Importance of *Alphitobius diaperinus* (Panzer) as a Reservoir for Pathogenic Bacteria in Algerian Broiler Houses

A. Agabou[1] and N. Alloui[2]

1. PADESCA Laboratory, Veterinary Institute, University of Constantine, 25000, Algeria
2. ESPA Laboratory, Veterinary Department, University of Batna, 05000, Algeria
* Corresponding author e-mail: amirveto@gmail.com

Abstract

During 2008, a study has been conducted on the potential of Alphitobius diaperinus as a reservoir of many poultry pathogenic bacteria, in twenty broiler houses in the North-East of Algeria. Many groups of bacteria 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. As this insect is an important source of poultry pathogenic bacteria in our region, serious measures must be implemented to control it.

Key words: 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.

Materials 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:

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

Incubation of main solutions (MS) at 37°C for 24h

0.1 ml of each MS into 10 ml of Rappaport –Vassiliadis enrichment broth. At 42°C for 24h.

XLD agar. At 37°C for 24h.

Hecktoen agar. At 37°C for 24h.

1 ml of each MS into 10 ml of Muller – Kauffman enrichment broth. At 37°C for 24h.

XLD agar. 37°C for 24h.

Hecktoen agar. At 37°C for 24h.

05 suspect colonies from each plate, streak on Nutrient agar. At 37°C for 24h.

Biochemical confirmation (API system 20 E)

Serological confirmation

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 4.5x107 aerobic bacteria/ml, 1.4x107 gram-negative bacteria/ml, 6.2x104 coliforms/ml, less than 20 Staphylococcus aureus/ml and 9x106 Streptococcus spp/ml. Only one of the seven tested samples was salmonella spp positive.

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

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

	Total Aerobic Bacteria	***Gram Negative Bacteria***	***Coliform spp***	***Staphylococci & Micrococci***	***Streptococci***	***Salmonella***	***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 Int*erior*	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 Ext*erior*	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
L*arvae* Ext*erior*	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

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.

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