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Evaluation of *Salmonella* Movement Through the Gut of the Lesser Mealworm, *Alphitobius diaperinus (Coleoptera: Tenebrionidae)*

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Abstract

Aims: The lesser mealworm, *Alphitobius diaperinus* is an important poultry pest prevalent during production that is capable of vectoring pathogens. This study was undertaken to determine the gut transit time of *Salmonella* for biosecurity risk analysis of pathogen dispersal into the environment. *Methods:* Adult and larval *A. diaperinus* were exposed to two concentrations of a fluorescently labeled *Salmonella enterica* for 15, 30, and 60 min time periods then externally disinfected to evaluate internal transfer of *Salmonella*. The insects were monitored every 30 min over 4 h and evacuated frass (feces) processed for the marker *Salmonella*. The minimum time monitored was 45 min (15 exposure + 30 min time point), and the maximum was 5 h (60 exposure + 4 h time point).
Results: Adults treated with 10^6 or 10^8 colony-forming units (cfu)/mL, which produced *Salmonella* positive frass within the 5 h experimental time, displayed a mean gut transit time of 144.4 min (range 90–270 min) and 186.3 min (range 120–300 min), respectively. Larvae treated with 10^6 or 10^8 cfu/mL displayed a mean gut transit time of 172.5 min (range 120–300 min) and 131.7 min (range 60–300 min), respectively.
Significance and Impact of Study: Understanding the sources and contribution of reservoir populations of pathogens in poultry production operations is important for development of biosecurity measures to mitigate their transfer. *A. diaperinus* are prevalent in production operations and difficult to suppress. Management standards accept the reutilization of litter in which insects survive between flock rotations. Removing litter and spreading it onto nearby fields results in the inadvertent dispersal of beetles. Few studies demonstrating the specific bacterial dispersal capacities of these insects have been performed. This study determined that *Salmonella* acquired internally, commonly transits the gut, allowed the insect to disperse viable pathogenic bacteria within 2–3 h.

Key Words: *Alphitobius diaperinus*—Bacteria—Darkling beetle—Gut transit time—Lesser mealworm—*Salmonella*.

Introduction

Salmonellosis in humans is largely contracted through the consumption of contaminated food (WHO 2005). The causative organisms, *Salmonella*, pass through the food chain from primary production facilities to households or food-service operations. These infections often result from a very small number of infectious agents unintentionally amplified by the improper handling of the commodities. Resulting foodborne disease is a public health burden and represents a significant cost to society. Although this issue is not solely a dilemma for the United States, reliable data related to the cost of foodborne diseases are not available from many countries. The World Health Organization estimated that about 1.4 million nontyphoidal *Salmonella* infections account for 168,000 hospital visits, 15,000 hospitalizations, and 580 deaths each year in the United States and a cost estimated at US$ 3 billion annually (WHO 2005).

The *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae) is a common inhabitant and a significant problem in

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many poultry production operations. They cause structural damage to production facilities when they burrow into the walls and insulation. In addition, all life stages of this insect inhabit the house floor and feed within commercial poultry operations (Pfeiffer and Axtell 1980, Stafford et al. 1988, Axtell and Arends 1990, Rueda and Axtell 1997). These insects transmit viral, fungal, and bacterial microbes (Harein et al. 1970, Despins et al. 1994, McAllister et al. 1994, 1995, 1996, Watson et al. 2000, Calibeo-Hayes et al. 2003, Strother et al. 2005, Templeton et al. 2006, Vittori et al. 2007). These omnivorous scavengers feed from many sources, such as, manure, spilled chicken feed, cracked eggs, chicken carcasses, house fly maggots, and detritus. In turn, they are often fodder for chickens, wild birds, other beetles, and opportunistic rodents within the facility (Pfeiffer and Axtell 1980, Axtell and Arends 1990, Rueda and Axtell 1997).

