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Salmonella Spp. and Serotypes of *Escherichia coli* Isolated from the Lesser Mealworm¹ Collected in Poultry Brooder Houses²

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ABSTRACT

Adult *Alphitobius diaperinus* (Panzer) were collected from the litter of poultry brooder houses in 1967 and 1968. One thousand of the lesser mealworms were individually surface disinfected, macerated, and cultured in thioglycolate enrichment broth at 37°C and streaked on eosin methylene blue agar or brilliant green agar plates for detection of *Escherichia coli* (Migula) Castellani & Chalmers and *Salmonella* spp., respectively. Five

species of *Salmonella* found within the lesser mealworms were identified as *S. heidelberg* Kauffman, *S. worthington* Edwards and Bruner, *S. saint paul* Kauffmann, *S. typhimurium* var. *copenhagen* Kauffmann, and *S. chester* Kauffmann and Tesdal. Forty-eight serotypes of *E. coli* were recovered from within 251 lesser mealworms. Twenty-six of these serotypes are known pathogens for man or animals.

The lesser mealworm, *Alphitobius diaperinus* (Panzer), is gaining importance as a contaminant of food and feed. Formerly it was known as a cosmopolitan and secondary infester of grain and related cereal products (Cotton 1956) but was later cited by Harding and Bissell (1958) as a chronic problem in poultry brooder houses. In this environment, the lesser mealworm breeds, feeds, and develops successfully in the mixture of warm litter and poultry droppings. Harris (1966) observed that the larval and adult stages also fed on the flesh and internal organs of dead and moribund birds. During this feeding they may become contaminated with the avian leukosis virus (Marek's disease) (Eidson et al. 1966, Lancaster and Simco 1967). The leukosis virus is transmitted to healthy poultry when they eat the contaminated lesser mealworms. Laboratory studies by De las Casas et al. (1969) indicated that the adult lesser mealworm was also a capable carrier of *Salmonella typhimurium* (Loeffler) and *Escherichia coli* (Migula) Castellani & Chalmers and should be considered a source of these and associated microorganisms. Subsequent studies were designed to examine field-collected lesser mealworms for different species of *Salmonella* and serotypes of *E. coli*. The results of these insect analyses are reported in this paper.

MATERIALS AND METHODS.—Lesser mealworms were collected during the summer of 1967 and 1968 from the litter in 5 poultry brooder houses situated within 100 miles of St. Paul, Minn. The poultry houses were of pole-frame construction with dirt floors covered with wood shavings for litter to a depth of ca. 10 cm. The houses ranged from 20 to 22 m wide and 60 to 120 m long.

Litter containing various stages of the lesser mealworm was removed from several infestation sites in each house. The infestation sites were usually adjacent to heating or watering areas or were under any object that would provide darkness, compacted litter, and relatively high moisture. The sample from each infestation site was isolated in a clean 4-liter glass jar and transported to the laboratory for analysis.

Only the adult lesser mealworms were tested for

bacteria. The litter and lesser mealworm mixtures were transferred from the collection jars to large pans. The adults were either picked up as they migrated out from the litter, or they were separated from the litter by sifting. Selected adults were confined to a 100×15-mm petri dish and killed by exposure to 6°C for 5–10 min. They were then washed immediately in 2 disinfecting solutions and sterile water as described previously (Harein and De las Casas 1968) to kill or remove bacteria from the exterior of their bodies. Effectiveness of the surface-disinfection technique was determined by dipping the treated insects into tubes of veal infusion broth. If the broth became turbid after a 24-hr incubation period at 37°C, the insect specimen was considered contaminated and was discarded.

The media used were thioglycolate enrichment broth, eosin methylene blue agar, and brilliant green agar. All media were prepared as described by Difco Laboratories, Inc. (1953).

A total of 1000 surface-disinfected adult lesser mealworms were individually and aseptically macerated in 2–3 ml of sterile saline solution using a mortar and pestle. Part of the resulting suspension was pipetted into tubes of thioglycolate enrichment broth and incubated at 37°C for about 20 hr. The broth was then streaked on eosin methylene blue agar plates for preliminary selection of *E. coli* colonies and on brilliant green agar for preliminary selection of *Salmonellae* colonies. The prepared plates were incubated 24 hr at 37°C. Suspect *E. coli* colonies were further analyzed by biochemical tests and serologically identified by a recognized antigen scheme (Kauffmann 1944, 1947, 1966; Edwards and Ewing 1962). Five suspect *Salmonellae* colonies were selected from each plate for biochemical identification followed by serological examination with "O" group antisera and the Spicer Edwards "H" antisera pools (Edwards and Ewing 1962, Kauffmann 1966). Final serological identification of the *Salmonella* species was done by the National Animal Disease Laboratory in Ames, Iowa.

RESULTS AND DISCUSSION.—*Salmonellae* were isolated from 22 of the adult lesser mealworms. This was 2.2% of the total population tested, and it represented 5 different *Salmonella* species (Kelterborn 1967) as listed in Table 1. Each species is a recognized pathogen for animals and humans (Oye 1964).

The percentage of lesser mealworms positive for *Salmonellae* may be significantly greater than the 2.2% reported. Because of the large number of sam-

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Table 1.—Species and frequency of occurrence of *Salmonella* recovered from 1000 surface-disinfected adult lesser mealworms collected in poultry brooder houses.

| Species of <i>Salmonella</i> | No. recoveries |
|---|----------------|
| <i>S. heidelberg</i> Kauffmann | 1 |
| <i>S. worthington</i> Edwards & Bruner | 3 |
| <i>S. saint paul</i> Kauffmann | 3 |
| <i>S. typhimurium</i> var. <i>copenhagen</i> Kaufmann | 4 |
| <i>S. chester</i> Kauffmann & Tesdal | 11 |

ples it was not practical to select more than 5 colonies from each plate. The *Salmonellae* colonies could have been missed, since *Citrobacter* spp. and *Proteus* spp. on brilliant green agar produce colonies resembling *Salmonella*.

