

Blood-Feeding Behaviors of *Anopheles stephensi* but not *Phlebotomus papatasi* are Influenced by Actively Warming Guinea Pigs (*Cavia porcellus*) Under General Anesthesia

Author(s): Jessica N. Buchta, Bethany S. Zarndt, Lindsey S. Garver, Tobin Rowland, Meng Shi, Silas A. Davidson and Edgar D. Rowton

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BLOOD-FEEDING BEHAVIORS OF *ANOPHELES STEPHENSI* BUT NOT *PHLEBOTOMUS PAPATASI* ARE INFLUENCED BY ACTIVELY WARMING GUINEA PIGS (*CAVIA PORCELLUS*) UNDER GENERAL ANESTHESIA¹

JESSICA N. BUCHTA,^{2,5} BETHANY S. ZARNDT,² LINDSEY S. GARVER,³ TOBIN ROWLAND,³
MENG SHI,⁴ SILAS A. DAVIDSON³ AND EDGAR D. ROWTON³

ABSTRACT. Animal models are often used to study hematophagous insect feeding behavior and evaluate products such as topical repellents. However, when these models are used the study animals often experience significant drops in core body temperature because of the effects of anesthesia. This study used a guinea pig model to investigate whether maintaining a normothermic core body temperature during anesthesia influenced the rate of *Anopheles stephensi* and *Phlebotomus papatasi* blood feeding. Experiments were conducted with anesthetized animals that had their body temperatures either maintained with a warming device or were allowed to drop naturally. Results showed that when guinea pigs were actively warmed by a heating device, *An. stephensi* feeding behavior was similar at the beginning and end of anesthesia. However, when a warming device was not used, fewer *An. stephensi* took a blood meal after the animals' temperatures had dropped. *Phlebotomus papatasi* were not as sensitive to changes in temperature and feeding rates were similar whether a warming device was used or not. These results are discussed and it is recommended that warming devices are used when conducting feeding experiments with insects sensitive to changes in host body temperature, such as *An. stephensi*.

KEY WORDS *Anopheles stephensi*, *Phlebotomus papatasi*, core body temperature, blood feeding, host seeking

INTRODUCTION

Small animals are frequently used to conduct basic and applied research with hematophagous insects. Animals are often used as surrogates for humans in repellent studies, and one of the most important factors when selecting an appropriate animal model is that the body temperature is similar to humans (Rutledge and Gupta 2006). Host body temperature is known to affect insect feeding behavior (Howlett 1910, Wigglesworth

and Gillett 1934, Peterson and Brown 1951, Davis and Sokolove 1975, Day and Edman 1984). It has been proposed that the warm, moist air surrounding mammals is an important short-range orientation cue for hematophagous insects (Gibson and Torr 1999).

Mosquitoes are attracted to the warmest skin surfaces when offered a temperature gradient (Smart and Brown 1956, Grossman and Pappas 1991). Some species are able to detect temperature differences as small as 1° (Gillett and Connor 1976). Mosquitoes are more attracted to pyrexia hosts (due to viremia) than lower-body-temperature hosts (Turell et al. 1984). The factors affecting host-seeking behavior in sand flies are less well understood. Body temperature of the host does play a role in sand fly host-seeking behavior, but is probably not the most important factor (Hoogstraal et al. 1962). Body odor and other skin emanations are likely to play a more significant role (Quinnell et al. 1992, Pinto et al. 2001).

Before laboratory animals can be used to feed hematophagous insects, they must be anesthetized for ease of handling and to minimize pain and distress. One of the most common complications of general anesthesia is hypothermia, due to inhibition of thermoregulatory control by the anesthetic (Kurz 2008, Lenhardt 2010). This is an even greater problem in small laboratory species because these animals have a larger surface area relative to body mass and there is a correspondingly greater loss of body heat (Kohn et al. 1997). Substantial body heat is lost from the tail, ears,

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² Veterinary Medicine, Walter Reed Army Institute of Research, 511 Robert Grant Avenue, Silver Spring, MD 20910.

