SCIENTIFIC RESEARCH *and* VETERINARY INFORMATION

Clinical, multicenter, pilot study

to evaluate the efficacy of a new otologic product (antimicrobial peptide AMP2041, tris-EDTA, chlorhexidine) in 30 dogs with either acute otitis externa or recurrent bacterial and/or yeasts otitis externa

Author and coordinator of the study:
Giovanni Ghibaudo Dr. Med. Vet.

Reserved for Veterinarians and Pharmacists

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Objectives of the study
The purpose of the study was to evaluate the clinical efficacy of a new gel otologic product with antimicrobial peptide AMP2041, tris-EDTA and chlorhexidine for the treatment of either acute otitis externa or recurrent bacterial and/or yeasts otitis externa in dogs.

Experimental design
This was an open field, multicenter controlled, pilot study. Enrolled dogs were first treated with Otoact solution. After 10 minutes the product was removed and the dogs were treated with a new gel otologic product with antimicrobial peptide AMP2041, tris-EDTA and chlorhexidine, applied every 48 hours for two weeks, according to the manufacturer instructions.

The efficacy and safety of the treatment, based on the clinical and cytological parameters, were evaluated by the veterinary surgeons at day 0, day 7, day 14 and day 28.

Thirty dogs were recruited (39 ears) by 16 co-investigators throughout Italy. Each investigator has enrolled at least 2-3 dogs during the study period.

*Thanks for collaboration to Doctors Francesca Aspidi, Danilo Bertozzi, Giovanna Cacciutto, Andrea Calderone, Lucia Covetti , Paola D'Intino , Lucia Marcella, Luca Luciani, Fabiano Necci, Claudia Pellicoro, Elena Picillo, Laura Porcari, Monica Pregliasco, Graziella Raviri, Annarita Talucci, Alessandro Priano.

The authors thank ICF for making this clinical study possible providing products to evaluated patients and supporting necessary analysis for the study

Animals
Affected dogs were recruited by the investigators in their daily clinical practice. Signalment, a full history and a consent form were collected for each patient. The study has been conducted according to GCP (Good Clinical Practice).

Inclusion criteria:
Dogs with acute otitis or exacerbations of chronic ceruminous and/or purulent otitis with Malassezia and/or bacterial overgrowth and/or a bacteria infection. Diagnosed was based on the clinico-pathological changes and the cytological examination of the cerumen/pus collected using a long cotton bud stick inserted in the cone of the otoscope all the way down to the junction between the vertical and the horizontal canal and then rolled on a glass slide. After air drying, the slides were fixed and then stained with the Wright's modified method (Hemacolor Merck) and examined under the microscope at oil immersion (1000x).

Exclusion criteria

- pregnant bitches, or nursing or scheduled for reproduction
- dogs with otoacariasis
- dogs with a perforated tympanic membrane
- dogs with ear canal neoplasia
- dogs with otitis lasting more than three weeks (chronic otitis)
- dogs with hyperplastic abnormalities of the ear canal walls (chronic otitis)
- dogs with generalized demodicosis
- dogs treated during the study or within 14 days prior been enrolled into the study with systemic antibiotics or antifungals. Heartworm prevention products, hyposensitation for atopic dermatitis, systemic antiparasite products and any other drug needed for the animal's survival (e.g. insulin, cardiac drugs, thyroxine, etc.) were allowed
- dogs which had received during the study or within 14 days prior to the time they were enrolled other otic treatments
- dogs that in an emergency would need systemic antibiotics or antifungals
- dogs who might develop adverse effects to the treatment (erythema, pruritus, drug reactions clinical signs)

Treatment

Products overview:
Otoact® solution contains: purified water, squalene, butyl chamomile extract, tannic and salicylic acid.

otologic product, hereinafter called ***MIX**** contains: purified water, polyvinylpyrrolidone, cellulose; active ingredients: AMP2041 (peptide), chlorhexidine digluconate, Tris-EDTA, Vitamin PP, Zinc PCA and GPI lysine.

