



Screening of starving Mosquitoes: Spreading Infectious Diseases From Gut Fluid

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Abstract:

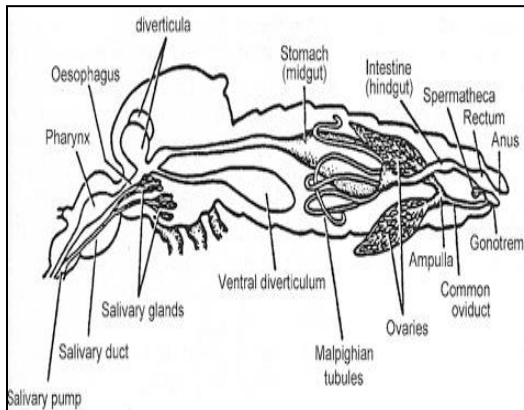
Mosquitoes act as vectors for transmission of many diseases since long period of time. Blood-feeding mosquitoes are much neater and more surgical; they insert a tube (proboscis) for drawing blood, by the time they are ready for their next meal, even on a second host after an interrupted meal, any bacteria or/and viruses from their first meal are safely stored away in their midgut. In the second mechanism, a mosquito would initiate the cycle by beginning to feed on any community disease carrier and be interrupted after it had successfully drawn blood. Instead of resuming the partial blood meal on its original host, the mosquito would select any disease free person to complete the meal. As it penetrates the skin of the new host, the mosquito would transfer virus/ bacteria particles that were adhering to the mouthparts from the previous meal. However, studies conducted by the CDC (centre for disease control and prevention) and elsewhere have shown no evidence of HIV transmission from mosquitoes. Lack of such outbreaks, despite intense efforts to detect them, supports the conclusion that HIV is not transmitted by insects as per CDC. Mosquitoes collected from local area at Warana valley were in larvae forms, which were kept in starving condition at artificial water hub. The quantities of gastro tract (fluid content/carrying capacity) of mosquitoes were evaluated by microtitration method; made in progress by sacrificing the vector. The vector nature of the same was verified by transfer of *Lactobacillus casein* (*non pathogenic*) bacteria from mosquitoes to microbial media of MRS (deMan Rogosa and Sharpy Media); where the bacteria get flourished acting as biomarker. The transfer of *Lactobacillus casein* at starvation made matter of concern in transfer of bacterial disease and even AIDS positive viruses.

Introduction:

The mosquito's digestive system is a closed system, with one long enclosed tube (alimentary canal) running lengthwise through the body. The alimentary canal is a one way street— food enters the mouth and gets processed as it travels toward the anus. Each of the three sections of the alimentary canal performs a different process of digestion.

Trypsin is synthesized less than 4 hours after the blood meal, but it is the "late trypsin", synthesized between 8 and 24 hours after the meal, which represents the major endoprotease involved in bloodmeal digestion. These enzymes play a role in parasite transmission and it has been shown that ookinete development is impaired after artificially feeding a mosquito on infected blood and trypsin inhibitors. The peritrophic membrane (PM) is an acellular sac produced by the cells lining the midgut, in response to food ingestion. It consists of proteoglycans and chitin. The PM completely surrounds the food bolus and contributes to the digestive process by compression. The role of the PM is not entirely clear: (1) act as physical protection against pathogens, (2) act as a filter for blood components, and (3) act as a barrier to avoid clogging the spaces between the microvilli of the epithelium. Malaria parasites have to complete their development, including mating and differentiation of ookinete, before the PM has reached maximum rigidity. To achieve this, the parasites have evolved mechanisms to penetrate the PM and traverse the gut to continue their development. The most important of these mechanisms is the secretion of a chitinase. The mosquito digestive system helps in this process, since the chitinase is actually secreted as an inactive enzyme which is activated by mosquito serine proteases.

Fig.1: Drawing of the digestive system of mosquito.



