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Efficacies of fenbendazole and albendazole in the treatment of commercial turkeys artificially infected with *Ascaridia dissimilis*

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ABSTRACT

The goal of this research was to determine the extent of resistance that turkey roundworms, *Ascaridia dissimilis*, have developed to anti-parasitic chemicals used in commercial poultry operations. Roundworm infections in turkeys have resulted in monetary losses for the poultry industry for years, generally due to poor feed conversion. The infection itself is subclinical and many turkeys have a light to moderate worm burden. Since parasitisms are light, this leads to the infections being noticed only during processing. *Ascaridia dissimilis* infections consist of adult worms and developing larvae with the latter comprising most of the worm burden and causing the most damage. In this study, eggs were collected from *A. dissimilis* found in turkeys previously treated with various parasiticides and combinations thereof. These eggs were in turn used to instill artificial infections in turkeys on site. These artificially infected turkeys were then treated with fenbendazole or albendazole. A third group of birds was left untreated as a control group. Drug efficacies were determined based on parasite loads post treatment (at necropsy). The results of this study will improve current knowledge of chemical resistance associated with these drugs.

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MEET THE STUDENT-AUTHOR

Jessica Perkins

I grew up in Siloam Springs, Arkansas and graduated from Siloam Springs High School in 2006. After graduation, I moved to Pennsylvania to study liberal arts. Upon realizing my mistake, I moved back home and began studying Animal Science at the University of Arkansas in 2009. I am the treasurer for the Pre-Veterinary club on campus and I hope to attend veterinary school in the fall of 2013. Currently I reside in Fayetteville with my two simple dogs and very malevolent cat. In my free time I enjoy knitting, advocating women's rights, and looking at funny pictures of cats on the internet.

I would like to thank Tom Yazwinski for his support and patience throughout this project, as well as Chris Tucker for teaching me how to properly dissect a turkey gut. I would also like to thank Nick Anthony and Duane Wolf for their encouragement throughout my time in the Honors program.

INTRODUCTION

The economic losses due to infections by *Ascaridia dissimilis* can be significant. Poor feed conversion makes it difficult for turkeys to gain weight. This results in low market weight and in some extreme cases, it can result in damaged livers that cannot be sold, contributing to even lower profits (Willoughby et al., 1995).

Turkey producers have been using numerous chemical parasiticides for treating *A. dissimilis* throughout the years, all with varying success rates. Poor results with anthelmintic drugs are often related to chemical resistance. Resistance results from a product killing off a large percentage of the worm population, but leaving a very small percentage that may not be susceptible to that particular product. This resistant population continues to reproduce, creating a new population of worms that are not affected by the product.

Ascaridia dissimilis has a direct life cycle with no intermediate host. The eggs (Fig. 1) are laid by adults in the intestines and are shed into the environment through droppings. Larvae develop inside the eggs and become infective within a few weeks (Seaton et al., 2001).

The first larval stage is developed inside the egg after approximately one week. After another 20 days, the larva develops into second-stage larvae (Fig. 2), which is infective to the turkey (Seaton et al., 2001). After ingestion, the larvae emerge from the eggs in the anterior portion of the intestinal tract, and localize against the mucosa in the small intestine. After approximately two weeks, they molt to the third-stage with some larvae migrating into the mucosa and submucosa ("tissue phase") while the remainder of the larvae stay in the mucus. After another two to three weeks, the larvae develop into fourth-stage larvae which in turn develop into adults. With naturally infected birds, a residual population of third-stage larvae is maintained.

Damage to the mucosa can result in hemorrhagic enteritis. Nutrients are not digested well, contributing to poor feed conversion and low market weight (Willoughby et al., 1995). Occasionally, *A. dissimilis* larvae have been observed in the livers of turkeys, causing an immune response that results in 1-mm white spots on the surface of the liver (Aziz and Norton, 2004). With heavy infections, the migration of developing larvae in the mucosa can result in death and adults can block the intestinal lumen (Yazwinski, 1999).

The piperazine salt anthelmintics are only partially effective against adult worms and repeat treatment is required for any real benefit to the bird. These drugs have been overused throughout the years and studies have revealed that piperazine severely lacks efficacy (Yazwinski, 1999).

Fenbendazole is a benzimidazole anthelmintic used to treat birds for helminth infections. It is FDA approved for turkeys and has long been considered effective against both the larvae and the adult worms. Recently, poultry producers have begun to notice increased resistance to it (pers. comm.). No studies have been published as of yet regarding the current efficacy of fenbendazole in turkeys. This study will therefore provide timely information on the effectiveness of this product.

Albendazole is another benzimidazole anthelmintic used to remove gastrointestinal worms. It is a "prescription only" drug for poultry and has not been evaluated by the FDA for use in commercial turkeys to treat ascarid infections (Tucker et al., 2007). Albendazole and fenbendazole both work through inhibiting microtubule polymerization. This restricts nematode glucose uptake and the worms lack the energy to survive.

