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Infection and Quantitative Recovery of *Salmonella Typhimurium* and *Escherichia Coli* From Within the Lesser Mealworm, *Alphitobius Diaperinus* (Panzer).^{1,2}

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THE lesser mealworm, *Alphitobius diaperinus* (Panzer) has been considered only as a minor pest of grain, cereal products and related agricultural commodities although it is widely distributed throughout the world (Cotton, 1956; Harris, 1966; Lancaster and Simco, 1967). Infestations are noted frequently in damp and mouldy grain, the product having lost most of its food value prior to the insect infestation. The lesser mealworm has been noted also as a secondary but chronic problem in poultry brooder houses where it breeds and develops in feed and in corn cob litter (Gould and Moses, 1951; Harding and Bissell, 1958). It is apparent, however, that its diet in this environment is not restricted to plant products (Harris, 1966). Harris noted that the lesser mealworm bore into and apparently feed on the flesh and internal organs of moribund and dead baby chicks. During this feeding they may become contaminated with the avian leukosis virus ("Marek's disease") (Eidson *et al.*, 1966). The leukosis is transmitted to healthy chickens when they eat the contaminated lesser mealworms.

The warm litter and poultry dropping mixture in brooder houses is an optimum environment for the culturing of many enteric bacteria especially *Salmonella* spp., *Escherichia coli*, and other microorganisms. It is important to determine the role that the lesser mealworm could play in the transmission of these two bacteria. The research reported in this paper covers laboratory experiments on the infection and quantitative recovery of *S. typhimurium* and *E. coli* (serotype Ola:H7) from lesser mealworms.

MATERIALS AND METHODS

Culture media. All media were prepared as described in the ninth edition of the Difco Manual of Dehydrated Culture Media and Reagents for Microbiological and Clinical Laboratory Procedures with the exception of 21% agar in the tomato juice medium.

Preparation of lesser mealworm. Lesser mealworm larvae were collected with the litter from several poultry brooder houses in Minnesota during the summer of 1967. The largest larvae were separated from the litter within 24 hours and each was placed in a separate 35 ml. glass vial. The larvae appeared nearly fully developed as indicated by size. Each vial was filled $\frac{1}{4}$ full with coarsely ground food composed of 50% Purina® dog food and 50% whole wheat flour. A freshly cut $\frac{1}{2}$ -inch cube of potato was placed in each vial every 2 days to provide moisture for the maturing larvae. The vials were then stored at $30 \pm 3^\circ\text{C}$. and a relative humidity of $75 \pm 25\%$ to await pupation of each insect.

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Only pupae 1-to-2-days old were selected for testing. Selected pupae were washed in various solutions for surface disinfection. The first washing was $1\frac{1}{2}$ to 2 minutes in 100 ml. of water containing 2% sodium hypochloride and 6 drops of Tween 80®, an emulsifier. The solution and pupae were both placed in a 100×15 mm. Petri dish. The Petri dish was closed, inverted quickly, and agitated by hand for 1 to 2 minutes. The solution was drained off by holding the dish on edge. Using this same procedure, the second washing was 20 seconds in 40 to 50% ethyl alcohol followed immediately by 3 to 4 washings of 20 seconds each in sterile water. To determine which pupae had not been disinfected, each was placed aseptically into separate Petri dishes containing sterile tomato juice agar. The plates were then incubated at 37°C. until the lesser mealworm emerged as adults 4 to 5 days later. Contaminated plates and insects were destroyed. Apparent surface disinfected adults remained on the tomato juice agar at 37°C. an additional 2 to 3 days to allow further opportunity to detect and remove contaminated specimens. Adult insects that still appeared surface disinfected were washed 1 minute with water containing 2% sodium hypochloride and 6 drops of Tween 80. After a final 3 to 4 washings in sterile water, the insects were used for testing.

Preparation of dog food medium. About 35 grams of coarsely ground Purina® dog food was placed in several 250 ml. Erlenmeyer flasks. To facilitate sterilization it was necessary to increase the moisture content of each flask of dog food by adding about 10 ml. of water. The flasks were closed with cotton plugs and loosely attached screwcap tops. Each flask was then autoclaved at 121°C. and 15 lbs. pressure for $\frac{1}{2}$ hour on two successive days. Between the 2 periods of autoclaving the prepared flasks were incubated at 37°C. to

enhance growth of any microorganisms present.

Preparation of inoculum. Glass test tubes containing 10 ml. of sterile nutrient broth were inoculated with *S. typhimurium* or *E. coli* or both. The tubes were stoppered with cotton plugs and incubated 16 to 18 hours at 37°C. After incubation 10 ml. of the designated bacterial growth suspension was introduced into the appropriate number of flasks containing the sterilized dog food. Each ml. of the *S. typhimurium* suspension contained 5 to 6 million viable bacteria and each ml. of the *E. coli* suspension contained 4 to 5 million viable bacteria. Untreated control flasks received only the sterile nutrient broth. A sterile cotton swab was used to mix each dog food and inoculant mixture. The flasks then received 2, 4, or 6 of the surface disinfected lesser mealworm adults after which they were incubated 4 days at 30°C. and 82% relative humidity in a small environmental chamber. During incubation the flasks were examined daily for contamination. Flasks free of extraneous contaminants were returned to the environmental chamber.

