Transmission of bacterial pathogens by the house fly *Musca domestica vicina*

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Abstract

To study the fate and distribution of normal bacterial flora in the developmental stages of the house fly *Musca domestica vicin* and to determine the external sites of the fly on which the pathogen(s) can adhere and mechanically transported through fly activity, the present study is carried out. Results suggest that bacteria and other microorganisms present in larval rearing media may play an important and specific role in the development of immature stages of the house fly *M. domestica*. Larvae reared in a microbe-free medium failed to pupate even after a long larval period (25 days). Larvae reared in a naturally contaminated medium pupated after 8-11 days post hatching. The results indicate that, if aseptic conditions are maintained, the development of house fly maggot, within limits, is linear with respect to the incubation time of larval rearing media and hence the amounts of bacterial products released during incubation and utilized by the house fly during growth and metamorphosis. Counts for normal bacterial gut flora in different larval stages were gradually increased with the increase of larval age. Mature maggots support populations of 6.5×10 bacteria. During the prepupal stage, the arvae lose more than 98% of their bacterial flora. The process of pupation is characterized by counts of 10 and 10. The newly formed fly shows a considerable reduction in bacterial florea, the majority of emerging flies 2 retain 10 bacteria but varying from 9 to 370 bacteria/fly. Thus two declines consistently appear during -the process of development in the prepupa and the newly emerged adult.

Keywords: pathogen, *Musca domestica vicin*, mechanically transported, bacteria, development of immature stages, microorganism.

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Introduction

The findings of infected flies in nature have been the main circumstantial and epidemiological evidence incriminating *Musca domestica* in the transmission of typhoid and dysentery. West (1951) cited 9 reports, and Steinhaus (1967) an additional 15, on the isolation of typhoid, paratyphoid and dysentery organisms from trapped flies. As many as 30 to 40% of fly pools cultured around human cases, or during epidemics, have yielded strains of Shiqella or Salmonella organisms (Floyd and Cook, 1953). The common house fly , *Musca domestica vicina* is a well known, cosmopolitan insect, which shares man his environment and is considered as one of the major insect vectors which transmits and disseminates different human pathogens, particularly in temperate and tropical countries. Accordingly, this insect may pause public health problems. In addition to their role in disease transmission, flies are usually regarded as indicator organisms, symptomatic of disposal problems and reflecting the sanitary level of the community. In the absence of valid statistical data, bacteriological information about an essential health situation.

We know almost nothing about the danger level of a fly population nor do we have well-tested criteria, as there are for Anopheles and malaria, for evaluating the vector role of the fly. The complexities and uniqueness of enzootic and endemic situations involving flies make generalization hazardous and often of limited value. The biggest gap in the logical development of incriminating evidence against flies is that which exists between our knowledge of the ability of flies to transmit and the actuality that they do (Greenberg, 1973). In Brazil, Imbiriba (1979) examined 14 samples of flies from three abattoirs; *Proteus sp*. and *Escherichia coli* were isolated from all samples and *Salmonella sp*. (in *Musca domestica* L.) from only one. Pseudomonas sp. (3 samples) and Aerobacter sp. (1 sample) were also isolated.In Mexico, Dosal and Garcia (1982) found a correlation between the time of flies peak infestation and the highest incidence of patients requiring treatment for infections potentially transmitted by adult muscoids. Gastrointestinal infections were the most numerous. Mumcuoglu and Rufli (1982), about the medical significance of insects and mites in Switzerland and adjacent regions, the house fly was among the most important passive transmitters of organisms harmful to man in the temperate zone. Echeverria et al. (1983) indicated that there is a correlation between the number of flies (predominantly Musca domestics) and the incidence of diarrgea in a Thailand village. Enterotoxigenic Escherichia coli. Shiqella spp., Vibrio cholera and Vibrio fluvalis were isolated from fly pools in yards (69%). In Nigeria, Adeymi and Dipeolu (1984), isolated seven genera of bacteria, some of

