PAPER

# Preliminary evaluation of two commercial ear solutions in the treatment of canine otitis externa

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**Objectives:** **Preliminary evaluation of the efficacy of two commercial ear solutions composed of (1) chlorhexidine-Tris-ethylenediaminetetraacetic acid (EDTA) or (2) medical grade honey, for the treatment of otitis externa in dogs.**

**Materials and Methods:** **Dogs affected with otitis externa housed in an animal shelter were eligible for inclusion. Treatment was applied daily for 10 days and effect was measured by otitis clinical scores and microbiological counts. One of the treatments was applied to affected left ears, while the other was applied to affected right ears.**

**Results:** **A total of 24 ears from 13 dogs were included in the study. During the treatment period, with both treatments it was observed an improvement in clinical scores and a decrease in microbiological counts. At the end of the study 22 of 24 ears were deemed to have mild (4 ears), or no (18 ears) pain, with only two ears still showing pruritus.**

**Clinical Significance:** **The application of ear solutions composed of chlorhexidine-Tris-EDTA or medical grade honey, in the absence of antimicrobial treatment, might be effective for the control of clinical signs and microbial colonisation in dogs with otitis externa. Additional randomised studies on clinical patients are required to validate these findings.**

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## INTRODUCTION

Otitis externa in dogs is one of the most common causes for consultation at veterinary practices. It is estimated that the prevalence of this disease ranges between four and 20% in different dog populations (Lund *et al.* 1999, O'Neill *et al.* 2014). Aetiologies of otitis externa (OE) have been divided into predisposing factors, primary and secondary causes, and perpetuating factors (Cole 2012). Successful treatment of OE depends on controlling or eliminating all of these factors and causes (Nuttall 2016). Bacterial and yeast infections represent the most frequent perpetuating factors associated with OE in dogs of which *Staphylococcus pseudintermedius*, β-haemolytic *Streptococcus* spp., *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Malassezia pachydermatis* are among the most common (Fernández *et al.* 2006, Lyskova *et al.* 2007).

Treatment options for secondary infections are varied and may include mechanical removal of cellular debris and microorganisms with ear flushing, together with topical or even systemic antimicrobials (Morris 2004, Paterson 2016). The use of topical antimicrobial therapy (mainly antibiotics and/or antifungals) for the treatment of OE is widespread among veterinarians, but there is a wide concern about the emergence of antibiotic resistance. Methicillin-resistant *S. pseudintermedius* (MRSP), which is often multidrug-resistant, as well as multidrug-resistant *P. aeruginosa* strains, are frequently isolated from canine OE (Subapriya *et al.* 2015). Hence, prudent use of antimicrobials is important from a public health standpoint whereby resistant bacteria or resistance genes can be transmitted from animals to humans (Hernando-Amado *et al.* 2019).

Recent reports suggest that topical administration of antiseptics, generally referred to as ear cleaners, could be a useful sole or



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adjunctive treatment for canine OE (Paterson 2016). Most products contain one or more ingredients with antimicrobial activity and other components such as ceruminolytics, astringents, stabilisers and surfactants, which increase the solubility and activity of the antiseptic. A number of studies have reported antibacterial and antifungal activity of commercial ear cleaners or their components (Lloyd *et al.* 1998, Cole *et al.* 2003, Rème *et al.* 2006, Swinney *et al.* 2008, Guardabassi *et al.* 2010, Mason *et al.* 2013, Chan *et al.* 2019), but most of these studies are *in vitro* while *in vivo* assays are few and far between.

The purpose of this study was a preliminary evaluation of the efficacy of two commercial ear antiseptic solutions, one composed of chlorhexidine-Tris-ethylenediaminetetraacetic acid (EDTA) and another of medical grade honey, in a group of dogs affected with OE.

