Alphytobius diaperinus: un vector de bacterias patógenas en los gallineros argelinos

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Este estudio se ha conducido con el fin de determinar el potencial del gusano de harina, *Alphitobius diaperinus* (Panzer) como un depósito y vector para muchas bacterias patógenas de las aves, en los gallineros.
Muchos grupos de las bacterias (aerobias totales, Gram-negative, *Coliforms*, *Estafilococos* y *Estreptococos*) se han enumerado. Las contaminaciones de *Salmonelas* y *Campylobacters* thermophilic se han buscado tambien.
Los adultos y las larvas presentaron múltiples especies de las bacterias patógenas de las aves. El 5% de las superficies de los adultos eran *Salmonelas Arizonae* - positivo y *Campylobacters thermophilic* no fueron identificados.
Los interiores de ambos adultos y larvas fueron contaminados más con bacterias Gram-negative, *Coliformes y Estreptococos* que sus superficies.
Este insecto es una fuente importante de las bacterias patógenas de las aves en nuestra región y las medidas serias se deben poner en ejecución para controlarla.

Palabras clave: *Alphitobius diaperinus* ; bacterias patogenas ; gallineros; Argelia

This study has been conducted on the potential of the lesser mealworm *Alphitobius diaperinus* (Panzer) as a reservoir and vector for many poultry pathogenic bacteria, in algerian broiler houses.
Many groups of bacteria (Total aerobic bacteria, Gram-negative bacteria, *Coliforms*, *Staphylococci* and *Streptococci*) have been enumerated; *Salmonella* and *Thermophilic Campylobacters* contaminations have been searched.
Both adults and larvae carried and harbored multiple species and numbers of poultry pathogenic bacteria. 5% of adults' surfaces were *Salmonella arizonae* - positive and no *Thermophilic Campylobacters* were identified. Interiors of both adults and larvae were more contaminated with Gram-negative bacteria, *Coliforms* and *Streptococci* than their surfaces.
This insect is an important source of poultry pathogenic bacteria in our region and serious measures must be implemented to control it.

Keywords: *Alphitobius diaperinus* ; pathogenic bacteria ; broiler houses; Algeria

Introduction

The lesser mealworm *Alphitobius diaperinus* is one of the most predominant poultry litters inhabiting insect species all over the world. Its biotope, behavior and feeding habits have incriminated it in carrying and accumulating large populations of poultry pathogenic organisms.

In this paper we have studied the interior and surface contaminations with poultry pathogenic bacteria of both adults and larvae *Alphitobius diaperinus* collected from twenty poultry houses in the

North-East of Algeria, with the aim to attract attention to this pest as a serious threat to poultry health in this region.

Material and methods

Samples collection: Insects were collected from twenty naturally infested poultry facilities located in the Province of Constantine (North-East of Algeria). On each poultry house, adults and larvae *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) were collected separately from the litter in three different locations, conditioned in sterile wide mouth bottles and frozen.

Detection of bacteria on the surface of adults and larvae: 1 g of adults and 1 g of larvae were placed separately with 10 ml of sterile buffered peptone water (BPW) and vortexed for 30 seconds to obtain main washing solutions. Dilutions were performed until 10^{-6} for plating on appropriate media. After that adults and larvae were moved from the washing solution and kept independently in other sterile wide mouth bottles.

Detection of bacteria in the interior of adults and larvae: The same 1 g of adults and 1 g of larvae used in the previous analysis were separately washed three times in 15 ml of sterile distilled water, then superficially disinfected by serial treatment of ethanol and hydrogen peroxide (As described by Crippen and Sheffield, 2006), after that they were rinsed three times in 15 ml of sterile distilled water to remove any traces of disinfectants. Each sample was macerated and homogenized with a hand blender in 9 ml of sterile BPW. Dilutions from these main macerating solutions were generated as described previously.

Bacteriological analysis: bacterial counts utilized standard plate counting techniques. Media used included tryptic soy agar (for total aerobic flora counts), MacConkey`s agar (for gram-negative bacteria and total coliforms counts), Baird-Parker agar (for Staphylococci + Micrococci counts) and Bile Esculine Azide agar (for Streptococci counts).

- Thermophilic campylobacters: 0.1 ml of each main washing and macerating solution was inoculated onto Campylobacter Selective agar (supplied with 5% of defibrinated blood/l of medium, 2 mg of vancomycine, 50 µg of polymixine and 1 mg of trimethoprim as selective additive) and incubated for 48h at 42°C under microaerobic conditions (using gas packs). For confirmation, the following tests have been used: oxidase, catalase, hippurate and Gram strain.
- *Salmonella spp* : The detection of salmonella in the washing and macerating main solutions of adults and larvae, was conducted according to the following scheme (**Table 1**):

Table 1: Horizontal detection of *Salmonella spp* from the main washing and macerating solutions of adults and larvae *A. diaperinus*.

