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## Studies on the Prevalence and Control of Parasitic Helminths in "Natural" Laying Hens

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**Studies on the Prevalence and Control of Parasitic Helminths in  
"Natural" Laying Hens**

An Honors Thesis submitted in partial fulfillment of the requirements of Honors  
Studies in Animal Sciences

By

Brittany Weir

Spring 2016

Bumpers College of Agricultural, Food, and Life Sciences

**The University of Arkansas**

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## Abstract

One societal trend that has been gaining much traction and popularity since the 21<sup>st</sup> century began is “organic” and/or “natural” food products. In 1999, the global market accounted for \$15.2 billion dollars worth of organic food and drink, compared to the market in 2014 where we consumed \$80 billion dollars worth (Willer et. al, 2016). With “natural” production of food animals however, “natural” parasite transmission may be a consequence. To that end, this experiment examines the prevalence of helminths in 110 “natural” laying hens from three regional farms and the efficacies of fenbendazole, piperazine, and levamisole on what should be naive helminths. The 3 regional farms were: Vital Farms in Evansville, Arkansas; Vital Farms in Westville, Oklahoma; and Arkansas Egg Company in Summers, Arkansas. Birds from each location were administered fenbendazole, levamisole, or piperazine and one additional group served as control. After 1 week the hens were sacrificed and processed for helminth qualification and quantification. The helminths that we collected and identified from the intestinal tracts were *Ascaridia galli*, *Heterakis gallinarum*, and *Raillietina cesticillus*. The results show that there were far more helminths in the control group than the other treatment groups. The results also show that there were far more *H. gallinarum* than *A. galli* in the intestines of these chickens. Overall these anthelmintics used could be successful in controlling “naïve” *A. galli* but could not be nearly successful in controlling “naïve” *H. gallinarum* in these “natural” laying hens.

## Introduction

One societal trend that has been gaining much traction and popularity since the 21<sup>st</sup> century began is “organic” and/or “natural” products. In 1999, the global market consumed \$15.2 billion dollars worth of organic food and drink, compared to the market in 2014 where we consumed \$80 billion dollars worth (Willer et. al, 2016). According to the U.S. Government Publishing Office under 7 C.F.R. § 205.105, the term “organic” is in reference to not being handled or produced with “synthetic substances and ingredients”, “nonsynthetic substances”, “nonorganic agricultural substances”, and “vaccines” (Allowed and prohibited substances, methods, and ingredients in organic production and handling, 2016). When using these guidelines to raise “organic” chickens the producer must not use any pesticides or herbicides on pastures and their food must be made from “organic” materials. They must also not use any vaccinations or anthelmintics on the chickens at any point. Finally when they are processed to sell they must be processed with “organic” materials and not have anything added to their meat. USDA’s definition of “natural” is as follows: “the product does not contain any artificial flavor or flavoring, coloring ingredient, or chemical preservative; synthetic ingredient; and the product and its ingredients are not more than minimally processed” (Food Safety and Inspection Service, 2005). When referencing this to “natural” chickens it means that the producer cannot use any chemicals or synthetic ingredients on/in the chicken, which includes the food and vaccines.

The poultry industry can be divided into different stages: primary breeders, hatchery, pullet farm, broiler farm, laying hen farm, processing/further-processing, and distribution. Primary breeders are the flocks that are responsible of developing and reproducing specific strain that a producer would like in their flocks. The hatchery is a facility where all of the eggs used to produce chicks are brought. This facility houses incubators that insure correct temperature and

humidity levels for the embryos to develop into a chick inside the egg (Hamre, 2013). Pullet farms are where the newly born chicks are held to grow into mature broilers or laying hens. Once the chicks become mature broilers they are transported to another farm where they are fed specific food to allow them to gain sufficient weight. Depending on the breed it could take 8 to 12 weeks for broilers to become mature enough for meat production (Jacob, 2015). Once mature they are taken to a slaughter facility and then processed for meat consumption. If the pullet chicks are raised to become laying hens, once mature they are transported to another farm to start laying eggs. When on the farm they will be exposed to 16 hours of light everyday which helps synchronize the egg production times (Patterson et. al, 2012). Workers collect the eggs which are then washed in water 10 degrees warmer than the egg (Clauer, 2009). This allows the egg contents to swell pushing the dirt and bacteria away from the pores. The eggs are then put in containers and stored at 50-55 degrees Fahrenheit (Clauer, 2009). Both broilers and laying hens can be in caged, non-caged, or free-range environments. The final step is processing of the meats or eggs to be sold as final products or for further processing.