## MATERIALS AND METHODS

### Selection of participants

The dogs included in this study were chosen from those housed in an animal shelter located in León (NW Spain). Most of the day dogs were housed in two adjacent concrete-floored pens, holding 50 to 60 dogs of the same sex. Occasionally, they were leash-walked. At night, dogs were kept indoors, in pairs, in heated and covered rooms. Based on previous history of OE or ear conformation predisposing to ear disease, 25 dogs were preselected and underwent general clinical examination, inspection of the pinnae and external ear canals, otoscopic examination and cytologic evaluation of otic exudate. Individuals that met the following inclusion criteria were included: (1) showing signs of inflammation of the inner pinna and/or the external ear canal, discharge, scratching or pain; (2) not being under treatment with antimicrobial drugs; (3) having a sufficiently visible tympanic membrane to determine its integrity; (4) not showing signs of systemic disease, facial nerve paralysis, Horner syndrome or deafness and (5) pathological numbers of yeast or bacteria (≥4 yeast or >16 bacteria cells per high-power oil field as reported by Ginel *et al.* 2002 and Angus 2004). Dogs which fulfilled these five selection criteria were classified with unilateral or bilateral otitis. Taking into account information of the anamnesis (previous history of otitis in the past 2 months) and findings indicative of chronicity in the physical examination (hyperplasia, stenosis and ulceration of the ear canal), each dog was preliminarily classified as having acute or recurrent-chronic OE.

Informed consent was obtained from the responsible staff at the animal shelter before administration of the treatments.

### Study design

On Day 0, before treatment, a sample was taken from each affected ear with a sterile swab and stored in AMIES® transport medium for identification of microorganisms. Samples were evaluated using direct microscopy of Gram-stained smears. Samples were cultured on blood agar (Oxoid) at 37°C when only bacteria were observed in the smear and in both blood agar and sabouraud dextrose agar (Scharlab) with chloramphenicol (0.05 mg/mL) at 32°C when yeast infection was also evident (Fernández *et al.* 2006). Subcultures were performed until pure cultures were obtained. Primary identification was based on cellular morphology using Gram stain, catalase and oxidase kit tests (Sigma-Aldrich) while confirmation was carried out using matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS) (Patel 2015).

Between Days 1 and 10, once-daily treatment with Otodine® (Industria Chimica Fine: Chlorhexidine digluconate 0.15%, Tris-EDTA 0.0048% with lactic acid at pH 8, propylene glycol and deionised water) or with Vetramil® Auris (BFactory Italia: Manuka honey 40%, propylene glycol, polysorbate-80 and deionised water) was applied to the affected ears following the instructions provided in the leaflet by the manufacturer. Briefly, Otodine® was poured in the auricular canal and allowed to reach the bottom of the canal by massaging the base of the external ear canal for 1 minute. After 10 minutes, the ear was dried using sterile gauze. In Vetramil® treated ears, 2 mL of the product were applied followed by a similar massaging of the base of the ear to distribute the product throughout the ear canal. In order to facilitate the application of the treatments at the animal shelter it was decided to apply the treatment with Otodine® in all the affected right ears (11 ears in total) and the treatment with Vetramil® in all affected left ears (13 ears).

Evaluation was based on clinical scores and microbiological count progression. In order to quantify the clinical signs and lesions of OE and assess response to treatment, a previously described clinical scoring system, named OTIS3, which included the evaluation of erythema, oedema/swelling, erosion/ulceration and exudate with a scale between 0 and 3 was used (Nuttall & Bensignor 2014). Pain and pruritus were also evaluated during the examination using a similar 0 to 3 grading scale. All the assessments were made by the same person to avoid subjective variations depending on the observer.

For the follow up, on study Days 0, 5 and 11, dogs underwent general clinical examination, specific clinical examination of the ear pinnae and external ear canals and otoscopy of each affected ear to score the OTIS3 index. On Days 0, 5 and 11 a sample was also collected before treatment by inserting a sterile swab into the external ear canal. This sample was smeared and stained with a commercial Diff-Quick® system (QCA) and cocci, bacilli and yeast was quantified. All the cytologies were blindly performed by the same person in order to avoid variations depending on the observer. The counts were made at ×1000 magnification with an immersion objective on a Nikon Eclipse E200 microscope, and selecting areas that allowed examination of the whole field. Yeast, cocci and bacilli counts were expressed as the mean of the counts of 10 microscopic fields.

The daily treatment of the dogs was carried out by veterinarians, trained to determine if a rescue treatment was necessary in case of an unfavourable evolution.

