FINAL REPORT

Evaluation of the safety of four over the counter shampoos, a skin powder, and an ear cleaner in healthy dogs

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1. Background:

Topical, over the counter, skin products for dogs should maintain a healthy skin and hair coat, be non-irritative and should not cause skin xerosis. The main clinical signs after using an irritant product are pruritus, erythema, and papules, whereas skin xerosis is typically manifested with fine scales.[1] Similarly, ear cleaners, should not cause irritation of the skin of the ear canal. If an irritant ear cleaner is applied, erythema, edema and increased cerumen/exudate in the ear canal is expected.[1]

The aims of this study are to a) evaluate the safety (i.e., lack of irritation and/or skin xerosis) and the effects on skin biophysical parameters (electrical capacitance, color, pH) of four over the counter shampoos, using a double-blinded, randomized, controlled study design, b) to evaluate the safety (i.e., lack of irritation and/or skin xerosis) and the effects on skin biophysical parameters (electrical capacitance, color, pH) of a skin powder, using an open-label study design, and c) to evaluate the safety (i.e., lack of irritation) of an ear cleaner, using a double-blinded, randomized, controlled, study design

2. Materials and methods

2.1. Dogs:

A total of six clinically healthy dogs were used.

The inclusion criteria were:

a) Age ≥ 6 months

b) No pregnancy or lactation

c) No clinical evidence of skin or ear disease (including absence of ear canal inflammation and presence of a normal-looking tympanic membrane on otoscopy) of any etiology at the time of enrolment

d) No historical evidence of skin or ear disease of any etiology during the previous six months

e) No clinical evidence of systemic disease of any etiology at the time of enrolment

f) No historical evidence of systemic disease of any etiology during the previous six months

g) No administration of systemic or topical medications that influence the gross appearance and the inflammation of the skin (e.g., glucocorticoids, ciclosporin, oclacitinib, lokivetmab, H1 antihistamines etc.) for the last 4 weeks (or for the last 8 weeks in the case of long-acting parenteral glucocorticoids) before enrolment. The administration of these drugs was prohibited for the duration of the study, except if it was considered necessary by the investigators due to moderate to severe irritation caused by test items or due to any other unrelated reason. However, in this case, the dog would drop-out for the remaining of the study.

h) No bath with shampoo for one week before the start of the trial and no use of any other shampoo except the test items for the duration of the trial

i) No administration of ear cleaners or any other topical ear products for the last 2 weeks before enrolment. The administration of these products, apart from the test items, was prohibited for the duration of the study, except if it was considered necessary by the investigators due to moderate to severe otitis caused by test items or due to any other unrelated reason. However, in this case, the dog was disqualified for the remaining of the study.

j) No planed changes in the dose/frequency of administration of any other medication that is administered on a long-term basis (e.g., ectoparasiticides, endoparasiticides, fatty acid supplements etc.) for the duration of the study. In the event of such change the dog was disqualified for the remaining of the study.

The fulfilment of these criteria was checked at the inclusion visit (time 0) that took place 1-2 weeks before the beginning of the study. In the same examination the following data were recorded for each dog: a) sex and neutering status, b) age, c) breed, d) hair length (short, medium, long), and e) body weight.

Adherence to the inclusion criteria f), g), h) and i) was checked at all subsequent examinations.

2.2. Test items:

For the randomized controlled study of the shampoos the following test items were used:

a) Chlorhexidine (Provet): contains chlorhexidine digluconate (0.27%), glycerin, and citric acid

b) Sebbaoric (Provet): contains sodium shale oil sulfonate (ichthyol), *Lonicera Japonica* liquid extract, L-carnitine HCl stabilized with orthosilicic acid, fatty acid-ceramide nano-emulsion complex, allantoin, unimoist U-125NP, vitamin E acetate, and D-panthenol

c) Sensitive skin (Provet): contains *Lonicera Japonica* liquid extract, L-carnitine HCl stabilized with orthosilicic acid, fatty acid-ceramide nano- emulsion complex, trimethyl glycine, allantoin, amisol trio, D-panthenol, and vitamin E acetate

d) Skin & Coat protect (Provet): *Rheum palmatum* root extract, *Lonicera Japonica* extract, liquid hydrolyzed silk peptides, *Populus tremuloides* (aspen) extract, fatty acid-ceramide nano-emulsion complex, liquidhydrolyzed silk ethyl ester, D-panthenol, glycerin, and allantoin

e) Allercalm shampoo (Virbac) as the control item

For the open label study of the skin powder, the following item was used:

Skin and coat powder (Provet): *Lonicera Japonica* extract, *Populus tremuloides* (aspen) extract, *Rheum palmatum* extract, hydrolyzed silk peptides, hydrolyzed silk ethyl ester, cetrimide, magnesium chloride, zeolite clinoptilolite, and kaolin

For the randomized controlled study of the ear cleaners the following test items were used:

a) Ear clean solution (Provet): contains *Lonicera Japonica* extract, *Rheum palmatum* extract, hesperidin, and fatty acid-ceramide nano-emulsion complex

b) Epi Otic (Virbac) as the control item

2.3. Randomization:

The order that the five shampoos were used in each dog was random. Randomization was done before the start of the study using a freely available random number generator (https://www.calculator.net/random-number-generator.html). Initially each of the five test items was assigned a random number (code) from 1 to 5 (Appendix II) and the five shampoos were provided in identical containers with the only indication on each container being the product code. The investigators remained masked to these codes until the end of the study and the statistical analysis. The order that each dog was

shampooed with each item depended on the order of enrolment into the study and is shown on Appendix III (the orders for each of the six dogs are random and have been produced by the above random number generator).

Randomization of the test and control ear cleaner was done before the start of the study, using the same random number generator, by assigning a random number (code) from 1 to 2 (Appendix IV). The two ear cleaners were provided in identical containers with the only indication on each container being the product code. The investigators were masked to these codes until the end of the study and the statistical analysis. The use of the test item in the right ear and of the control item in the left ear or the reverse was depending on the order of each dog enrolment into the study and is shown on Appendix V (the selection of the ears for each of the six dogs is random and has been produced by the above random number generator).