<table>
<tr><td colspan="4">Incubation of main solutions (MS) at 37°C for 24h</td></tr>
<tr><td colspan="2">0.1 ml of each MS into 10 ml of Rappaport –Vassiliadis enrichment broth. At 42°C for 24h.</td><td colspan="2">1 ml of each MS into 10 ml of Muller – Kauffman enrichment broth. At 37°C for 24h.</td></tr>
<tr><td>XLD agar. At 37°C for 24h.</td><td>Hecktoen agar. At 37°C for 24h.</td><td>XLD agar. 37°C for 24h.</td><td>Hecktoen agar. At 37°C for 24h.</td></tr>
<tr><td colspan="4">05 suspect colonies from each plate, streak on Nutrient agar. At 37°C for 24h.</td></tr>
<tr><td colspan="4">Biochemical confirmation (API system 20 E)</td></tr>
<tr><td colspan="4">Serological confirmation</td></tr>
</table>

Results and discussion

The present results prove that both adults and larvae *A. diaperinus* carry and harbor high levels of multiple poultry pathogenic bacteria (**Table 2**). Interiors of both adults and larvae were more contaminated with gram-negative bacteria, coliforms, and streptococci; these bacteria could be natural

inhabitants of the intestinal tract of both adults and larvae of this insect. Larvae exteriors were more contaminated with staphylococci + micrococci. 5% of adults' surfaces were *Salmonella arizonae* positive and no thermophilic campylobacters were identified. The fact that external surfaces of both adults and larvae were more infected with total aerobic flora, suggests the presence of other bacteria groups not searched in our study.

Our results are in agreement with other studies. Goodwin and Waltman (1996) found within 75 adults macerated in 30 ml of sterile buffered saline: up to $4.5x10^7$ aerobic bacteria/ml, $1.4x10^7$ gram-negative bacteria/ml, $6.2x10^4$ coliforms/ml, less than 20 *Staphylococcus aureus*/ml and $9x10^6$ *Streptococcus spp*/ml. Only one of the seven tested samples was *salmonella spp* positive.

Table 2: Average external and internal contamination levels of adults and larvae *A. diaperinus*.

	Total Aerobic Bacteria	***Gram - Negative Bcteria***	***Coliforms***	***Staphylococci & Micrococci***	***Streptococci***	***Salmonella spp***	***Thermophilic Campylobacters***
Adults Exterior	9.4 Log UFC/g	5.3 Log UFC/g	4.8 Log UFC/g	5.7 Log UFC/g	6.3 Log UFC/g	5% *S. arizonae* positive	Negative
Adults Interior	8.4 Log UFC/g	7.5 Log UFC/g	7.4 Log UFC/g	6.2 Log UFC/g	8 Log UFC/g	Negative	Negative
Larvae Exterior	9.8 Log UFC/g	4.7 Log UFC/g	4.4 Log UFC/g	6.4 Log UFC/g	7.4 Log UFC/g	Negative	Negative
Larvae Interior	8.3 Log UFC/g	7.8 Log UFC/g	7.3 Log UFC/g	6.1 Log UFC/g	8.1 Log UFC/g	Negative	Negative

A wide range in the number of bacteria were found within a single adult beetle and some harbored several thousand colonies of *Micrococcus spp*, *Streptococcus spp* and *Bacillus subtilis* which were the most gram-positive isolated bacteria (De Las Casas *et al*., 1972). Segabinazi *et al.* (2005) isolated 14 species of the Enterobacteriacae family (including *Escherichia coli*, *Yersinia enterocolitica and Salmonella sp*...). The bigger numbers and the greater diversity were recorded from the external surface of the adult beetles (in contradiction with our results and which can be explained by the use of a different surface disinfection method).

- *E.coli*: Adults and larvae *Alphitobius diaperinus* have been shown able to harbor *E. coli (Migula)* on their external and internal body for 12 days. Consuming infected larvae caused more positive chicks than feeding on infected adults (McAllister *et al*. 1996). Up to 48 serotypes of this bacterium have been isolated from adult beetles and 26 serotypes among them are known to be pathogenic for animals and men (Harein *et al*., 1970).

***-Campylobacter spp*:** Even though we haven't found any campylobacter in this study; Bates *et al*. (2003*)* isolated a large number of campylobacter serotypes with some genetically common isolates between the broiler flock and the beetles. All campylobacter-positive beetles were always related to a campylobacter-positive flock (Skov *et al*., 2004). This bacterium has been recovered from the interior of larvae for 72 hours and from their exterior for 12 hours post exposure. 90% of birds that consumed 10 infected beetles or larvae became campylobacter-positive (Strother *et al*., 2005).

-Salmonella Spp: Skov *et al*. (2004) found the identical genotype of *S. indiana* in broilers and beetles collected from the same poultry house. They could also isolate *Salmonella spp* from beetles collected during the empty period between flocks. The bacterium has been isolated from the interior and the exterior of both adults and larvae through 16 days of exposure (McAllister *et al*., 1994). Other salmonella serotypes have also been identified from the lesser mealworm: *S. heidelberg*, *S. worthington*, *S. saint paul*, S. *typhimurium* var. Copenhagen and *S. chester* (Harein *et al*., 1970). Baggesen *et al*. (1992) concluded that the elimination of salmonella infections from a contaminated poultry house could not be successful until eradication of *A. diaperinus* was complete.

Conclusion

In conclusion; both adults and larvae *A. diaperinus* can acquire and harbor high amounts of several poultry pathogenic bacteria then transmit them to chicken flocks and participate to their spread on and between poultry farms. Serious control measures seem to be a necessity in our region, to reduce infectious agents' transmission risks by this insect.

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