### Statistical analysis

The values of OTIS3 were transformed into a categorical variable considering that an OTIS3 cut-off value ≥4 has been recommended for the identification of OE affected ears while a cut-off value ≤3 should indicate clinical success of the treatment (Nuttall & Bensignor 2014). Assessment of OTIS3 evolution was carried out using chi-squared test. Likewise, the counts of microorganisms were estimated as the mean of the observations in a total of 10 fields and analysed by non-parametric Kruskall-Wallis tests (because the

conditions for a classical analysis of variance were not fulfilled [Kolmogorov–Smirnov test for normality and Levene test for equality of variances]). Statistical analysis was carried out with the Epi Info™ 7 software (CDC) for Windows (version 7.2.1.0) and SPSS Statistics (version 24.0.0.1) taken $P<0.05$ as the level of significance.

## RESULTS

Among 25 preselected dogs, a diagnosis of OE was determined in a total 24 ears from 13 animals after the examination on Day 0: seven males and six females with a median age of 7 years (range one to 15 years) and median weight of 25.0 kg (range 8.6 to 36.5 kg). Most of the OE-affected dogs were mixed-breeds (n=9) followed by English setter (n=3) and carea Leonés (n=1). Eleven dogs presented with bilateral otitis and two with unilateral otitis. According to anamnesis and signs of chronicity, seven dogs were preliminarily classified as having chronic/recurrent otitis while the remaining six were classified with acute otitis (Table 1).

On Day 0 an OTIS3 value ≥4 was observed in 15 of the 24 affected ears included in the study. The clinical sign leading to higher scores was exudate, abundant and dark in colour, generally of an erythroceruminous nature, followed by erythema and oedema/swelling. On Day 0, the pain was classified as zero (Score 0) or mild (Score 1) in 19 of the 24 studied ears and only one ear out of 24 presented with severe pain (Score 3). In addition, five of the 24 ears showed pruritus which was classified as moderate (Score 2) in three and as severe (Score 3) in two ears (Table 1).

There were no apparent differences in OTIS3 in the ears classified as chronic/recurrent otitis compared with those identified as acute otitis. In contrast, pain was observed more frequently among chronic/recurrent OE affected ears (10 of 12) as compared with acute otitis (four of 12). Similarly, no pruritus was reported in any of the acutely affected ears while it was recorded in five of 12 chronically affected ears. Finally, stenosis of the auricular canal was detected in two of the chronically affected ears.

Microbiological counts at the beginning of the study (Day 0) are shown in Table 2. The highest counts were for yeast, followed by bacilli and cocci. In all the affected ears in which a significant number of yeast cells was observed, this diagnosis was confirmed by the isolation of *Malassezia pachydermatis* (19 isolates). *S. pseudintermedius* was the most common isolated coccus (n=4), followed by *S. schleiferi* (n=2), *Enterococcus faecalis* (n=1), *Neisseria mucosa* (n=1) and *Macrococcus caseolyticus* (n=1). In spite of higher rod counts, only four isolates were recovered; *P. mirabilis* (n=2), *Pseudomonas aeruginosa* (n=1) and *Moraxella atlantae* (n=1).

Evolution of OTIS3 index as well as scores of each of the parameters which make up the OTIS3 index, pain and pruritus through the follow-up period for the two groups is shown in Table 1. Clinical evaluation using OTIS3 index showed a clear progression towards improvement in both groups. The proportion of treated ears with an OTIS3 index ≥4 on Day 11 was significantly lower when compared with Day 0 for the two products (Otodine®: $\chi^2=4.70$, P=0.030; Vetramil Auris®: $\chi^2=5.85$, P=0.016) but not on Day 5 (Fig 1). Moreover, at the end of the study (Day 11) 22 of 24 ears were deemed to have mild (four ears with Score 1), or no (18 ears with Score 0), pain. On Day 11 only two ears (one with score 1 and the other with Score 2) continued to show pruritus.