The epidemiology of *Salmonella* within a poultry facility is not yet fully understood; and many elements are likely at play, including *A. diaperinus*. Davies and Breslin (2003) found that *Salmonella* Enteritidis persisted in the litter, bird feces, feed, and the ground beetle, *Amara aulica* Panzer, 26 months after removal of birds from the poultry production facilities. Harein et al. (1972) found that 27% of *A. diaperinus* samples collected from a turkey brooder house were positive for *Salmonella*. An infestation of *A. diaperinus* can be difficult to control, and these pests are often inadvertently dispersed to neighboring poultry houses and residences by the spreading of beetle-containing spent litter onto fields as fertilizers (Armitage 1986, Calibeo-Hayes et al. 2005). Consequently, they are potential participants in the dissemination of pathogenic bacteria into the environment. In addition, their transmission of *Salmonella* may play a role in the maintenance of these bacteria in the poultry house between flock rotations.

The beetle's ability to harbor bacteria internally, not just externally, further complicates the dilemma facing producers to limit the spread of infectious pathogens (Crippen et al. 2009). Internalization of bacteria might allow the establishment of a viable colony and long-term dispersal of bacteria into the environment by the beetle. However, little is known about the uptake and subsequent dispersal potential of pathogenic bacteria by this beetle. We used a validated external disinfection method allowing the differentiation of internally carried bacteria and *Salmonella* with a chromosomally inserted fluorescent tag, thus allowing the long-term tracking of specifically introduced bacteria (Crippen and Sheffield 2006). This study determined the minimal gut transit time of *Salmonella* in *A. diaperinus*. These data will be useful in determining whether *Salmonella* can colonize the alimentary canal of these insects and determine the spatiotemporal variations of pathogen dispersal by this insect around the poultry facility. In this study, the gut transit time of *Salmonella* is represented by the first appearance of the bacteria in the frass produced by the beetle, monitored at specific time intervals after oral acquisition of *Salmonella* and surface disinfection of the insects.

Materials and Methods

Beetles

The Southern Plains Agricultural Research Center (SPARC) starter colony of *A. diaperinus* was a generous gift from a colony originally isolated from a poultry farm located in Wake County, NC, and maintained by Dr. D. W. Watson (North Carolina State University, Raleigh, NC). The SPARC colony was initiated and has remained in production since 2004. Beetles were reared in 1000 mL wheat bran (Morrison Milling Co., Denton, TX) in plastic containers ($15 \times 15 \times 30$ cm) with screen tops and held at 30°C in an 8:16 h (light:dark) cycle. Additionally, each cage contained a 6 cm^2 sponge moistened with deionized water and a 0.5 cm thick slice of a medium sized apple replenished twice per week, and 30 mL of fishmeal (Omega Protein, Inc., Hammond, LA) was added to the wheat bran once per week.

Experimental design

Three replications of each experimental exposure protocol were conducted by using three beetles per treatment regime in each experiment. A fluorescent marker bacteria, *Salmonella enterica* serovar Typhimurium-green fluorescent protein (ST-GFP), a generous gift from Drs. Roy J. Bongaerts and Jay Hinton, Norwich Research Park, Norwich, Umited Kingdom, was used to track the movement of bacteria through the beetle (Hautefort et al. 2003).

Ingestion threshold determinations

Beetles of at least 4 weeks of age and larvae of ~6 weeks of age were selected from the colony and exposed to a lawn of bacteria in exposure tubes, where they ingest bacteria on the agar. These exposure tubes were produced by placing 8 mL Brain Heart Infusion agar (Biolink Scientific, Austin, TX) into a 17×100 mm (14 mL) snap-cap polypropylene round-bottom tube (Fisher Scientific, Pittsburgh, PA). Ten microliters of phosphate-buffered saline (PBS) or ST-GFP (treatment) were added to the top of the agar. Two beetles or larvae were added per tube and allowed to move freely at 30°C in the dark. After appropriate time periods (15, 30, and 60 min), insects were sterilely collected, externally disinfected by washing in SporGon® (Decon Labs, Inc. Bryn Mawr, PA), and used for experimentation. The disinfection was validated by exposure to and culture of randomly selected insects placed into buffered peptone water (BPW), as previously described (Crippen and Sheffield 2006). After exposure to the marker *Salmonella* and surface disinfection, three of the study insects were homogenized in BPW to determine initial bacterial ingestion by the insect, as previously described (Crippen and Sheffield 2006). Enumeration was performed by serial dilution, in triplicate, onto plates containing the selective media, Brilliant Green agar (Biolink Scientific) with 1.2% chloramphenicol (BGA-C), and incubation at 37°C for 18–24 h to determine bacterial concentration. Postexposure, ST-GFP was cultured from random samples of the exposure tubes to assure that the insects were exposed to viable bacteria.

