Video Article

The Madagascar Hissing Cockroach as an Alternative Non-mammalian Animal Model to Investigate Virulence, Pathogenesis, and Drug Efficacy

Jennifer Chua[1], Nathan A. Fisher[2], Shane D. Falcinelli[1], David DeShazer[1], Arthur M. Friedlander[3]

[1]Bacteriology Division, United States Army Medical Research Institute of Infectious Diseases

[2]Southern Research

[3]Headquarters Division, United States Army Medical Research Institute of Infectious Diseases

Correspondence to: Jennifer Chua at jennifer.chua.ctr@mail.mil

URL: https://www.jove.com/video/56491
DOI: doi:10.3791/56491

Keywords: Immunology, Issue 129, Madagascar hissing cockroach, *Gromphadorhina*, *Burkholderia mallei*, *Burkholderia pseudomallei*, *Burkholderia thailandensis*, type 6 secretion system, insect, animal model, host-pathogen interaction, virulence, drug toxicity, drug efficacy

Date Published: 11/24/2017

Citation: Chua, J., Fisher, N.A., Falcinelli, S.D., DeShazer, D., Friedlander, A.M. The Madagascar Hissing Cockroach as an Alternative Non-mammalian Animal Model to Investigate Virulence, Pathogenesis, and Drug Efficacy. *J. Vis. Exp.* (129), e56491, doi:10.3791/56491 (2017).

Abstract

Many aspects of innate immunity are conserved between mammals and insects. An insect, the Madagascar hissing cockroach from the genus *Gromphadorhina,* can be utilized as an alternative animal model for the study of virulence, host-pathogen interaction, innate immune response, and drug efficacy. Details for the rearing, care and breeding of the hissing cockroach are provided. We also illustrate how it can be infected with bacteria such as the intracellular pathogens *Burkholderia mallei, B. pseudomallei, and B. thailandensis*. Use of the hissing cockroach is inexpensive and overcomes regulatory issues dealing with the use of mammals in research. In addition, results found using the hissing cockroach model are reproducible and similar to those obtained using mammalian models. Thus, the Madagascar hissing cockroach represents an attractive surrogate host that should be explored when conducting animal studies.

Video Link

The video component of this article can be found at https://www.jove.com/video/56491/

Introduction

The use of insects as alternative non-mammalian animal models to study bacterial pathogenesis and innate host defense has been gaining momentum in recent years. Logistically, this is due to their relatively inexpensive cost and the ease in obtaining, handling, and caring for insects compared to mammals. There is also no regulatory policy governing the use of insects in research; it is not subject to the purview or restrictions set forth by any animal use committee or government agency. Insects as surrogate animal models are particularly amenable to comprehensive screening studies for virulence factors, host-pathogen interactions, and assessments of anti-microbial drug efficacy. Their use can reduce the number of mammals used for research thereby overcoming some of the ethical dilemmas inherent to the conduct of animal experimentation [1,2].

Insects may serve as surrogate hosts because there is a high degree of commonality between the innate immune systems of insects and mammals [1,3]. Both insect plasmatocytes and mammalian macrophages phagocytose microorganisms [4]. The insect counterpart to the neutrophil is the hemocyte [5,6]. Intracellular oxidative burst pathways in insect and mammalian cells are similar; reactive oxygen species in both are produced by orthologous $p47^{phox}$ and $p67^{phox}$ proteins [5]. The signaling cascades downstream of Toll receptors in insects and Toll-like receptors and Interleukin-1 in mammals are also remarkably similar; both result in production of antimicrobial peptides, such as defensins [7]. Thus, insects can be utilized to study general innate immune mechanisms that are shared by metazoans.

An insect called the Madagascar hissing cockroach from the genus *Gromphadorhina,* is one of the largest cockroach species that exists, typically reaching 5 to 8 cm at maturity. It is native only to the island of Madagascar and is characterized by the hissing sound it makes - a sound that is produced when the hissing cockroach expels air through respiratory openings called spiracles [8]. The distinctive hiss serves as a form of social communication among hissing cockroaches for courtship and aggression [9] and can be heard when a male is disturbed in its habitat. The Madagascar hissing cockroach is slow moving compared to the American cockroach and other urban pest species. It is easy to care for and breed; a pregnant hissing cockroach can produce 20 to 30 offspring at a time. A baby hissing cockroach, called a nymph, reaches sexual maturity in 5 months after undergoing 6 molts and can live up to 5 years both in the wild and in captivity [8].

