THE LESSER MEALWORM BEETLE (*ALPHITOBIUS DIAPERINUS*) AS VECTOR OF *SALMONELLA* TO POULTRY: PRELIMINARY RESULTS

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Abstract

Lesser mealworm beetles (*Alphitobius diaperinus*) are frequently present in large numbers in poultry houses. They may be important as a reservoir and vector of various pathogens including *Salmonella* spp., which are responsible for many of the humane food-borne disease outbreaks. Previously it was shown by McAllister et al (1994) that under controlled laboratory conditions, *Salmonella* can survive in the digestive system of beetles for over 14 days. In controlled trials we were able to show that by force-feeding of *Salmonella*-free turkey poults or chicks with *Salmonella*-infected adult *Alphitobius diaperinus* beetles, the infection was distributed to the birds which subsequently showed clinical salmonellosis. The beetles were left for 7 days on mash feed contaminated with 10^6 CFU/g *Salmonella infantis*. On the 8th day, before feeding the birds with infected beetles, the beetles were externally disinfected with 70% ethyl alcohol followed by 2% sodium hypochlorite, which was proven to kill all *Salmonella* on the beetle's integument. The intestinal tract of the beetles still contained the pathogen. Each bird from a group of 10-15 was force-fed with 10 infected beetles, while 5-10 other birds served as non-contaminated controls and were fed non-contaminated beetles. Cloacal shedding of *Salmonella* started 1 or 2 days post infection, as mortality of poults due to *Salmonella* (none of the chicks died). To conclusively determine whether infected beetles can transmit *Salmonella* between consecutive flocks in a poultry house despite the cleaning and disinfection process, more trials need to be done. These would include determining the ability of the pathogen to survive the molting stages and metamorphosis of the host beetle, and examining the means and duration of *Salmonella* adherence to the cuticle of the beetle. This preliminary study suggests that beetles in poultry houses may be a potential vector of *Salmonella* to birds.

INTRODUCTION

The lesser mealworm beetles (Alphitobius diaperinus) are frequently present in large numbers in poultry houses (Kingsolver and Andrews, 1991; McAllister et al 1996). They may be a reservoir and vector of various pathogens of humans and animals, including *Salmonella* spp. which is known as one of the most important bacterial diseases of poultry and a problem of economic concern to the poultry industry (Snoeyenbos and Williams 1991, Barrow 2000), responsible for more microbial food-borne diseases outbreaks than any other bacteria (Todd 1985). Two studies preformed in Israel illustrated a strong correlation between the types and the frequency of *Salmonella* isolations from poultry sources with the type and frequency in humans (Sechter et al, 1978; Sechter and Hirschmann, 1988).

When the beetle larvae look for a place to pupate they burrow into any soft material including polyurethane thermal insulation. The adult beetles hide in the burrow and in crevices until a new poultry flock is introduced (Arends 1991, Axtell and Arends 1990, Samish and Argamann 1991). The adult starts to lay eggs on ground after the broiler house has been filled with chicks (Samish et al, 1994). Larvae and adults wander almost exclusively at night (Geden and Axtell, 1987).

Thus, the pupal and adult stages, hidden in the empty poultry house between cycles of birds, can evade the chemical control that is applied between flocks. This persistence between flocks may be in part due to the beetles carrying the pathogen from one flock to the following flock. It was shown that under controlled laboratory conditions, *Salmonella* may survive in the digestive system of adult beetles for more than two weeks (McAllister et al, 1994).

In the present study we demonstrated that the beetles can transmit *Salmonella* to chicks up to 4-7 days after their exposure to a contaminated environment. Therefore, one of the most interesting questions is whether the beetles serve merely as mechanical carriers of *Salmonella* in a contaminated environment, or whether they play an active role in enhancing the epidemiological effect of the pathogen. The experimental model is a simulation of a process by which infected beetles in a previously infected poultry house survive the cleaning and disinfection processes usually performed between flocks.

Actually this is an initial, preliminary step, in clarifying the possible role of the mealworm beetle in *Salmonella* epidemics in poultry houses. The main objectives of the present study were to learn whether *Salmonella*-infected beetles will transmit the infection to chicks thus start a flock infection process, and for how long after the exposure of beetles to the pathogen they will still be able to distribute the pathogens further.

MATERIALS AND METHODS

Bacteria. A known isolate of *Salmonella infantis* of avian origin was used as the pathogenic agent in all trials. This isolate was selected for the preliminary set of experiments because of its relatively low virulence to humans. The bacteria were cultured overnight on a nutrient broth, reaching concentration of $7x10^8$ CFU/ml.

Beetles. The lesser mealworm beetles (*Alphitobius diaperinus*) were collected in a commercial chicken house. Before and during the experiments the beetles were kept at an average temperature of 26°C with mash feed based on pelleted dog food with additives. The beetles were examined and found to be free of *Salmonella* before every experiment.

