Efficacy of Boric-Complexed Zinc and Acetic-Complexed Zinc Otic Preparations for Canine Yeast Otitis Externa

The purpose of this 2-week, double-blinded, controlled clinical trial was to evaluate the efficacy of topical amino acid-complexed zinc gluconate formulated with boric acid (ZGB) or acetic acid (ZGA) versus a topical placebo in the treatment of yeast otitis externa in dogs. Included in the study were dogs with otitis externa and a cytopathological finding of yeast organisms in the affected ear. Ears were treated with the placebo, ZGA, or ZGB medications. Yeast counts as well as clinical appearance of the ears were monitored. Results revealed that ZGB significantly reduced the number of yeast organisms in cases of otitis externa. **J Am Anim Hosp Assoc 2005;41:12-21.**

Colleen L. Mendelsohn, DVM, Diplomate ACVD

Craig E. Griffin, DVM, Diplomate ACVD

Wayne S. Rosenkrantz, DVM, Diplomate ACVD

Larry D. Brown, DVM, MPVM, PhD, Diplomate ACVPM

Mona J. Boord, DVM, Diplomate ACVD

Introduction

A large variety and quantity of canine topical otic preparations are available and serve as a testament to the prevalence of ear disease in dogs. Otitis externa is one of the most common reasons for seeking veterinary care, accounting for up to 15% of all dogs presented.[1,2] Primary causes of otitis externa include hypersensitivity disorders (e.g., atopy, food allergy), parasitic diseases, and metabolic disorders (e.g., primary keratinization defects, hypothyroidism, hyperadrenocorticism).[1,2] Perpetuating factors such as proliferative changes, excessive cleaning, or the use of inappropriate cleaning products also contribute.[1,2] Otitis externa may also occur secondary to infections. *Malassezia pachydermatis* is the most common isolate from diseased ears and often requires therapy.[1,2]

Identifying the cause and controlling the primary diseases are the goals of treating most cases of canine otitis externa. However, even if the primary cause is identified and addressed, many cases require recurrent, long-term systemic and topical therapy for secondary factors.[1,2] In some cases of atopic disease, controlling secondary infections (especially *Malassezia* spp.) helps alleviate the clinical signs. Most otic preparations are combinations of corticosteroids and antimicrobials. Frequent and repeated use of these products is often indicated; however, when such products are used repeatedly for management and prevention, problems of antibiotic resistance and glucocorticoid side effects (both cutaneous and systemic) may occur.

Products containing mild cleansers or disinfectants are valuable in the treatment of otitis externa, and they do not have as many potential risks as the long-term antibiotic or glucocorticoid therapies. Many otic products are available that are combinations of mild cleansers, drying agents, and disinfectants, with or without antimicrobial agents. In the authors' experience, some of these products can be used as maintenance therapy to prevent the recurrence of otitis externa. Common cleansing ingredients

From the Animal Dermatology Clinics, 2965 Edinger Avenue, Tustin, California 92780 and 5610 Kearny Mesa Boulevard, San Diego, California 92111 (Mendelsohn, Griffin, Rosenkrantz, Boord); and the Addison Biological Laboratory, Inc. (Brown), 507 North Cleveland Avenue, Fayette, Missouri 65248.

include boric acid, acetic acid, lactic acid, malic acid, fatty acids, enzymes, chelating agents, and minerals. Of these agents, acetic and boric acids have received attention for their abilities to kill yeast and bacterial organisms.

Acetic acid has been reported to be effective at concentrations from 0.5% to 5% in treating and preventing otic yeast infections.[1] Acetic acid has been beneficial in people in the management of chronic suppurative otitis media. The effect was not solely related to pH, as other acidic products have not been as effective.[3,4] Acetic acid may irritate already damaged otic epithelium, and its unpleasant odor creates problems with patient acceptance and client compliance.[5]

Boric acid has also been shown to be effective against yeast infections.[6-8] One study reported that boric acid was as efficacious as topical antibiotics in treating human otitis externa.[6] In another study, 95% of vaginal yeast infections in people were eliminated with boric acid vaginal suppositories.[7] The mechanism of action of boric acid is unknown.[7,8] It has been proposed that boric acid may cleanse the lipids from the epithelium, which removes the metabolic substrates for *Malassezia* spp. or may inactivate a hygroscopic, neutrophilic chemo-attractant protein elaborated by *Malassezia* spp.[5,6] In addition, an *in vitro* study of canine otitis isolates showed that a combination of 0.5% boric acid and 0.5% acetic acid was lethal to *Staphylococcus intermedius*.[8]