Gut transit time

Three beetles or larvae were exposed per time point and concentration, to PBS or a mean of 4.03×10^8 (10^8) or 4.15×10^6 (10^6) colony-forming units (cfu)/mL of ST-GFP. Treated insects were harvested at 15, 30, and 60 min postexposure; control (PBS) insects were harvested at 60 min postexposure and externally disinfected. Postexposure, three beetles or larvae were randomly removed, and initial bacterial ingestion was determined. Each of the remaining insects were placed

into the first well of an eight well (beetles) or four well (larvae) chamber slide (LAB-TEK®; Nalgene Nunc International, Naperville, IL) and held at 30°C in the dark. Beetles were transferred to successive wells at 30 min intervals, and harvested after 240 min (8 transfers). The minimum time monitored was 45 min (15 exposure + 30 min time point), and the maximum was 5 h (60 exposure + 4 h time point). The insects were externally disinfected, homogenized in BPW, and internally carried ST-GFP was enumerated by serial diluted onto BGA-C plates. An aliquot of 1.0 mL BPW was used to wash each chamber slide well to collect the deposited frass, these samples were incubated 24 h at 37°C and then enriched for *Salmonella*. After 24 h, the sample was transferred into 9 mL tetrathionate broth (BVA Scientific, San Antonio, TX) and incubated at 37°C for 24 h, at which time 100 μL was transferred into 5 mL Rappaport-Vassiliadis Medium (BVA Scientific) and incubated at 42°C for 24 h. An aliquot of 100 μL was then plated onto BGA-C and incubated at 37°C for 24 h. Resulting colonies were checked for the presence of ST-GFP by using fluorescent microscopy. Three replications of each experiment were conducted.

Data analysis

Data were analyzed by using commercially available statistical software (Prism ver. 5.01; GraphPad Software Inc., La Jolla, CA). Descriptive statistics were generated by using the geometric mean (GM) calculated as the n^{th} root of the product of (n) numbers and 95% confidence intervals (CIs) and presented in table formats. Within each exposure concentration and sample type, a means comparison of time point was performed by using a two-way analysis of variance followed by Bonferroni post tests to determine least square means ($p < 0.05$). Comparison of initial bacterial ingestion to post-counts within each treatment group was performed by Mann-Whitney tests.

Results

Initial bacterial ingestion

Postexposure to ST-GFP, three beetles or larvae were randomly collected; and the geometric mean of initial bacterial ingestion was determined. The concentration of bacteria acquired by the insect during treatment in exposure tubes (initial bacterial ingestion) was compared over time of exposure and by concentration of bacteria (Table 1). A significant interaction between time of exposure and concentration was found. PBS exposed insects were all negative for ST-GFP. Larvae exposed to10^8 cfu/mL acquired significantly more bacteria than larvae or adults exposed to 10^6 cfu/mL at all time points and adults exposed to 10^8 cfu/mL for 15 and 30 min. No other significant differences were measured in bacterial ingestion.

