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TRANSMISSION OF EIMERIA, VIRUSES, AND BACTERIA TO CHICKS: DARKLING BEETLES (ALPHITOBIIUS DIAPERINUS) AS VECTORS OF PATHOGENS

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SUMMARY

Darkling beetle homogenates (DBH) were prepared from beetles collected from seven premises (farms). DBH were shown to contain myriad infectious organisms including bacteria (e.g., Salmonella), viruses (e.g., reovirus), and Eimeria (the causative agents of intestinal coccidiosis).

The present study establishes the fact that darkling beetles serve as vectors for common avian pathogens. Darkling beetles must be considered on a list of other vectors known to transmit common poultry pathogens. The risk posed by beetles with respect to dissemination of diseases is of immense importance to the poultry industry. The possibility of severe adverse economic impact as a result of these diseases should not be overlooked or casually dismissed.

Keywords: Alphitobius diaperinus, bacteria, chickens, darkling beetles, pathogens, poultry, Salmonella, virus

DESCRIPTION OF PROBLEM

Darkling beetles (also called litter beetles or black bugs) are the adult stage of the lesser mealworm Alphitobius diaperinus [1]. These beetles reside and reproduce within today’s modern poultry microenvironments, where their populations can reach astronomical proportions [2].

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Excessive populations of darkling beetles can cause financial losses for the poultry producer [2, 3, 4, 5, 6]. These losses center on the ability of darkling beetles to transmit (vector) poultry pathogens including bacteria (e.g., Salmonella, Escherichia coli), fungi (Aspergillus), and viruses such as infectious bursal disease virus (birnavirus), fowl poxvirus, Newcastle disease virus (paramyxovirus),...
and avian leukosis virus (herpesvirus). In addition, excessive beetle populations can adversely affect feed conversion ratios and chick body weight gains and can cause damage to poultry house insulation. Furthermore, excessive dry litter conditions created, at least in part, by the use of nipple-type drinkers has resulted in nocturnal darkling beetle attacks on live chickens as the beetles seek any and all sources of water [5]. As a consequence, beetle bites of the skin around feather follicles may cause lesions that resemble skin leukosis. Lastly, when beetle-infested litter is spread on pastures, large populations of adult beetles may reach near-by residences. Complaints and health concerns are passed from the homeowner to local health officials [7].

Poultry health and production personnel have recently become increasingly concerned about the spread of poultry diseases within and among poultry in their companies. The frequency with which darkling beetles serve as vectors for common poultry pathogens is not known. The purpose of the present study was to test darkling beetles for common poultry pathogens.

**MATERIALS AND METHODS**

**CASE MATERIALS**

The farms selected for the present study were known to harbor large populations of darkling beetles. At least seventy-five darkling beetles were collected from one broiler chicken house on each of seven premises (farms). Beetles were collected by hand into clean plastic bags, labeled with the farm name, chilled on ice, shipped frozen to our laboratory, and stored at -80°C. The seven beetle samples were always kept separate.

**EXPERIMENTAL DESIGN**

Specific pathogen-free chicks (SPAFAS, Inc, Storrs, CT) were hatched from eggs in a biosecure incubator and hatcher, wing-banded with a numerical code, placed into sterilized bioisolators, and given feed and water *ad libitum*.

Each bag of frozen beetles was assigned an alphanumerical code. Thawed beetles were weighed *en masse*, then placed into coded sterile plastic centrifuge tubes to which 30 mL of sterile physiologic buffered saline (PBS) were added. Beetles were macerated and homogenized with a hand held blender (Braun, Lynnfield, MA). A coded 10 mL aliquot was reserved for bacterial culture. Plate count methods were used to enumerate the bacterial profile.

**Aerobic Plate Count:** Beetle homogenate was inoculated onto tryptose agar and incubated at 35°C for 48 hr. All bacterial colonies were counted. Counts represent CFU/mL of homogenate.

**Coliform Count:** Beetle homogenate was inoculated onto MacConkey agar and incubated at 35°C for 48 hr. All pink-red (lactose fermentative) bacterial colonies were counted. Counts represent CFU/mL of homogenate.