The yeast, cocci and bacilli counts recorded during the treatment with the two products are shown in Table 2. Affected ears treated with Otodine® showed a significant reduction in the number of yeast (H=8.52, P=0.014) and a non-significant reduction in the number of bacilli (H=5.68, P=0.058). No significant decrease in cocci counts was found (H=4.66, P=0.102). A significant reduction in yeast counts occurred after treatment with Vetramil® Auris (H=7.59, P=0.022) but no change in bacilli and cocci counts were observed in this group (H=3.59, P=0.166 and H=2.69, P=0.261, respectively).

## DISCUSSION

The results suggest that the application of Otodine® or Vetramil® Auris for 10 consecutive days, in the absence of classical antibiotic or antimycotic agents, may be effective for the control of clinical signs and reduce the number of yeast in affected dogs with OE. In order to assess the efficacy of these products, we have used a previously proposed clinical index, OTIS3, which quantifies each of erythema, oedema/swelling, erosion/ulceration and exudate with a 0 to 3 score (Nuttall & Bensignor 2014). A cut-off value ≥4 was recommended for the identification of affected ears while a cut-off value ≤3 should indicate clinical success of the treatment.

A significant clinical improvement measured using OTIS3 after a 10-day treatment period was shown for both treatments with only two affected ears remaining with OTIS3 scores higher than 3 in each group at the end of the follow-up period. However, no clinical improvement was reported for either treatments by Day 5 suggesting that the duration of the treatment may be a critical factor. A longer treatment period, up to 21 days, was used in a previous report (Maruhashi *et al.* 2016) evaluating the efficacy of medical grade honey in OE management while a treatment between seven and 14 days is recommended in the technical leaflet for Vetramil® Auris.

Clinical results were confirmed using cytology assessment. On Day 0 and using previously proposed cut-off limits (Ginel *et al.* 2002, Angus 2004), 22 of 24 ears showed pathological levels of yeast (≥4 yeast cells *per* field) while an abnormally increased population of bacteria (>16 bacterial cells *per* field) was observed in six of 24 ears. At the end of the period, yeast counts were within the normal range in 18 of 24 ears, after use of either tested product. However, neither of the two products significantly reduced bacilli or cocci counts.

Otodine® ear solution contains chlorhexidine and Tris-EDTA. Previous studies have shown their *in vitro* effectiveness against microorganisms commonly associated with canine OE (Cole *et al.* 2006, Cole *et al.* 2007, Swinney *et al.* 2008, Guardabassi *et al.* 2010, Steen & Paterson 2012, Banovic *et al.* 2013, Mason *et al.* 2013, Boyd *et al.* 2019, Chan *et al.* 2019). In three of these studies *in vitro* evaluation of the same product used in this study (Otodine®) was carried out and the results obtained showed moderate activity against *M. pachydermatis* (Mason *et al.* 2013); *Pseudomonas* spp. (Steen & Paterson 2012) and *S. pseudinterme-*

**Table 1. Values of the clinical parameters at the beginning (Day 0) and over the follow-up period (Days 5 and 11) in the 13 dogs included in this study (24 ears affected) treated with Otodine® (11 ears) or Vetramil® Auris (13 ears)**

