

# Mechanical Transmission of *Bacillus anthracis* by Stable Flies (*Stomoxys calcitrans*) and Mosquitoes (*Aedes aegypti* and *Aedes taeniorhynchus*)

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We evaluated the potential of stable flies, *Stomoxys calcitrans*, and two species of mosquitoes, *Aedes aegypti* and *Aedes taeniorhynchus*, to transmit *Bacillus anthracis* Vollum 1B mechanically. After probing on Hartley guinea pigs with a bacteremia of ca.  $10^{8.6}$  CFU of *B. anthracis* per ml of blood, individual or pools of two to four stable flies or mosquitoes were allowed to continue feeding on either uninfected guinea pigs or A/J mice. All three insect species transmitted lethal anthrax infections to both guinea pigs and mice. Both stable flies and mosquitoes transmitted anthrax, even when they were held at room temperature for 4 h after exposure to the bacteremic guinea pig before being allowed to continue feeding on the susceptible animals. This study confirms that blood-feeding insects can mechanically transmit anthrax and supports recent anecdotal reports of fly-bite-associated cutaneous human anthrax. The potential for flies to mechanically transmit anthrax suggests that fly control should be considered as part of a program for control of epizootic anthrax.

During the recent outbreak of anthrax in Zimbabwe, there were numerous anecdotal reports of human cutaneous anthrax associated with "fly" bites (3, 4, 12, 15, 20). Similarly, Krishna Rao and Mohiyudeen (10) reported human cutaneous anthrax cases associated with insect bites during an outbreak in India in 1957. Because of the high bacteremia titers produced during *Bacillus anthracis* infections, as high as  $10^{8.1}$  CFU/ml of blood in sheep (14), and the relative stability of spores of this agent, other workers considered cases associated with insect bites to be epidemiological evidence of mechanical transmission of *B. anthracis* by biting arthropods.

Although there are numerous references to the mechanical transmission of anthrax before 1920 (6, 16, 17), we are unaware of any current studies that convincingly demonstrate mechanical transmission of *B. anthracis* by biting insects. Therefore, in an attempt to confirm the earlier studies, we evaluated the potential of stable flies, *Stomoxys calcitrans*, and two species of mosquitoes, *Aedes aegypti* and *Aedes taeniorhynchus*, to transmit *B. anthracis* mechanically.

## MATERIALS AND METHODS

**Insects.** Stable flies, *S. calcitrans*, were provided as pupae by the U.S. Livestock Insects Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Kerrville, Texas. Two mosquito species, the Rockefeller strain of *A. aegypti*, provided by G. Craig, Jr., of the University of Notre Dame, and the Vero Beach strain of *A. taeniorhynchus*, were from long-established laboratory colonies. All insects used in this study were provided with apple slices as a carbohydrate source and maintained in environmentally controlled incubators held at  $26 \pm 1^\circ\text{C}$  until used in the transmission tests.

**Bacteria and bacterial assay procedures.** *B. anthracis* Vollum 1B was used in all experiments. This virulent (*cap*<sup>+</sup> *tox*<sup>+</sup>) strain contains plasmids pX01 and pX02 and was obtained from the U.S. Army Medical Research Institute of

Infectious Diseases culture collection (9). Spore suspensions used to infect guinea pigs were prepared from colonies on blood agar plates harvested at 4 days as previously described (13).

Heart blood was removed from dead and moribund animals and checked for *B. anthracis* by routine microscopic and culture techniques. Serial 10-fold dilutions of the samples were plated on blood agar and on nutrient agar containing 0.9% NaHCO<sub>3</sub>. These plates were incubated for 24 h in a 5% CO<sub>2</sub> incubator at 37°C. The presence of large, nonmotile, gram-positive rods and of typical nonhemolytic colonies on blood agar and mucoid (capsule-forming) colonies on medium containing bicarbonate (11) was considered evidence of the presence of *B. anthracis*. In addition, virulence was verified by injecting a suspension of these bacteria intramuscularly into guinea pigs.

**Vertebrates.** Female Hartley guinea pigs (*Cavia cobaya*) and A/J mice (*Mus musculus*) were obtained from Charles River Breeding Laboratories, Inc., (Portage, Mich.) and Jackson Laboratory (Bar Harbor, Maine), respectively. To facilitate insect feedings, a 4- by 6-cm patch was shaved on the side of each guinea pig with an electric hair clipper. Mice were used unshaven.

**Experimental design.** Guinea pigs were infected by intramuscular inoculation of  $10^3$  *B. anthracis* spores. About 48 h after inoculation, guinea pigs were anesthetized by inoculating them with 0.5 ml of a ketamine hydrochloride and xylazine (1:1) solution in the caudal thigh muscle and used as the source of infection in the transmission attempts.

For the transmission tests, flies or mosquitoes were placed individually in small Plexiglas (Rohm & Haas Co., Philadelphia, Pa.) cages (6 by 3 by 2 cm) with screening on both large surfaces. A cage was placed on the shaved area of the infected guinea pig, and the insect was observed. Once the mouthparts made contact with the skin, the insect was allowed to probe for about 1 min and was then removed. Insects with visible blood in their abdomens were removed immediately, even if they had fed for less than 1 min. These exposed insects were either transferred to and allowed to feed on an anesthetized susceptible animal or held at room

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TABLE 1. Mechanical transmission of *B. anthracis* Vollum 1B by stable flies and mosquitoes to guinea pigs and mice

Vector	Host	No. of animals dead/no. tested (no. of insects tested) [minimal transmission rate (%) at time (in h) between feeding and transfer]		
		<1	4	24
<i>S. calcitrans</i>	Guinea pig	3/4 (16) [19]	2/2 (8) [25]	0/2 (5) [0]
	Mouse	1/8 (12) [8]	NT <sup>a</sup>	NT
<i>A. aegypti</i>	Guinea pig	2/3 (12) [17]	0/1 (4) [0]	0/2 (8) [0]
	Mouse	2/4 (12) [17]	NT	NT
<i>A. taeniorhynchus</i>	Guinea pig	1/3 (12) [8]	1/2 (8) [13]	0/2 (8) [0]
	Mouse	1/4 (12) [8]	NT	NT

<sup>a</sup> NT, Not tested.

temperature (ca. 22°C) for 4 or 24 h before being transferred to a susceptible animal.

