

The effect of different types of skin surfaces on the transmission of *Brugia pahangi* infective larvae by the mosquito *Aedes aegypti*

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Abstract

The transmission of *Brugia pahangi* from *Aedes aegypti* into *Meriones unguiculatus* was compared for four different skin surfaces (HAIRY, CLOTH, CLEAN, GREASY). Unshaven jirds reduced the feeding avidity of infective mosquitoes. The loss of larvae from infective mosquitoes was greatest when these insects fed on "exposed" skin surfaces. Significantly fewer infective larvae penetrated the host when infective mosquitoes fed on a jird through a thin layer of cloth.

Introduction

The vector-host interface is of vital importance in the transmission of infective larvae of filarial worms from vector to mammalian host (MCGREEVY *et al.*, 1974). LINDSAY *et al.* (1984) have demonstrated the importance of environmental humidity in this context and this paper describes how the characteristics of the mammalian host's surface can influence filarial transmission.

When an infective mosquito feeds on a host the filarial larvae emerge from the proboscis and lie on the skin in a small quantity of insect haemolymph. The haemolymph is considered to have two main functions with regard to transmission.

Firstly, it is protective. Filarial larvae are incapable of penetrating intact skin and they gain access to the host via the puncture wound made by the feeding mosquito (YOKOGAWA, 1939; GORDON & CREWE, 1953; EWERT, 1967). The haemolymph prevents the worms from drying out on the skin surface before they enter the puncture wound. Disappearance of the haemolymph droplet either by evaporation and/or absorption should, therefore, reduce transmission.

Secondly, the haemolymph also acts as a medium for larval locomotion. MCGREEVY *et al.* (1974) considered that penetration would be optimal when the haemolymph formed a dome-shaped droplet with its basal centre directly over the puncture wound.

Spreading of the haemolymph would, therefore, not only reduce transmission because the increased surface area would result in a greater rate of evaporation but also because of the less suitable droplet shape.

In our present study an attempt was made to investigate whether these considerations were of importance to the transmission of filariasis. The experiment was performed by feeding infective *Aedes aegypti* mosquitoes on jirds (*Meriones unguiculatus*) presenting four different types of skin surfaces. These were normal (HAIRY), clean and bald covered with a thin layer of cotton (CLOTH), clean and bald (CLEAN) and greasy and bald (GREASY). The two "covered" surfaces (HAIRY and CLOTH) were used chiefly to test the importance of absorption of haemolymph in relation to filarial transmission and the two "exposed" surfaces (CLEAN and GREASY) to test the effect of droplet shape on larval penetration. However, trans-

mission is not solely dependent on the success of larval penetration but is also affected by the readiness of infective mosquitoes to feed and the subsequent loss of larvae from these insects. These other factors were also investigated.

Materials and Methods

The method for rearing infective mosquitoes was described by DENHAM (1982) and the experimental protocol for transmission was similar to that described by LINDSAY *et al.* (1984). Dried fruit was removed from a cage of infective mosquitoes 24 hours before feeding. Equal numbers of between 60 and 100 infective female mosquitoes were placed into five separate 20 × 20 × 20 cm gauze cages (one control cage and four test cages). Experiments were performed 12 days after infection when a large number of larvae have accumulated in the proboscis (labium).

The different skin surfaces upon which the mosquitoes fed were prepared as follows. The HAIRY surface was left untouched. The remaining three skin surfaces were shaved with electric clippers and then depilated with "Nair" (Carter-Wallace Ltd., Folkestone, Kent, UK) three days before feeding. These surfaces were rinsed with 70% ethanol and dried with paper tissues 10 min before feeding. In addition a small quantity of petroleum jelly ("Vaseline", Chesebrough Ponds Ltd., London) was rubbed into the GREASY surface. The material used for the CLOTH surface consisted of a clean, white, cotton handkerchief, with fibres 0.11 to 0.20 mm thick, spaced at 0.09 to 0.15 mm intervals.