This study will determine the presence of helminths in natural chickens. Their life cycles are described below. All of these helminths cause anemia, enteritis, and other physiological problems in the digestive tract of chickens.

-*Capillaria obsignata* has a direct lifecycle. The eggs of the adult nematodes are expelled from body along with feces and “develop into first larval stage in 9 to 14 days” inside the eggs until ingestion (Permin et. al, 1998). Once ingested the eggs hatch in the small intestine and develop into adult worms. These nematodes have a prepatency time of “approximately 3 weeks” (Permin et. al, 1998). Prepatency

time is referring to the amount of time between the host getting infected with the parasite to detection of the parasite, such as oocytes or eggs in the blood or feces.

-*Ascaridia galli* undergo 4 molts in their life time after the eggs are excreted with the feces from the chicken and eaten by another. 2<sup>nd</sup> stage larvae develop in the egg before it is eaten by the bird, then when ingested it will hatch in the proventriculus, gizzard, or small intestine (Permin et. al, 1998). From this stage the larva will gravitate towards the mucosa and molt to the 3<sup>rd</sup> stage after 7-17 days post infection (Permin et. al, 1998). 3<sup>rd</sup> stage *A. galli* will reside in the mucosal epithelium while the rest will stay in the mucus. All of them will then molt into 4<sup>th</sup> stage larvae in the mucus and 10 days later will molt again into adults (Permin et. al, 1998). *A. galli* has a prepatency time of at least 30 days.

-The life cycle of *Heterakis gallinarum* starts when the eggs are shed in the feces from an infected bird. They develop into 2<sup>nd</sup> stage larva in 3 weeks and hatch inside the intestinal tract of a chicken when the egg is ingested. *H. gallinarum* larva will migrate to the ceca where they will develop into 3<sup>rd</sup>, 4<sup>th</sup>, and adult stages of life. They have a prepatency of 24- 30 days (Permin et. al, 1998).

-*Raillientina cesticillus* cestodes use an intermediate host, specifically “darkling beetles”, in the maturation of their infective stages (Permin et. al, 1998). These beetles eat the eggs dropped in the feces of chickens and the larvae hatch in the intestines. Chickens eat the beetles and the tapeworms will migrate to the mucosa in the small intestine where they will attach to the walls by their scolices and mature. (Permin et. al, 1998). The prepatency time of tapeworms is 2 to 3 weeks.



In this research experiment we used 3 different anthelmintics: Fenbendazole, Levamisole, and Piperazine. Fenbendazole, trade name SafeGuard®, is the only ingredient in the Benzimidazole group that is approved to be used in organic livestock (USDA, AMS, Agricultural Analytics Division for the USDA National Organic Program, 2015). Benzimidazoles have been found to selectively inhibit the formation of microtubules in nematodes (Martin, 1997). Microtubules have many functions in the cell such as structure and movement of items in the cell so if they cannot be formed cells will die. Levamisole, trade name Prohibit®, and Piperazine, trade name Wazine®, are not approved for use in organic livestock production. Levamisole is an agonist at “nicotinic acetylcholine receptors of nematode muscle,” whereas Piperazine is an agonist of GABA receptors. Both lead to a state of paralysis (Martin, 1997). Paralysis will eventually lead to the death of the nematodes since they are not able to acquire the nutrients they need. All of these anthelmintics are orally given for one dose with a volumetric rate of 0.6 ml/kg.

Research studies involving chickens and the use of anthelmintics are performed to look at the presence and enumeration of helminths. In one study, 10 chickens were left unmedicated and 7 other chickens (all 77 to 79 weeks old) were treated with Fenbendazole (Yazwinski et. al, 2013). In this study it was found that fenbendazole was 85.5 and 89.5% effective for the removal of *Ascaridia galli* and *Heterakis gallinarum* populations, respectively. Another experiment was conducted to look at the types of nematodes in 19 free-range egg-laying flocks where 9 were organic flocks and 10 were non-organic (Sherwin et. al, 2013). They collected fecal samples and counted nematode eggs. They found that 89% of the 17 flocks had *Heterakis* eggs, 84% of 16 flocks had *Ascaridia*, 47% of 9 flocks had *Trichostrongylus*, and 32% of 6 flocks had *Syngamus* (Sherwin et. al, 2013). In Europe, a widespread study covered 8 countries looking at the

