Behavior of *Wuchereria bancrofti* (Filariidea: Onchocercidae) Infective Larvae in the Vector *Aedes polynesiensis* (Diptera: Culicidae) in Relation to Parasite Transmission

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ABSTRACT In French Polynesia, *Aedes polynesiensis* Marks is the major vector of human filariasis caused by subperiodic *Wuchereria bancrofti* (Cobbold). Factors affecting transmission of infective larvae from vector to humans were assessed. The 66–100% loss of infective larvae during a blood meal was independent of the initial vector parasite burden. Infective larvae were able to migrate to the mouthparts during a blood meal. Blood feeding by mosquitoes to repletion was one important aspect in the escape of larvae. Infective larvae were not transmitted (lost) without a blood meal and may move in the vector's body. *Ae. polynesiensis* may retain infectivity potential during several days and may infect several hosts during one blood meal session or on several gonotrophic cycles. In terms of parasite transmission, these findings were interpreted as efficient adaptations of the filaria to the *Ae. polynesiensis* biting habits.

KEY WORDS *Wuchereria bancrofti, Aedes polynesiensis,* infective larvae, loss of parasites, blood feeding

Aedes polynesiensis Marks is distributed widely in the South Pacific region (Evenhuis and Gon 1989) and is involved in the transmission of subperiodic *Wuchereria bancrofti* (Cobbold) (White 1989). In French Polynesia this disease is of public health importance and *Ae. polynesiensis* has long been recognized as the major vector (Rosen 1955).

Some aspects of the *W. bancrofti*/mosquito relationship may have important effects on transmission dynamics (Nelson 1964). Parasite development within the vector has often been examined and most studies on vector efficiency have dealt with the success of ingested microfilariae to become infective L3 larvae (Bryan and Southgate 1988 a, b; Bryan et al. 1990; Southgate and Bryan 1992). These aspects of transmission have been well documented for *W. bancrofti* in *Ae. polynesiensis* in French Polynesia (Pichon 1974; Pichon et al. 1974, 1980; Prod'hon et al. 1980). After maturation in the vector, the loss of infective larvae is an important aspect of transmission efficiency. Usually in epidemiological studies on filariasis, it is assumed that all infective larvae of *W. bancrofti* in the mosquito are capable of initiating a new infection when the vector blood feeds on a suitable host. However, Hairston and de Meillon (1968) have shown indirectly that, in the field, the number of infective larvae entering the human population is not equal to that in the mosquito population. Rozeboom et al. (1968) discussed factors that would affect the efficiency of transmission of *W. bancrofti* L3 by southern house mosquito, *Culex quinquefasciatus* Say, and laboratory studies were carried out to quantify the loss of mature *W. bancrofti* larvae during blood feeding (Pratt and Newton 1946, Jordan 1959, de Meillon et al. 1967, Zielke 1976). Moreover, infective larvae may be lost without blood feeding (Wharton 1957a, 1960; Lavoipierre and Ho 1966; Ramachandran 1966; Ramachandran and Zaini 1968; Bosworth and Ewert 1971; Rifaat et al. 1971; Zielke 1976). This loss was generally progressive, very slow and sometimes did not occur (Brengues 1975). Newton and Pratt (1945), Gordon and Crewe (1953) and Wharton (1957b) demonstrated that L3 larvae are very motile and may affect vector biology. Lavoipierre and Ho (1966) observed that L3 larvae moved to the mosquito's head during a weekly cycle. These previous studies indicated the importance of the behavior of infective larvae in the vector and, in particular, movements of larvae to the head and loss rates of L3 larvae during blood feeding. Unfortunately, epidemiological studies on transmission of *W. bancrofti* by *Ae. polynesiensis* lack these kinds of data. In particular, the rate of escape of infective larvae from *Ae. polynesiensis* has never been assessed despite its importance in computing transmission potential.

This research was carried out on the transmission of *W. bancrofti* by *Ae. polynesiensis* to quantify the rate of loss of infective larvae during a blood meal, the movement of infective larvae from all parts of the mosquito's body to the proboscis at the time of blood feeding, the effect of a replete blood meal on the loss of infective larvae, the loss of infective larvae without any blood meal, and the

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pattern of movement by infective larvae in the mosquito's body between blood meals.