Twenty-six pathogenic serotypes of *E. coli* were isolated from 151 of the lesser mealworms (Table 2). Most of these are pathogenic to poultry as originally isolated, tested, and classified by Siccardi (1965⁵). Thirty-five of the lesser mealworms carried other serotypes that are pathogenic to new-born calves and pigs (Glantz et al. 1959, Moon et al. 1966).

E. coli serotypes pathogenic to humans were also recovered from 30 of the lesser mealworms. The

⁵ F. J. Siccardi. 1965. Identification of disease producing ability of *Escherichia coli* associated with *Escherichia coli* infection of chickens and turkeys. M.S. thesis. University of Minnesota, St. Paul 104 p.

Table 2.—Serotypes and frequency of occurrence of pathogenic *E. coli* recovered from 1000 surface-disinfected adult lesser mealworms collected from poultry brooder houses.

| Serotypes | | No. recoveries |
|------------------------|--------------------------------|----------------|
| O antigen | H antigen ^a | |
| 026 ^{e,d} | H30 | 1 |
| 045 ^b | NM | 1 |
| 078 ^{b,c} | NM | 1 |
| 086 ^d | H48 | 1 |
| 0107 ^b | NM | 1 |
| 0119 ^{e,d} | H4 | 1 |
| 020 ^e | NM | 2 |
| 035 ^b | H8 | 2 |
| 064 ^b | H30 | 2 |
| 0105 ^b | H32 | 2 |
| 0140 ^{b,c} | NM | 2 |
| 0123 ^b | — | 3 |
| 0128 ab ^d | NM | 3 |
| 0141 ab ^{e,f} | — | 3 |
| 0826 | H8 | 4 |
| 09 ^e | NM | 5 |
| 017 ^e | NM (1) H18 (4) | 5 |
| 0102 ^b | — | 6 |
| 02 ab ^b | H31 (3) NM (4) | 7 |
| 0112 ab ^{b,d} | — | 7 |
| 0127 ^{b,d} | H29 | 8 |
| 0124 ^{b,d} | — | 9 |
| 088 ^b | NM (8) H8 (1) | 10 |
| 01 ab ^b | H10 (5) NM (1) H16 (6) H30 (2) | 14 |
| 08 ^{b,c} | H10 (13) NM (1) H11 (6) | 20 |
| 0135 ^b | H10 (29) NM (2) | 31 |

^a No. in parentheses is total recoveries with different "H" antigen.

^b Pathogenic to chickens (embryos and/or chicks).

^c Associated with diarrheal disease of pigs or calves.

^d Associated with diarrheal disease of humans.

^e Edema disease in pigs.

^f Enteritis of weaned pigs.

Table 3.—Serotypes and frequency of occurrence of non-pathogenic *E. coli* recovered from 1000 disinfected adult lesser mealworms collected in poultry houses.

| Serotypes | | No. recoveries |
|-----------|--------------------------------|----------------|
| O antigen | H antigen ^a | |
| 011 | H15 | 1 |
| 016 | H10 | 1 |
| 019 ab | H2 | 1 |
| 033 | H10 | 1 |
| 043 | — | 1 |
| 059 | H21 | 1 |
| 079 | — | 1 |
| 0109 | H10 | 1 |
| 0117 | H42 | 1 |
| 0131 | H2 | 1 |
| 029 | H10 | 2 |
| 049 | H10 | 2 |
| 05 | H4 | 4 |
| 0120 | H21 | 4 |
| 0×9 | H10 | 5 |
| 040 | — | 5 |
| 0110 | — | 5 |
| 0×6 | H10 | 6 |
| 025 | H18 (1) NM (3) H10 (1) H34 (1) | 6 |
| 0132 | NM (7) H2 (2) | 9 |
| 021 | H19 (3) H4 (7) H2 (2) | 16 |
| | H12 (1) H18 (2) H26 (1) | |
| 068 | H7 (23) H10 (1) | 26 |
| | H33 (1) H38 (1) | |

^a No. in parentheses is total recoveries with different "H" antigen.

potential importance of these serotypes to human diseases is emphasized by outbreaks of diarrhea in hospitalized humans resulting from enteropathogenic *E. coli* (Page and Stuiberg 1962). In addition, serologically distinct types of *E. coli* have been associated with epidemic and sporadic outbreaks of diarrhea in infants (Ewing 1956, 1962; Whitaker et al. 1958).

Twenty-two nonpathogenic serotypes of *E. coli* also were found in 100 lesser mealworms (Table 3). Serotypes 021, 068, and 0131 were present most frequently. Researchers in the Department of Veterinary Microbiology and Public Health at the University of Minnesota indicated that nonpathogenic serotypes such as 09, 021, 025, 040, and 068, as well as pathogenic serotypes, can be recovered also from air samples removed from poultry houses.⁶

In summary, the relatively constant migration of lesser mealworms in search of optimum feeding or breeding areas ultimately results in their exposure to most of the environment within poultry houses. As they concentrate in select areas their metabolic activity contributes to the temperature and moisture within the litter. This feature creates favorable sites for the survival and reproduction of microorganisms. Consequently, the lesser mealworm serves as an indicator for microorganisms in the poultry environment and can be considered a potential source and effective disseminator of pathogenic bacteria for man and animals.

⁶ Personal communication. 1969. Sadiq Mohamed. Present address: Department of Medical Microbiology—College of Medicine, University of Manitoba, Canada.

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