| Treatment | Patient | Type of otitis | Erythema | | | Oedema | | | Erosion | | | Exudate | | | Pain | | | Pruritus | | | OTIS3 index | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Day 0 | Day 5 | Day 11 | Day 0 | Day 5 | Day 11 | Day 0 | Day 5 | Day 11 | Day 0 | Day 5 | Day 11 | Day 0 | Day 5 | Day 11 | Day 0 | Day 5 | Day 11 | Day 0 | Day 5 | Day 11 |
| Otodine® | 1 | Chronic | 2 | 2 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 3 | 3 | 2 | 2 | 2 | 1 | 2 | 0 | 0 | 6 | 6 | 2 |
| | 4 | Acute | 2 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 2 | 2 | 1 | 2 | 1 | 0 | 0 | 0 | 0 | 5 | 4 | 3 |
| | 5 | Chronic | 1 | 1 | 2 | 1 | 0 | 2 | 1 | 0 | 0 | 2 | 0 | 2 | 1 | 0 | 1 | 0 | 0 | 0 | 5 | 1 | 6 |
| | 6 | Chronic | 1 | 2 | 2 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 0 | 3 | 2 | 0 | 2 | 5 | 5 |
| | 8 | Acute | 2 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 4 | 2 |
| | 9 | Acute | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 2 | 0 |
| | 10 | Acute | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 1 |
| | 11 | Chronic | 2 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 3 | 2 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 6 | 4 | 3 |
| | 12 | Acute | 2 | 1 | 1 | 2 | 1 | 0 | 1 | 0 | 0 | 2 | 2 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 7 | 4 | 2 |
| | 13 | Acute | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 2 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 4 | 2 |
| | 14 | Chronic | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 3 | 1 | 0 | 1 | 0 | 0 | 2 | 2 | 0 | 5 | 3 | 0 |
| Vetramil® Auris | 1 | Chronic | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 2 | 1 | 3 | 0 | 0 | 2 | 2 | 2 | 2 | 3 | 2 |
| | 2 | Chronic | 3 | 3 | 1 | 2 | 2 | 1 | 3 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 0 | 0 | 0 | 9 | 7 | 4 |
| | 3 | Chronic | 2 | 2 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 5 | 3 |
| | 4 | Acute | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 2 | 3 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 5 | 5 | 1 |
| | 5 | Chronic | 1 | 2 | 1 | 2 | 2 | 1 | 2 | 2 | 0 | 2 | 2 | 2 | 1 | 3 | 3 | 0 | 0 | 0 | 7 | 8 | 4 |
| | 6 | Chronic | 2 | 1 | 1 | 1 | 2 | 1 | 0 | 2 | 0 | 2 | 2 | 1 | 2 | 2 | 0 | 3 | 2 | 0 | 5 | 7 | 3 |
| | 8 | Acute | 2 | 1 | 0 | 1 | 1 | 0 | 2 | 1 | 0 | 2 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 7 | 4 | 1 |
| | 9 | Acute | 2 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 0 |
| | 10 | Acute | 2 | 1 | 1 | 2 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 3 | 3 |
| | 11 | Chronic | 2 | 1 | 1 | 2 | 1 | 0 | 0 | 0 | 0 | 3 | 2 | 2 | 1 | 0 | 0 | 0 | 1 | 0 | 7 | 4 | 3 |
| | 12 | Acute | 1 | 2 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 2 | 1 | 1 | 0 | 0 | 1 | 0 | 2 | 0 | 3 | 5 | 3 |
| | 13 | Acute | 0 | 2 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 4 | 3 |
| | 14 | Chronic | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 0 |

Each parameter was graded on a scale of 0 to 3; the OTIS3 index is the result of the sum of the values of erythema, oedema, erosion and exudate.

**Table 2. Values of yeast, cocci and bacilli counts (expressed as the mean of the counts of 10 microscopic fields) at the beginning (Day 0) and over the follow-up period (Days 5 and 11) in the 13 dogs included in this study (24 ears affected) treated with Otodine® (11 ears) or Vetramil® Auris (13 ears)**

