The epidemiological effectiveness of a vector species of mosquito depends on, among other factors, the proportion of the vector population that survives for completion of the cyclical development of the pathogen in the mosquito body; in this case the larvae of *W. bancrofti*. These results show for *C. fatigans* in the endemic zone of Ceylon that, if adulticides are used to supplement larval control operations in a filariasis control programme, the adult mosquitos should be killed before they have completed their third gonotrophic cycle. Although the time factor concerned is still under investigation, the present data indicate that their kill must be effected not later than about nine days after first ingesting microfilariae. Since transmission appears to be mainly by mosquitos which take up microfilariae in their first blood meal, this means killing adult *C. fatigans* before they themselves are about ten days old. This is not a generous time factor for a species of vector widely known for its tolerance of insecticides in the adult stage.

The present study also shows that in all filariasis units along the endemic belt the age structure of the infected mosquitos follows the same pattern. Although the number of infected mosquitos is not great in each filariasis unit, infective *C. fatigans* were found at one station, Matara, in every month during the period of this study. Transmission of filariasis therefore takes place throughout the year in Matara, and probably also in all other filariasis units along the coastal belt of Ceylon.

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A Rapid Method for Screening Blood Smears for the Presence of Microfilariae and Subsequent Identification of Species by Staining*

by N. AHAD, M.B.,B.S., *Director, Pasteur Institute of the Union of Burma, Rangoon; and Bacteriologist to the Government of the Union of Burma*

With the techniques generally used it may be difficult for field workers engaged in mass surveys to examine a sufficiently large number of blood smears daily for the presence of microfilariae. Accordingly the writer has devised a rapid screening method based on the known fact that microfilariae can be detected in blood films after dehaemoglobinization. The advantages of the method are the possibility it affords of a rapid first screening of blood films and subsequent economy of time and materials from the fact that only those slides preliminarily screened as positive need be examined for identification of the microfilarial species.

The writer has also developed a modified staining method by which, in his experience, the structural details of microfilariae are well defined and which appears suitable for species identification.

By this method the Pasteur Institute of the Union of Burma has in 30 days, working at four hours a day, screened 6000 blood smears received from the Corporation of Rangoon, and stained and identified 235 positive slides from that total.

Preparation of slides

The slides for the above purpose are prepared as follows:

1. The blood is collected between 8 p.m. and 12 midnight.

2. Under aseptic precautions the tip of any finger, except the little finger or the thumb, is pricked boldly with a sterile needle and the finger tip pressed to get a good-sized blood drop.

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3. Four such drops are collected on the surface of a glass slide on the four corners of an imaginary ½-inch (1.5-cm) square.

4. The four drops of blood are then joined by means of a corner of another slide to form a uniform film.

5. The films thus made are labelled properly and then dried overnight in a closed dish to avoid their being affected by insects or dust.

Screening technique

Stage 1 :

1. As soon as the dry blood films for microfilariae are received in the morning they are serially numbered on the same side of the slide as the film.

2. A series of staining dishes filled with tap water, and which can take about 10 slides each, is then arranged on the right-hand side of the microscope.

3. A slide box with a capacity of 20 or more slides is placed on the left-hand side of the microscope together with a tray to receive the negative slides.

4. All slides serially numbered are now immersed serially from right to left in the staining dishes filled with tap water and allowed to remain in water till he examination is over.

5. After five minutes, which is the minimum time for dehaemoglobinizing a slide, and while the slides are still wet, start taking out the slides according to serial number.

6. Quickly wipe with a piece of gauze the back side of the slides.

7. Quickly examine under the low power of the microscope ($^2/_3$ objective) with the condenser slightly lowered. The examination should be completed quickly while the blood film is still wet.

The microfilariae are seen very clearly as gracefully curved, homogeneous, shining, light-yellowish, refractile structures against a colourless background.

White blood cells are seen as refractile, yellowish, globular bodies.

The dehaemoglobinized red blood cells appear as homogeneous, colourless structures.

Cotton fibres and blotting fibres, if any, are seen as irregular blackish structures with a yellowish central core.

8. After completion of screening, the negative slides are discarded into the tray on the left-hand side of the microscope and the positive slides are arranged serially from left to right in the slide box on the same side.

Stage 2 :

The positive slides can further be verified, if necessary, by immersion for 10 seconds in a 1 : 30 dilution of Ziehl-Neelsen carbol fuchsin in distilled water, followed by re-examination while wet in the same manner as before.

The microfilariae are seen as colourless or slightly coloured (pink), gracefully curved structures against a homogeneous pinkish background of dehaemoglobinized red blood cells.

White blood cells are stained light pink with deep pink nucleii.

The slides are then dried and kept for staining by Leishman's stain for identification at any convenient time up to a maximum of six months.

Stage 2 has three particular advantages.

First, if any worker wants further to verify his finding after dehaemoglobinization (Stage 1), he can do so.

Secondly, the fuchsin and carbolic acid present in the Ziehl-Neelsen carbol fuchsin works out at 0.3% fuchsin and 0.17% carbolic acid when the stain is diluted 1:30 with distilled water. This definitely prevents fungal growth when the slides are treated with it, dried and preserved. The writer has had the opportunity of observing this effect up to 6 months only.