2.4. Timing of interventions, historical information, clinical and otoscopic examinations, and measurement of the biophysical parameters of the skin:

All examinations were performed in a room with stable temperature (23 ± 1°C) and relative humidity (50 ± 5%). Initially, historical information was obtained and then the dog entered the examination room for clinical and otoscopic examination. This was followed by the measurement of skin biophysical measurements; these measurements were performed after the dog had been acclimatized to the examination room for at least 30 min.

On each of the six weeks of the trial, each dog was first examined on Tuesday afternoon (history, clinical examination, otoscopic examination, measurement of biophysical parameters of the skin). Then the two ear cleaners were applied by the investigators (until filling each ear canal followed by massage for 1 min) and the dog was returned to the owner. The owner returned home to bath the dog with the randomly selected shampoo (weeks 1-5) or to apply the skin powder (week 6). Owners were instructed to a) bath the dog (weeks 1-5) in the way they usually do, to avoid bathing the head (including ear pinnae), and to try to leave the shampoo in contact with the skin for approximately 5 min, b) to apply the skin powder (week 6) uniformly on the body trunk in a way that it will cover the whole skin and hair coat without building up.

Subsequent examinations were performed on Wednesday afternoon (i.e., approximately 24 h after bathing or application of the skin powder), Friday afternoon (i.e., 3 days after bathing or application of the skin powder) and next Tuesday afternoon (i.e., one week after bathing or application of the skin powder). The same procedures as those described for Tuesday were repeated on Wednesday and Friday, except for the application of the ear cleaner that was performed only on Tuesday (Appendix VI).

2.5. Historical information:

At each examination the owner was asked to:

a) Report any adverse health events noticed since the previous examination (except for the Tuesday of week 1, because no test item will have been applied by that time). The probability of the association between any adverse health events and the use of test items will be evaluated using the Naranjo adverse drug reaction probability scale[2]

b) Score the pruritus of the dog during the previous 24 h (except pruritus on the ears that will be scored separately) using the validated Pruritus Visual Analogue Scale (PVAS)[3,4] (Appendix VII)

c) Score the pruritus and other signs of ear irritation/otitis (head shaking, discomfort, pain) during the previous 24 h, separately for the right and the left ear, using a modified PVAS (Appendix VIII)

d) Score the quality of the hair coat compared to the last Tuesday as: 0 (greatly improved), 1 (moderately improved), 2 (remaining the same), 3 (moderately deteriorated) or 4 (greatly deteriorated)

e) Score skin dryness compared to the last Tuesday as: 0 (greatly improved), 1 (moderately improved), 2 (remaining the same), 3 (moderately deteriorated) or 4 (greatly deteriorated)

f) Score skin scaling compared to the last Tuesday as: 0 (greatly improved), 1 (moderately improved), 2 (remaining the same), 3 (moderately deteriorated) or 4 (greatly deteriorated)

g) Score skin odor compared to the last Tuesday as: 0 (greatly improved), 1 (moderately improved), 2 (remaining the same), 3 (moderately deteriorated) or 4 (greatly deteriorated)

h) Score ear odor compared to the last Tuesday, separately for the right and the left ear as: 0 (greatly improved), 1 (moderately improved), 2 (remaining the same), 3 (moderately deteriorated) or 4 (greatly deteriorated)

2.6. Clinical and otoscopic examination:

In addition to the general physical examination for the detection of possible systemic adverse health events, a detailed dermatological examination was performed to score the extent and severity of skin lesions indicative of irritation (e.g., erythema, papules) and dryness (e.g., scales) caused by the test items. To this aim the body (except for the head and ear pinnae) was separated to 19 areas (Appendix IX) and in each of them erythema, papules, scales, and any other skin lesion were scored as 0 (absent), 1 (mild), 2 (moderate) or 3 (severe)[5] (Appendix X).

The examination of the ears included a) scoring of the extend and severity of skin lesions indicative of irritation (e.g., erythema, papules, edema) and dryness (e.g., scales) caused by the ear cleaners, separately for the right and the left ear pinnae, and b) the otoscopic examination of the two ear canals and the scoring of erythema, edema, erosion, and exudate on a 0 to 3 scale according to the validated otitis clinical scoring system OTIS3[6] (Appendix XI).

2.7. Measurement of the biophysical parameters of the skin:

At each examination, electrical capacitance, color of the skin (erythema), and pH, were measured with the Courage-Khazaka (Koln, Germany) MPA 580 device and the probes corneometer SM825, colorimeter CL-400, and skin-pH-meter pH905, respectively. Measurements were done in four areas of the skin (right axillae, right inguinal area, right lateral thorax over the last rib and in the middle between the spine and the sternum, lumbar area between iliac crests-Appendix XII) after atraumatic clipping with an electrical blade with 1 mm scissors. Clipping was done immediately after entrance of the dog into the examination room, so that a period of 30 min was passing from clipping until measurements of skin biophysical parameters (in re-examinations clipping were repeated if the previously clipped hair had regrown to a longer than 1 mm length). A circle with a diameter of approximately 1 cm was drawn, with an atraumatic marker pen, on each clipped area, to delineate the exact site of the measurements.

After 30 min that were devoted to clinical and otoscopic examination, the biophysical parameters of the skin were measured as follows:

a) All measurements were completed first in the right axillae, then in the inguinal area, then in right lateral thorax, and finally in the lumbar area

b) In each body area the electrical capacitance will be measured first, followed by the color, and finally the pH

c) All these measurements were repeated five consecutive times and the mean values for each parameter were calculated and recorded.