We have utilized the Madagascar hissing cockroach as a surrogate host for infection with the intracellular pathogens *Burkholderia mallei, B. pseudomallei*, and *B. thailandensis* [10,11]. The virulence of these pathogens in hissing cockroaches was compared to their virulence in the benchmark animal model for *Burkholderia*, the Syrian hamster. We found that the 50% lethal dose (LD_{50}) of *B. pseudomallei* and *B. mallei* was similar in both models [11]. Interestingly, *B. thailandensis,* although avirulent in the rodent model, is lethal in the hissing cockroach [11]. This difference with respect to *B. thailandensis* infection underscores the utility of the hissing cockroach model; *B. thailandensis* attenuating mutants

can be more readily resolved in the hissing cockroach than in rodent models. Furthermore, as *B. thailandensis* is often used as the model organism for *B. pseudomallei* and *B. mallei* [10,12,13], identifying attenuating mutations in it could lead to similar targets in its more virulent relatives.

Despite the difference in virulence of *B. thailandensis* in the hissing cockroach versus the Syrian hamster, mutations in critical virulence factors, such as those in the type 6 secretion system-1 (T6SS-1), which are attenuating in *B. mallei* and *B. pseudomallei*, are similarly attenuating for *B. thailandensis* [11]. The hissing cockroach model is further validated in that individual T6SS mutants (T6SS-2 to T6SS-6) in *B. pseudomallei*, which have no bearing on virulence in Syrian hamsters, remain virulent in the hissing cockroaches [11]. Thus, the hissing cockroach is a viable surrogate animal model for the three *Burkholderia* species. We recently utilized the hissing cockroach as a surrogate animal model to examine the efficacy of the anti-malarial drug chloroquine (CLQ) against *Burkholderia* infection [10] and its toxicity.

Here, we describe the rearing and care of the Madagascar hissing cockroach and provide details on how to infect this insect with three *Burkholderia* species. Furthermore, we illustrate that the hissing cockroach is a viable surrogate model to study virulence and drug efficacy in *Burkholderia* infections and that it likely can also serve as a surrogate host for other bacterial pathogens in similar studies.

Protocol

1. Preparations for Maintaining a Hissing Cockroach Colony

1. Prepare cages for the hissing cockroaches to live in. Apply a thin layer of petroleum jelly, approximately 20 to 30 mm in width, to the circumference of the inner walls near the top of the cage to prevent the hissing cockroaches from climbing out of the cage and escaping.
   NOTE: Hissing cockroaches can be housed in a variety of containers that have a large floor space, are of sufficient height, and have lids. Use mouse cages (~43 cm x 23 cm x 20 cm). For cages destined for 37 °C, do not apply petroleum jelly.
2. Include a cardboard egg carton placed upside down inside the cage to provide a hiding place for the naturally shy insects. Do not substitute the cardboard egg carton with one made of polystyrene foam to prevent ingestion of plastic.
3. Do not provide bedding for easier clean-up and to increase the visibility of newly hatched nymphs.
4. Obtain dry dog food which contains a composition of ~20% crude protein. Coarsely grind the dog food with a food processor or blender and store at 4 °C.
5. Obtain several shallow dishes and aquarium stones or a sponge for food and water.
   NOTE: Petri dishes are recommeded.

2. Hissing Cockroach Care and Breeding

1. Obtain outbred Madagascar hissing cockroaches (~5 cm) from a commercial breeder. The species used in this protocol is *Gromphadorhina laevigata*. Unpack the shipping box containing the hissing cockroaches immediately upon receipt.
2. Follow appropriate institutional guidelines for the use of personal protective equipment in handling animals.
   NOTE: Use thick disposable gloves for handling cockroaches as the tarsal claws of a hissing cockroach are sharp.
3. Transfer up to 75 large hissing cockroaches (>3 cm) per large cage. Transfer the newly hatched and smaller nymphs (<3 cm) to a separate cage.
   NOTE: Keeping significantly more than 75 large hissing cockroaches per mouse cage could lead to deteriorating health of the colony and could also result in deaths.
4. Handle a newly molted cockroach, which is off-white in color, gently and avoid squeezing it. Alternatively, allow the exoskeleton to darken and harden before handling.
   NOTE: It is not necessary to remove the mites, *Gromphadorholaelaps schaeferi,* that often accompany hissing cockroaches upon receipt from breeders. The beneficial mites keep the hissing cockroaches clean and are harmless to humans.
5. Feed hissing cockroaches with ground dry dog food in a shallow dish once or twice a week. Provide enough dog food to ensure that the hissing cockroaches have sufficient food until the next feeding. Discard any food that has become wet and moldy.
6. In addition to dog food, provide cut-up fruits and vegetables, such as apples, potatoes, or lettuce, in a shallow dish. Discard moldy or rotten food.
7. Provide drinking water once or twice a week in a shallow dish but do not overfill the dish to prevent spillage in the cage. Place either small aquarium stones or a sponge in the dish to provide a landing pad for small nymphs. Use reverse osmosis water, if available.
8. Keep hissing cockroaches in the dark at temperatures ranging from 21 °C to 30 °C. Keep large hissing cockroaches destined for experimentation at a lower temperature (~21 °C) for ~2 months to decrease breeding and pregnancy. In contrast, keep nymphs at higher temperatures (28 °C to 30 °C) to hasten growth.
   NOTE: Keep cages at room temperature (21 °C) in a dark cabinet.
9. Provide humidity by including a separate pan of water if hissing cockroaches are kept in an incubator.
10. Clean the cage by scooping out dry feces regularly or by transferring hissing cockroaches to a clean cage every 2 to 3 weeks. Keep the bottom of the cage dry. Clean the cage immediately if excess water is allowed to accumulate and mix with the excrement at the bottom of the cage.