**Gram Negative Bacterial Count:** Beetle homogenate was inoculated onto MacConkey agar and incubated at 35°C for 48 hr. All bacterial colonies were counted. Because of the bile salts and crystal violet dye, this medium is inhibitory to most all Gram + bacteria. Counts represent CFU/mL of homogenate. **S. aureus Count:** Beetle homogenate was inoculated onto Baird-Parker agar and incubated at 35°C for 48 hr. Only black colonies with zones were counted as *S. aureus*. Counts represent CFU/mL of homogenate.

**Enterococcal Count:** Beetle homogenate was inoculated onto bile esculin azide agar and incubated at 35°C for 48 hr. All black colonies were counted and represent putative enterococci. Counts represent CFU/mL of homogenate.

**Fungal (mold) Count:** Beetle homogenate was inoculated onto Sabaroud dextrose agar supplemented with 20 µg/mL chloramphenicol and incubated at 35°C for 3–5 days. All fungal colonies were counted. Counts represent CFU/mL of homogenate.

**Yeast Count:** Beetle homogenate was inoculated onto Sabaroud dextrose agar supplemented with 20 µg/mL chloramphenicol and incubated at 35°C for 3–5 days. All yeast colonies were counted. Counts represent CFU/mL of homogenate.

**Sorbitol Negative E. coli:** Beetle homogenate was inoculated onto MacConkey agar base supplemented with sorbitol and incubated at 35°C for 48 hr. Ten clear (sorbitol negative) colonies were inoculated onto MacConkey agar and incubated at 35°C for 24 hr. All pink-red colonies were confirmed biochemically to be *E. coli.*
**Research Report**

GOODWIN and WALTMAN

**Clostridium Count:** Beetle homogenate was inoculated into cooked meat media undiluted, and at a 1:100 dilution. The medium was incubated at 35°C for 48 hr. Tubes were observed for the presence of gas. All tubes were further checked for *Clostridium* by Gram stain.

**Salmonella:** Beetle homogenate was inoculated (1:10) into tetrathionate enrichment broth and incubated at 35°C for 24 hr. The broth was inoculated onto brilliant green agar with 20 μg/mL novobiocin and xylose lysine tergitol four agar. These plates were incubated at 35°C for 24 hr and observed for typical colonies. Three typical *Salmonella* colonies were identified and inoculated into triple sugar iron agar and incubated at 35°C for 24 hr. Each *Salmonella* culture was confirmed biochemically and serologically. If the original plating was negative, the original tetrathionate broth was left at room temperature for five days and 0.5–1.0 mL of broth was transferred to a fresh tube of tetrathionate broth and incubated once again at 35°C for 24 hr. The culture was then processed as described above.

Antibiotics (gentamicin sulfate, 0.25 mg/mL; penicillin, 1,000 u/mL; and streptomycin, 1 μg/mL) were added to the remaining beetle homogenate portions. The homogenates were frozen and thawed twice. Each of seven day-old chicks were given 0.5 mL of beetle homogenate by transpharyngeal cannulation of the esophagus (PO) and by intraperitoneal injection (IP). Control chicks were sham-inoculated with 0.5 mL antibiotics in PBS.

At 10 days post-infection (DPI), feces were collected by nylon loop catheterization of the rectum and examined for parasite ova/oocysts [8]. At 21 DPI, chicks were gently restrained, and blood samples were collected from medial ventral wing veins. Serum was obtained and processed for detection (ELISA, Kirkegaard and Perry Laboratories, Gaithersburg, MD) of common avian pathogen (reovirus [REO], coronavirus [IBV, IB], paramyxovirus [NDV, ND], birnavirus [IBDV, IBD], and herpesvirus [ILT]) antibodies. Findings were inventoried and tabulated.

