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Wings of the common house fly (*Musca domestica* L.): importance in mechanical transmission of *Vibrio cholerae*

Yap, K.L., Kalpana, M. and Lee, H.L[1].
Department of Biomedical Science, Faculty of Allied Health Sciences Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia
[1]Division of Medical Entomology, Institute for Medical Research, Jalan Pahang, Kuala Lumpur
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Abstract. The importance of house fly (*Musca domestica* L) wings in mechanical transmission of bacteria was studied. A droplet of phosphate-buffered saline containing *Vibrio cholerae* was rolled along one wing of each house fly. None adhered to the wings but small proportions of the bacterium were isolated from about half the wings. *Vibrio cholerae* was spread onto the ventral wing surfaces of each unconscious house fly which then was placed inside a bottle. When it regained consciousness, the types of activity it performed over five minutes were noted before the house fly was killed and the bacteria on its wings numerated. Control were house flies killed before inoculation. The proportion of house flies with bacteria on their wings and the mean number of bacteria remaining were significantly less on live house flies than killed controls. Among the live house flies, bacteria were detected on fewer house flies which flew (25%) than those which did not fly (81%). In addition, the mean number of bacteria on the former was significantly less than the latter (5 against 780 colonies). However, both these parameters were not significantly different between the group which performed and the group which did not perform wing grooming; takeoff and alighting over short distances, and somersaulting. Wings of unconscious house flies tethered by their thoraxes were inoculated with *V. cholerae*. After regaining consciousness, the house flies were allowed to move their wings in flight motions for up to 30 seconds. Small proportions of bacteria remained on all the house flies. House flies were placed in a chamber containing a liquid bait spiked with *V. cholerae*. After two hours, 10 were removed sequentially and cultured for *V. cholerae*. The bacterium was isolated from four house flies: two from the legs, and two others from their bodies minus legs and wings. In conclusion, house fly wings do not play an important role in mechanical transmission of bacteria suspended in a non-adhering liquid medium because of the low transfer rate of the bacteria to the wings and poor retention of bacteria on the wings during normal house fly activities.

INTRODUCTION

Transmission of microbes by house flies can either be biological or mechanical (Greenberg, 1973). In mechanical transmission of microbes carried on the vector's exterior, all exposed surfaces are potential carriage sites for microbes. In this regard, the efficiency of a particular part of a house fly in transmission of microbes depends on its ability to perform actions: picking up microbes, retaining the microbes, maintaining infectivity of microbes during travel, depositing the infectious microbes on a new host or a surface likely to come into contact with the host.

House flies in their daily lives encounter a wide variety of materials in various physical forms including liquids. A key factor in the transfer of microbes to house flies is the medium in which the microbes are suspended. An earlier study revealed contact between macroscopic liquid droplets and house fly wings has two possible outcomes: entire droplets attached to house fly wings causing the wings to collapse, and the whole droplets did not adhere to house fly wings on contact but numerous residual

microscopic droplets were retained between the tiny hair-like microtrichae (Kalpana *et al.*, 2004). If non-adhering droplets contained pathogens, these pathogens could be transferred to the wings in the microdroplets left behind following contact. The importance of this mode of transmission has yet to be established.

For microbes transferred to house fly wings, transmission of pathogens by the wings is possible only if the pathogens are retained during normal house fly activities such as flight and 'grooming'. An earlier study concluded that house fly wings are unlikely to be important in the transmission of rotavirus because most virus particles were removed from the wings early during wing movement of tethered house flies (Tan *et al.*, 1997). However, the different shape and size of bacteria from viruses and the presence of appendages such as flagella and pili on some bacteria might produce a different retention pattern. In contrast to retaining the bacteria on the house fly's exterior during transportation, bacteria that managed to remain attached to the wings must be dislodged when the house fly reaches a suitable surface. Certain activities including wing grooming may be important.

This study examined the importance of house fly wings in mechanical transmission of enteropathogenic bacteria by using *V. cholerae* as the model. The use of *V. cholerae* is relevant because it has been isolated from the exterior of house flies (Sukontason *et al.*, 2000; Fotedar, 2001). The first part of this study ascertained the efficiency of the transfer of *V. cholerae* to house fly wings following contact with non-adhering liquid droplets carrying *V. cholerae*. The second part determined the role of a number of house fly activities on the retention of *V. cholerae* on house fly wings. Finally, the extent of *V. cholerae* contamination of wings of house flies exposed to a liquid spiked with *V. cholerae* was determined.

