N O T E

A Comparison of Hamster Anesthetics and Their Effect on Mosquito (Diptera: Culicidae) Blood Feeding[1]

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Laboratory hamsters are routinely used in studies of insect pests, including tests for efficacy of repellants and pesticides, and vector pathogen transmission studies (Moncayo et al. 2009, J. Med. Entomol. 46:862 - 865; Turell et al. 2008, J. Med. Entomol. 45:102 - 108; Svobodova and Votypka 2003, Microb. and Infect. 5:471 - 474; Ghosh and Mukhopadhyay 1998, Int. J. Parasitol. 28:275 - 281). U.S. Government principles for the use of vertebrate animals in research require that pain, discomfort, and distress should be avoided when consistent with sound scientific principles (Guide for the Care and Use of Laboratory Animals, 1996, National Research Council, National Academy Press, Washington, DC). Anesthetic agents are often used to control pain and manage stress, as well as to immobilize hamsters used for vector transmission trials (Turell et al. 2008), however, these agents can cause profound physiological changes such as altered peripheral circulation and decreased body surface temperatures that could affect the success of the feeding model or alter the experimental results.

A commonly used anesthetic is ketamine (Ketamine-HCl), a rapid, centrally acting, dissociative anesthetic (Curl 1988, Laboratory. Anim. 22: 309 - 312) that by itself gives incomplete pain relief and poor muscle relaxation (Green et al. 1981, Laboratory Anim. 15:163 - 170). It is often used in conjunction with other sedatives or pain relievers during surgical or laboratory procedures. Xylazine (Xylazine-HCl) is a centrally-acting sedative, analgesic, and muscle relaxant (Curl 1988), and gives improved pain relief compared with ketamine, but incomplete anesthesia. In addition, xylazine can cause a drop in peripheral blood flow and body temperature as a secondary pharmacological effect of bradycardia and decreased blood pressure (Knight 1980, J. Am. Vet. Med. Assoc. 176: 454 - 455). A combined use of ketamine and xylazine is often used for surgical procedures and pain management of laboratory rodents (Green

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et al. 1981). We hypothesized that the use of ketamine alone would result in better mosquito feeding as compared with the combination with xylazine, due to the expected better peripheral blood flow.

Mosquitoes (Diptera: Culicidae) are nuisance pests and are well-known vectors of diseases such as malaria, dengue fever, and Rift Valley fever. Females take a blood meal to provide essential protein for egg production. Host seeking is initiated and affected by a number of factors, but mosquitoes that feed on mammals are generally attracted to body heat from warm-blooded animals, and carbon dioxide has been identified as a universal activator and attractant of mosquitoes to their hosts (Dekker et al. 2005, J. Exp. Biol. 208:2963 - 2972). Two genera of mosquitoes used in this study- *Aedes* and *Anopheles*- are known to feed preferentially (> 93%) on mammalian hosts (Molaei et al. 2008, J. Med. Entomol. 45: 1143 - 1151). Members of the *Culex* genera are highly ornithophilic (Burkett-Cadena et al. 2008, Am. J. Trop. Med. Hyg. 79(5): 809 - 815; Juliusson et al. 2009, J. Med. Entomol. 46(2): 380 - 390; Dow et al. 1957, Am. J. Trop. Med. Hyg. 6(2): 294 - 303), but may feed on reptiles or mammals if the preferred host is not available. The objective of this study was to evaluate blood feeding by mosquitos on hamsters anesthetized with either ketamine alone or ketamine with xylazine.

All procedures and experiments described herein were in accordance with approved University of Wyoming Institutional Animal Care and Use Committee protocols. Hamsters were females of matched age, housed in 28x35.5 cm plastic cages, and given *ad-libidum* access to standard rodent chow and water.

Hamsters ($n = 6$) were assigned to 1 of the 2 anesthesia treatments for each of 3 mosquito species - *Anopheles quadrimaculatus* Say, *Aedes aegypti* (Linnaeus), and *Culex tarsalis* Coquillett - in a completely randomized design. *Anopheles quadrimaculatus* specimens were purchased as 1st and 2nd instar larvae from Benzon Research (http://www.benzonresearch.com/insectlist.htm, Carlisle, PA). *Culex tarsalis* were from a colony maintained at the Arthropod Borne Animal Diseases Research Unit that was originally established in California in 1951 by Bill Reisen. *Aedes aegypti* were obtained as eggs from a colony at Colorado State University (Fort Collins). All mosquitoes were raised to adults and maintained at 75°F +/− 3°F on a 10% sucrose solution with a 12-h photoperiod. Mosquitoes were allowed to age differentially to allow for maximum feeding (Kristine Bennett, pers. obs.): *Ae. aegypti* and *An. quadrimaculatus* adults were used 2 - 3 d postemergence, and *Cx. tarsalis* adults were used 5 - 7 days postemergence. Twenty-four hours prior to being offered a hamster blood-meal, mosquitoes were anesthetized using CO_2 for 10 - 15 sec and placed in clear polycarbonate Nalgene jars (125 ml, 70 mm cap) with a disk of Whatman filter paper covering the bottom of the jar and a fine mesh top held in place with a canning jar ring. Approx. 5 - 20 females and 5 - 10 males were placed in each jar. While in these jars, mosquitoes were deprived of sucrose but were provided water and were otherwise maintained in the same conditions described previously.