2.8. Statistical analysis

2.8.1. Study power analysis:

- Assuming that the a) baseline PVAS (except pruritus on the ears) and b) baseline modified PVAS for pruritus and other signs of ear irritation/otitis, will be 1.5 with a standard deviation of 0.2,[3,4] the study had a power of 80% to detect a 15% increase of PVAS/modified PVAS at 0.05 level of significance.
- By definition (see inclusion criteria) the quality of hair coat, skin dryness, skin scaling, skin odor and ear odor at baseline will be 2 for all dogs. The study will have a power of 100% to detect an increase or decrease by 1 point of owner's assessment of hair coat quality, skin dryness, skin scaling, skin odor and ear odor.
- Assuming that the baseline cumulative score of the extent and severity of each lesion (erythema, papules, scales) in the 19 body areas will be 8 with a standard deviation of 1,[5] the study had a power of 80% to detect an 18% increase in the cumulative score.
- Assuming that the baseline score of extend and severity of each lesion (erythema, papules, edema, scales) on each ear pinnae will be 0.8 with a standard deviation of 0.1,[5] the study had a power of 80% to detect a 14% increase in this score.
- Assuming that the baseline OTIS3 score for each ear will be 0.8 with a standard deviation of 0.1,[6] the study had a power of 80% to detect an 14% increase in this score.
- Assuming that the baseline electrical capacitance of the skin will be 16.03 corneometer units with a standard deviation of 0.88,[7] the study had a power of 80% to detect a 7% increase or decrease in electrical capacity.
- Assuming that the baseline skin pH will be 8.02 with a standard deviation of 0.09,[7] the study had a power of 80% to detect a 2% increase or decrease in skin pH.

2.8.2. Statistics

The distribution of continuous variables was examined using Kolmogorov-Smirnov test with Lilliefors correction. Variables following normal distribution are presented as means ± standard deviation (SD), whereas variables not following normal distribution are presented as medians and range.

The possibility of a carry-over effect of the use of a shampoo to the outcomes of the next shampoo (PVAS, erythema, papule, and scale scores, electrical capacitance, color, pH) was tested by comparing these parameters between day 0 and day 7 after the use of each of the five shampoos. If the outcome followed normal distribution paired-samples t test was used, whereas, if the outcome did not follow normal distribution Wilcoxon signed rank test was used.

The outcome measures (PVAS, modified PVAS, owner's assessments of the quality of hair coat, skin dryness, skin scaling, skin odor and ear odor, erythema, papule, and scale scores, pinnal erythema and OTIS3 scores, electrical capacitance, color and pH of the skin) were compared among the day of use of a shampoo or of skin powder (day 0) and days 1, 3, and 7, as well as between the day before the first application of an ear cleaner and days 0, 1, 3, 7, 8, 10, 14, 15, 17, 21, 22, 24, 28, 29, 31, 35, 36, 38 and 42, using repeated measures ANOVA (data following normal distribution) or with Friedman two-way ANOVA (data not following normal distribution).

Owner's assessments of the quality of hair coat, skin dryness, skin scaling, and skin odor were compared among the five shampoos for the same day after use of each of them (days 1, 3 and 7) using repeated measures ANOVA (data following normal distribution) or with Friedman two-way ANOVA (data not following normal distribution). The same

comparison was done between the two ear cleaners for the same day after their first application (days 1, 3, 7, 8, 10, 14, 15, 17, 21, 22, 24, 28, 29, 31, 35, 36, 38 and 42).

2.9. Ethics

Study protocol was approved by the Ethical Committee of the Faculty of Veterinary Sciences, University of Thessaly (license No 128/13-11-21)

3. Results

3.1. Dogs:

A total of six mix-breed dogs were used in the study. They included two intact males and four females (three spayed and one intact). Their median age was 1.3 years (range: 9 months-8.5 years) and their body weight was 8.4 ± 4.2 Kg. Three (50%) of the dogs were short-coated and the other three (50%) had a medium length of hair coat.

All dogs completed the trial, there were no deviations from the protocol, and it was not necessary to administer systemic or topical medications that influence the gross appearance and the inflammation of the skin or additional ear products. General physical examination did not show any systemic adverse effects associated with the use of the topical products.

3.2. Double-blinded randomized controlled trial of shampoos

3.2.1. Adverse health events:

The reported adverse health events after using the four test shampoos and the control shampoo are presented on the following table

Table. Adverse health events reported by the dog owners 1, 3 and 7 days after the use of five shampoos

Shampoo	Day	Adverse effect	Number of dogs
Chlorhexidine (Provet)	1	-	-
	3	-	-
	7	-	-
Sebbaoric (Provet)	1	Unpleasant smell	2/6 (33.3%)
	3	-	-
	7	-	-
Sensitive Skin (Provet)	1	-	-
	3	-	-
	7	-	-
Skin & Coat Protect (Provet)	1	-	-
	3	-	-
	7	-	-
Allercalm (Virbac)	1	Pruritus	1/6 (16.7%)
	1	Strong odor	1/6 (16.7%)
	1	Oily feeling	1/6 (16.7%)
	3	-	
	7	Pruritus	1/6 (16.7%)

With the exception on an unpleasant smell (that was characterized as "tea-like") in 2/6 (33.3%) of the dogs on day 1 after using Sebbaoric (Provet), no other adverse effects were recorded. On the contrary the control shampoo was associated with pruritus, strong odor and oily feeling of the skin and hair coat (one dog each on day 1) and with pruritus (one

dog on day 7). All these adverse events had a Naranjo adverse drug reaction probability scale score of 5 and thus they are classified as probable.

3.2.2. Pruritus

The distribution of PVAS scores was normal with two exceptions: day 0 on Chlorhexidine shampoo (P=0.004), and day 3 on Skin & Coat Protect shampoo (P=0.024). Subsequently it was considered appropriate to present all data as normally distributed (i.e., mean ± SD) and use parametric tests for their analysis.

There was no carry-over effect from the use of the previous shampoo to the PVAS scores recorded for the next shampoo, because there was no significant difference between day 0 and day 7 for all five shampoos (paired samples t test; all P values ≥0.117).