prevalence and magnitude of helminths in organic laying hens. Mr. Thapa, and colleagues, studied 892 hens from 55 flocks and found a “mean prevalence of 69.5%” for *A. galli*, mean prevalence of 29% for *Heterakis spp.*, and mean prevalence of 13.6% for *Raillietina spp* (Thapa et. al, 2015). Another study was conducted with the use of laying hens in Sweden. Mr. Jansson, and colleagues, found that ascarids were present in non-caged birds at a rate of 16.7 to 48.6% in 2004 compared to 28.6 to 77.1% in 2008 (Jansson et. al, 2010). The current study examines the prevalence of helminths in “natural” laying hens from three regional farms and the efficacies of fenbendazole, piperazine, and levamisole on what should be naive helminths.

## Methods

### A. Housing

On September 22<sup>nd</sup>, 2015 we obtained 36 random “natural” laying hens from Vital Farms in Evansville, Arkansas; 37 from Vital Farms in Westville, Oklahoma; and 37 from Arkansas Egg Company in Summers, Arkansas. All birds were “spent hens” obtained at “clean out”. “Spent hens” are those birds that have gotten old and their “egg production, shell quality, and internal egg quality” has declined (Webster et al, 2014). All of these birds were transported to the University of Arkansas Parasitology Farm and housed for 6 days in a 15 X 30 feet pen within a poultry house. Birds were provided food and water ad-libitum. All of the hens were marked with 3 specific colors on their back to identify farm origin; green, orange, and yellow from Summers, Arkansas; Westville, Oklahoma; and Evansville, Arkansas, respectively

### B. Treatment

All birds were acclimated for 6 days prior to treatment on September 28<sup>th</sup>, 2015. All birds were weighed on the day of treatment to determine dosage. Control chickens were banded with purple or grey leg bands and received no treatment. Birds treated with Fenbendazole (trade name Safeguard®) were banded with blue leg bands and dosed at 0.6 ml/kg body weight at an effective rate of 5 mg/kg. Birds treated with Piperazine (trade name Wazine®) were banded with black leg bands and dosed at 0.6 ml/kg body weight with an effective dosage rate of 100 mg/kg. Bird were treated with Levamisole (trade name Prohibit®) were banded with red or pink leg bands and dosed at 0.6 ml/kg body weight with an effective dosage rate of 12 mg/kg.

### C. Necropsy

All birds were euthanized via cervical ligation at 7 days post treatment. The intestinal tracts, from gizzard to vent, were removed. Tracts (and ceca) were straightened by removing mesentery tissues and opened lengthwise. For each bird, gut contents and gut were placed into a labeled container and refrigerated overnight. The intestinal tract was then removed by pulling through a clenched fist and discarded. Content plus soak slurry was sieved through a 120 mesh (125 micron aperture) sieve and the residue formalized until later examination.

### D. Counting helminths

Each container was sieved through a 120 mesh sieve to remove the formalin. The entire residue was examined in a black pan to remove and count any large adult ascarids. The total was recorded on a data sheet. The residue was then made up to 1000 ml by adding water and a 10% aliquot removed for helminth identification and quantification. Images of the helminths that were being identified are found in the Appendix section of this paper.

## Results

### Key

Farm group	Location	Farm Name
Yellow Farm	Evansville, Arkansas	Vital Farms
Orange Farm	Westville, Oklahoma	Vital Farms
Green Farm	Summers, Arkansas	Arkansas Egg Company

**Table 1**

<i>Arithmetic Mean and Standard Deviation of Yellow Farm Helminths Per Treatment Group</i>					
Drug	Total Number of Chickens	<i>Ascaridia galli</i>		<i>Heterakis gallinarum</i>	
		Mean	SD	Mean	SD
Fenbendazole	8	1.25	3.5	85	192.9
Piperazine	9	1.11	3.3	191.11	269.22
Levamisole	10	0.2	4.5	22	46.6
Control	9	6.89	28.1	81.11	70.6

**Table 2**

<i>Arithmetic Mean and Standard Deviation of Orange Farm Helminths Per Treatment Group</i>					
Drug	Total Number of Chickens	<i>Ascaridia galli</i>		<i>Heterakis gallinarum</i>	
		Mean	SD	Mean	SD
Fenbendazole	11	5.45	8.2	15.45	38.3
Piperazine	9	0	0	55.56	87.6
Levamisole	8	2.5	7.4	1.25	3.5
Control	9	12.22	52.7	233.33	268.6

