Effects of an Ivermectin Otic Suspension on Egg Hatching of the Cat Ear Mite, *Otodectes cynotis*, in Vitro*

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ABSTRACT
An otic suspension containing 0.01% ivermectin (Acarexx™; Blue Ridge Pharmaceuticals, Greensboro, NC) controls adult ear mites (*Otodectes cynotis*), but there are no data published on its efficacy against the eggs of this parasite. Three in vitro trials were conducted to examine the efficacy of this product for prevention of hatching of *O. cynotis* eggs. Eggs were placed in Petri dishes on 1 ml agarose only, agarose with 50 µl of the ivermectin otic suspension (low dose), or agarose with 0.5 ml of the otic suspension (full dose). In Trials 1 and 2, eggs were held at 28˚C; in Trial 3, eggs were kept at 37˚C. After 6 days, 22% to 38% of the eggs hatched in the control cultures, compared with 0% to 14% in the low-dose medium, and 0% to 8% of the eggs exposed to the full-dose medium. In the controls, larvae hatched throughout the 6 days and crawled away from the empty eggshells. Only one other larva (from a low-dose plate in Trial 2) crawled away from its eggshell. At the full dose of ivermectin, larvae hatched only within the first day of culture, and those few that did hatch died immediately. The 0.01% ivermectin otic suspension prevented the hatching of larvae from eggs or immediately killed the small number of mites that hatched.

INTRODUCTION
Otitis externa is one of the most common reasons that pets are presented for veterinary attention. The ear mite (*Otodectes cynotis*) is very annoying and is one of the most frequently encountered causes of otitis externa in cats. Clinically, affected animals exhibit head shaking, scratching, and rubbing of the ears, and the presence of thick, brown cerumen is a classic sign of infestation with *O. cynotis*. Rarely, hypersensitive reactions may cause severe pruritus on the head and neck.

Treatment for ear mites should be preceded by thorough irrigation to clear away all cerumen from the ear canal. Numerous products are available as miticidal agents including thiabendazole, rotenone, carbaryl, and pyrethrins. Ivermectin has been used off label for a number of years to treat feline ear mite infestations. Different treatment regimens have been de-
scribed for the use of ivermectin off label including 300 µg/kg given orally, 250 µg/kg by subcutaneous injection, or 1 to 5 mg applied directly in the ear canal. In all cases, treatments were repeated for up to three treatments.

Recently, an ivermectin otic suspension (Acarexx™; Blue Ridge Pharmaceuticals, Greensboro, NC) was approved for treatment of adult mites in cats and kittens 4 weeks of age or older. The product contains 0.01% ivermectin in a water-based liposomal formulation and is applied directly in the ear canal at the rate of 0.5 ml per ear. The suspension has been shown to be efficacious for treatment of adult mites; however, effectiveness against eggs and immature stages of O. cynotis has not been established. The purpose of the study described here was to examine the ability of the product to prevent the hatching of mite eggs after application. The study was designed and methods were developed so that individual tests could be performed in vitro.

**MATERIALS AND METHODS**

**Collection of Mite Eggs**

Veterinarians at several private practices (Pilot Mountain Animal Hospital, Pilot Mountain, NC; Rural Hall Animal Hospital, Rural Hall, NC; Friendly Animal Clinic, Greensboro, NC; Loma Linda Animal Hospital, Inc., San Bernardino, CA) collected mites (O. cynotis) from naturally infested cats for the development of the assay and for use in Trial 1. The staff of a private research facility (CHK-R&D, Stanwood, MI) collected mites used in Trials 2 and 3 from two naturally infested cats at their facility. The mites were collected on cotton swabs and shipped to Cornell University where the assays were performed.

Eggs were collected from swabs by teasing the detritus apart with fine forceps and needles on glass slides in several milliliters of mineral oil. Eggs were then picked up individually using a fine sable-hair brush and transferred to a Petri dish (35 × 10 mm) containing 1 ml of 1% agarose in deionized water. The eggs were rolled around on the surface of the agarose to remove any excess mineral oil and then transferred to a second Petri dish of agarose where the rolling process was repeated. The washed eggs were then transferred to a third dish of agarose. All eggs used were from vials of mites that were collected and shipped the day prior to culturing, and all eggs for each trial were collected over the period of several hours at a single sitting.

**Test Plates**

Two levels of the 0.01% ivermectin otic suspension, plus a control, were evaluated for their effectiveness in preventing hatching of O. cynotis. One ml of the liquid agarose was added to each of the 35-mm Petri dishes designated to be control plates and was allowed to harden. For culture plates containing the maximum dose, 0.5 ml of ivermectin otic suspension (the contents of one plastic ampule) was added to the bottom of the dish, and the warmed liquid agarose was added. The agarose was swirled while in liquid form to mix it with the formulation. The white nature of the test product made it possible to ascertain when complete mixing had occurred. For plates containing the low dose, 50 µl of the ivermectin suspension from a plastic ampule was added to the plate before the addition of the liquid agarose, and again the plate was swirled to induce mixing. The full-dose plates were fairly opaque; the low-dose plates appeared to contain a number of suspended droplets; and the control plates were clear. Gloves were changed between preparation of the different plates to minimize the carryover of ivermectin between test chambers. Also, plates were made with increasing ivermectin concentrations (i.e., controls, low-dose, and then full-dose plates).

