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Feasibility of Inducing Overlap Immunologic Competence in Gallinaceous Birds with Ascardia dissimilis and A. galli

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Feasibility of inducing overlap immunologic competence in gallinaceous birds with *Ascaridia dissimilis* and *A. galli*

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ABSTRACT

Chickens and turkeys are routinely infected with the roundworms *Ascaridia galli* and *A. dissimilis*, respectively. The current study was conducted to gather basic information on these worms and to determine whether heterologous infections (chicken worms in turkeys and turkey worms in chickens) would be successful. Chickens and turkeys were obtained at day of hatch, brooded to 7 days of age, and placed in pens (25/pen) according to infection as received at 7 days of age: homologous, heterologous and control (no infection). Bird weights, mortalities, and feed efficiencies were monitored for 3 weeks postinfection, at which time all birds were killed for parasite collection and counting. Feed efficiency, a parameter more adequately measured in large-scale studies, did not vary between experimental groups. Bird weights at necropsy varied significantly ($P < 0.05$) between groups only for the turkeys, with homologous infection (*A. dissimilis*) birds weighing less than controls. All induced, homologous, and heterologous infections were successful. Rates of establishment, however, were significantly ($P < 0.05$) depressed for each heterologous model. Total *A. dissimilis* numbers were only 55% as great as those for *A. galli* in chickens [geometric means (GMs) of 13.2 versus 24.2], and total *A. galli* numbers were only 56% as great as *A. dissimilis* numbers in turkeys (GMs of 5.6 versus 10.0). Given the fact that heterologous infections were successful, albeit reduced, in both types of birds (infections that included tissue-phase forms), additional studies are planned to determine whether these infections might induce interspecies (overlapping) immune competence in the host and aid in reducing natural parasitisms to levels with no economic impact.

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INTRODUCTION

Parasites of poultry are traditionally grouped as internal or external. Given current housing, management, and control practices, the external parasites have been all but eliminated in the United States. The internal parasites remain a problem around the world. These parasites are primarily found in the intestinal tract and are of two very diverse groups: the protozoan coccidia and the helminths (roundworms). Our study involved the latter.

Chickens and turkeys are a contrast in regard to the roundworms they naturally "carry" (Yazwinski *et al.*, 1996). Depending on production type and/or bird age, four different worms can be routinely found in the chicken, including *Ascaridia galli* Schrank. As for the turkey, only the ascarid (*A. dissimilis* Vigueras) can be found under normal, commercial conditions. One would think that in this country, with its highly developed technologies and capabilities, an ancient affliction such as "worms" would have been conquered years ago. The fact is, however, that worms are alive and well in our chickens and turkeys, and only now is a thoroughly researched and proven medication on the verge of being approved for routine commercial use in turkeys, i.e., fenbendazole (Yazwinski, 1999).

Chemical means of roundworm control are necessary if optimal production is to be achieved in poultry (Willoughby *et al.*, 1995). Alternative measures of control, in addition to basic biology governing these worms, should be researched as well. In the current study, chickens and turkeys were given roundworms naturally occurring in the other species so that the development of these unnatural parasitisms could be documented; this is an initial step in evaluating the feasibility of reducing roundworm disease in poultry by developing protective, immune responsiveness. In a recent article, the scientific foundation for this research was outlined (Meeusen and Balic, 2000). Parasites occur in "permissive" hosts and not in "nonpermissive" ones. Factors that determine the status of a host are for the most part found in the host's innate (naturally occurring) inflammation and immune responses. In our research, we challenged these innate responses and measured the effect.

MATERIALS AND METHODS

Chickens and turkeys (150 of each) were obtained on the day of hatch from two local (Springdale and Gentry, Ark., respectively), commercial hatcheries. Chickens were of Ross **x** Cobb breeding (Tyson Hatchery) and turkeys were Honeysuckle White (Cargill Hatchery). All birds were male.

Within the 3 months immediately preceding the study, intestinal tracts were obtained from area chicken and turkey processing plants. These tracts were opened, and gravid female ascarids were isolated for dissection, uteri maceration, and egg harvest. The eggs were allowed to embryonate at room temperature for 30 days in a shallow layer of water. At the end of the embryonation period, the eggs were counted in accordance with presence or absence of infective (L2) larvae. At the time of infection induction (birds at 7 days of age), the eggs were diluted so that a 1-ml dose would deliver 1000 larvated eggs to each bird. (Table 1 describes the schedule followed for the conduct of this study.)

To determine the success and degree of damage of natural (homologous) and unnatural (heterologous) infections, we used the design shown in Table 2.

