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Report

Exposure of Laboratory Mice to Bed Bug Bites and Salivary Gland Extract

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Abstract: An animal model to study cutaneous reactions to bed bug biting is currently not available. This study was initiated to evaluate Swiss-Webster (outbred) mice as a model organism for research on reactivity to bed bug bites and bed bug salivary gland extract (SGE). In this study, 1-2 bed bugs were fed on each of eight mice in a location on their backs which had been shaved; eight mice were injected intra-dermally (ID) with SGE in a similar location, and four control mice had phosphate buffered saline (PBS) injected also in a similar location. This procedure was repeated 3 times using the same mice over a 48-day period, after which all mice were humanely euthanized, bled, and punch biopsies taken from the locations on their backs. During the study, none of the mice developed any outward signs of illness or distress. Further, none showed cutaneous reactions attributable to bed bug bites or SGE injection. After the mice were euthanized, pathology of the skin punch biopsies revealed no specific allergy or inflammatory reactions in any of the sections. Also, there were no specific differences reported by the pathologist in skin samples among mice. All mice skin biopsies had sentinel (resident) numbers of mast cells, lymphocytes, and macrophages. Enzyme-linked immunosorbent assay (ELISA) testing for presence of antibodies was negative as well, with no reactivity by sera of any mice to SGE used in the ELISA; all results were no different from that of the negative controls used in the test. Results of this study indicate that either: 1) thresholds of bed bug biting and/or exposure to SGE proteins to elicit an immune response in mice are much higher than that in humans, or 2) Swiss-Webster mice are not very reactive to bed bug bites or SGE proteins. If the latter is true, then Swiss-Webster mice are not a good model to pursue for studies of cutaneous reactions to bed bugs.

Key Words: Bed bugs; Bite reactions; Laboratory Mice; Saliva; Lesions: Mice

Introduction

Bed bugs have increasingly been reported as causes of cryptic insect biting in hotels, apartments, and single-family dwellings (May 2007, Miller 2008, Goddard and de Shazo 2009a, Potter et al. 2010, Doggett et al. 2012). Cutaneous reactions to bed bug bites historically have been considered little more than a nuisance; however, a recent study revealed that bed bugs sometimes cause a local, highly destructive,

cutaneous reaction similar to that occurring in Churg-Strauss vasculitis syndrome (a rare disease that involves inflammation of medium and small blood vessels) (de Shazo et al. 2012). Accordingly, new information is needed to delineate the pathophysiologic mechanisms related to bed bug bite lesion development in both humans and animals. Our lab has been studying the health effects of bed bugs for some time (Goddard and de Shazo 2009b, Goddard et al. 2011, Goddard and de Shazo 2012), and recently developed a technique to remove salivary glands from bed bugs for in-vitro analysis of cytokines and chemokines produced by immune cells in response to salivary proteins (Goddard et al. 2013). Further, we have studied effects of bed bug saliva on human skin using bed bugs with and without intact salivary glands (Goddard and Edwards 2013). In this current study, we evaluated laboratory mice as models for studies of cutaneous reactions to bed bugs and to compare murine skin reactions to those we have already reported in people.

Materials and Methods

Mice procedures. Since laboratory mice (*Mus musculus*) have been the major model for allergy studies over many years, and since commercial reagents/antibodies are readily available, we chose Swiss-Webster (outbred) mice as our model organism. Twenty healthy, 6-week-old female Swiss-Webster (*Mus musculus*) mice, purchased from a USDA licensed dealer and ranging in weight from 20-30 grams, were housed and cared for according to our Mississippi State University Institutional Animal Care and Use Committee (IACUC) approval (MSU IACUC # 13-039) from May 15, 2013 through July 18, 2013. We used all female mice because they are thought to be more tractable and using a single sex reduces the likelihood of interactions among the mice. These mice were “outbred” meaning they are more reflective of animals in the wild. Dr. Lucy Senter, University Laboratory Animal Veterinarian, MSU Laboratory Animal Resources and Care (LARAC), provided oversight and medical care for the mice throughout the study.