³ Entomology Branch, Walter Reed Army Institute of Research, 503 Robert Grant Avenue, Silver Spring, MD, 20910.

⁴ Division of Medical, Audio, Visual, Library and Statistical Services, Walter Reed Army Institute of Research, Silver Spring, MD 20910.

⁵ To whom correspondence should be addressed.

and feet of rodents to the environment (Rembert et al. 2004).

Hairless guinea pigs (*Cavia porcellus*) are useful models for entomological biting studies because of the physiological similarity in the nonhaired skin of the guinea pig to human skin (Sueki et al. 2000), the observation that many insects have a preference for feeding on hairless skin (Walker and Edman 1985), and the similar body temperature maintained by guinea pigs and humans (Spector 1956). Rutledge and Gupta (2006) list 14 studies that have evaluated insect repellents with a guinea pig model system.

In this study we sought to discern whether hypothermia, induced by general anesthesia in guinea pigs, decreases blood feeding in anopheline mosquitoes and phlebotomine sand flies. An active warming device was used to maintain a normothermic core body temperature in guinea pigs under anesthesia and to measure the rate of sand fly and mosquito blood feeding on these animals compared to unwarmed counterparts.

MATERIALS AND METHODS

Animals: Twelve male Institute Armand Frapier (IAF) hairless guinea pigs ranging in age from 3 months to 1 year were used in this study. Their weights ranged from 527 to 1,027 g. The guinea pigs were purchased from Charles River (Wilmington, MA) and certified free of common laboratory diseases. Guinea pigs were fed ad libitum on guinea pig diet (Guinea Pig 5P18 diet, Purina Mills) and water (on-site reverse-osmosis reserve) and supplemented with a rotation of spinach and kale daily. Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals*, 8th edition. (National Research Council 2011). All procedures used in this study were approved by the Institutional Animal Care and Use Committee. The facility where this research was conducted is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International.

Insects: *Phlebotomus papatasi*, originating from north Sinai, were reared in the Walter Reed Army Institute of Research (WRAIR) insectary according to the methods described in Modi and Rowton (1999). Sand flies were maintained at 25°C and 80% relative humidity (RH). Adults were provided 30% sucrose solution ad libitum. Female sand flies were 3–5 days postemergence and starved 24 h before feeding.

Anopheles stephensi were raised in the (WRAIR) insectary and maintained at 26°C, 80% RH, and a 12:12 light:dark cycle. Larvae were raised on a diet of ground Tetramin® (Tetra,

Blacksburg, VA) fish food. Adults were provided 10% sucrose solution ad libitum. Female mosquitoes were 4–7 days postemergence at the time of experimentation and starved 24 h before exposure to the guinea pig.

Warming device: A MouseSTAT™ (Kent Scientific, Torrington, CT) was used as the warming device in this study. This device uses a warming pad that emits far-infrared radiation (FIR) to regulate body temperature based on real-time feedback from a rectal temperature probe. The device adjusts the amount of FIR emitted to maintain a desired core body temperature that is programmed into the machine. For this study the desired temperature was set at 39.5°C. The warming pad was placed under each guinea pig without interfering with insect biting sites (Fig. 1). Because these guinea pigs were hairless, a thin layer of 100% polypropylene microfilm material (The Safety Zone, LLC, Centerbrook, CT) was placed between the guinea pig and the FIR warming device to prevent superficial thermal injuries. Temperature was recorded every 5 min throughout the experiments.

Feeding assay: Guinea pigs were divided into 2 groups (6 animals for mosquito feedings and 6 animals for sand fly feedings) and groups were assayed separately. Each guinea pig served as its own control by undergoing trials both with and without the warming device. Guinea pigs were anesthetized during feeding experiments by an intramuscular injection of ketamine (100 mg/ml) and xylazine (100 mg/ml). The animals were allowed at least 1 wk of rest between each feeding trial.