3. Cockroach Preparation for Experimentation

1. Transfer the appropriate number of hissing cockroaches in a cage to a 37 °C humidified incubator 1 to 3 weeks prior to an experiment for acclimation. Include a control group for injection. This acclimation period is critical for avoiding temperature shock during experimentation.
2. Do not apply petroleum jelly to cages destined for 37 °C because the higher temperature melts the jelly.
3. Check the hissing cockroaches every 1 to 2 days to replace food and water and clean the cage.
4. Obtain clear disposable plastic food containers with lids for grouping the hissing cockroaches during experimentation. For ventilation, punch holes in the lid or the side of the container with a nail and hammer.

NOTE: Use screw cap containers over snap cap containers in the biosafety level 3 setting. Snap cap containers can be reinforced with tape, if necessary.

5. On the day of injection, distribute 6 to 12 acclimatized hissing cockroaches into groups per container, ensuring equal distribution by sex and body mass.
6. Determine the sex of individual hissing cockroaches. Determine the sex by the prominence of horns found on males and the lack thereof on females.
7. Weigh the individual hissing cockroaches. To facilitate the weighing of a hissing cockroach on the balance, enclose it within two weigh boats that have been tared.
   NOTE: For consistency between experiments, use hissing cockroaches with a weight of 4 to 8 g. However, no difference in survival after infection have been found between small (1.5 to 2 g) and large (6 to 8 g) hissing cockroaches. For drug studies, use hissing cockroaches with a weight of ~5 g to obtain a more consistent drug concentration per body mass.
8. In lieu of a water dish, include high water content fruit or vegetables, such as a slice of apple or potato. Discard rotten food. Do not provide additional water to keep the container dry.
9. Return hissing cockroaches to 37 °C until injections.

4. Bacterial Culture and Preparations

NOTE: The bacterial species used in this protocol are *B. mallei, B. pseudomallei, and B. thailandensis.* All manipulations with *B. mallei* and *B. pseudomallei* must be performed in Class II or Class III biological safety cabinets located in a biosafety level (BSL) 3 laboratory. Perform manipulations with *B. thailandensis* in similar biological safety cabinets located either in a BSL2 or BSL3 laboratory. Follow institutional standard operating procedure for BSL3 work. Follow institutional guidelines for use of personal protective equipment when handling bacteria.

1. Prepare a master plate of *Burkholderia* at least 3 days prior to infection. Use Luria-Bertani (Lennox) (LB) agar for *B. pseudomallei* or *B. thailandensis* and use LB agar supplemented with 4% glycerol for *B. mallei*. Streak bacteria from a 25% glycerol stock stored at -80 °C.
   NOTE: Always streak the master plate directly from the frozen glycerol stock; avoid serial passage of the bacteria from plate to plate as this may cause reduced virulence.
2. Inoculate 10 to 20 mL LB broth with several colonies of *B. pseudomallei* or *B. thailandensis* from the master plate. Similarly inoculate LB broth supplemented with 4% glycerol for *B. mallei*.
3. Shake the broth culture at 175 to 250 rpm at 37 °C for ~18 h.
4. Centrifuge 2 to 3 mL of culture at 5,000 x g for 10 min.
5. Discard the supernatant and resuspend the bacterial pellet in sterile phosphate buffered saline (PBS, 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2PO_4, 1.8 mM KH_2PO_4, pH 7.4).
6. Dilute the bacteria with PBS to achieve an optical density (OD) of 0.5, measured at an absorbance at 600 nm in a spectrophotometer.
7. Serially dilute the bacteria ten-fold in PBS from the starting OD of 0.5. Use these suspensions for infection.
8. Determine the colony forming units (CFU) of the inoculum by serial dilution and plating of 100 µL aliquots on agar media. Incubate plates at 37 °C for 24 to 48 h.