MATERIALS AND METHODS

House flies

The Kuala Lumpur strain of *Musca domestica* L. used was from breeding colonies established at the Medical Entomology Division of the Institute for Medical Research, Kuala Lumpur. Breeding house flies were maintained on granular sugar, water and ground mouse pellets at a photoperiod of 12:12 (L:D) hours. Mouse pellets soaked overnight with water were placed in a tray and put inside a cage of house flies. Trays with eggs laid on the pellets were removed, covered with fine netting and placed in an empty cage. A small amount of water was added when the medium became dry. Room temperature was maintained between 28 to 30° C and relative humidity at 85%. Five to six days after appearance of larvae, dry clean woodchips was added on top of the medium to facilitate pupation. Larvae were removed into a plastic bowl after all had pupated and house flies emerged two to three days later.

Vibrio cholerae

The bacterium used, a non O1 serotype, was isolated from a cholera patient from the Kuala Lumpur Hospital. A working stock was prepared from a lyophilized culture and kept at room temperature.

House flies feeding chamber

The feeding chamber was a 2-litre rectangular orange juice bottle with a narrow neck (height: 30 cm, base: 12x7 cm). A tray of 2 ml water used to rinse a gutted fish and then spiked with $5x10^7$ colonies forming units (cfu) of *V. cholerae* was placed inside the bottle through a horizontal cut on a side near the base. The mouth of the bottle was closed with an opened inverted plastic universal bottle in place of bottle cap.

Preparation of *V. cholerae* for experiments

Vibrio cholerae from the working stock culture was streaked on a thiosulfate-citrate bile salts-sucrose (TCBS) agar plate and incubated overnight at 37° C. An isolated yellow colony from the plate was inoculated into 10 ml alkaline peptone water and incubated overnight for 16-18 hours at 37° C. The culture was centrifuged at 9700 *g* for 15 minutes and the bacterial pellet

resusupended in PBS. The bacterial suspension was adjusted to an absorbance reading of 0.564-0.565 at 420 nm. This range of values corresponded to about $5.5x10^7$ cfu/ml.

Viable count of *V. cholerae*

Viable *V. cholerae* bacteria were numerated in cfu by the spread plate method. Suitable dilutions of bacterial suspension were prepared in PBS and 50 ml from each dilution was spread onto a TCBS agar plate. Each dilution was plated in 4 replicates. The plates were incubated overnight at 37° C. The yellow colonies were counted and the average cfu/sample calculated.

Experimental protocols

Transfer of *V. cholerae* to house fly wings on contact with PBS droplets containing *V. cholerae*

House flies were killed by exposure to -20° C for 10 min. Each dead house fly was held by the thorax using a forceps. A slight pressure on the forceps caused the wings to extend from the body. A 10 ml drop of PBS containing *V. cholerae* was drawn into a micropipette tip using a Gilson micropipette (Gilson S.A.S. Villiers Le Bel, France) and expelled until it formed a droplet at the end of the micropipette tip. The droplet was rolled along the ventral surface of a wing from the base to the tip. The wing was detached, placed into 1 ml PBS and the number of *V. cholerae* cfu determined. In the first group, six house flies were tested with PBS containing an estimated $1x10^5$ cfu /drop; in the second group, 12 were tested with $5x10^5$ cfu /droplet.

Effect of unrestricted activities on retention of *V. cholerae* inoculated on house fly wings

Each house fly was rendered unconscious and the wings extended as described earlier. A 1 ml drop of PBS containing 10% meat peptone (Hi Media Labs Ltd., Mumbai, India) and with an estimated $5x10^3$ cfu of *V. cholerae* in suspension was spread onto the ventral surfaces of both wings. The unconscious house fly was placed on its back in a plastic universal bottle. On regaining consciousness, the activities of the house fly were observed and recorded for the next five minutes before it was killed by placing the bottle at -20° C for 10 min. Both wings were removed and the number of *V. cholerae* cfu on them determined. A total of 20 house flies were studied. The 3rd pair of legs of the last 12 house flies was excised to prevent wing grooming. Controls were 14 house flies treated similarly but killed immediately following inoculation and viable bacterial counts determined.