Gut transit time

Time required for the *Salmonella* to first appear in the frass (gut transit time) was compared over time and by concentration of exposure (Table 2). PBS exposed insects were all negative for ST-GFP. The gut transit time of all ST-GFP treatments (15, 30, and 60 min) was significantly different from the control (PBS) group. The concentration of bacteria (10^8 or 10^6 cfu/mL) to which the adults and larvae were exposed did not significantly affect the gut transit time of *Salmonella*, except after 15 min of exposure of larvae to 10^8 cfu/mL. Otherwise, there was no significant difference between gut transit time of *Salmonella* related to the time of exposure of the larvae or adult to the bacteria. Overall, adults treated with 10^6 or 10^8 cfu/mL, which produced ST-GFP positive frass within the 5 h experimental time, displayed a mean gut transit time of 144.4 min (range 90–270 min) and 186.3 min (range

Table 1. The Initial Bacterial Ingestion (cfu/mL) of *Salmonella* Through the Alimentary Canal of *Alphitobius diaperinus* Was Determined by Collection of Random Insects Exposed to Identical Conditions as the Experimental Insects

		Initial bacterial ingestion		
	cfu/mL	*Exposure (min)*	*GM[a] (cfu/mL)*	*95% CI (cfu/mL)*
Adult	10^8	15	2.01E+04[A]	3.01E+04
		30	1.30E+03[A]	3.75E+04
		60	8.37E+04[B]	5.89E+03
Larvae	10^8	15	3.15E+04[B]	3.26E+04
		30	1.25E+05[B]	1.86E+05
		60	9.32E+04[B]	2.90E+05
Adult	10^6	15	8.66E+02[A]	2.87E+03
		30	4.50E+03[A]	9.55E+03
		60	2.09E+03[A]	3.10E+03
Larvae	10^6	15	7.42E+02[A]	1.08E+03
		30	2.17E+02[A]	1.16E+02
		60	1.67E+03[A]	2.18E+03

[a]GM calculated as the nth root of the product of (n) numbers and 95% CIs.
[A,B]Samples with the same letter are not significantly different ($p < 0.05$). All sample groups included in the comparison.
GM, geometric mean; CIs, confidence intervals; cfu, colony-forming unit.

Table 2. The Gut Transit Time (min) of *Salmonella* Through the Alimentary Canal of *Alphitobius diaperinus* Was Determined by Addition of Exposure Time Plus Time to Production of First Frass Containing *Salmonella*

				Range	
	Treatment (cfu/mL)	*Exposure (min)*	*Mean (min)*	*Shortest time*	*Longest time*
Adult	10^8	15	177	135	225
		30	190	180	210
		60	186	120	300
Larvae	10^8	15	105[A]	75	135
		30	133	60	210
		60	157	90	300
Adult	10^6	15	147	105	225
		30	126	90	210
		60	154	120	270
Larvae	10^6	15	195	135	225
		30	158	120	240
		60	160	120	300

Three hundred minutes was the maximum time tested.
[A]Significantly different from larvae 10^6 cfu/mL 15 min exposure ($p < 0.05$).

120–300 min), respectively. Larvae treated with 10^6 or 10^8 cfu/mL displayed a mean gut transit time of 172.5 min (range 120–300 min) and 131.7 min (range 60–300 min), respectively. Those that had not produced positive frass within the 5 h experimental period were not used to calculate mean gut transit time.

Bacterial postcounts

Presence of bacteria within the insects after the 5 h experimental period was determined by externally disinfecting, homogenizing, and culturing the experimental insects immediately after removal from the chamber slides. These postcounts were compared over time and by concentration of exposure (Table 3). PBS exposed insects were all negative for ST-GFP. There were no significant differences associated with specific times or concentrations of exposure after the 5 h experimental period in any of the treatment groups comparing insects that produced *Salmonella* positive frass. However, 37% of the adults and 0.2% of the larvae did not produce frass containing ST-GFP within the 5 h experimental period. The remaining internal bacterial load of the non-producing adults ($n=20$) was determined to be a GM of 4.49×10^3 cfu/mL (95% CI $=1.37\times10^4$), and the larvae ($n=1$) was 2.36×10^5 cfu/mL bacteria in postcounts. There was no significant difference in the initial bacterial ingestion (Table 1) and in the postcount concentration of *Salmonella* remaining within the insects (Table 3) for each treatment group.