Sampling method. Dog food and insect samples were removed 4, 13, and 23 to 24 days after the above 4-day observation period. The dog food sample was about 1 gram measured volumetrically and was immediately diluted with a sterile saline solution. Two lesser mealworms were removed from each flask, placed aseptically in individual sterile Petri dishes and killed by exposure to -18°C . for 1 minute. Each was then washed separately in water containing 2% sodium hypochloride plus 6 drops of Tween 80, rinsed twice in sterile water, and mascerated individually in 10 ml. of sterile saline solution. Effectiveness of the technique was determined periodically by dipping some of the treated insects in test tubes of nutrient broth. If the broth became turbid within 24 hr, the in-

TABLE 1.—Number of *Salmonella typhimurium* colonies per adult lesser mealworm, *Alphitobius diaperinus* (Panzer) and per volumetric measurement (ca 1 gram) of Purina® dog food

Flask	Sampling Time days	Number of <i>S. typhimurium</i> colonies (×1000)			
		Dog food		Lesser mealworm ¹	
		Rep. 1	Rep. 2	Rep. 1	Rep. 2
1	4	59,000	11,800	900 ²	1,035
2	4	21,500	8,000	117	1,040
3	4	14,000	18,800	736	2,700
Untreated Control	4	0	0	0	0
1	13	17,100	8,900	3 ²	2,840 ²
2	13	6,500	14,500	1,282	3,535
3	13	4,000	10,100	947	1,300
Untreated Control	13	0	0	0	0
1	24	7,300	7,400	—	1,718
2	24	5,328	23,800	—	2,732
3	24	5,000	15,300	2	5,260 ²
Untreated Control	24	0	0	0	0

¹ Average of two samples from separate insects.

² One sample.

sect sample was considered contaminated and was discarded.

Serial dilutions of the mascerated insects and the dog food were pipetted into sterile Petri dishes. Brilliant Green agar was used to culture *S. typhimurium* and Desoxycholate Lactose agar was used to culture *E. coli*. The serial dilutions and selected medium or media were mixed by swirling the Petri dishes. The agar was allowed to solidify at room temperature after which the preparation was incubated at 37°C. for 24 to 48 hours.

Immediately following the 24 day sampling, six adult mealworms were removed from the flasks, killed by exposure to sub-zero temperatures and stored separately at room temperatures in sterile Petri dishes. After 45 days they were surface disinfected, mascerated, and checked for *S. typhimurium*.

RESULTS AND DISCUSSION

About 50% of the pupae subjected to the initial surface disinfection procedure were still contaminated with various microorganisms. The predominant contaminants were *Aspergillus flavus*, several fungi

in the *A. glaucus* group and yeasts identified as *Candida* spp. The relatively coarse surface of the pupa along with its many invaginations provides unlimited sites for the collection and harborage of various extraneous debris and associated microorganisms. The surface disinfection procedure had no apparent ill effect on the insects as indicated by their subsequent mortality rate or behavior.

The number of *S. typhimurium* colonies in the inoculated dog food decreased an average of 50% between the 4th and the 13th day after which it remained relatively constant (Table 1). Over the same period *S. typhimurium* counts increased from about 1 to 2.4 million per insect. Although the averages illustrate trends, individual counts per insect varied widely between replicates. This wide variation in counts was also apparent with several species of bacteria in insects immediately following their removal from natural field infestations. No explanation can be offered at this time for these variations.

S. typhimurium was recovered from each of the six mealworm adults that had been killed and stored in a sterile environment

TABLE 2.—Number of *Escherichia coli* (serotype 01a:H7) colonies per adult lesser mealworm, *Alphitobius diaperinus* (Panzer) and per volumetric measurement (ca 1 gram) of Purina® dog food

Flask	Sampling Time days	Number of <i>E. coli</i> colonies (×1000)			
		Dog food		Lesser mealworm ¹	
		Rep. 1	Rep. 2	Rep. 1	Rep. 2
1	4	50,000	80,000	5,590 ²	1,045
2	4	36,100	47,000	3,145	2,535
3	4	40,500	58,000	2,550	1,042
Untreated Control	4	0	0	0	0
1	13	40,000	21,000	330 ²	3,240 ²
2	13	96,000	2,700	1,095	9
3	13	67,000	13,700	187	1,427
Untreated Control	13	0	0	0	0
1	23	6,300	5	—	0 ²
2	23	3,238	107	—	46 ²
3	23	9,000	6,555	2,075	1,240 ²
Untreated Control	23	0	0	0	0

¹ Average of two samples from separate insects.² One sample.

for 45 days. The colony counts ranged from 50 to 435,000 per insect.

The number of *E. coli* colonies from the insects and dog food also varied considerably (Table 2). Average counts per insect appeared to decrease markedly between the 4th and the 13th day while average counts in the dog food decreased with increases in the sampling time.

Mixing cultures of *S. typhimurium* and

E. coli in the dog food apparently had no deleterious effect on the growth of the bacteria in the dog food or in the insects exposed (Table 3).

The capability of the lesser mealworm, alive or dead, as a carrier of *S. typhimurium* and *E. coli* is evident. Consequently this insect should be considered as a source of these and associated microorganisms in feed for animals and food for humans.

TABLE 3.—Number of *Salmonella typhimurium* and *Escherichia coli* (serotype 01a:H7) colonies per adult lesser mealworm, *Alphitobius diaperinus* (Panzer) and per volumetric measurement (ca 1 gram) of Purina® dog food

Flask	Sampling time days	Number of bacterial colonies (×1000)			
		<i>S. typhimurium</i>		<i>E. coli</i>	
		Dog food	Lesser mealworm ¹	Dog food	Lesser mealworm ¹
1	4	40,000	2,795	56,000	2,475
2	4	89,000	4,390	47,000	25
3	4	37,000	2,793	23,000	1,555
Untreated Control	4	0	0	0	0
1	13	98,000	5,100 ²	132,000	4,500 ²
2	13	127,300	9,800	115,000	11,380
3	13	50,000	10,192	125,000	7,125
Untreated Control	13	0	0	0	0

¹ Average of two samples from separate insects.² One sample.

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