Anesthetized guinea pigs were placed in a polycarbonate glove box with an interior environment maintained at 70–80% RH and 17–26°C. Mosquitoes or sand flies (8–10 insects per feed) were loaded into feeding chambers (capacity 45 ml, diam 2.5 cm) constructed from a 40-dram clear plastic vial topped with fine-gauge nylon mesh screen. The feeding chambers were placed on the guinea pig's thoracic region in an area away from the warming pad and secured by rubber bands. The chambers were first placed on the animals from 0 to 5 min, with 0 min corresponding to when the animals stopped moving and were considered fully sedated. A new cohort of mosquitoes or sand flies were loaded in a fresh feeding chamber and placed on the abdomen of the animals from 25–30 min postanesthesia. At the end of 30 min the guinea pigs were removed from the glove box and returned to their cages where they were monitored until fully recovered. There was no appreciable difference between the average basal body temperature of the 6 guinea pigs used for sand fly feeding (39.4°C) and the 6 used for mosquito feeding (39.3°C).

The feeding chambers were placed in a laboratory freezer for 15–20 min to ensure insect death.

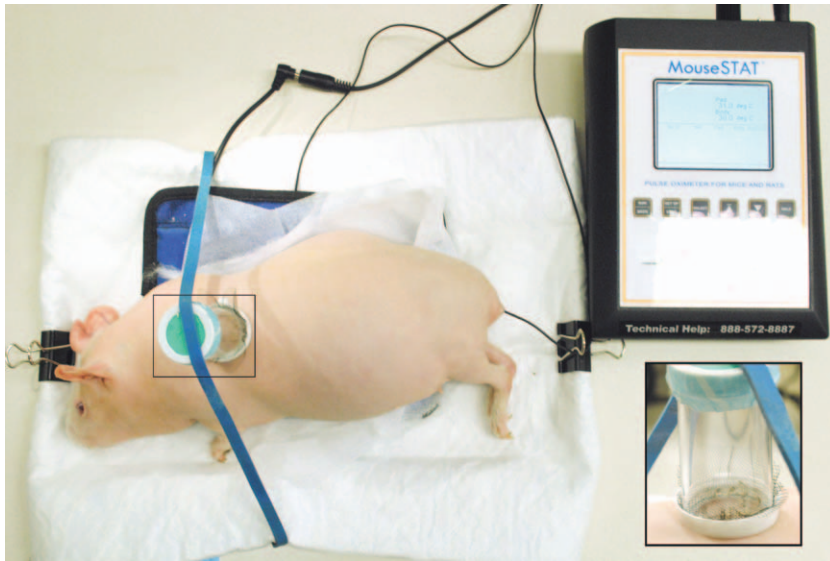


Fig. 1. Pictorial representation of biting experiments. Actively warmed guinea pigs positioned atop MouseSTAT warming pad with microfilm material as a barrier against superficial burns. Rectal probe gauges core temperature, observable in real time via display. Elastic banding secures clear-walled feeding chamber with mesh-side contacting skin. Biting events are viewed through walls of chamber (inset photo).

The mosquitoes and sand flies were then separated into 2 groups: blood-fed or non-blood-fed. Blood feeding was characterized by the presence of any amount of blood within the abdomen of the mosquito or sand fly. The midguts of both species were evaluated under a stereomicroscope for evidence of blood. Sand flies with no detectable blood in the abdomen were crushed on white paper and inspected for a blood smear.

Statistical analysis: The data were analyzed with the use of SAS software by 3 comparison groups of feeding behaviors per insect species: 1) blood feeding on the control group at 0 and 25 min, 2) blood feeding on the actively warmed group at 0 and 25 min, and 3) blood feeding at 25 min between the actively warmed group and the control group. A generalized linear mixed model was used to evaluate the effect of guinea pig core body temperature on mosquito and sand fly blood-feeding behavior. The model assumed that the data were under binomial distribution, and used the logistic link function.

RESULTS

During sand fly experiments, actively warmed guinea pigs maintained their core temperature, and control animals experienced a significant drop in core body temperature. Control (unwarmed) guinea pigs presented to sand flies showed a mean difference in core temperature of 2.4°C degrees between the 0-min feeding (39.4°C) and the 25-min feeding (37°C) (Fig. 2A). In this group, the largest drop in core body temperature of any individual

was 3.3°C and the mildest drop was 2.2°C. In contrast, guinea pigs maintained by FIR warming experienced a 0.1°C difference (39.4°C at 0 min to 39.3°C at 25 min). Among warmed individuals, the greatest temperature fluctuation was a 0.5°C decrease in temperature and the slightest was a 0.1°C increase in temperature.