Materials and Methods

Infection of Mosquitoes. The same protocol was used in all experiments. *Ae. polynesiensis* females were blood fed on the forearm of a microfilaremic volunteer from Tahiti (Paea) who harbored a high microfilariae density (>5,000 microfilariae per 1 ml of blood as estimated with 5–10 10-μl blood drops). Because the parasite "success rate" in a mosquito is optimum when both parasites and mosquitoes come from the same geographic area (Failloux et al. 1995), a Tahiti (Paea) strain of *Ae. polynesiensis* was colonized from females collected from this locality. After the infective blood meal, engorged females were kept in an insectary (≈26°C, ≈80% RH, natural photoperiod of ≈12:12 [L:D] h). A 5% honey solution on damped cotton was supplied until the number of infective larvae in head, thorax, and abdomen of mosquitoes was recorded (i.e., ≈ 2 wk). In all mosquito groups, the high microfilaremic level of the blood used for feeding ensured a 100% L3 infestation of mosquito females.

Loss of Infective Larvae After a Blood Meal. The loss of infective larvae during blood feeding was estimated by comparing the mean number of infective larvae in batches of mosquitoes before and just after a blood meal on a mouse after the mosquitoes fasted for 6 h. A random sample of unfed mosquitoes first was removed and then a mouse was introduced into the cage. Mosquitoes were allowed to feed until repletion. This experiment was carried out on 3 occasions (denoted as experiments 1, 2, and 3). In experiments 2 and 3, some females were maintained 1 additional week on sugar solution before blood feeding. These experiments were referred to as experiments 4 and 5, respectively.

Influence of Repletion on Loss of Infective Larvae. During blood feeding in experiment 2, some mosquitoes were ≈½ fed and were used to compare the loss of infective larvae in completely and incompletely engorged females.

Migration of Infective Larvae in Mosquito Body Before Blood Feeding. Mosquitoes used for this experiment came from another study designed to determine a degree-day model of filarial larvae development in the vector (unpublished data). As such, mosquitoes were kept in environmental chambers (MIR 250, Sanyo, Tokyo) set at 20, 22.5, 25.0, and 27.5°C, and every day 20 females were dissected to count *W. bancrofti* larvae in mosquito head, thorax, and abdomen. Experiments lasted until the last mosquito was killed in each cage. Infective larvae observations lasted 6 d at 20°C (i.e., from day 28 to day 33), 11 d at 22.5°C (from day 20 to day 30), 6 d at 25°C (from day 13 to 18), and 9 d at 27.5°C (from day 12 to 20). The percentage of L3 in head, thorax, and abdomen was compared every day, using chambers as replicates.

Loss of Infective Larvae without a Blood Meal. Mosquito colonies were maintained either on sugar-damped cotton or on a 5% honey solution in a small petri dish covered with a gauze to prevent insects from falling into the solution. Daily loss of infective larvae during sugar feeding was assessed by examining the sugar-damped cotton and honey solutions for L3 larvae using a Leica M3 binocular microscope at 400× magnification. Moreover, a comparison of the mean number of infective larvae between experiments 2 and 4 before the blood meal on mouse and between experiments 3 and 5 also was used to investigate the loss of infective larvae without a blood meal at 1-wk intervals. Experiments that investigated migration of larvae also permitted the analysis of loss of larvae without a blood meal by comparing decreases in daily mean numbers of infective larvae per mosquito.

Statistical Analysis. Statistical analysis was performed using the CSS:Statistica software (CSS:Statistica 1993).