Each jird was anaesthetized with 0.06 ml of Sagatal (Sodium pentobarbitone, 60 mg/ml) intraperitoneally and placed on one of the test cages. The mosquitoes were allowed to feed for 15 min through a rectangular area 5.0 × 2.5 cm cut from a paper towel, on the abdomen of the jird. Feeding took place between 10.30 and 12.30 hours at 28 ± 1°C, 70 to 80% relative humidity on russet-coloured, female, jird litter-mates (obtained from Intersimian Ltd., Abingdon, Oxon., UK).

Jird autopsies were performed to determine how many larvae had penetrated the animal, using the method of LINDSAY *et al.* (1984). 50 mosquitoes were taken from each test cage at random as were 30 from the control cage, and then dissected as described by LAVOPIERRE & HO (1966) to determine the number of infective larvae within each mosquito. In addition, the abdomens of the remaining mosquitoes from the test cages were dissected to determine if they had fed.

The experiment was performed on six occasions. The order in which the test cages were fed on the different skin surfaces was changed at random between replicates.

The percentage of mosquitoes feeding, the percentage loss of larvae from those mosquitoes and the percentage of the larvae escaping from those mosquitoes and successfully penetrating the host were calculated using the formulae described by LINDSAY *et al.* (1984).

Initially each set of data was analysed using the Friedman two-way analysis of variance by ranks in order to test that there was no difference between results for the four different skin surfaces. When $p < 0.05$ the null hypothesis was rejected. Further comparisons between the skin surfaces were made by combining the data of similar groups and using either the Friedman two-way analysis of variance by ranks or the Wilcoxon matched-pairs signed-ranks test to test for significance between these groups. When $p < 0.05$ the difference was considered significant.

Although we have previously been unable to demonstrate that mosquitoes with large numbers of infective larvae are less likely to feed (LINDSAY *et al.*, 1984), many authors have found this to be true (WHARTON, 1957; LAVOPIERRE, 1958; NELSON, 1964; ZIELKE, 1976). If indeed this were the case fewer larvae would be lost from the feeding mosquitoes and consequently the calculated values of larval loss and larval penetration would be underestimated. The Mann-Whitney U-test was used to test the significance of the difference between the number of larvae in each control mosquito and the number of larvae in each non-feeding test mosquito, for the four different groups. Non-parametric tests have been applied to all the data because the distribution of larvae within our mosquitoes is overdispersed.

As a corollary to this study the behaviour of infective larvae in small droplets was observed. Individual larvae were placed in 2 μ l droplets of PBS on a glass slide and observed under a dissecting microscope at a magnification $\times 40$, using a cold-light source for illumination. These experiments were performed at room temperature.

Results

The results of feeding infective mosquitoes on the different skin surfaces are presented in Table I. The null hypothesis that all groups were similar was rejected ($0.05 < p < 0.02$). However the percentage of mosquitoes feeding on CLOTH, CLEAN and GREASY surfaces were similar ($P = 0.252$). Significantly fewer infective mosquitoes fed on the HAIRY surface compared with the other three surfaces.

Between 64 and 86% of the control mosquitoes were infected and the pooled mean larval load in the control group was 6.2 (range 3.6 to 9.6, between replicates). By the Mann-Whitney U-Test, two of the test groups, HAIRY ($\bar{x} = 7.8$, $p = 0.002$, $n = 123$) and CLEAN ($\bar{x} = 7.8$, $p = 0.046$, $n = 60$) had significantly greater worm burdens in their non-feeding mosquitoes compared with the control group. The other two test groups were marginally non-significant by comparison (CLOTH, $\bar{x} = 7.6$, $p = 0.061$, $n = 59$; GREASY, $\bar{x} = 8.3$, $p = 0.087$, $n = 73$). However as the percentage of mosquitoes feeding on CLOTH, CLEAN and GREASY surfaces were similar the non-feeders in all three test groups were pooled and compared with the control group. The difference between the two groups was highly significant ($p = 0.008$), greater numbers of worms occurring in the non-feeding mosquitoes ($\bar{x} = 7.9$).