**Table3**

<i>Arithmetic Mean and Standard Deviation of Green Farm Helminths Per Treatment Group</i>					
Drug	Total Number of Chickens	<i>Ascaridia galli</i>		<i>Heterakis gallinarum</i>	
		Mean	SD	Mean	SD
Fenbendazole	9	2.22	6.7	7.78	12.0
Piperazine	10	1	3.2	39	61.2
Levamisole	9	4.44	3.9	5.56	8.8
Control	9	31.11	33.0	292.22	394.1

**Table 4**

<i>Arithmetic Mean of the Nematodes over all Farms by Treatment Group</i>		
Drug	<i>Ascaridia galli</i>	<i>Heterakis gallinarum</i>
Fenbendazole	3.0	36.1
Piperazine	0.7	95.2
Levamisole	2.4	9.6
Control	16.7	606.7

## Results

In this research experiment we were studying the prevalence of helminths in the intestines of “natural” laying hens coming from three area farms that were put into four different treatment regimens. The 4 different anthelmintic regimens we used in this research experiment were: fenbendazole, piperazine, levamisole, and untreated control. Overall it was shown that *A. galli*, *H. gallinarum*, and *R. cesticillus* were the only helminths found in these “natural” laying hens, but no real inference on *R. cesticillus* efficacy could be made in this study.

Table 1 shows the means and standard deviation of each helminth found on the yellow farm for each specific anthelmintic regimen. This research has shown that there were very few *A. galli* in the intestines after the treatments, which is shown by the means ranging from 0.2 to 6.89. The standard deviations for *A. galli* were small ranging from 3.3 to 4.5, except for the Control group being 28.1. This means that the values were relatively close to the overall mean except for the control group. The *H. gallinarum* were substantially more prevalent in the intestines with the means ranging from 22 to 191.11. The standard deviations were substantially large as well with the largest being 269.22 in the piperazine treatment group. This means that the values were very spread out with much variety from the overall mean. We can also conclude that the levamisole treatment was the could be the best at controlling *A. galli* and *H. gallinarum* on the yellow farm since they had the lowest means of 0.2 and 22 respectively.

There is an interesting result found in Table 1 when comparing the means of *H. gallinarum* in the control and piperazine treatment regimens. The control group had a mean of 81.11 compared to piperazine having a 191.11 mean. I would have expected the control to have the highest means for all of the helminths on this farm since it was not treated with any anthelmintic. These results show that the birds with higher means could have been infected with

far more helminths than the others. It could also mean that the *H. gallinarum* found in the higher mean group could have more “resistant” genes. Finally these results could mean that the proper dose of anthelmintic was not given or the hen did not swallow all of it. So I believe that further testing would need to be done to see if similar results are found.

Table 2 shows the means and standard deviations of each helminth found on the orange farm for each specific anthelmintic regimen. We can conclude that piperazine was the most effective in controlling *A. galli*, while levamisole was most effective towards *H. gallinarum*. The standard deviations for the *A. galli* and *H. gallinarum* are shown to be large. This is an indication that there was wide variation between the chickens with how many helminths were in their intestines which shows the inconsistency of the anthelmintics. Overall the control group had the most helminths found in all of the groups which is consistent with my predictions since it was not treated with any anthelmintic.

Table 3 shows the means and standard deviations of each helminth found on the green farm for all of the anthelmintic regimens. Overall the means for *A. galli* were similar for fenbendazole, piperazine, and levamisole treatments with means of 2.22, 1, and 4.44, respectively. The standard deviations were more spread out with values of 6.7, 3.2, 3.9, and 33.0, respectively. This shows the inconsistency of the anthelmintics on treating the helminths. It can be seen that all of the treatment regimens were the least successful in controlling *H. gallinarum* since they had the largest means in each category. The means of all the helminths in the control group were substantially larger, which is consistent with what is expected since it was not treated with any anthelmintic.

Table 4 shows the means of all the nematodes from all farms by treatment group. *A. galli* records show very low averages with 3.0, 0.7, 2.4, and 16.7 for the fenbendazole, piperazine,

levamisole, and control groups, respectively. *H. gallinarum* records, on the other hand, show much larger averages with 36.3, 95.2, 9.6, and 606.7 for those treatment groups, respectively. So overall it can be surmised that there are far more *H. gallinarum* in these areas and that the anthelmintics could not be sufficient in controlling.



