treatment (incorrect dosing or missed doses)	1	10
Lack of compliance with scheduled visits (owner did not return on day 14 ±1 day)	3	3
Extraction of foreign bodies from the ear canal after inclusion	1	0
Onset of concomitant disease (skin lesions) requiring unauthorised treatment (systemic antibiotics)	2	1
Loss to follow-up after the first visit	2[a]	1[a]

[a] *These dogs could not be included in the safety analysis either*

Table 3*. Characteristics of dogs at baseline (Day 0)*

Parameter	Easotic (n=79)	Otomax (n=73)	P-value
Age (years): mean ±SD	5.95 ±3.47	5.76 ±3.36	0.769[a]
Weight (kg): mean ±SD	21.50 ±12.60	21.36 ±12.81	0.918[a]
Sex			
Male	39	37	0.871[b]
Female	40	36	
Ear type			
Pendulous	61	59	0.586[b]
Erect	18	14	
Living habits			
Indoors	52	53	0.366[b]
Outdoors	27	20	
Other pets in the same household			
Yes	31	20	0.122[b]
No	48	53	
Otitis history			
Primary episode	57	55	0.655[b]
Recurrent episode	22	18	
Otitis location			
Right ear	21	16	0.771[b]
Left ear	14	15	
Bilateral	44	42	
Otitis type			
Erythemato-ceruminous	54	56	0.250[b]
Purulent	25	17	
Total clinical score: mean ±SD	10.99 ±3.85	10.34 ±3.87	0.254[a]

[a] *Wilcoxon rank sum test,* [b] *Chi-square test*

ed: serious deviation in visit times, otitis due to foreign body, administration of forbidden treatment for skin problems or loss to follow up after the initial visit (Table 2). A total of 152 dogs were thus included in the evaluation of efficacy (79 with the test product and 73 with the control product).

No significant difference was detected between groups on day 0 for demographic characteristics of animals, medical history and type of otitis, as well as total clinical score (Table 3) or microbial scores (Table 4). The sex ratio was balanced in both groups. A majority of dogs had pendulous ears (78.9%) while otitis was most often bilateral (56.6% of cases) and classified as erythemato-ceruminous (72.4% of cases). About one fourth of the animals (26.3%) had suffered previous episodes of inflammation in the ear canal. Clinical signs recorded in nearly all dogs included excessive quantity of exudate (99.3%), erythema of the ear canal (97.4%) and pruritus (96.7%). Pain (88.8%) and oedema of the ear canal wall (76.3%) were also frequently reported. Excoriations and crusts were noted in 68.4% of cases. Few dogs presented with head shaking (17.8%). Average values of the total clinical score in both groups exceeded 10 out of a maximum of 22 (Table 3). Mean cytology scores for *Malassezia* exceeded those for Cocci or rods in both groups (Table 4).

Bacteriological and fungal culture results

A positive bacterial culture was obtained from ear swabs in 64.6% of cases on day 0. Ninety-eight bacterial isolates were identified, often as a single isolate (Table 5). *Staphylococcus pseudintermedius* was most frequently cultured (46.6% of isolates), followed by *Bacillus spp* (10.3%), *Pseudomonas aeruginosa* (8.6%), *Streptococcus* group D (6.9%), *Escherichia coli* and *Proteus mirabilis* (each 5.2%). Other species isolated less frequently (1 to 3 isolates each) belonged to the following genera: *Pasteurella, Streptococcus, Staphylococcus, Pseudomonas, Stenotrophomonas, Burkholderia, Corynebacterium, Enterococcus, Pantoea* and *Rhanella*. More than 80% of bacterial isolates were susceptible to gentamicin in vitro, with no significant difference between groups (Table 5).

A positive fungal culture was recorded from 32.9% of ear samples on day 0 (22.4% of which in conjunction with a positive bacterial culture). Fifty yeast isolates were cultured, all identified as *Malassezia pachydermatis*. Few yeasts were found resistant to miconazole (12%) or clotrimazole (10%), with no significant difference between groups (Table 5).

Clinical efficacy

Significant reduction of the total clinical score was recorded in both groups from day 5 (Table 6). An average improvement of the clinical score from baseline higher than 80% was achieved with both the test and control product on days 7 and 14. The lower limit of the 95% confidence interval of the difference in clinical score reduction (test product – control product) was not less negative than -10%, thus the test product was not inferior to the control product for the main efficacy parameter on any of the examination time points (Table 6).