Choosing a disinfectant for surface sterilization of beetles. Rubber network sealed plastic tubes were filled with 15 adult and 15 larval beetles and dipped (with manual shaking) for 10 s in a nutrient broth (with 0.001% triton-x) of *Salmonella infantis* ($7X10^4$ CFU/ml). After a light wiping and 5 min at room temperature, the beetles were dipped (with manual shaking) for another 10 s if not otherwise said in one of the following disinfection treatments:

a. twice in 70% ethyl alcohol with 0.01% triton-x (with a delay of 10 s between immersions).
b. in 2% sodium hypochlorite with 0.01% triton-x.
c. in 70% ethyl alcohol with 0.01% triton-x, followed by a short rinse in sterile water and a second immersion in 1% sodium hypochlorite with 0.01% triton-x for 1 min.
d. the same as treatment c., but with a second immersion for 2 min in 2% sodium hypochlorite.
e. in 70% ethyl alcohol with 0.01% triton-x, followed by a short rinse in sterile water and a second immersion in 0.1% sodium benzoate with 0.01% triton-x for 5 min.

After each treatment the beetles were briefly rinsed in sterile water.

In addition, there were two control groups of adult or larval beetles: a negative control consisting of water with triton-x, and a positive control consisting only immersion in a *Salmonella* broth.

Thereafter, all the tubes with beetles were shaken strongly in a Rappaport-Vassiliadis medium for the enrichment of *Salmonella*. It may be assumed that most *Salmonella* microorganisms from the cuticle of the beetles were thus rinsed into the broth, which was incubated (without the beetles) for 24-48 h at 42°C. A drop of the medium surface was plated on brilliant green agar at 24 h, and again at 48 h, for a further 18-24 h incubation at 37°C. Colonies suspected to be *Salmonella* (red-colored) were plated on triple sugar iron agar for confirmation of the organism.

Treatment d. was the only one that totally sterilized the beetle's cuticle and therefore used in further experiments.

Testing the effect of disinfection agents on the viability of beetles. To ensure that the disinfection agent would not harm the beetles, 5 adult beetles and 5 larvae were subjected to all of the above experimental procedures without immersion in *Salmonella* broth. They were then kept for a few weeks in bottles with food and their viability recorded. All beetles remained always alive.

Preparing Salmonella-infested beetle's food and infested beetles. ~~and its examination of the mash and the beetles for Salmonella.~~ Ten ml of 1:100 diluted nutrient broth with *Salmonella* were mixed with 100g beetle's food, to a final dilution of about 10^6 CFU/g. The beetle's food was re-infected with *Salmonella* broth *(*half of the original dose) every 2 days. The same procedure was conducted without *Salmonella* broth to provide a control. The infected beetle's food and the control were divided into 2g bottles, each containing 10 adult beetles. The beetles were kept for 7 days with the contaminated or the control non-contaminated food before being fed to chicks (day of feeding is time 0).

Examining the beetle's food and the beetles for Salmonella. T~~T~~he beetle's food and the beetles were examined by immersing them into a Rappaport-Vassiliadis medium and plating a drop of the medium on brilliant green agar as described above. The beetle's cuticle was examined externally by strongly shaking of about 10 beetles in the medium and then incubating it. The internal parts of these beetles were examined after crushing them in the medium followed by incubation.

Birds: Turkey poults (Exp. 1) or chicks (Exp. 2) were examined by cloacal swabs and were free of *Salmonella* before the trials. They were kept in isolation units with water and food (starter mash No. 201) ad lib.

The experimental infection was performed by force-feeding of 15 turkey poults, 8-12-days-old (Exp. 1) or 10 chicks, 14-20-days-old, with 10 surface disinfected adult beetles to each bird, by inserting the insects directly into the crop with a cut plastic pipette. Control groups of 10 poults or 5 chicks, were force-fed with 10 adult beetles kept with non-contaminated food. Concurrently to the infection, the remainders beetles were surface sterilized and removed to new containers with clean beetle's food, on time 0, 7 days and 14 days in Exp. 1, or time 0, 2 days, 4 days and 6 days in Exp. 2. At these time intervals some of the beetles and their food were checked for *Salmonella* contamination. The beetle's food and the beetles (non-exposed and exposed) were examined for *Salmonella* before every experimental infection.

Examining the birds for

***Salmonella*.** Before infection, cloacal swabs were taken from all birds and were always free of *Salmonella*. Following infection, swabs were taken from the cloaca, between 2 and 12 days post infection in Exp. 1, and between 1 and 4 days post infection in Exp. 2, to test for *Salmonella*. Dead birds were necropsied and assessed for *Salmonella* infection by culturing liver swabs on blood and MacConkey agars, and culturing the guts as described for food and beetles. The surviving poults were euthanized 15 days post infection, and the surviving chicks, 7 days post infection, and were also assessed for *Salmonella* infection.

