Fenbendazole resistance in Heterakis gallinarum, the vector of Histomonas meleagridis, on a broiler breeder farm in South Carolina

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Abstract

Background: Due to their ubiquity, management of parasites is a common and important factor for profitable production of poultry. *Heterakis gallinarum*, a cecal nematode, is the most common nematode parasite of poultry. While typically causing no direct pathology, *H. gallinarum* is the vector of *Histomonas meleagridis*, a protozoan parasite that causes blackhead disease in poultry. *Histomonas meleagridis* is highly pathogenic in turkeys, often leading to high mortality within flocks. In contrast, disease caused by *H. meleagridis* is much less severe in chickens, where it primarily reduces productivity without manifestations of clinical disease. There are no approved treatments for *H. meleagridis*, making control reliant on control of the helminth vector. In the United State, the benzimidazole anthelmintic fenbendazole (FBZ) is the only drug labeled for treatment of *H. gallinarum*, whereas flubendazole is approved in several other countries. We were contacted by an industry veterinarian regarding concerns in a broiler-breeder house due to histomoniasis, despite frequent anthelmintic treatments. Since we had recently diagnosed resistance to FBZ in *Ascaridia dissimilis*, a closely related nematode of turkeys, we were interested to determine if *H. gallinarum* had also evolved resistance to FBZ.

Methods: *Heterakis gallinarum* eggs were isolated from litter collected from the breeder house and used to infect 108 Cobb 500 chicks. Treatment groups included a non-treated control, a label-dose, and a 2X-label dose of FBZ, with 36 birds per group divided into two replicate pens of 18 birds each. Birds were placed at 1-day post hatch, and at 3 weeks of age were infected with 150 embryonated eggs via oral gavage. Two weeks post infection treated birds were administered a minimum of either a label- or 2X label-dose of FBZ in water for 5 days (SafeGuard® Aquasol, 1mg/kg BW). To increase the likelihood that all birds consumed the full intended dose at a minimum, the dosage was calculated using 1.25 times the average body weight. One-week post treatment, birds were euthanized, ceca removed, and parasites enumerated. Efficacy was calculated by comparing the total numbers of worms recovered from each treatment group to the numbers recovered in the non-treated control group.

Results: There were no significant differences in worm numbers recovered from any of the three groups (p-value=0.3426), indicating that both dosage levels of FBZ failed to provide expected levels of efficacy.

Conclusions: These data provide strong evidence that *H. gallinarum* has developed resistance to FBZ on this farm. Consequently, on this farm and any others with FBZ-resistant *H. gallinarum, H. meleagridis* will be able to cycle through the birds in an unrestricted manner. Further investigation is needed to determine the prevalence of resistance in *H. gallinarum on* chicken farms, but it is clear this has the potential to have a large-scale economic impact on the poultry industry. These data when viewed together with our recent findings of FBZ resistance in *A. dissimilis*, suggest that drug resistance in ascarid nematodes may be an important emerging problem on poultry operations. Additionally, drug-resistant poultry ascarids can serve as an important resource for studying drug resistance in the important ascarid of humans, *Ascaris lumbricoides*.

1. Background
The near ubiquity of parasites on poultry farms makes the management of parasites a common and important factor effecting profitable production of poultry. A study of birds from 10 different production companies in the southeastern United States reported that 98.6% of birds were infected by parasitic helminths, with 96% being infected with the cecal worm, *Heterakis gallinarum* (1). *Heterakis gallinarum* belongs to the family Ascarididae, which also contains the closely related *Ascaridia galli* and *Ascaridia dissimilis*, important small intestinal nematodes of chickens and turkeys, respectively. Helminth eggs from this family are resistant to environmental pressures such as temperature, dehydration, and pH extremes, as well as chemical disinfectants, causing a cycle of continuous infection and transmission within the house environment (2, 3). *Heterakis gallinarum* is a small nematode that rarely causes significant levels of direct pathology, but it serves as the vector for *Histomonas meleagridis*, a highly pathogenic protozoan parasite that is the causative agent of Blackhead disease in poultry.

