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SHORT COMMUNICATION

DAYTIME INFECTION OF *CULEX QUINQUEFASCIATUS* MOSQUITOES WITH THE HUMAN FILARIA PARASITE *WUCHERERIA BANCROFTI* BY IN VITRO FEEDING

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Introduction

Culex quinquefasciatus Say is the dominant natural vector of the human filaria parasite *Wuchereria bancrofti* Cobbold in Sri Lanka. The vector normally becomes infected when it takes a nocturnal blood meal on a microfilaraemic person. Laboratory colonies of *Cx. quinquefasciatus* are routinely blood fed at night with mice or guinea pigs. The night blood feeding habit of this mosquito, coupled with the appearance of infective microfilariae in the peripheral blood of man only at night, has limited infectivity and other studies on the *Cx. quinquefasciatus* - *W. bancrofti* association to late night hours. At this time mosquitoes are fed directly on infected volunteers. Laboratory studies on this important parasite-vector combination are therefore mostly confined to day-time feeding laboratory vectors of *W. bancrofti*, such as *Aedes togoi* Theobald[1] and *Anopheles tessellatus* Theobald,[2] and other anophelines[3] which are infected with the parasite by *'in vitro'* membrane feeding. *Cx. quinquefasciatus* has however been infected with arboviruses by membrane feeding[4]. The present communication is, to our knowledge, the first report of the infection of the natural vector *Cx. quinquefasciatus* with *W. bancrofti* by membrane feeding during day time.

Materials and Methods

A colony of *Cx. quinquefasciatus* derived from wild caught mosquitoes in Peliyagoda, an urban locality endemic for filariasis in Sri Lanka, was maintained in our laboratory, at 28 ± 1^{0}C, 70-80% relative humidity and natural day-night conditions. Larvae were fed soya protein and adults on 10% glucose supplemented with multivitamins. The colony was blood fed overnight using guinea pigs or mice.

Five to seven day old mosquitoes used for blood feeding were deprived of sugar solution for 24 hr and water for the next 20 hr. Mosquitoes were held in paper cups covered with nylon netting. Males were removed as they interfered with feeding. For normal feeding, heparinized rabbit blood was presented to the mosquitoes, through a water-jacketed membrane (Baudruche membrane) feeder of 12mm diameter with circulating water at 40-41^{0}C. Feeding was carried out at different times between 0900 hr and 1400 hr. *W. bancrofti* infected blood from adult volunteers (approximately 5 microfilariae per 20 μl) was collected in the evening and kept in a refrigerator at 4^{0}C overnight. Such storage did not damage microfilariae as observed in a thick blood smear stained with Giemsa. The infected blood was then presented to mosquitoes through the membrane feeder the following morning. Blood fed mosquitoes were provided with glucose and maintained in paper cups. The mosquitoes were examined for L_3 larvae 14 days after the blood meal by dissecting the head, thorax and abdomen separately in 0.01M phosphate-buffered saline, pH 7.4.

Results and Discussion

Mosquitoes were readily attracted to the membrane and fed to engorgement with feeding being completed in 15-20 min. In six different trials, 8/24, 4/8, 2/11, 2/7, 6/12 and 6/12 females took a blood meal through the membrane. Engorged females frequently remained on the membrane, preventing non-fed females from feeding. It was therefore necessary to limit the number of females in a cup to 10-12 to ensure good blood feeding. It was observed that other *Cx. quinquefasciatus* starved in a similar manner and held in cages, were not attracted to feeding on live guinea pigs, mice or a human arm presented to them during the same time of day. It is possible that some aspect of the biological rhythm of vertebrates prevents *Cx. quinquefasciatus* from feeding on such hosts during the day. No mortality or obvious physiological changes were seen in females engorged by *in vitro* feeding. The longevity and fecundity (as measured by the number of mature oocytes produced) in membrane fed mosquitoes were not different from mosquitoes fed at night on live hosts. The infection rates among blood fed mosquitoes in three groups of feeds were 2/4, 2/5 and 2/5. On dissecting *Cx. quinquefasciatus* fed on infected human blood, only live infective L_3 larvae were present. These larvae were distributed in the head, thorax and abdomen. Although the number of observations made was small, the results nevertheless demonstrate that *Cx. quinquefasciatus* can be infected with *W. bancrofti* during the day by *in vitro* feeding. However, no attempt was made to infect *Cx. quinquefasciatus* with *W. bancrofti* by *in vitro* feeding at night. Since *Cx. quinquefasciatus* is the dominant natural vector of *W. bancrofti* in South Asia, the ability to infect the mosquitoes during the day, facilitates a variety of studies on this important human parasite-vector relationship. This method of infecting *Cx. quinquefasciatus* with *W. bancrofti* also has ethical and other practical advantages since it avoids the use of human volunteers for direct feeding of mosquitoes.

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