Four mice each in two replicates (8 total) were used for bed bug feeding; four mice each in two replicates (8 total) for dermal injection of bed bug saliva; and two mice each in two replicates for controls (4 total) were used in this study. Overall, during each event, 1-2 bed bugs were fed on each of eight mice; eight separate mice were injected intra-dermally (ID) with extract from bed bug salivary glands in a location on their backs which had been shaved, and four control mice had phosphate buffered saline (PBS) injected in a similar location (Figure 1). “Injections” consisted of dispensing a small drop on the tip of a 28-gauge insulin syringe and then puncturing the mouse’s skin within the shaved location. To restrain mice during bed bug feedings and blood collection, they were initially anesthetized by isoflurane inhalation (Figure 2), 4-5% for induction, and 1-2% maintenance, followed by ketamine (80-120 mg/kg) plus xylazine (Rompun®) 5-10 mg/kg IP (Table 1). Once anesthetized, the location to be injected or for placing bed bugs was shaved with electric clippers and the assigned treatment procedure was performed. After the first round of feeding or injection, mice were monitored for 19 days for development of skin lesions and then fed upon/injected again. After another 13 days, the procedure was repeated. All animals in this study were monitored daily and to determine whether they were distressed (lethargic, dull or matted hair coat, decreased consumption of food and water, weight loss, trembling, twitching, panting, isolation, or decreased movement). At the end of the study (another 16 days, total = 48 days), all mice were euthanized and punch biopsies taken at the actual bite or injection site(s) for sectioning and pathology studies (Figure 3). In addition, blood was collected from each mouse via an intra-cardiac puncture for an enzyme-linked immunosorbent Assay (ELISA) to estimate antibody titers against bed bug salivary proteins (see next section).

Figure 1. Injection of bed bug salivary gland extract and bed bug adults feeding.

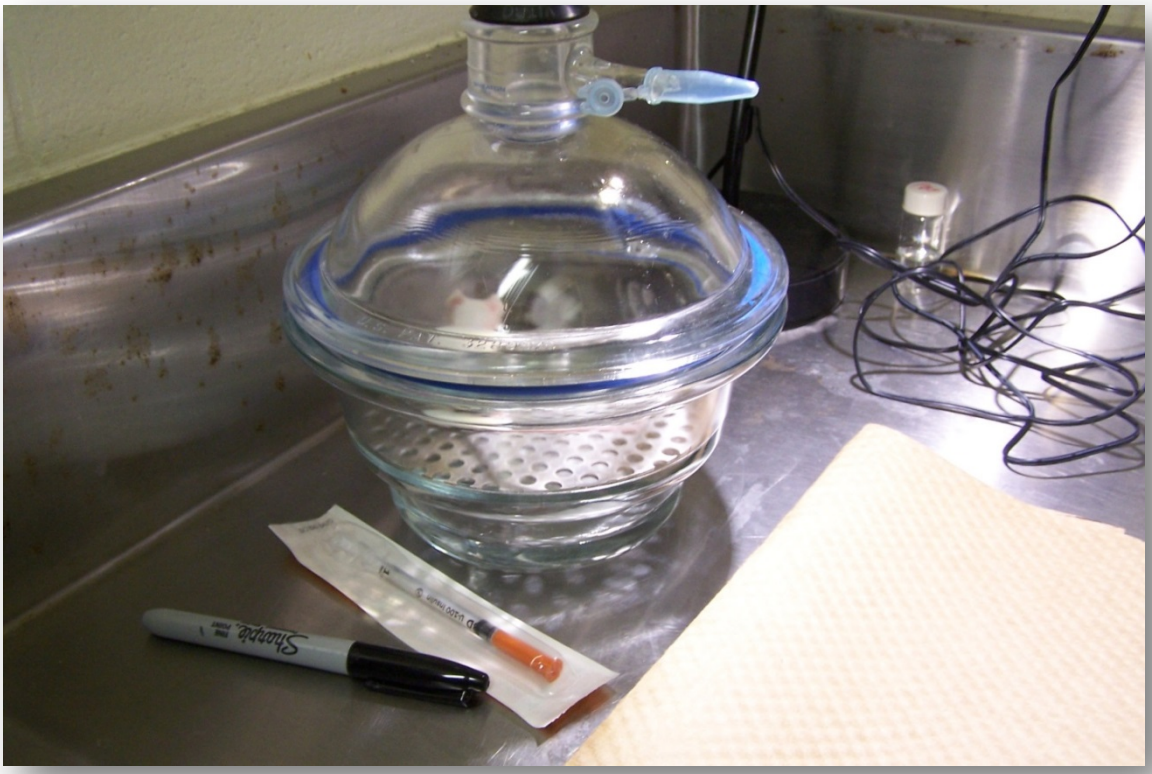

Figure 2. Administration of isoflurane for anesthesia.