A high proportion of dogs (about 70%) had recovered clinically by day 14 whatever the treatment group (Table 7). With both products, the clinical recovery rate tended to be higher for erythemato-ceruminous versus purulent otitis and in primary episodes versus recurrent ones. The probability of clinical recovery was not significantly different between groups across the different types of otitis (Table 7).

None of the dogs showing clinical recovery on day 14 had relapsed by day 49.

Antimicrobial efficacy

Microbial scores recorded at cytology were reduced over the study period with no significant difference between groups, except on day 5 where a lower *Malassezia* score was obtained with the test product (Table 4). Cytological recovery was achieved in a higher proportion of dogs with the test product (61.3%) than with the control product (37%) by day 14. Additional benefit of the test treatment over the control product was

***Table 4**. Microbial scores determined from cytological examination of ear swabs over the study period*

Microbial score[a]: Mean ± SD		Easotic (n=79)	Otomax (n=73)	P-value
Malassezia	Day 0	1.6 ± 1.23	1.3 ±1.23	0.235
	Day 5	0.6 ± 0.77	1.1 ±1.14	0.027
	Day 7	0.5 ± 0.79	0.9 ± 1.05	0.208
	Day 14	0.5 ± 0.81[b]	0.9 ± 1.14	0.190
Cocci	Day 0	0.6 ± 0.99	0.3 ± 0.80	0.124
	Day 5	0.2 ± 0.51	0.2 ± 0.42	0.945
	Day 7	0.2 ± 0.43	0.1 ± 0.17	0.244
	Day 14	0.2 ± 0.55[b]	0.1 ± 0.28	0.230
Rods	Day 0	0.6 ± 1.03	0.4 ± 0.86	0.520
	Day 5	0.2 ± 0.62	0.3 ± 0.63	0.069
	Day 7	0.2 ± 0.55	0.2 ± 0.58	0.624
	Day 14	0.3 ± 0.83[b]	0.3 ± 0.53	0.325

[a] *Average from 10 oil immersion fields using the semi-quantitative scale of table 1*
[b] *Missing data for 4 dogs*

***Table 5**. Results of bacterial and fungal culture from ear swabs on day 0*

Parameter	Easotic (n=79)	Otomax (n=73)	P-value
Number of bacterial species isolated			
0	27 (34.2%)	27 (37%)	0.911[a]
1	42 (53.2%)	38 (52.1%)	
2	10 (12.7%)	8 (11%)	
Susceptibility to gentamicin			
Resistant	5 (8.1%)	5 (9.3%)	0.658[b]
Intermediate	2 (3.2%)	4 (7.4%)	
Susceptible	55 (88.7%)	45 (83.3%)	
Number of yeast species isolated			
0	52 (65.8%)	50 (68.5%)	0.658[b]
1	27 (34.2%)	23 (31.5%)	
Susceptibility to miconazole			
Resistant	4 (14.8%)	2 (8.7%)	0.451[b]
Intermediate	1 (3.7%)	3 (13%)	
Susceptible	22 (81.5%)	18 (78.3%)	
Susceptibility to clotrimazole			
Resistant	3 (11.1%)	2 (8.7%)	0.387[b]
Intermediate	2 (7.4%)	5 (21.7%)	
Susceptible	22 (81.5%)	16 (69.6%)	

[a] *Chi-square test,* [b] *Fisher's exact test*

detected in the subgroups of erythemato-ceruminous otitis and in case of primary episode (Table 8).

Safety

Out of the 173 dogs considered in the evaluation of safety, 4/86 dogs (4.7%) treated with the test product and 7/87 dogs (8.0%) treated with the control product showed suspicion of product-related adverse events during the study period. There was no statistically significant difference between groups for the number of adverse events (P=0.360). Two dogs showed temporary deafness respectively 1 and 6 days after initiation of treatment in the control group. These adverse events lasted respectively for 3 and 14 days and the outcome was complete recovery without the need for corrective treatment. Increased erythema of the ear in the first days of product administration was observed with the test (4 cases) and control product (5 cases). The observation of redness of the ear was short lived (from several hours to 2 days), benign to moderate in severity, and did not require treatment interruption.

DISCUSSION

In this study, the test topical ear treatment administered daily for 5 days was not inferior to the reference topical ear treatment administered twice daily for 7 days in the management of otitis externa in dogs.