The PVAS scores before and after using the four test shampoos and the control shampoo are presented on the following table. None of the shampoos resulted in increased pruritus (all P values ≥0.222)

Table. Pruritus visual analogue scale (PVAS) scores before (day 0) and 1, 3 and 7 days after the use of five shampoos (ear pruritus is not considered)

Shampoo	Day	PVAS (mean ± SD)	P value
Chlorhexidine (Provet)	0	0.5 ± 0.7	0.222
	1	0.3 ± 0.3	
	3	0.4 ± 0.3	
	7	0.3 ± 0.3	
Sebbaoric (Provet)	0	0.4 ± 0.3	0.525
	1	0.4 ± 0.3	
	3	0.5 ± 0.6	
	7	0.6 ± 0.5	
Sensitive Skin (Provet)	0	0.5 ± 0.4	0.483
	1	0.3 ± 0.2	
	3	0.3 ± 0.3	
	7	0.2 ± 0.2	
Skin & Coat Protect (Provet)	0	0.2 ± 0.2	0.342
	1	1.1 ± 1.6	
	3	1.1 ± 1.5	
	7	0.7 ± 0.6	
Allercalm (Virbac)	0	0.4 ± 0.4	0.333
	1	0.3 ± 0.3	
	3	0.2 ± 0.2	
	7	0.4 ± 0.3	

3.2.3. Owner's assessment of quality of hair coat, skin dryness, scaling an odor

The distribution of owner's scores for the 3 re-examinations after the use of each shampoo are presented on the following table

Table. Owner's assessment of quality of hair coat, skin dryness, scaling an odor 1, 3 and 7 days after the use of five shampoos

Shampoo	Day	Great improvement	Moderate improvement	Same	Moderate deterioration	Great deterioration
Quality of hair coat						
Chlorhexidine (Provet)	1	2/6 (33.3%)	2/6 (33.3%)	1/6 (16.7%)	1/6 (16.7%)	
	3		1/6 (16.7%)	4/6 (66.7%)	1/6 (16.7%)	

	7		1/6 (16.7%)	5/6 (83.3%)		
Sebbaoric (Provet)	1		2/6 (33.3%)	3/6 (50%)	1/6 (16.7%)	
	3			5/6 (83.3%)		1/6 (16.7%)
	7		1/6 (16.7%)	5/6 (83.3%)		
Sensitive Skin (Provet)	1		4/6 (66.7%)	2/6 (33.3%)		
	3	1/6 (16.7%)	1/6 (16.7%)	4/6 (66.7%)		
	7			6/6 (100%)		
Skin & Coat Protect (Provet)	1		1/6 (16.7%)	3/6 (50%)	2/6 (33.3%)	
	3			4/6 (66.7%)	2/6 (33.3%)	
	7			6/6 (100%)		
Allercalm (Virbac)	1	3/6 (50%)	2/6 (33,3%)	1/6 (16.7%)		
	3		4/6 (66.7%)	2/6 (33.3%)		
	7	1/6 (16.7%)	2/6 (33.3%)	3/6 (50%)		
Skin dryness						
Chlorhexidine (Provet)	1			6/6 (100%)		
	3		1/6 (16.7%)	5/6 (83.3%)		
	7		1/6 (16.7%)	5/6 (83.3%)		
Sebbaoric (Provet)	1		1/6 (16.7%)	4/6 (66.7%)	1/6 (16.7%)	
	3			4/6 (66.7%)	2/6 (33.3%)	
	7		1/6 (16.7%)	5/6 (83.3%)		
Sensitive Skin (Provet)	1			6/6 (100%)		
	3			6/6 (100%)		
	7			6/6 (100%)		
Skin & Coat Protect (Provet)	1		1/6 (16.7%)	3/6 (50%)	2/6 (33.3%)	
	3			5/6 (83.3%)	1/6 (16.7%)	
	7			5/6 (83.3%)	1/6 (16.7%)	
Allercalm (Virbac)	1		1/6 (16.7%)	5/6 (83.3%)		
	3			6/6 (100%)		
	7		1/6 (16.7%)	5/6 (83.3%)		
Scaling						
Chlorhexidine (Provet)	1			6/6 (100%)		
	3			6/6 (100%)		
	7		1/6 (16.7%)	5/6 (83.3%)		
Sebbaoric (Provet)	1			6/6 (100%)		
	3			6/6 (100%)		
	7		1/6 (16.7%)	4/6 (66.7%)	1/6 (16.7%)	
Sensitive skin (Provet)	1			6/6 (100%)		
	3			6/6 (100%)		
	7		1/6 (16.7%)	5/6 (83.3%)		
Skin & Coat protect (Provet)	1			6/6 (50%)		
	3		1/6 (16.7%)	5/6 (83.3%)		
	7			6/6 (100%)		
Allercalm (Virbac)	1		1/6 (16.7%)	5/6 (83.3%)		
	3		1/6 (16.7%)	5/6 (83.3%)		
	7		1/6 (16.7%)	5/6 (83.3%)		
Skin odor						
Chlorhexidine (Provet)	1	1/6 (16.7%)	3/6 (50%)	2/6 (33.3%)		
	3			4/6 (66.7%)	2/6 (33.3%)	

	7			5/6 (83.3%)	1/6 (16.7%)	
Sebbaoric (Provet)	1		2/6 (33%)	3/6 (50%)	1/6 (16.7%)	
	3			5/6 (83.3%)	1/6 (16.7%)	
	7		1/6 (16.7%)	5/6 (83.3%)		
Sensitive Skin (Provet)	1		1/6 (16.7%)	5/6 (83.3%)		
	3			6/6 (50%)		
	7			6/6 (50%)		
Skin & Coat Protect (Provet)	1	1/6 (16.7%)		5/6 (83.3%)		
	3		1/6 (16.7%)	5/6 (83.3%)		
	7			5/6 (83.3%)		1/6 (16.7%)
Allercalm (Virbac)	1	2/6 (33.3%)	1/6 (16.7%)	2/6 (33.3%)	1/6 (16.7%)	
	3		1/6 (16.7%)	5/6 (66.7%)		
	7			5/6 (66.7%)	1/6 (16.7%)	

