COMPARISON OF FOUR MEMBRANES FOR ARTIFICIALLY BLOODFEEDING MOSQUITOES[1]

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ABSTRACT. Four species of mosquitoes, *Aedes aegypti*, *Ae. triseriatus*, *Culex pipiens* and *Cx. tarsalis*, were allowed access to defibrinated rabbit blood via 4 different membranes and a standard membrane feeder. Natural animal skins (mouse or quail) were the most effective. More than 50% of the *Aedes* mosquitoes fed within 5 min and approximately 90% fed within 20 min on either mouse or quail skin. *Culex* species fed best on quail skin, but the difference in feeding on quail skin and sheep intestine was not significant at 10 or 20 min. In general *Culex* mosquitoes fed less readily on natural animal skins than *Aedes*.

Artificial bloodfeeding of arthropods is frequently utilized in physiological, behavioral and vector-agent studies. In our laboratory, artificially feeding mosquitoes has facilitated the infection of mosquitoes with parasites and pathogens, especially *Dirofilaria immitis* and several mosquito-borne viruses, and has eliminated the need for carefully timed infective animals. Furthermore, artificial feeding permits manipulation of the dosage of infectious agents administered.

A variety of methods has been developed for artificially bloodfeeding mosquitoes through membranes. Early studies recognized that warming the blood was beneficial to attract and initiate feeding by mosquitoes. Greenberg (1949) warmed blood with electrically heated resistance wires, and Tarshis (1959) maintained blood at a constant temperature with a water bath. A water-jacketed membrane feeder developed by Rutledge et al. (1964) allows water to flow through the jacket chamber to maintain blood at a constant temperature. This apparatus or some modification of it is commonly used today.

Different types of membranes have been used (with varying success) to bloodfeed mosquitoes (Tarshis 1958). Natural membranes include chicken skin (Bishop and Gilchrist 1944), sausage casings (Eyles 1952, Kartman 1953, Wirtz and Rutledge 1980) and condoms made from sheep intestine (Bailey et al. 1978). Plastic wrap and Parafilm® (Rutledge et al. 1964) have also been tested for utility in artificially feeding mosquitoes. Obtaining the highest feeding success in the shortest time is an important consideration in many of our experiments. The objective of this study was to determine mosquito bloodfeeding success through several commonly used membranes.

The feeding success of 4, typically colonized mosquito species was compared on 4 easily obtained membranes. *Aedes aegypti* (Linn.), *Ae. triseriatus* (Say), *Culex pipiens* (Linn.) and *Cx. tarsalis* Coq. were used to evaluate feeding responses of mosquitoes with a wide variability in host preference. Membranes tested were unshaven mouse skin; plucked bobwhite quail, *Colinus virginianus* (Linn.), skin; condoms made from sheep intestine (Naturalamb®); and latex condoms (Trojan®). Blood was obtained from rabbits via heart puncture, mechanically defibrinated and maintained at 37–38°C in feeders similar to those described by Rutledge et al. (1964). Mosquitoes were exposed to membranes for different periods of time.

Mosquitoes were reared by following common laboratory procedures; 30–50 female pupae were placed in 0.5-liter emergence cartons. Adults were afforded access to 0.3 M sucrose in saturated cotton pads. Sucrose was removed from cartons 12–24 h before bloodfeeding, and 7-day-old mosquitoes were exposed to the membranes with exposure surfaces 3.5 cm in diam. *Aedes* mosquitoes were bloodfed 3–4 h preceding normal lights off, and *Culex* were fed less than 2 h before lights off. Room lighting was decreased to facilitate feeding of *Culex* mosquitoes.

Mouse and quail membranes were prepared by euthanizing animals[2] and immediately removing their skins. Mouse skin was obtained by cutting along the ventral midline and separating the skin from connective tissue. Skin from the back of the mouse was used as the membrane. Bobwhite quail were plucked, and the skin was removed from each breast. Sheep intestine (Naturalamb) and latex condoms were washed in a dilute detergent solution and thoroughly rinsed to remove lubricants and perfumes.

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[2] Authors have adhered to *Journal's* policy for animal care and use.

Cartons of mosquitoes were exposed to membranes for 5, 10 or 20 min. Each exposure (species × membrane × time) was repeated 3–5 times. Differences in the percentage of mosquitoes bloodfeeding were compared using PROC GLM and Tukey's studentized range test. Data were normalized prior to analysis using Bartlett's arc sine transformation (Snedecor and Cochran 1980). Differences in feeding preference of each species, utility of each membrane, and time of exposure were considered significant at $P < 0.05$.

Each species responded differently to the membranes (Table 1). *Aedes aegypti* fed better on the natural animal skins, feeding readily on both mouse and quail skin. At 20 min, the number feeding on the sheep intestine membrane was significantly lower than the number feeding on either animal skin. Very few *Ae. aegypti* fed on the latex membrane. *Aedes triseriatus* also fed well on natural animal skins. A significantly higher percent fed on quail skin during the 5-min exposures; however, there was no significant difference in the percent engorged after exposure to quail or mouse skins for 10 or 20 min (Table 1). *Aedes triseriatus* did not feed on the latex membrane, but similar to *Ae. aegypti*, more than 50% fed on the sheep intestine condom during the 10- and 20-min exposures. *Culex pipiens* fed best on quail skin, but the difference between quail skin and sheep intestine was not significant at 10 or 20 min. *Culex pipiens* did not feed on the latex membrane, and less than 10% fed on the mouse skin. *Culex tarsalis* fed best on the quail skin and sheep intestine membranes. There were no statistically significant differences among these membranes at any exposure time. *Culex tarsalis* were not exposed to a latex membrane because of poor feeding by other species.

