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Proportion of *Wuchereria bancrofti* larvae released by *Culex pipiens* (Diptera: Culicidae) during the second blood feeding

Belal Soliman[1*] and Ahmed Shoukry[2]

1. Department of Biological Sciences, Faculty of Education, Suez Canal University, Suez, Egypt.
2. Department of Plant Protection, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt.

ABSTRACT

We investigated the parasite buden, the release and the pattern of redistribution of infective larvae (L_3) of the parasite *Wuchereria bancrofti* in its main vector *Culex pipiens* L. after a second blood meal. Infected mosquitoes were allowed to feed on the blood of the vertebrate host on the 14^{th} day after the infected blood meal, at a time when L_3 larvae were present in the mosquite head. The prevalence of infection and the mean number of infective stage larva decreased considerably after taking the second blood meal. The greatest loss of infective larvae (65 %) was in mosquitoes dissected one day after blood feeding, while a marked loss (47 %) was noted among those females dissected immediately after the blood feeding. The distribution pattern of L_3 larvae changed completely in infected mosquitoes after the second blood meal. A high percentage of infective larvae migrated from the thoracic muscles toward the head and the proboscis regions, this pattern stabilising one day after blood feeding.

KEYWORDS: Bancroftian filariasis, *Wuchereria bancrofti*, *Culex pipiens*, infective larvae release

INTRODUCTION

Several studies have been made of the quantitative analysis of transmission by mosquitoes of filarial larvae to the definitive host. They discuss several factors that might affect the efficiency of filarial transmission, such as the feeding mechanisms of mosquitoes, the parasite burden, mechanisms of egress of the filaria larvae and the proportion of infective-stage larvae released from the mosquitoes associated with blood feeding (Warton 1957; De Millon 1967; Ewert & Ho 1967; Ho & Ewert 1967; Lavoiperre & Ho 1973, Gwads & Chernin 1973; Soliman *et al.* 1993, Soliman 1995).

The release of infective larvae from mosquitoes has epidemiological implications, and should be taken into consideration in evaluating the efficiency of filaria transmission (Lavioperre & Ho 1973; Soliman 1990).

* Address for correspondence

The mechanism causing the release of third-stage filarial larvae from the mosquito host is very interesting, and requires more investigation. Such information is crucial in understanding the transmission dynamics of the filarial parasites, and for describing the epidemiology of *W. bancrofti*. This study estimates the numbers of infective larvae released during the second blood meal.

MATERIALS AND METHODS

Culex pipiens mosquitoes originated from larvae from a filarial area in El-Kashish village (Qalubiya Governorate) in the Nile Delta of Egypt. Larvae were reared in small enamel pans containing tap water under controlled conditions (27°C ± 2, 70-80% RH and 12:12hr. L:D photoperiod). Larvae were fed daily on Tetramine (Falks-pets Pacific Inc. Honolulu, Hawai, USA, 96819). Pupae were collected daily and were housed in 30 × 30 × 30 cm gauze cages. Emerging adults were provided with 10% sucrose solution on cotton pads until given a meal of blood infected with *Wuchereria bancrofti*.

Adult female mosquitoes 4-5 day old were allowed to feed on microfilaremic volunteers (with 25 microfilariae/ 20µl blood) in the field between 2200-2400hr. The engorged females were separated and maintained under the previously described laboratory conditions. To evaluate the comparative uptake of microfilariae by mosquitoes, samples were dissected immediately after feeding on infected blood. Starting on the 10^{th} day post infection, samples of mosquitoes were dissected daily to locate and determine the infective larvae and their distribution throughout the mosquito body.

After the appearance of infective-stage larvae in the head region, mosquitoes were separated individually in tubes: sugar-soaked cotton pads and all liquids were removed to limit release of infective larvae. Then females were divided into two equal groups: females of the first (group A) were dissected in saline solution (0.9%) immediately before the second blood feeding to determine parasite load and the pattern of filarial distribution at the time of feeding; females of the second group were fed a second blood meal (pigeon blood).

Engorged fed females of the second group were further divided in two: one (group B) was dissected immediately after imbibing the second blood meal, and the other (group C) was maintained for 24 hr without water and sugar, then killed and dissected to determine the redistribution of filariae after the second blood meal. All mosquitoes killed in these experiments were kept in a freezer at - 70 °C until dissected.