When AIDS was first recognized, there were many newspaper reports on the possibility of mosquitoes being involved in its transmission. Almost half of 6625 men and women interviewed in Zaire in 1988 to determine existing levels of knowledge regarding AIDS believed in the transmission of AIDS by mosquitoes. A recent survey involving 1500 high school students (grade 10) from 14 schools in 4 different provinces in Papua New Guinea revealed that more than a third of them (34%) considered mosquitoes to be carriers of HIV. Although mosquitoes are known to be vectors of certain death-threatening viral diseases such as yellow fever, dengue fevers. We review the theoretical mechanisms which would allow blood-sucking insects such as mosquitoes to transmit virus and discuss the evidence against the transmission of HIV by mosquitoes.

Theoretical mechanisms for the possible transmission of HIV by mosquitoes:

I. Biological transmission:

A mosquito feeding on a person infected with HIV could ingest the virus which then undergoes multiplication within the mosquito and migrates to its salivary glands. When the infected mosquito seeks a second blood-meal from an uninfected person, it could transfer the HIV from its salivary gland during the course of feeding. This is the mechanism involved in the transmission of yellow fever and dengue.

II. Mechanical transmission:

Human defensive behaviour may prevent a partially blood-fed mosquito from completing a blood-meal on a person infected with HIV. However, instead of

resuming feeding on the same person the mosquito may select an uninfected person to complete its feeding on. As it penetrates the skin of the new host, the mosquito could transfer HIV particles that were adhering to the mouthparts from the previous partial blood-meal. This is purely mechanical transmission because the virus does not undergo any multiplication or development in the mosquito.

Transmission through mosquito squashes:

Squashing mosquitoes is thought of by many people as an instinctive reaction triggered by the sight of the creatures on human skin. A mosquito that had had a partial blood-meal from an HIV carrier and resumed feeding on an uninfected person may be squashed into a cut in the skin. Viral infection could be initiated in this way depending on the quantity of viral particles contained in the partial blood-meal. For the cultivation of the mosquito flora mimicking and starving mosquitoes are needed. GI content of the starving mosquito if microtitrated along with the microbial assay will reveal the vector properties of the mosquitoes.

Materials and Methods:

Microtitration of mosquito's GI content

Two different methods were employed to estimate the GI content. The Microtitration of mosquito's GI content in starving condition and Microbial assay of GI content of mosquito flora

Micro-titration:

Larvae of the mosquito were taken from the pond and were grown in sterile water. Due to the lack of nutrients in sterile water, larvae that undergo metamorphosis into mosquito arrive to starving state. They are grown into adult mosquitoes with source of nutrient as *lactobacillus casein* which also acts as biomarker for further study.

Mosquitoes suck the bacteria as their nutrient medium till their Gastro tract is fully occupied. Two groups of Mosquitoes were made. All Mosquitoes groups were collected, sacrificed and the abdominal content was oozed out. First group of Mosquitoes were kept in water at its pre stage i.e. larvae. The adult metamorphosed Mosquitoes content were latter microtitrated by using NaOH and HCl in presence of phenolphthalein as an indicator. These groups lacked the bacteria in the media where they were grown. It

had given the concentration of the water that present in its Gastro tract.

Second groups Mosquitoes were also collected and the GI content was oozed and subjected for micro titration. These groups contained the bacteria in their GI obtained from the media where they were grown. This was followed by blank titration using the same procedure, wherein instead of Mosquitoes content plain 0.01 N HCl solutions was titrated to check the effectiveness of the reagent.

Fig 2: Starving



Microbial Load Evaluation Study:

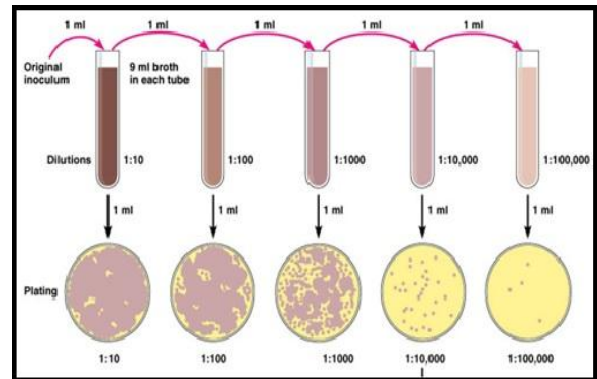
The amount of the Gastro fluid content which was determined by method I is utilized to screen the microbial content growth rate study. “X” ml of the fluid quantified in method I is loaded with the bacterial count of *lactobacillus casein*. This is taken as blank reading by plating the bacteria on MRS media. To determine the concentration of viable bacteria present inside the stomach of mosquito, the content of the sacrificed Mosquito is spread over the MRS Media by the spread plate technique. Growth of bacteria is seen as per specific time interval and the colonies are counted. Similarly the concentration of viable bacteria present inside the stomach of mosquito with HCl as gastro component are utilized for the study, this involved the collection of the content of the sacrificed Mosquitoes and spreading it over the MRS media by the spread plate technique.