5. Drug Preparations

1. Determine the appropriate amount of drug to be given per body mass. The CLQ dosage given to the hissing cockroach was based on the standard dosage for mammals, which is 50 mg/kg/day.
2. Resuspend or dilute the drug of interest in vehicle. For example, dilute CLQ in PBS at a concentration of 12 mg/mL. This concentration provides 300 µg of drug to a ~6 g hissing cockroach in a 25 µL injected volume.
3. If necessary, sterilize the drug solution by passing it through a 0.22 µm syringe filter.
4. Store the drug solution at 4 °C until use. Warm the drug solution to at least 21 °C (room temperature) prior to injections.

6. Assembly of the Injector

1. Set the pointer to the desired volume (25 µL) by rotating the adjustment screw on a repetitive pipette.
2. Push the release bar of the repetitive pipette inward and pull the pusher outward. The repetitive pipette is now ready to accommodate a loaded syringe.
   NOTE: Calibrate the volume ejected by the repetitive pipette by measuring the amount of water ejected on a balance.
3. Fill a 1 mL syringe with suspension containing either bacteria or drug.
4. Attach syringe to a sterile 26 or 27 G x ½ inch (or shorter) needle.
5. Tap the syringe to float the air bubbles to the top and expel the bubbles and some suspension into a container filled with 10% bleach.
6. Snap the syringe onto the syringe clip of the repetitive pipette with the needle bevel facing up.
7. Pull the release bar outward and press the dispenser button firmly to perform blank injections into a container filled with 10% bleach until the syringe plunger is against the pusher.
8. Perform 1 or 2 additional blank injections to ensure liquid is being ejected out of the syringe. The repetitive pipette is ready for injections.

7. Cockroach Injections

1. Perform all cockroach injections in a Class II or Class III biological safety cabinet in a BSL2 or BSL3 setting using institutional recommended personal protective equipment.
2. Clean work surfaces in the safety cabinet that may come in contact with the hissing cockroaches. Use 10% bleach followed by 70% ethanol to remove residual bleach. Allow the surface to air dry.

3. With one hand, grip the hissing cockroach by its side. Immobilize a hissing cockroach such that it is unable to recoil during injection. Bend the hissing cockroach slightly so that the cutaneous membranes between the abdominal terga are exposed.
4. With the other hand, hold the repetitive pipette such that the needle is at a 0° to 30° angle from the dorso-ventral midline of the hissing cockroach. Puncture the cutaneous membrane adjacent to the 3rd, 4th, or 5th tergum from the posterior end.
   NOTE: Entry of the needle into the hissing cockroach at the angle indicated ensures that the needle does not go through and exit out of the hissing cockroach. The injection site ensures that the ejected material is contained within the abdominal cavity filled with hemolymph. Practice holding the hissing cockroaches and injecting with water before undertaking injections with live bacteria or drug.
5. Push the dispense button firmly to inject the volume. Gently withdraw the needle at the same angle as used for entry.
6. Place the injected hissing cockroach in a separate container to distinguish it from the hissing cockroaches that have not yet been injected. Wipe off any hemolymph that may have oozed out from the injection site.
7. Continue injecting other hissing cockroaches within a group using the same syringe and needle. Stop injecting before the syringe plunger reaches the bottom of the barrel to prevent incomplete dosing in the last injection.
8. For multiple injections in a single hissing cockroach, such as those with bacteria and drug, inject at different sides of a tergum on the dorsal side of the hissing cockroaches. Alternatively, perform multiple injections by injecting at different terga (3rd to 5th).
9. Ensure that the lids of the containers are securely fastened. Reinforce the lids with tape, if necessary.
10. Incubate hissing cockroaches in a humidified 37 °C incubator.

8. Recording Hissing Cockroach Morbidity and Mortality

1. Perform all hissing cockroach examinations in a Class II or Class III biological safety cabinet with institutional recommended personal protective equipment. Clean the surfaces in the safety cabinet with 10% bleach followed by 70% ethanol. Allow the work area to air dry.
2. Score hissing cockroaches once or twice daily over a period of 1 to 2 weeks using the morbidity scoring table (**Table 1**).
3. Remove dead hissing cockroaches from the container and record the number of survivors.
4. Transfer remaining live hissing cockroaches to a clean container if excess moisture has accumulated at the bottom of the container. Alternatively, use disposable paper towels to wipe off excess moisture.
5. Replace rotten food daily and return hissing cockroaches to the humidified 37 °C incubator.
6. At the end of study, place remaining survivors in a biohazard bag and freeze at -80 °C to euthanize.
7. Statistically analyze the data[14] to determine LD_{50} and by Kaplan-Meier and Log-Rank analysis for survival. As with all insect experimentation, some deaths may occur in the control group. These deaths are attributed to the natural mortality of insects [15]. For a more detailed discussion on how to account for deaths in the control group, see reference[15].

