Mosquitoes and Malaria

Wellcome Trust Film Unit, 1988.

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1953 film material from the Wellcome Trust Film Archive.

Colour
Duration: 00:13:23:24

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<**Opening credits**>

<**Barry Paine narrates over close shot of mosquito on human skin**>

Malaria is transmitted by the bite of a mosquito. Not all mosquitoes can transmit malaria, only one genus, anopheles, is responsible, and only the female feeds on blood. This female anopheles is feeding on blood drawn from the capillaries in a man's wrist. If she can't find blood, she can feed on fruit juices, but without a blood meal she can't make eggs and can't reproduce. Given the opportunity, she'll feed on blood every two or three days and each time she takes in more than her own weight of blood. Her abdomen swells up and as she feeds, she concentrates the blood and

the clear fluid separated is excreted. If a mosquito feeds on someone infected with malaria […]

<**Paine over microscopic view of a mosquitoes blood meal, then animated illustrations showing mosquito reproduction following blood meal**>

[…] parasites will be taken up with the blood.

Here is a mosquito's blood meal. These are red cells and these are the sickle shaped gametocytes of *Plasmodium falciparum*; falciparum means sickle shaped.

A male and a female. The male gametocyte exflagellates to produce the motile, thread-like microgametes. When a male contacts a female, the two fuse to produce an invasive, motile zygote, the ookinete; this swims through the blood meal and penetrates the stomach wall where it grows rapidly into an oocyst.

After ten to twelve days, the oocyst is mature and packed with sporozoites; this mosquito is carrying a very heavy infection. The oocysts rupture into the body cavity and the sporozoites move through the body of the mosquito to enter its salivary glands. When the mosquito feeds again, the sporozoites are injected with the saliva into another person and the transmission cycle is complete.

To summarise the cycle: the gametocytes are ingested with the blood meal; the male exflagellates; fertilisation takes place; the ookinetes form oocysts on the stomach wall; and sporozoites leave the oocysts and migrate to the salivary glands.

To show that a mosquito is infected with malaria, it's necessary to find oocysts on its stomach wall or sporozoites in its salivary glands. This can be done by dissection under a microscope, and the following demonstration, filmed in 1953, was carried out by PG Shute and RS Bray.

<**Paine over film of mosquito dissection**>

If a recently-fed mosquito is captured, its stomach will be distended with blood. It's best, therefore, to keep it for a day or two to allow the blood to digest and to prevent the distended stomach from being broken during the dissection. During this time the oocysts will mature.

Dissecting a mosquito is a quick and simple matter after a little practise. In addition to the dissecting microscope, the only instruments needed are two very sharp stainless steel dissecting needles, stainless steel because the dissection is carried out in saline which is corrosive to ordinary steel.

The mosquito is placed in a narrow test tube, shaken to the bottom of the tube and then stunned by tapping the tube sharply on the hand. The insect is killed by pressing it lightly on the thorax; not too hard or it will be crushed. The insect is then held by one wing while the legs are pulled off one at a time. The other wing is pulled off and the wing that has been held is cut off. The head is then cut off and the body of the mosquito is then ready for dissection.

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The salivary glands are removed first, a magnification of about ten times is used. The insect is placed on the microscope stage with its thorax to the right. A drop of saline is put on the slide, close to the thorax, but not touching it. Holding the thorax firmly with one needle, at a point just behind the glands, they are pressed gently out into the saline drop with the other needle. This is how the operator sees the process.

Each gland has three lobes, usually they are about a third of a millimetre long, but of course they'll vary between insects. If sporozoites are present, they'll be found in the cells and ducts of the glands. A microscope cover slip is placed over the glands, dropping it just heavily enough to rupture without shattering them. The material is examined under a standard microscope with a good-quality, high power objective, and if sporozoites are present, they'll be seen as relatively inactive thread-like organisms, drifting slowly in the surrounding fluid.