Application

The first treatment was always done in presence of the owner so as to show the correct procedure. Otoact solution was applied by filling the external ear canal (without touching the skin with the nozzle of the bottle), and massaging the ear canal for 10 seconds.

After 10 minutes the ear canal was dried by inserting the index finger wrapped in a cotton pad. The same procedure was used between the ear folds (by using cotton buds). Soon after ****MIX*** was applied in the ear canal as follows: two sprays in small and medium size dogs (up to 25 kg); 3 sprays in large size dogs. The ear canal was then massaged for 10 seconds.

This treatment was repeated every 48 hours for 2 weeks.

****MIX** Otologic product in gel with purified water, polyvinyl pyrrolidone, cellulose; active ingredient: AMP2041 (peptide), chlorhexidine digluconate, Tris-EDTA, Vitamin PP, Zinc PCA, GPI lysine.*

Treatment

Day 0

All dogs included in the study had a clinical and otoscopic examination, and cytology of the cerumen/exudate. If there was a tympanic membrane perforation the dogs were not included in the study.

In dogs with bilateral otitis, the ears have been evaluated and treated separately.

Before doing the cytology a microscopic analysis of the cerumen was done to rule out the presence of mites. If present, the dog was not included in the study. In the case a foreign body was detected in the external ear canal, the dog was included only after removing the foreign body and assessing the integrity of the tympanic membrane.

The veterinary surgeon has always carried out the first application as described above.
The owner had to evaluate the dog for the presence of pruritus and pain using the VAS analogue scale.

Day 7

Dogs undergoing treatment were re-examined by the veterinary surgeon and a full clinical and otoscopic examination, and cytology were done.

Day 14 and day 28

The dogs treated were re-examined by the veterinary surgeon and a full clinical and otoscopic examination, and cytology were done.
The parameters to be assessed with the otoscopic examination and the cytology were the same as done at day 0.
The owner evaluation of the pruritus and pain was also recorded using the VAS analog scale. The owner also reported on the safety of the product.
Both the owner and the veterinary surgeon evaluated the overall efficacy of the treatment.

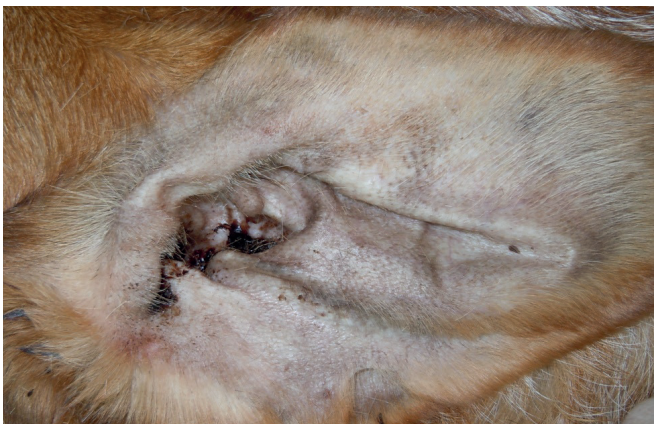


Photo 1 (day 0) dog with ceruminous otitis caused by Malassezia spp.

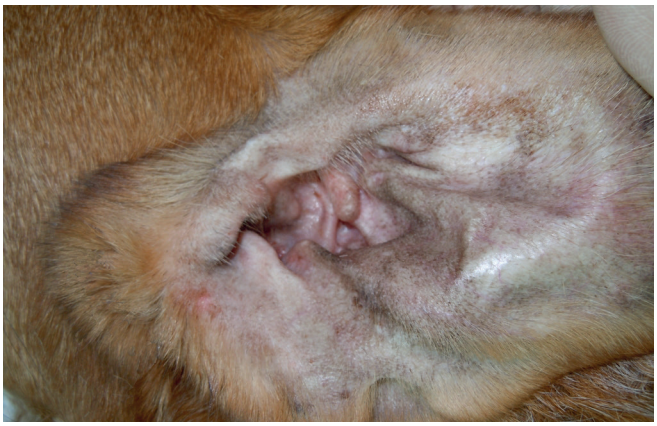


Photo 2 (day 14) the same dog after 2 weeks of therapy with a new gel otologic product with antimicrobial peptide AMP2041, tris-EDTA and chlorhedine l

Data recording
The medical history and clinical data were recorded on appropriate forms by the investigators.

