GOAL:
The primary project goal is to assess the efficacy of two treatment regimens of a water-based product containing fenbendazole (FBZ) in eliminating pinworm infections in mice. A secondary goal of the project is to compare diagnostic detection methods for parasite diagnoses.

PROJECT DESCRIPTION:
MediDrop FBZ is formulated to be a stable solution that contains uniform concentrations of fenbendazole (150 ppm). As mice drink their daily requirement of water, they will receive therapeutic doses of FBZ. MediDrop FBZ was provided free choice via water bottles for either 4 or 8 weeks to mice naturally-infected with pinworms (*Syphacia obvelata* and *Aspiculuris tetraptera*). MediDrop FBZ was added at a rate of 250 mL per sterile water bottle. Bottles were replaced weekly. Each treatment group consists of 5 cages with three 6-7 wk-old female mice per cage. The experimental design of the project is described in Table 1.

Table 1: Summary of Experimental Design

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th># cages</th>
<th>Collection Time Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBZ8:– 8 wk continuous tx</td>
<td>5</td>
<td>Day 0, 14, 28, 56, 84, 112, 140, 168</td>
</tr>
<tr>
<td>FBZ4:– 4 wks continuous tx</td>
<td>5</td>
<td>Day 0, 14, 28, 56, 84, 112, 140, 168</td>
</tr>
<tr>
<td>Control - untreated</td>
<td>5</td>
<td>Day 0, 14, 28, 56, 84, 112, 140, 168</td>
</tr>
</tbody>
</table>

RESULTS:

Pre-treatment Confirmation of Mite and Pinworm Infestation:
Samples collected from mice prior to the onset of treatment revealed that all mice had pinworms. Tape test and fecal float results were variably positive for pinworm ova, with 20-80% of the mice in each group shedding pinworm eggs. When fecal samples were tested by PCR, all were positive for pinworm DNA.

Figure 1. Pinworm Prevalence in Mice at Onset of Study

<table>
<thead>
<tr>
<th>Day</th>
<th>Traditional Test Results</th>
<th>PCR Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Syphacia</em></td>
<td><em>Aspiculuris</em></td>
</tr>
<tr>
<td>Day 0</td>
<td>1/5</td>
<td>2/5</td>
</tr>
<tr>
<td>8 wk tx</td>
<td>1/5</td>
<td>2/5</td>
</tr>
<tr>
<td>4 wk tx</td>
<td>1/5</td>
<td>4/5</td>
</tr>
<tr>
<td>no tx</td>
<td>4/5</td>
<td>4/5</td>
</tr>
</tbody>
</table>

Observations on Product Delivery via Water Bottle:
For the first 2 weeks of the study, mice were monitored twice daily for physical appearance, body condition and hydration to be assured they were drinking the medicated water. MediDrop FBZ volumes in water bottles were spot-checked daily during this time frame to assure delivery through the drilled hole in the bottle’s shoulder. All mice remained hydrated and exhibited normal behavior and grew while on medicated water.

Pinworm Results: Traditional Tests (perianal tape test and fecal float)
Study mice have both *Aspiculuris* and *Syphacia* pinworm infections, so a combination of traditional diagnostic test methods was used to detect ova. *Syphacia* gravid females migrate from the cecum to the rectum, exit the anus and release their eggs which cling to the perianal hairs. The perianal tape test is used to capture ova in this region and the tape is fixed to a glass slide and examined microscopically. A tapetest was performed on each mouse in the cage and
the results reported as a cage positive – that is, if one mouse or all mice were positive for Syphacia ova, the cage was considered *Syphacia* positive.

*Aspiculuris* female worms do not migrate, but rather release their eggs into the colonic contents, and the eggs pass out with the feces. A fecal flotation method is used for identification of the eggs. Pooled fecal samples from each cage were collected, hydrated and subjected to fecal flotation. The presence of ova was recorded as a positive result for that cage.

For both pinworms, the peak shedding of ova occurs in juveniles to young adults. Even with peak infection, the release of ova can be intermittent based on the reproductive biology of the pinworm. A resistance to pinworm re-infection occurs as mice age, so the pinworm burden in the gut declines, further limiting ova shedding. Taken together, the intermittent release of ova may result in false negative test results.

The results of the exams for pinworm ova as detected by traditional tests are illustrated in Figure 1. The Y axis is the number of cages tested, and the X axis is the time course of the study in weeks. The variability in detection of pinworm ova is evidenced by data at the onset of the study, in which all cages of mice assigned to the control group were pinworm positive and most, but not all cages, in the treatment groups were positive. As mentioned earlier, all cages were determined to be positive for pinworm by PCR testing.

After the onset of treatment, detection of pinworm ova in feces declined in a similar manner in both treatment groups. At the four week time point, all samples evaluated microscopically were negative for pinworm ova. No ova were detected at the remaining time points in the study from treated mice. From traditional test data, the results from 4 weeks of MediDrop FBZ treatment were equal to those from mice receiving 8 weeks of treatment. Control mice shed ova intermittently throughout the study, with two to four positive cages at every time point past day 0.

**PCR Evaluation of Fecal Samples**

Real-time PCR assays were used to detect DNA from the pinworms *Syphacia* and *Aspiculuris*. The initial PCR screen of feces is a generic “pinworm” assay that amplifies DNA from both *Syphacia* and *Aspiculuris*. Individual *Syphacia* and *Aspiculuris* PCR assays are also performed for pinworm identification. All results are reported from the pinworm PCR assay.

At the onset of the study, all cages of mice were positive for pinworms (see Figure 2). All cages in the control group were positive by PCR for pinworms until week 12. From this time point til week 24, four of five cages tested positive
for pinworms. The one cage that tested negative by PCR from 12 to 24 weeks was also consistently negative by traditional tests.

A decline in amplification of pinworm DNA occurred at 2 weeks of treatment in both treatment groups. By week 4, all treated cages were negative for pinworms and remained negative until the end of the study. Importantly, mice treated for 4 weeks on MediDrop FBZ remained negative for pinworms until study end.

**Pinworm Exam at Study End**

Mice were removed from the study were euthanized and cecum and colonic intestinal tracts were examined for pinworms. All treated mice were negative for pinworms. In the control group, the one cage of mice that tested negative for pinworms in mid-study was also negative for worms by the direct exam method. The other 4 cages of mice were pinworm positive and the adult burden was low.

**Study Conclusions**

MediDrop FBZ was safe for use in water delivery. No treated mice displayed avoidance of water and no adverse side effects were observed.

MediDrop FBZ was convenient to use. The ready-to-use formulation added to the convenience. The recommendation was to replace water bottles weekly, which matched the bottle replacement frequency in our standard husbandry practices.

MediDrop FBZ eradicated pinworms in mice treated for 8 weeks, and also in mice treated for 4 weeks. The shorter effective treatment period will offer great savings in cost of product, labor and time that treated mice are in isolation/quarantine.

PCR tests outperformed traditional tests in identifying pinworm infections in mice. Intermittent shedding of pinworm ova renders traditional tests less sensitive in detecting infected mice.