30th EUROPEAN VETERINARY DERMATOLOGY CONGRESS ORGANIZED BY ESVD-ECVD 27-29 SEPTEMBER 2018 DUBROVNIK CROATIA

Comparative Time-kill Kinetics of two commercial ear cleaners

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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.

STUDY OBJECTIVE

The purpose of this study was to compare the bactericidal activity in relation to time of two ear cleaners (Sonotix®: Vetoquinol, Lure, France and Epi-otic® Advanced: Virbac, Carros, France) against *Staphylococcus pseudintermedius, Pseudomonas aeruginosa* and *Malassezia pachydermatis*.

MATERIAL AND METHODS

○ *Culture*

The strains used in the study consisted of: 3 *Malassezia pachydermatis* (yeast), 3 *Pseudomonas aeruginosa* (bacteria) and 3 *Staphylococcus pseudintermedius* (bacteria). The strains were isolated from canine otitis externa.

P. aeruginosa and *S. pseudintermedius* strains were cultured twice on Tryptone Soya Agar (TSA) and incubated at 35 ± 2 °C for about 18 to 24 h and *M. pachydermatis* strains were cultured on Sabouraud glucose agar (SGA) at 35 ± 2 °C for about 24 to 72 h.

○ *Inoculum preparation*

For *M. pachydermatis* strains, the inoculum was prepared by performing a 7 ± 0.3 McF suspension with some colonies from the second culture, in 3 mL of sterile physiological serum (NaCl 0.85%) in order to reach 10^8 CFU/mL.

For *P. aeruginosa* and *S. pseudintermedius* strains, the inoculum was prepared by performing a 0.5 ± 0.1 McF suspension with some colonies from the second culture, in 3 mL of sterile physiological serum in order to reach 10^8 CFU/mL.

○ *Inoculum count*

The inoculum size was determined by plating 2 spots of 25 µL of each inoculum on Sabouraud glucose agar for *M. pachydermatis* strains and on Columbia agar with 5% sheep blood for *P. aeruginosa* and *S. pseudintermedius* strains. Then, the Petri dishes were incubated at 35 ± 2 °C for about 24 to 72 hours for *M. pachydermatis* strains and for about 18 to 48 hours for *P. aeruginosa* and *S. pseudintermedius* strains.

○ *Bactericidal activity determination*

2 mL of each product (Sonotix® and Epi-otic® Advanced) were put in a sterile tube with 20 µL of inoculum previously prepared.

Moreover, for each strain, a viability control was performed: 20 µL of the inoculum were added to 2 mL of sterile physiological serum in a sterile tube.

○ *Killing rate determination*

All the tubes were closed and incubated at room temperature (19-25 °C), without agitation until the end of the trial.