*Histomonas meleagridis* is currently tied for the highest research priority in broilers of any parasite of poultry (4). *Histomonas meleagridis* is carried within the eggs of *H. gallinarum*, and histomonads are released into the gut when the larvae hatch from the nematode egg in the intestine. *Histomonas meleagridis* causes the disease histomoniasis, which is characterized by necrosis of the mucosal tissues in the ceca and liver, and may cause dark discoloration of the head, hence the name Blackhead. Historically, infections in turkeys often produced high levels of mortality, whereas in chickens, infections were largely asymptomatic. Recently, this view has shifted, as studies show that both chickens and turkeys may demonstrate clinical signs such as apathy, depression, and ruffled feathers, with decreased feed and water uptake often being the only signs of infection in chickens (5). *Histomonas meleagridis* is now thought to be a reemerging problem impacting chickens in multiple different production systems, causing reduced feed conversion in broilers, and decreased egg quality and production in layers and breeders (6–9). Despite these effects on health and production, there are currently no drugs approved by the United States Food and Drug Administration for the treatment of histomoniasis, making control of this disease dependent on control of the *H. gallinarum* vector.

Currently, fenbendazole (FBZ) is the only anthelmintic approved for use against ascarids of poultry in the United States. Fenbendazole belongs to the benzimidazole class of anthelmintics, a drug class used widely across multiple livestock species. In registration studies for SafeGuard® Aquasol®, a formulation of FBZ that is suspended in water for delivery, average efficacy against adults of *H. gallinarum* was 96.2%, similar to that of *Ascaridia galli*, at 97.6% (10). Additionally, in registration trials for the EU, this same formulation was shown to be 97.2% effective at eliminating L5 stage *H. gallinarum* (11). In registration studies for the feed additive formulation of SafeGuard®, average efficacy against *H. gallinarum* was 97.85% in growing turkeys (12). Both formulations are delivered from a central ration, or medication tank, and then distributed throughout the house. These methods of administration, along with human error, may result in poor delivery of treatment, leading to underdosing. Underdosing is recognized as one of the major contributors to the development of anthelmintic resistance, (13, 14) and combined with the high frequency of treatment, as often as every four weeks, development of resistance is a major concern (15).
Resistance to benzimidazoles in many of the most economically important strongyloid nematodes of livestock is highly prevalent (16–18), however reports of resistance in ascarid nematodes is rare. Reports in the human ascarid, *Ascaris lumbricoides*, indicate reduced efficacy in certain populations, but no definitive cases of resistance currently exist (19). Reduced efficacy in ascarids of poultry was first reported in the turkey nematode *Ascaridia dissimilis*, leading to speculation that drug resistance may have developed (1). This suspicion was recently confirmed in a controlled efficacy study, where FBZ resistance was clearly demonstrated in *A. dissimilis* (20). This confirmation of resistance highlights the potential of ascarid nematodes of poultry to develop resistance to FBZ, and since birds treated with FBZ may be infected with both *A. dissimilis* and *H. gallinarum*, FBZ resistance in *H. gallinarum* may already exist.

Given the recent concern of increased infection and disease from *H. meleagridis* in breeder chickens and having recently demonstrated FBZ resistance in one ascarid species of poultry, we were interested in determining whether FBZ resistance had also developed in *H. gallinarum*. Through collaboration with an industry veterinarian, we identified a farm with suspected-resistant *H. gallinarum* and conducted a controlled efficacy trial to determine if the worms on that farm were in fact resistant to FBZ.

### 2. Methods

#### 2.1 Chickens

One hundred eighteen, Cobb 500, chicks were hatched and placed the following day in housing at the Poultry Science Farm at the University of Georgia. Nipple drinkers and hanging feeders were used to provide water and feed *ad libitum*. Birds were fed a diet of non-medicated Nutrena® NatureWise® Chick Starter Grower feed.

#### 2.2 Parasite Isolates

A potentially resistant isolate of *Heterakis gallinarum*, AmFa 1.0, was identified through collaboration with an industry veterinarian. Prior to May of 2017, the farm of origin for this parasite isolate treated birds with a variety of treatments including FBZ, but after May of 2017, exclusively treated six flocks at 10 days, 6 weeks, 10 weeks, and 20 weeks post-hatch with FBZ. In March 2020, a small pilot study was performed to investigate a suspicion of FBZ resistance. Ceca from 10 individual birds were harvested both prior to, and 10 days after, treatment with FBZ. Parasites were recovered and enumerated at both time points, and efficacy of FBZ treatment was determined. Results suggested that the treatment was ineffective. Based on these preliminary data, litter was obtained from the suspect farm, and *H. gallinarum* eggs were isolated using previously established protocols (20) for use in a controlled efficacy study. Briefly, litter was washed through a series of sieves to remove debris, and then the remaining sediment was added to a saturated salt solution (sodium nitrate) with specific gravity of 1.15 and centrifuged at 433 x g for 7 mins. Eggs within the fluid phase were collected on a 32μM sieve, rinsed with deionized water, and stored in tissue culture flasks in deionized water containing 0.5% formalin at 10°C. Approximately four weeks prior to the controlled efficacy study, this parasite isolate was cycled through
several chickens to generate a large supply of fresh eggs. Eggs were then incubated in non-coated cell culture flasks at 30°C until they reached the fully developed infective stage.

