The comparative cerumenolytic activity of otic preparations, an *in vitro* study

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The comparative cerumenolytic activity of otic preparations, an *in vitro* study

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Canine Cerumen [1,2]

It covers the epithelial surface of the external ear canal

- Cerumen is formed of the secretions of sebaceous and ceruminous glands together with exfoliated cells

The lipid component of earwax can vary widely (18.2-92.6%)

Canine cerumen is mainly composed of ***exfoliated cells, waxes, oils, free fatty acids (margaric, stearic, oleic, linoleic acid), esters, immunoglobins and proteins***

Actions:

1. Facilitates the capture and excretion of debris
2. It maintains the moisture content through a repulsive effect on water
3. Antimicrobial activity (oleic and linoleic acid)

[1] **Anatomy and physiology of the canine ear**
Lynette K. Cole; Vet Derm, 2009

[2] **Ceruminal diffusion activities and ceruminolytic characteristics of otic preparations - an *in vitro* study**
Stahl J et al. BMC Veterinary Research. 2013

Histological changes in the external ear canal of dogs with otitis externa [3]

- Canine otitis externa (OE) is one of the most common diseases in small animal practice
- It is a syndrome with a multifactorial aetiology
- In chronic OE, due to the inflammatory process, ceruminous glands show hyperplasia, increase secretory activity and, as a result, the amount of earwax produced.

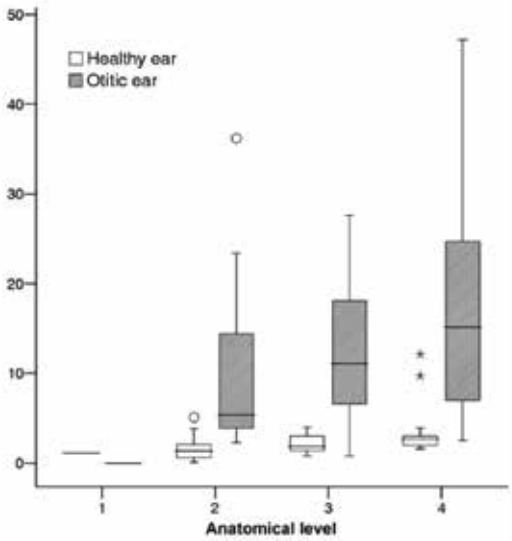

Figure 5. The mean proportion (%) of each section occupied by ceruminous glands in the aural skin at four anatomical levels in healthy ears (n = 28) and ear with otitis externa (n = 15). Outliers (circles) and extreme values (asterisks) are indicated.

Pathological changes in the external ear canal of dogs with COE [4]

Earwax changes:

1. Reduction of lipid content
2. Increased humidity
3. Decreased antibacterial activity
4. Epithelial migration (EM) absent
5. Excess of wax material
6. Production of earwax plugs

Cerumen and ceruminous canine otitis [5]

An excess of cerumen may be irritating, prevent medications from contact with the ear canal wall and create a favourable environment for microorganisms to proliferate leading to ceruminous otitis externa and secondary yeast (*Malassezia*) and bacterial infections

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[3] **Histological changes in the external ear canal of dogs with otitis externa**
Hui-Pi Huang et al. Vet Dermatol. 2009

[4] **Breed variations in histopathologic features of chronic severe otitis externa in dogs: 80 cases (1995-2001)**
Angus JC et al. J Am Vet Med Assoc. 2002

[5] ***In vitro* investigation of ceruminolytic activity of various otic cleansers for veterinary use**
Sánchez-Leal et al. Vet Dermatol. 2006

Reasons for the use of ceruminolytic agents [6]

- Ear cleaning can be beneficial in maintaining the normal otic environment, especially in 'seborrhoeic' or allergic dogs with excessive cerumen in their ears
- Cerumenolytic activity and diffusion through cerumen residues helps to make the disintegration of earwax and its detachment from the ear epithelium faster and more effective

Ceruminolytic ear cleaners [7]

• The ability to effectively clean ears to remove wax is an important part of the treatment and then ongoing maintenance therapy for otitis
• Diocytl sodium sulphosuccinate (DOSS), calcium sulphosuccinate, urea or carbamide peroxide are potent ceruminolytics. Urea and carbamide peroxide are foaming agents that release oxygen in situ to help break up debris
• Cerumino-solvent ear cleaners contain organic oils and solvents that soften and loosen cerumen. Examples of these are butylated hydroxytoluene, cocamidopropyl betaine, glycerine, lanolin, propylene glycol and squalene

The comparative cerumenolytic activity of otic preparations, an *in vitro* study
• The cerumenolytic activity of some otic preparations represents a valuable aid for the management of this condition
• The study aimed to evaluate the wax removal efficacy, *in vitro*, of various veterinary otic preparations used in Europe

Materials and Methods
• evaluate the effectiveness of the otic preparations:
Otoprof ®, Otoact ®, Epiotic ®, Diclorex oto ®, Otifree ®, Aurinet ® Malacetic ®, Otodog ®, Otolane ®