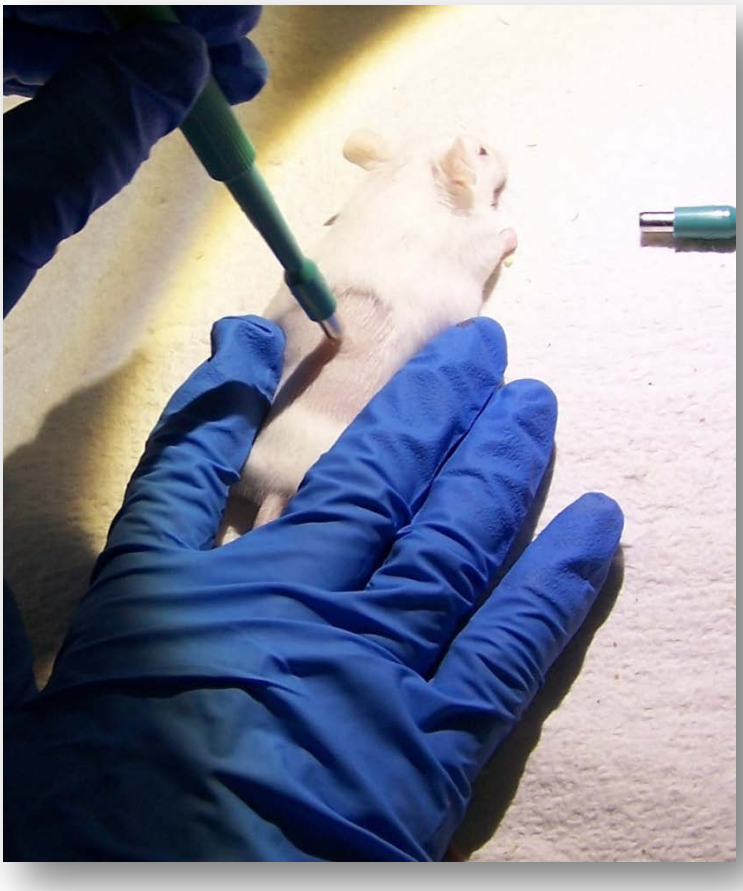

Figure 3. Punch biopsies of bite and injection sites.

ELISA procedures. For detecting antibodies in mouse sera, 96-well Costar high-binding EIA plates were coated with SGE at a concentration of 0.5ug per 100µl of carbonate (50 mM $NaHCO_3)_{buffer}$ and incubated overnight at 4°C. Plates were washed 3 times with 200 µl PBS/Tween. Blocking was performed by adding 200 µl of wash buffer + 5% nonfat dry milk and placing the plate on a rotator for 2 hours at 150 rpm. Plates were again washed (3x 200 µl wash buffer) and 100 µl of mouse serum diluted to 1:200 was added to the wells and the plate was placed on a rotator for 1 hour at 150 rpm. Plates were again washed (3x 200 µl wash buffer) and anti-mouse IgG, IgM, IgA. Secondary antibody (Life Technologies) was added at a dilution of 1:1000 in 200 µl of blocking buffer. Following another wash (3x 200 µl wash buffer), equal parts of TMP Peroxidase Substrate + Peroxidase Substrate Solution B (Kirkegaard & Perry Labs) were added at 100µl/well. Exactly 10 minutes later, 100µl stop buffer (1M HPO_4) was added. Plates were read at 450nm on an ELISA plate reader (Biotek instruments).

Pathology procedures. Cutaneous punch biopsies from the mice were submitted to the Mississippi State University College of Veterinary Medicine for standard pathologic examination by the third author. Samples were fixed in 10% neutral buffered formalin for 7 days, then cross-sectioned, processed, embedded in paraffin, sectioned in 5µm slices, and stained with hematoxylin and eosin. In addition, all skin samples were also stained with Giemsa stain.

Results and Discussion

One of the control mice (# 1.3) died during anesthesia on the first day (day 0) of the experiment. Otherwise, on that day, all intradermal injections with SGE or PBS were successful (11 mice) and 1 bed

bug each fed on 7 mice. No bed bugs fed on mouse # 4.5 (repeated attempts were unsuccessful). At the second feeding or injection day (Day 19), all intradermal injections with SGE or PBS were successful (11 mice) and 1 bed bug each fed on 7 mice; mouse #4.5 had 2 bed bugs feed on it. At the third feeding or injection day (Day 32) all intradermal injections with SGE or PBS were successful (11 mice) and 1 bed bug fed on mouse #4.2; all other mice with bed bugs placed on them had 2 bed bugs each feeding on them.

During the 48-day study, none of the mice developed any outward signs of illness or distress. Further, none developed cutaneous reactions to the PBS "injections" or bed bug SGE (Figure 4), with the exception of one mouse which seemed to develop a transient erythematous response at the site of needle stick (Figure 5). After the mice were euthanized, pathology of skin punch biopsies revealed no specific allergy or inflammatory reactions in any of the sections (Figure 6). Also, there were no specific differences in skin samples among mice. All mice skin biopsies had sentinel (resident) numbers of mast cells, lymphocytes, and macrophages. It is possible, however, that there might have been an initial reaction upon each exposure that subsequently healed before pathological analysis. ELISA testing was negative as well. There was no reactivity of any sera from bitten or SGE-injected mice to SGE proteins used in the ELISA, and all results were no different from that of the negative controls used in the test, meaning that the mice failed to produce measurable antibodies to SGE or bed bug bites during the 48 day exposure(s).