Evaluation

Primary outcomes: evaluation of clinical and cytological improvements, effectiveness, ease of administration and tolerability of the product on day 14 and clinical and cytological examination 2 weeks after the last dose (day 28).

- Evaluation of the clinical signs according to the OTI3 scale recently validated and published by Nuttall et al, 2014.
- Owner evaluation of the pruritus using the visual analogue scale (VAS) validated by Hill et al, 2007, on days 0, 7, 14 and 28.
- Overall evaluation done by the veterinary surgeon and the owner (0-3) on day 14 and 28.
- Owner evaluation of the tolerability (0-3) on day 14 and 28.
- Evaluation of the number of bacteria and yeasts by using the semiquantitative scale (range 0-4) validated and published by Budach et al, 2012, on day 0, 7, 14 and 28:

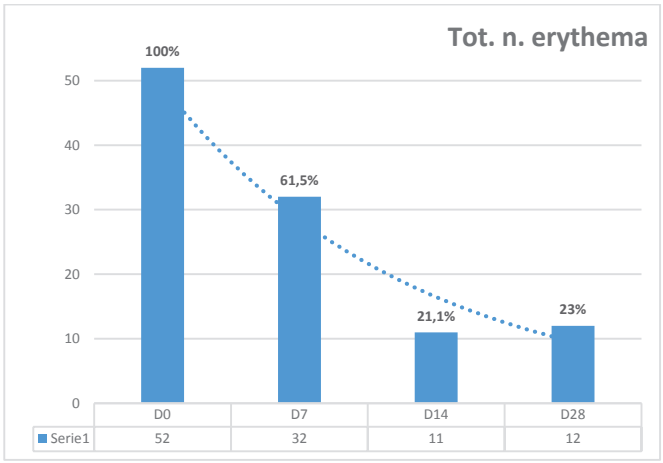
0. absence of bacteria and/or yeasts
1. occasional presence of bacteria and/or yeasts seen only after a careful search
2. bacteria and/or yeasts present in small quantities but easily seen
3. bacteria and/or yeasts present in large amounts and easily seen
4. large number of bacteria and/or yeasts.

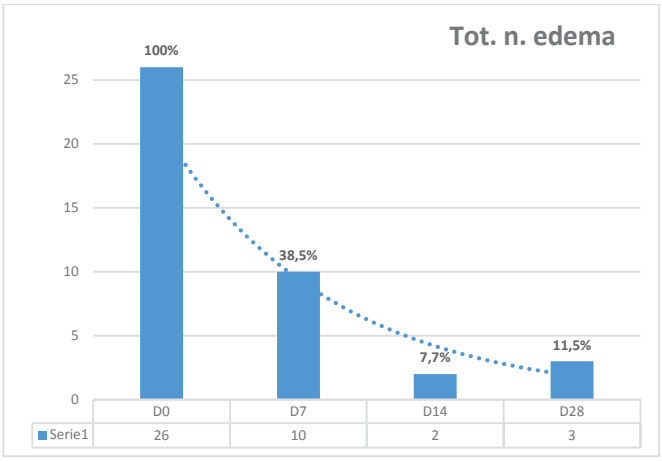
Adverse events
Any adverse event had to be recorded on the clinical form (Annex no. 1) and immediately communicated to the manufacturer. In case of a severe reaction the dog was withdrawn from the study and treated accordingly.

