Fate of Bacteria in the Developmental Stages of the House Fly, *Musca Domestica Vicina*

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Abstract: Bacterial distribution in different developmental stages of house fly *Muscadomesticavicina* revealed that in the majority of maggots and prepupae, the hemolymph is sterile. Fat cells and trachea are like-wise sterile. Bacteria was distributed through the digestive tract of immature stages of the fly. In larvae/ the largest bacterial count was found in the fore gut then the hind gut and lastly the anterior portion of the mid-gut. The normal flora was diminished slightly as it passed down the mid-gut. The hind-gut of the prepupa harbors more bacteria than the mid gut. In the pupa rearrangement of the intestinal flora of the prepupa to the inner surface of the puparium, the molting membrane, and the surface of the pupa takes place whereas the pupal gut retained very fewnumber of bacteria. During adult emergence, most bacteria are retained in the puparium and the adult emerged with relatively few number of bacteria. External bacterial contamination was studied in four sites of the house fly. Bacterial contamination was found in all sites. However, according to the comparative bacterial density of these sites, the body (thorax and abdomen) was the site which demonstrated the highest density, followed by the legs, head and then the wings.

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1.Introduction

Little attention has been given to the fate of bacteria in the house fly (Dipeolu*et al.,* 1977; Shoukry and Radi, 1988).Greenberg *et al.*(1970) found that the fate of Salmonella in an adult flyis significantly influenced by the following factors (i) species of fly (ii) size of input: low inputs can result in massive multiplication, but the percentage of successful implantation increases with a dose up to 10 (iii) microbial condition of fly gut-interspecies antagonism leading to rapid elimination of Salmonella, or more gradual suspension by a mixed flora, may markedly affect the natural vector capacity of the fly. In case of house fly maggots, Greenberg (1973) found that, the digestive tract of an actively feeding 3rd instar maggot contains about 10 microbes. House fly maggots breeding in manure, garbage, and privies have similar number of organisms.These are distributed throughout the tract, but the largest number is lodged in the crop. When the maggot stop feeding and enters the prepupal stage, the contents of the crop diminish rapidly. This reduction occurs throughout the tract as the last meal is absorbed or eliminated and the gut constricts. The molt plays an important role in the elimination of bacteria from the fore and hind gut during pupation. Microorganisms left in themidgut are destroyed by pupal epithelium and phagocytized. Shoukry and Radi (1988) reported a significant increase of *Escherichia coli* counts ingested by the house fly, which reflects bacterial propagation inside the gut. The author stated that, the idea of considering the possibility of biological transmission may exist due to bacterial propagation which may change either the bacterial density and/or virulence. This assumption is in accordance with that reached by Emerson *et al*. (1999) who indicates a multiplication of *Salmonella typhimurium* in the fly gut but on ingesting high concentration inoculum.**Pruss and Mariotti (2000)** fed groups of the house fly, *Muscadomesticasorbens*, on sugary solution contaminated with an a flagellated strain of *Escherichia coli*. Multiplication of this pathogen was detected during one week after the ingestion of coli contaminated food. Bacterial propagation in the fly gut was found to be accompanied by a change of the a flagellated *E. coli* to the flagellated form. The gaining of flagellae during incubation in the fly gut was found to be stable even after being ingested again by another group of flies. The authors concluded that, propagation of *E. coli* in the fly gut as well as flagelation development could indicate a biological means of transmission of enteric bacteria by *Muscadomesticasorbens*. During the First World War, it was observed that the wounds of injured men left unattended in the field were sometimes infested with fly larvae. Baer (1929, 1931) showed that the condition of myiasis appeared to benefit the healing of the wounds and described the civilian use of larvae of *Musca* and *Lucilia* to help in the treatment of long-standing cases of osteomyelitis. Livingstar and Prince (1932) claimed to have, demonstrated the *in vitro* bacteriostatic activity of crushed larvae of

Luciliasericata, but their results could not be confirmed by Robinson and Norwood (1933).