Mean numbers of infective larvae were compared using Student t-test for 2 independent means or by analysis of variance (ANOVA) when >2 means were analyzed. Assumptions underlying these statistical analyses were verified as follows: before t-test computations, the distribution of larval counts was tested for normality by a chi-square test. Homogeneity of variances was checked by Bartlett's chi-square test. Counts (x) were transformed by square root ($x + 0.5$) to normalize the distribution and stabilize the variance (Lison 1968). When >2 means were compared, the relationship between means and variances was analysed using Taylor's power law (Taylor 1961). This enabled a more effective transformation of the data. When necessary (i.e., when variances were proportional to a fractional power of the arithmetic means), the appropriate transformation to normalize the data and stabilize the variances was to replace each count x by x^p, where $p = 1 - b/2$ and b is the slope of the log–log regression line between variances and means. In addition to satisfying assumption requirements for the 2-way ANOVA, data transformation also may remove the interaction effect which was never of interest in the various analyses performed in this study. In tables, means and variances were back-transformed for presentation. When the ANOVA was significant, means were grouped using the Student–Newman–Keuls method (Scherrer 1984), which assesses the probability of obtaining greater differences for each pair of ranked means under the null hypothesis (no difference between means in the population).

Global comparisons of >2 proportions were performed by G tests. When homogeneity of a set of proportions was rejected, G tests were used in

multiple comparisons to pool proportions into homogenous groups (Sokal and Rohlf 1981).

Results

Loss of Infective Larvae After a Blood Meal. In experiment 1, the percentage of reduction in the mean number of infective larvae per infective female after a blood meal on a mouse was 79.6% (Table 1). A 2-way ANOVA was carried out to investigate blood meal (this paragraph) and location (i.e., head, thorax, and abdomen) effects (next paragraph). There was a significant difference in mean numbers of L3 per female before and after blood feeding (i.e., a significant loss of larvae) ($F = 57.15$; df = 1, 114; $P < 0.0001$). In experiments 2 and 3 the percentages of reduction were 96.2 and 66.3%, respectively, and ANOVA indicated a significant difference before and after the blood meal ($F = 46.09$; df = 1, 120; $P < 0.0001$ and $F = 12.21$; df = 1, 84; $P < 0.001$, respectively). In experiments 4 and 5, where infective mosquitoes were maintained an additional 1 wk on sugar solution before the blood meal, the percentages reduction of larvae were 100 and 72.8%, respectively, indicating that larvae were still motile after a 1 wk delay. ANOVA indicated a significant effect of blood meal ($F = 14.04$; df = 1, 33; $P < 0.001$ and $F = 10.14$; df = 1, 60; $P < 0.01$, respectively). To gain precise insight into the duration of infectivity of larvae, results in experiments 2 and 4 were compared by 2-way ANOVA, which indicated that there was a significant difference before and after the blood meal ($F = 23.2$; df = 1, 50; $P < 0.0001$) but not between experiments ($F = 0.07$; df = 1, 50; $P = 0.79$), and there was no interaction between experiments and blood meal effect ($F = 0.24$; df = 1, 50; $P = 0.62$). A similar comparison between experiments 3 and 5 gave identical ANOVA results indicating a reduction of parasites after 1 wk of waiting (blood meal effect: $F = 15.9$; df = 1, 48; $P < 0.001$; experiment effect: $F = 0.002$; df = 1, 48; $P = 0.96$ and interaction: $F = 0.06$; df = 1, 48; $P = 0.79$).

Active Migration of Infective Larvae During a Blood Meal. Experiments 1–5 were analyzed to determine the location of infective larvae within the mosquito before and after the blood meal (Table 1). Here, 2-way ANOVAs investigated a location effect (i.e., in head, thorax, or abdomen) and all were significant ($P > 0.05$). Student–Newman–Kuels comparisons were carried out between the mean larval burden in heads, thoraces, and abdomens before and after blood meals, to indicate which body segment was responsible for the significance following ANOVA. As an example, Table 2 gives Student–Newman–Kuels results for experiment 1. In all experiments, significant differences existed between heads and in experiments 1, 2, and 4, between thoraces, indicating a significant reduction in parasites in these segments before and after blood feeding. The abdomens did not exhibit sig-

Table 1. Distribution of mean number ± SD of *W. bancrofti* infective larvae in head, thorax, and abdomen of infective *Ae. polynesiensis* before and after a blood meal