## **Discussion**

In this research experiment it can be seen that all of the birds from the three regional farms were still infected with some combination of *A. galli*, *H. gallinarum*, and *R. cesticillus* after undergoing the treatment regimens. Overall there were far more helminths in the *A. galli* and *H. gallinarum* control groups, with 16.7 and 606.7 as the means, compared to the means of fenbendazole, piperazine, and levamisole groups for both nematodes. This could correspond to diminished bird performance and health since these helminths actively attack and harm the intestines and/or ceca of the hens.

There are substantially far more *H. gallinarum* in the intestines of all the hens from these 3 regional farms after undergoing treatment regimens. From this it can be concluded that further research on other products is necessary to deduce if they are better in controlling *H. gallinarum* or if the 3 anthelmintics used in this experiment are the best. Overall there were far less *A. galli* in all 110 hens after treatment, which shows that all of these products would be satisfactory in controlling these nematodes.

Finally it is shown that fenbendazole, the only product able to be used in organic production, is not the most sufficient at controlling *A. galli* and is the second best at controlling *H. gallinarum* when comparing just these three anthelmintics.

## **Conclusions**

Given these current research results further research must be undergone. First further inquiring must be made to see if handling laying hens “naturally” is truly helping the animals or if it is hurting them just as much as other producing environments. This is needed because of the health affects that could be induced from having *A. galli*, *H. gallinarum*, and *R. cesticillus* in their intestines compared to being treated with an anthelmintic. Researchers can look at the incidence of disease in “natural” laying hens compared to treated hens in caged and non-caged environments. Further research on the anthelmintics used in this project must also be researched in “natural” laying hens not only in Arkansas but in other major “natural” laying hen producing states to see if similar results are obtained. This will help strengthen the results found and theories provided in this paper. Finally other anthelmintics should be researched on “natural” laying hens to see if they are better in controlling helminths than the anthelmintics used in this research study. Overall these anthelmintics used could be successful in controlling “naïve” *A. galli* but could not be nearly successful in controlling “naïve” *H. gallinarum* in these “natural” laying hens.

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## Appendix

Image 1: Life stages of *A. galli*

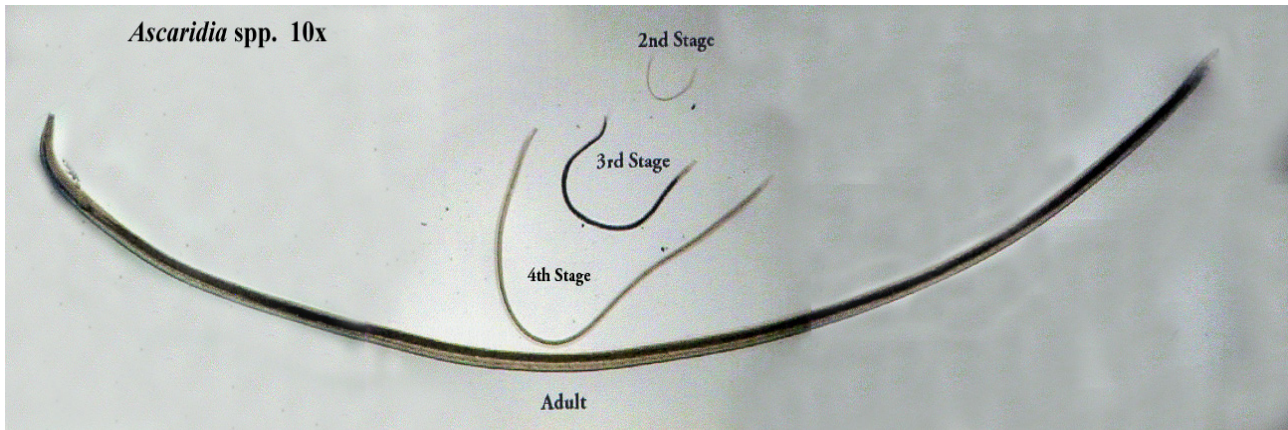


Image 2: Image showing a male (top) and female (bottom) *H. gallinarum*



Image 3: Image of Raillietina cesticillus with specific details.