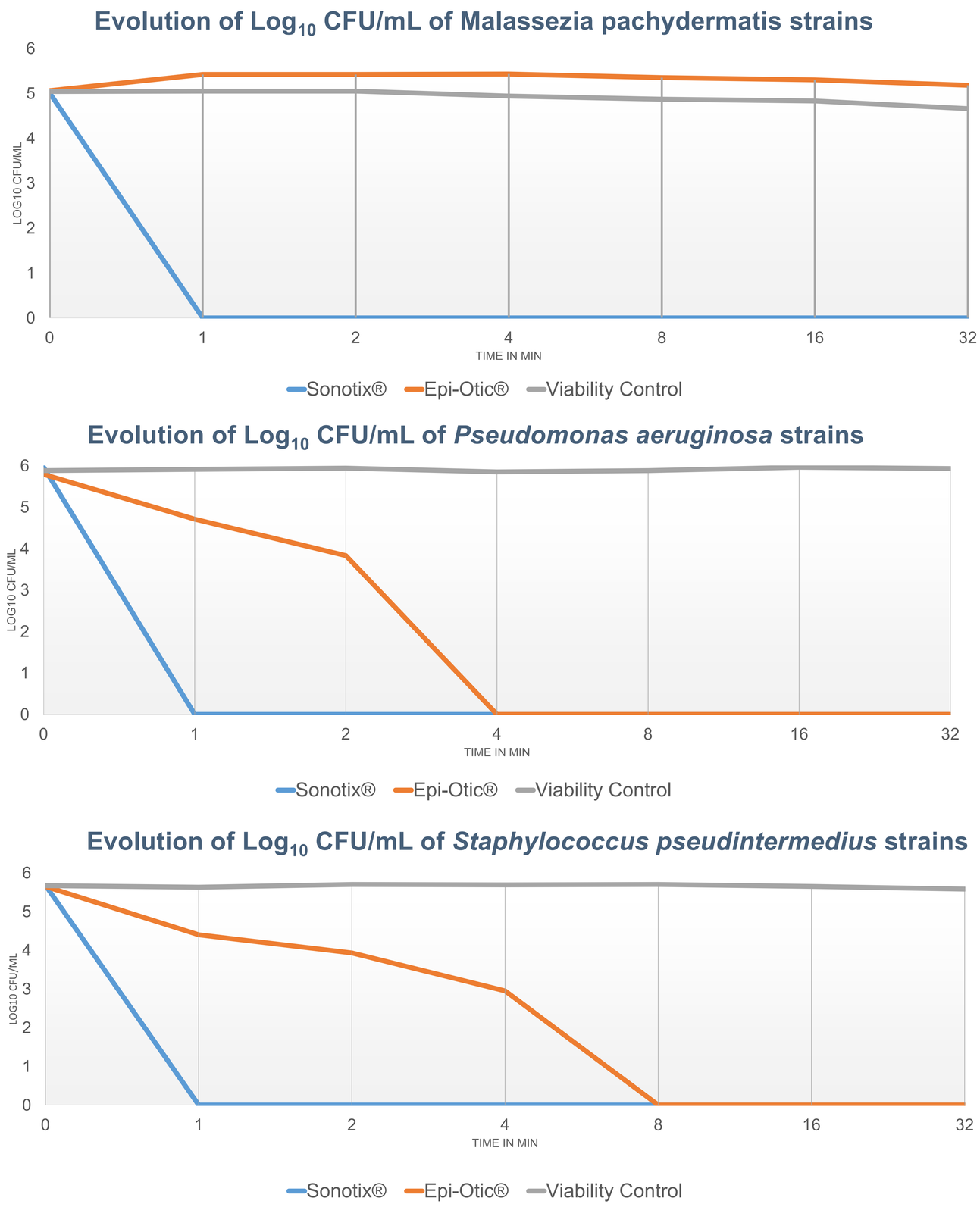
For each product and for the viability control, a 50 µL sample from each test tube was taken on the following occasions: 1, 2, 4, 8, 16 and 32 minutes. Then, 2 spots of 25 µL were plated on SGA for *M. pachydermatis* strains and on Columbia agar with 5% sheep blood for *P. aeruginosa* and *S. pseudintermedius* strains. Finally, the Petri dishes were incubated at 35 ± 2 °C for about 24 to 72 hours for *M. pachydermatis* strains and for about 18 to 48 hours for *P. aeruginosa* and *S. pseudintermedius* strains.

○ *Reading and interpretation of results*

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 or 1.90 Log_{10} CFU/mL. If no colony has grown on plate, the value of counting was set at < 80 CFU/mL or < 1.90 Log_{10} CFU/mL.

RESULTS

In this *in vitro* study, the bactericidal activity of two commercial ear cleaners was tested during 32 min against *M. pachydermatis*, *P. aeruginosa* and *S. pseudintermedius.*
Bactericidal activity was defined as no growth on media.
Results are shown in *Figures* 1, 2, 3**.**
Both undiluted ear cleaners exhibited 100% bactericidal activity against all strains of *S. pseudintermedius* and *P. aeruginosa.* However, only Sonotix® ear cleaner showed 100% bactericidal activity against *M. pachydermatitis.*
For the 3 pathogens, Sonotix® ear cleaner exhibited a knock-out effect with 100% activity at 1 min whereas such activity was reached only at 8 minutes for *S. pseudintermedius* and 4 min for *P. aeruginosa* with Epi-otic® Advanced.



Figures 1, 2, 3: Mean values in Log_{10} CFU/mL obtained at each sampling time for *M. pachydermatis, P. aeruginosa* and *S. pseudintermedius* strains.

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* study time-kill kinetic results shows that Sonotix® achieves very fast bactericidal activity against common pathogens in veterinary dermatology. 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. Further studies evaluating the absolute efficacy values, adequately designed *in vivo* studies, should be performed.

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