which were pathogenic to humans, were isolated from legs,, wings, mouth parts and mid gut of the flies. Low numbers of bacteria were isolated from flies caught in areas where hygienic condition prevailed. Bacillus spp. were the most numerous of the bacteria isolated. The greatest numbers of bacteria were on the legs. Shane et al., (1985) infected the house fly (Musca domestica) with a liqued suspension of Campylobacter jejuni; 20% of the bacteria were recovered from the feet and ventral surface of the body and 70% from the viscera. These findings demonstrated the potential role of flies in the dissemination of avian campylobacteriosis.

Keiding (1986) stated also that there are many possibilities for flies to transport and mechanically transmit pathogens, usually connected with a low standard of hygiene. However, in each situation the question is: How important a factor are flies for spreading infections as compared to other ways of transmission, e.g. directly by food, water, air or dirty fingers and other direct contact from person to person. Radi et al. (1988), the role of house fly in the transmission of bacterial diseases in four Egyptian hospitals has been studied by Labib (1990), and the interaction between bacteria and house fly was studied by EL-Sobky and Hanan (1990).

Keeping in mind all of the above mentioned studies, the capability of the flies as a factor for spreading infections as compared to other ways of transmission, must be seen as a quantitative problem and depending on many factors. The intimate association between *Staphylococcus sp*. and the house fly was also observed by Labib (1990), where 136 isolates of this species were isolated from the house fly samples collected from four Egyptian hospitals. The following strains of *Streptococci* were isolated by Merdan and Allam (1974), *Streptococcus agalactiae, Strept. salivaius, Strept. lactis, Strept. equines, Strept. pyogenes* and *Strept. faecalis* . Also Umeche and Mandah (1989), arrived to the same observations. Cholera, the causative organism of which is Vibrio comma, was among the first disease in which house fly was incriminated as a vector. Though, flies have the mechanism and habits for the transmission of the tubercle bacillus, no conclusive work has established the relationships of the flies to such transmission (Fotedar, 2001; Nazni, 2005) . The importance of the house fly wings in mechanical transmission of vibrio cholera was discussed by Yab et, al (2008) because of the low transfer rate of the bacteria to wings.

Material and Methods

1. Entomological procedures

A colony of the house fly, *Musca domestica* vicina Macq., was raised in a valk-in insectary at the Biology Department, Faculty of Science for girls, King Abdulaziz University.

Rearing technique was followed according to a modified technique from Hafez, M. (1948). A petri dish (9 cm in diameter and 2.5 cm in height) containing a piece of cotton moderately soaked in 10% milk solution was placed in the breeding cage (length, 18 inches; breadth, 9 inches; height, 10 inches) with the adult flies. Adults were fed on the milk and the females laid their eggs on the milk pad. . The latter was replaced by a fresh pad every 24 hours. After the eggs had been laid, the pad was transferred to one pound jam jar containing a fresh milk pad to provide food for the hatching larvae. The jar was then covered by a piece of chess cloth using a rubber band.

Prior to pupation, the larvae usually collected at the upper and drier surface of the milk pad which by this time became blown up into a more or less spongy mass due to the continual tunneling I ; of the larvae inside and probably to the onest of fermentation. At this upper surface, the larvae usually pupated. Pupae were either collected or preferably left in the jar to completed development. The emerging adult flies were then transferred to the breeding cage.

The grinding apparatus was housed in a glass hood equipped with an ultraviolet light and a gas flame. The grinder is a variable speed motor stirrer to which is bre attached a glass rod. The rod is sterilized by flaming. Grinding tubes are 15 ml glass tubes. During the grinding on process, the tube was placed in a cub containing a large piece of cotton.

