The Dissection of a Mosquito for Malaria Parasite

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Mosquito diagram prepared from a drawing by B Jobling.
Diagrams executed by Goodliffe-Wilson Ltd.

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<**Opening credits**>

<**David Lloyd James narrates over moving images of mosquitoes**>

Mosquitoes of the genus Anopheles are the transmitters of malaria to man. This is a female *Anopheles atroparvus* feeding on blood drawn from the capillary bed in the wrist of its victim. The blood meal is necessary to the female Anophelean before the eggs can mature, but it is also the means whereby malaria is transferred from man to insect and then on to man again.

<**James narrates over animated diagrams of mosquito anatomy**>

The female may feed about every 48 hours or so, ingesting each time slightly more than her own weight of blood so that the stomach becomes greatly distended. At the

same time, clear serum which is separated from the blood in the stomach is passed along the gut to the exterior. At the start of any anti-malarial work, one of the first essential steps is the investigation of the local Anophelean mosquitoes to find which species are the important vectors of malaria. To do this, the mosquitoes must be dissected and malaria parasites looked for, either in the stomach or mid gut of the mosquito or in the salivary glands.

The gametocytes or sexual forms of the malaria parasite enter the stomach of the mosquito with the blood from an infected human. Fertilisation occurs there and ookinetes are formed which penetrate the stomach wall. The blood meal is digested in about 48 hours by which time the ookinetes have developed oocysts on the outer surface of the stomach. The oocysts grow and by the 10th or 12th day after infection until they are mature. They then rupture and liberate thousands of sporozoites into the body of the mosquito. Many of these enter the salivary glands and when the mosquito next feeds they are carried with the saliva into the body of the victim. Thus, sporozoites in the salivary glands and oocysts on the stomach wall are the evidence of infection.

<**James narrates over image of mosquito stomach**>

This stomach is exceptionally heavily infected, seldom would such a large number of oocysts be found during field surveys. These are 7 days old and are fairly large, about 40 microns in diameter. Sporozoites are long, thin organisms which are seen moving here near to an unruptured oocyst. They are about 14 microns long and to show their structure they should be stained. This is not however done during field work since they are readily recognisable unstained.

<**Intertitle: Technique of Dissection, 1. Removal of Salivary Glands**>

<**James narrates over moving images of mosquito**>

If the stomach of the captured mosquito is filled with blood, the insect should be kept for a day or two, until the blood has been digested. This is because the distended stomach ruptures easily and the oocysts may be obscured by blood.

<**James narrates over demonstration of dissection of a mosquito**>

Special dissecting needles with very sharp cutting edges are used and they are made of stainless steel because the dissection is carried out in saline. The mosquito is killed before dissection: the live insect is shaken to the bottom of a narrow test tube and is stunned by giving the tube a few sharp raps, then a light pressure on the thorax kills it without crushing it. It is then held by a wing while the legs are pulled off one at a time, followed by one of the wings. The other wing is cut off and so is the head and the mosquito is ready for dissection.

00:04:35:02

<**James narrates over images of mosquito dissection**>

The salivary glands are removed first. A dissecting microscope magnifying 10 times is used and the insect is placed on the stage with its thorax on the operator's right. A drop of saline is put on the slide, close to the thorax but not touching it. Then the thorax is held with a needle at a point just before where the glands lie and with another needle the glands are gently pressed out and into the saline. <*Shows same from the perspective of the dissector*> This is as the operator would see the process.

Each gland has 3 lobes – the length of these varies but it averages about a third of a millimetre. The sporozoites are present in the cells and ducts of the glands. A microscope coverslip is now dropped cornerwise onto the glands, just heavy enough to rupture them without distressing them, which makes for easy examination. The sporozoites are observed with a 4mm objective, they are relatively inactive organisms, drifting slowly in the saline and body fluids.

<**Intertitle: 2. Removal of Stomach**>

<**James narrates over images of mosquito dissection**>

To remove the stomach, the body of the mosquito is placed on a slide, in a drop of saline, with its thorax to the operator's left. The thorax is held with a needle while the cutaneous abdominal covering is nicked above and below at the level of the sixth or seventh segment. The detached segments are then gently pulled and the gut comes away with them.

At higher magnification it is seen that first the Malpighian tubes and the hind gut emerge, then the stomach or mid gut and finally part of the oesophagus. For convenience the slide is turned round and the Malpighian tubes are cut off. Then a coverslip is lowered very gently onto the stomach and if the correct amount of saline has been used, the stomach is just flattened without being ruptured. The oocysts can be seen with a 16mm objective. These specimens are fairly heavily infected – the oocysts are well developed and easily identified.

The percentage of mosquitoes found to have sporozoites is termed the sporozoite rate for the species. The oocyst rate may be similarly defined, however, oocysts on the stomach wall only show the susceptibility of the mosquito to infection, they do not confirm it as a vector. The sporozoite rate on the other hand is the most important measurement of field malariology. Its average value in highly endemic areas is about 4%, but for one species the rate may vary from 30%, during an epidemic, to .06% where the malaria transmission is occurring in the presence of a high mosquito population.

It is therefore the combination of the sporozoite rate with the density of the species that determines the importance of that species as a vector in a particular locality.

<**End credits**>