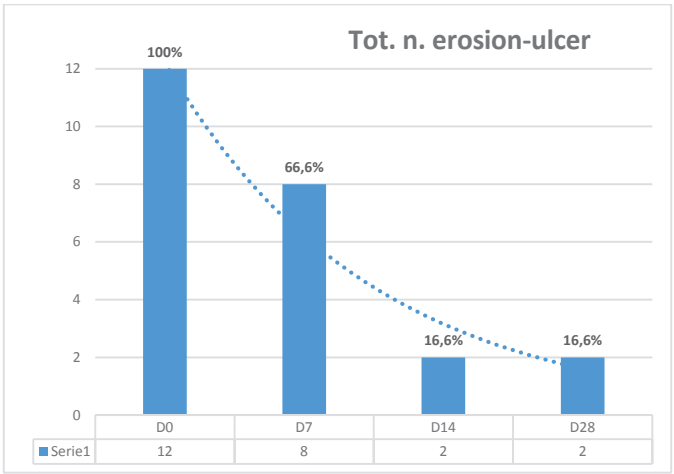
The possible correlation between the adverse event and the otic products used in this study was assessed.

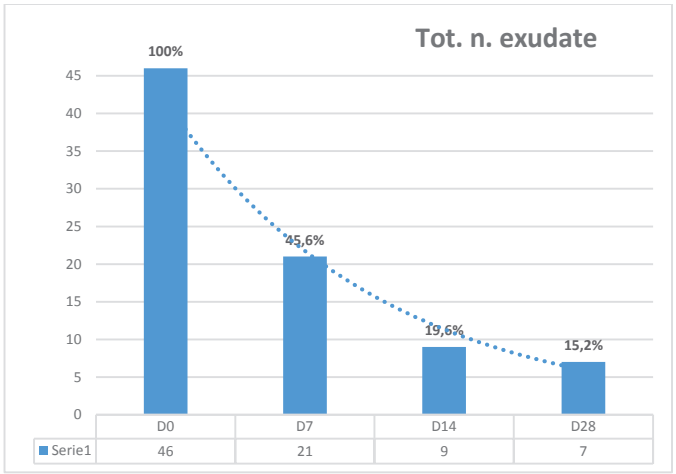
Results

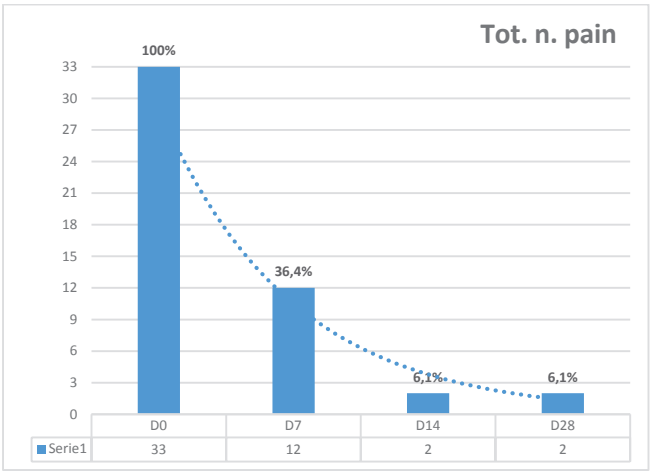
Thirty dogs of different breeds were enrolled into the study: 16 crossbreds, 5 English bulldogs, 2 French bulldogs, 2 Boxers, 1 Pointer, 1 Maltese, 1 Labrador retriever, 1 Cocker spaniel, 1 Weimaraner. The average age was 5.9 years (5 months to 13 years). The gender distribution was as follows: 22 females (18 sterilized) and 8 entire males. There average weight was 18.7 kg (3 to 36.5 kg). The results of the clinical and cytological parameters are shown in the following charts:

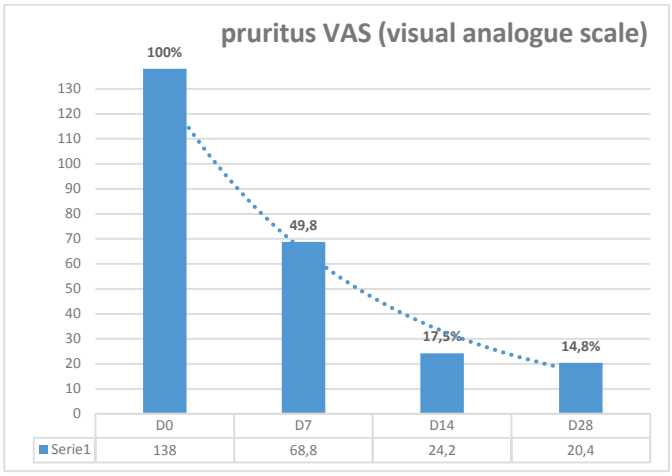


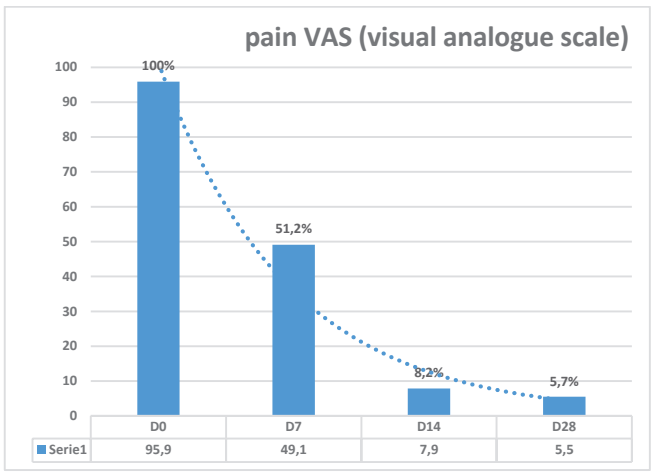


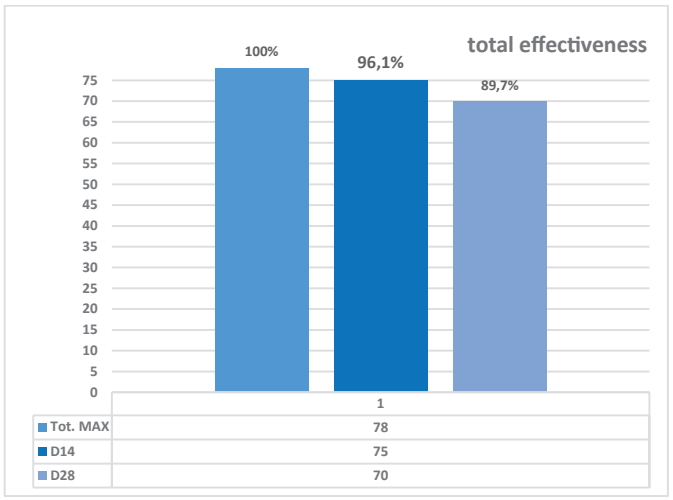


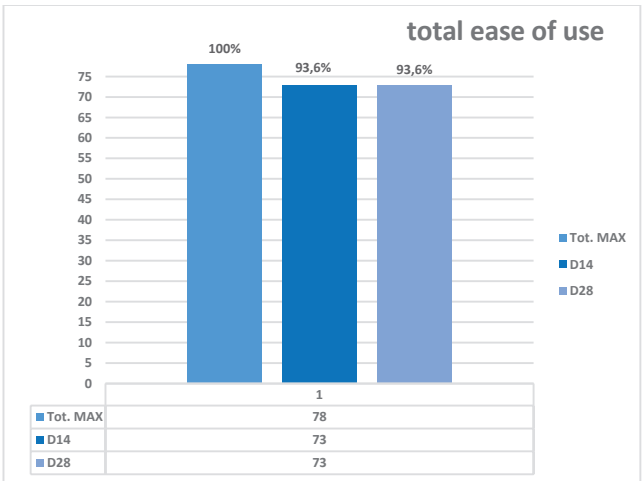


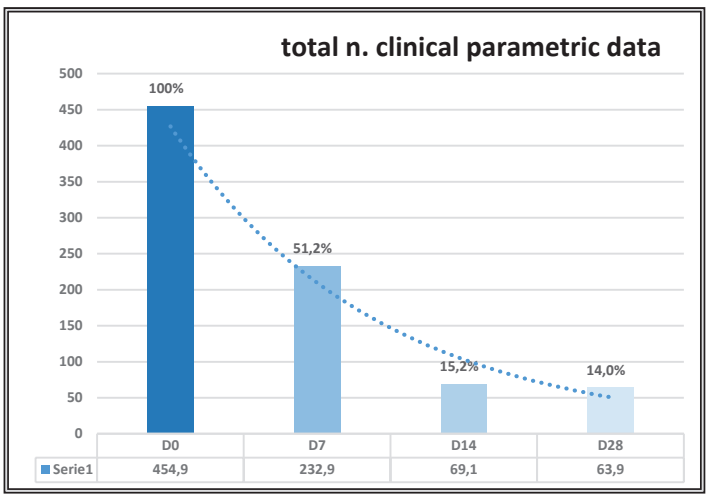

Tot. n. pain
100%
36,4%
6,1%
6,1%
D0
D7
D14