Effect of tethered flight in the house fly on the retention of the *V. cholerae* inoculated on the wings

A fine wire was attached to its dorsal thorax of a unconscious house fly using U-Hu glue. The ability of the house fly to move its wings in flight motion was ascertained when the house fly regained consciousness. If positive, the house fly was made unconsciousness again and 1 ml drop of 10% meat peptone-PBS mixture containing an estimated $1x10^5$ cfu of *V. cholerae* was spread onto the ventral surfaces of both wings. The unconscious house fly was held in the centre of a plastic universal bottle and the bottle mouth closed with a piece of plastic which had a slit to accommodate the wire. The house fly was discarded if no wing movement occurred within five minutes of regaining consciousness. If wing movement occurred, the duration and pattern of movement were recorded. Thirty seconds after commencement of wing movement in flight motion, the house fly's head was quashed with a fine tip forceps, the wings detached and the number of *V. cholerae* cfu on them determined. A total of seven replicate test house flies and seven controls were used.

Isolation of *V. cholerae* from wings, legs and bodies of house flies kept with a *V. cholerae*-contaminated liquid in a feeding chamber

Twenty-two house flies were released simultaneously into the feeding chamber which held a tray of liquid spiked with *V. cholerae*. The liquid was water used to rinse a gutted fish and was shown to attract house flies and adhere to their exteriors. Two hours

later, the plastic universal bottle which functioned as the chamber cap was replaced with another bottle. The first bottle was rinsed with 1 ml PBS and the numbers of *V. cholerae* cfu in the rinsing determined. At different times thereafter, over a period of about 2 hours, 10 house flies were removed singly by being allowed to enter into a universal bottle. Each house fly was killed by low temperature exposure. All the legs and wings were removed from the house fly using two different sterile forceps and placed into two different Eppendoff tubes, each containing 1 ml PBS. The body without its extremities was placed into another tube of PBS. The numbers of *V. cholerae* cfu on the three different parts of the house fly were determined.

Statistical analysis

Data were analyzed using SigmaStat for Windows 2.0 statistical software (Jandel Scientific, San Rafael, CA). The proportions of different groups were compared for significant difference using Fisher exact test. The median values of two groups were compared for significant difference by the Mann-Whitney rank sum test. Significant correlation between 2 variables was tested using Pearson product moment correlation.

RESULTS

Transfer of *V. cholerae* to house house fly wings on contact with PBS droplets containing *V. cholerae*

All PBS droplets with $1x10^5$ suspended *V. cholerae* per droplet did not adhere when rolled along house fly wings. However, *V. cholerae* was transferred to half the number of wings following contact with the droplets (Table 1). A 5-fold increase in bacteria number did not alter significantly the proportion of house flies with bacteria transferred to them or the average number of bacteria transferred. The numbers of bacteria transferred were very small proportions of the total bacteria in the droplets. The range of the number of bacteria transferred to different wings was very wide.

Table 1. Transfer of *V. cholerae* to house fly wings following non-adherence contacts with phosphate-buffered saline droplets which contained *V. cholerae*

Total *V. cholerae* in PBS droplet	Proportion wings with *V. cholerae* isolated	Colonies of *V. cholerae* on wings	
		median	range
$1x10^5$	3/6 (50.0%)	380 (0.38%)[a]	60/920
$5x10^5$	7/12 (58.3%)	53 (0.01%)[a]	7/1220

[a]Median number of bacteria transferred to wings expressed as percentage of the median number of bacteria in the whole droplets.

Effect of unrestricted activities on retention of *V. cholerae* inoculated on house fly wings

All the 20 house flies inoculated with *V. cholerae* walked after regaining consciousness. Seven (35%) groomed their wings and 1 in this group flew. Among the 13 house flies which did not groom their wings, three flew. Other activities observed were rapid wing flicking (20%), somersaulting (30%), takeoff (appeared as jumping) and alighting (appeared as dropping) over a short distance (65%). All house flies performed more than one activity.

After five minutes of unrestricted activities, there was significant reduction in proportion of *V. cholerae*-inoculated house flies which retained bacteria on their wings ($p = 0.031$). Furthermore, the average number of bacteria retained on the wings per house fly compared to control house flies was significantly reduced (< 87.3%) ($p < 0.001$).