### 2.3 Infection & Treatment

Birds were divided into two replicates of 18 birds each for the following three treatments: non-treated control, label dosage of FBZ, and 2X label dosage of FBZ. An additional group of 18 birds were infected for infection monitoring and a smaller group of 10 birds remained uninfected as environmental controls to confirm that there was no additional infection occurring beyond the controlled infections performed in the current study. Birds were allowed to grow to three weeks of age before being infected with approximately 150 embryonated eggs via oral gavage. Mesh curtains were placed between pens to prevent cross-over of birds between treatment groups. Prior to treatment, two birds from an additional infection monitoring group were euthanized and parasites recovered to confirm successful infection.

Two weeks post-infection, birds in the treated groups were treated with either a label dose (1 mg/kg BW x 5 days), or 2X the label dose (2 mg/kg BW x 5 days), of SafeGuard® Aquasol®, a FBZ formulation designed for delivery in water. To increase the likelihood that every bird received the target dosage at a minimum, the average bird weight of each group on the day before treatment plus 25% was used to calculate the dose administered. The total dosage of FBZ was mixed into 90% of the volume of water estimated to be consumed, as per production guidelines. For delivery of the FBZ, water lines were connected to carboys with both replicates of each treated group receiving water from the same carboy. As per label directions, treatment was administered over the course of five days, resuspending the drug daily.

### 2.4 Worm Recovery

Seven days after the last day of treatment, all birds were humanely euthanized using CO₂ followed by cervical dislocation, and then necropsied for worm recovery. Ceca were removed, opened, and submerged in physiological saline. Samples were incubated overnight at 37°C to aid in the recovery of tissue associated nematodes. Cecal contents were then washed over a 50μM mesh sieve to remove small debris, and cecal cores if present were manually disrupted. Cecal contents were then examined under a dissecting microscope, and all nematodes were recovered and enumerated.

### 2.5 Statistical Analysis

#### Analysis of Initial Pilot Data

Statistical analysis of data from birds in a pilot study of 20 birds was performed using count regression models (21). Based on the log-likelihood values and exploratory analysis (Supplemental File 1), zero-inflated negative binomial distribution was used to analyze the data. Using the chosen distribution, the following model was fitted to the data

\[
\log(\text{population mean for a treatment group } i) = M_1 + B_i, \ i = 1, 2
\]

and

\[
\log(\frac{\text{probability of zero-inflation}}{1-\text{probability of zero inflation}}) = M_2 + G_i, \ i = 1, 2.
\]
In the above, $M_1$ represents the overall population mean and $M_2$ represents the overall population mean modeled for zero inflation, $B_i$ represents the mean for untreated and treated groups and $G_i$ represents the mean for untreated and treated groups adjusted for zero inflation, and $i = 1$ represents the untreated group and $i = 2$ represents the treated group. The following two hypotheses ($H$) were tested using the likelihood ratio procedure: $H1: B_1 = B_2$ and $H2: G_1 = G_2$. Hypothesis $H1$ is concerned with if there are statistical differences in the treatment effect while the hypothesis $H2$ is concerned with if there are statistical differences in the zero-inflation probability.

Finally, using the formula:
Efficacy = $1 - \frac{\text{post treatment mean}}{\text{pre-treatment mean}}$

efficacy will be estimated and appropriate confidence interval will be provided. All statistical comparisons were evaluated at 5% level of significance using the SAS software version 9.4 (Cary, North Carolina).