D28
Serie1
33
12
2
2


pruritus VAS (visual analogue scale)
100%
49,8
17,5%
14,8%
D0
D7
D14
D28
Serie1
138
68,8
24,2
20,4

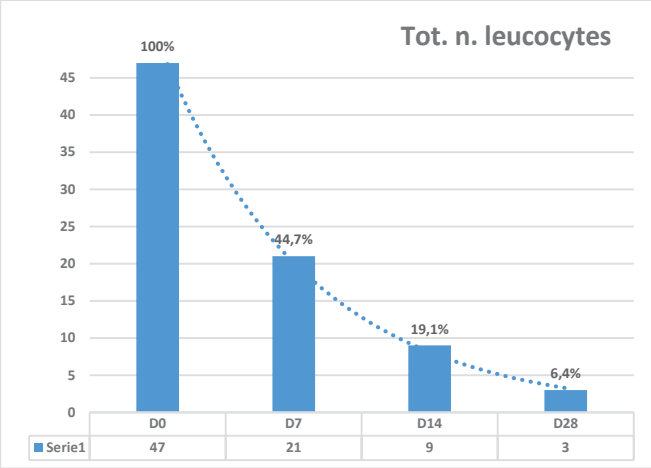

pain VAS (visual analogue scale)
100%
51,2%
8,2%
5,7%
D0
D7
D14
D28
Serie1
95,9
49,1
7,9
5,5