Despite the drop in average core temperature of the control group of guinea pigs, there was no difference in blood-feeding behavior of sand flies between the group accessing actively warmed guinea pigs and the group accessing control guinea pigs (P value = 0.5299) at 25 min (Fig. 2B).

Furthermore, when feeding at 0 and 25 min on either the control (unwarmed) or the actively warmed guinea pigs, sand flies showed no statistically significant difference in blood-feeding behavior on the same animal between time points (P = 0.6844 for control; P = 0.2402 for warmed) (Fig. 2C).

For the mosquito-feeding experiment, control guinea pigs exhibited a mean difference in core temperature of 1.8°C between the 0-min feeding (39.4°C) and the 25-min feeding (37.6°C). The greatest individual loss of body heat among the control group was 2.4°C and mildest loss was 1.0°C. Guinea pigs maintained by FIR warming showed no difference (39.2°C at 0 min and at 25 min) (Fig. 3A). In these warmed animals, the largest temperature swing was a 1.2°C, and 1 animal exhibited a 0.1°C decrease.

Among mosquitoes, there was a weak but statistically significant difference in blood-feeding behavior between those that fed on control and

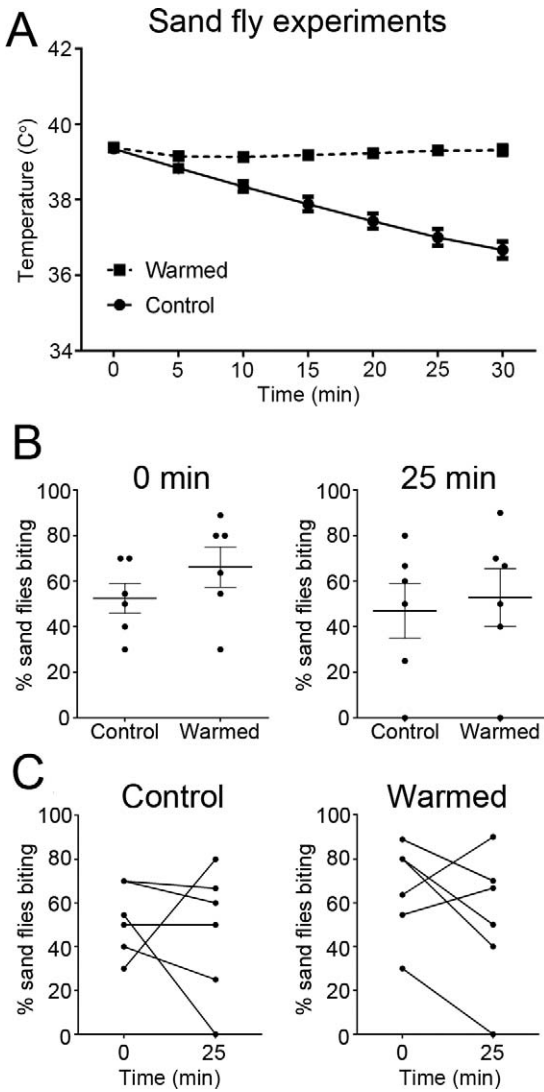


Fig. 2. Sand flies do not display differential biting behaviors on warmed or unwarmed guinea pig hosts. (A) Warmed (dashed line with square time points) and control (solid line with circle time points) guinea pig core temperature readings via MouseSTAT. Error bars represent SEM among 6 replicates. (B) Propensity of sand flies to take a blood meal on control and warmed guinea pigs at onset of experiment (left panel) and after 25 min of anesthesia (right panel). Dots represent proportion of sand flies feeding on each individual guinea pig; black horizontal line represents median of 6 replicate feeds (individual animals) and error bars represent SEM. (C) Propensity of sand flies to take a blood meal on the same guinea pig when cooling is allowed (control, left panel) or prevented (warmed, right panel). Dots represent proportion of sand flies feeding on each individual guinea pig; black lines connect feeds performed on the same animal.