**Analysis of Controlled Efficacy Study Data**

Statistical analysis of data from birds in the controlled efficacy study was performed using count regression models. Based on the log-likelihood values and exploratory analysis (Supplemental File 1), zero-inflated negative binomial distribution was used to analyze the data. Using the chosen distribution, the following model was fitted to the data

$$
\log(\text{population mean for a treatment group } i/200) = M_1 + B_i, \ i = 0, 1, 2
$$

and

$$
\log(\text{probability of zero-inflation}/(1-\text{probability of zero inflation})) = M_2 + G_i, \ i = 0, 1, 2
$$

The above is identical to the modeling used in the pilot study, but the three treatment groups, noted by $i$, are as follows: $0 =$ control, $1 =$ label dosage, and $2 =$ 2X dosage. The following hypotheses ($H$) were tested using the likelihood ratio procedure: $H1: B_0 = B_1 = B_2$; $H2: B_0 = B_1$; $H3: B_0 = B_2$; $H4: B_1 = B_2$; $H5: G_1 = G_2 = G_3$. Hypothesis $H1$ is concerned with if there are statistical differences in the treatment effect. Hypotheses $H2$ and $H3$ evaluate if there are statistically significant differences in the mean worm reductions after treatment with the Label and 2X dosage compared to the untreated groups respectively. Hypothesis $H4$ compares the Label treatment and 2X treatment. Finally, the hypothesis $H5$ is concerned with statistical differences in the zero-inflation probability.

Finally, the relative risk of negative outcomes, with the appropriate confidence interval, was estimated using the formula:

$$RR_{ij} = \frac{\text{mean for birds receiving treatment } i}{\text{mean for birds receiving treatment } j} \ i,j = 0,1,2,$$

where $i$ and $j$ represent the three treatments: $0 =$ control group, $1 =$ label dosage, and $2 =$ 2X dosage. All statistical comparisons were evaluated at 5% level of significance using the SAS software version 9.4 (Cary, North Carolina).
3. Results

3.1 Results of Pilot Experiment

Based on the likelihood ratio test, there were statistically significant differences between the means of the untreated and treated groups (p-value = 0.0304). However, there were no statistically significant differences in the zero-inflation probability (p-value = 0.332). The least squares means are provided in Table 1. Efficacy of FBZ treatment was estimated to be 54.09% and the 95% confidence interval was determined to be (0.12, 0.76). Raw parasite count data is available in Supplemental File 2.

3.2 Results of Controlled Efficacy Study

Based on the likelihood ratio test, there were no statistically significant differences between the three groups (Hypothesis H1) (p-value = 0.3426) and there were no differences in the zero-inflation probability between the three groups (p-value = 0.5726). Turning to Hypothesis H2, H3 and H4, concerned with effects of treatment when compared to controls, there were no significant treatment effects (p-values: 0.1691, 0.2765, and 0.8016 respectively). Distribution of worm counts is shown in Fig. 1, summary worm count data is shown in Table 2, and raw data is available in Supplemental File 2. The least squares mean differences for treatment comparisons are given in the following Table 3, where the Difference in LSMeans represents the differences in the log scale. Table 4 reflects the same data on the original scale with 95% Confidence Intervals.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>LSmeans</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>2.40</td>
<td>0.20</td>
</tr>
<tr>
<td>Treated</td>
<td>1.62</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th># of birds with no worms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.69</td>
<td>18.64</td>
<td>15</td>
</tr>
<tr>
<td>Label Dose</td>
<td>6.86</td>
<td>9.60</td>
<td>13</td>
</tr>
<tr>
<td>2x Dose</td>
<td>6.58</td>
<td>11.42</td>
<td>16</td>
</tr>
</tbody>
</table>
Table 3
Differences in least square means (LSmeans) on the log scale for the controlled efficacy study.

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Difference LSmeans</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control VS Label</td>
<td>0.653</td>
<td>0.4749</td>
</tr>
<tr>
<td>Control VS 2X</td>
<td>0.5345</td>
<td>0.4912</td>
</tr>
<tr>
<td>Label VS 2X</td>
<td>0.1185</td>
<td>0.4719</td>
</tr>
</tbody>
</table>

Table 4
Ratios of least square means (LSmeans) on the original scale for the controlled efficacy study.