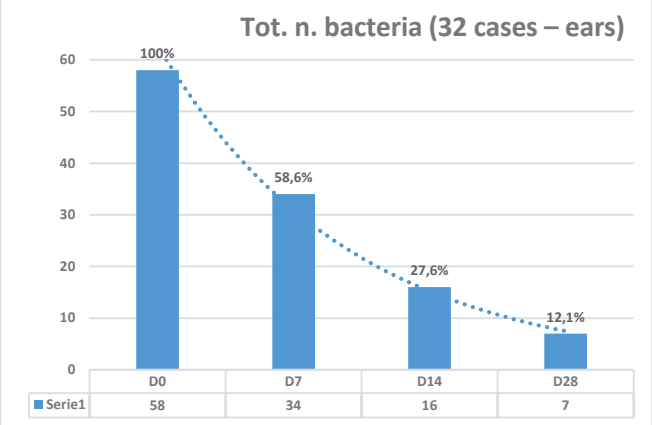

total effectiveness
100%
96,1%
89,7%
1
Tot. MAX
78
D14
75
D28
70

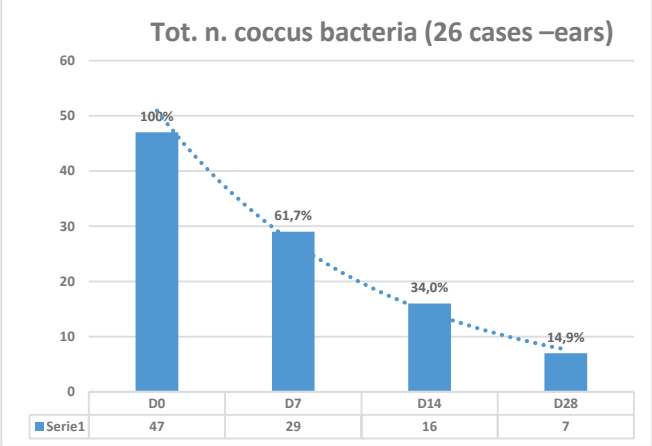

total ease of use
100%
93,6%
93,6%
Tot. MAX
D14
D28
1
Tot. MAX
78
D14
73
D28
73

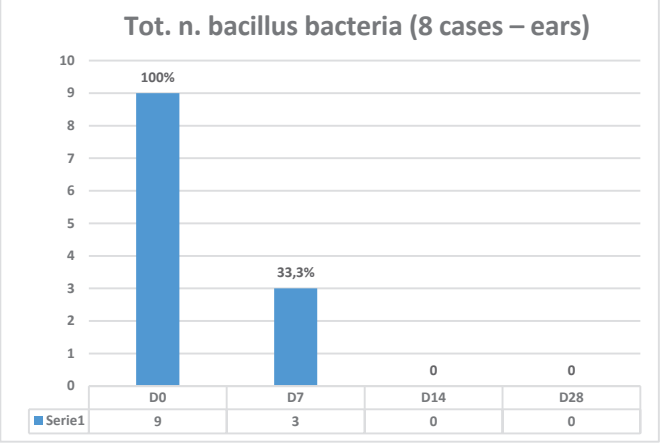

total n. clinical parametric data
100%
51,2%
15,2%
14,0%
D0
D7
D14
D28
Serie1
454,9
232,9
69,1
63,9

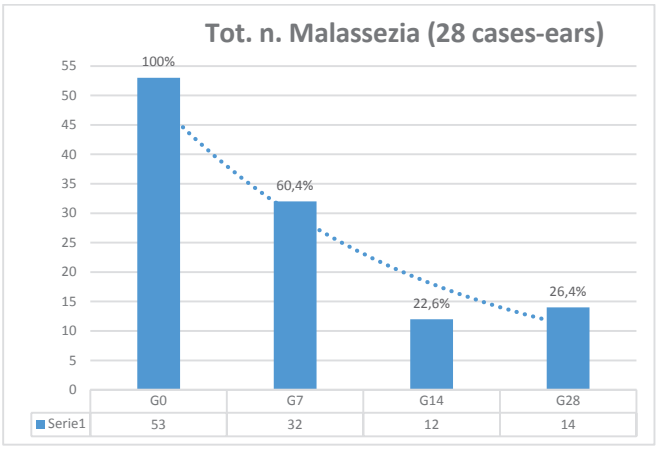
Clinical improvement
- day 7 - improvement 48.8%
- day 14 - improvemente 84.8% with 13/16 dogs cured and 3/16 much improved.
- day 28 - improvement 86% compared to day 0