Discussion

Larvae of many insects are specialized active feeders, eating voraciously to fuel their transition into adult form. In our study, *A. diaperinus* larvae fed 10^8 cfu/mL bacteria acquired significantly more bacteria than adults, except adults exposed for 60 min. Although no studies could be found quantitatively measuring the feeding behavior of adults versus larval *A. diaperinus*, this is likely a result of the increased feeding behavior associated with the larval stage in many insects.

Many factors affect gut passage of food in insects. Dagg and Wyman (1983) and Ham and Peterson (1988) reported on the effects that temperature had on gut clearance time and remarked that ingestion and egestion rates were not at equilibrium over short periods of time, thus making it difficult to determine a rate constant. Various species of aquatic caddisflies were evaluated at 12 h intervals for rates of gut clearance at two different water temperatures, after starvation for 48 h (Sangpradub and Giller 1994). A higher environmental temperature was found to increase the rate of gut clearance of this freshwater insect. Although times varied slightly for different species, all acquired food was within the foregut by the first 12 h observation, and most had cleared their digestive systems by the 60 h observation at lower temperatures and the 36 h observation at higher temperatures. The researchers concluded that the interval between gut examinations might not have been short enough to allow precise measurement. We studied gut transit over short periods of time (30 min) at a constant temperature (30°C); however, the fluctuation in transit time was large even at a constant temperature.

Time required for gut transit is also influenced by food concentration and food type (Murtaugh 1985), by ingestion rate (Murtaugh 1984), and by the state of digestion (Persson 1984). The beetles used in our experiments were raised on identical diets of wheat bran, apples and fish meal; however, the concentration of food previously consumed by an individual insect and the state of digestion before use in experimentation was unknown. We were interested in determining the gut transit time of the average beetle found inhabiting a poultry house. Therefore, we did not starve the insects before experimentation. We measured an average passage time given the normal varied states of digestion one would

Table 3. The Postcounts of the *Salmonella* Remaining in Alimentary Canal of *Alphitobius diaperinus* (Internal Bacterial Load), of Insects That Excreted *Salmonella* with Their Frass (*Salmonella enterica* Serovar Typhimurium-Green Fluorescent Protein Positive), and Those Which Did Not Pass *Salmonella* with Their Frass (Negative) Within 4 h After Initial Exposure

			Internal bacterial uptake					
			ST-GFP positive frass			*Negative frass*		
	Treatment (cfu/mL)	*Exposure (min)*	*GM[a] (cfu/mL)*	*95% CI[a] (cfu/mL)*	n	*GM (cfu/mL)*	*95% CI (cfu/mL)*	n
Adult	10^8	15	9.45E+03	5.49E+03	5	1.07E+04	6.68E+03	4
		30	3.92E+04	3.21E+05	3	4.59E+04	4.81E+04	6
		60	3.23E+04	1.24E+05	5	1.24E+04	1.00E+04	4
Larvae	10^8	15	5.62E+04	8.74E+04	8	2.63E+05	-	1
		30	4.61E+04	2.25E+05	9			
		60	1.04E+04	1.18E+05	9			
Adult	10^6	15	1.50E+03	4.89E+03	6	1.14E+03	1.28E+04	3
		30	1.18E+03	1.86E+04	7	2.65E+02	6.85E+03	2
		60	1.62E+04	3.25E+04	8	4.45E+03	-	1
Larvae	10^6	15	7.32E+03	1.18E+04	9			
		30	7.95E+02	4.44E+04	9			
		60	1.08E+03	1.04E+04	9			

Bacterial concentration was determined by serial dilution and culture of externally disinfected, homogenized experimental insects immediately after the final time point in the chamber slides.

[a]GM calculated as the nth root of the product of (n) numbers with 95% CI.

ST-GFP, *Salmonella enterica* serovar Typhimurium-green fluorescent protein.

encounter from a random sample of insects with continuous access to a food source. The type and complexity of food ingested by insects in natural settings may affect bulk flow through the digestive system and ultimately the timing of waste elimination in comparison to the findings in this study where insects only had access to an artificial diet.