RESULTS

Of 53 females of group A dissected immediately before the second blood meal, 66% contained infective larvae of *W. bancrofti*, but the prevalence of infection of groups B and C decreased to 41% (group B, dissected immediately after the second blood meal) and 42% (group C, dissected one-day later). Statistically, there was a significant difference in prevalence between group A and both groups B and C ($p < 0.001$).

The intensity of infection in mosquito females, the parasite burden, was significantly different among tested groups (Table 1). The control group (5.9 larvae/ female) and group B (3.2) differed by approximately twice that between groups B and C (2.1) ($p < 0.001$). This indicates that 47% and 65% of L_3 larvae were released by the mosquitoes of both experimental groups respectively during their second blood-feeding

attempt. Thus a 24-hr delay (group C) after blood feeding caused about 20% loss in the parasite burden, whereas 40% were lost during blood feeding itself. The data indicate that after infective mosquitoes have fed again only once, they still harbor about 35% of their original infective-stage larval load.

Table1: Mean number and the expected proportion of *Wuchereria bancrofti* infective larvae(L3) released by *Cx. pipiens* after the second blood feeding

Tested group	No. of female tested	No. and % of infective mosquitoes		Average No. / female	No. of L_3 larvae released	
		Observed	Expected	(Range)	No. observed	% expected loss
A	53	35 (66.01)	35 (66.01)	5.9 (0-23)	207	-
B	58	25 (43.1)	38 (65.5)	3.2 (0-13)	79	46.6
C	76	32 (42.1)	50 (65.7)	2.1 (0-9)	66	65.1

A: Mosquito females dissected before imbibed the second blood meal (control).
B: Mosquito females dissected immediately post imbibing the second blood meal.
C: Mosquito females dissected after one day post the second blood meal
% Expected loss = $\frac{\text{expected - observed X 100}}{\text{expected}}$

The pattern of the distribution of infective larvae within the mosquito body regions (Figure 1) changed in the different groups. It shows clearly that, immediately after taking the second blood meal (group B), the number of infective larvae decreased and the percentage of larvae in the head and thoracic muscles decreased also, with similar values. In addition, the percentage of L_3 larvae found in the abdomen increased. Group C females showed a different pattern, since infective larvae were distributed approximately equally in the head and proboscis (14 and 15 % respectively). A similar pattern was observed also in both thorax and abdomen regions (34 %). It appears that infective larvae of *W. bancrofti* move from all parts of the host into the head and labium.

From the results it can be seen that a second blood meal given to an infective mosquitoes two weeks after its infected blood meal resulted in a large proportion of infective larvae being released during the feeding process, approximately 65%.

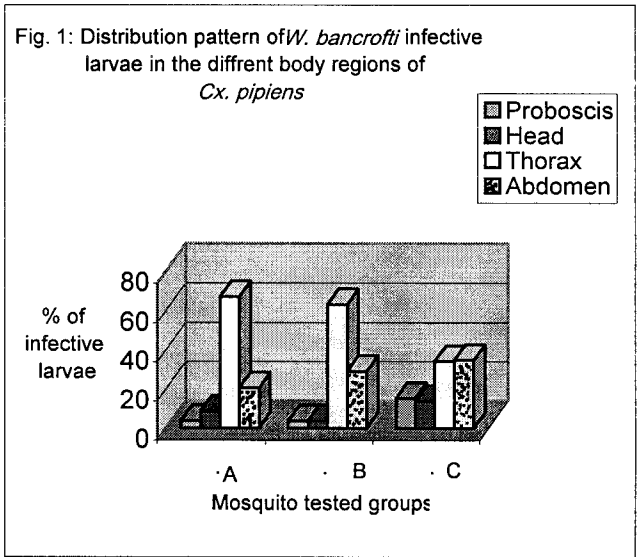

Fig. 1: Distribution pattern of *W. bancrofti* infective larvae in the diffrent body regions of *Cx. pipiens*

DISCUSSION

In the present study, the number of infective larvae of *W. bancrofti* was considerably reduced in all parts of infected *Cx. pipiens* after feeding. A markedly high percentage (60 %) of infective larvae was lost from the proboscis after feeding process. Similar results were obtained by De Millon *et al.* (1967) in *Culex pipiens fatigans* infected with *W. bancrofti*, and by Ewart & Ho (1967); Ho & Ewert (1967) and Lavoiparre & Ho (1973) dealing with *Brugia pahangi* in *Aedes togoi*.