The main efficacy criterion was based on clinical findings. The parameters and scoring system used to evaluate severity of the ear condition are similar to those used in a variety of clinical studies on dogs with otitis externa.[15,30,31,32] Erythema, exudation and oedema of the ear canal lining were frequently reported on initial presentation, as found in previous otitis trials.[33] On average, both products rapidly reduced the clinical score from day 5, with the full magnitude of the effect being seen at day 7 (around 80% reduction from baseline) and maintained up to day 14. This is consistent with rapid anti-inflammatory activity of the glucocorticoid compounds contained in both formulas (reduction of oedema, erythema and ceruminous gland hyperplasia), acting in conjunction with antibiotic and antifungal components that address complicating microbial infection. The effect of medical treatments alone was evaluated here since no prior ear cleaning was allowed, while the later has been considered as a factor for increased likelihood of therapeutic success in practice.[34] The test treatment was as effective as the reference topical product in reducing the clinical score over time. The margin of tolerance used in this study to evaluate the equivalence of treatments on the main efficacy parameter (ie 10% difference) was severe, likely below what is clinically detectable. A difference between groups lower than 20% for the odds ratio of clinical recovery was used in a recent comparative study of two antimicrobial/anti-inflammatory formulations in the treatment of canine otitis externa.[15]

By day 14 in the present trial, 70% (control product) to 72% (test product) of dogs had clinically recovered. Numerous clinical studies of topical products containing antibiotics, antifungals and glucocorticoids for the management of canine otitis externa have reported variable degrees of clinical improvement in 71% to 97% of cases.[15,32,35,36,37,38] It is difficult to compare results between studies because different definitions are used for clinical improvement (eg. “excellent to good”, “treated successfully and improved”, “satisfactory to partial improvement”, “decrease of at least one level on the scale between the pre- and post-treatment period”, “normalization of 50% or more of the initial parameters”,…). In a recent study over a 2-week period, 95% of dogs treated with the control product improved clinically.[32] In the present study, the criterion for clinical recovery (eg >75% reduction of the total clinical score from baseline) was deemed as reflecting a very important reduction of the clinical scores, likely associated with some stabilisation of the condition beyond the treatment period, as suggested by the fact that none of the dogs classified as clinically recovered on day 14 had relapsed after further follow-up up to day 49. While no

Table 6. *Percentage reduction from baseline of the total clinical score over the study period*

% reduction of the clinical score from Day 0: mean ±SD	Easotic (n=79)	Otomax (n=73)	95% confidence interval of difference
Day 5	66.5 ± 20.2	59.0 ± 23.1	0 ; 13.89
Day 7	80.9 ± 19.5	80.2 ± 21.2[a]	-4.76 ; 5.26
Day 14	83.2 ± 22.3	86.2 ± 18.3	-6.67 ; 0

[a] *n=72, missing data for one dog*

Table 7. *Clinical recovery rate on day 14 based on the type of otitis externa*

Clinical recovery[a] rate: No. of dogs (% of total)	Easotic	Otomax	Odds ratio (OR)	95% confidence interval of OR
All types of otitis (n=152)	57 / 79 (72.2%)	51 / 73 (69.9%)	1.118	0.554 ; 2.254
Erythemato-ceruminous otitis (n=110)	42 / 54 (77.8%)	40 / 56 (71.4%)	1.400	0.590 ; 3.324
Purulent otitis (n=42)	15 / 25 (60%)	11 / 17 (64.7%)	0.818	0.228 ; 2.933
Primary episode (n=112)	43 / 57 (75.4%)	39 / 55 (70.9%)	1.260	0.545 ; 2.913
Recurrent episode (n=40)	14 / 22 (63.6%)	12 / 18 (66.7%)	0.875	0.236 ; 3.241

[a] *>75% reduction of the clinical score from baseline (day 0), with no additional treatment given*

Table 8. Cytological recovery rate on day 14 based on the type of otitis externa

Number of dogs (% of total) showing cytological recovery[a]	Easotic	Otomax	Odds ratio (OR)	95% confidence interval of OR
All types of otitis (n=148[b])	46 / 75 (61.3%)	27 / 73 (37%)	2.702	1.390 ; 5.253
Erythemato-ceruminous otitis (n=107)	34 / 51 (66.7%)	21 / 56 (37.5%)	3.333	1.506 ; 7.380
Purulent otitis (n=41)	12 / 24 (50%)	6 / 17 (35.3%)	1.833	0.511 ; 6.572
Primary episode (n=109)	36 / 54 (66.7%)	19 / 55 (34.5%)	3.790	1.715 ; 8.379
Recurrent episode (n=39)	10 / 21 (47.6%)	8 / 18 (44.4%)	1.136	0.321 ; 4.022