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Ratio. LSmeans</th>
<th>Standard Error</th>
<th>Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control VS Label</td>
<td>1.9213</td>
<td>0.4749</td>
<td>(0.7574, 4.8737)</td>
</tr>
<tr>
<td>Control VS 2X</td>
<td>1.7066</td>
<td>0.4912</td>
<td>(0.6517, 4.4692)</td>
</tr>
<tr>
<td>Label VS 2X</td>
<td>1.1259</td>
<td>0.4719</td>
<td>(0.4465, 2.8388)</td>
</tr>
</tbody>
</table>

4. Discussion

Evidence of benzimidazole resistance was first found in a small pilot study. Ten birds each were euthanized before and after treatment, and the ceca were recovered, and worm burdens enumerated. Finding a reduced efficacy, we screened this isolate in a controlled efficacy study. We confirm here, for the first time, resistance to FBZ in an isolate of the poultry nematode, *Heterakis gallinarum*. There were no significant differences between the untreated control and groups receiving either the label or a 2X dosage of fenbendazole (p-value = 0.3426). Observed differences between groups are likely an effect of random variation between birds and groups, as the worm counts were highly over-dispersed with many zeros in all three groups (Table 2). No parasites were recovered from environmental sentinel birds, confirming the apparent lack of efficacy as a failure of treatment, as opposed to potential environmental contamination and reinfection with parasites.

In a previous passage of this isolate, cecal cores, associated with histomoniasis, were seen in infected birds, and we observed that few or no worms were recovered from birds with cecal cores. This observation is consistent with results of a study that demonstrated a negative interaction between *H. meleagridis* and *H. gallinarum* within the ceca (22). Given this issue, we felt that it would be optimal to treat the birds early in the course of infection to limit confounding effects on worm numbers due to formation of cecal cores. Thus, treatment was administered on day 14 post-infection, a timeframe where the worms would be L5-stage (immature adults). High efficacy against L5-stage parasites was previously documented in registration trials for Panacur® Aquasol®, the label name for SafeGuard® Aquasol® in the European Union. Average efficacy, reported from registration trials, against L5 *H. gallinarum* was 97.2% (11). As efficacy against L5s was validated in European registration trials, the decision was made to treat at this earlier stage to limit negative effects associated with *H. meleagridis*. Lund *et al.* initially
documented completion of the final molt to the L5 stage at 14 days post infection (23), and thus Day 14 was used as the start date for treatment in order to limit effects on worm burdens due to histomonaiasis. Our efforts to limit effects of secondary infection were not entirely successful, as cecal cores due to histomoniasis were still seen in many infected birds. No parasites were recovered from birds with large cecal cores, whether in the non-treated control or treated groups, leading many birds to having no worms at necropsy. Nevertheless, we were able to clearly demonstrate a lack of efficacy of FBZ against this isolate of *H. gallinarum*. Treatment with FBZ at the label dose and 2X label dose groups failed to produce a significant reduction in worm burdens as compared to controls. Moreover, there was no significant improvement in efficacy of the 2X label dose as compared to the label dose.

Ideally, in this study we would have included a susceptible isolate to serve as a baseline for comparison, however we did not obtain such an isolate for the current study. While we lack this isolate for comparison, our results strongly indicate that this isolate is truly resistant. Our treatment protocol has previously been validated in our screenings of *A. dissimilis* as an effective means of accurately delivering treatment in order to discriminate for resistant and susceptible isolates (20), and thus inaccurate dosing is unlikely to play a role in our results. By dosing at 25% more than the average pen weight, we increased the likelihood that the label dosage group received the full dosage at a minimum. Furthermore, the 2X dosage ensured that this group received a dosage greatly exceeding the label dosage, and lack of efficacy at this dosage acts as confirmation of resistance. This, combined with the pilot data and our previous work with *A. dissimilis*, supports the notion that this isolate is truly resistant to fenbendazole.

The farm of origin for this isolate of *H. gallinarum* has a history of FBZ use, further highlighting the risks for resistance associated with having only one approved compound for use against helminths of poultry. As compared to *A. dissimilis, H. gallinarum*, by itself, poses less disease risk to its host. However, due to its role as a vector for *H. meleagridis*, FBZ resistance in *H. gallinarum* poses important health challenges in poultry operations. *Histomonas meleagridis* is one of the most concerning disease pathogens of poultry production today, due to its severe impact on animal productivity and welfare. Historically only a severe problem in turkeys, histomoniasis has re-emerged as a problem in chickens, causing significant impacts in production of both broilers and layers (6–9), including effects on feed conversion and egg quality. Since there are no approved drugs for the treatment and control of *H. meleagridis*, prevention of histomoniasis relies heavily on the control of *H. gallinarum* using anthelmintics. The long-term survival of histomonads in the environment, and from one flock to the next, is dependent on protection provided by the helminth egg (24). Consequently, failure to control FBZ-resistant *H. gallinarum* is likely to lead to a continuous cycle of infection and disease with *H. meleagridis*. This then presents a scenario of production loss and animal welfare concerns that cannot be readily prevented.