Zinc has been studied for its effects on wound healing, and at the time this article was written, it was not incorporated into any commercial veterinary otic products. Zinc administered topically has had beneficial effects on wound healing, regardless of an individual's systemic zinc levels.[9] Like trisethylenediaminetetraacetic acid and silver-sulfadiazine, zinc also has chelating effects on cells.[10] In humans, topical zinc oxide accelerated healing of diabetic leg ulcers.[11] In pigs and mice, the topical application of zinc oxide enhanced reepithelialization of partial- and full-thickness wounds, and was as effective as streptokinase-streptodornase in removing necrotic tissue from pressure ulcers.[12-14] When embedded in occlusive dressing, zinc decreased the inflammatory reaction typically seen during the formation of granulation tissue.[15] Zinc has also been demonstrated to have antimicrobial properties, specifically related to the topical application of zinc. Zinc gluconate lozenges have been shown to decrease the duration of cold symptoms, and the efficacy of zinc gluconate lozenges increases with the length of time the lozenge is present in the oral cavity.[16-18] An *in vitro* study demonstrated that herpes simplex virus was inactivated after treatment with zinc gluconate.[19] Zinc gluconate also specifically decreased the expression of certain inflammatory mediators by keratinocytes exposed to nickel, an allergen responsible for some cases of contact dermatitis.[20]

The purpose of this double-blinded, placebo-controlled clinical trial was to evaluate the efficacy of topical amino acid-complexed zinc gluconate formulated with boric acid (ZGB) or acetic acid (ZGA) versus a placebo in the treatment of yeast otitis externa in dogs.

Materials and Methods

Dogs that were presented with clinical signs of otitis externa and a cytopathological finding of yeast in the affected ear(s) were eligible for the study. On examination of swabs of the ear, an average count of three yeast per oil-immersion field (OIF) was considered the minimum criterion for a diagnosis of yeast otitis [Figure 1].[21] Initially each slide was scanned on low power (100×) to select areas with keratinocytes or wax. Examination under oil immersion (1000×) was then performed, and yeast organisms were counted in 10 separate OIFs that contained keratinocytes. The numbers from these 10 counts were then averaged. Dogs were excluded from the study if they had received any topical or systemic antimicrobials or corticoidsteroids in the 2 weeks prior to presentation.

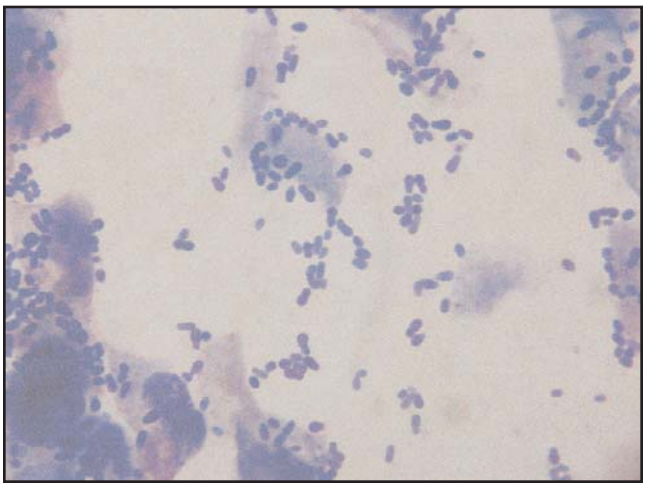


***Figure 1*—**Numerous yeast organisms and keratinocytes on an oil-immersion field (1000×) as seen on cytopathology from case no. 30 on day 0.

Each diseased ear was considered an individual case. An otoscopic examination was performed, a visible tympanum was verified, and the ear was randomly assigned to a ZGB,[a] ZGA, or placebo control group. After cleaning the ear with a petrolatum-based squalene cleanser,[b] 1 to 3 mL (depending on the size of the ear canal) of either ZGB, ZGA, or the placebo was applied to the ear twice daily for the entire study period. The ear was reevaluated and cleaned again on days 7 and 14 of the study by one of the available authors/investigators. The authors/investigators and the owners were blinded to the type of solution administered until the end of the study.

All study solutions contained deionized water, methylparaben (500 ppm), propylparaben (100 ppm), and propylene glycol at 1%. The placebo solution contained only these compounds. The zinc solutions also contained zinc gluconate, L-lysine, taurine, and either 1% acetic acid or 1% boric acid. The ZGB solution and the ZGA solution were compounded by Addison Biological Laboratory, Inc., but the ZGB solution has now become commercially available. The pH of all three test solutions was determined by a calibrated,

hand-held electronic pH meter.[c] The pH of ZGA and ZGB solutions was 4.5, while the pH of the placebo solution was slightly higher at 4.93.