RESULTS

The results are presented in 3 categories: a. The recovery of *Salmonella* inside beetles, on their suface and/or in their food (excreted bacteria) (Table 1). b. The recovery of *Salmonella* from live birds (cloaca) (Table 2). c. The recovery of *Salmonella* from dead birds (liver, guts) (Table 3).

Table 1 summarizes the recovery of *Salmonella* from beetle's food and from surface sterilized crushed beetles immediately after keeping the beetles for 7 days (time 0) in *Salmonella*-contaminated or non-contaminated (control) food, and 7 and 14 days later in Exp. 1, or 2, 4 and 6 days later in Exp. 2. As mentioned, at each time interval (starting from time 0), the beetles were transferred after surface sterilization to new *Salmonella*-free food.

Table 1. *Salmonella recovered from beetle's food, beetles surface or from the inside of the beetles, immediately after keeping on contaminated food (time 0), and after various time intervals, with concurrent non-exposed control groups*

		NON-CONTAMINATED FOOD			CONTAMINATED FOOD		
Experiment no.	*Days after separating from contaminated food*	*Beetle's food*	*Beetles: External*	*Beetles: internal*	*Beetle's food*	*Beetles: external*	*Beetles: Internal*
1	0	-	-	-	+	-	+
	7	-	-	-	+	-	-
	14	-	-	-	-	-	-
2	0	-	-	-	+	-	+
	2	-	-	-	+	-	+
	4	-	-	-	+	-	+
	6	-	-	-	+	-	+

- = negative for *Salmonella*.
+ = positive for *Salmonella*.

Table 1 demonstrates internal self-purification of the beetles from infestation with *Salmonella* within 7 days after their separation from the contaminated food (Exp. 1). The beetles in Exp. 2 still contained the bacteria 6 days after this separation. The beetles shed bacteria into their food at least up to 7 days after separation from contaminated food but not after 14 days (Exp. 1). The surface sterilization was successful throughout the experiments as demonstrated by the negative results of the external bacteriological tests of the beetles.

Table 2 presents the shedding of *Salmonella* by chicks based on cloacal examination. The chicks were fed with beetles immediately after separation of the later from *Salmonella*-contaminated food or non-contaminated (control) (time 0) (Exp. 1, 2) or 2, 4 and 6 days post separation (Exp. 2). The birds, force-fed with 10 beetles each (non-exposed or *Salmonella*-exposed), shed *Salmonella* up to 12 days at least (Exp. 1, only one feeding) or up to 2-4 days (Exp. 2, four feedings).

Table 2. *Number of Salmonella-positive birds (shedding from the cloaca)/total tested after force-feeding with 10 beetles each (non-exposed or Salmonella-exposed), in various times after separation of the beetles from the non-contaminated or contaminated food*

Time post separation		*0*		*2 days*		*4 days*		*6 days*	
Experiment no.	*Days post force-feeding of birds*	*Non-exposed beetles*	*Exposed beetles*	*Non-Exposed Beetles*	*Exposed Beetles*	*Non-exposed beetles*	*Exposed beetles*	*Non-exposed beetles*	*Exposed beetles*
1	0	0/7	0/9						
	2	NT	2/4						
	3	0/7	2/11						
	6	0/5	1/9						
	9	0/6	0/7						
	12	0/5	2/5						
2	0	0/8	0/8	0/4	0/4	0/5	0/5	0/6	0/6
	1	0/5	1/9	0/5	2/10	0/5	1/10	0/5	0/10
	2	0/5	1/9	0/5	1/10	0/5	1/10	0/5	0/10
	4	0/5	0/9	0/4	2/10	0/5	2/10	0/5	0/10

NT = Not tested.

As demonstrated in table 2, the exposed birds started to shed *Salmonella* already 1 day post exposure (Exp. 2). Apparently most birds in both experiments did not shed the pathogen. In Exp. 2, only beetles during the first 4 days after their separating from contaminated food, were capable to infect the birds with *Salmonella*. The control non-exposed chicks were free of *Salmonella* in all groups in both experiments.

Table 3 presents the number of dead birds and *Salmonella* isolations among the birds force-fed with 10 non-exposed or *Salmonella*-exposed beetles each, post exposure of the poults or at the day of their euthanasia (15 days post exposure in Exp. 1, 7 days post exposure in Exp. 2). In Exp. 2, none of the chicks died after the exposure, therefore all the isolations are from euthanized chicks.