The present results revealed changes in the mean number and the distribution pattern in infective mosquitoes after imbibing the second blood meal. The mean number of infective larvae per mosquito decreased considerably. Immediately after feeding, the proportion of *W. bancrofti* larvae decreased in both head and thoracic muscles of mosquitoes, increasing in the abdomen. The distribution of infective larvae changed completely one day after feeding, the proportion in the thorax dropping by half, but increasing in the proboscis and head.

It therefore seems likely that the third-stage larvae that develop in the thorax may move first into the abdomen before migrating towards other sites, as mentioned before (Wharton 1957; De Meillon *et al.* 1967). It is clear that infective larvae that remain in the mosquito after a second blood meal start to migrate and become redistributed almost immediately, their levels in each site of mosquito body not fully returning to their original values. Such differences in the mean number and in the distribution of infective larvae are due to the number of the larvae lost as a result of filling the stomach with blood during the second meal. The same conclusion has been reported by many previous investigations (Lavoipiere & Ho 1966; McGreevy *et al.* 1974; Ho & Lavoipierre 1975; Zielk 1976 & 1979; Lindsay & Denham 1986). One day after feeding on the second blood meal, the remaining infective larvae apparently stabilised their distribution in the mosquito body. The difference in larval distribution between the female mosquitoes dissected immediately after feeding and those dissected one day later was due to the short time required for redistribution after the second blood meal (De Millon *et al.* 1967).

The changes in mean number and distribution were mainly caused by the pressure of the blood meal pushing the larvae in the abdominal cavity towards the thorax and other regions of the body. Many investigators have reported similar conclusions, but some believed that filling of the stomach with blood does not constitute the single significant factor in causing the release of infective larvae from the insect host. Ho & Lavoipierre (1975) concluded that the binding of the labium during the feeding process is the most important stimulus leading to the escape of infective larvae, because they emerged whether mosquito fed to repletion or only probed the skin of the experimental animal. On the other hand, some investigators reported that filarial larvae were lost when mosquitoes fed only on sugar solution (Wharton 1957; Rifaat *et al.* 1971; Gwads & Chernin 1973; Zielke 1976; Lardeux & Cheffort 1996). It can be concluded that the second blood meal is a significant factor in the release of infective larvae, but obviously there are other factors which act as stimuli for release of infective filaria larvae, which need further investigation.

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الملخص العربى

معدلات خروج يرقات الطور المعدى لطفيلى *ووشيريريا بانكروفتى* من بعوض *كيولكس بيبينز* (رتبة ثنائية الآجنحة - كيوليسيدى) خلال تناول وجبة الدم الثانية

بلال سليمان[1] و أحمد شكرى[2]

١- قسم العلوم البيولوجية - كلية التربية - جامعة قناة السويس -السويس مصر
٢- قسم وقاية النبات - كلية الزراعة - جامعة قناة السويس - الإسماعيلية - مصر

تم دراسة الحمل الطفيلى ونمط إعادة توزيع يرقات الطور المعدى الطفيلى "*ووشيريريا بانكروفتى* " فى الناقل الأساسى لبعوض "*كيولكس بيبينز* " بعد تناول وجبة الدم الثانية، ولذلك فقد تم تغذية البعوض على وجبات دم تحتوى على يرقات الطفيلى وذلك عقب إتمام فترة حضانة الطفيلى لتقييم معدلات النمو لليرقات المعدية ومعدلات الخروج خلال الإغتذاء. ولقد تبين أن معدلات الإصابة ومتوسط عدد يرقات الطور المعدى لكل أنثى قد تناقص بمعدل متوسط بعد وجبة الدم الثانية وكانت أكبر نسبة لفقدان هذه اليرقات هى ٦٥% بعد يوم واحد من تناول وجبة الدم. كذلك فإن نمط إعادة إنتشار يرقات الطفيلى قد تغيرت بشكل واضح. ولقد لوحظ أن أكبر عدد من اليرقات قد هاجرت من منطقة عضلات الصدر فى إتجاهها إلى منطقة رأس وخرطوم الأناث وذلك خلال يوم واحد بعد تناول وجبة الدم.