2- Bacteriological procedures

a- Disinfecting procedure

Different stages of the house fly, *M. domestica* were disinfected using the following procedure: A sample was removed from the breeding medium, rinsed twice in distilled water and placed in a test tube containing a solution of 10% Na Cl. After 10 minutes of immersion, the solution was poured off and the sample was washed twice in distilled water then immersed for 5 minutes in 5% formalin and washed twice in sterile water. The sample was agitated at. intervals by shaking the tube.

b- Grinding procedure

The grinding apparatus was housed in a glass hood equipped with an ultraviolet light and a gas flame. The grinder is a variable speed motor stirrer to which is br attached a glass rod. The rod is sterilized by flaming. Grinding tubes are 15 ml glass tubes.

The specimens was ground in a tube containing a pinch of sand and 0.1 ml of a 0.9'% saline solution. After grinding 0.9 ml of saline was added, to the tube. The homogenates were kept cold until dilutions were made.

c- Plating and counting procedures

Appropriate dilutions of the homogenates were mixed with nutrient agar as pour plates. Plates were incubated for 2 days at 37 c and the final count for a specimen was the average of 2 replicates.

Experimental Methods

To determine the effect of bacteria and other microorganisms on the development of immature stages of the house fly *Musca domestica vicina*, the following experiment was planned. Several 250 cc. conical flasks containing cotton pads, moderately soaked in diluted milk were plugged and divided into four groups (each of five flasks). The first group was autoclaved immediately after preparation, the second group was autoclaved after 24 hours' incubation at 37 C, the third group was autoclaved after 48 hours incubation at 37 C. Another group was left without incubation or autoclaving as a control group.

House fly eggs were thoroughly agitated in detergent in a 50 test tube to clean and separate them. After several rinses with distilled water the aseptic sequence is: 10 min. in Naocl; 2 rinses in distilled water; 5 min. in formalin; 2 rinses in distilled water. While in these solutions, the eggs were agitated at intervals by shaking the tubes.

Groups of eggs were introduced into each flask under complete aseptic conditions. Sterility tests were run for eggs and medium at this time, using nutrient broth which was incubated at 37 C and held for 48 hours. Aluminum foil was used to cap the cotton plugs of the flasks.

All flasks were kept under laboratory conditions (27 f 3 C and R.H. of 55-75%). Larval and pupal durations as will as percentage of egg hatching, pupation and adult emergence were recorded for all groups.

Results and discussion

1- Effect of bacteria on the development of the house fly *Musca domestica vicina* Macq

To study the effect of bacteria and other microorganisms on the development of immature stages of the house fly, four groups of flasks containing larval media were treated as follows: The first group was autoclaved as soon as it was prepared (without incubation) so that bacteria were eliminated from this group; the 2nd and 3rd groups were incubated for 24 and 48 hours respectively, incubation of the media will give the chance to the existing bacterial flora to multiply and produce vitamins and/or accessory food substances which may be needed for developing flies. The last group was left without incubation or autoclaving as a control group. All groups were seeded with disinfected eggs under complete aseptic conditions.

Disinfection of house fly eggs using a solution of 10% Na Cl for 10 min, then 5% formalin for 5 min. resulted in sterile, viable and undamaged eggs. Eggs hatchability reached about 71.2%. The best results were obtained by using young eggs, old eggs are more contaminated and are not disinfected by this treatment. The data in table (1) summarizes the results of these experiments. The data in table (1) indicate the following:

In the control group, full grown healthy 3rd instar larvae were formed 6-9 -days post hatching. Pupation takes place in the cotton plugs and at the upper surface of the cotton pads. Fully formed flies began appearing 4 days after pupation so that the minimum developmental period was 12 days and the maximum period was 17 days. Percentages pupation and adult emergence were 75.7 and 88.6 respectively.

Larvae in the medium autoclaved as soon as it was prepared had very long life span (about 25 days). Only 2 larvae were pupated (1.1%) after 7 and 13 days post hatching. Other maggots were aggregated at the prefer of the cotton plug and died as mature 3rd instars without further development.