Table 3. *Number of dead or euthanized birds and isolations Salmonella out of all birds force-fed with 10 beetles previously exposed to Salmonella or non-exposed (control) beetles, post exposure of the chicks or on the day of their euthanasia.*

No mortality or *Salmonella* isolations were found in the non-exposed (control) group.

a. Exp. 1: All birds fed with beetles on day 0 post exposure of the beetles to *Salmonella*-contaminated food.

Days post feeding of birds	1	2	3	4	7	11	12	15 (eutanized)	Total
Total No. of birds	14	13	11	10	9	7	6	5	
No. of dead birds	1	2	1	1	2	1	1	5 eutanized	9
No. of dead birds positive to *Salmonella*	NT	2	0	0	0	1	NT	1	4

NT = Not tested.

* Euthanized birds were not included.

b. Exp. 2: Four groups of birds according to the day post *Salmonella*-exposure of the beetles. Since no birds died, all birds were euthanized 7 days post feeding.

Days post exposure of beetles to *Salmonella*	0	2	4	6	Total
Total No. of euthanized birds	9	10	10	10	39
No. of *Salmonella* – positive birds	8	9	5	0	22

No *Salmonella* isolations were found in the control non-exposed groups (Exp. 1 & 2).

As demonstrated in table 3, mortality of the foece-fed turkey poults started already 1 day post feeding. In poults, four *Salmonella* isolations were found out of 9 dead birds after the feeding with *Salmonella*-infected beetles. In the chicks, only those up to the third exposure (i.e. chicks exposed to beetles that their exposure to *Salmonella* ended 4 days ago) were positive at the day of euthanasia (about 90% of the chicks in the first two groups, and 50% in the third group were *Salmonella*-positive).

DISCUSSION AND CONCLUSIONS

This preliminary study confirms the assumption that eating of beetles exposed to *Salmonella* inside their food is capable to transmit the infection into chicks and to start a flock infection process. Though the most important *Salmonella* species are the human pathogens *S. enteritidis* and *S. typhimurium* (Le Bacq et al 1994, Berrang et al 1998), we used in this preliminary research *S. infantis* as a model species. The main objective of this preliminary study was to demonstrate for how long beetles that were previously infected with *Salmonella* can still transmit the pathogen to chicks through feeding. Several questions still arise: Is there a minimal dose of the microorganism that beetles have to eat in order to infect chicks? For how long can *Salmonella*-contaminating beetles remain pathogenic to chicks? Is there a process of cleaning an infected beetle after exposure to the pathogen? To what extant external factors (temperature, beetles activity etc.) may influence *Salmonella* survival (e.g. for how long can the beetle serve as vector of the organism if not surface sterilized?) Some of these questions were addressed and preliminary answers are presented, however more detailed experiments are required.

The preliminary trial, designed to find the best disinfectant for outer sterilization of beetles, without harming the beetles themselves, enabling to test the "internally infected beetles". Dipping the beetles in 70% ethyl alcohol followed by 2% sodium hypochlorite for 2 min was sufficient for absolute external sterilization. Still these beetles were infected with *Salmonella* in their guts and were carriers of *Salmonella*. Nevertheless, the question remains of whether the beetles serve merely as carriers of the pathogen in a contaminated environment, or whether they play a more active role in enhancing the epidemics by supporting propagation of the pathogen somewhere within their bodies. As the beetles loose the bacteria within a few days, its propagation within the beetles seems to be negligible if exists at all.

The experimental design that included the crushing of insects was appropriate to detect *Salmonella* inside the insects. The results obtained with turkey poults indicate that under that experimental design, one week was enough for the infected beetles to become free of *Salmonella*, when they were not further re-exposed to this pathogen. Chicks became infected and started shedding *Salmonella* already 1 or 2 days after feeding with 10 beetles that have been exposed to the

pathogen, as found also by McAllister et al (1994). An additional week was enough for the cessation of *Salmonella* secretion outside. When the first chicks in the group become infected, they may infect the others, thus it is difficult to discriminate between infection from the beetles or from infected chicks. Only part of the poults in the first experiment died due to the infection, while the others became carriers of this organism. In view of the results in both trials, it may be assumed that some of the birds, but not all of them, became infected already at the first days after been fed with infected beetles, and these birds distributed the infection further.

Shorter intervals between the exchanges of infected beetle's food by clean food, as was carried in the second experiment, resulted in infected beetles on day 6 after the first infection, which was the end of this experiment, but not in infected chicks. This probably means that the pathogen load in the 10 crushed beetles was too low to cause an efficient flock infection. McAllister et al (1994) showed viability of *S. typhimurium* in the digestive tract of the same beetles for over 14 days. These assumptions will be examined in further detailed experiments. In poultry houses, exposed beetles will probably be infected by *Salmonella* for a longer time, due to their hiding in holes and crevices, thus avoid the disinfecting agents used and in addition, avoid staying with their *Salmonella*-infected secretions.

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