Blood meal on mouse	Experiment 1		2		3		4		5	
	Before	After	Before	After	Before	After	Before	After	Before	After
No. mosquitoes dissected	20	20	26	16	15	15	8	5	9	13
L3 in head	3.3 ± 2.0	1.1 ± 1.4	3.2 ± 3.6	0.1 ± 0.3	4.1 ± 3.2	0.8 ± 1.5	2.4 ± 2.6	0.0 ± 0.0	3.7 ± 3.5	0.2 ± 0.4
L3 in thorax	6.5 ± 5.5	0.9 ± 1.3	3.9 ± 4.5	0.2 ± 0.5	1.9 ± 2.1	0.8 ± 1.1	1.8 ± 2.1	0.0 ± 0.0	2.8 ± 3.5	0.4 ± 0.8
L3 in abdomen	1.3 ± 1.7	0.3 ± 0.6	1.4 ± 2.2	0.0 ± 0.0	0.6 ± 1.3	0.6 ± 1.3	0.9 ± 1.5	0.0 ± 0.0	0.3 ± 0.5	1.2 ± 1.7
L3 in whole mosquito	11.05 ± 7.3	2.3 ± 2.5	8.5 ± 7.5	0.3 ± 0.7	6.5 ± 4.9	2.2 ± 3.0	5.0 ± 4.7	0.0 ± 0.0	6.8 ± 5.8	1.8 ± 2.5

Table 2. Student–Newman–Keuls probabilities for comparisons of mean numbers of *W. bancrofti* infective larvae in head, thorax, and abdomen of vector before and after a blood meal (BM) in experiment 1

	Before blood meal			After blood meal	
	Head	Thorax	Abdomen	Head	Thorax
Thorax before BM	0.0700				
Abdomen before BM	0.0003*	0.0001*			
Head after BM	0.0005*	0.0001*	0.9107		
Thorax after BM	0.0002*	0.0001*	0.6274	0.4198	
Abdomen after BM	0.0001*	0.0001*	0.0581	0.0431*	0.1069

* Significant difference at $P = 0.05$.

nificant differences before and after blood meal, probably because of the low number of infective larvae in abdomen at this time after the infectious blood meal.

Influence of Parasite Burden on Parasite Loss. There was a significant difference in the percentages of parasite reduction among the 5 experiments (G test = 68.6, df = 4, $P < 0.0001$). Multiple comparisons grouped experiments 1, 3, and 5 into a homogenous group and experiments 2 and 4 into another. There was a significant difference among means of infective larvae in each experiment before blood feeding ($F = 2.56$; df = 4, 77; $P = 0.04$). Student–Newman–Kuels procedure grouped experiments 1 and 2, and experiments 2, 3, 4, and 5 into 2 groups, showing that in experiment 1 mosquitoes were more heavily parasitized. As groupings were different in the 2 analyses, infective larval burden did not influence the parasite loss rate.

Influence of Blood Feeding to Repletion on Loss of Infective Larvae. Before and after fully engorging on a mouse, the mean numbers of infective larvae per mosquito were 8.50 (SD = 7.45) and 0.32 (0.70), respectively (Table 1, experiment 2). In this same experiment, the mean number of larvae remaining in half-engorged mosquitoes was 2.50 (2.08), significantly more than in fully engorged females. The larval loss was 70.5% in the half-engorged females, less than the 96.2% loss seen in fully engorged females. Mean numbers of infective larvae in half-engorged mosquitoes were 0.50 (0.57) in head, 1.50 (1.29) in thorax, and 0.50 (0.60) in abdomen. Student–Newman–Kuels comparisons indicated that the mean number of larvae in the head was significantly different from females before blood meal ($P < 0.05$), but not from fully engorged females ($P = 0.51$). By contrast, the mean number of larvae in thorax was not different from females before blood meal ($P = 0.79$), but was significantly different from fully engorged females ($P < 0.05$). The means for abdomen were not statistically different before or after fully or half engorging.

Loss of Infective Larvae without a Blood Meal. Infective larvae were not found in sugar-damped cotton, and only 1 was found in the petri dishes with honey solution. Moreover, there was no significant difference between means of infective larvae before the blood meal in experiment 2 and in experiment 4 (t-test = 1.24, df = 32, $P = 0.22$) and between means in experiment 3 and experiment 5 (t-test = 0.11, df = 14, $P = 0.46$), indicating that there was no loss of infective larvae in mosquitoes during this 1-wk holding period. In addition, the mean numbers of infective larvae per female did not decrease over time in any of the 4 temperatures studied ($P > 0.08$) (Table 3).