## RESULTS

**Stable fly experiments.** Blood from each of the inoculated guinea pigs contained  $10^{8.7}$  CFU of *B. anthracis* per ml. Flies partially feeding on these guinea pigs transmitted *B. anthracis* to 3 of 4 and 2 of 2 susceptible guinea pigs after being held at room temperature for less than 1 h or for 4 h, respectively (Table 1). As 4 flies fed on each guinea pig, a total of 24 flies were used. Thus, the minimal transmission rate was 5 of 24 (21%) for flies held for 4 h or less after their initial exposure. None of the five flies transmitted *B. anthracis* 24 h after their initial exposure. Transmission to mice, although slightly lower, with 1 of 8 mice for 1 of 12 flies (minimal transmission rate, 8%), was not significantly different from transmission to guinea pigs. Blood removed from the heart of each animal shortly after death contained bacteria confirmed as *B. anthracis* by criteria described earlier.

**Mosquito experiments.** The two infected guinea pigs contained  $10^{8.4}$  and  $10^{8.5}$  CFU of *B. anthracis* per ml of blood. Both species of mosquito mechanically transmitted *B. anthracis* to uninfected guinea pigs and mice (Table 1). Transmission rates were similar for the two mosquito species, for the different times after exposure (<1 h or 4 h), and for transmission to guinea pigs or mice. However, none of 16 mosquitoes transmitted *B. anthracis* after being held for 24 h after their initial exposure before being allowed to continue feeding. The overall minimal transmission rate for mosquitoes held  $\leq 4$  h was 7 of 60 (12%) (Table 1). Again, *B. anthracis* was recovered from all mosquito-infected animals that died during the experiment.

In both the stable fly and mosquito experiments, all surviving animals died when challenged with  $10^3$  spores of *B. anthracis*, thus indicating their susceptibility to this agent.

## DISCUSSION

This study confirms that lethal *B. anthracis* infections can be mechanically transmitted by hematophagous diptera. For both stable flies and mosquitoes, transmission rates were similar at either <1 h or 4 h after the initial exposure to the bacteremic guinea pig. Thus, transmission could occur for at least several hours after the insects had fed on a bacteremic animal. Although we did not demonstrate transmission after 24 h, our sample sizes were too small to preclude this possibility. Bacteremia titers in sheep are similar to those in the donor animals used in this study (14). Thus, hematophagous insects feeding on naturally infected domestic animals

would be exposed to a similar dose of *B. anthracis* as that used in the current study, and they should be able to transmit *B. anthracis* mechanically.

Although transmission rates were relatively low in this study, 17% for stable flies and 12% for mosquitoes, the high population densities of many hematophagous diptera and the numerous bites they inflict on humans and domestic animals imply that mechanical transmission may play a role in the dissemination of this agent. In particular, transmission by biting flies may explain the cutaneous anthrax cases in individuals in Zimbabwe with no known animal contact (12).

The insect species used in this study have also been shown to be able to transmit Rift Valley fever virus mechanically (7). Also, studies have shown that many other agents can be mechanically transmitted by hematophagous insects. These include vesicular stomatitis virus (New Jersey) transmitted by numerous *Tabanus* and *Chrysops* species and four species of mosquitoes (5), rinderpest virus transmitted by the tsetse fly *Glossina morsitans* (8), and swine pox virus transmitted by the hog louse *Haematopinus suis* (19). In addition to the species tested in our study, *B. anthracis* has also been mechanically transmitted by *Tabanus striatus* (16), *Haematobia irritans* (17), and various species of mosquitoes (17).

In addition to transmission by hematophagous diptera, the potential for transmission of *B. anthracis* has also been shown for nonbiting flies, either on contaminated body parts (1) (e.g., feet or mouthparts) or in their feces (2). *B. anthracis* has been recovered from fly feces at least 20 days after the insects had fed on secretions of a bacteremic animal; spores remain alive and virulent in dead infected flies for at least 3 years (6). Also, if an individual is contaminated with *B. anthracis*, the itching and scratching resulting from insect bites may allow this agent to pass through previously unbroken skin.

Various forms of evidence strongly suggest that flies play a role in the transmission of *B. anthracis* to humans and domestic animals during an anthrax outbreak. This evidence includes the cutaneous anthrax cases associated with insect bites in both Zimbabwe (3, 4, 12, 15, 20) and India (10); the demonstrated ability of several species of hematophagous diptera to transmit *B. anthracis* mechanically for at least 4 h after contact with an infected animal; and the ability of other diptera to contaminate surfaces with *B. anthracis*, either with spores in their feces or by direct contamination with spores or vegetative forms on their body surfaces. Also, there are often enormous populations of flies associated with anthrax epidemics in domestic animals (2, 10, 18). Therefore, fly control should be considered as part of an anthrax control program.

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