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The Madagascar hissing cockroach as a novel surrogate host for *Burkholderia pseudomallei, B. mallei and B. thailandensis*

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Burkholderia *pseudomallei* and *Burkholderia* mallei are gram-negative pathogens responsible for the diseases melioidosis and glanders, respectively. Both species cause disease in humans and animals and have been designated as category B select agents by the Centers for Disease Control and Prevention (CDC). Burkholderia thailandensis is a closely related bacterium that is generally considered avirulent for humans. While it can cause disease in rodents, the B. thailandensis 50% lethal dose (LD_{50}) is typically ≥ 10^4-fold higher than the B. pseudomallei and B. mallei LD_{50} in mammalian models of infection. Here we describe an alternative to mammalian hosts in the study of virulence and host-pathogen interactions of these Burkholderia species.

Madagascar hissing cockroaches (MH cockroaches) possess a number of qualities that make them desirable for use as a surrogate host, including ease of breeding, ease of handling, a competent innate immune system, and the ability to survive at 37^0C. MH cockroaches were highly susceptible to infection with B. pseudomallei, B. mallei and B. thailandensis and the LD_{50} was <10 colony-forming units (cfu) for all three species. In comparison, the LD_{50} for Escherichia coli in MH cockroaches was >10^5 cfu. B. pseudomallei, B. mallei, and B. thailandensis cluster 1 type VI secretion system (T6SS-1) mutants were all attenuated in MH cockroaches, which is consistent with previous virulence studies conducted in rodents. B. pseudomallei mutants deficient in the other five T6SS gene clusters, T6SS-2 through T6SS-6, were virulent in both MH cockroaches and hamsters. Hemocytes obtained from MH cockroaches infected with B. pseudomallei harbored numerous intracellular bacteria, suggesting that this facultative intracellular pathogen can survive and replicate inside of MH cockroach phagocytic cells. The hemolymph extracted from these MH cockroaches also contained multinuclear giant cells (MNGCs) with intracellular B. pseudomallei, which indicates that infected hemocytes can fuse while flowing through the insect's open circulatory system in vivo.

The results demonstrate that MH cockroaches are an attractive alternative to mammals to study host-pathogen interactions and may allow the identification of new Burkholderia virulence determinants. The importance of T6SS-1 as a virulence factor in MH cockroaches and rodents suggests that the primary role of this secretion system is to target evasion of the innate immune system.

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