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Comparative time-kill kinetics of two commercial ear cleaners and comparison of their *in vitro* ceruminolytic activity

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ABSTRACT

The aim of these studies was to compare the ceruminolytic properties of two ear cleaners [Sonotix® (Vetoquinol; Lure, France) and Epiotic® SIS (Virbac; Carros, France)] and their antimicrobial activity against three isolates of each of the following pathogens, Staphylococcus pseudintermedius, Pseudomonas aeruginosa, Proteus mirabilis and Malassezia pachydermatis. Strains of each organism were incubated 32 min with each ear cleaner. Aliquots were taken from each test tube at multiple time points (1, 2, 4, 8, 16 and 32 min) and spread on different agar media for colony counting. Both ear cleaners exhibited 100% bactericidal activity against all pathogens; however, only Sonotix® exhibited an inhibitory effect with 100% bactericidal activity at 1 min against all S. pseudintermedius, P. mirabilis, M. pachydermatitis strains and between 1 and 4 min against P.aeruginosa. Epiotic® SIS showed bactericidal activity after 32-min exposure but no fungicidal activity. The ceruminolytic activity was assessed in vitro *based on dissolution of synthetic canine cerumen (SCC). Two ml of each ear cleaner was incubated with SCC for 20 min in a shaking water bath at 35°C. Test tubes were inverted for one hour to allow the dispersed SCC to slide out of the tubes and then weighed. This operating procedure was repeated 4 times in total for both products. The final percentage of SCC elimination was 60% for Sonotix®, whereas Epiotic® SIS showed 0% ceruminolytic activity. These* in vitro *pilot studies show that Sonotix® achieves very fast bactericidal activity and an efficient* in-vitro *ceruminolytic activity.*

INTRODUCTION

Canine ear disease is a common issue among dogs and represents about 15% of veterinary visits. The causes of otitis externa are conventionally classified as: predisposing factors that bias an individual to ear disease, primary factors that trigger the otitis, and perpetuating factors that exacerbate the disease and prevent resolution. Bacterial and yeast infections are particularly important secondary factors as they quickly complicate most cases of otitis in dogs. In otitis externa, ear cleaning is frequently performed to maintain normal otic environment, help treat otitis and prevent recurrence in dogs prone to otitis [1,2,3]. A wide range of ear cleaning preparations and procedures aimed to remove exudates and ceruminous debris has become very popular in veterinary practice [4]. Among other components, some of the commercially available products contains bactericidal agents and ceruminolytics agents.

STUDY OBJECTIVE

The aim of these studies was to compare the ceruminolytic properties of two ear cleaners [Sonotix® (Vetoquinol; Lure, France) and Epiotic® SIS (Virbac; Carros, France)] and their antimicrobial activity against three isolates of each of the following pathogens, *Staphylococcus pseudintermedius, Pseudomonas aeruginosa, Proteus Mirabilis and Malassezia pachydermatis*.

MATERIAL AND METHODS

Bactericidal activity and killing rate determination

- Three strains, isolated from canine otitis externa, of each of the following pathogens, *Malassezia pachydermatis, Pseudomonas aeruginosa, Staphylococcus pseudintermedius and Proteus mirabilis,* were used in this study.
- For each strains, an inoculum was prepared in order to reach 10^7 CFU/mL for *M. pachydermatis and* 10^8 CFU/mL for *P. aeruginosa, S. pseudintermedius and Proteus mirabilis* strains.
- Inoculum of each organism were incubated for 32 min with 2 mL each ear cleaner. (Sonotix® and Epiotic® SIS). Moreover, for each strain, a viability control was performed.
- Aliquots were taken from each test tube at multiple time points (1, 2, 4, 8, 16 and 32 min) and spread on different agar media for colony counting.
- After incubation, the number of colonies per spot was counted and the mean of two spots in CFU/mL was calculated. A transformation of the CFU/mL average to Log_{10} CFU/mL was performed before graphic interpretations. The limit of detection of the method was 80 CFU/mL, 1.90 Log_{10} CFU/mL. If no colony has grown on plate, the value of counting was set at 0.