Quality of the hair coat: the distribution of the data did not follow the normal distribution in the majority (8/15) of the data sets and for this reason non-parametric tests were considered appropriate. There were no significant differences among days 1, 3 and 7 for any of the five shampoos ($P \geq 0.091$). When the five shampoos were compared on day 1, there was a significant difference ($P=0.049$) among them; in the pairwise comparisons the quality of the hair coat was better for Allercalm compared to Sebbaoric ($P=0.025$) and to Skin & Coat Protect ($P=0.025$). When the five shampoos were compared on day 3, there was a significant difference ($P=0.032$) among them; in the pairwise comparisons the quality of the hair coat was better for Allercalm compared to Chlorhexidine ($P=0.046$), to Sebbaoric ($P=0.046$) and to Skin & Coat Protect ($P=0.046$); also on day 7, it was better for Sensitive Skin compared to Skin & Coat Protect ($P=0.046$). When the five shampoos were compared on day 7, there was no significant difference among them ($P=0.155$)

Skin dryness: the distribution of the data did not follow the normal distribution in the majority (9/15) of the data sets and for this reason non-parametric tests were considered appropriate. There were no significant differences among days 1, 3 and 7 for any of the five shampoos ($P \geq 0.097$). When the five shampoos were compared, there was no difference among them on day 1 ($P=0.736$), day 3 ($P=0.16$) or day 7 ($P=0.406$).

Skin scaling: the distribution of the data followed the normal distribution in the majority (8/15) of the data sets and for this reason parametric tests were considered appropriate. There were no significant differences among days 1, 3 and 7 for any of the five shampoos ($P \geq 0.363$). When the five shampoos were compared, there was no difference among them on day 1 ($P=0.363$), day 3 ($P=0.363$) or day 7 ($P=0.444$).

Skin odor: the distribution of the data did not follow the normal distribution in the majority (10/15) of the data sets and for this reason non-parametric tests were considered appropriate. When the three re-examinations (days 1, 3, and 7) after the use of each shampoo were compared there was a significant difference for the Chlorhexidine shampoo ($P=0.009$); in the pairwise comparisons skin odor was lower on day 1 compared to day 3 ($P=0.025$) and day 7 ($P=0.025$). No significant differences among days 1, 3 and 7 were found for the remaining four shampoos ($P \geq 0.146$). When the five shampoos were compared, there was no difference among them on day 1 ($P=0.565$), day 3 ($P=0.208$) and day 7 ($P=0.507$).

3.2.4. Clinical examination

Disregarding the examinations where all scores were zero, the distribution of erythema, papule and scale scores was non-normal ($P \leq 0.008$) with two exceptions: erythema on the day of use of Sebbaoric ($P=0.2$) and erythema on the day of use of Allercalm ($P=0.094$). Subsequently it was considered appropriate to present all data as non-normally distributed (i.e., median, range) and use non-parametric tests for their analysis.

There was no carry-over effect from the use of the previous shampoo because there was no significant difference between day 0 and day 7 for all five shampoos regarding erythema (P≥0.157), papule (P=1 for all shampoos) or scale (P≥0.317) scores.

Erythema, papule, and scale scores before and after using the four test shampoos and the control shampoo are presented on the following table. None of the shampoos resulted in increased erythema (P≥0.241), papule (P≥0.392), or scale (P≥0.261) scores.

Table. Erythema, papule, and scale scores before (day 0) and 1, 3 and 7 days after the use of five shampoos

Shampoo	Day 0	Day 1	Day 3	Day 7
Erythema				
Chlorhexidine (Provet)	0 (0-7)	0 (0-5)	0 (0-2)	0 (0-2)
Sebbaoric (Provet)	0 (0-3)	1 (0-2)	0 (0-1)	0 (0-4)
Sensitive Skin (Provet)	0 (0-4)	0 (0-5)	0 (0-7)	0 (0-7)
Skin & Coat Protect (Provet)	0 (0-2)	0 (0-5)	0 (0-2)	0 (0-3)
Allercalm (Virbac)	0.5 (0-2)	0 (0-3)	0 (0-3)	0 (0-2)
Papules				
Chlorhexidine (Provet)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Sebbaoric (Provet)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Sensitive Skin (Provet)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Skin & Coat Protect (Provet)	0 (0-0)	0 (0-1)	0 (0-0)	0 (0-0)
Allercalm (Virbac)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Scales				
Chlorhexidine (Provet)	0 (0-0)	0 (0-1)	0 (0-0)	0 (0-0)
Sebbaoric (Provet)	0 (0-1)	0 (0-1)	0 (0-3)	0 (0-0)
Sensitive Skin (Provet)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-2)
Skin & Coat Protect (Provet)	0 (0-0)	0 (0-5)	0 (0-0)	0 (0-0)
Allercalm (Virbac)	0 (0-2)	0 (0-0)	0 (0-1)	0 (0-0)

No other skin lesions except mild (+1) hypotrichosis in 1/19 skin areas on the day of use of Allercalm shampoo were witnessed.

3.2.5. Biophysical parameters of the skin:

3.2.5.1. Electrical capacitance:

The distribution of 75/80 data sets (5 shampoos x 4 examinations x 4 body areas) followed the normal distribution. The exceptions were the inguinal area on day 1 after shampooing with Skin and Coat Protect (P=0.042), the lumbar area on day 1 after shampooing with Chlorhexidine (P=0.031), the lumbar area on day 0 (day of shampooing) of Sensitive skin (P=0.023), and the lumbar area on day 0 (day of shampooing) and day 7 of Allercalm (P=0.003 and P=0.04, respectively). Therefore, use of parametric tests for analysis was considered appropriate.

The comparisons between day 0 and day 7 for each of the five shampoos did not show significant differences with the only exceptions of lateral thorax that had a lower electrical capacitance on day 7 compared to day 0, after shampooing with Chlorhexidine (P=0.032) and Sebbaoric (P=0.023). This isolated evidence of a carry-over effect was not considered problematic for subsequent analysis.

None of the shampoos resulted in significant changes in the electrical capacity of the skin of right axillae (P≥0.09), inguinal area (P≥0.305), right lateral thorax (P≥0.094) or lumbar area (P≥0.261).