Yeast counts in the affected ears were evaluated (precleaning) on days 0, 7, and 14. An average yeast count of ≤3 organisms per OIF was considered normal and given a score of 0. An average count of 3.1 to 8 organisms per OIF was assigned a score of 1; an average count of 8.1 to 14 organisms per OIF was given a score of 2; and yeast counts ≥15 yeast per OIF were assigned a score of 3.[21] Bacteria present at levels >5 organisms per OIF were considered significant and were recorded. An aerobic bacterial culture and sensitivity were subsequently performed on these samples.[22] The presence of bacteria in the original cytology and a positive culture did not preclude entrance of the case into the study.

For clinical scoring, the ear was divided into two regions. The external ear included the external aural orifice and the adjacent pinna. The ear canal included both the vertical and horizontal portions. The external ear was evaluated by visual inspection, while the ear canal was evaluated by otoscopic examination.[d] Erythema, exudate, and stenosis of each region were evaluated by a single examiner and given scores of 0, 1, 2, or 3 representing none, mild, moderate, or severe changes, respectively. The scores for these three parameters were summed so that the highest possible clinical score was 9 for each region of the ear.

At each recheck visit, clients were questioned about their use of the test solutions and their perceptions as to response to treatment. In the event that the animal was worse (as determined by the owner) on day 7, the dog was removed from the study, and using the "intent to treat rule," the values from day 7 were carried over to day 14.[23-25]

Statistical Analysis

The three treatment groups included a control group, a ZGA treatment group, and a ZGB treatment group. Differences between groups were analyzed by analysis of variance (ANOVA) using a commercially available statistical software program.[e] Three sets of scored data (i.e., yeast score, external ear clinical score, ear canal clinical score) were evaluated by Model I ANOVA for the control versus ZGA groups, the control versus ZGB groups, and the ZGA versus ZGB groups. A three-way (placebo control versus ZGA versus ZGB) analysis was also performed, comparing scores between all three groups. The statistical significance level (P=alpha or probability of committing a Type 1 error) applied to all comparisons was $P \leq 0.05$.

Results

Twenty-one dogs were included in the study. Fourteen had bilateral disease and seven had unilateral disease based on the inclusion criteria, resulting in a total of 35 ears. In 12 dogs (20 ears), atopic dermatitis was the primary condition (based on history, distribution of lesions, responsiveness to therapy, and absence of other hypersensitivity disorders), and 10 of these 12 dogs were under treatment with allergen-specific immunotherapy. The remaining two atopic dogs had mild seasonal signs and were not on any therapy. One dog (one ear) had both atopic dermatitis and food allergy; this dog was on allergen-specific immunotherapy and a restricted diet. One dog (two ears) had primary idiopathic seborrhea. Three dogs (five ears) had chronic recurrent otitis externa of undetermined cause, although atopic dermatitis was suspected in one. Four dogs (seven ears) were presented with no prior history of skin or ear disease.

Breed distribution was as follows: mixed-breed dogs (n=4), Labrador retriever (n=3), rottweiler (n=2), standard poodle (n=2), American cocker spaniel (n=2), dachshund (n=2), Dalmatian (n=2), and one each of beagle, golden retriever, Akita, and red bone coonhound. Ages ranged from 1.5 to 10 years, with a mean age of 4.3 years and a median of 4.0 years.

Control Group

Ten ears were treated with the placebo solution. These cases were assigned numbers 1 through 10 after the study was completed. The average yeast scores for the control dogs on days 0, 7, and 14 were 1.6, 1.1, and 1.1, respectively [Table 1]. The mean external ear clinical scores for days 0, 7, and 14 were 4.5, 3.5, and 2.7, respectively [Table 2]. The mean ear canal clinical scores for days 0, 7, and 14 were 5.0, 3.6, and 3.2, respectively [Table 2]. In case nos. 7 and 10, bacterial otitis and a worsening of the clinical signs were found on day 7, and systemic and topical antimicrobial treatments were initiated. These three cases were subsequently removed from the study.

Seven ears remained in the study for the full 14-day follow-up period. Yeast scores were increased in two cases on day 14, three scores remained the same, and five scores improved. In three cases (case nos. 3, 4, and 7), significant bacterial otitis was present on day 0. *Staphylococcus intermedius* was identified on day 0 in case nos. 4 and 7, but no organisms were initially isolated in case no. 3. In case no. 3, the bacterial component resolved by day 14. In case no. 7, the bacterial counts worsened and *Pseudomonas aeruginosa* was cultured on day 14. Case no. 10 was withdrawn from the study and had *Staphylococcus intermedius* isolated on day 14.