Studies have not been done examining the transit time of pathogenic microbes within the gut of these scavenger beetles. Hurst and Jackson (2002) monitored the fate of a marker bacterium, *Serratia entomophilia*, in the grass grub, *Costelytra zealandica* (White). They found bacteria throughout the gut in the luminal fluid, associated with food particles and adhering to the crop lining. The bacteria appeared to pass through the gut and be voided into the environment, demonstrating the capability of insects to disperse pathogenic bacteria from their digestive system.

Many studies tracked a variety of nonpathogenic elements within the digestive systems of insects. Woolfolk et al. (2004) explored the common lacewing, *Chrysoperla rufilabris* (Burmeister), alimentary canal, and the accumulation of ingested particles throughout the digestive system. Fluorescent particles were used to monitor movement and all sizes (0.1, 4.0, and 10.0 μm) had similar distribution patterns. Within the first 4 h postingestion, all sized particles accumulated within the diverticulum of the foregut. By 20 h, a few particles remained in the foregut, but were concentrated within the midgut, with some reaching the pyloric region and rectum. Preliminary work by Mumcuoglu et al. (2001) suggested that the gut transit time for food in Green Bottle fly maggots, *Lucilia sericata* (Meigen), was 1 to 1.5 h. According to Chapman (1998), a freshly eaten meal of solid food by a grasshopper, *Schistocerca* sp., takes about 15 min to reach the midgut, 60–90 min to reach the hindgut, and about 90 min for first production of feces. Pollen grains were excreted in the frass 2 h after feeding of Boll weevils, *Anthonomus grandis* (Boheman) (Cate and Skinner 1978).

Our study determined the minimal time required to transit through the digestive tract of *A. diaperinus* and begin dispersal of viable pathogens into the environment. We could find no studies that evaluated the very short-term gut transit in beetles; most monitored total evacuation or clearance times. In our study, most treated adults (63%) and larvae (98%) passed viable *Salmonella* into the environment within the 5 h experimental time period and displayed an average gut transit time of 2–3 h. As previously noted, the voracious and presumably frequent feeding behavior of the larval stage may account for quantitative differences in the number of insects egesting *Salmonella* within 5 h. The turnover of solid particles in the foregut of grasshoppers, *Schistocerca* sp., under continuous feeding conditions takes about 90 min; however, without further exposure to food, a single freshly eaten meal takes about 5 h to clear the foregut completely, 8 h to clear the midgut, and 20 h to clear the alimentary system (Chapman 1998).

The fate of a marker *Escherichia coli* was monitored in the digestive tract of *L. sericata* used in maggot debridement therapy. The concentration of bacteria decreased as it moved posterior from the crop to the mid- and hindgut (Mumcuoglu et al. 2001). However, the mechanical vectoring, the internal carriage, and the passage of viable microbes through the digestive tract or the colonization of the alimentary canal and subsequent deposition of viable microbes by insects are of the most concern for livestock and poultry production facility biosecurity. In our study, at least some of the marker *Salmonella*, which passed through the *A. diaperinus* digestive system and were voided in the frass, survived and were viable. The viable bacterial concentration could not be precisely measured, due to the use of enrichment protocols to assure detection of low levels of egested *Salmonella*. However, the amount of *Salmonella* remaining internally in the whole insect after the 5 h experimental period was quantified. There was no significant difference between the amount acquired during initial bacterial ingestion and the amount still in the insect 5 h later. Presumably, these insects would continue to shed viable bacteria. In addition, several insects, all of which had acquired bacteria, had not yet discharged any in their frass and would also likely be a subsequent source of contamination. Hurst and Jackson (2002) recovered *S. entomophilia* from the grass grub as long as 14 days after exposure. Future studies with the *A. diaperinus* will examine the internal retention of *Salmonella* by *A. diaperinus* and longevity of potential dispersal. The challenge will be to separate the insect from the possibility of recontamination by its own frass, so that baseline values can be obtained.