[a] *Mean Malassezia and cocci scores ≤1 and mean rod score =0 on 10 oil immersion fields, no neutrophils seen on microscopic slides*

[b] *Missing data for 4 dogs*

significant difference was detected between treatments in the chance of recovery across the different types of otitis, lower recovery rates (60-67%) were recorded with both treatments in purulent and recurrent cases. The later may correspond to more complex or chronic situations that sometimes require a longer treatment period, additional cleaning and proper management of the primary cause to achieve clinical resolution.

Bacterial organisms isolated from ear specimens on day 0 are those usually reported in infected ears: coagulase-positive staphylococci, *Pseudomonas aeruginosa, beta-hemolytic streptococci, Proteus, enterococci, Escherichia coli* and *Corynebacterium*.[34] *Staphylococcus pseudintermedius* was by far the most common bacterial isolate, which is consistent with previous reports.[13,15,39] *Staphylococci* and *Corynebacterium* may also be isolated from normal ears as they are commensal organisms, but the number of organisms isolated would generally be lower than those obtained from infected ears.[34] The significance of *Bacillus* isolation can be questioned because it is a common environmental organism. A single bacterial isolate was cultured in a majority of cases, as found in a previous large scale retrospective study on canine ear specimens presented to a microbiological laboratory.[34] Mixed infections with 2 bacterial isolates were less frequent, probably reflecting the lower number of recurrent otitis cases included in that study, since multiple isolates are especially common from animals with chronic infections.[34] More than 80% of bacteria were sensitive in vitro to gentamicin, the antibiotic used in both ear treatments evaluated. This is in agreement with earlier reports of gentamicin sensitivity in 83 to 95% of *Pseudomonas* and *staphylococci* isolates from dogs with otitis externa,[17,22,23,40] although some studies report a higher incidence of resistant *Pseudomonas* strains.[15] *Malassezia* was the only fungal pathogen isolated, either alone or in mixed infection with bacteria. High isolation rates of this yeast are similarly reported in other trials.[13,15,32] As with *staphylococci*, with which it is frequently associated in infected ears, *Malassezia pachydermatis* belongs to the normal flora and confirmation of infection often relies on large numbers of organisms being observed at cytology in stained smears. A similar low level of resistance to miconazole (antifungal in the test product) or clotrimazole (antifungal of the control product) was detected, confirming good activity of imidazole compounds reported elsewhere.[18,41] The clinical relevance of resistance findings from disc susceptibility testing is difficult to appreciate in the early management of otitis externa, as very high antibiotic or antifungal drug levels can be achieved in the ear canal using topical products, whereas disc susceptibility data tend to reflect concentrations achieved in the course of systemic treatment.

Cytological examination of otic exudates was used in this study to evaluate the antimicrobial efficacy of treatments, since this method provides greater diagnostic information about the participation of bacteria in the ear disease, and yeast overgrowth is also identified accurately.[9] To reduce subjectivity and variability between investigators, microbial counts were performed by the same person in the central laboratory, although factors like quality of sampling and sample transportation to the laboratory may still have influenced microbial results. Both ear treatments reduced yeast, cocci and rod counts in smears after application, consistent with direct local activity in the ear canal of topically delivered antibiotic and antifungal ingredients in the products. There is debate about the number of organisms per microscopic field that would be considered indicative of infection and therefore any definition of cytological recovery is somewhat arbitrary. The cut-off for the cytological diagnosis of *Malassezia* overgrowth in this study (≥3 organisms/oil immersion field, ie *Malassezia* score>1) is in agreement with an earlier semiquantitative cytologic study on healthy and pathological samples from the ear canal of dogs[9] and recent clinical studies of antimicrobial preparations.[31,33] A higher threshold was set for cocci overgrowth (≥9 organisms/oil immersion field, ie cocci

score>1) consistent with the findings of the former cytological study.[9] Since *Pseudomonas* bacteria are not routinely isolated from healthy ears,[34] any rod score higher than 0 (≥2 organisms/oil immersion field), as well finding inflammatory cells in smears, was considered pathological. With these limits set, microbial recovery was detected in a higher number of dogs with the test product (61%) than with the control product (37%) at day 14, especially in the subgroup of dogs with erythemato-ceruminous otitis externa, or in primary episodes, that may be more susceptible to short-term treatment. Lower recovery rates were recorded for antimicrobial efficacy versus clinical efficacy in both groups. This may be related to cytological and clinical criteria used. Correlation of microbial populations with clinical signs is difficult because of the secondary role of microbial pathogens and the multifactorial nature of canine otitis externa.