The current study was conducted so that a current evaluation of drug efficacies might be obtained. This information is essential for the control of turkey ascaridiasis, a common condition of turkeys that is of animal health and producer profit significance.

MATERIALS AND METHODS

Collection and Incubation of Ascaridia Dissimilis Eggs. Approximately one hundred intestinal tracts containing *A. dissimilis* were shipped overnight to the University of Arkansas from four separate turkey operations in the USA. Each turkey operation used a different chemical regimen for treating ascarid infections. The intestinal tracts were cut open lengthwise, the contents removed and sieved, and all mature female *A. dissimilis* were collected. Eggs were removed from the uteri of the female *A. dissimilis*, washed and incubated at 30 °C in water until reaching the infective larval stage.

Brooding and Infection of Turkeys. One hundred and fifty, one-day-old turkeys were obtained from Cargill in Gentry, Ark. and brought back to a University of Arkansas poultry growing facility. At 7 days of age, each bird was wing tagged with a unique identification number. Throughout brooding, the turkeys were moved into new pens to allow for adequate space.

At 14 days of age, and again at 28 days of age, birds were orally gavaged with source specific infective eggs of *A. dissimilis* (approximately 40 birds per source and 1000 eggs per dose). At 45 days of age, birds were treated according to random selection within each infection source group. Treatments equally applied per group were fenbendazole suspension (5 mg/kg BW), albendazole suspension (5 mg/kg BW) and control (no treatment) (Table 1). For each benzimidazole, a stock solution with a concentration of 5 mg/ml was made from commercially available products (10% fenbendazole suspension [Safeguard], 11.36% albendazole solution [Valbazen]). On the day of treatment, each bird was weighed and treated at the volumetric rate of 1 ml/kg BW, with the total dose given in halves with a 6-hr interval. Humane euthanasia by cervical dislocation occurred at 52 days of age. The intestinal tracts were removed from the duodenal/gizzard junction to the rectum as per World Association for the Advancement of Veterinary Parasitology (WAAVP) efficacy evaluation guidelines (Yazwinski et al., 2003) and sent to the parasitology lab at the University of Arkansas to be processed for nematode enumeration.

Quantification of Ascaridia dissimilis. Intestinal tracts from the study birds were cut open lengthwise and soaked overnight to optimize nematode collection. After soaking, the intestines were drawn through a tightly clenched fist to remove all contents. These contents were then washed over a number 200 sieve (aperture of 75 μm). All *A. dissimilis* recovered were identified and counted to developmental stage with stereoscopic microscopes at 4-30× magnification.

Statistics. Individual bird infections by *A. dissimilis* (adult and larval forms combined) were transformed to the $\log_{10}(X + 1)$ prior to analysis of variance to test for group mean separations at the 5% level of probability. This transformation is the norm for data sets where large variability is encountered among individual counts within populations. Data are presented as arithmetic means and geometric means, with the latter given with significant mean separations. The following standard equation was used to calculate efficacy percentages:

$$\% \text{ efficacy} = \frac{[\text{control group geometric mean} - \text{treatment group geometric mean}]}{\text{control group geometric mean}} \times 100$$

RESULTS AND DISCUSSION

Data were organized per infection source and treatment group with attention given to total larval ascarids, total adult ascarids, total (combined larval and adult stages) worm burdens, average worm burdens and overall efficacy of treatment based on geometric means for total worm burdens. In Table 2, the nematode burden arithmetic means by infection source and treatment group are presented for adult, larval and combined infections. Light infections were seen for all stages of *A. dissimilis*, regardless of source or infection group. Judging from the data in Table 2, it is apparent that good infections were obtained for all source infections and that considerable range in worm counts was seen for each source × treatment group population (standard deviations were usually equal to arithmetic means). Geometric means for total worm burdens as seen for the 12 experimental groups are presented in Table 3. Significant ($P < 0.05$) treatment effect was seen for infections sourced from producers A and B. Worm burdens from all 4 source infections were not significantly different dependent on benzimidazole evaluated, indicating that the two anthelmintics used in this study did not significantly differ in efficacies.

According to WAAVP standards, to be considered "effective", anthelmintic drugs must have a >90% efficacy (Yazwinski et al., 2003). As seen in Table 4, only albendazole treatment given for source B infections can be considered efficacious in the current study. In previous studies, fenbendazole was demonstrated to be 97% to 99% effective against all stages of *A. dissimilis* (Yazwinski, 1993 and 2009). In comparison, birds treated with fenbendazole in this study had dramatically lower efficacy percentages (source dependent efficacies of 26.8%, 54.9%, 80.9% and 83.6%). Albendazole efficacies displayed in the current study by infection source were 0, 5.1%, 78.3% and 92.3%.