Ceruminolytic activity

- The ceruminolytic activity was assessed *in vitro* based on dissolution of a synthetic canine cerumen (SCC) (ref) mimicking the lipidic composition and texture of canine cerumen.
- 500 mg of melted SSC was weighed and placed into individual 10 x 75 mm polypropylene test tubes.
- Once the SSC cold and solidified in the tubes, 2 ml of each ear cleaner was incubated with SCC for 20 min in a shaking water bath at 35°C. Test tubes were inverted for one hour to allow the dispersed SCC to slide out of the tubes and then weighed. This operating procedure was repeated 4 times in total for both products.
- The percentage of SSC removed was then calculated by comparing the initial and final weights of the tubes and SSC.
- This *in vitro* model was originally designed to simulate the diameter of a dog's ear canal, and the temperature, contact time and head shaking that would occur in a real ear canal. The volume of ear cleaner used and number of cleaning repetitions is compatible with recommended ear cleaning practice for owners at home.

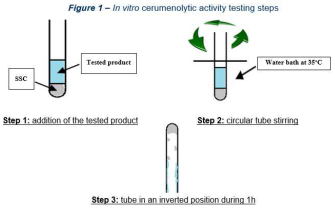
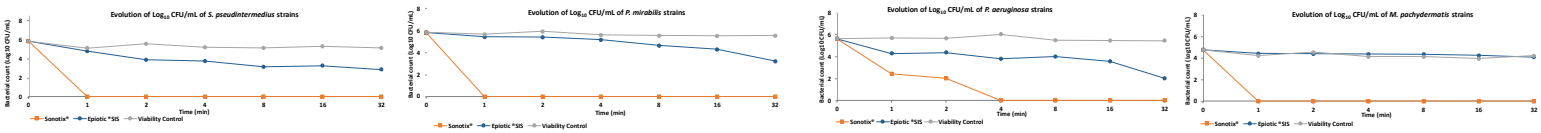


***Figure 1** – In vitro* cerumenolytic activity testing steps

RESULTS

Bactericidal activity and killing rate determination

Both ear cleaners exhibited 100% bactericidal activity (decrease of $3Log_{10}$ CFU/mL) against all pathogens; however, only Sonotix® exhibited an inhibitory effect with 100% bactericidal activity at 1 min against all *S. pseudintermedius, P. mirabilis, M. pachydermatitis* strains and between 1 and 4 min against *P. aeruginosa*. Epiotic® SIS showed bactericidal activity after 32-min exposure. No fungicidal activity was shown after 32 min incubation.



Ceruminolytic activity

In the present study, the ceruminolytic activity was defined as the weight loss of SSC after consecutive incubation and rinsing of otic preparations. Ceruminolysis causes disintegration and elimination of cerumen and so it is characterized by weight loss.
The final percentage of SCC elimination was 60% for Sonotix® whereas Epiotic® SIS showed 0% ceruminolytic activity.

DISCUSSION - CONCLUSION

Secondary bacterial and yeast infections occur as a consequence of alteration of the skin microclimate by these factors and act to exacerbate otitis and prevent clinical resolution [1, 2]. Ear cleaning in diseased ears allows to remove debris and purulent material, therefore optimizing penetration and diffusion of topical medication to the deeper parts of the horizontal canal. Antiseptics primarily act at the site of application and are currently not thought to select for microbial resistance at the high concentrations used on skin. In a previous study antimicrobial efficacy of some ear cleaners has been shown to be highly variable and it was difficult to draw many conclusions about the efficacy based only on composition [5].

In this *in vitro* time-kill kinetic study, results shows that Sonotix® achieves very fast bactericidal and fungicidal activity against common pathogens in veterinary dermatology an efficient *in vitro* ceruminolytic activity. One of the studies limitation is that an *in vitro* study may not replicate antimicrobial efficacy *in vivo*, where the presence of inflammatory exudate and cerumen may affect activity, and where anti-adhesives and other non-killing mechanisms can play a role. Moreover, the synthetic canine cerumen doesn't mimic the composition of cerumen in diseased ear which contains more keratin, inflammatory cells and serum than normal ears. Further studies evaluating the absolute efficacy values, adequately designed *in vivo* studies, should be performed.

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