Representative Results

This section illustrates the results that were obtained when Madagascar hissing cockroaches were infected with *B. mallei, B. pseudomallei,* or *B. thailandensis*; the results show that this insect is a tractable animal model for different species of *Burkholderia* in studying virulence, drug toxicity, and drug efficacy against bacterial infection. More hissing cockroaches survived in groups that were infected with the attenuated mutants (Δ*hcp1*) than in groups that were infected with wildtype *B. pseudomallei* K96243, parental *B. mallei* SR1, or *B. thailandensis* DW503 (**Figure 1**). Conversely, infection with virulent mutants (Δ*hcp2* or Δ*hcp3*) killed the hissing cockroaches similarly to the wildtype *B. pseudomallei* (**Figure 1**). Infection with the mammalian avirulent *Burkholderia* species, *B. thailandensis* E264, and its aminoglycoside sensitive derivative DW503, show that the hissing cockroach model is particularly suitable for elucidating mutations in *B. thailandensis* that lead to attenuation (**Figure 2**). Thus, it is a more fitting animal model for *B. thailandensis* studies than rodent models. Increasing concentrations or multiple injections of CLQ did not kill the hissing cockroaches; this illustrates that drug toxicity can also be tested in the hissing cockroach model **(Figure 3)**. Further, the efficacy of CLQ against *B. thailandensis* infection is shown in **Figure 4**. Important aspects of hissing cockroach care and infection are shown in **Figure 5**. **Table 1** can be used to score the morbidity of hissing cockroaches during experiments.

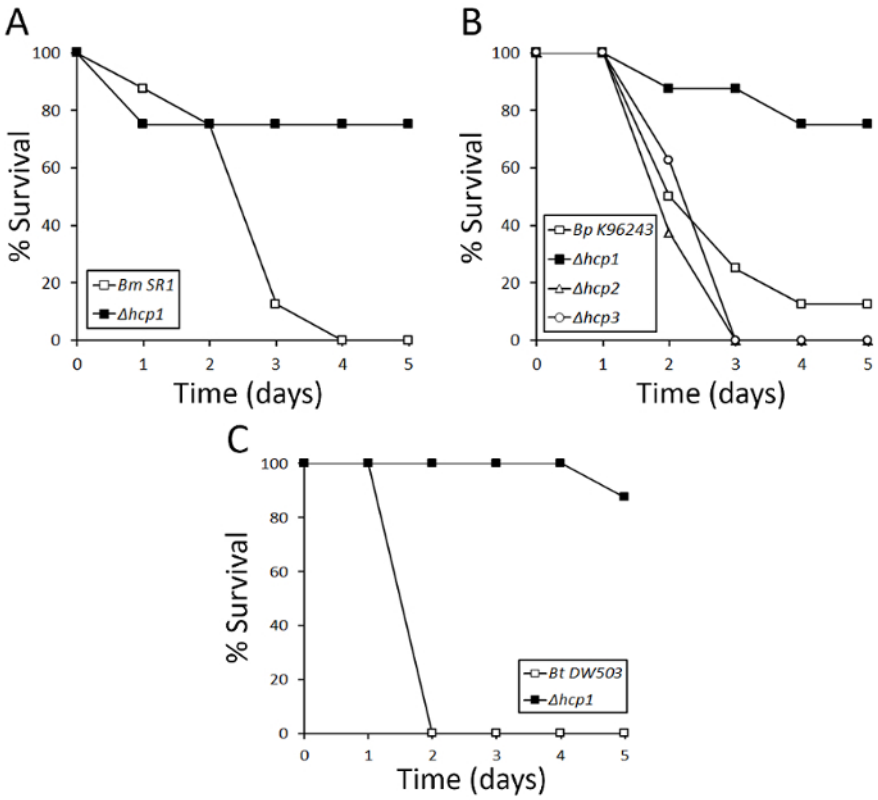

Figure 1: Hissing cockroach survival after injection with virulent and attenuated *Burkholderia*. Eight hissing cockroaches per group were injected with 25 µL of bacterial suspension. Hissing cockroaches were checked for survival once a day for 5 days. (**A**) Hissing cockroaches were injected with parental *B. mallei* SR1 (open square) or the Δ*hcp1* mutant (closed square) at 100 CFUs. (**B**) Hissing cockroaches were injected with wild type *B. pseudomallei* K96243 (open square), Δ*hcp1* (closed square), Δ*hcp2 (open triangle)*, or Δ*hcp3* (open circle) mutant at 10 CFUs. (**C**) Hissing cockroaches were injected with parental *B. thailandensis* DW503 (open square) or Δ*hcp1* mutant (closed square) at 100 CFUs. Figure originally published in reference [11]. Please click here to view a larger version of this figure.