Table 2 shows of the four house flies which flew, one retained an extremely small number of *V. cholerae* on its wings. In contrast, in the group of 16 house flies which did not fly, a high proportion retained bacteria on the wings. When house flies of this group was examined for bacteria retention on their wings based on specific activity, it was observed that there was no significant difference in the proportion of house flies which retained bacteria on their wings between the subgroup which performed takeoffs, alighting and

Table 2. Effect of unrestricted activities on retention of *V. cholerae* inoculated on house fly wings

House fly's activities	Proportion wings with *V. cholerae* isolated (%)	Colonies of vibrios on wings	
		median	range
none[a]	14/14 (100%)	6,163	1180/10625
all activities	14/20 (70%)	778	5/6886
flying			
+	1/4 (25%)	5	0
–	13/16 (81%)	780	25/6886
wing grooming (*no flight*)			
+	5/6 (83%)	775	25/6886
–	8/10 (80%)	830	55/6195
takeoff, alighting, somersaulting (*no flight*)			
+	9/11 (82%)	780	6886/55
–	4/5 (80%)	545	4370/25

[a] The house flies inoculated with the same bacteria inocula used on test house flies were killed immediately after inoculation and cfu on the wings determined.

somersaulting, and the subgroup which did not. The mean bacteria numbers on the wings of the two subgroups were not significantly different too. In the same group of the 16 house flies, similar patterns were observed for the group which groomed their wings and the group which did not. Of the six house flies which groomed their wings and did not fly, frequency of grooming ranged from one to 13 times. There was no significant correlation between number of times wing grooming was performed and the numbers of bacteria retained on the wings.

Effect of tethered flight in the house fly on the retention of the *V. cholerae* inoculated on the wings

Table 3 shows the average number of bacteria retained on the wings of tethered house flies after they have moved their wings in flight motions was a very small proportion of the figure of control house flies. The wings of all the tethered house flies inoculated with *V. cholerae* retained bacteria but the proportion of bacteria retained on different house flies varied widely. The numbers retained in some house flies were relatively high. Two types of wing movements were observed: continuous and intermittent. There was no significant correlation between number of bacteria retained and duration of wing movement.

Isolation of *V. cholerae* from wings, legs and bodies of house flies kept together with a *V. cholerae*-contaminated liquid in a feeding chamber

At 2 hours after the release of the 20 house flies into the feeding chamber, 15 *V. cholerae* colonies were isolated from the inside surface of the chamber cover. Among the ten house flies sampled sequentially thereafter, *V. cholerae* was isolated from 4 house flies: from the legs of two house flies sampled at 2 hours 30 minutes (10 colonies) and 3 hours 45 minutes (5 colonies), and from the house fly bodies minus the legs and wings of two house flies sampled at 3 hours 5 minutes (5 colonies) and 3 hours 50 minutes (30 colonies) later.

DISCUSSION

V. cholerae suspended in a liquid medium (PBS) was transferred to house fly wings during contact between droplets of the liquid and the wings even though the droplets did not adhere to the wings. Bacterial transfer most likely was caused by microscopic liquid

Table 3. Effect of house fly wings in flight motions on retention of *V. cholerae* inoculated onto the wings

House fly	Wing Movement		Colonies of vibrios on wings	
	Type	Duration (sec)	median	range
Control house flies				
C1 to C7	NA	NA	111,250[a] (100%)	87750/115400
Test house flies				
T1-T7			685[a] (0.61%)[b]	15/4190
T1	3 short bursts	7	2050 (1.84%)	
T6	3 short bursts	15	4190 (3.77%)	
T3	continuous	15	15 (0.01%)	
T7	continuous	20	565 (0.51%)	
T5	continuous	20	685 (0.62%)	
T2	continuous	20	2885 (2.59%)	
T4	continuous	30	185 (0.17%)	

NA: Not applicable. Duration for movement was limited to a 30 sec period.
[a] Significantly different (Mann-Whitney test, T = 77.00, p =<0.001).
[b] Percentage of median number of bacteria on control house flies' wings.

particles carrying *V. cholerae* which became detached from the droplets during contact and were trapped between the tiny hairs that cover the wing surface. Trapping of microscopic droplets during contact between non-adhering macroscopic droplets and house fly wings was reported previously (Kalpana *et al.*, 2004). This mode of bacterial transfer from a liquid which did not adhere to the house fly was inefficient because about half of the contacts resulted in bacterial transfer. In addition, the proportions the bacterial transferred were very small.