Greater weight should be attached to the p values obtained when comparing the larval loads of the control group and the various non-feeding mosquitoes in the test groups because the control group also contains a number of mosquitoes which would have been non-feeders. Therefore, the real percentages of larvae entering the host are greater than indicated

from the results. It should be emphasized, though, that some mosquitoes even with a low infection or none at all will not feed when given the opportunity.

The loss of larvae from infective mosquitoes feeding on the four surfaces as shown in Table II was not the same and the null hypothesis was rejected ($0.05 < p < 0.02$). Significantly more larvae were lost from infective mosquitoes feeding on the two "exposed" skin surfaces (CLEAN and GREASY) than from those feeding on "covered" skin surfaces (HAIRY and CLOTH).

The pooled mean larval recovery from jirds for the four different skin surfaces were as follows; HAIRY, 40.7 (range 12 to 91, between replicates), CLOTH, 8.5 (range 2 to 22), GREASY, 70.8 (range 38 to 122) and CLEAN, 48.7 (range 17 to 119). The percentage of larvae on the skin surface which successfully penetrated the host (Table III) was not the same for each group and the null hypothesis was once more rejected ($0.01 < p < 0.001$). However the larval penetration of CLEAN, GREASY and HAIRY surfaces was similar

Table I—Results showing the percentage of mosquito feeding on different skin surfaces

Expt. No.	HAIRY	CLOTH	CLEAN	GREASY
1	54.2	78.9	65.7	73.9
2	51.4	42.9	55.6	63.3
3	40.5	74.3	79.6	56.2
4	47.6	70.7	76.3	62.5
5	51.6	86.4	92.9	82.5
6	83.3	86.0	89.5	76.8
\bar{X}	54.8	73.2	76.6	69.2

Table II—Results showing the percentage loss of larvae from mosquitoes feeding on different skin surfaces

Expt. No.	HAIRY	CLOTH	CLEAN	GREASY
1	80.3	80.6	86.7	80.4
2	86.4	88.2	95.3	90.2
3	66.1	50.2	91.1	98.5
4	89.5	56.2	85.6	96.1
5	90.1	76.4	91.9	88.6
6	77.9	44.1	83.8	87.4
\bar{X}	81.7	66.0	89.1	90.2

Table III—Results showing the percentage of larvae penetrating jirds with different skin surfaces

Expt. No.	HAIRY	CLOTH	CLEAN	GREASY
1	35.5	2.0	6.0	42.6
2	4.0	0.7	5.5	14.2
3	39.5	5.0	35.7	28.9
4	30.7	8.3	21.6	25.9
5	19.4	8.5	14.7	14.8
6	12.7	4.0	14.2	41.1
\bar{X}	23.6	4.8	16.3	27.9

($p = 0.43$). The shape of the haemolymph droplet, therefore, has no significant effect of the ability of filarial larvae to penetrate the host. When the data for these three groups were combined and compared with the results for the CLOTH surface a significant difference was found. This means that larval penetration was significantly reduced when infective mosquitoes fed through cloth.

Our *in vitro* observations demonstrated that larvae are not constrained by the limits of the droplet and can move rapidly across a surface when covered by a thin film. Work by WALLACE (1958) with *Heterodera schachtii* in different thicknesses of water showed that the maximum speed of this nematode occurred when the water-film was slightly less than half the thickness of the worm. It is likely that filarial larvae moving across a damp skin surface stand little chance of locating the puncture wound unless there is direct contact with serum from the puncture wound. It is possible that the larvae may use slight differences in skin-surface temperature as orientation cues, but in any case such movement would be comparatively short-lived due to the evaporation of the thin-film and trail-wetting.

Our *in vitro* observations also demonstrated that the worms obtain purchase from the sides of the droplet and in particular the top of the dome. This invariably results in attempts by larvae to penetrate the substratum either using a persistent tapping or pushing motion of the anterior end. As the droplet shrinks the worm uses more of its body to gain leverage from the side of the droplet. Even in thin films the larvae make repeated attempts to penetrate the substratum but in larger droplets the larvae have more time and a greater freedom of movement with which to locate the puncture wound. Such "searching" behaviour is probably a manifestation of the worms intrinsic movement. Basically this consists of a series of sinusoidal waves being propagated from the anterior end of the worm producing forward movement. A less frequent occurrence is the production of a forward directed wave which results in reversing.