Migration of Infective Larvae in the Mosquito's Body Not Related to Blood Feeding. G tests indicated significant heterogeneities among the distribution of larvae in head, thorax, and abdomen in mosquitoes maintained at 20, 22.5, 25, and 27.5°C (Table 3) (G test > 41.8, df = 10 to 20, $P < 0.0001$). Multiple comparisons did not indicate any cyclic localization of infective larvae in the head. The proportion of infective larvae in the abdomen decreased with time at all temperatures (Table 3).

Discussion

Loss of Infective Larvae After a Blood Meal. Our experiments were conducted with vectors feeding on mice and should be considered as animal models which mimic the loss of infective *W. bancrofti* from their vector. Transmission to humans was not studied directly. In our experiments, the percentage reduction of infective larvae in mosquitoes after a blood meal ranged from 66% to 100%. This agreed with experiments carried out with other mosquito hosts and filaria, where the percentage reduction ranged from 20 to 100% (Lavoipierre and Ho 1966, de Meillon et al. 1967, Ewert and Ho 1967, Zielke 1976). Maintaining mosquitoes for 1 wk did not affect the loss of larvae during blood feeding. Thus, the mosquito retains infectivity for several days, allowing synchronization between *W. bancrofti* larval maturation and the vector gonotrophic cycle. In French Polynesia, *Ae. polynesiensis* is also the vector of *Dirofilaria immitis* Leidy, the dog heart worm, indicating that this mosquito feeds on at least 2 host species. Because females do not lose all their larvae after the 1st blood meal and because larvae are still infective 1 wk later, this species remains potentially infective even if the 1st blood meal was not taken on a suitable host or if the gonotrophic cycle length was

Table 3. Number of *W. bancrofti* infective larvae in vector at 4 different temperatures, beginning at 1st d (D) of appearance of that stage

Temp, °C	1st d of infective L3		D	D + 1	D + 2	D + 3	D + 4	D + 5	D + 6	D + 7	D + 8	D + 9	D + 10
20.0	D 28	No. mosquitoes	3	3	5	3	3	2					
		Mean L3 ± SD	4.0 ± 3.0	4.3 ± 4.2	6.4 ± 4.3	10.0 ± 4.0	6.7 ± 2.1	6.5 ± 4.9					
		% L3 in head	33.3	7.7	21.9	73.3	50.0	30.8					
		% L3 in thorax	41.7	23.0	31.3	20.0	45.0	53.8					
		% L3 in abdomen	25.0	69.3	46.8	6.7	5.0	15.4					
22.5	D 20	No. mosquitoes	9	10	8	9	5	9	6	8	9	12	6
		Mean L3 ± SD	13.8 ± 7.4	13.5 ± 5.9	11.4 ± 7.0	9.0 ± 4.7	12.2 ± 8.8	9.7 ± 4.5	6.8 ± 4.0	9.1 ± 5.9	6.3 ± 3.8	8.6 ± 5.9	6.7 ± 3.0
		% L3 in head	44.4	40.0	44.4	51.9	32.8	33.3	39.0	46.6	59.6	59.8	30.0
		% L3 in thorax	35.4	44.5	35.5	40.7	45.9	40.3	53.7	41.1	36.9	31.5	57.5
		% L3 in abdomen	20.2	15.5	20.1	7.4	21.3	26.4	7.3	12.3	3.5	8.7	12.5
25.0	D 13	No. mosquitoes	10	10	15	9	12	6					
		Mean L3 ± SD	10.5 ± 7.6	8.8 ± 4.9	9.1 ± 6.5	7.3 ± 4.9	7.8 ± 6.4	6.5 ± 6.1					
		% L3 in head	26.7	44.3	38.2	42.5	42.6	41.0					
		% L3 in thorax	43.8	47.8	54.5	46.3	55.3	59.0					
		% L3 in abdomen	29.5	7.9	7.3	11.2	2.1	0.0					
27.5	D 12	No. mosquitoes	11	9	10	7	10	8	8	7	10	4	
		Mean L3 ± SD	9.3 ± 4.8	9.2 ± 7.2	11.6 ± 5.0	9.6 ± 5.0	8.3 ± 5.2	8.9 ± 5.8	6.1 ± 2.4	6.9 ± 5.1	6.3 ± 2.9	4.0 ± 1.4	
		% L3 in head	29.4	34.9	47.4	23.9	38.6	57.7	30.6	50.0	30.2	43.7	
		% L3 in thorax	36.3	49.5	38.0	71.6	54.2	26.8	53.1	47.9	63.5	50.1	
		% L3 in abdomen	34.3	15.6	14.6	4.5	7.2	15.5	16.3	2.1	6.3	6.2	