No discomfort occurred in association with medication application in any case. In two ears (case nos. 4, 9), owners felt their dog's condition had slightly improved. For the remaining eight ears, owners reported either worsening or no change (even though clinically they had apparent improvement). An acute episode of lumbosacral pruritus occurred in case no. 8 on day 6.

Zinc Gluconate in Acetic Acid Treatment Group

Thirteen ears were treated with ZGA (assigned case numbers 11 through 23). The mean yeast scores on days 0, 7, and 14 were 2.4, 1.3, and 1.2, respectively [Table 3]. The mean external ear clinical scores for days 0, 7, and 14 were 4.2, 2.5, and 3.0, respectively [Table 4, Figures 2A, 2B]. The mean ear canal clinical scores for days 0, 7, and 14 were 5.2, 4.5, and 4.1, respectively [Table 4]. On day 7, case no. 23 had a significant increase in both yeast counts and clinical

Table 1

Yeast Counts and Scores for 10 Dogs in the Control Group

Case No.	Day 0 Average Yeast Count (per OIF)*	Day 0 Assigned Score†	Day 7 Averaged Yeast Count (per OIF)	Day 7 Assigned Score	Day 14 Averaged Yeast Count (per OIF)	Day 14 Assigned Score
1	>20	3	>20	3	>20	3
2	3.3	1	0.2	0	0	0
3	4.1	1	0	0	0	0
4	8.5	2	2.1	0	1.4	0
5	6.0	1	14.1	3	>20	3
6	5.0	1	>20	3	>20	3
7‡	8.5	2	0	0	0	0
8	>20	3	4.0	1	0	0
9	3.5	1	0	0	3.0	1
10‡	5.0	1	2.0	1	2.0	1
Mean ± Standard Deviation		1.6 ± 0.84		1.1 ±1.37		1.1 ± 1.37
Mean ± Standard Error of Means		1.6 ± 0.27		1.1 ± 0.43		1.1 ± 0.43

* OIF=oil-immersion field; 10 OIFs per ear were counted and averaged
† See text for definition of Assigned Scores
‡ Bacterial otitis and worsening of clinical signs were detected on day 7; case was withdrawn from study for auxiliary treatments. Day 7 scores were carried over to day 14 under 'intent to treat' rule.

scores, so it was removed from the study and started on systemic and topical anti-yeast therapies. One yeast score was increased at day 14, three scores remained the same, and nine scores improved.

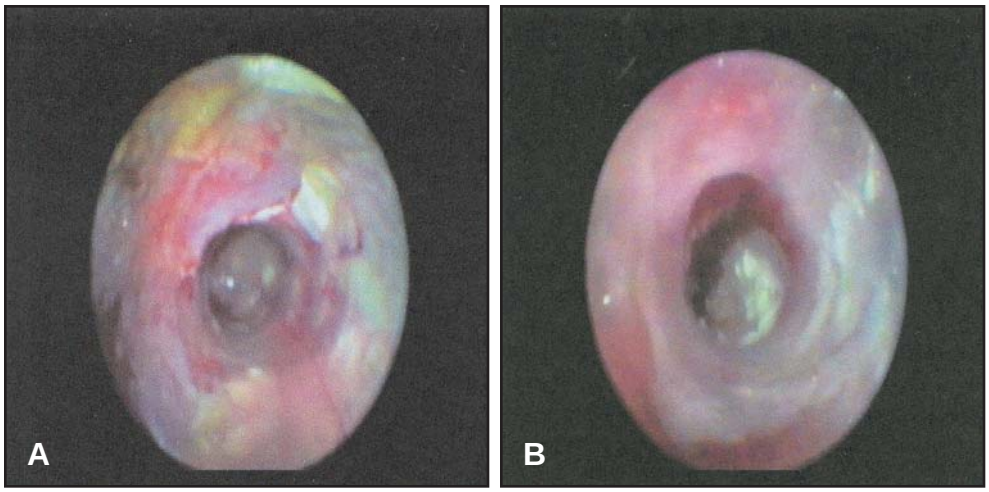
Case nos. 11, 14, and 21 had significant bacterial otitis at the start of the study, and *Staphylococcus intermedius* was cultured from all of the affected ears. Seven owners reported improvement by day 14, and five owners reported no change at day 14.

In seven cases, significant discomfort occurred in association with application of the medication at various times during the study. However, by day 14, four of the owners reported that the initial discomfort associated with application of the medication had resolved. All owners reported the medication had an unpleasant odor similar to vinegar. In three cases, generalized pruritus continued throughout the study period.