All procedures used for parasite collection, isola-

Table 1. Schedule of infection induction.

Bird age (days)	Procedures
0	Birds obtained from hatcheries
0 – 7	Birds brooded
7	Birds, according to random selection, placed into pens at the rate of 25/pen; total pen weight of birds obtained as well as weight of starting feed
	Infections given to all birds in pen according to pen designation (see Table 2); record and weight of all mortalities and feed intakes started on this day and continued for the remainder of study
14	Bird pen weights and weekly feed consumption obtained
21	Bird pen weights and weekly feed consumption obtained
28	All birds euthanized, weighed, and necropsied for parasite retrieval
	Final feed consumptions obtained.

Table 2. Study design.

Pen No.	Bird	Infection given
1	Chicken	None (control)
2	Chicken	*A. dissimilis* (heterologous)
3	Chicken	*A. galli* (homologous)
4	Turkey	*A. dissimilis* (homologous)
5	Turkey	None (control)
6	Turkey	*A. galli* (heterologous)

tion, and counting have been published previously (Yazwinski *et al.*, 1986). Briefly, intact small intestines were removed from each euthanized bird, opened lengthwise, and the contents collected. The cleaned intestine was then soaked overnight for the release of worms in tissue-dwelling stages, with soak fluids collected the day after posting. For each bird's content and soak collection, all worms were isolated and counted according to stage of development.

All roundworm data were analyzed according to currently recognized appropriate procedures. All counts were transformed (logarithmic) to obtain "normal" distributions, and significance of difference between experimental groups was determined at the probability level of 5% by the repeated *t*-test.

Specimens were initially cleared in lactophenol for 24 hours. Illumination and magnification were provided by a Nikon Labophot compound microscope (Nikon, Garden City, N.Y.). Specimen images were then received through a JVC color video camera and digitized using a SNAPPY attachment (Play Incorporated) on line to a Gateway E 4200 – 700 MHz computer (Gateway, North Souix City, S.D.).

RESULTS AND DISCUSSION

Mortality rates by experimental group ranged from 16 to 24% for the chickens and 27 to 37% for the turkeys (Table 3). No correlation was observed between rate of mortality and infection status (data not shown). Average weekly feed efficiencies are also given in Table 3. For both the chickens and the turkeys, experimental groups with homologous infections had the poorest feed efficiencies. This was seen as a consequence of the natural parasitisms (homologous) being the most successful and, correspondingly, the most harmful to the host.

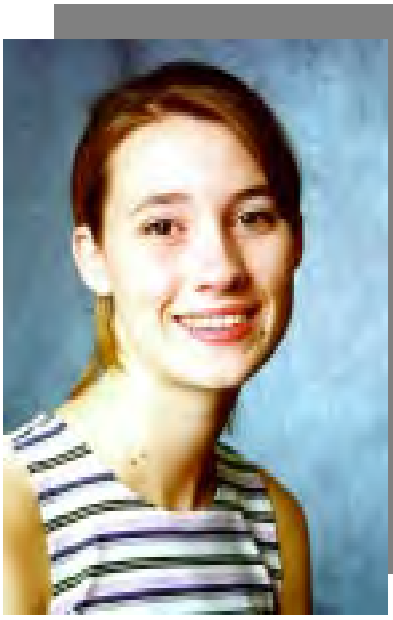
All *Ascaridia* counts are given as geometric means in Table 4. Parasitic larval stages for *Ascaridia* are second, third, and fourth, progressing from earlier to later stages prior to the fourth larval molt into adults. For the second larval stage, heterologous (turkey worm) infections in the chicken produced the greatest burdens. This suggests that the turkey worms were retarded in development in the unnatural chicken host. Further evidence of retarded development of *A. dissimilis* in the chicken was seen at the third larval stage, with significantly ($P < 0.05$) greater levels seen in this experimental group than

Meet the Student-Author

I am from Cabot, Ark., and a graduate of Cabot High School. Currently, I am a junior majoring in animal science. I have received the M.S. Offut Scholarship, the Whitaker Scholarship, and the G. Brown Scholarship. I plan to finish my B.S. degree at the University of Arkansas and then continue my education until I receive a Ph.D. in animal science.

I chose this research project because it was interesting and my professor expressed the need for research in this area. This experience gave me insight into what I might expect from graduate school, while allowing me to feel more confident about my decision to continue my education. I feel that I am now more "competitive" than less experienced students and that I am more capable of applying information from the classroom, dealing with the unexpected, and expressing the results both orally and in writing. It was a great experience for me.

I would like to express my appreciation to Dr. Yazwinski and Dr. Tucker for their availability and direction during this research project.