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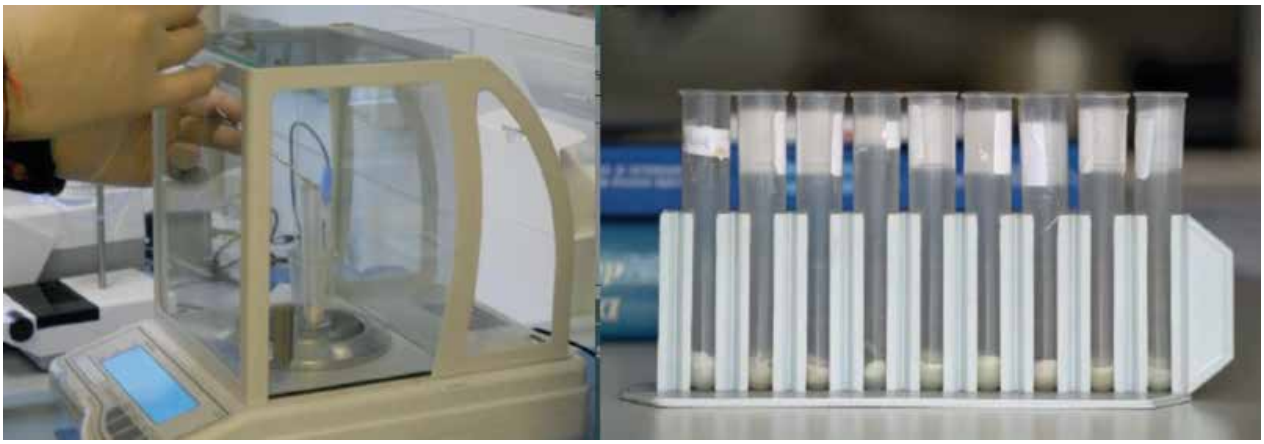
6 ***In vitro* investigation of ceruminolytic activity of various otic cleansers for veterinary use** Sánchez-Leal et al. Vet Dermatol. 2006

7 **Paterson S. Topical ear treatment—options, indications and limitations of current therapy.** J Small Anim Pract 2016

Products	Agents
Otoprof ®	Aqua, Dioctyl Sodium Sulphosuccinate, Carbamide peroxide, Propylene Glycol
Otoact ®	Squalene, Salicylic acid, Butyl extract of chamomile, Tannic acid
Epiotic ®	Aqua, Disodium EDTA, PCMX, Diethylhexyl sodium sulfosuccinate, Salicylic acid, Glycotechnology (Rhamnose, Galactose, Mannose), Defensin technology (Peumus boldus leaf extract, Spiraea ulmaria extract).
Diclorex oto ®	Glycerin, Dipropylene glycol, Isopropyl myristate, Cetearyl alcohol, Oleic acid, Cetrimonium chloride, Hydroxyethylcellulose, Glycyrrhetinic acid, Chlorhexidine digluconate 0,3%, BHT, Disodium, EDTA, water
Otifree ®	Calendula 1%, 40% propylene glycol, water, emulsifiers, basil oil. Neutral pH solution
Aurinet ®	Aqua, paraffinum liquidum, polysorbate 60, ethylhexyl palmitate, sorbitan stearate, polyacrylamide, benzyl alcohol, c13-14 isoparaffin, cetearyl stearate, cetearyl alcohol, thymus zygis herb oil, zinc oxide, laureth-7, eucalyptus globulus leaf/twig oil, dehydroacetic acid, peg-20 stearate, salicylic acid, eugenia caryophyllus bud oil, eugenol, linalool, limonene, sodium hydroxide, citral, citric acid
Malacetic ®	Aqua, 2% acetic acid, 2% boric acid
Otodog ®	Aqua; fermented vegetable extract; sodium laureth sulfate; citric acid; sodium benzoate; cupric sulfate; parfum; methylchloroisothiazolinone
Otolane ®	Acetic acid: 0.5 %, Chlorhexidine digluconate: 0.2%

An analytical and experienced protocol was used
Standardized synthetic cerumen (SCC) was prepared, weighed in test tubes, and left to solidify