Figure 4. Mouse number 4.4 after one month showing no reaction to bed bug bites

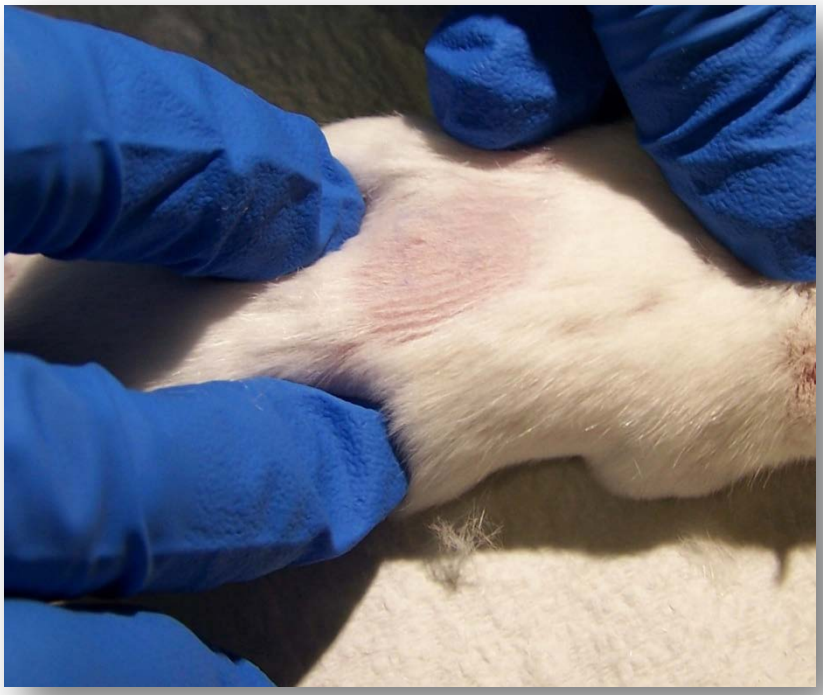

Figure 5. Mouse number 2.3 developed a transient small lesion at site of injection.

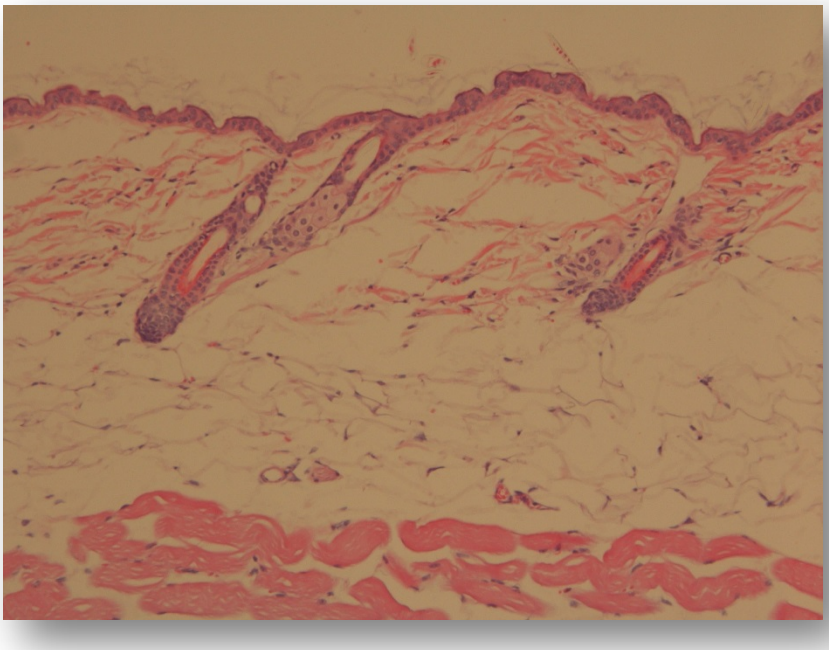

Figure 6. Example of pathology of mouse skin biopsies, showing no inflammation or allergic reaction (H&E staining 100X).

Results of this study indicate that either: 1) thresholds of bed bug biting and/or exposure to SGE proteins to elicit an immune response in mice are much higher than that in humans, or 2) Swiss-Webster mice are not reactive to bed bug bites or SGE proteins. If the latter is true, then Swiss-Webster mice are not a good model to pursue for studies of cutaneous reactions to bed bugs.

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Table 1. Drugs, route of delivery, dose, and schedule used during the study.

Drug	Route of Delivery	Dose	Schedule
Isoflurane	Inhalation	4-5% for induction;1-2% maintenance	Days 0, 19, 32, and 48
Ketamine	Intraperitoneal	80-120 mg/kg	Days 0, 19, 32, and 48
Xylazine	Intraperitoneal	5-10 mg/kg	Days 0, 19, 32, and 48

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