The less than efficacious anthelmintic efficacies of fenbendazole and albendazole as seen in this study may indicate a rising resistance to benzimidazoles in *A. dissimilis* populations. Resistance in a pathogen population to chemicals used for control is a natural result of most intervention programs. Given the apparent reduction of benzimidazole efficacies in the treatment of *A. dissimilis* infections, altered or different treatments must be investigated so that this common disease condition of commercial turkeys might once again be effectively controlled.

ACKNOWLEDGEMENTS

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Fig. 1. *Ascaridia dissimilis* egg.

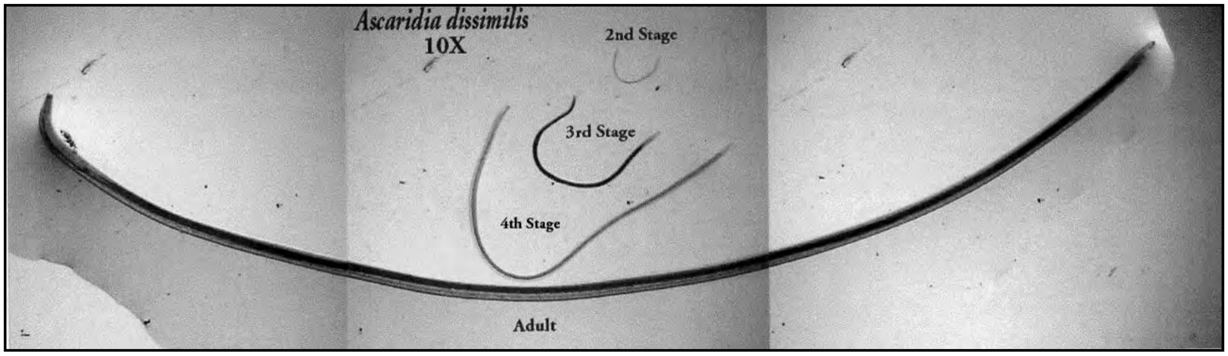

Fig. 2. Parasitic, life stages of *Ascaridia dissimilis*.

Table 1. Description of experimental groups used in this study.

Source*	Field treatment	Birds per group	Study treatment
A	Albendazole	11	Control
		13	Fenbendazole
		13	Albendazole
B	Fenbendazole	12	Control
		12	Fenbendazole
		13	Albendazole
C	Piperazine	12	Control
		12	Fenbendazole
		13	Albendazole
D	Piperazine and Albendazole	12	Control
		13	Fenbendazole
		13	Albendazole

*Letters denote the commercial turkey operation from which the source infections originated.

Table 2. Arithmetic means (SD) for adult and larval (L4 and L3) *Ascaridia dissimilis* populations by source of infection and treatment group.

Source*	Treatment Group	*A. dissimilis* as: Adult	Larvae	Total
A	Control	11.1 (12.6)	17.9 (16.5)	28.2 (22.4)
	Albendazole	2.8 (1.8)	2.3 (3.4)	5.1 (4.4)
	Fenbendazole	2.3 (2.4)	1.8 (2.2)	4.1 (2.9)
B	Control	11.4 (15.0)	15.8 (12.3)	27.3 (19.6)
	Albendazole	2.1 (3.1)	1.5 (2.0)	3.5 (4.8)
	Fenbendazole	2.3 (1.9)	3.5 (5.1)	5.8 (5.8)
C	Control	5.3 (3.6)	11.8 (13.3)	17.1 (12.5)
	Albendazole	20.2 (26.9)	5.2 (5.9)	25.5 (27.3)
	Fenbendazole	8.3 (10.8)	7.5 (12.7)	15.8 (16.1)
D	Control	6.3 (9.4)	1.5 (1.7)	7.8 (9.1)
	Albendazole	4.6 (5.1)	2.0 (2.6)	6.6 (5.1)
	Fenbendazole	2.6 (4.2)	1.6 (2.4)	4.2 (5.1)

*Letters denote the commercial turkey operation from which the source infections originated.

Table 3. Geometric means for *Ascaridia dissimilis* (adult and larval stages combined) by source of infection and treatment group.

	Treatment Group		
Source*	**Control**	**Albendazole**	**Fenbendazole**
A	17.06[a]	3.71[b]	3.26[b]
B	23.11[a]	1.74[a]	3.79[b]
C	13.82	17.45	10.14
D	5.10	4.84	2.30

[a,b] Means in the same row with different superscripts are different ($P \leq 0.05$).
*Letters denote the commercial turkey operation from which the source infections originated.

Table 4. Percent efficacies against *Ascaridia dissimilis* by source of infection and treatment (based on geometric means).

	% efficacy for	
Source*	**Albendazole**	**Fenbendazole**
A	78.3	80.9
B	92.3	83.6
C	0.0	26.8
D	5.1	54.9

* Letters denote the commercial turkey operation from which the source infections originated.