Dilution technique:

One bead of a deep-frozen culture was transferred into MRS broth and incubated overnight at 37°C. This broth was then used to inoculate the final broth,

which was subsequently incubated at 37°C for 24 hour. Cells were harvested and washed once with PBS buffer pH 7.0. After washing, the pellet was re-suspended in the final carrier solution. The dilution was carried out in saline water of dextrose 5% or 0.9% w/v to maintain the viability of the microbes and also to maintain cell osmosis. The Strain microbes were diluted in the ratio of 1:100, 1:1000, 1:10000 etc for ease to get the viable count by plating method. The dilution is must and if it is not carried out, would result in the heavy crowd, overlapping colonies; which cause to get the inappropriate cell count and failure in its determination

Fig 3: Serial dilution technique to gain appropriate cell count.



Result & discussion:

Observation- Table I: Without bacteria

Without adding the bacteria to the dwelling habitat (water) of mosquitoes, the burette readings (titration) was taken with the time interval of 12 to 48 hours. The readings observed after 12 hrs were shown in the table below. The result was observed after 24 to 48 hrs which showed a constant burette reading

Sr. No.	Time	Titration by HCl (Burette Reading)
1.	12	0.13
2.	24	0.11
3.	36	0.10
4.	48	0.10

Observation- Table II: With bacteria

By adding the bacteria to the dwelling habitat of mosquitoes, the burette readings were taken with the

specific time interval of 12 to 48 hours. The reading observed after 12 hrs are shown in the table below which indicated a constant reading. With the addition of the bacteria concentration of the HCl was decreased.

Sr.No.	Time	Titration by HCl (Burette Reading)
1.	12	0.13
2.	24	0.12
3.	36	0.11
4.	48	0.11

Observation-Table III:

The above observational method was carried out on many groups of the mosquitoes and the mean was taken to quantify the exact content of mosquitoes Gastro tract. From the observations it was concluded that, after bacterial feeding the concentration of HCl decreases with increase in bacterial concentration indicating the utility of HCl by bacteria.

S.No	Time	Burette reading /Mosquitoes					CBR (ml)
		1	2	3	4	5	
1.	With bacteria after 24 hrs	0.104	0.108	0.098	0.098	0.100	0.10
2.	Without bacteria after 24 hrs	0.106	0.118	0.110	0.116	0.102	0.11

Observation- Table IV: Bacteria load IN-VITRO

Dilution	10 ⁰	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵
No of colonies	Un	Un	Un	332	98	45

Blank reading to verify bacterial load in 0.09ml quantity, *Un = Uncountable

Fig.5: Uncountable bacterial growth without dilution technique



Observation- Table V: bacteria in mosquitoes IN-VIVO in absence of acid HCl

S.No	Time (hrs)	10 ⁰	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵
1.	12	Un	Un	Un	110	75	25
2.	24	Un	Un	Un	70	40	20
3.	36	Un	Un	Un	40	12	6
4.	48	Un	Un	Un	40	12	6

*Un = Uncountable.