| Treatment | Patient | Type of otitis | Mean number of yeast | | | Mean number of cocci | | | Mean number of bacilli | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Day 0 | Day 5 | Day 11 | Day 0 | Day 5 | Day 11 | Day 0 | Day 5 | Day 11 |
| Otodine® | 1 | Chronic | 58.2 | 75.3 | 0 | 19.5 | 17.7 | 5.7 | 71.4 | 75.3 | 23 |
| | 4 | Acute | 8.9 | 9.9 | 0.2 | 0 | 0.4 | 0 | 8.1 | 4.3 | 0 |
| | 5 | Chronic | 7.2 | 3.3 | 1.2 | 7.3 | 2.0 | 0 | 0 | 0.3 | 0 |
| | 6 | Chronic | 12.1 | 6.0 | 1.0 | 0 | 0.2 | 0 | 0.1 | 0.5 | 0 |
| | 8 | Acute | 15.9 | 25.4 | 24.6 | 0.1 | 0 | 0 | 0.2 | 0 | 0 |
| | 9 | Acute | 12.0 | 7.3 | 1.1 | 0 | 0.3 | 4.6 | 6.0 | 0.3 | 0.2 |
| | 10 | Acute | 4.0 | 0 | 5.2 | 0 | 0 | 0 | 0.2 | 0 | 0 |
| | 11 | Chronic | 7.9 | 9.6 | 1.0 | 0.3 | 0 | 0 | 0 | 0 | 0 |
| | 12 | Acute | 4.0 | 0 | 1.1 | 0.8 | 0 | 0 | 0.6 | 0 | 0 |
| | 13 | Acute | 24.2 | 17.7 | 8.0 | 8.0 | 0 | 0 | 0 | 0 | 0 |
| | 14 | Chronic | 11.8 | 4.4 | 0 | 7.2 | 6.9 | 0 | 37.9 | 13.8 | 0 |
| | Total | Mean (sd) | 15.11 (15.39) | 14.45 (21.55) | 3.95 (7.29) | 3.93 (6.17) | 2.50 (5.44) | 0.94 (2.10) | 11.32 (22.86) | 8.59 (22.51) | 2.11 (6.93) |
| | | Median (range) | 11.8 (56.2) | 7.3 (75.3) | 1.1 (24.6) | 0.3 (19.5) | 0.2 (17.7) | 0 (5.7) | 0.2 (71.4) | 0.3 (75.3) | 0 (23) |
| Vetramil® Auris | 1 | Chronic | 43.4 | 32.6 | 0 | 23.9 | 30.8 | 63.0 | 30.1 | 32.6 | 0 |
| | 2 | Chronic | 0.6 | 0.2 | 1.0 | 8.8 | 3.3 | 0 | 39.0 | 32.6 | 14.6 |
| | 3 | Chronic | 8.5 | 7.1 | 0.6 | 0.2 | 0 | 0.1 | 9.4 | 7.8 | 0.2 |
| | 4 | Acute | 0.4 | 0 | 1.1 | 8.5 | 7.1 | 0.2 | 33.3 | 33.5 | 0.1 |
| | 5 | Chronic | 6.4 | 0.2 | 2.1 | 0 | 0.1 | 0 | 0 | 0.2 | 0 |
| | 6 | Chronic | 22.6 | 23.4 | 11.0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 8 | Acute | 21.5 | 22.2 | 10.2 | 36.1 | 37.7 | 21.7 | 0.3 | 0 | 0 |
| | 9 | Acute | 11.4 | 8.1 | 0.2 | 1.9 | 1.6 | 0.1 | 0.4 | 0.5 | 0 |
| | 10 | Acute | 9.9 | 11.6 | 14.9 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 11 | Chronic | 27.5 | 24.4 | 1.8 | 0.3 | 0 | 0 | 0 | 0 | 0 |
| | 12 | Acute | 11.6 | 9.4 | 0.8 | 0.3 | 0 | 0 | 0.1 | 0 | 0 |
| | 13 | Acute | 6.0 | 9.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 14 | Chronic | 13.3 | 8.3 | 0 | 1.0 | 0 | 0 | 0 | 0 | 0 |
| | Total | Mean (sd) | 14.08 (11.98) | 12.06 (10.41) | 3.36 (5.09) | 6.23 (11.27) | 6.20 (12.69) | 6.55 (17.99) | 8.66 (14.86) | 8.25 (14.22) | 1.15 (4.04) |
| | | Median (range) | 11.4 (43) | 9.3 (32.6) | 1.0 (14.9) | 0.3 (36.1) | 0 (37.7) | 0 (63.0) | 0.1 (39.0) | 0 (33.5) | 0 (14.6) |

Total values are expressed as mean and median with standard deviation and range, respectively, given in parentheses.

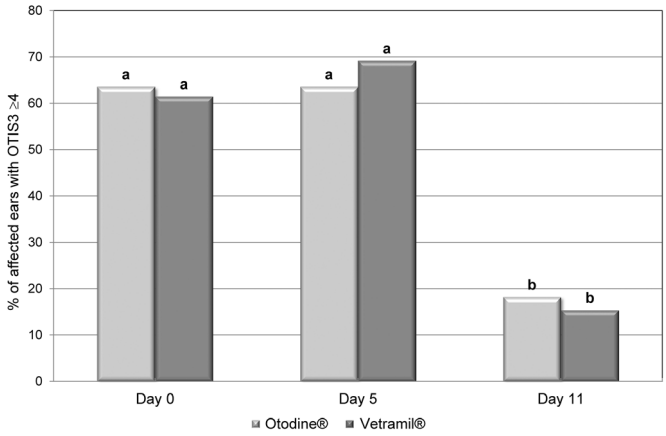


**FIG 1. Response of affected ears treated with Otodine® or Veramil Auris® throughout the study. Bars represent the percentages of ears having an OTIS3 index ≥4. Different letters show statistically significant differences**