The eggs were transferred in groups of ten, using a dissecting microscope, to the center of each test plate by means of a fine sable-hair
brush. The brush was washed with soap and water between plates within the same dose and then air-dried. Between plates of different doses, the brush was washed with soap and water followed by several changes of 70% alcohol. After the transfer of the eggs, the plates were sealed by wrapping the sides with self-sealing film. Sealed plates were then transferred to plastic boxes with a dampened paper towel on the bottom. Mite eggs from the controls and low-dose plates were examined for hatching using an inverted microscope; those from the full-dose plates were examined with a dissecting microscope because of the opacity of the agarose. The eggs were checked daily for 6 days to determine the number of mites that hatched. The status of the recently hatched mites (dead or alive) also was determined daily by observing movement or the ability of the larvae to crawl away from their eggshells. Six days after culture initiation was the period selected because preliminary trials showed that after this time, eggs that had not yet hatched tended not to hatch. Even though there were no data to support the procedure, it was believed that the eggs might develop more efficiently if they were turned like chicken eggs, so the eggs were rolled about with a brush at the time of observation and then rearranged into groups in the center of the plate.

For Trial 1, five test plates (two controls, two low-dose, and one full-dose) were prepared. In Trials 2 and 3, 15 test plates were prepared, including five of each dilution. Plates in Trials 1 and 2 were kept at 28˚C; those in Trial 3 were kept at 37˚C.

Efficacy of each level of ivermectin exposure was calculated using the following formula:

\[
\text{Percent efficacy} = \frac{\text{Total live mites in control plates} - \text{Total live mites in treated plates} \times 100}{\text{Total live mites in control plates}}
\]

■ RESULTS

Hatching rates for controls on individual plates ranged from 20% to 50%. The overall hatch rate for control mites across all three trials was 30% (Table 1). Of the 20 control eggs in Trial 1, 6 (30%) hatched. In Trial 2, 11 of 50 (22%) control eggs hatched, and in Trial 3, 19 of 50 (38%) control eggs hatched. In Trials 1 and 2 (28˚C), larvae hatched out of 0% and 8%, respectively, of the eggs exposed to the low dose of ivermectin. In these two trials, 0% and 6% of the eggs in the full-dose plates hatched. At 37˚C, 14% of the eggs in the low-dose and 8% of the eggs in the full-dose ivermectin otic solution hatched.

Larvae hatching in control dishes were motile and moved away from the empty eggshells (Table 1). In the dishes with ivermectin at the low dose, only one larva (Day 2 in Trial 2) moved away from the eggshell after hatching. In the full-dose ivermectin plates, all hatching occurred on Day 1, and all larvae that hatched died immediately adjacent to the eggshell from which they had hatched.

If efficacy of the product is based on mean number of actual egg hatches per plate across all trials, the controlled efficacy for exposure of eggs to the low dose (50 µl) was 69.4% and for the full dose (0.5 ml) was 78.8%. However, according to the criterion used to determine efficacy (i.e., the number of mites that hatched and survived in treated plates relative to controls), the efficacy of the low dose was 97.2% (one hatching mite was alive and motile) and the full dose was 100% effective (none of the hatched mites lived long enough to crawl away from the empty eggshells) (Table 1).

■ DISCUSSION

Treatment of eggs by simply placing them on agarose that had been mixed with the 0.01% ivermectin suspension resulted in a complete reduction in the ability of the eggs to
A small number of the eggs placed in contact with 0.5 ml of the ivermectin suspension did hatch, but the larvae coming from these eggs died instantly. In addition, the only hatching observed in this full-dose group occurred on the first day of culture. It appears that these larval mites may have already developed to the point where hatching was imminent as they were placed. After the eggs were in the presence of the liposomal formulation of ivermectin for 24 hours, none of the eggs cultured in the presence of the ivermectin suspension were capable of hatching. With the low-dose exposure (50 µl), there were eggs capable of hatching for a longer period, however, almost all of these larvae died as soon as they came out of the eggshells and came in contact with the medium containing ivermectin suspension. Originally, it was planned to make the visualization of the eggs under these conditions extremely difficult. Thus, it was decided that eggs would simply be placed on a suspension of the ivermectin in agarose. The agarose was used as a cushion and to provide moisture for the eggs during development. The test system actually seems a more natural assessment of the treatment efficacy.

