ORAL INFECTION OF *AEDES POLYNESIENSIS* BY *WUCHERERIA BANCROFTI* BY USING PARAFILM MEMBRANE FEEDING

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ABSTRACT. In order to construct a cDNA library from third-stage larvae (L3) of *Wuchereria bancrofti* var. *pacifica*, the Parafilm™ membrane feeding method is proposed for the oral infection of *Aedes polynesiensis*. Heparinized blood supplemented with $5 \cdot 10^{-3}$ M ATP was put in the feeder with carbon dioxide provided as additional phagostimulant. The results of this artificial infection feeding method were compared with those obtained when mosquitoes fed directly on the forearm of a microfilaremic patient. The number of females feeding through the artificial membrane was smaller than on the patient's forearm (32.1 vs. 84.8%). The mean number of L3s obtained per female was not statistically different between the 2 feeding methods; however, the total number of L3s obtained from 100 females allowed to feed in each group was twice as high in the natural feeding method.

The infection of laboratory bred vectors is often used to study parameters involved in the biology of the vector-parasite relationship or to produce parasites that are infective for vertebrate hosts. The artificial membrane bloodfeeding technique has been used with success for many hematophagus insects like *Aedes aegypti* (Linn.), *Culex annulirostris* Skuse, *Haematobia irritans exiqua* de Miejere, *Culicoides marksi* Lee and Reye, *Culicoides bunyensis* Lee and Reye, and *Culicoides victoriae* Macfie (Owens 1981).

Aedes (*Stegomyia*) *polynesiensis* Marks, the main vector of *Wuchereria bancrofti* var. *pacifica* (Galliard et al. 1949), pathogen of lymphatic filariasis in French Polynesia, is a rural mosquito that breeds in natural cavities such as crab or tree holes and in coconut shells. Because this mosquito is a very selective feeder in the laboratory, artificial membrane feeding has never been very successful. Due to the lack of an experimental animal model for *W. bancrofti*, it is usually necessary to infect this mosquito species by feeding females on the forearm of a microfilaremic patient (Duke et al. 1967). This raises important ethical questions when infecting a large number of mosquitoes, so it would be desirable to have an artificial membrane feeding technique for this purpose.

In this paper we describe the Parafilm™ membrane bloodfeeding method for the oral infection of *Ae. polynesiensis* by *W. bancrofti*, and compare its efficiency in producing infective third-stage parasites (L3) with natural bloodfeeding on the forearm of a microfilaremic patient.

The Raiatea strain of *Ae. polynesiensis* which was used has been maintained in the laboratory since April 1990. Larvae were reared to the pupal stage in a 40 × 30 × 5 cm pan filled with 2 liters of water supplemented with liver extract (1 g/liter) and a few yeast grains. An average of 250 stage one larvae were put in each pan to obtain females of a homogeneous size. Pupae were collected and placed in cages (30 × 30 × 30 cm) until emergence. The adults were fed on a 10% sucrose solution. Microfilariae (mf) of *W. bancrofti* were obtained from a patient in Tahiti having a microfilaremia of 4,600 mf/ml of blood.

The mosquitoes were infected in parallel on the patient's forearm and through a membrane with venous blood from the same patient. The blood was collected in heparinized sterile tubes, kept at room temperature, and used within 2 days. The membrane feeder was similar to the one described by Rutledge et al. (1964) which consists of an inverted water-jacketed glass funnel maintained at 37 ± 1°C, with a base opening of 3.5 cm in diameter (Pasteur Institute, Paris). The Parafilm (American Can Co.) was commonly used as a membrane for artificial feeding (Bunner et al. 1989). It was stretched to about twice its original size and placed over the opening. The feeder was placed on the top of a plastic cup covered with nylon net which contained about fifty 6-day-old females that were starved for 24 hours. Three ml of parasitized blood were put into the feeder, and the mosquitoes were allowed to feed for 1 h through the membrane. All mosquitoes that fed on patient's forearm and through the Parafilm membrane were treated identically thereafter. Only engorged females were kept. They were maintained in cages at 26 ± 1°C with a 12 h light/dark photoperiod, and fed on a 10% sucrose solution. Thirty females were individually dissected immediately to estimate the number of mf ingested and about 30 from each group thereafter every day as possible to determine if larval development was proceeding normally. The effects of the phagostimulants adenosine triphosphate (ATP $5 \cdot 10^{-3}$ M) (Sigma Chemical Co., #A-3377) and carbon dioxide (CO_2) in the form of a piece of dry ice above the feeder were evaluated.

