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TRANSMISSION OF ENTERIC PATHOGENS OF TURKEYS BY DARKLING BEETLE LARVA (ALPHITOBIUS DIAPERINUS)

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Primary Audience: Researchers, Veterinarians, Flock Supervisors

SUMMARY

Larvae of the darkling beetle (lesser mealworm) were exposed to turkey feces from an enteritis-affected flock and determined to contain turkey enterovirus and rotavirus. Growth depression and increased mortality were observed in turkey poults which fed on the exposed larvae. Exposed larvae which had been surface-sterilized also produced clinical signs of enteritis after consumption by the poults, indicating that pathogens were able to survive within the larvae. This experiment demonstrated the capacity of the larva of the darkling beetle to serve as a mechanical vector for enteric pathogens of turkeys.

Key words: *Alphitobius diaperinus*, **darkling beetle, lesser mealworm, poult enteritis**
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DESCRIPTION OF PROBLEM

The darkling beetle, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae), also known as the lesser mealworm and litter beetle, is a common inhabitant in the litter of commercial turkey brooder and growout houses [1, 2]. This beetle species is a major health concern to poultry producers. Many species of pathogenic bacteria and fungi have been recovered from the insect [3, 4, 5]. It has been shown to be a potential carrier of avian viruses [6, 7]. Snedeker *et al.* [8] determined the presence of the viral agent of infectious bursal disease in darkling beetles which were collected from a house in which an outbreak of the disease had occurred. A nutrient broth solution of the collected beetles inoculated into 3-wk-old chicks resulted in typical lesions of infectious bursal disease.

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MacCreary and Catts [9] observed broiler chickens scratching in densely infested litter for darkling beetles. It was hypothesized that consumption of the insects could compromise the health of a flock by ingestion of pathogens or parasites that might have been present inside or on the body surface of the darkling beetle. Up to this point there have been no detailed studies demonstrating the transmission of a disease agent to poultry following ingestion of infected darkling beetles. Therefore the objective of this experiment was to determine if the larvae of the darkling beetle can serve as vectors for pathogens associated with acute enteritis in turkeys.

MATERIALS AND METHODS

AMPLIFICATION OF FECAL PATHOGENS

Turkey droppings were collected from a commercial flock of poults which exhibited signs of acute enteritis characterized by watery intestinal contents and thin-walled, gas-filled intestines. Amplification of the pathogens in the fecal samples was conducted at the College of Veterinary Medicine, NCSU. Feces were centrifuged and the liquid was separated from the solid fraction. The liquid material was filtered through a 0.45 μm filter and diluted 1:10 with minimal essential media (MEM). A 1 ml aliquot of this liquid was introduced into the crop of 5-day-old turkey poults. Feces were collected daily from the poults for seven days; each collection was frozen at -70°C. The original inoculum and material collected on days 2, 3, and 4 post-inoculation were mixed and diluted 1:10 in MEM and used in a second passage in poults. The feces were shown by electron microscopy to contain rotavirus and enterovirus particles. The feces were negative for coccidia by fecal flotation [10], cryptosporidia by auramine-O stained fecal smears [11], and *Salmonella* by tetrathionate enrichment and culture [12]. Feces collected on days 2, 3, and 4 post-exposure had the highest concentration of virus particles and were used in the transmission experiment.

EXPERIMENTAL DESIGN

Three hundred female hatchling BUTA (British United Turkeys of America) poults were obtained from a commercial hatchery and were fed unmedicated turkey starter mash and water *ad libitum*. Poults were not beak- or toe-trimmed. The starter feed was formulated by the North Carolina State University, Department of Poultry Science and mixed in the departmental feed mill. The ration met or exceeded the National Research Council nutrient requirements for turkey poults [13].

The pen in which the turkey poults were housed was a hard plastic wading pool (1 m diameter, 0.2 m height) with a hardware cloth cylinder (1.4 cm^2 mesh, 61 cm height) inserted around the margin to contain the birds; twenty poults were placed in each pen and grown to six days of age. A wood shavings litter base, ca. 5 cm deep, was placed on the bottom of each pen. Recommended floor area, feeder and watering space requirements, as well as guidelines for care of turkeys were followed [14].

On the sixth day of life, the poults were weighed, individually identified by a wing band, and randomly assigned into five treatment groups comprising ten poults per pen and three pens per treatment. On day 7, the poults were subjected to these treatments: 1) poults which were fed no beetle larvae (negative control); 2) poults fed "clean" beetle larvae (grown in culture and not exposed to infected fecal material); 3) poults fed larvae which were exposed to infected fecal material and had been subsequently surface-sterilized; 4) poults fed larvae which were exposed to infected fecal material and were not surface-sterilized; and 5) poults orally dosed with 1 ml infected fecal material diluted 1:10 with 0.9% physiological saline (positive control).