In conclusion, *A. diaperinus* is abundant in poultry production facilities. In addition to being a structural pest, this insect has been shown to be capable of carrying many different microbial pathogens. We exposed these insects to a marker *Salmonella* and then monitored the uptake and discharge of viable pathogenic bacteria to determine the minimal gut transit time of *Salmonella*, as well as the potential for dispersing viable bacteria into the environment. The larval stage of the insect acquired significantly more bacteria than the adult stage when exposed to a dose of 10^8 cfu/mL. The minimal time required for pathogenic bacteria to transit the gut of *A. diaperinus* was 1 h; and the maximal time is unknown, as not all exposed insects discharged marker bacteria within 5 h; the average transit time of those which did was 2–3 h dependent on dose and developmental stage. This study demonstrates that *Salmonella* acquired internally can rapidly transit the gut, and the insect can become a contaminating source dispersing viable pathogenic bacteria.

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Disclosure Statement

No competing financial interests exist.

References

Armitage, DM. Population changes of four species of insects in three deep pit poultry houses. Entomol Mon Mag 1986; 122:75–77.

Axtell, RC, Arends, JJ. Ecology and management of arthropod pests of poultry. Annu Rev Entomol 1990; 35:101–126.

Calibeo-Hayes, D, Denning, SS, Stringham, SM, Guy, JS, et al. Mechanical transmission of turkey coronavirus by domestic

houseflies (*Musca domestica linnaeaus*). Avian Dis 2003; 47: 149–153.

Calibeo-Hayes, D, Denning, SS, Stringham, SM, Watson, DW. Lesser mealworm (Coleoptera: Tenebrionidae) emergence after mechanical incorporation of poultry litter into field soils. Vet Entomol 2005; 98:229–235.

Cate, JR, Skinner, JL. The fate and identification of pollen in the alimentary canal of the boll weevil. Southwest Entomol 1978; 3:263–265.

Chapman, RF. Ailmentary canal, digestion and absorption. *The Insects Structure and Function*, 4th edition. Cambridge, UK: Cambridge University Press, 1998:38–68.

Crippen, TL, Sheffield, C. External surface disinfection of the lesser mealworm beetle, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). J Med Entomol 2006; 43:916–923.

Crippen, TL, Sheffield, CL, Esquivel, SV, Droleskey, RE, et al. The acquisition and internalization of *Salmonella* by the lesser mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). Vector Borne Zoonot Dis 2009; 9:65–71.

Dagg, MJ, Wyman, K. Natural ingestion rates of copepods neocalanus *plumchrus* and *n. Cristatus* calculted from gut contents. Marine Ecol Prog Ser 1983; 13:37–46.

Davies, RH, Breslin, M. Persistence of *Salmonella* Enteritidis phage type 4 in the environment and arthropod vectors on an empty free-range chicken farm. Environ Microbiol 2003; 5: 79–84.

Despins, JL, Axtell, RC, Rives, DV, Guy, JS, et al. Transmission of enteric pathogens of turkeys by darkling beetle larva (*Alphitobius diaperinus*). J Appl Poult Res 1994; 3:61–65.

Ham, HG, Peterson, WT. The effect of temperature on the gut clearance rate constant of planktonic copepods. J Exp Marine Biol Ecol 1988; 123:1–14.

Harein, PK, De las Casas, E, Larsen, CT, Pomeroy, BS. Microbial relationship between the lesser mealworm and its associated environment in a turkey brooder house. Environ Entomol 1972; 1:189–194.

Harein, PK, de las Casas, E, Pomeroy, BS, York, MD. *Salmonella* spp. and serotypes of *Escherichia coli* isolated from the lesser mealworm collected in poultry brooder houses. J Econ Entomol 1970; 63:80–82.

Hautefort, I, Proenca, MJ, Hinton JCD. Single copy green fluorescent protein gene fusion allows accurate measurement of *Salmonella* gene expression *in vitro* and during infection of mammalian cells. Appl Environ Microbiol 2003; 69:7480–7491.