elongated because of temperature or difficulty in host location.

Active Migration of Infective Larvae During a Blood Meal. During blood feeding, infective larvae in head and thorax were able to move to the mosquito's proboscis and leave the vector. We feel that infective larvae in the abdomen most likely behave the same (experiments 1, 2, and 4, Table 1), but because of the small number of larvae remaining in the abdomen in experiments 3 and 5 before and after blood feeding, we could make no conclusion about movement after a 1-wk delay. The proportion of infective larvae in the abdomen is low and if an active migration from the abdomen exists, it is difficult to demonstrate without a large number of mosquito dissections. Moreover, because individual larvae were not labeled, it is not possible to conclude if the number of larvae before and after blood feeding remained the same. For example, loss from the thorax could mean addition to abdomen, even if initial larvae in abdomen had moved elsewhere. In our experiments, the number of larvae in the head and thorax decreased significantly after blood feeding and the (small) number of larvae in abdomen remained the same indicating that most larvae may have left the vector, even if some may have migrated in other directions or remained in posterior compartments. Jordan (1959) estimated that *W. bancrofti* infective larvae may be transmitted by *Cx. quinquefasciatus* to humans during a complete blood meal irrespective of larval location. We think that it is likely to be the same for *Ae. polynesiensis.*

Influence of Parasite Burden on Parasite Loss. There was no influence of infective larval burden on the rate of parasite loss during a blood meal. The experiment where parasite loss was lowest was not the experiment with the highest initial larval burden.

Influence of Repletion on Loss of Infective Larvae. When mosquitoes take only a partial blood meal, the duration of the blood meal may be shortened and infective larvae may not have enough time to migrate to the proboscis and escape into the host. Newton and Pratt (1945) estimated that an infective larvae took 5 min to migrate from the abdomen to the proboscis. Our results indicate that the process of loss is relatively slow and continuous during blood feeding. As such, the shortening of the duration of the meal is the simplest hypothesis to explain the reduction in parasite loss during partial meals. Obviously, larvae in the head are more likely to succeed in leaving the vector as indicated by the high rate of loss from this area. Moreover, *Aedes* species often probe their host and may take several partial meals on different hosts before satiating. Because loss of larvae may take several minutes, disturbance, probing, and multiple blood feeding may distribute infective larvae to several hosts and increase the transmission rate. This also may decrease the efficiency of transmission by reducing the chance of a mated pair of worms occurring in a host, especially in areas where the proportion of infective vectors is low and where vectors are poorly infected. Despite some investigations (McGreevy et al. 1974), mechanisms which stimulate the L3s to move to the head during blood feeding are still unclear. A mechanical stimulus induced by the proboscis at the time of the bite may be suggested, as well as an activation induced by mosquito hormones while blood feeding. If so, partial meals, in addition to a shortened duration, may not stimulate enough hormonal production to result in a significant loss of parasites.

Loss of Infective Larvae without a Blood Meal. Our results are in agreement with Brengues (1975) who did not observe any significant loss of larvae without a blood meal. *Ae. polynesiensis* retains infective larvae for more than the time required to complete the gonotrophic cycle and find a suitable host. However, in natural conditions frequent blood meals are the rule (Rivière 1988) and mosquitoes may transmit the parasite as soon as the infective stage is reached.