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The efficiency of the 2 methods of infection was compared by calculating the following parameters: (1) the percentage of engorged females, (2) the percentage of infected females (i.e., containing microfilariae) among the engorged females, (3) the average number of mf ingested per infected female, (4) the percentage of infective females (containing L3 larvae) among the number of alive mosquitoes on day 15, (5) the average number of L3 per female, (6) the number of L3 per 100 females allowed to engorge obtained as followed: (1) × (2)/100 × (4)/100 × (5), and (7) the percentage of cumulated mortality at day 15.

The percentages were compared using χ^2 contingency tables or Fisher's exact test. The means were compared by the Mann and Whitney test. The data were analyzed by using Statgraphics (Plus Ware Products, USA) and a IBM PC compatible computer.

The effects of carbon dioxide (CO_2) and adenosine triphosphate (ATP) on the Parafilm feeding method are reported in Table 1. Separately ATP and CO_2 significantly improved the percentage of females that engorged. When both ATP and CO_2 were used, the percent engorgement increased significantly.

The comparative results of the 2 methods of infection are given in Table 2. Because of the large number of engorged females obtained using the natural infection method, about 30 females were dissected on days 0 through 11 and day 15, whereas dissections only on days 0, 4, 9 and 15 were carried out for mosquitoes infected through the membrane. These dissections indicated that the migration and molts of the parasite followed a normal and identical course of development in the vector irrespective of the method of infection.

The oral infection of *Ae. polynesiensis* with *W. bancrofti* through an artificial Parafilm membrane has been achieved successfully. The presence of CO_2 and the addition of ATP in the blood were necessary for the engorgement of the females. These phagostimulants have been used previously for the artificial blood feeding of other hematophagus vectors (Owens 1981, Bunner et al. 1989). In our case, the percentage of engorgement using the artificial membrane was significantly lower than with the method of feeding directly on the human (32.1 vs. 84.8%, $P < 0.05$). This result stressed the difficulty of artificially feeding *Ae. polynesiensis*, a highly selective mosquito that is well adapted to feeding on humans. Previous experiments using chicken skin and pig intestine failed (unpublished data), but finally the Parafilm membrane method gave improved results. Moreover, Parafilm is convenient because it is readily available, cheap and easy to handle. Similar feeding success rates were obtained with *Culicoides* spp. using one-day-old duck skin and condoms as a membrane (Owens 1981). Other authors have tried with success other kinds of membranes such as collagen sausage casing for the bloodfeeding of *Ae. aegypti*, *Ae. taeniorhynchus* Wied., *An. albimanus* Wied., *An. quadrimaculatus* Say, *An. stephensi* Liston (Wirtz and Rutledge 1980) and nylon gauze covered with an acrylic resin for *Ae. aegypti* (Hagen and Grunewald 1990).

Table 1. Effect of CO_2 and ATP on the engorgement of *Aedes polynesiensis* through a Parafilm membrane.

ATP	CO_2	Percentage engorgement (number)
−	−	0.0 (0/90)
−	+	7.6 (9/118) a
+	−	18.4 (9/49) b
+	+	37.3 (59/158) c

Comparison a,b: $P < 0.05$.
Comparison b,c: $P < 0.05$.

Although the percentage of infected females (containing mf) was not different between the 2 feeding methods, the mean number of mf ingested per engorged female was higher in the natural feeding method (14.13 vs. 3.13, $P < 0.05$). This difference may be explained either by the smaller volume of blood ingested when using the Parafilm method, as visually observed, or by the higher microfilaria density in capillary blood than in venous blood (Eberhard et al. 1988, Lowrie et al. 1989).

The percentage of females containing stage 3 larvae and the mean number of L3s obtained per female was not statistically different between the 2 methods. However, the efficiency of producing L3s is more than twice higher by the natural feeding method than by artificial feeding. Consequently, for the mass collection of infective third-stage parasites, the natural feeding method is recommended. The high mortality observed in the artificial feeding method may be related to the increased handling of the mosquitoes.

We concluded that *Aedes polynesiensis* can be successfully infected by *Wuchereria bancrofti* using the Parafilm feeding method. To avoid ethical problems, this method is proposed for the mass collection of L3 from mosquitoes which have been infected artificially.

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Table 2. Oral infection efficiency of *Aedes polynesiensis* with *Wuchereria bancrofti* using the natural and artificial bloodfeeding techniques.

	% Engorged females	% Infected females	Average no. mf ingested	% Infective females	Average no. L3/female	No. L3/100 females allowed to feed	% Cumulated mortality at day 15
Natural feeding	84.8 (652/769)	90.0 (27/30)	14.13 (424/30)	47.1 (8/17)	4.06 (69/17)	146	44.8 (292/652)
Artificial feeding	32.1 (291/906)	86.7 (26/30)	3.13 (94/30)	81.0 (17/21)	2.67 (56/21)	60	64.6 (188/291)
Significance	*	NS	*	NS	NS		*

* $P = 0.05$.

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