Graczyk*et al.* (2001) showed that, not only did the scavenging action of the larvae play an important part in the success of "myiasis treatment" but calcium carbonate excreted by the stimulated phagocytosis by alkalinizing the wound. Greenberg (1954) stated that, although blow fly maggots !develop equally well on germ-free or contaminated meat, house fly maggots quickly die in their standard medium (CSMA) if microbs are excluded. Greenberg and Burkman (1963) made another study on the same subject and found that microbes play a specific role only in the nutrition of larvae, germ free house flies have normal longevity. In the absence of bacteria, maggots of the house fly do not grow on blood agar slants contain 25% beef blood, 38.5 beef pepton broth, 35% yeast extract, and,1.5% agar. The author does not identify the limiting factor.De Jesus *et al.*(2004)has found activity against haemolytic Streptococci, *Staphvlococcusaureus* and *Staph. albus* from larvae of house fly.With -some exceptions, the general features of maggotsbactericides are the following: They are active against gram-negative and gram-positive bacteria, the active substances seems to originate in the gut and is stable to heat, drying, and proteolysis **(Greenberg, 1973).**Any attempt to analyze this bactericidal phenomenon requires a precise data on pH in the maggot tract. **Greenberg (1968)** determined gut pHs of conventional and gonobiotic maggots of *Muscadomestica L., Phormiaregina* (Meigen)*, Sarcophagabullato*Parker*, and Calliphoravicina*, using micro-electrode. Crop pH was unbuffered and fluctuates with pH of the food which is influenced by microbial activity. However, the 3 regions of midgut appear buffered,, and their pH is microbe independent. Many investigators looked for the role of house flies in transmitting pathogens, Zureke*t al.*(2001), Antonio *et al.*(2004) and others.Isolation of *Campylobacter* and *Salomonella* from houseflies (*Muscadomestica*)in university campus and a poultry farm in Selangor, Malaysia was investigated by Choo*et al.* (2011), they concluded that 60% of collected samples were carried the objective pathogens.

2- Material and methods

Entomological procedures

A colony of the house fly, *Muscadomesticavicina* Macq., was raised in a valk-in insectary at the Biology Department, Faculty of Science for girls, King Abdulaziz University. To obtain a continuous picture of the quantitative distribution of bacteria harbored by the house fly, from early maggot to young adult, the following categories were undertaken. Distribution of bacteria in the gut of mature maggots A mature maggot was surfacely decontaminated and placed on a sterile slide. Using a sterile forceps and needles, the larval body was cut longitudinally and the gut was squeezed out into a drop of sterile saline. The fat cells and tracheae attached to the gut were easily teased away and plated on nutrient agar.

The gut was then transferred to another well-slide containing a drop of sterile saline and separating the gut into the fore gut, anterior and posterior mid gut and the hind gut. Each portion of the gut was separately rinsed, ground and plated on nutrient agar.

The above steps were repeated five times using maggots ofthe same age and size..

Distribution of bacteria in the gut of the prepupae: Prepupae weresurfacely decontaminated and treated as follows: The extreme anterior and posterior ends of the specimen were; snipped transversely and the gut was squeezed out posteriorly I into a drop of saline. Clusters of fat cells and tracheae were removed and plated on nutrient agar. The gut was then separated from the hind gut at the juncture of the malpighian tubules.Each portion of the gut was separately rinsed in sterile saline, ground and plated. Results of five series of such dissections were recorded.

Distribution of bacteria in the gut of pupae:

Three days old pupae were removed from the disinfected puparium in the following way: The anterior thrid of the puparium was cut away and the entire pupawas gently withdrawn while the puparium was held with the forceps. The gut was then freed of adhering fat and tracheae, and processed in the same way as the prepupa Results of five series of such dissections were recorded.Distribution of bacteria in the gut of a newely emerged house fly: Disinfected pupae were kept singly in pteridishes at room temperature till emergence. The newly emerged flies were immobilized by cold, ground and plated on nutrient agar. The empty puparia were also ground and plated. Less than 72 hours old pupae were difficult to remove because of the fluid nature of its contents at this time. The experiment was repeated five times and the results were recorded.

Distribution of bacteria at different external sites of the adult house fly:

Three days old flies were collected from the rearing cages and treated as follows:

Samples of legs, wings, mouth parts (with the heads) and abdomens were removed from a group of 5 flies. The detached parts of each group were aseptically o transferred to 5 ml. nutrient broth tubes and incubated at 37 C for 24 hours. The bacterial growth of the tested groups was measured by plate cell counts to compare between the bacterial densities of each site. The test was replicated 3 times each of 5

flies,(all procedures used in the bacteriological procedures were sterile).

3- Results and discussion

Quantitative distribution of bacteria in the developmental stages of the house fly *Muscadomesticavicina* Macq.

The results concerning the fate of normal bacterial gut flora in the developmental stages of the house fly disclose two significant declines manifest in the prepupa and newly emerged adults. The mechanisms of this reduction may be explained by studying the quantitative distribution of bacteria in different developmental stages of the house fly.

Distribution of bacteria in the gut of mature maggots:

Mature maggots were surfacely decontaminated, dissected in sterile saline and each portion of the gut was separately rinsed, ground and plated. The results of five specimens of such dissections are shown in table (3).

Table (1): Location and number of bacteria in mature maggots ofthe house fly *Muscadomesticavicina*Macq.