Darkling beetle larvae (7–13 mm in length) produced in culture were used in this experiment. Larvae were deprived of food and water for three days prior to being given to the poults. Larvae used in Treatments 3 and 4 were also exposed for 21 hr to 60 g lots of the amplified infected feces (a mixture of the collections from days 2, 3, and 4). After exposure to the feces, the larvae were separated from the feces using a sieve (no. 10, 2.0 mm aperture). After separation, no feces were seen adhering to the larvae.

Larvae were surface-sterilized for Treatment 3 using the washing procedure adapted from Harein and De las Casas [15]. Larvae were swirled in a 2% sodium hypochlorite solution for 2 min. Next, the sodium hypochlorite solution was drained by pouring the larvae and solution into a sieve (no. 12, 1.7 mm aperture) followed by rinsing with warm tap water for

2 min. Then the larvae were dried on paper toweling.

Live larvae which were fed to poults in Treatments 2 and 4 were placed directly on a 2.5 cm thick base of wood shavings litter in each pen for the poults to consume. Larvae in Treatment 3 were killed or immobilized by the sterilization procedure and were placed in the feed pans for the poults to consume. For Treatment 5, the diluted fecal suspension was introduced by syringe into the crop of each poult. During this 19-hr exposure period, the feed was removed from all pens and the drinkers were suspended 15 cm above the litter to prevent the larvae from finding refuge from the poults.

Poults were weighed and examined daily for clinical abnormalities during the 14-day experimental period. Fecal samples were collected from poults in each treatment on each of the first seven days after exposure. The fecal samples were later examined for presence of specific viral, bacterial, and protozoan pathogens. Examination for coccidia was by fecal flotation [10], cryptosporidia by auramine-O stained fecal smears [11], *Salmonella* spp. by tetrathionate enrichment and culture [12], and enteric viruses by electron microscopy [16].

Necropsy using standard techniques was performed on each dead poult and on three live poults from each treatment replicate at the end of the experiment. All viscera were examined for gross lesions.

Poult body weight data beginning on day 1 post-exposure were subjected to analysis of variance with treatment groups as the independent variable. Significant differences ($P = 0.05$) among treatment means were determined by Tukey's test.

RESULTS AND DISCUSSION

After being deprived of food and water for three days, the larvae were highly attracted to the infected turkey feces. The insects immediately began to crawl over the feces and actively feed. Poults in Treatments 2 and 4 actively searched the litter for the larvae which were avidly consumed. The dead surface-sterilized darkling beetle larvae placed in feed pans in Treatment 3 were also readily eaten by the poults. Each poult in the Treatments 2, 3, and 4 consumed about 360 larvae during the 19-hr exposure period.

Clinical signs (watery feces, inactivity, reduced weight gain) in poults which had ingested exposed larvae were observed on day 2 post-exposure (Table 1). Poults which fed on exposed darkling beetle larvae (Treatment 4) or on the exposed, surface-sterilized larvae (Treatment 3) showed a significant depression in growth in comparison to those poults which received either feed only (Treatment 1) or clean laboratory-reared darkling beetle larvae (Treatment 2). On day 9 after exposure, there was a decline in body weight in Treatments 4 and 5. By the end of the experiment, the body weight of the Treatment 4 poults was about the same as the Treatment 5 poults. The growth of poults in Treatment 3 was also significantly suppressed and this group was intermediate in

TABLE 1. Mean (±SD) body weight (g) of poults at 1 day pre- and 1–14 days post-treatment

TREATMENT NUMBER	MEAN (±SD) WEIGHT (g) OF POULTS AT DAYS PRE- AND POST-TREATMENT										
	-1	1	2	3	4	5	6	7	9	11	14
1	133.4[a]	178.8[a]	201.3[a]	224.2[a]	245.7[a]	273.6[a]	306.4[a]	336.4[a]	402.1[a]	506.0[a]	538.3[a]
	(7.8)	(12.2)	(13.1)	(13.9)	(15.9)	(18.7)	(21.9)	(23.9)	(29.1)	(39.6)	(44.4)
2	134.0[a]	163.5[b]	188.6[b]	209.3[b]	235.1[a]	264.0[a]	298.8[a]	329.6[a]	393.4[a]	465.4[b]	497.4[b]
	(6.7)	(9.7)	(12.4)	(12.2)	(12.9)	(13.5)	(15.0)	(16.0)	(22.3)	(40.3)	(43.9)
3	133.7[a]	166.9[b]	177.7[c]	194.6[c]	213.9[b]	237.8[b]	259.7[b]	274.5[b]	302.0[b]	372.6[c]	390.5[c]
	(7.5)	(10.8)	(10.1)	(12.8)	(15.5)	(21.5)	(27.1)	(27.8)	(35.5)	(38.4)	(71.6)
4	131.7[a]	161.1[b]	174.6[c]	190.5[c]	211.9[b]	225.6[b]	242.3[b]	254.9[b]	248.6[c]	279.0[d]	296.1[d]
	(8.1)	(10.5)	(11.7)	(13.0)	(15.5)	(22.4)	(34.3)	(44.6)	(51.5)	(34.8)	(33.8)
5	135.1[a]	177.6[a]	175.5[c]	178.9[d]	180.0[c]	185.6[c]	196.0[c]	204.4[c]	195.0[d]	282.6[d]	305.0[d]
	(8.2)	(11.4)	(15.6)	(20.9)	(25.1)	(26.6)	(30.3)	(34.0)	(39.2)	(46.9)	(15.5)