3.2.5.2. Skin color (redness):

The distribution of 78/80 data sets (5 shampoos x 4 examinations x 4 body areas) followed the normal distribution. The exceptions were the axillae on day 3 after shampooing with Skin and Coat Protect (P=0.041), the thorax on day 1 after shampooing with Allercalm (P=0.036) and the lumbar area on day 3 after shampooing with Skin and Coat Protect (P=0.024). Therefore, use of parametric tests for analysis was considered appropriate.

The comparisons between day 0 and day 7 for each of the five shampoos did not show significant differences (P≥0.055).

None of the shampoos resulted in significant changes in the redness of the skin of right axillae (P≥0.419), inguinal area (P≥0.328), right lateral thorax (P≥0.524) or lumbar area (P≥0.172).

3.2.5.3. Skin pH

The distribution of 71/80 data sets (5 shampoos x 4 examinations x 4 body areas) followed the normal distribution. The exceptions were the axillae on day 1 after shampooing with Sensitive skin (P=0.038), the axillae on day 1 after shampooing with Skin & Coat Protect (P=0.009), the inguinal area on day 3 after shampooing with Chlorhexidine (P=0.03), the inguinal area on day 7 after shampooing with Sebbaoric (P=0.032), the inguinal area on the day 0 (day of shampooing) of Sensitive Skin (P=0.009), the inguinal area of day 3 after shampooing with Sensitive skin (P=0.039), the thorax on day 0 (day of shampooing) of Sensitive skin (P=0.044), the thorax on day 1 after shampooing with Skin & Coat Protect (P=0.014), and the lumbar area on day 3 after shampooing with Skin & Coat Protect (P=0.005). Therefore, use of parametric tests for analysis was considered appropriate.

The comparisons between day 0 and day 7 for each of the five shampoos did not show significant differences with the only exceptions of lateral thorax that had a lower pH on day 7 compared to day 0, after shampooing with Chlorhexidine (P=0.027) and Skin & Coat Protect (P=0.041). This isolated evidence of a carry-over effect was not considered problematic for subsequent analysis.

None of the shampoos resulted in significant changes in the pH of the skin of right axillae (P≥0.078), inguinal area (P≥0.092), right lateral thorax (P≥0.29) or lumbar area (P≥0.215).

3.3. Open trial of the skin powder

3.3.1. Adverse health events:

The only adverse event reported was a yellowish pigmentation of the hair coat in one dog (16.7%) on day 3.

3.3.2. Pruritus

The distribution of PVAS scores was normal and, thus, data are presented as means ± SD and were analyzed using parametric tests.

The PVAS scores before and after using the skin powder are presented on the following table. The use of the skin powder did not result in increased pruritus (P=0.339)

Table. Pruritus visual analogue scale (PVAS) scores before (day 0) and 1, 3 and 7 days after the use of skin powder (ear pruritus is not considered)

Day	PVAS (mean ± SD)	P value
0	0.6 ± 0.5	0.339
1	0.4 ± 0.4	
3	0.4 ± 0.5	
7	0.3 ± 0.3	

3.3.3. Owner's assessment of quality of hair coat, skin dryness, scaling an odor

The distribution of owner's scores for the 3 re-examinations after the use of the skin powder are presented on the following table

Table. Owner's assessment of quality of hair coat, skin dryness, scaling an odor 1, 3 and 7 days after the use of the skin powder

Day	Great improvement	Moderate improvement	Same	Moderate deterioration	Great deterioration
Quality of hair coat					
1		1/6 (16.7%)	4/6 (66.7%)	1/6 (16.7%)	
3			6/6 (100%)		
7			6/6 (100%)		
Skin dryness					
1			5/6 (83.3%)	1/6 (16.7%)	
3			6/6 (100%)		
7			6/6 (100%)		
Skin scaling					
1			6/6 (100%)		
3			6/6 (100%)		
7			6/6 (100%)		
Skin odor					
1		2/6 (33.3%)	3/6 (50%)	1/6 (16.7%)	
3			6/6 (100%)		
7			5/6 (83.3%)	1/6 (16.7%)	

Quality of the hair coat: the distribution of the data followed the normal distribution in the majority (2/3) of the data sets and for this reason parametric tests were considered appropriate. There was no significant difference among days 1, 3 and 7 (P=1).

Skin dryness: the distribution of the data followed the normal distribution in the majority (2/3) of the data sets and for this reason parametric tests were considered appropriate. There was no significant difference among days 1, 3 and 7 (P=0.363).

Skin scaling: the distribution of the data followed the normal distribution in all (3/3) data sets and for this reason parametric tests were considered appropriate. There was no significant difference among days 1, 3 and 7 (P=1).

Skin odor: the distribution of the data followed the normal distribution in the majority (2/3) of the data sets and for this reason parametric tests were considered appropriate. There was no significant difference among days 1, 3 and 7 (P=0.694).

3.3.4. Clinical examination

Disregarding the examinations where all scores were zero, the distribution of erythema, papule and scale scores was non-normal ($P \leq 0.001$). Subsequently, all data are presented as non-normally distributed (i.e., median, range) and non-parametric tests were used for their analysis.

Erythema, papule, and scale scores before and after using the skin powder are presented on the following table. The application of the skin powder did not result in increased erythema (P=0.392), papule (P=1), or scale (P=1) scores.

Table. Erythema, papule, and scale scores before (day 0) and 1, 3 and 7 days after the use of skin powder

Parameter	Day 0	Day 1	Day 3	Day 7

Erythema	0 (0-4)	0 (0-2)	0 (0-1)	0 (0-1)
Papules	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Scales	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)

3.3.5. Biophysical parameters of the skin:

3.3.5.1. Electrical capacitance:

The distribution of 13/16 data sets (4 examinations x 4 body areas) followed the normal distribution. The exceptions were the lateral thorax on day 1 (P=0.045), and the lumbar area on day 1 (P=0.002) and day 3 (P=0.001). Therefore, use of parametric tests for analysis was considered appropriate.

Application of the skin powder did not result in significant changes of the electrical capacity of the skin of right axillae (P=0.107), inguinal area (P=0.555), right lateral thorax (P=0.518) or lumbar area (P=0.212).