In spite of the high rate of larval mortality recorded in the medium autoclaved after 24 hour incubation, normal larval growth takes place and under sized pupae were formed 9-14 days after hatching. Adult emergence was very low, only 6 adults were emerged (6.5%). With a 48 hours incubation, larvae grow as rapidly as the controls. Full grown third instar larvae were formed from 8 to 12 days post hatching. Fully formed flies began to appear 5 days after pupation, so that the minimum developmental period was 13 days and the maximum one was 21 days. Percentage pupation and adult emergence was 81.9 and 89.3 respectively. Pruss and

Mariotti (2000) suggested that the bases of trachoma was through person – to person contact and flies appear to constitute the major transmission pathways. Hence, houseflies need to be regarded as important mechanical vectors of gastrointestinal diseases such as campylobacteriosis and salmonellosis.(Wales, et, al. 2010, Choo, et, al. 2011).

Table (1); Effect- of bacteria on the development of the house fly (at 27 ± 3oC and R.H. of 55-75 %)

Rearing media	A	B	C	D	E	F	G	H	I	J
Natural contaminated control	191	133	69.63	8--11	114	85.71	4--6	101	88.59	12--17
Autoclaved without any incubation	243	179	73.66	25	2	101	–	–	–	–
Autoclaved after 24h incubation	210	156	74.28	9--14	92	58.97	11--14	6	6.2	20--28
Autoclaved after 48h incubation	221	149	67.42	8--12	122	81.87	5--8	109	89.3	13--20

A: No. of eggs; B: No. of larvae hatched; C: % hatchability; D: Larval periods (Days); E: No. of pupae formed; F: % pupation; G: Pupal periods (Days); H: No. of adult emerged; I: % adult emergence; J: Total duration from egg to adult (Days).

2- Fate of bacteria in the developmental stages of the house fly *Musca domestica vicina*

In order to trace the fate of the normal bacterial gut flora and pattern of bacterial survival in all fly stages, samples of different larval ages, prepupae, pupae and newly emerged adults were eliminated from the natural breeding media, treated as previously mentioned in material and methods page.

The availability of abundant samples of each stage enabled us to follow the progress of bacterial gut flora in all fly stages. The data of the 5 experiments are tabulated in table (2).

The data in table (2) revealed the following:

- Counts for normal bacterial gut flora in different larval stages were gradually increased with the increase in larval age. 4 one-day old larva supported a population of 9.5×10 bacteria whereas a full grown 3rd instar larva supported a population of bacteria.

- When a mature 3rd instar larva stops feeding and reaches the prepupal stage, it loses more than 98% of its bacteria. This stage exhibiting more variability than the larval stage.

-The process of pupation is characterized by counts of 10 and 4 10, with some variation at the upper and lower limits. This range is maintained during the 4 days of pupal development.

-Newly formed flies show a considerable reduction, this stage exhibiting far more variability than other stages, with the 2 majority of 10 , but varying from 9 to 370 bacteria.

Table (2): Fate of bacteria in the developmental stages of the house fly *Musca domestica vicina* Macq