[a–d]Within each column, means followed by different letters are significantly different, Tukey's test, alpha = 0.05.

body weight by day 14 post-exposure. Poults in Treatments 4 and 5 were very vocal ("distress" peeping) on day 9. On day 10 after exposure, Treatment 4 poults became relatively quiet and were inactive.

Mortality was observed in Treatment 4 on days 11, 12, and 14 after exposure (4, 1, and 1 poults, respectively) and in Treatment 5 on day 6 after exposure (1 poult). Necropsies of the birds revealed gross lesions in the digestive tract. The crop, proventriculus, and ventriculus of all birds were devoid of feed. The intestinal tracts were thin-walled and filled with fluid. The ceca were distended with gas and fluid. No gross lesions were found in the samples of surviving poults on routine necropsy at the end of the experiment.

Enteroviruses were detected at least one time by electron microscopy in the feces of all the treatment groups by day 2 post-exposure, and these viruses were detected through the end (day 7 post-exposure) of the fecal collection period (Table 2). Rotaviruses were detected only in the feces from poults orally inoculated with the feces collected from poults with enteritis (Treatment 5), from poults fed surface-sterilized larvae exposed to feces (Treatment 3), and from poults fed unsterilized larvae exposed to feces (Treatment 4). Rotavirus was detected more frequently in feces from poults in Treatmemt 3 (feces-exposed surface-sterilized larvae) than in other treatments, being detected on days 3 to 7 post-exposure. Rotaviruses were not detected in feces of untreated poults (Treatment 1) or poults fed clean larvae (Treatment 2).

All of the fecal samples were negative for coccidia and cryptosporidia. Either *Salmonella reading* or *Salmonella livingstone* were isolated several times from the feces in Treatments 1 and 2 and only once from Treatments 3, 4, and 5. These *Salmonella* are common contaminants in turkeys and are regarded as relatively non-pathogenic.

The results of this experiment demonstrate that the larva of the darkling beetle can serve as a mechanical vector of enteric pathogens of turkey poults. In this experiment poults showed symptoms of enteritis after feeding on larvae which had come in direct contact with feces from infected poults. Larvae which were surface-sterilized following exposure to the infected feces also produced enteritis in poults, indicating that pathogens were able to survive in the gut of the darkling beetle larvae. It is not known how long the pathogens can survive inside the larva and whether adults developing from exposed larvae are infective.

These findings indicate that larvae can serve as mechanical vectors for transmission of rotavirus. The role of larvae in the transmission of enterovirus cannot be determined by this study because this virus was detected in all treatments. Apparently the poults were already infected with enterovirus when obtained from the hatchery.

This study suggests that darkling beetles play a significant role in production facilities in the transmission of pathogens which pro-

TABLE 2. Detection of viruses in feces collected from turkey poults in Treatments 1–5 through day 7 post-exposure

DAYS POST-EXPOSURE	TREATMENT				
	1	2	3	4	5
1	N[A]	E[B]	N	E	E
2	E	E	E	N	N
3	E	N	E, R[C]	E	E, R
4	E	E	E, R	E	E, R
5	E	E	E, R	E, R	E, R
6	E	N	E, R	E	N
7	E	N	E, R	E, R	N

[A]N = negative

[B]E = enterovirus

[C]R = rotavirous

duce acute enteritis in turkey poults. Our data support the hypothesis that larvae of the darkling beetle transmit pathogens within a flock of poults. Feces collected from poults affected with enteritis were attractive to larvae. Contact with this material results in infection of larvae which become vectors for transmission to other poults.

CONCLUSIONS AND APPLICATIONS

1. Larvae of darkling beetles exposed to turkey feces from infected poults transmitted acute enteritis following ingestion by healthy turkey poults.
2. Rotavirus was associated with enteritis in poults under controlled experimental conditions.
3. The findings emphasize the need to control populations of darkling beetles in turkey brooder and growout houses.

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