3.3.5.2. Skin color (redness):

The distribution of all 16 data sets (4 examinations x 4 body areas) was normal. Subsequently, parametric tests were used for analysis.

Application of the skin powder did not result in significant changes of the color (redness) of the skin of right axillae (P=0.786), inguinal area (P=0.935), or lumbar area (P=0.746), but the redness of the right lateral thoracic skin was significantly reduced after the application of the skin powder(P=0.004); however, post-hock tests did not show significant differences between any two time points.

3.2.5.3. Skin pH

The distribution of 13/16 data sets (4 examinations x 4 body areas) followed the normal distribution. The exceptions were the inguinal area on day 1 (P=0.046) and day 3 (P=0.039), and the lumbar area on day 0 (P=0.03). Therefore, use of parametric tests for analysis was considered appropriate.

Application of the skin powder did not result in significant changes of the pH of the skin of right axillae (P=0.323), inguinal area (P=0.919), right lateral thorax (P=0.074) or lumbar area (P=0.282).

3.4. Double-blinded randomized controlled trial of ear cleaner

3.4.1. Adverse health events:

No local adverse health events were reported by the owners in the 18 re-examinations for the ears where the Ear Clean Solution (Provet), as well as for the ears where the control ear cleaner was applied.

3.4.2. Pruritus

The distribution of modified PVAS scores for ear pruritus and other signs of ear irritation/otitis (head shaking, discomfort, pain) was normal for both ears on days 0, 1, 3, 7, 8, 10, 14, 15, 17, 21, 22, 24, 28, 29, 31, 35, 36, 38 and 42. Subsequently, data are presented as mean ± SD and parametric tests were used for analysis.

The modified PVAS scores before and after using the two ear cleaners are presented on the following table. None of the ear cleaners resulted in increased ear pruritus or other signs of ear irritation/otitis (both P values = 0.637).

Table. Modified pruritus visual analogue scale (PVAS) scores before (day 0) and 1, 3, 7, 8, 10, 14, 15, 17, 21, 22, 24, 28, 29, 31, 35, 36, 38 and 42 days after the weekly use of two ear cleaners

Ear cleaner	Day	Modified PVAS (mean ± SD)	P value
Ear Clean Solution (Provet)	0	0.4 ± 0.5	0.637
	1	0.4 ± 0.3	
	3	0.3 ± 0.3	
	7	0.3 ± 0.3	
	8	0.2 ± 0.2	
	10	0.4 ± 0.3	
	14	0.3 ± 0.3	
	15	0.3 ± 0.2	
	17	0.2 ± 0.2	
	21	0.3 ± 0.3	
	22	0.3 ± 0.3	
	24	0.3 ± 0.3	
	28	0.3 ± 0.2	
	29	0.3 ± 0.3	
	31	0.3 ± 0.2	
	35	0.3 ± 0.3	
	36	0.3 ± 0.3	
	38	0.4 ± 0.5	
	42	0.2 ± 0.2	
Epi Otic (Virbac)	0	0.4 ± 0.5	0.637
	1	0.3 ± 0.2	
	3	0.6 ± 0.5	
	7	0.3 ± 0.2	
	8	0.2 ± 0.2	
	10	0.3 ± 0.3	
	14	0.2 ± 0.2	
	15	0.3 ± 0.2	
	17	0.3 ± 0.2	
	21	0.3 ± 0.3	
	22	0.4 ± 0.4	
	24	0.2 ± 0.2	
	28	0.3 ± 0.2	
	29	0.3 ± 0.3	
	31	0.3 ± 0.2	
	35	0.3 ± 0.3	
	36	0.3 ± 0.3	
	38	0.4 ± 0.4	
	42	0.2 ± 0.2	

3.4.3. Owner's assessment of ear odor

The distribution of the data did not follow the normal distribution in the majority (27/36) of the data sets and for this reason non-parametric tests were considered appropriate. There was no significant difference among days 1, 3, 7, 8, 10, 14, 15, 17, 21, 22, 24, 28, 29, 31, 35, 36, 38 and 42 for the Ear Clean Solution (P=0.836) or the Epi Otic (P= 0.35). When the two ear cleaners were compared, there was no difference between them on day 1 (P=0.564), day 3 (P=1), day 7 (P=0.157), day 8 (P=0.371), day 10 (P=1), day 14 (P=1),

day 15 (P=0.317), day 17 (P=1), day 21 (P=1), day 22 (P=1), day 24 (P=1), day 28 (P=1), day 29 (P=1), day 31 (P=0.317), day 35 (P=0.317), day 36 (P=1), day 38 (P=1) and day 42 (P=1)

3.4.4. Clinical and otoscopic examination

None of the dogs developed papules, edema, or scales on either of the ear pinnae. Disregarding the examinations where all scores were zero, the distributions of pinnal erythema and OTIS3 were non-normal (P≤0.002 for erythema and P≤0.004 for OTIS3). Subsequently, all data are presented as non-normally distributed (i.e., median, range) and non-parametric tests were used for their analysis.

Pinnal erythema and OTIS3 scores before the first use of ear cleaners (day 0) and after 1, 3, 7, 8, 10, 14, 15, 17, 21, 22, 24, 28, 29, 31, 35, 36, 38 and 42 days are presented on the following table. The severity of pinnal erythema differed among the 19 time points in the ears treated with Ear Clean Solution (P=0.04) and was significantly lower on days 10, 17, 28, 29, 31, 35, 36 compared to day 0 (P=0.046). The severity of pinnal erythema did not differ among the 19 time points in the ears treated with Epi Otic (P=0.456). OTIS3 did not differ among the 19 time points in the ears treated with either Ear Clean Solution (P=0.309) or Epi Otic (P=0.844).