Specimen no.	1	2	3	4	5	Average
Life stage						
1 day old larva	$8.1x10^3$	$1.6x10^4$	$4.2x10^5$	$9.5x10^4$	$2.4x10^4$	$7.3x10^3$
2 day old larva	$9.1x10^4$	$3.4x10^5$	$7.11x10^6$	$3.18x10^6$	$8.2x10^6$	$1.9x10^5$
3 day old larva	$6.3x10^5$	$1.2x10^7$	$8.1x10^6$	$1.07x10^7$	$8.8x10^6$	$2.4x10^7$
4 day old larva	$1.3x10^7$	$8.1x10^6$	$2.0x10^8$	$6.5x10^7$	$4.0x10^7$	$6.2x10^7$
Prepupa	$4.0x10^6$	$6.3x10^4$	$3.7x10^4$	$8.5x10^5$	$1.0x10^5$	$7.1x10^4$
Newly formed pupa	$6.0x10^4$	$8.0x10^4$	$9.3x10^3$	$5.69x10^5$	$8.0x10^5$	$1.9x10^6$
1 day old pupa	$6.2x10^{10}$	$8.1x10^3$	$1.4x10^4$	$1.5x10^5$	$6.3x10^5$	$7.3x10^4$
2 day old pupa	$2.4x10^4$	$1.9x10^4$	$7.1x10^3$	$2.3x10^4$	$4.4x10^3$	$6.2x10^4$
3 day old pupa	$7.1x10^2$	$1.4x10^4$	$3.2x10^3$	$1.9x10^4$	$1.7x10^4$	$8.4x10^3$
4 day old pupa	$8.1x10^2$	$2.4x10^3$	$6.8x10^3$	$8.1x10^3$	$6.3x10^3$	$2.4x10^4$
Newly formed fly	9	$6.9x10^1$	$2.8x10^2$	$1.6x10^2$	$8.6x10^1$	$3.7x10^2$

Results of the present study suggest that microbial flora and/or vitamins that they may produce and the decomposition products of rearing medium may play a role in the nutrition of larvae as well as on the metamorphosis process, i.e. the transformation of larvae to pupae and adult emergence.

Larvae, reared in a medium autoclaved as soon as it was prepared (free from bacteria),

failed to pupate even after a long larval periods (25 days). Whereas, larvae reared in a naturally contaminated medium pupated after 8-11 days post hatching. This indicates that bacteria and/or their products may have a specific role in the process of metamorphosis.
Larvae, reared in a medium incubated for 24 hours before autoclaving, developed to the pupal stage but they died as such without further development. Larvae reared in a medium autoclaved after 48 hours incubation had normal longevity and development. So that, it may be concluded that, within limits, the development of maggots is linear with respect to the incubation time and hence bacterial products in the medium.

Inhibition of larval growth in the absence of bacteria was observed by Radvan .(I960), who fed house fly maggots on blood agar slants containing 25% beef blood, 38.5% beef-peptone broth, 35% yeast extract, and 1.5% agar, no larval growth was observed and the author couldn't identify 'the limiting factoThe route of transmission of *Helicobacter pylori* from individual to individual remains undefined it has recently been reported that the domestic housefly, *M. domestica*, when fed pure cultures of *H. pylori*, was able to harbor the organism in its mid gut for up to 30 hr. (Osata, et al. 1998) .

Also Nazni et al. (2005) examined flies from various breeding sites such as food courts, dumping ground, food processing area and poultry farm. *Bacillus sp*., *Coccobacillus sp*., *Staphylococcus sp*., *Micrococus sp*., *Streptococcus sp*., *Acinetobacter sp*., *Enterobacter sp*., *Proteus sp*., *Escherichia sp*., *Klebsiella sp*., and yeast cells were isolated from feaces. Vomitus external surfaces and internal organs of house fly. Newly emerged housefly did not harbor any bacteria. Currently, *M. domestica* is recognized as the mechanical vector of a wide variety of viral, bacterial and protozoa pathogens. Fly control is still an important public health measure in the 21st century especially in developing countries (Cirillo, 2006). Holt *et, al*. (2007) reinforced the findings that the fliesresding in environments contaminated with human pathogens become contaminated themselves.Babak, *et, al*.(2008),were isolated and identification bacteria that are pick up by house fly over the human and animal permises. Forster, *et al*. (2009) and Hamid *et, al*. (2012) arrived to the same conclusion.

In conclusion, with such emphasis given to flies as a mechanical vector in spread of disease, hence, the health problem but the presence of flies would indicate sanitary deficiency and unhygienic condition. Possible breeding sites for flies should be eliminated and flies should be prevented from gaining access to contaminate human material.

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