

ISSN: 1936-6019

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## Research article

# Antibody titers to bed bug salivary proteins in chickens from an infested broiler breeder house

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Received: 30-III-2016 Accepted: 31-III-2016

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### Abstract

Bed bugs (Hemiptera: Cimicidae) might be significant pests in poultry operations, especially in broiler breeder houses. In this study, chickens in breeder houses, known to be infested with bed bugs, demonstrated antibodies to bed bug salivary gland extract, while those not exposed to bed bugs, did not. Antibody titers ranged from 1:160 to 1:640. These results document for the first time that chickens in commercial production, when exposed to bed bug biting, produce antibody responses to proteins contained in bed bug saliva.

**Key Words:** Poultry, broiler breeder houses, bed bugs, antibodies, immune response

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### Introduction

Bed bugs (Hemiptera: Cimicidae) are blood-sucking insects that feed on blood of warm-blooded animals such as humans, bats, and birds (Usinger 1966, Miller 2008). There has been a dramatic increase and spread of these pests since the 1980's (Potter 2006, Anderson and Leffler 2008) and now they are frequently reported in U.S. hotel rooms, dorms, and apartments. Bed bugs primarily feed at night, hiding in crevices during the day, and take about 5 to 10 minutes to ingest a full blood meal. They can survive long periods without feeding, and when their preferred human hosts are absent they might take a blood meal from other animals, including pets and poultry (Little and West 2008, Steelman et al. 2009). Bed bugs (*Cimex lectularis* L.) might become significant pests in poultry houses, especially broiler breeder houses, where they cause anemia and decreased egg production (Cater et al. 2011). The objective of this study was to evaluate antibody titers to bed bug salivary proteins in the blood of 6 chickens sampled during August 2013 in a house known to have heavy bed bug infestations versus titers of 6 chickens in a house without bed bugs.

### Methods

Both breeder houses are located in central Mississippi. The poultry house that was infested had been visited on several occasions due to a severe bed bug problem (bed bugs plainly visible inside the house). Blood was collected from the wing vein of birds located in the middle of each house, bled with a 22 gauge 1-inch needle on a 3 cc syringe, and placed in serum separator tubes (no anticoagulant). Blood samples were allowed to clot at room temperature for 2 hours, and then refrigerated prior to centrifugation. Sera

were screened by the indirect fluorescent antibody test (IFA) test according to previously published protocols (Edwards et al. 2011) using extract from macerated bed bug salivary glands (SGE). SGE was smeared on 12-well slides as antigen, with various dilutions of chicken sera in the primary; and for the secondary, fluorescein-labeled goat anti-chicken IgG (KPL Labs, Gaithersburg, MD) diluted 1:80. For a positive control, we applied high-titered human serum (diluted 1:80) from a previous project (Goddard et al. 2015) to the SGE smears followed by 1:100 fluorescein-labeled goat anti-human IgG (KPL Labs, Gaithersburg, MD). The negative control was identical except tick gut smears were used as antigen on the slides.

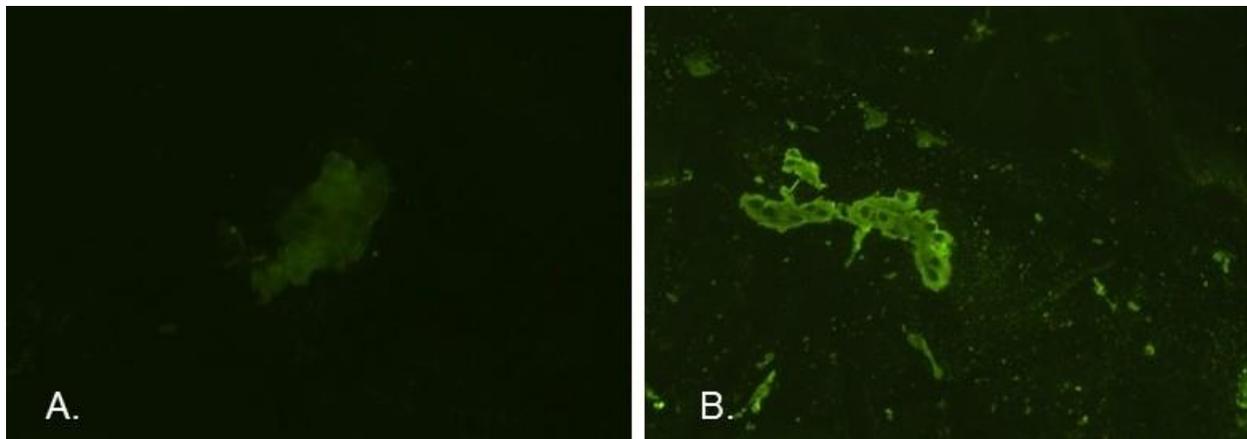
**Results and Discussion**

None of the chickens in the non-infested breeder house showed antibodies to bed bug SGE by the IFA method (Table 1). In the negative samples, salivary gland tissue could be visualized, but showed no specific fluorescence (Figure 1). One of the 6 serum samples from the infested house had to be excluded due to low volume, but of the 5 tested, 4 of them (80%) showed antibodies to SGE ranging from 1:160 to 1:640 (Figure 1). Sera were not evaluated below 1:160, so theoretically even our negative sample (slide #2-4) might have contained some antibodies. These results document for the first time that chickens in commercial production, when exposed to bed bug biting, produce antibody responses to the proteins contained in bed bug saliva. This information might be useful for diagnostic purposes if bed bug infestations are not severe enough for the parasites to be observed. However, to what extent, if any, antibody titers to SGE correspond to lower egg production is unknown at this time, and deserves further investigation. Persons involved in poultry production throughout the U.S. should be aware of this new and increasing threat, and establish plans for bed bug prevention and control should an infestation occur.

**Table 1.** Results of IFA analysis on 6 chicken sera from non-bed bug infested (1) and 6 from bed bug-infested breeder houses (2).

Slide	1:160	1:320	1:640	1:1280
1-1	-	-	-	-
1-2	-	-	-	-
1-3	-	-	-	-
1-4	-	-	-	-
1-5	-	-	-	-
1-6	-	-	-	-
2-1	n/a*	n/a	n/a	n/a
2-2	+	-	-	-
2-3	+	-	+	-
2-4	-	-	-	-
2-5	+	+	-	-
2-6	+	+	-	-

\*Not enough serum to test



**Figure 1.** Indirect fluorescent antibody staining of bed bug salivary gland extract using chicken serum from a non-infested house (A) and an infested house (B).

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