Gut area	Specimen number					Mean
	1	2	3	4	5	
Fore –gut	$7.2x10^9$	$4.1x10^8$	$9.3x10^8$	$6.4x10^9$	$3.1x10^9$	$3.6x10^9$
Anterior Mid-gut	$4.7x10^7$	$7.3x10^6$	$1.2x10^7$	$1.2x10^7$	$7.2x10^6$	$2.3x10^7$
Posterior Mid-gut	$5.3x10^6$	$1.3x10^7$	$4.2x10^6$	$6.1x10^6$	$2.2x10^7$	$1.0x10^7$
Hind gut	$3.5x10^9$	$8.4x10^8$	$2.1x10^8$	$7.7x10^6$	$9.2x10^7$	$9.3x10^8$

The results presented in table (1) above indicate that the largest bacterial count was found in the fore gut followed by the hind gut and the anterior portion of the midgut. The normal bacterial flora diminished slightly as it passes down the midgut.

Distribution of bacteria in the gut of prepupae:

Prepu'pae were surfacely sterilized, dissected and each portion of the gut, fat cells and trachea was separately rinsed and plated. The results of five series of such dissections are given in table (2).

Table (2): Distribution of bacteria in House fly, *Muscadomesticavicina*prepupae

Gut area	Specimen number					Mean
	1	2	3	4	5	
Mid-gut	$5.2x10^2$	$1.1x10^4$	$3.6x10^2$	$3.2x10^4$	$3.2x10^3$	$9.4x10^3$
Hind gut	$6.7x10^3$	$3.1x10^5$	$1.5x10^6$	$4.3x10^5$	$9.3x10^5$	$6.3x10^5$

The results in table (2) indicate that the hind gut is more heavily contaminated than the midgut. Fat cells and trachea were almost sterile.

Distribution of bacteria in the gut of pupae

Three days old pupa was removed from the disinfected puparium, dissected and each' portion of the gut was rinsed, ground and plated. Results of five series of this experiment are shown in table (3).

Table (3): Distribution of bacteria in the gut of pupa *Muscadomesticavicina*

Gut area	Specimen number					Mean
	1	2	3	4	5	
Mid-gut	2	-	1	2	-	1.0
Hind gut	10	-	-	4	2	3.2
Total	12	-	1	6	2	4.2

It is- clear- from the data in table (3) that the gut of 3 days old pupae retain relatively few number of bacteria (about 4.2 bacteria/pupal gut). The hind gut is more contaminated than the mi d gut. The marked disappearance of most bacteria from the pupal gut indicated a need for further study on the location and number of bacteria in other structures of the pupa.

Three days old pupae were treated as previously mentioned in the part of materials and methods.

The data in table (4) indicate that the inner surface o£ the puparium becomes the repository for the largest number of bacteria followed by the molting membrane, the surface of the pupa with gut removed, and lostely the gut itself. The results of 3 series of the above experiment are shown in table (4).Less than 72 hours old pupae were difficult to remove because of the fluid nature of its contents at this time.

Distribution of bacteria in the newly emerged fly

The newly emerged flies were immobilized by cold, ground and plated. Their empity puparia were also ground and plated.

Results of 5 series of this experiments are shown in table (5).

The data in table (5) indicate that most bacteria

are retained in the puparium and the adult emerged with relatively few number of bacteria.

Comparative bacterial density at different external sites of the house fly *Muscadomesticavicina*

Plate counts of the bacterial contamination of the four tested sites are presented in table (6).

Table (4): Location and number of bacteria in the pupa of the house fly *Muscadomesticavicina*

Location	Specimen number			Mean
	1	2	3	
Inner surface of puparium	$3.8x10^3$	$6.7x10^4$	$4.5x10^5$	$1.7x10^5$
Moulting membrane	$9.8x10^2$	$7.8x10^3$	$3.5x10^5$	$1.2x10^5$
Surface of pupa	$2.4x10^4$	$1.8x10^3$	$7.2x10^2$	$8.8x10^3$
Pupa minus gut	$1.8x10^3$	$3.4x10^2$	$2.2x10^3$	$1.4x10^3$
Gut of pupa	$1.1x10^2$	$1.8x10^1$	$2.5x10^1$	$5.1x10^1$

Table (5): Bacterial counts of newly emerged flies *Muscadomesticavicina*and their puparia

Location	Specimen number					Mean
	1	2	3	4	5	
Fly	14	61	7	320	170	$1.14x10^2$
Puparium	$4.9x10^4$	$2.6x10^5$	$4.2x10^5$	$1.7x10^4$	$8.2x10^3$	$1.5x10^5$

Table (6): Comparative bacterial denisty at different external sites of the house fly*Muscadomesticavicina*

Location	Average no. of viable cells/loopefull
Wings	$1.2 X 10^{3*}$
Legs	$4.1 X 10^3$
Mouth parts	$3.2 X 10^3$
Thorax & abdomen	$7.1 X 10^3$

The data in table (6) indicate the presence of the bacterial contamination in all the four sites. However, according to the comparative bacterial density of these sites, the body (thorax Viand abdomen) was the site which demonstrated the highest density, followed by legs, head and then the wings.