This behaviour is unlikely to be influenced by gravity since the force exerted by surface tension on a nematode in an aqueous film is 10^4 to 10^5 times stronger (CROFTON, 1954). Therefore larval penetration will be the same whether the droplet hangs from or rests on a skin surface.

Discussion

Hairy skin partially deters mosquitoes from feeding on a host, but it is not fully protective. Although LEWIS (1933) was unable to feed mosquitoes on hairy guinea-pigs it is our experience that this is possible. A heavy growth of hair on man may also deter feeding but again it is not protective (GORDON, 1922).

The tendency of mosquitoes with high density infections not to feed is a further demonstration of the debilitating effect that filariasis can have on mosquitoes. Other manifestations include reduced egg production (JAVADIAN & MACDONALD, 1974; GAABOUB, 1976; CHRISTENSEN, 1981), impaired flight activity (PAIGE & CRAIG, 1975; HOCKMEYER *et al.*, 1975; WIJERS & KULU, 1977; amongst others) and an increase in mortality (TOWNSON, 1970; BECKETT, 1973; SAMARAWICKREMA & LAURENCE, 1978).

The greatest loss of larvae from mosquitoes feeding on exposed surfaces occurs when the labium is most severely bent (LAVOPIERRE & HO, 1966, 1973). However, our direct observations of mosquitoes feeding through cotton cloth show that they bend their labiums more acutely than when feeding on an exposed surface; the tip of the labium (labellae) being positioned on the surface of the fibres. One would expect that mosquitoes feeding on the skin through material would have to sink their stylets deeper in order to feed on the same capillaries as they would on an uncovered surface. As a consequence the mosquito pushes its heads closer to the surface and thus the labium is bent more acutely. In such a position the labium appears to be pinched-shut at one or possibly two points at its junction with the head capsule. If this is so it is probable that only those larvae in the proboscis are lost during feeding, the pinched labium effectively preventing recruitment of larvae from other areas of the body.

Larval penetration is clearly affected by the nature of the skin surface on which the infective mosquito feeds. The failure of larvae to penetrate the host through the CLOTH surface is probably due to a combination of factors. Possibly the most important is that the haemolymph is absorbed by the cotton cloth fibres, because in the other "covered" surface (HAIRY), where larval penetration is relatively good, absorption of haemolymph by the pelage is minimal. Moreover, the lack of continuity between the haemolymph droplet and serum from the puncture wound would also reduce larval penetration. This is important for several reasons. It seems probable that larvae will move up both a serum and temperature gradient as occurs when the haemolymph comes into contact with the warm serum from the puncture wound. The physical shape of the haemolymph droplet will also facilitate penetration.

Any proposals to encourage individuals in areas of endemic filariasis to keep their bodies covered when vectors are most likely to feed must be tempered by two facts. Firstly, in a tropical environment body coverings are kept to a minimum simply because it is more comfortable. Secondly, mosquitoes preferentially feed on exposed areas of the body, so efficient protection cannot be given to an individual unless that person is fully covered by an absorptive material.

These results demonstrate that filariasis transmission is a subtle biological phenomenon and that entry of larvae into a host is not guaranteed when an infective mosquito feeds. In general the risk of infection is increased when the host's skin surface is "exposed".

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INTERNATIONAL FILARIASIS ASSOCIATION (SOCIETE INTERNATIONALE DES FILARIOSES)

The IFA was founded over 20 years ago to encourage and facilitate mutual acquaintance and collaboration between persons of all nationalities concerned with work on filariasis (including onchocerciasis) and facilitate knowledge of filariasis and its control.

In the past it has arranged workshops at international conferences, circulated a newsletter, has representation on various international bodies and has awarded the O'Connor prize on four occasions to distinguished workers in the field.

Recently, at the XI International Congress for Tropical Medicine and Malaria in Calgary, Canada, it was decided to ascertain whether there is still support for maintaining the Association.

So, if you are interested (even if already a member), please contact:

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