Zinc Gluconate in Boric Acid Treatment Group

Twelve ears were treated with ZGB (case nos. 24 through 35). The mean yeast scores on days 0, 7, and 14 were 2.0, 1.0, and 0.3, respectively [Table 5]. The mean external ear clinical scores for days 0, 7, and 14 were 2.8, 1.3, and 1.8, respectively [Table 6]. The mean ear canal clinical scores for days 0, 7, and 14 were 3.6, 2.0, and 2.7, respectively [Table 6]. No scores increased for any of the ears in this group. No yeast counts increased, one count remained the same, and 11 counts improved.

Case nos. 24, 26, and 27 had significant bacterial otitis on day 0, but the bacteria had subsided by days 7 or 14. Bacteria were isolated from only one culture (case no. 26) and were identified as *Micrococcus* spp. In nine cases, the owners reported significant improvement, while in three cases the owners reported no change. No cases were withdrawn from the study. In case no. 27, discomfort occurred



***Figures 2A, 2B*—**Affected ear canal before **(A)** and after **(B)** cleaning on day 0, demonstrating an intact tympanum, moderate erythema, and mild exudate from case no. 13 on day 7.

after the medication was applied. In case no. 32, the owner reported continued generalized pruritus.

Statistical Results

At day 0, no statistical difference was found between groups for any of the scores evaluated. Within each treatment group, the yeast score and ear canal score gave the most obvious evidence of improvement. Within the ZGB group, a significant decrease in yeast score occurred over 2 weeks of treatment ($P \leq 0.00002$). Within the ZGA group, yeast scores also decreased significantly, but to a lesser degree ($P \leq 0.013$). The yeast scores in the control group did not change significantly.

When comparing results between groups, changes in yeast scores with ZGB were significantly better at day 14 than ZGA ($P \leq 0.034$) but not statistically significantly less than the placebo ($P \leq 0.085$) [Figure 3]. Day 14 yeast scores were not significantly different from each other for the control and ZGA groups.

The ZGB group had a reduced ear canal score on day 14, as compared to day 0 (baseline); however, the result was not statistically significant. Statistical comparisons (two- and three-way ANOVA) for all other within- or between-group yeast scores, external ear clinical scores, and ear canal clinical scores were not significant ($P > 0.05$).

Discussion

Both acetic and boric acid have been effective in treating yeast infections.[2-9] In the study reported here, use of ZGA did not significantly eliminate or reduce yeast numbers when compared to placebo. Use of ZGB, however, did result in a significant decrease of yeast numbers. No significant differences in

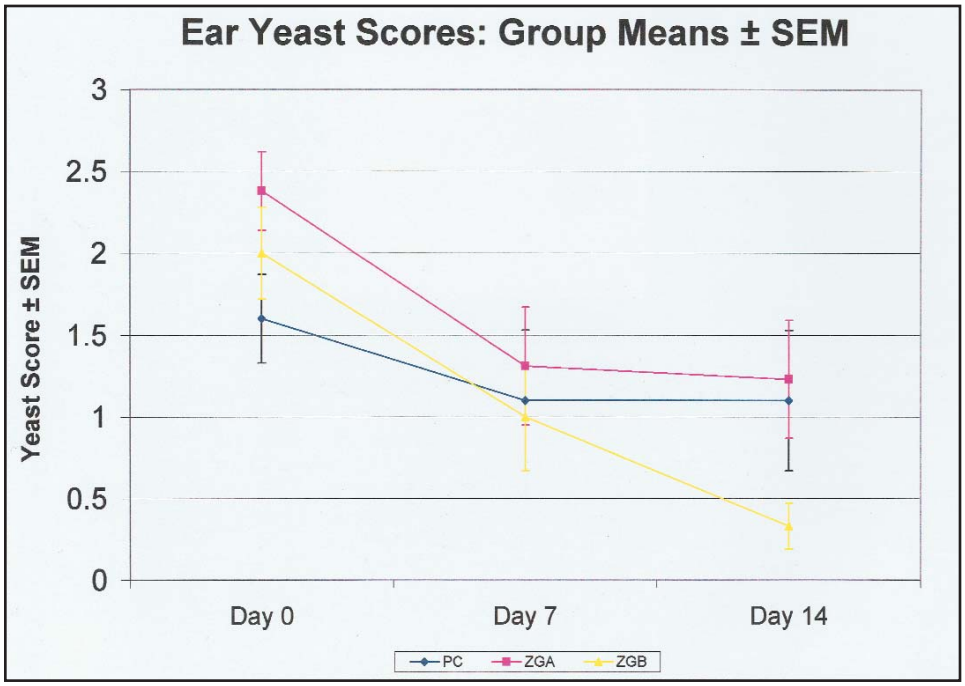


Figure 3—Ear yeast scores at three time points. SEM=standard error of means; PC=placebo control group; ZGA=zinc gluconate formulated with acetic acid treatment group; ZGB=zinc gluconate formulated with boric acid treatment group.