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any other (same occurrence as with the second stage larvae). Levels of larvae at the fourth stage of development were highest in experimental groups given homologous infections. In the case of chickens infected with *A. galli*, these numbers reflected normal, successful patterns of development. For fourth larval stage numbers in turkeys, the comparatively high count was also consistent with the natural situation, wherein fourth stage counts are characteristically of greatest magnitude for naturally infected turkeys (Yazwinski *et al.*, 1993).

Numbers of roundworms at the adult stage were significantly ($P < 0.05$) greater in naturally infected chickens than in any other group. Chickens normally harbor the majority of their ascarids as adults, whereas turkey infections are predominately of the fourth-stage variety. The current data clearly reflect the normal distribution. In regard to total worm burdens (all stages combined), homologous infections were the most successful. For both chickens and turkeys, heterologous infection numbers were only 55 to 56% as great as homologous infection numbers. Life-cycle stage specimens of *Ascaridia* isolated in this research project are presented in Fig. 1.

Table 3. Mortality rate, feed efficiency,[z] and mean necropsy weight by experimental group.

Pen No.	Bird host	Infection status	Mortality rate (%)	Feed efficiency	Mean necropsy weight (g)
1	Chicken	None	24	2.27	574
2	Chicken	*A. dissimilis*	16	2.22	559
3	Chicken	*A. galli*	24	2.30	614
4	Turkey	*A. galli*	37	2.39	396 ab
5	Turkey	None	36	2.40	433 a
6	Turkey	*A. dissimilis*	27	2.54	376 b

Means for experimental groups of the same host species with unlike superscripts were significantly different ($P < 0.05$).

[z] Units consumed per units gained (weekly values averaged over the 3 weeks postinfection).

Table 4. *Ascaridia* geometric means by stage of parasite and experimental group.

Pen No.	Bird host	Infection status	Geometric mean for *Ascaridia* as: Second	Third	Fourth	Adult	Total
1	Chicken	None	0 b	0 c	0 d	0 c	0 d
2	Chicken	*A. dissimilis*	2.0 a	7.2 a	2.2 b	0.1 c	13.2 b
3	Chicken	*A. galli*	0.1 b	1.2 b	5.6 a	15.6 a	24.2 a
4	Turkey	*A. galli*	0.2 b	1.5 b	1.0 c	1.5 b	5.6 c
5	Turkey	None	0 b	0 c	0 d	0 c	0 d
6	Turkey	*A. dissimilis*	0 b	1.6 b	6.4 a	1.0 b	10.0 b

Means in the same column with unlike superscripts were significantly different ($P < 0.05$).

CONCLUSIONS

In our study, homologous infections of chickens and turkeys by *Ascaridia* were consistent with previous observations. Heterologous infections were successful, but with clearly reduced rates of development as well as infection rate. Given the fact that the heterologous infections were successful, additional studies are planned to investigate the degree to which these unnatural infections impart resistance in the host to their natural parasitisms. In addition, the specimens and data collected from the current research will be further scrutinized so that more insight into the basic life styles of these parasites in our birds might be gained.

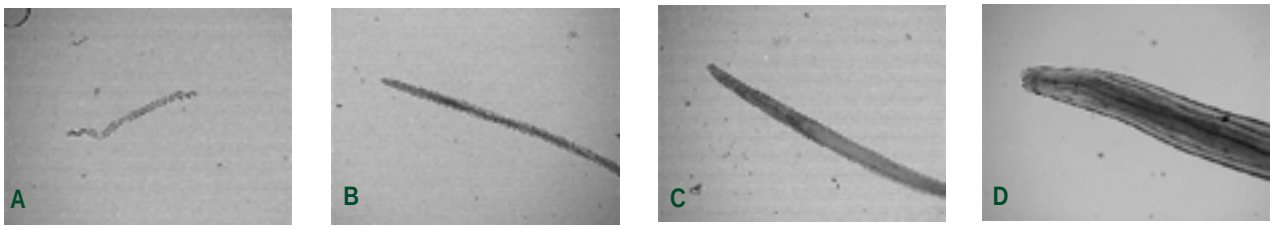

Fig. 1. Parasitic stages of *Ascaridia dissimilis* as isolated in this study. *A.* Second-stage larva. *B.* Third-stage larva. *C.* Fourth-stage larva. *D.* Adult. All specimens at 40X magnification. Actual sizes of the roundworms ranged from 1 mm to 4 cm for second-stage larvae and adults, respectively.

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Julie Hamilton wishes to thank her Mom and Dad, again.

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