### TABLE 1. Number of Mites Hatching Each Day Following Placement of Ten Eggs in Culture Plates with Different Levels of 0.01% Ivermectin Otic Suspension

<table>
<thead>
<tr>
<th>Trial &amp; Temperature</th>
<th>Treatment</th>
<th>Number of Plates</th>
<th>Newly Hatched Mites/10 Eggs per Plate</th>
<th>Total Hatched Mites</th>
<th>Total Surviving Mites</th>
<th>Efficacy*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
<td>Day 3</td>
<td>Day 4</td>
</tr>
<tr>
<td>Trial 1 28°C</td>
<td>Control</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Low dose</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Full dose</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Trial 2 28°C</td>
<td>Control</td>
<td>5</td>
<td>4†</td>
<td>1†</td>
<td>2†</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Low dose</td>
<td>5</td>
<td>1†</td>
<td>1†</td>
<td>2†</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Full dose</td>
<td>5</td>
<td>3†</td>
<td>0†</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Trial 3 37°C</td>
<td>Control</td>
<td>5</td>
<td>7</td>
<td>5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Low dose</td>
<td>5</td>
<td>3†</td>
<td>1†</td>
<td>0†</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Full dose</td>
<td>5</td>
<td>4†</td>
<td>0†</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Percent efficacy = \( \frac{\text{Total live mites in control plates} - \text{Total live mites in treated plates}}{\text{Total live mites in control plates}} \times 100 \)

†Mites hatched but died immediately next to the eggshells from which they hatched.

Control = agarose only in plate; low dose = 50 µl ivermectin otic suspension in agarose medium; full dose = 0.5 ml ivermectin otic suspension in agarose medium.
ment, because there is a good chance that the lotion will contact eggs by the permeation of the various waxy sheets of detritus to which the eggs are attached in the ears of infested cats.

It also seems reasonable to suspect that if eggs had come in direct contact with the ivermectin suspension in the test plates, the effects would have been even more marked in the current study. There was no attempt to stage the development of the eggs prior to setting up the test plates, although this could be done if individual eggs were examined using an inverted microscope. The agarose in the plates is very clear, and it would be possible to select and grade the eggs as to developmental state prior to sorting for testing. The sorting and individual selection of eggs could have identified eggs for which hatching was imminent. As well, the sorting of eggs would probably increase the percentage of mites hatching at various times in control plates after test initiation.

Many practitioners have used off-label ivermectin to treat ear mites in cats by direct application to the ear canal. However, very little information has been published on the actual efficacy of the treatment. In one report, cardrops with 1 mg or 2 mg of ivermectin eliminated mites from four of five cats receiving one to three treatments, and 5 mg of ivermectin eliminated mites from all ten cats given one to three treatments. In another report, mites were cleared from a research colony of cats and kittens by treating all animals with ivermectin applied directly into the ear canal three times, 8 to 10 days apart. The product used in this study, a 0.01% ivermectin otic suspension, has a reported efficacy of 94% in treating adult ear mite infestations after a single treatment with 0.5 ml (50 µg ivermectin) of the product applied within the ear canal. This treatment applies much less active ingredient than what was used in the published reports of off-label use of other formulations of ivermectin, and the work presented here shows that the treatment would also prevent the hatching of eggs of the mites within the ear canals, or at least kill the mites immediately after hatching. It seems reasonable to suggest that the ear canal be massaged well after administration so the formulation reaches as much of the surface of the ear canal as possible, thus increasing the opportunity for the eggs to come into contact with the formulation, but this hypothesis has not been tested.

The number of plates established in each trial was determined based on the availability of mite eggs in the different clinical samples. All the mites used in Trial 1 came from a single clinical source, and the mites used in Trials 2 and 3 were from one laboratory source. Before the study was initiated, it was unclear what would be the optimal temperature for incubation of the mite cultures. Preliminary trials conducted before initiation of the pivotal study reported here included evaluations of several temperatures, including room temperature, 28°C, 37°C, and 41°C. Although mites did tend to hatch more reliably at 37°C, this temperature tended to be associated with fungal overgrowth of the cultures associated with the detritus; however, this was markedly reduced later by the cleaning methods wherein the eggs were rolled about on the agarose surface. There was a concern that the 150 eggs collected for Trial 2 might not complete the trial because of fungal overgrowth; thus, Trial 2 was performed at 28°C. The availability of a large number of eggs allowed another trial to be performed at 37°C. In earlier work on the biology of the mite, eggs were transferred to vials containing sterilized epidermic debris and hair from the ears of positive animals and maintained at 35°C with 80% relative humidity. The eggs in this earlier work were noted to hatch within 4 days, but no information was presented on the percentage of eggs that successfully completed the
development and hatching process. Further work would be required to develop a system to monitor the development of the eggs and the percentage produced by a female that would hatch under in vitro conditions.

When exposed to the full dose (0.5 ml) of the ivermectin otic suspension, larvae hatched only within the first day of culture, and those few that did hatch died immediately.

REFERENCES