Table 2

Sum of Clinical Scores* for 10 Dogs in the Control Group

Case No.	External Ear			Ear Canal		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
1	9	6	7	9	7	7
2	2	0	0	2	1	1
3	6	2	0	4	1	0
4	3	2	0	5	2	3
5	7	6	1	6	4	4
6	3	6	5	4	7	5
7†	6	9	9	7	8	8
8	4	3	0	7	3	0
9	2	0	2	3	1	2
10†	3	1	1	3	2	2
Mean ± Standard Deviation	4.5 ± 2.37	3.5 ± 3.06	2.5 ± 3.31	5.0 ± 2.21	3.6 ± 2.76	3.2 ± 2.78
Mean ± Standard Error of Means	4.5 ± 0.75	3.4 ± 0.97	2.5 ± 1.05	5. ± 0.70	3.6 ± 0.87	3.2 ± 0.88

* Score=sum of erythema, exudate, and stenosis scores; maximum=9
† Bacterial otitis and worsening of clinical signs were detected on day 7; case was withdrawn from study

the degree of inflammation were noted between the treatment groups, and this result may have indicated continuing inflammation caused by the underlying primary disease, which was usually atopic dermatitis. This supposition was supported by the observation that the pinnae, which were not treated, remained more erythematous than the ear canal in the ZGB group.

Although acetic acid has been effective against many of the organisms that perpetuate canine otitis externa, the discomfort and inflammation induced by application of the acetic acid into the ear canal can result in worsening of the infection or inflammation.[1-3] In this study, seven of the 13 owners in the ZGA group reported mild to severe discomfort with application of the eardrops, even after improvement of the infection. In the ZGB group, only one of the 12 ears had a mildly painful reaction to the topical medication.

The improvement noted in the control group on day 7 was an interesting finding and may have been related to the ear cleaning that was performed and/or the decreased pH of the placebo agent. Unlike the ZGB and ZGA treatment groups, bacterial growth appeared to worsen in some cases within the control group. It is possible that increased moisture in the absence of an antimicrobial agent contributed to this complication.[1] The inability to demonstrate bacteria on cultures from the cases in which bacteria were visualized on cytopathology may have arisen from differences in the specific sites in the ear canal from which the samples were taken, from relatively low numbers of bacteria, from alterations in transport media, or from laboratory handling errors.

In clinical trials assessing the use of topical zinc to treat pressure wounds and diabetic foot ulcers, the duration of the clinical trials ranged from 6 to 12 weeks.[11,12,14] It is possible that the 2-week duration of topical therapy used in the study reported here was not long enough to induce or recognize clinical improvement in the inflammatory component of otitis externa. In addition, the primary cause of otitis was not addressed during this study, as demonstrated by several instances of continued generalized or localized pruritus during the study period. Another possible explanation for lack of improvement in the clinical scores was the small sample size, which would not have

Table 3

Yeast Counts and Scores for 13 Dogs Treated With Zinc Gluconate in Acetic Acid

Case No.	Day 0 Average Yeast Count (per OIF)*	Day 0 Assigned Score†	Day 7 Average Yeast Count (per OIF)	Day 7 Assigned Score	Day 14 Average Yeast Count (per OIF)	Day 14 Assigned Score
11	14.0	3	9.3	2	4.9	1
12	>20	3	1.9	0	3.9	1
13	9.2	2	5.1	1	2.0	0
14	>20	3	6.1	1	6.0	1
15	10.7	2	14.9	3	19.9	3
16	5.0	1	1.0	0	0	0
17	4.0	1	1.0	0	2.0	0
18	4.0	1	0	0	0	0
19	>20	3	0.1	0	0	0
20	>20	3	15.7	3	>20	3
21	>20	3	8.6	1	4.8	1
22	15.5	3	14.0	3	>20	3
23‡	15.0	3	>20	3	>20	3
Mean ± Standard Deviation		2.4 ± 0.87		1.3 ± 1.32		1.2 ± 1.30
Mean ± Standard Error of Means		2.4 ± 0.24		1.3 ± 0.36		1.2 ± 0.36