*dius*, *M. pachydermatis*, *Streptococcus canis* and *Corynebacterium auriscanis* (Guardabassi *et al.* 2010). Here we have shown that the application of Otodine® decreased the counts of microorganisms throughout the treatment, although the differences were only statistically significant for yeast counts.

However, to our knowledge, only one study has shown the *in vivo* efficacy of Otodine® in the treatment of OE in dogs (Bouassiba *et al.* 2012), in which Otodine® was followed by an ear medication containing marbofloxacin, dexamethasone and clotrimazole. Tris-EDTA has been described as a bacteriostatic product capable of potentiating other antimicrobial agents, including antibiotics, and its synergistic effect against resistant bacteria associated with otitis has specifically been described (Wooley & Jones 1983, Farca *et al.* 1997, Boyd *et al.* 2019). In our study we have shown the efficacy of Otodine® alone, without the administration of any other antimicrobial or anti-inflammatory agents in the management of canine OE.

A recent open pilot study evaluated the efficacy of a medical grade honey gel in the management of canine OE (Maruhashi *et al.* 2016). *In vitro* assays of the biocidal activity of medical grade honey showed activity against all bacterial isolates recovered from 26 affected ears including methicillin-resistant *S. pseudintermedius* (MRSP). *In vivo* medical honey promoted rapid clinical progress, with 70% of dogs achieving clinical cure between Days 7 and 14 and over 90% having resolved by Day 21. Recently, another study (Oliveira *et al.* 2018) determined the *in vitro* activity of a honey-based gel against methicillin-susceptible *S. pseudintermedius* (MSSP), MRSP and *M. pachydermatis*. Bactericidal effect was observed at 20% w/v (weight/volume) and no difference was observed between MSSP and MRSP isolates while antifungal effect was observed at 10% (w/v); time-kill test showed the effectiveness of honey gel as quickly as after 1 hour exposure while all tested isolates were killed after 4 hours of exposure.

In agreement with these results, in the present pilot study we have demonstrated the *in vivo* efficacy of a 10-day treatment with a commercial product based on 40% Manuka honey (Vetramil® Auris) in the management of OE in dogs in which yeasts (*M. pachydermatis*) are involved. However, the efficacy of this product in those OE in which bacteria are the main secondary cause should be further evaluated. It is interesting to note that Manuka honey has also been described as having a synergistic effect with some antibiotics (Jenkins & Cooper 2012).

Taking into account the type of otitis, acute or chronic, clinical improvement measured by OTIS3 was achieved with both commercial products at the end of the follow up for acute otitis but not for chronic/recurrent cases. However, there were slight differences in the behaviour of both commercial products. There was a clinical improvement of OE measured by OTIS3 in six of six acute otitis cases treated with Otodine® compared to four of six of those treated with Vetramil® Auris. In contrast, the evolution of chronic otitis showed better results for Vetramil® Auris, which reduced OTIS3 index in six of seven cases compared to three of five for Otodine®.

In view of the overall results of this work, it appears that Vetramil® Auris might be beneficial for the treatment of OE in dogs. However, some of its results do not match the efficacy of a conventional chlorhexidine-Tris-EDTA-based ear cleaner such as Otodine® in a 10-day treatment. Regarding the aetiology of the process, Otodine® and Vetramil® showed adequate behaviour for otitis associated with *Malasezzia pachydermatis*. Our study was small without prestudy sample size calculations, implying that statistical testing has unknown reliability; further studies will be required to support these findings.

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## Conflict of interest

Fatro Ibérica S.L. provided the ear solutions evaluated in this work though playing no part in the design of the study, the analysis of the results or the preparation of the manuscript.

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