The second stage in the dissection is to remove the stomach of the mosquito. The insect's body is transferred to another slide into a new drop of saline. The thorax is held with one needle and the other used to knick the abdominal covering, just above the tail, at the level of the sixth or seventh abdominal segment. These detached segments can then be gently teased away and the gut pulled away with them – it needs a little care and patience. This is the operator's view. First the malpighian tubules, then hind gut, now the stomach or midgut and, finally, the oesophagus. The slide is turned round and the malpighian tubules cut off. A cover slip is lowered very gently onto the specimen, just to flatten the stomach without breaking it.

<**Paine over microscope images of dissected mosquito**

The slide is transferred to the ordinary microscope and examined under the low power. Oocysts are seen as tiny translucent vesicles on the stomach wall, this specimen is heavily infected; the oocysts are well developed and easy to identify. If oocysts are found on the wall of the stomach, this only shows that the mosquito is susceptible to infection. To confirm that it is acting as a vector, sporozoites must be found.

The sporozoite rate is the percentage of mosquitoes with sporozoites in their salivary glands; it's an important component of epidemiological studies.

<**Paine over still image of mosquito on human flesh**>

It shows which species are likely to transmit malaria and is essential in judging the effectiveness of control measures. In highly endemic areas, the sporozoite rate may be up to 4%. In epidemic areas it may vary between 30% during a severe outbreak, to almost zero when there's very little transmission. But dissection <*shots of mosquito dissection*> is labour intensive. A trained technician can process about a hundred mosquitoes a day and nowadays immunological techniques are helping to reduce the labour, increase the output and to detect very light infections.

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<**Paine over illustrations of sporozoites**>

The protein on the surface of a sporozoite has a central region of the molecule made up of repeated amino acid sequences unique to each malaria species. *Plasmodium falciparum* has a group of four amino acids, repeated forty-one times; and *Plasmodium vivax*, a group of nine amino acids, repeated nineteen times. Monoclonal antibodies that recognise these specific repeat regions have been prepared and are used in an enzyme linked, immunosorbent assay (ELISA) to detect sporozoites.

<**Paine over shots demonstrating how the ELIZA technique works**>

Using this technique, as few as fifteen parasites have been detected in a single mosquito and most infected mosquitoes contain more than five hundred.

Mosquitoes that have been collected in surveys weeks or months before can be used for the test. With the aid of a miniature pestle and mortar, mosquito heads and thoraces are ground up with a little detergent to extract the protein. Samples of the fluid are then distributed into the wells of a plastic plate already coated with monoclonal antibody. After standing a while to allow the sporozoite protein, if present, to stick to the antibody, the plate is washed. Monoclonal antibody labelled with an enzyme is then added and this is captured by any sporozoite protein in the wells. The plate is washed again. A colour producing substrate for the enzyme is added and where the enzyme has stuck, the colour develops.

This is how it works. Here are two wells on the plate, coated with specific monoclonal antibody. Protein extracts from mosquitoes are added, the one on the left contains sporozoite protein and it sticks. After washing, antibody labelled with an enzyme is added and this also sticks to the sporozoite protein. The plate is washed again and the enzyme substrate is added. The enzyme releases the colour in the left hand well, but nothing has stuck in the right hand well and no colour develops. The depth of

colour depends on how much sporozoite protein, and therefore how much enzyme, has stuck.

By using the ELIZA technique, our technician can process about three thousand mosquitoes in batches of twenty every day. He can also determine, using appropriate plates, whether *Plasmodium falciparum* or *Plasmodium vivax* are present.

In our demonstration, we have featured the simplest ELIZA equipment. Sophisticated automatic machines for processing and reading the plates are now available and are in everyday use in laboratories around the world. ELIZA is used for a wide range of tests, including AIDS. But in areas where ELIZA equipment may not be available, mosquito dissection and keen eyesight will continue to play an important part in the epidemiology of malaria.

<**End credits**>

<In addition to those at the beginning of transcription>

With special thanks to:

Dr Alister Voller.
Dr Dennis Bidwell.
Institute of Zoology, Zoological Society of London.

Prof GAT Targett.
Martin Looker.
Neil Rogers.
John Williams.
London School of Hygiene and Tropical Medicine.

Walter Reed Army Institute of Research, Washington DC, USA.