* OIF=oil-immersion field; 10 OIFs per ear were counted and averaged
† See text for definition of Assigned Scores
‡ Worsening of clinical signs was detected on day 7; case was withdrawn from study

compensated for subjective differences between investigators. The presence of otitis media and its potential impact on the clinical scores also could not be ruled out, although most of the dogs in this study had mild to moderate otic disease, and not all dogs had chronic otitis.[22]

Conclusion

Based on the results of this study, amino acid-complexed zinc gluconate with boric acid was efficacious against yeast otitis externa in dogs. It reduced ear canal inflammation, which is essential to promoting epithelial healing. Further studies are needed to examine the success of using boric acid alone by evaluating both organism numbers and the inflammatory response, and to determine whether concurrent use of low-potency topical corticosteroids alters the inflammation attributed to the primary disease, especially atopic dermatitis.

[a] Zn4.5 Otic; Addison Biological Laboratory, Inc., Fayette, MO 65248
[b] Cerumine; EVSCO, Buena, NJ 08310
[c] Extech Instruments, Waltham, MA 02451
[d] With a 10× magnifying lens
[e] Microsoft Excel XP statistical tools, Microsoft Corporation

References

1. Scott DW, Miller WH, Griffin CE. Diseases of eyelids, claws, anal sacs and ears. Muller & Kirk's Small Animal Dermatology. 6th ed. Philadelphia: WB Saunders, 2001:1203-1232.
2. White PD. Medical management of chronic otitis in dogs. Compend Contin Educ Pract Vet 1999;21:716-728.

Table 4

Sum of Clinical Scores* for 13 Dogs Treated With Zinc Gluconate in Acetic Acid

Case No.	External Ear			Ear Canal		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
11	6	1	3	5	4	4
12	9	1	6	9	6	7
13	1	2	0	4	4	1
14	6	3	0	6	5	1
15	3	2	4	5	5	6
16	3	1	1	5	4	3
17	0	1	1	4	3	3
18	1	2	2	1	3	2
19	5	1	4	4	1	2
20	0	1	1	3	5	2
21	9	3	2	9	3	4
22	6	6	7	7	6	9
23†	6	8	8	5	9	9
Mean ± Standard Deviation	4.2 ± 3.14	2.5 ± 2.18	3.0 ± 2.65	5.2 ± 2.23	4.5 ± 1.94	4.1 ± 2.81
Mean ± Standard Error of Means	4.2 ± 0.87	2.5 ± 0.61	3.0 ± 0.73	5.2 ± 0.62	4.5 ± 0.54	4.1 ± 0.78

* Score=sum of erythema, exudate, and stenosis scores; maximum=9
† Worsening of clinical signs was detected on day 7; case was withdrawn from study

3. Aminifarshidmehr N. The management of chronic suppurative otitis media with acid media solution. Am J Otol 1996;17:24-25.
4. Matousek JL, Campbell KL, Kakoma I. The effects of four acidifying sprays, vinegar, and water on canine cutaneous pH levels. J Am Anim Hosp Assoc 2003;39:29-33.
5. Merchant SR. Medically managing chronic otitis externa and media. Vet Med 1997;92:518-534.
6. Slack RW. A study of three preparations in the treatment of otitis externa. J Laryngol Otol 1987;101:533-535.
7. Swate T, Weed J. Boric acid treatment of vulvovaginal candidiasis. Obstet Gynecol 1974;43:893-895.
8. Bensen CE. Susceptibility of selected otitis externa pathogens to individual and mixtures of acetic and boric acids. Proc Annu Am Acad Vet Derm/Am Coll Vet Derm 1998;14:121.
9. Agren MS. Studies on zinc in wound healing. Acta Derm Venereol Suppl 1990;154:1-36.
10. McCarthy TJ, Zeelie JJ, Krause DJ. The antimicrobial action of zinc ion/antioxidant combinations. J Clin Pharm Ther 1992;17:51-54.
11. Stromberg HE, Agren MS. Topical zinc oxide treatment improves arterial and venous leg ulcers. Br J Dermatol 1984;111:461-468.
12. Agren MS, Chvapil M, Franzen L. Enhancement of re-epithelialization with topical zinc oxide in porcine partial-thickness wounds. J Surg Res 1999;50:101-105.
13. Kietzman M. Improvement and retardation of wound healing: effects of pharmacological agents in laboratory animal studies. Vet Derm 1999;10:83-88.
14. Agren MS, Stromberg HE. Topical treatment of pressure ulcers. A randomized comparative trial of Varidase and zinc oxide. Scand J Plast Reconstr Surg 1985;19:97-100.
15. Wetter L, Agren MS, Hallsman G, *et al*. Effects of zinc oxide in an occlusive, adhesive dressing on granulation tissue formation. Scand J Plast Reconstr Surg 1986;20:165-172.
16. McElroy BH, Miller SP. Effectiveness of zinc gluconate glycine lozenges (Cold-eeze) against the common cold in school-aged subjects: a retrospective chart review. Am J Ther 2002;9:472-475.
17. Turner RB, Cetnarowski WE. Effect of treatment with zinc gluconate or zinc acetate on experimental and natural colds. Clin Infect Dis 2000;31:1202-1208.
18. Marshall S. Zinc gluconate and the common cold. Review of randomized controlled trials. Can Fam Physician 1998;44:1037-1042.