actively warmed guinea pigs ($P = 0.0483$) at 25 min (Fig. 3B). The least-square estimate probability that mosquitoes would blood feed on the control guinea pig was 58.53% with 95% confi-

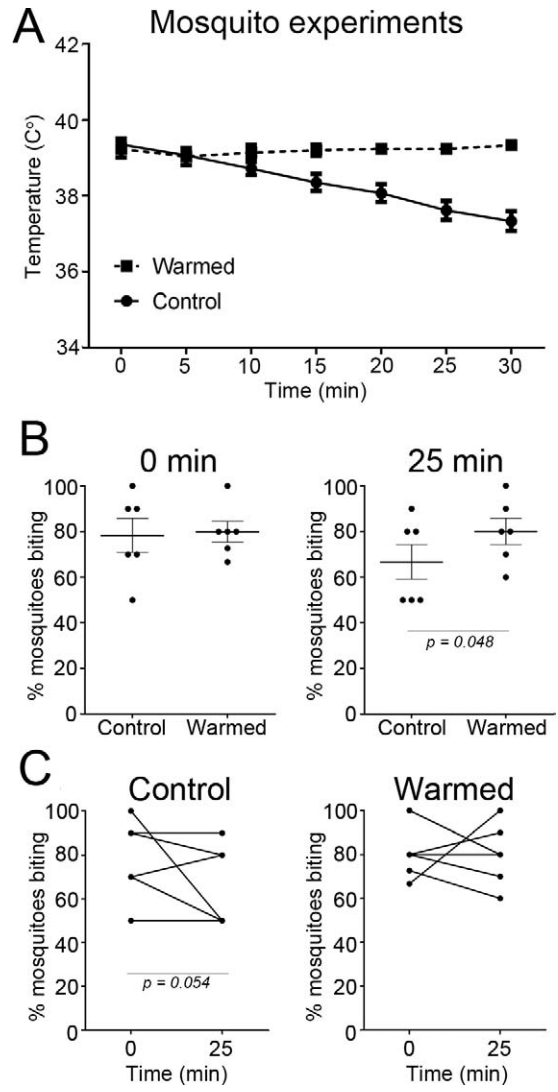


Fig. 3. Mosquitoes display slight differential biting behaviors on warmed or unwarmed guinea pig hosts. (A) Warmed (dashed line with square time points) and control (solid line with circle time points) guinea pig core temperature readings via MouseSTAT. Error bars represent SEM among 6 replicates. (B) Propensity of mosquitoes to take a blood meal on control and warmed guinea pigs at onset of experiment (left panel) and after 25 min of anesthesia (right panel). Dots represent proportion of mosquitoes feeding on each individual guinea pig; black horizontal line represents median of 6 replicate feeds (individual animals) and error bars represent SEM. (C) Propensity of mosquitoes to take a blood meal on the same guinea pig when cooling is allowed (control, left panel) or prevented (warmed, right panel). Dots represent proportion of mosquitoes feeding on each individual guinea pig; black lines connect feeds performed on the same animal.

dence interval of 33.76%, 79.63%. The least-square estimate probability that mosquitoes would blood feed on the actively warmed guinea pig was 81.23% with 95% confidence interval of

58.03%, 93.12%. Furthermore, when comparing blood-feeding propensity of mosquitoes on the control guinea pigs at 0 min and at 25 min, there was an effect that approached statistical significance ($P = 0.0544$). This mild effect disappears when blood feeding on the actively warmed guinea pig, as there was no statistically significant difference in blood-feeding behavior between 0 and 25 min ($P = 0.4776$) when core temperature is maintained for the duration of the experiment (Fig. 3C).

DISCUSSION

The purpose of this study was to discern whether hypothermia induced by general anesthesia in the guinea pig decreases blood feeding in anopheline mosquitoes and phlebotomine sand flies.