Several factors may account for equal clinical performance, and increased antimicrobial activity, of the test product administered daily for 5 days only compared to the reference product administered twice daily for 7 days. The total daily volume of the test product applied in the ear canal of dogs (1 mL whatever the size of the dog) was more than two-fold that delivered by the control product (8 to 16 drops according to the animal body weight, ie 0.2 to 0.4 mL according to label). While both products are oily suspensions, the former therefore may have covered in greater quantities an extended surface area in the ear canal, possibly increasing product persistence locally. In addition, the pre-set pump delivery system of the test product ensured constant reliable administration of the 1mL dose, while some variability in actual doses administered by owners cannot be ruled out for the drop-dosed control product. The concentrations of active ingredients delivered daily per label are also higher with the test product (1mL contains 15.1mg of miconazole, 1505 IU of gentamicin and 1.11mg of hydrocortisone aceponate) than with the control product (16 drops contains 3.568 mg of clotrimazole, 1070 IU of gentamicin and 0.3568mg of betamethasone). This also may contribute to level clinical efficacy between treatments, despite a shorter 5-day regimen applied in the test product group. Pharmacokinetic data from the European registration file of the test product (unpublished) indicate that significant concentrations of gentamicin (1.3 to 7.6µg/mL) and miconazole (1.5 to 2.7µg/mL) can still be recovered in water, propylene glycol and methanol extracting solutions after flushing the ear canal of dogs 10 days post cessation of a 5-day treatment. A higher persistence of antimicrobials in the ear canal may account for the higher cytological recovery rate recorded with the test product on day 14. Hydrocortisone aceponate is a non-halogenated diester glucocorticoid classified as moderately potent to potent based on results of the standard vasoconstriction assay.[42] Acetate esterification at C21 of the parent hydrocortisone molecule increases stability, whereas propionate esterification at C17 enhances affinity for the corticosteroid receptor and anti-inflammatory activity.[28,29] Double esterification (which significantly increases lipophilicity) greatly enhances penetration of the stratum corneum. Significant radiolabelling of 3H-hydrocortisone aceponate is detected on skin biopsies by photographic revelation and coloration 6h after topical application on the skin surface in dogs. Silver grains are visible in the epidermis and superficial dermis. The intensity of radioactivity, but not its distribution, is increased with repetition of applications over several days indicating a reservoir effect in the upper layers of the skin.[43] A high dose of hydrocortisone aceponate delivered daily in the ear canal, together with good penetration properties and possible accumulation of that active ingredient at the site of inflammation, may also play a favourable role in the clinical results obtained with the test treatment despite the short 5-day course.

Both products were well tolerated over the study period with few adverse events. Increased erythema reported in some dogs in the first days of application of the products may be related to the severity of the

initial condition, requiring several days of treatment before abatement is perceptible. Temporary reduced hearing experienced by two dogs with the control product can result from consequences of the infection, residue build-up, mechanical effect of product application in ears or ototoxicity in relation to alteration of the tympanic membrane. In practice, ototoxicity is rare in small animals.[44] Early studies have reported ototoxic effects in animals of experimental transtympanic or middle ear infusions of gentamicin,[45] the antibiotic used in both ear formulations. However the concentrations tested (3 to 10%) and mode of administration were very different from clinical practice. By contrast in a more recent trial, 3 weeks of twice daily topical ear treatment with a gentamicin sulphate solution (3 mg/mL) to dogs with surgically ruptured tympanic membranes did not induce alteration of vestibular or auditory function (as assessed by neurologic examination and determination of brain stem auditory evoked potentials).[46]

CONCLUSIONS

In this study, two-thirds of cases of canine otitis externa (initial presentation or acute relapse) responded successfully to 5-day treatment with the test product, which demonstrated clinical and antimicrobial efficacy. In addition, the once a day short-term treatment and convenient mode of application (pump delivery system) of the product favour owner compliance. Therefore, the test treatment is an interesting option to consider for the first line management of infectious otitis externa in dogs.

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