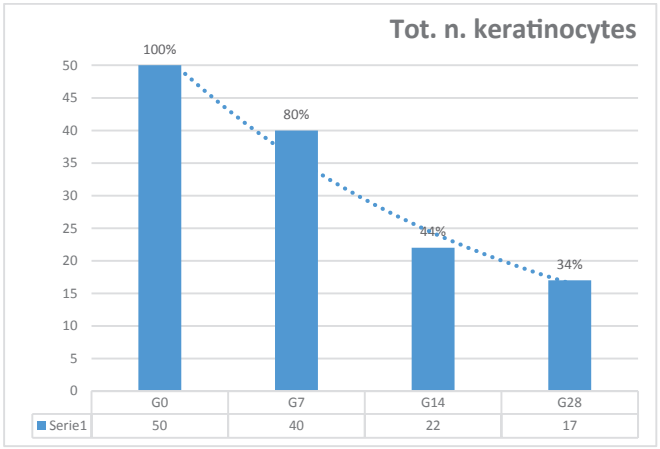


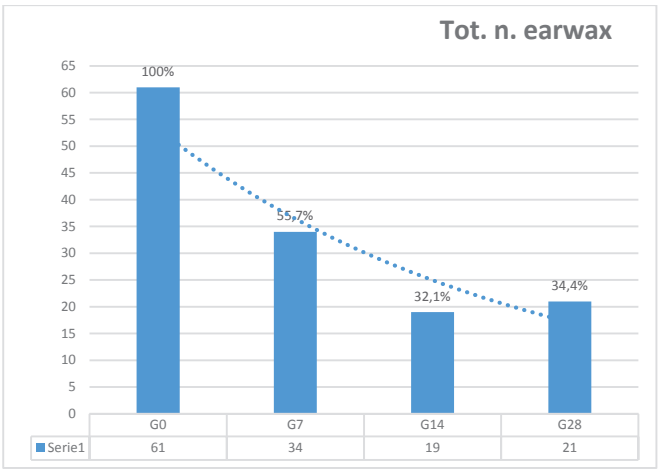


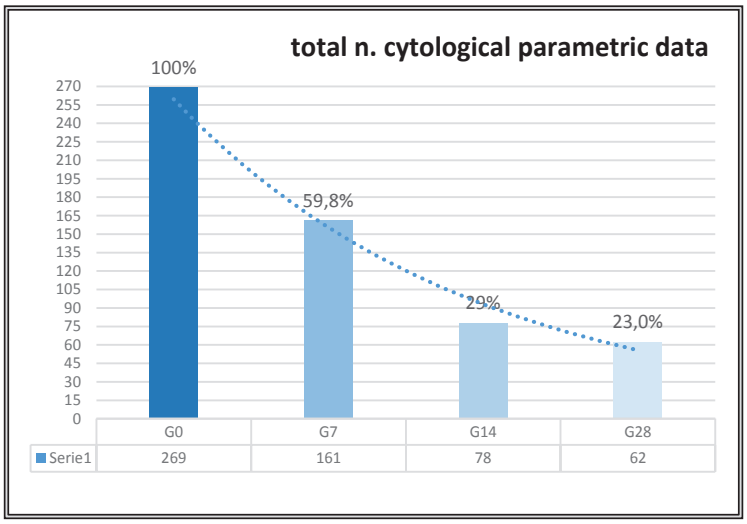












Cytological improvement

- day 7 - 40.2% improvement.
- day 14 - 71% improvement in 8/8 ears with a rod- caused otitis; 72.4% reduction in 32 ears with bacterial (cocci) ear infections; 77.4% reduction in yeast number in 28 ears with Malassezia otitis.

After 2 weeks (day 28) from the last dose there was a 77% improvement when compared with day 0. These data show the antimicrobial efficacy of the ingredients contained in ***MIX**: AMP2041 (peptide), chlorhexidine digluconate, and Tris-EDTA. The efficacy of the product was considered to be 96.1% by the investigators and by the owners on day 14; and, of course in a lower percentage, 89.7% on day 28 (after 14 days from the last dose!).

The VAS for the pruritus showed a reduction of 82.5% of the clinical sign on day 14 and of 85.2% on day 28. The VAS for pain showed a reduction of 91.8% of the clinical signs on day 14 and of 94.3% on day 28 suggesting that the ingredients present in ***MIX** (Vitamin PP, Zinc PCA, GPI lysine) may have had an anti-inflammatory and analgesic effect.

Both investigators and owners considered the ***MIX** easy to use (93, 6%) on both day 7 and 14.

This product has shown a 100% tolerability in all 30 dogs recruited in this pilot study. More randomized double blind controlled studies with a large number of dogs is needed to confirm these preliminary findings.

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