While the current study uses chickens as the host for *H. gallinarum*, these results are of great concern to all poultry, as *H. gallinarum* readily infects most gallinaceous birds. Resistance to treatment is of even greater concern in turkey production, where histomoniasis often causes fatal disease. Similar treatment schedules exist in many of the major poultry production systems and underscores the risk of anthelmintic resistance developing in parasites infecting birds from any production system undergoing intensive
anthelmintic treatment. Due to evidence seen in other production systems, it is possible that anthelmintic resistance in the poultry industry is already common. If the prevalence of resistance is as high as we believe it could be, it is possible that anthelmintic resistance is playing a large role in the recent resurgence of *H. meleagridis* as a concern in chickens (6–9). To this end, we are investigating the genetic mechanisms of FBZ resistance in poultry ascarids which will facilitate the development of a diagnostic test, which would facilitate the measurement of resistance prevalence on a wide geographic scale. In addition to ongoing work for diagnostics, our data further supports the need for new alternative treatments for both for *H. gallinarum* and *H. meleagridis*.

Beyond the poultry industry, these resistant isolates of *A. dissimilis* and *H. gallinarum* are among the first cases of resistance to benzimidazoles in ascarids to be confirmed in controlled studies. Studying these resistant isolates becomes even more important when put in a One Health the context. Up to 1.2 billion people worldwide are infected with *Ascaris lumbricoides*, making it the most prevalent soil transmitted helminth in humans (25). Ascariasis causes hundreds of thousands Disability Adjusted Life Years (DALYs) each year (26), and control primarily relies on mass administration of benzimidazole anthelmintics. Resistance in these ascarids of poultry highlight the very real risk of resistance developing in ascarids of humans if it has not occurred already. We are currently working to further develop these resistant ascarids of poultry into a model system to elucidate the genetic mechanisms of benzimidazole resistance in ascarids, develop diagnostics, and screen new treatments. Current swine models are often cost prohibitive, and poultry offers many benefits in terms of cost and ease of handling. This model offers an ideal system for studying resistance and screening new compounds for use against ascarids.

5. Conclusions

In conclusion, we have identified resistance to FBZ in two different species of poultry nematodes in two successive trials. These findings highlight the possibility that FBZ resistance is much more common on poultry farms than is currently appreciated. Drug resistance in poultry ascarids may have important impacts both directly and, in the case of the *H. meleagridis* life cycle, indirectly on animal welfare and production loss. Given the ease with which we have found farms with drug-resistant ascarids, there is an important need to determine the scope and magnitude of this problem by investigating the prevalence of FBZ resistance in nematodes of poultry.

Declarations

Ethics approval and consent to participate

All birds were handled under protocols approved by the University of Georgia Institutional Animal Care and Use Committee (IACUC) under animal use policy A2019 01-005-Y2-A3

Consent for Publication

Not Applicable
Availability of Data and Materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing Interest

The authors have no competing interest to declare.

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Author Contributions

JC performed day to day research, some data analysis, and drafting of manuscript. BJ facilitated all research using avian hosts. AV performed all modeling and statistical analysis of the models, as well as creating the stats report used in preparation of this manuscript. AB served as an industry collaborator that allowed procurement of the subject parasite isolate. RK acted as a supervisor to JC’s research and worked with JC to organize all aspects of this work. All authors have reviewed this submission prior to submitting.

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References


2. Cauthen GE. Some studies on the viability and development of the ova of Ascaridia lineata (Schneiler). 1931.


**Figures**

![Plot of worm counts from each bird in each treatment group of the controlled efficacy study, shown with mean (mid-line) and standard deviation presented as error bars.](image-url)

**Figure 1**

Plot of worm counts from each bird in each treatment group of the controlled efficacy study, shown with mean (mid-line) and standard deviation presented as error bars.
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- 20220321Supplemental1StatsReport.docx
- 20220321Supplemental2RawData.xlsx