Hamsters were weighed and anesthetized with intraperitoneal injection of either ketamine (100 mg/kg, Ketaset, Fort Dodge Animal Health, Ft. Dodge, IA) alone or with xylazine (10 mg/kg, AnaSed, BenVenue Laboratories, Bedford, OH) prior to mosquito feeding. The abdominal area was shaved, and the hamster was placed on the mesh top of mosquito canisters for 20 min. Hamsters that began to move before the end of the feeding period were gently restrained by hand. Mosquitoes were then removed from the feeding container, sorted according to feeding status, and counted.

Data were analyzed as a logistic ANOVA according to a completely randomized design using the GLIMMIX procedure of SAS® version 9.2 (SAS Institute, Cary, NC).

The proportion of mosquitoes fed on hamsters was fitted to a generalized linear mixed model treating anesthetic and species factor combinations as fixed effects and hamsters within treatment and species as the random effect. The model also included all possible interactions. Data are presented as the proportions of blood-fed mosquitoes.

Aedes aegypti fed on hamsters anesthetized with ketamine or ketamine+xylazine were 6 and 1% blood-fed, respectively (Fig. 1), but with a high variance. This 6-fold increase was interesting but not significant ($P = 0.126$). The percentage of adult *An. quadrimaculatus* that fed on hamsters anesthetized with ketamine or ketamine and xylazine was 37% and 38% respectively, and the percentage of adult *Cx. tarsalis* that fed on hamsters anesthetized with ketamine or ketamine and xylazine was 7% and 8%, respectively (Fig. 1). *Anopheles quadrimaculatus* had significantly higher percentage adults that blood fed than the other species ($P = 0.003$).

This study used a uniform dosage of anesthetics based on weight for all hamsters. A broad range of individual hamster sensitivity to ketamine was observed in this study, as has been previously reported (Curl 1988), resulting in variable anesthetic depth. Some hamsters exhibited movement before the end of the 20-min feeding period. This likely contributed to some of the observed variation in fed mosquito proportions. Under actual vector feeding experiments, dosage would be individually adjusted to yield a uniform depth of anesthesia. Within each species of mosquito there was also a broad range of feeding success on individual hamsters that is not accounted for by hamster movement and may be explained by mosquito feeding selection preference for individual host animals (Dow et al. 1957).

The higher proportion of *Anopheles* that fed on the hamsters, regardless of anesthetic choice, may be a reflection of the preference of *Culex* species for avian and ectothermic hosts (Kent et al. 2009, J. Med. Entomol. 46(2): 380 - 90; Burkett-Cadena et al. 2008), and *An. quadrimaculatus* may have a higher intrinsic host preference for hamsters when compared with *Ae. aegypti* (Lardeux et al. 2007, Malaria J. 6: 1 - 14).

This study compared the mosquito blood-feeding success of 3 species of mosquitoes on hamsters anesthetized with either ketamine or ketamine/xylazine to determine if these anesthetic choices affect feeding efficiency in laboratory trials. The results suggest that for *Ae. aegypti*, ketamine alone results in better feeding efficiency,

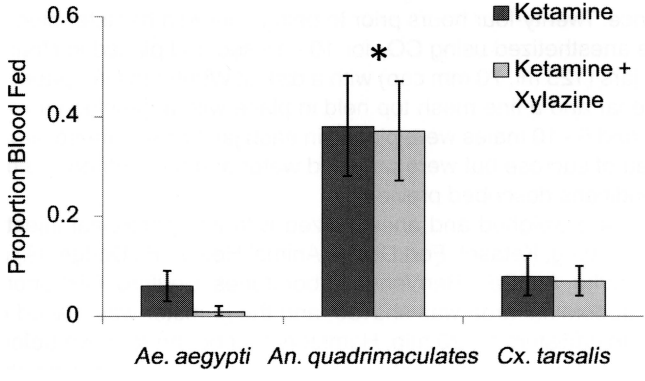

Fig. 1. Proportion of adult mosquitoes by species that blood fed on hamsters anesthetized with ketamine or ketamine and xylazine.

but due to large variability this failed to reach significance at the 90% confidence level. Further investigation will be needed to determine if this is indeed significant.

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