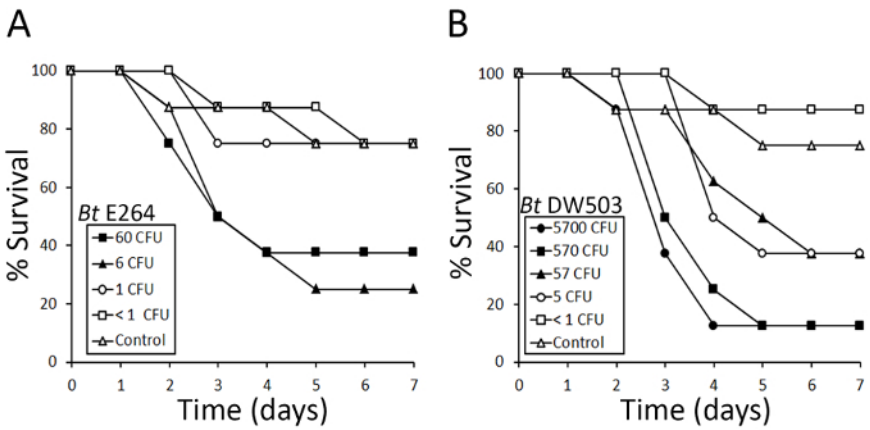

Figure 2: Hissing cockroach survival after injection of increasing concentrations of *B. thailandensis* for LD_{50} determination. Eight hissing cockroaches per group were injected with wildtype *B. thailandensis* E264 (**A**) or the aminoglycoside sensitive derivative DW503 (**B**) and survival was scored for 7 days. The LD_{50} is 3 CFUs for E264 and 6 CFUs for DW503. Please click here to view a larger version of this figure.

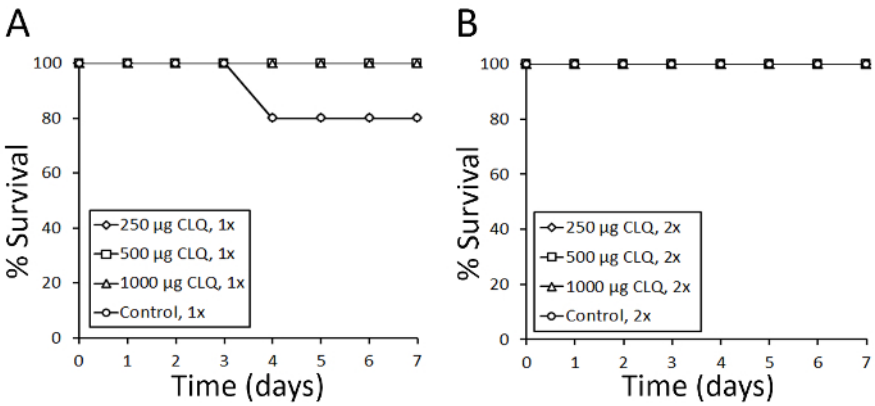

Figure 3: Hissing cockroach survival after injection with chloroquine. Five hissing cockroaches per group were injected once (**A**) or twice on two consecutive days (**B**) with 250 (diamond), 500 (square), or 1,000 µg (triangle) CLQ or PBS (circle) and survival was scored for 7 days. Please click here to view a larger version of this figure.

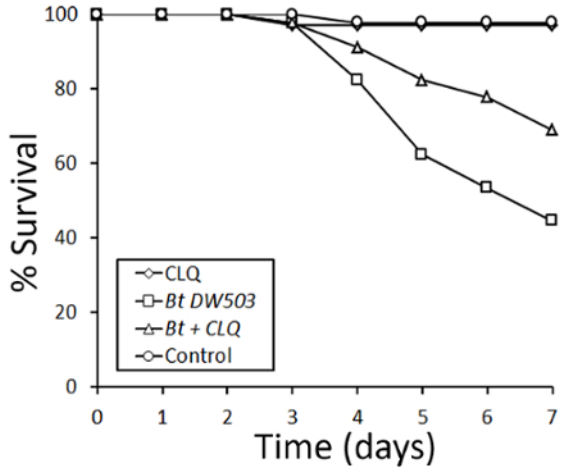

Figure 4: Hissing cockroach survival after infection with *B. thailandensis* and treatment with chloroquine. 10 to 12 hissing cockroaches per group were infected with *B. thailandensis* DW503 and not treated (square), infected with *B. thailandensis* DW503 and treated with CLQ (triangle), treated with CLQ alone (diamond), or were uninfected and untreated (circle). Survival was recorded for 7 days. The survival curve, a composite of 4 separate experiments, is expressed as a percentage equal to the total number of survivors divided by the total number of hissing cockroaches for each treatment on the days indicated. The CFU inoculum given ranged from 10 to 20 LD_{50}. Figure originally published in reference [10]. Please click here to view a larger version of this figure.