Migration of Infective Larvae in the Mosquito's Body Before Blood Feeding. Unlike Lavoipierre and Ho (1966) but in agreement with Brengues (1975), no cyclic migration of infective larvae was found to the mosquito's head. Nevertheless, head and thorax concentration of larvae facilitates parasite loss during blood feeding. As such, the decrease of larvae in abdomen with time may increase their number in the head and thorax and may facilitate escape during blood feeding. The daily changes in the proportion of larvae found in the head, thorax, and abdomen indicate that larvae are motile in the mosquito's body. Under laboratory conditions, it may affect the ability of the vector to survive by disturbing wing muscles and modifying flight activity (Townson 1970; Husain and Kershaw 1971; Brunhes and Brunhes 1971, 1972; Brunhes 1975), especially when the larval burden is high. In addition, more heavily parasitized mosquitoes have higher mortality rates, in particular when L3 larvae move to the mosquito's head (Brengues 1975). With *Ae. polynesiensis,* differential mortality between low and high mosquito infection rates has been observed under laboratory conditions (Failloux et al. 1995). However, in field-collected *Ae. polynesiensis,* Lardeux et al (1995) did not observe high infection rates indicating either a limitation phenomenon just after the intake of microfilariae (Pichon 1974, Pichon et al. 1974) or a greater mortality rate in over-parasitized females shortly after blood feeding. This study's estimated a range of 1–12 infective larvae in infected mosquitoes, similar to Mataika et al. (1971) and Rakai et al. (1974) for *Ae. polynesiensis.* Because of the small number of L3 larvae found in wild *Ae. polynesiensis,* it is likely that the motility of infective larvae is not a major cause of vector mortality, even if it may disturb

Table 4. Adaptations of *W. bancrofti* behavior to *Ae. polynesiensis* biting habits

Possible deficient interaction for filarial transmission	Filarial adaptation	Consequence for transmission
Low infection of mosquitoes as a result of limitation phenomenon	No loss of infective larvae without a blood meal	Good vectorial capacity at low infection rates
	Low mortality of infective larvae in the vector	No damage of vector muscles, no disturbance of vector behavior and low vector mortality caused by overparasitism
	Low mortality of larvae from L1 to L3 in thoracic muscles	
Blood meal taken while larvae are not yet infective	Persistent vector infectivity	As soon as infective larvae appear, infective bites may be given, whatever the length of gonotrophic cycle
Several host species for blood meals (e.g., human, dog) (i.e., lower probability of finding a suitable host)	Not 100% parasite reduction	At least 2 gonotrophic cycles may be infective
	Not an immediate loss of all larvae	Multiple partial blood meals on different hosts in 1 blood feeding session may result in multiple transmission

flight activity of the vector, disturbing its vital activities such as feeding and reproduction.

The survival of a parasite during evolution is troublesome, because the parasite yield during transmission should be sufficient to maintain the parasite, but low enough to maintain the host. Adaptive phenomena resulting in coevolution of the host(s) parasite system are the cue to species survival (Chabaud et al. 1986). Adaptations may arise to facilitate the dissemination of the filariae, some occurring within the vector phase of the life cycle. Table 4 summarized those found for the *W. bancrofti/Ae. polynesiensis* relationship in this study. More quantitative studies are needed to understand better the efficiency of filarial transmission once larvae are lost during blood feeding. For example, the rate of larval penetration into the human circulatory system is not well quantified. The inefficiency of transmission of *W. bancrofti* from mosquito to humans has been discussed by Hairston and de Meillon (1968) and later emphasized by Brunhes (1975). Even if the vector phase may appear to be a weak point in the transmission cycle, we do not totally agree with these authors. In contrast, we think that there have been several sophisticated adaptations between the parasite and its vector which clearly optimize transmission and improve the fitness of the parasite to its environment. For example, the low natural infection of *Ae. polynesiensis* may appear inefficient, but the parasite adaptive strategy leads in fact to an increase in the parasite yield (i.e., 1 ingested microfilariae may give 1 infective larvae in most cases) and the parasite may be transmitted even in low-prevalence or low-density areas (Pichon et al. 1974).

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