Blood occasionally congealed on the sheep intestine membrane after several minutes; if this occurred, mosquitoes were unable to feed. Mechanically defibrinated blood did not congeal on any other membrane. A preservative or lubricant may have caused the congealing, even though the membranes were thoroughly washed and rinsed several times. When congealing occurred the results were discarded, and the experiment was repeated with a different membrane. Rutledge et al. (1964) reported a similar occurrence with Baudruche membranes.

Natural animal skins proved to be the most effective membranes for bloodfeeding mosquitoes. More than 50% of the *Aedes* mosquitoes fed within 5 min on either mouse or quail skin. Approximately 90% of the *Aedes* mosquitoes had engorged on either of these membranes after 20-min exposures. *Culex* mosquitoes fed less readily than *Aedes* on natural animal skins and showed little difference between the quail skin and sheep intestine membranes. Twenty-minute exposures were adequate for 50% feeding to occur on the sheep intestine membrane. Other factors such as blood temperature, time of feeding, starvation before feeding, etc. may alter feeding success. Modification of one or more of these parameters may increase the success of mosquitoes bloodfeeding on these membranes.

Additional considerations may affect the choice of membrane. Wirtz and Rutledge (1980) demonstrated the cost-effectiveness of main-

Table 1. Bloodfeeding of mosquitoes on 4 different membranes.

Mosquito species	Membrane	Percentage bloodfed[1] (±SD) 5 min	10 min	20 min
Aedes aegypti	Mouse skin	65 (8)a	94 (6)a	92 (12)a
	Quail skin	52 (17)ab	84 (15)ab	93 (11)a
	Sheep intestine	40 (12)b	62 (19)b	59 (12b)
	Latex condom	0c	0c	2 (4)c
Aedes triseriatus	Mouse skin	60 (9)a	76 (2)a	87 (4)a
	Quail skin	79 (4)b	80 (13)a	92 (5)a
	Sheep intestine	29 (10)c	52 (20)a	56 (7)b
	Latex condom	0d	0b	0c
Culex pipiens	Mouse skin	4 (4)a	7 (9)a	7 (5)a
	Quail skin	40 (10)b	56 (16)b	70 (16)b
	Sheep intestine	23 (7)c	38 (7)b	60 (18)b
	Latex condom	0a	0a	0a
Culex tarsalis	Mouse skin	0a	4 (5)a	16 (17)a
	Quail skin	43 (34)b	58 (31)b	64 (15)b
	Sheep intestine	27 (19)b	45 (17)b	63 (15)b

[1] Statistical analysis compared mean feeding percentages (normalized with Bartlett's arc sine transformation) within each exposure time for each species. Means in each group (4 membranes, 1 species, 1 exposure time) followed by a different letter are significantly different (PROC GLM and Tukey's studentized range test, $P < 0.05$).

taining mosquito colonies using reusable sausage casings compared with maintaining laboratory animals for such purposes. However, for small-scale bloodfeeding, our data indicate that the use of sheep intestine membranes is a practical choice only if a 50% feeding success rate is tolerable.

We have not observed difficulties in infecting mosquitoes using fresh animal skin membranes. Mouse skins have been used to infect *Ae. aegypti* and *Ae. trivittatus* with *Dirofilaria immitis* (Berry et al. 1987). The titer of La Crosse virus does not decrease over the feeding period when *Ae. triseriatus* is exposed to La Crosse encephalitis virus through a mouse skin in our laboratory (Berry, unpublished data).

REFERENCES CITED

Bailey, D. L., D. A. Dame, W. L. Munroe and J. A. Thomas. 1978. Colony maintenance of *Anopheles albimanus* Wiedemann by feeding preserved blood through natural membrane. Mosq. News 38:403–408.

Berry, W. J., W. A. Rowley and B. M. Christensen. 1987. Influence of developing *Dirofilaria immitis* on the spontaneous flight activity of *Aedes aegypti* (Diptera: Culcidae). J. Med. Entomol. 24:699–701.

Bishop, A. and B. M. Gilchrist. 1944. A method for collecting sporozoites of *Plasmodium gallinaceum* by feeding infected *Aedes aegypti* through animal membranes. Nature 153:713–714.

Eyles, D. E. 1952. Studies on *Plasmodium gallinaceum*. II. Factors in the blood of the vertebrate host influencing mosquito infection. Am. J. Hyg. 55:276–290.

Greenberg, J. 1949. A method for artificially feeding mosquitoes. Mosq. News 9:48–50.

Kartman, L. 1953. Factors influencing infection of the mosquito with *Dirofilaria immitis* (Leidy, 1856). Exp. Parasitol. 2:27–78.

Rutledge, L. C., R. A. Ward and D. J. Gould. 1964. Studies on the feeding response of mosquitoes to nutritive solutions in a new membrane feeder. Mosq. News 24:407–419.

Snedecor, G. W. and W. G. Cochran. 1980. Statistical methods, 7th ed. Iowa State Univ. Press, Ames, IA.

Tarshis, I. B. 1958. Feeding techniques for bloodsucking arthropods. Proc. Tenth Int. Congr. Entomol., Montreal, 1956. 3:767–784.

Tarshis, I. B. 1959. Feeding *Culex tarsalis* on outdated whole human blood through animal-derived membranes (Diptera: Culicidae). Ann. Entomol. Soc. Am. 52:681–687.

Wirtz, R. A. and L. C. Rutledge. 1980. Reconstituted collagen sausage casings for the blood feeding of mosquitoes. Mosq. News 40:287–288.