* Average of 3 replicates each of 5 flies.

6- Fate of bacteria in the developmental stages of the house fly*Muscadomesticavicina*

In order to trace the fate of pathogenic bacteria which may be ingested by maggots of the house fly, the fate of the countless bacteria swallowed in the normal course of feeding was initially studied. Successful passage of such organisms from larva to adult would enhance the vector potential of the fly by aiding in the survival and dissemination of those organisms. Counts for normal bacterial gut flora in different larva 1 stages were gradually increased with increase of larval age. This may be due to the increased amount of food (bacteria) consumed by larger larvae. Normal bacterial gut flora is distributed throughout the digestive tract of mature maggots, but the largest number was / found in the fore gut. This may be due to its high capacity and the similarity of the pH of larval medium and the fore gut.

Low bacterial count in the midgut may be due to its acidic medium. High bacterial density in the hind gut could be attributed to its neutral medium which may enhance the multiplication of bacteria. Greenberg (1968) found that, in *Muscadomestica* L., the crop pH is unbuffered and fluctuates with pH of the food which is influenced by microbial activity. However, the 3 regions of midgut appear buffered. The fore gut is slightly acidic or neutral, the mid-mid gut is most strongly acid. The hind-mid gut is near neutrality. When the maggot stops feeding and enters the prepupal stage, it loses more than 98% of their bacteria. In this stage, most bacteria are lodged in the hind gut.

It seems likely that the primary causes for this decrease is the continuous digestion and elimination of mostbacteria present in the mid gut. Most bacterial flora of the fore and hind gut may be eliminated during the molt.

Reduction in bacterial flora throughout the tract at this stage may be due to bacteriostatic activities of the fly gut. Bactericides are of widespread occurrence in flies, many investigators demonstrated bactericidal effects in flies among them are ***(Picado, 1935, Landi, 1960, & Beesley, 1968).***

Greenberg (1973) who stated that, bactericides are active against gram-negative and gram-positive bacteria. Other investigators including Slocum *et al.* (1933), Maseritz (1934), Paven (1949) and others disproved this bactericidal activity of flies. In spite of the very few number of bacteria recovered from the gut of 3 days old pupa in the present work, heavy bacterial contamination was found in the intact pupa. This may indicate a relocation of the prepupa's

bacterial flora. The shedding of the cuticular lining of the fore and hind gut displaces the majority of bacteria from the gut to the inner surface of the puparium, the molting membrane,, and the surface of the pupa itself. During the remainder of the pupation period, these bacteria are able to maintain their numbers situated in a relatively neutral environment, beyond phagocytic and other inimical host factors. Greenberg (1959) stated that, bacteria can persist undiminished within the empty pupa case at least 2 days after eclosion, when kept at 100% R.H. The eclosion of the fly with a very few number of bacteria from the heavy contaminated pupa is of considerable epidemiological significance. This auto sterilization however, is not a consistent process, and while some flies are sterile, others from the same batch retain high number of viable.This phenomena (autosterilization) has been observed in many muscoid flies, midges and mosquitoes **(Greenberg, 1973)** but nothing is known that explains the emergence of saints and' sinners among flies.

It is well known that the house flies pick up bacteria from their surroundings very easily. The hairy body, the tarsi with their sticky hairs, the wings and grooved proboscis all provide hide ways for bacterial stowage. The results of the present work indicate that all of the tested sites of the house flies were externally contaminated by bacteria. However, according to the comparative bacterial density of these sites, the body was the site which demonstrated the highest density, followed by the legs, head (mouth parts), and then the wings.The relatively high bacterial density of the abdomen may be due to its large surface area. High bacterial density on the legs may be due to the behavior of flies which constantly preening their wings and brushing their mouth parts with their legs. **Shoukry and Radi (1988)** contaminated *Muscadomesticasorbens* with *E. coli*; their results revealed high external, contamination densities of the mouth parts, followed by the abdomen surface/ legs and then wings. The presence of large number of bacteria on legs and mouth parts may increase the fly capability of pathogen transmission during resting period on man and his belongings and through feeding activity. High bacterial density on the abdomen may be of little public health importance except at accidental falling of flies in food or drink or at accidental crushing by hands. Our results were completely agree with that obtained by Nazani*et al*. (2005), Banjo *et al.* (2005) that they weredetermined the type of pathogen, it was gram positive bacteria. Also, Babak*et al*. (2008), identify the bacteria which possible transmitted by *Muscadomestica*. Yap *et al*. (2008), Jerry *et al.* (2012), Choo*et al.*(2011) and Hamid*et al*. (2012) investigate the role of house fly wings in mechanical transmission of *Vibrio cholera*and many serious pathogenic bacteria.

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