In the sand fly feeding experiment, our results showed there was no statistically significant difference in blood-feeding behavior at 25 min between actively warmed guinea pigs and control guinea pigs during general anesthesia (Fig. 2B). Additionally, sand fly blood-feeding patterns on individual animals were not measurably different at the beginning or end of the anesthesia period, regardless of whether guinea pigs were actively warmed or allowed to cool through the duration of the experiment (Fig. 2C). This suggests body heat is not a predominant determinant of sand fly feeding success in a laboratory model using rodents as blood sources and may suggest sand flies have more balance in their multifactorial host-seeking behavior compared to mosquitoes, or that another host-seeking cue trumps the lure of heat.

The mosquito feeding model shows a different outcome: that mosquitoes' propensity to take a blood meal from a guinea pig is somewhat influenced by the animal's body temperature. This is supported by 2 lines of evidence. First, more mosquitoes are slightly more willing to take a blood meal from a guinea pig that has been actively warmed during a 25-min sedation than from one that has been allowed to cool (as is normally observed without warming intervention) (Fig. 3B). Second, mosquitoes display disparate blood-feeding patterns when exposed to control guinea pigs that are allowed to cool under general anesthesia from the beginning of the anesthetic period when their core body temperature is normothermic, versus the end of the anesthetic period (25 min) when their core body temperature had become hypothermic. In this group, mosquitoes were slightly more likely to feed at the 0-min time point, when the average core temperature is 39.4°C, than at the 25-min time point, when the temperature had dropped to 37.6°C; 3 animals elicited a reduced feeding rate at 25 min and 2 showed no change (Fig. 3C). It is

also worth noting that the proportions of mosquitoes feeding on each control animal varies more than when warmed animals are used, suggesting that active warming may reduce experimental variability from one feeding to the next. This experiment uses the exact same guinea pig for both feedings, ruling out any other host cues that may be individual-specific, such as skin microflora or body size. Correspondingly, there was no statistically significant difference in mosquito blood-feeding behaviors toward the actively warmed guinea pig between the beginning and end of the anesthetic period. This is most likely because the core body temperature remained normothermic throughout the entire anesthetic period and further supports the supposition that loss of rodent body heat can be a factor influencing mosquito feeding rate in a laboratory model. The insects were also fed on 2 different areas of the guinea pig's body to avoid any confounders from insects feeding on the same area twice.

In these experiments, body temperature appears to be less important as a host-seeking factor for sand flies than it is for mosquitoes. It should be noted that this conclusion is made with the experimental design caveat that host species used, insect species used, changes in ambient environment, length of time given for blood feeding, length of time for body-heat loss, size of feeding container, and other elements of this design may influence the effect of body heat on feeding behaviors. Nonetheless, this experimental setup is one commonly used by entomologists, and therefore it is important to consider how anesthesia-induced cooling may affect such an experiment.

Our data suggest that maintenance of a normothermic body temperature of the host for mosquito blood feeding is an important factor to consider when conducting reliable repeatable research. Not only can experiments with rodent models benefit from enhanced feeding rates when the host is kept warm, but they can benefit from mosquitoes taking blood meals of a consistent temperature. A warmer blood meal can cause physiological responses within the mosquito that do not occur when the mosquito takes a blood meal from a cooler host (Benoit et al. 2011, Lahondere and Lazzari 2012). When host animals are allowed to cool during blood feeding, mosquitoes from the same cohort that are feeding at different times after anesthesia is administered are taking different-temperature blood meals, possibly confounding research results. The data presented here show that actively warming rodent hosts during general anesthesia controls such temperature fluctuations, thereby mitigating this confounding influence on mosquito-rodent feeding studies.

As our results show, these temperature preferences for blood feeding were not exhibited by the sand flies. For the purposes of sand fly blood feeding, active warming of the rodent host appears not to be important. However, for the health and well-being of the rodent, active warming is highly recommended during general anesthetic events (Kurz 2008).

Overall, we conclude that mosquitoes do show a preference for blood feeding on guinea pigs with a normothermic core body temperature when compared to a hypothermic core body temperature due to general anesthesia.

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