Fig 6: CFU count of (*Lactobacillus casein*) bacteria



Observation- Table VI: HCl + bacteria in mosquitoes

S.No	Time (hrs)	10 ⁰	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵
1.	12	Un	Un	Un	120	79	20
2.	24	Un	Un	Un	70	40	20
3.	36	Un	Un	Un	42	22	6
4.	48	Un	Un	Un	42	22	6

*Un = Uncountable

Conclusion:

Mosquitoes act as vectors for various organisms. These vectors collected from local area at Warana valley were obtained in larvae forms; these forms gave the virgin vectors for the study. These vectors kept in starving condition were alive in hub containing mixture of 0.01N HCl and bacteria (*Lactobacillus casein*) in water. HCl was used as biomarker which did not affect the study. The quantities of gastro tract (fluid content/carrying capacity) of mosquitoes were evaluated by microtitration method; made in progress by sacrificing the vector. The vector nature of the vector was verified by transfer of *Lactobacillus casein* (non pathogenic) bacteria from mosquitoes to microbial media of MRS (deMan Rogosa and Sharpy Media). These bacterial loads were easily flourished in MRS Media. The transfer of *Lactobacillus casein* at starvation gave evidence that shift of bacterial disease occur easily from mosquitoes to host. This also arrives the question of transfer of AIDS positive viruses from vector to host.

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Bibliography:

- 1) Moses j. Bockarie1 and Raymond parul, Can mosquitoes transmit AIDS?, PNG Med J 1996; 39: 205-207
- 2) Wilson D, Sibanda P, Greenspan R, Wilson C. Knowledge about AIDS among Zimbabwean teacher-trainees before and during the public awareness campaign. Cent Afr J Med 1989; 35:306-309.
- 3) Friesen H, Danaya R, Doonar P, Kemeki A, Lagani W, Mataio G, Mokela D, Rongap T, Vince J. 19-22 Sep 1995:21.
- 4) Crans WJ. Why mosquitoes cannot transmit AIDS. Wing Beats 1994; 5:23-24.
- 5) Piot P, Schofield CJ. No evidence for arthropod transmission of AIDS. Parasitol Today 1986; 2:294-295.
- 6) Hudson CP, Hennis AJ, Kataaha P, Lloyd G, Moore AT, Sutehall GM, Whetstone R, Wreghitt T, Karpas A. Risk factors for the spread of AIDS in rural Africa AIDS 1988;2:255-260.
- 7) Morse SS, Schluederberg A. From the National Institute of Allergy and Infectious Diseases, the Fogarty International Center of the National Institutes of Health, and the Rockefeller University. Emerging viruses: the evolution of viruses and viral diseases. J Infect Dis. 1990 Jul; 162(1):1-7.
- 8) Morse SS. Emerging viruses: defining the rules for viral traffic. Perspect Biol Med. 1991 Spring; 34(3):387-409.
- 9) Soares S, Kristinsson KG, Musser JM, Tomasz A. Evidence for the introduction of a multiresistant clone of serotype 6B Streptococcus pneumoniae from Spain to Iceland in the late 1980s. J Infect Dis. 1993 Jul;168(1):158-163.

- 10) Rogers DJ, Packer MJ. Vector-borne diseases, models, and global change. *Lancet*. 1993 Nov 20;342(8882):1282–1284.
- 11) Myers G, MacInnes K, Korber B. The emergence of simian/human immunodeficiency viruses. *AIDS Res Hum Retroviruses*. 1992 Mar;8(3):373–386.
- 12) Bertrand JT, Makani B, Hassig SE, Niwembo KL, Djunghu B, Muanda M, Chirhamolekwa C. AIDS-related knowledge, sexual behavior, and condom use among men and women in Kinshasa, Zaire. *Am J Public Health* 1991; 81:53- 58.
- 13) Allan JS, Short M, Taylor ME, Su S, Hirsch VM, Johnson PR, Shaw GM, Hahn BH. Species-specific diversity among simian immunodeficiency viruses from African green monkeys. *J Virol*. 1991 Jun;65(6):2816–2828.
- 14) Gao F, Yue L, White AT, Pappas PG, Barchue J, Hanson AP, Greene BM, Sharp PM, Shaw GM, Hahn BH. Human infection by genetically diverse SIVSM-related HIV-2 in West Africa. *Nature*. 1992 Aug 6;358(6386):495–499.
- 15) Barbour AG, Fish D. The biological and social phenomenon of Lyme disease. *Science*. 1993 Jun 11;260(5114):1610–1616.
- 16) Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y. Evolution and ecology of influenza A viruses. *Microbiol Rev*. 1992 Mar; 56(1):152–179.