Table. Pinnal erythema and OTIS3 scores before (day 0) and 1, 3, 7, 8, 10, 14, 15, 17, 21, 22, 24, 28, 29, 31, 35, 36, 38 and 42 days after the weekly use of two ear cleaners

Ear cleaner	Day	Erythema	OTIS3
Ear Clean Solution (Provet)	0	1 (0-1)	0 (0-1)
	1	0 (0-1)	0 (0-1)
	3	0 (0-1)	0 (0-0)
	7	0 (0-1)	0 (0-0)
	8	0 (0-1)	0 (0-1)
	10	0 (0-0)	0 (0-0)
	14	0 (0-1)	0 (0-0)
	15	0 (0-1)	0 (0-1)
	17	0 (0-0)	0 (0-1)
	21	0 (0-1)	0 (0-1)
	22	0 (0-1)	0 (0-1)
	24	0 (0-1)	0 (0-0)
	28	0 (0-0)	0 (0-0)
	29	0 (0-0)	0 (0-1)
	31	0 (0-0)	0 (0-0)
	35	0 (0-0)	0 (0-0)
	36	0 (0-0)	0 (0-0)
	38	0 (0-1)	0 (0-0)
	42	0 (0-1)	0 (0-0)
Epi Otic (Virbac)	0	0.5 (0-1)	0 (0-2)
	1	0 (0-1)	0 (0-1)
	3	0 (0-1)	0 (0-0)
	7	0 (0-1)	0 (0-1)
	8	0 (0-1)	0 (0-1)
	10	0 (0-0)	0 (0-0)
	14	0 (0-1)	0 (0-1)
	15	0 (0-1)	0 (0-1)
	17	0 (0-0)	0 (0-1)
	21	0 (0-1)	0 (0-0)

	22	0 (0-1)	0 (0-1)
	24	0 (0-1)	0 (0-1)
	28	0 (0-0)	0 (0-0)
	29	0 (0-0)	0 (0-1)
	31	0 (0-1)	0 (0-0)
	35	0 (0-0)	0 (0-1)
	36	0 (0-0)	0 (0-1)
	38	0 (0-1)	0 (0-1)
	42	0 (0-1)	0 (0-0)

4. Conclusions

Chlorhexidine shampoo (Provet): a single bath in healthy dogs was not associated with systemic or topical adverse effects. There was no pruritus and most owner's assessments denoted a stable or improved cosmetic appearance of the skin and hair coat, except for a moderate deterioration of the quality of hair coat in 1/6 (16.7%) dogs on days 1 and 3, and a moderate deterioration of skin odor in 2/6 (33.3%) dogs on day 3 and in 1/6 (16.7%) dogs on day 7. The shampoo did not result in signs of irritation (erythema, papules) or xerosis (scales) and did not change the electrical capacitance, color (redness), and pH of the skin. Subsequently, under the conditions of this study, the shampoo was found to be safe.

Sebbaoric shampoo (Provet): a single bath in healthy dogs was not associated with systemic or topical adverse effects (except an unpleasant smell in 2/6 dogs that was reported by their owners one day after use and was not reported two days later). There was no pruritus, and most owner's assessments denoted a stable or improved cosmetic appearance of the skin and hair coat, except for a moderate deterioration of the quality of hair coat in 1/6 (16.7%) dogs on day 1 and a great deterioration in 1/6 (16.7%) dogs on day 3, a moderate skin dryness in 1/6 (16.7%) dogs on day 1 and in 2/6 (33.3%) dogs on day 3, a moderate scaling in 1/6 (16.7%) dogs on day 7, and a moderate deterioration of skin odor in 1/6 (16.7%) dogs on day 1 and in 1/6 (16.7%) dogs on day 3. The shampoo did not result in signs of irritation (erythema, papules) or xerosis (scales), and did not change the electrical capacitance, color (redness), and pH of the skin. Subsequently, under the conditions of this study, the shampoo was found to be safe.

Sensitive Skin shampoo (Provet): a single bath in healthy dogs was not associated with systemic or topical adverse effects. There was no pruritus, and all owner's assessments denoted a stable or improved cosmetic appearance of the skin and hair coat. The shampoo did not result in signs of irritation (erythema, papules) or xerosis (scales), and did not change the electrical capacitance, color (redness), and pH of the skin. Subsequently, under the conditions of this study, the shampoo was found to be safe.

Skin & Coat Protect shampoo (Provet): a single bath in healthy dogs was not associated with systemic or topical adverse effects. There was no pruritus, and most owner's assessments denoted a stable or improved cosmetic appearance of the skin and hair coat, except for a moderate deterioration of the quality of the hair coat in 2/6 (33.3%) dogs on day 1 and 2/6 (33.3%) dogs in day 3, a moderate skin dryness in 2/6 (33.3%) dogs on day 1, 1/6 (16.7%) dogs on day 3 and 1/6 (16.7%) dogs on day 7, and a greatly deteriorated skin odor in 1/6 (16.7%) dogs on day 7. The shampoo did not result in signs of irritation (erythema, papules) or xerosis (scales), and did not change the electrical capacitance, color

(redness), and pH of the skin. Subsequently, under the conditions of this study, the shampoo was found to be safe.

Skin and coat powder (Provet): a single application in healthy dogs was not associated with systemic or topical adverse events, except for yellowish pigmentation of the hair coat in 1/6 (16.7%) dogs on day 3. There was no pruritus, and most owner's assessments denoted a stable or improved cosmetic appearance of the skin and hair coat, except for a moderate deterioration of the quality of the hair coat in 1/6 (16.7%) dogs on day 1, a moderate skin dryness in 1/6 (16.7%) dogs on day 1, and a moderate deterioration of skin odor in 1/6 (16.7%) dogs on day 1 and 1/6 (16.7%) dogs on day 7. The powder did not result in signs of irritation (erythema, papules) or xerosis (scales), and did not change the electrical capacitance, color (redness), and pH of the skin. Subsequently, under the conditions of this study, the powder was found to be safe.

Ear Clean Solution (Provet): weekly application in the external ear canal of healthy dogs for 6 weeks was not associated with systemic or topical adverse events, increased ear pruritus or ear odor. It was not associated with skin lesions on the ear pinnae indicative of irritation (erythema, papules, edema) or xerosis (scales) and it did not induced inflammation of the ear canal. Subsequently, under the conditions of this study, the ear solution was found to be safe.

5. References

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