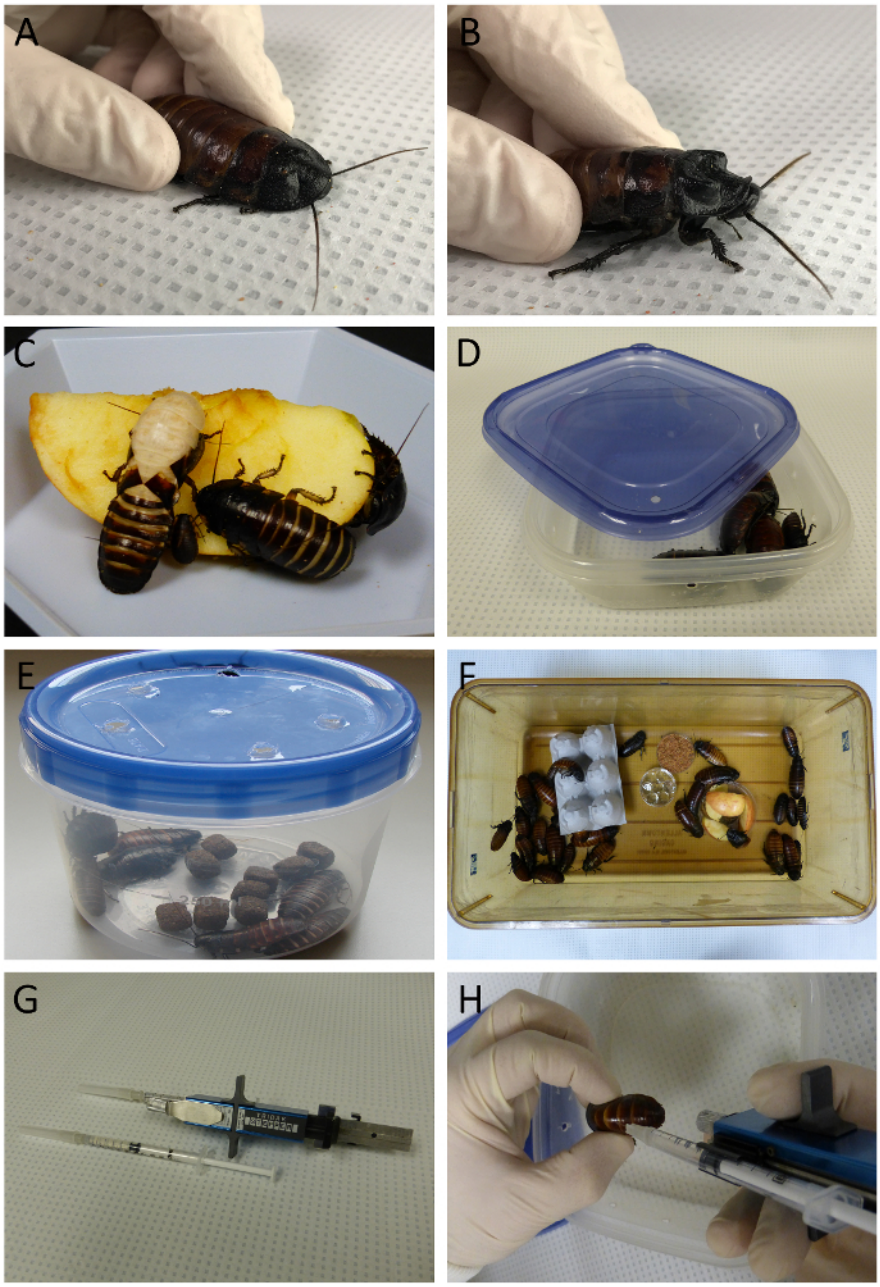

Figure 5: Images related to the hissing cockroach model. (**A**) A female hissing cockroach lacks protrusions on its head. (**B**) A male hissing cockroach can be identified by the presence of horns. (**C**) A hissing cockroach must molt out of its exoskeleton to grow. The emerging insect is white in color but gradually darkens as the new exoskeleton hardens. (**D**) Hissing cockroaches may be housed in a snap cap plastic container with ventilation holes during an experiment. (**E**) Under BSL3 conditions, hissing cockroaches are housed in screw cap plastic containers. (**F**) A large mouse cage is used to house a hissing cockroach colony. It should contain food, water and a cardboard egg carton for hiding. (**G**) Hissing cockroaches are injected with a 1 mL syringe attached to a repetitive pipette. (**H**) A hissing cockroach is inoculated by injection through the cutaneous membrane between the abdominal terga. Please click here to view a larger version of this figure.