**RESULTS AND DISCUSSION**

The present study establishes the fact that darkling beetles serve as vectors for common avian pathogens (Tables 1 and 2), a finding supported by the works of others [9, 10, 11, 12, 13, 14]. In addition, we have found that the frequency with which these beetles can carry detectable pathogenic viruses, coccidia, or bacteria is unexpectedly high. Darkling beetles must be considered on a list of vectors known to transmit common poultry pathogens. These pathogens also include bacteria such as *Salmonella*. The risk posed by beetles as disseminators of diseases is of immense importance to the poultry industry, and the threat of severe adverse economic

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**TABLE 1. Results from testing darkling beetle (*Alphitobius diaperinus*) homogenates for bacterial, fungal, and yeast pathogens**

| LABORATORY COUNTS | TEST RESULTS BY FARM |
|-------------------|----------------------|
|                   | 1        | 2        | 3        | 4        | 5        | 6        | 7        |
| Aerobes           | 1.2 x 10⁶ | 2.6 x 10⁶ | 3.3 x 10⁶ | 4.5 x 10⁷ | 3.6 x 10⁶ | 3.6 x 10⁶ | 1.4 x 10⁷ |
| Coliforms         | 4.0 x 10⁴ | 1.2 x 10⁴ | 9.0 x 10³ | 5.0 x 10⁴ | 2.0 x 10⁴ | 20       | 6.2 x 10⁴ |
| Gram negatives    | 4.5 x 10⁴ | 1.2 x 10³ | 1.8 x 10⁴ | 3.3 x 10⁵ | 8.2 x 10⁴ | 800      | 1.4 x 10⁷ |
| *S. aureus*       | >20      | <20      | <20      | >20      | >20      | <20      | <20      |
| Strep⁵⁵           | 5.9 x 10⁵ | 2.8 x 10⁵ | 1.3 x 10⁴ | <10⁵    | 1.9 x 10⁴ | 8.9 x 10⁵ | <10⁶    |
| Fungi             | <10⁴    | 400      | 20       | <10⁴    | 160      | 20       | 40       |
| Yeasts            | 400      | 600      | <20      | 400      | <20      | <20      | 4.8 x 10³ |
| E. coli           | <20      | <20      | <20      | <20      | <20      | <20      | <20      |
| Clostridium       | <200     | <200     | >200     | >200     | <200     | >200     | >200     |
| *Salmonella*      | negative | negative | positive⁶ | negative | negative | negative | negative |

⁵ Group D
⁶ Group C
impact from these diseases should not be overlooked or casually dismissed. Follow-up studies are warranted, perhaps to calculate the impact of beetle homogenate on production performance parameters and financial cost/loss estimates. One practical implication of our findings is that poultry producers should seriously consider incorporating darkling beetle abatement programs into their disease control programs.

| LABORATORY TEST          | TEST RESULTS BY FARM^A |
|--------------------------|------------------------|
|                          | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Viruses                  |   |   |   |   |   |   |   |
| reovirus (REO)           | Y | N | Y | Y | N | N | N |
| coronavirus (IBV, IB)    | N | N | N | N | N | N | N |
| paramyxovirus (NDV, ND)  | N | N | N | N | N | N | N |
| birnavirus (IBDV, IBD)   | Y | N | N | Y | Y | Y | N |
| herpesvirus (ILT)        | N | N | N | N | N | N | N |
| Protozoa                 |   |   |   |   |   |   |   |
| Eimeria sp.              | N | Y | Y | Y | Y | Y | Y |

^A Y = yes, N = no

**CONCLUSIONS AND APPLICATIONS**

1. Darkling beetles serve as vectors for common avian pathogens.
2. The incidence of beetle-vectored pathogens is unexpectedly high.
3. These pathogens include immunosuppressive viruses (birnavirus, the agent of infectious bursal disease) and bacteria such as Salmonella.
4. There is substantial risk posed by beetles with respect to dissemination of diseases. The poultry industry should consider the implications of this risk seriously.
5. The threat of severe adverse economic impact from beetle-vectored diseases should not be overlooked or casually dismissed.
6. Darkling beetle abatement programs should be incorporated into disease control programs and should eliminate beetle-vectored pathogens, thus decreasing poultry production costs and increasing poultry production profits.

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