Table 5

Yeast Counts and Scores for 12 Dogs Treated With Zinc Gluconate in Boric Acid

Case No.	Day 0 Average Yeast Count (per OIF)*	Day 0 Assigned Score†	Day 7 Average Yeast Count (per OIF)	Day 7 Assigned Score	Day 14 Average Yeast Count (per OIF)	Day 14 Assigned Score
24	12.0	2	8.1	2	4.1	1
25	16.5	3	1.4	0	<1	0
26	12.0	2	12.0	2	0.1	0
27	3.4	1	0	0	0.1	0
28	3.1	1	19.5	3	3.0	1
29	5.0	1	0	0	0	0
30	>20	3	10.0	2	5.0	1
31	5.0	1	5.0	1	0	0
32	>20	3	0.7	0	0	0
33	5.5	1	1.9	0	1.5	0
34	14.7	3	10.2	2	7.9	1
35	15.0	3	<1	0	0	0
Mean ± Standard Deviation		2.0 ± 0.95		1.0 ± 1.13		0.3 ± 0.49
Mean ± Standard Error of Means		2.0 ± 0.28		1.0 ± 0.33		0.3 ± 0.14

* OIF=oil-immersion field; 10 OIFs per ear were counted and averaged
† See text for definition of Assigned Scores

19. Arens M, Travis S. Zinc salts inactivate clinical isolates of herpes simplex virus *in vitro*. J Clin Microbiol 2000;38:1758-1762.
20. Sainte-Marie I, Jumbou O, Tenaud I, *et al*. Comparative study of the *in vitro* inflammatory activity of three nickel salts on keratinocytes. Acta Derm Venereol 1998;78:169-172.
21. Ginel PJ, Lucena R, Rodriguez JC, *et al*. A semiquantitative cytological evaluation of normal and pathological samples from the external ear canal of dogs and cats. Vet Derm 2002;13:151-156.
22. Cole LK, Kowchka KW, Kowalski JJ, *et al*. Microbial flora and antimicrobial susceptibility patterns of isolated pathogens from the horizontal ear canal and middle ear in dogs with otitis media. J Am Vet Med Assoc 1998;212:534-538.
23. Sheiner LB, Rubin DB. Intention-to-treat analysis and the goals of clinical trials. Clin Pharmacol Ther 1995;57(1):6-15.
24. Sheiner LB. Is intent-to-treat analysis always (ever) enough? Brit J Clin Pharmacol 2000;54:203-211.
25. Olivry T, Dunston SM, Rivierre C, *et al*. A randomized controlled trial of misoprostol monotherapy for canine atopic dermatitis: effects on dermal cellularity and cutaneous tumor necrosis factor-alpha. Vet Derm 2003;14:37-46.

Table 6

Sum of Clinical Scores* for 12 Dogs Treated With Zinc Gluconate in Boric Acid

Case No.	External Ear			Ear Canal		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
24	5	1	3	5	3	5
25	6	0	0	5	3	1
26	1	0	1	2	1	2
27	6	2	0	4	1	1
28	0	1	2	5	2	2
29	3	2	0	2	2	0
30	3	1	2	3	2	5
31	1	2	3	1	2	6
32	4	1	4	3	0	2
33	0	0	0	2	1	0
34	0	2	1	6	3	4
35	5	4	6	5	4	4
Mean ± Standard Deviation	2.8 ± 2.37	1.3 ± 1.15	1.8 ± 1.90	3.6 ± 1.62	2 ± 1.13	2.7 ± 2.06
Mean ± Standard Error of Means	2.8 ± 0.68	1.3 ± 0.33	1.8 ± 0.55	3.6 ± 0.47	2 ± 0.33	2.7 ± 0.59

* Score=sum of erythema, exudate, and stenosis scores; maximum=9