1	Live, Normal	-actively mobile -able to grasp and hold onto fingers when hissing cockroach is picked up
2	Live, Lethargic	-immobile but crawls when prodded
3	Live, Moribund	-immobile with legs tucked in -does not move when prodded -antennae and/ or legs move when prodded
4	Dead	-immobile with legs tucked in -antennae do not move when prodded -legs do not move when prodded

Table 1: Hissing cockroach morbidity scoring system. The overall score for a group of hissing cockroaches is based on the hissing cockroach with the highest score in the group.

Discussion

Optimal experimental conditions begin with a healthy hissing cockroach colony, which requires a minimal but consistent time commitment. Although hissing cockroaches can go for a relatively long period of time (~weeks) without food and water, weekly or bi-weekly cage maintenance must be provided. This includes checking the food and water supply and ensuring that the cage is dry. Maintaining dry living conditions is especially important during acclimation and incubation at higher temperatures; we find that more hissing cockroaches die and at a faster rate at higher temperatures when containers were not cleaned daily.

The key to consistent dosing or inoculation of the hissing cockroach is to press the dispenser button on the repetitive pipette firmly. We recommend practicing this technique, loading of the syringe onto the repetitive pipette, and performing blank injections. The most time-consuming step of the procedure for an operator new to the technique is holding or immobilizing the hissing cockroach during injection. Therefore, we also highly recommend practicing the technique of holding and injecting multiple hissing cockroaches before tackling a more ambitious project. This can be achieved by maintaining a small group of hissing cockroaches that is used exclusively for injection practices. Although we have found that injection can be performed quickly when holding the hissing cockroach on its side, other techniques for holding hissing cockroaches (*e.g.* immobilizing a hissing cockroach on a smooth curved surface; perching the hissing cockroach on the middle finger while the index finger and thumb immobilize it) may be preferred and should be explored by different operators.

The use of the hissing cockroach model affords several advantages over other insect models (e.g. the wax worm larva *Galleria mellonella* and the fruit fly *Drosophila melanogaster*) that have been previously used as animal models with *Burkholderia* infection [16,17,18]. For example, the experimental window for a hissing cockroach ranges from months to years allowing flexibility to the researchers, whereas that for a wax worm larva is only five days [19,20]. For a wax worm larva, the five day period also coincides with cocoon encasement; removal of cocoons is a labor intensive process that may cause physical trauma to the larvae [20]. More importantly, a *B. thailandensis* T6SS-1 mutant that is attenuated in both the Syrian hamster and the hissing cockroach [11], was virulent in *Galleria*, suggesting that *Galleria* is not a good model for the study of some mutants such as T6SS in *B. thailandensis* (data not shown).

The use of the hissing cockroach presents several advantages over the fruit fly. The hissing cockroach is large and of a substantial body mass with a tough exoskeleton that allows it to be easily handled during injections. In contrast, the fruit fly is small and requires specialized equipment for inoculation. Also, whereas the hissing cockroach naturally lives in temperatures that are similar to or exceed human body temperature, the optimal temperature for the fruit fly is between 22 to 28 °C. This makes the fruit fly of limited use in the context of studying processes that are dependent on human body temperature (such as multi-nucleated giant cell formation in *Burkholderia* [10]).

Some disadvantages to the use of hissing cockroaches do exist. The genetics of the hissing cockroach are not as well studied as those of *Drosophila* or even *Galleria*. The hissing cockroach also has a substantial "ick" or gross factor. However, the hissing cockroach remains an attractive and viable surrogate host for *Burkholderia* by providing clear advantages to its use in research that are unique to the species. As we have illustrated that the Madagascar hissing cockroach is a tractable surrogate host for *Burkholderia*, it very likely can also serve as a surrogate host for other bacterial pathogens and we are currently utilizing it in such studies.

Disclosures

The authors have nothing to disclose.

Acknowledgements

J. Chua, N.A. Fisher, D. DeShazer and A.M. Friedlander designed the procedures described in the manuscript. J. Chua, N.A. Fisher, S.D. Falcinelli and D. DeShazer performed the experiments. J. Chua wrote the manuscript.

The authors thank Joshua J. W. Roan, Nora D. Doyle, Nicholas R. Carter and Steven A. Tobery for excellent technical assistance and David P. Fetterer and Steven J. Kern for statistical analysis.

The work was supported by the Defense Threat Reduction Agency Proposal #CBCALL12-THRB1-1-0270 to A.M.F and #CBS.MEDBIO.02.10.RD.034 to D.D.

Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the U.S. Army.

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