Hurst, MRH, Jackson, TA. Use of green fluorescent protein to monitor the fate of *Serratia entomophila* causing amber disease in the New Zealand grass grub, *Costelytra zealandica* J Microbiol Methods 2002; 50:1–8.

McAllister, JC, Steelman, CD, Newberry, LA, Skeeles, JK. Isolation of infectious bursal disease virus from the lesser mealworm, *Alphitobius diaperinus* (panzer). Poult Sci 1995; 74:45–49.

McAllister, JC, Steelman, CD, Skeeles, JK. Reservoir competence of the lesser mealworm (Coleoptera: Tenebrionidae) for *Salmonella* Typhimurium (Eubacteriales: Enterobacteriaceae). J Med Entomol 1994; 31:369–372.

McAllister, JC, Steelman, CD, Skeeles, JK, Newberry, LA, et al. Reservoir competence of *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) for *Escherichia coli* (Eubacteriales: Enterobacteriaceae). J Med Entomol 1996; 33:983–987.

Mumcuoglu, KY, Miller, J, Mumcuoglu, M, Friger, M, et al. Destruction of bacteria in the digestive tract of the maggot of *Lucilia sericata* (Diptera: Calliphoridae). J Med Entomol 2001; 38:161–166.

Murtaugh, PA. The influence of food concentration and feeding rate on the gut residence time of Daphnia. J Plankton Res 1985; 7:415–420.

Murtaugh, PA. Variable gut residence time: problems in inferring feeding rate from stomach fullness of a mysid crustacean. Can J Fish Aquat Sci 1984; 41:1287–1293.

Persson, L. Food evacuation and models for multiple meals in fishes. Environ Biol Fish 1984; 10:305–309.

Pfeiffer, DG, Axtell, RC. Coleoptera of poultry manure in caged layer houses in North Carolina. Environ Entomol 1980; 9:21–28.

Rueda, LM, Axtell, RC. Arthropods in litter of poultry (broiler chicken and turkey) houses. J Agric Entomol 1997; 14:81–91.

Sangpradub, N, Giller, P. Gut morphology, feeding rate and gut clearance in five species of caddis larvae. Hydrobiologia 1994; 287:215–223.

Stafford, KC, Collison, CH, Burg, JG, Cloud, JA. Distribution and monitoring lesser mealworms, hide beetles, and other fauna in high-rise, caged layer poultry houses. J Agric Entomol 1988; 5:89–101.

Strother, KO, Dayton Steelman, C, Gbur, EE. Reservoir competence of lesser mealworm (Coleoptera: Tenebrionidae) for *Campylobacter jejuni* (Campylobacterales: Campylobacteraceae). J Med Entomol 2005; 42:42–47.

Templeton, JM, De Jong, AJ, Blackall, PJ, Miflin, JK. Survival of *Campylobacter* spp. in darkling beetles (*Alphitobius diaperinus*) and their larvae in Australia. Appl Environ Microbiol 2006; 72:7909–7911.

Vittori, J, Schocken-Iturrino, RP, Trovó, KP, Ribeiro CAM, et al. *Alphitobius diaperinus* as a vector of *Clostridium perfringens* in broiler houses in the state of São Paulo—Brazil. Ciência Rural 2007; 37:894–896.

Watson, DW, Guy, JS, Stringham, SM. Limited transmission of turkey coronavirus in young turkeys by adult *Alphitobius diaperinu*s (Coleoptera: Tenebrionidae). J Med Entomol 2000; 37:480–483.

[WHO] World Health Organization. 2005. Drug-resistant *Salmonella*. Available at www.who.int/mediacentre/factsheets/fs139/en/ (accessed September 2011).

Woolfolk, SW, Cohen, AC, Inglis, GD. Morphology of the alimentary canal of *Chrysoperla rufilabris* (Neuroptera: Chrysopidae) adults in relation to microbial symbionts. Ann Entomol Soc Am 2004; 97:796–808.

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