If house fly wings became contaminated with bacteria a subsequent condition for successful transmission must be met. This is the retention of significant proportions of the bacteria on the wings in the interval between transport and deposition of the bacteria on a suitable site. In this study, the proportion of house fly with the inoculated bacteria remaining on the wings and the mean number of inoculated bacteria on the wings after 5 minutes of unrestricted activities were significantly reduced. Since certain activities on their own, i.e. takeoffs and alighting, somersaulting, and wing grooming did not cause significant reduction in bacteria numbers or the proportion of house flies which retained bacteria, it is likely that it was the combination of all these activities and others not assessed in this study which resulted in the significant decrease. However, another key factor in mechanical transmission may also be involved, i.e. maintenance of infectivity of the pathogens during transmission. In this regard, inactivation of significant proportion of the inoculated bacteria during the period taken for the inoculated house flies to regain consciousness and the subsequent 5 minutes of activities may have occurred and this would have contributed to the significant reduction in both the proportion of house flies which retained the inoculated bacteria on their wings and the number -retained on the house fly wings.

Flying causes vigorous wings movements (West, 1951; Clausen, 1954) and therefore likely to remove bacteria from house fly wings. This was supported by the observation that although a large proportion of house flies which did not fly retained bacteria on their wings, almost all houseflies which flew had no vibrios on their wings. The

loss of bacteria from the wings might not be caused solely by flying because all the four house flies which flew also performed other activities. To prevent activities, other than wing movements, that could affect bacterial detachment, house flies were tethered to wires which allowed them to move their wings in flight motions. Unlike most unrestricted house flies which lost the entire bacterial inoculum during flight, all tethered house flies retained small proportions of the inoculated bacteria after moving their wings in flight motions. This may be caused by an inoculum size 20 folds larger than that used in unrestricted flight. The actual numbers of bacteria retained by some house flies were relatively high. The implication is very heavy bacterial contamination of wings can result in relatively high number of bacteria retained on the wings of some house flies which flew. Thus in this specific situation, the house fly wings may have a role in mechanical transmission, especially if flight to a suitable surface for transmission is short. Takeoffs and alighting over very short distances, and somersaulting also involved wing movements. The lack of significant reduction in retention of *V. cholerae* on house fly wings during these activities could be because of very limited wing actions and low wing beat frequency.

Wing grooming which keeps the wings in good condition for flight is a characteristic activity of house fly (West 1951). In this study, wing grooming was performed by almost all house flies with all 3 pairs of legs intact. If wing grooming is able to dislodge contaminating bacteria from house fly wings, its has 2 contrasting effects on the mechanical transmission of pathogens depending on where it occurred. At the site of contamination, it removes bacteria from the house fly's body and therefore effects mechanical transmission negatively. On the other hand, at a suitable site which will lead to infection, wing grooming promotes mechanical transmission by releasing the bacteria carried by the house fly. The results of this study indicated wing grooming was not efficient in removing bacteria suspended in a liquid medium from house fly wings.

The importance of house fly wings in mechanical transmission of *V. cholerae* suspended in a liquid was assessed in a more natural setting by ascertaining wing contamination of house flies which were kept in a chamber containing a *V. cholerae*-contaminated liquid medium. The medium was water after it was used to rinse a gutted fish. This medium was reported to attract house flies (Boonchu *et al.*, 2004) and to adhere readily to house fly wings (Kalpana *et al.*, 2004). The presence of *V. cholerae* in the chamber cover which was 30 cm away from the tray of *V. cholerae*-contaminated liquid revealed that some of the house flies translocated *V. cholerae* from the liquid. This was supported by the isolation of *V. cholerae* from 40% of house flies tested. However, it is noteworthy that none of the isolates was from the wings. This outcome probably reflects the difficulty of direct contact between house fly wings and pathogen-containing materials the house fly came to rest since house fly's wings are held above ground. In addition, the ventral wing surface is partially concealed of by the thorax and the abdomen when the house fly is at rest (West, 1951). If bacteria were deposited on the wings, they probably were removed during flight.

In conclusion, liquid droplets that did not adhere to house fly wings were able to transfer suspended bacteria to the wings, albeit inefficiently. House fly wings play only a minor role, at most, in the mechanical transmission of bacteria suspended in a liquid medium because of the low transfer rate of the bacteria to the wings and poor retention of bacteria on the wings during normal house fly activities.

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