Finally, if a dehaemoglobinized slide is treated with 1 : 30 dilution of carbol fuchsin, dried in air and finally stained with Leishman's stain, it gives, in the experience of the writer, better definition of the structures and hence makes for easier identification of the microfilarial species.

Identification of species by staining

The positive slides treated with a 1 : 30 dilution of carbol fuchsin as mentioned above are now stained with Leishman's stain for 8 minutes or with double-strength of Leishman's stain for 5 minutes in the usual way and then dried and examined under the microscope with an oil-immersion lens.

The sheath of a microfilariae does not stain and stands out as a colourless covering of the microfilarial body, which has a pinkish-violet colour.

The nucleii of microfilariae stain deep violet.

The musculo-cutaneous tissue shows clear pinkish to violet striation.

The dehaemoglobinized red blood cells stand out as homogeneous pinkish clouds.

White blood cells, including eosinophils, can be identified clearly. The nuclei stain deep violet like

those of microfilariae and the cytoplasm stains a very light violet.

Cotton wool and blotting fibres, if any, stand out as dirty, blackish, irregular threads.

The most important points in identification of species are to ascertain: (1) the source from which the microfilariae were obtained (blood, or tissue); (2) the presence or absence of a sheath; and (3) the position of the nuclei in relation to the tail end of the microfilariae, i.e., whether they extend right up to tip or not.

* * *

By this method, two medical officers at the Pasteur Institute of the Union of Burma, working about four hours a day, have been able to examine 6000 slides in 30 working days, which works out to an average of 200 slides in four hours per day. The time taken from the receipt of the slide to completion of the examination works out to a little over two minutes per slide.

Out of the 6000 blood smears examined, 235 were found positive for microfilariae, 3.93% of the total specimens received. All positive slides showed presence of *Microfilaria bancrofti* only.

Human Infection with Filariae of Non-Human Hosts *

by ERNEST CARROLL FAUST, *Emeritus Professor of Parasitology, Tulane University School of Medicine, New Orleans, La., USA*

As in the case of human exposure to hookworms adapted to non-human hosts and to the dog ascarid *Toxocara canis* or *Gnathostoma* spp., so in human exposure to non-human filariae, these nematodes are rarely able to develop to maturity in man but in an advanced larval or adolescent stage are trapped in subcutaneous or visceral tissues, in which they may migrate but eventually die, at times causing considerable tissue reaction.

The essential life-history difference between the non-human hookworms and *Toxocara canis* on the one hand and the filariae on the other is that the former mature to the infective stage on the ground, whereas the filariae have a required period of development from microfilaria to infective larval stage in the body of a blood- or tissue-sucking insect, which is the vector to the vertebrate host. The filarial larvae in the inoculum of the proboscis of the insect are introduced into the skin of the definitive host when the insect takes its next meal and usually seek out cutaneous lymphatic channels in which to migrate.

Man as definitive host for filariae primarily or exclusively adapted to other vertebrates is probably on most occasions entirely refractory to growth of larvae to the adult stage when introduced by the insect; otherwise the number of clinical reports of recovery of these nematodes from the tissues would be far in excess of the cases on record. For the most part published data on authentic diagnoses describe subcutaneous filarial infection with immature females, with or without a history of migration.[a-d] Rarely, the worms have been demonstrated in granulomatous lesions in the viscera.[e] Finally there are three authentic cases of adult filariae in blood vessels or in the heart.[f, g, h]

The original designation of the immature filaria in man was *Filaria conjunctivae*, because of the anatomical site where the worm was discovered.[i] Then, on the basis of common morphological characters of a majority of these immature filariae they were assembled in the genus *Dirofilaria* and referred to as *Dirofilaria conjunctivae*.[j] Certainly a majority of the extracted worms which have been

* Note submitted to the WHO Expert Committee on Filariasis, July 1961.

[a] Faust, E. C. (1957) *Z. Tropenmed. Parasit.*, **8**, 59.

[b] Sams, W. N. & Beck, V. (1959) *A.M.A. Arch. Dermat.*, **79**, 294.

[c] Lenth, V. T. J. (1960) *J. Amer. med. Ass.*, **173**, 1124.

[d] Jung, R. C. & Harris, F. H. (1960) *A.M.A. Arch. Path.*, **69**, 371.

[e] Dashiell, G. F. (1961) *Amer. J. trop. Med. Hyg.*, **10**, 37.

[f] Blanchard, E. (1895) *Les vers du sang.* In: Laveran, A. & Blanchard, R., *Les hématozoaires de l'homme et des animaux*, Partie 2, Paris.

[g] Faust, E. C., Thomas, E. P. & Jones, J. (1941) *J. Parasit.*, **27**, 115.

[h] Teixeira de Freitas, J. F. & Mayall, R. (1953) *Rev. bras. Med.*, **10**, 463.

[i] Addario, C. (1885) *Ann. Ottal.*, **14**, 135.

[j] Desportes, C. (1939-40) *Ann. Parasit. hum. comp.*, **17**, 380, 515.