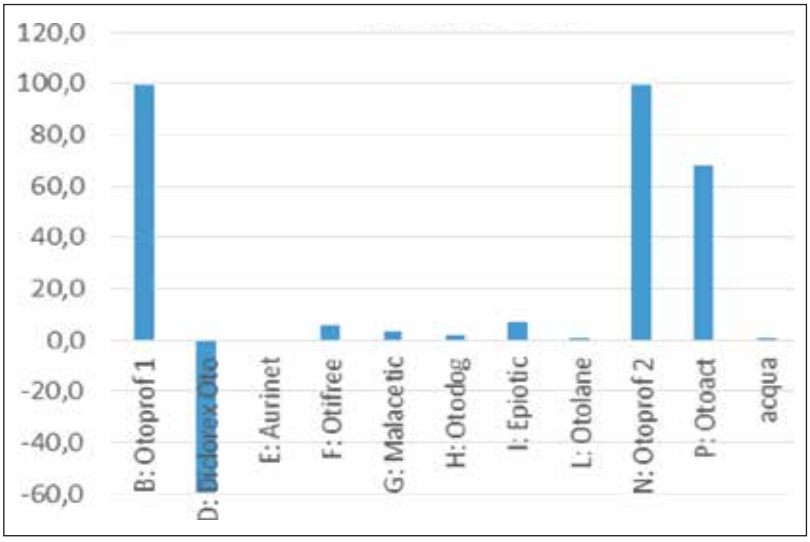
- An aliquot part of the otological product equal to two milliliters (ml) was added
- The tubes were shaken moderately at a temperature of 35 ° C for five minutes, and subsequently, were inverted
- After 24 hours each test tube was weighed, and another two ml of the product were added
- Five washes per tube were repeated and the test was performed in triplicate

Results

Standard artificial ear wax removal rate for each test; expressed as a ± confidence limit at the 80th percentile, according to a normal distribution

Sample	test 1	test 2	test 3	test 4	test 5
B: Otoprof 1	19,7 ± 2,1	33,6 ± 10,5	52,8 ± 12,2	82,8 ± 12,0	99,4 ± 0,3
D: Diclorex Oto	-54,9 ± 2,1	-59,3 ± 11,0	-56,2 ± 6,6	-56,1 ± 9,1	-59,6 ± 5,0
E: Aurinet	-2,2 ± 1,5	-1,0 ± 1,3	-8,4 ± 3,1	-6,1 ± 1,7	-0,1 ± 5,9
F: Otifree	-0,8 ± 0,9	1,7 ± 1,6	1,9 ± 1,8	6,4 ± 1,5	5,8 ± 1,6
G: Malacetic	-0,2 ± 0,3	1,3 ± 0,5	1,0 ± 0,5	3,0 ± 1,9	3,7 ± 0,5
H: Otodog	0,3 ± 0,1	1,1 ± 0,2	1,2 ± 0,1	1,8 ± 0,1	1,7 ± 0,2
I: Epiotic	1,4 ± 0,9	-0,9 ± 6,5	4,0 ± 1,3	6,8 ± 1,2	7,0 ± 1,2
L: Otolane	0,0 ± 0,5	1,1 ± 0,7	-0,4 ± 1,3	0,4 ± 1,2	0,2 ± 1,4
N: Otoprof 2	20,2 ± 1,6	43,6 ± 2,6	63,3 ± 3,7	95,8 ± 4,0	99,7 ± 0,4
P: Otoact	-18,3 ± 1,4	-5,7 ± 1,4	13,3 ± 1,5	31,5 ± 2,1	68,0 ± 3,2
acqua	-1,4 ± 0,2	0,0 ± 0,2	-0,1 ± 0,4	1,4 ± 0,1	0,4 ± 0,1

Standard artificial earwax percentage removal histogram after the fifth wash. All tested products are listed



• 99.4% of removed cerumen was obtained only with the product containing carbamide peroxide and DOSS (Otoprof ® ICF Srl, Palazzo Pignano, Italy)
• A good cerumenolytic property, 65%, was obtained from the product containing squalene (Otoact ® ICF Srl, Palazzo Pignano, Italy)
• Other products containing Disodium EDTA, PCMX, diethylhexyl sodium sulfosuccinate (like Epiotic ® Virbac Srl, Milano, Italy) showed less cerumenolytic activity with 7% and the remaining ones with a range from 5 to 1% of activity

Conclusions
This *in vitro* comparison study demonstrated that the molecules with the most cerumenolytic activity are *carbamide peroxid*e and DOSS (**Otoprof ®**) and *squalene* (**Otoact ®**).
Clinical studies in dogs with ceruminous otitis are required to confirm the *in vivo* efficacy of these formulations.

Discussion
Investigators used synthetic cerumen based on the average results of human and canine cerumen studies:
- Bortz JT et al. *Composition of cerumen lipids.* J Am Acad Dermatol. 1990
- Masuda A. et al. *Study of lipid in the ear canal in canine otitis externa with Malassezia pachydermatis*. J Vet Med Sci. 2000
- Sánchez-Leal J. *In vitro investigation of ceruminolytic activity of various otic cleansers for veterinary use*. Vet Dermatol. 2006
- Stahl J et al. *Ceruminal diffusion activities and ceruminolytic characteristics of otic preparations - an in-vitro study.* BMC Vet Res. 2013

No DOSS, urea or carbamide peroxide cleaners were tested in these *in vitro* and *in vivo* studies.

Our *in vitro* study was the first that demonstrates the DOSS and carbamide peroxide cerumenolytic efficacy and confirm that squalene has a good activity too.