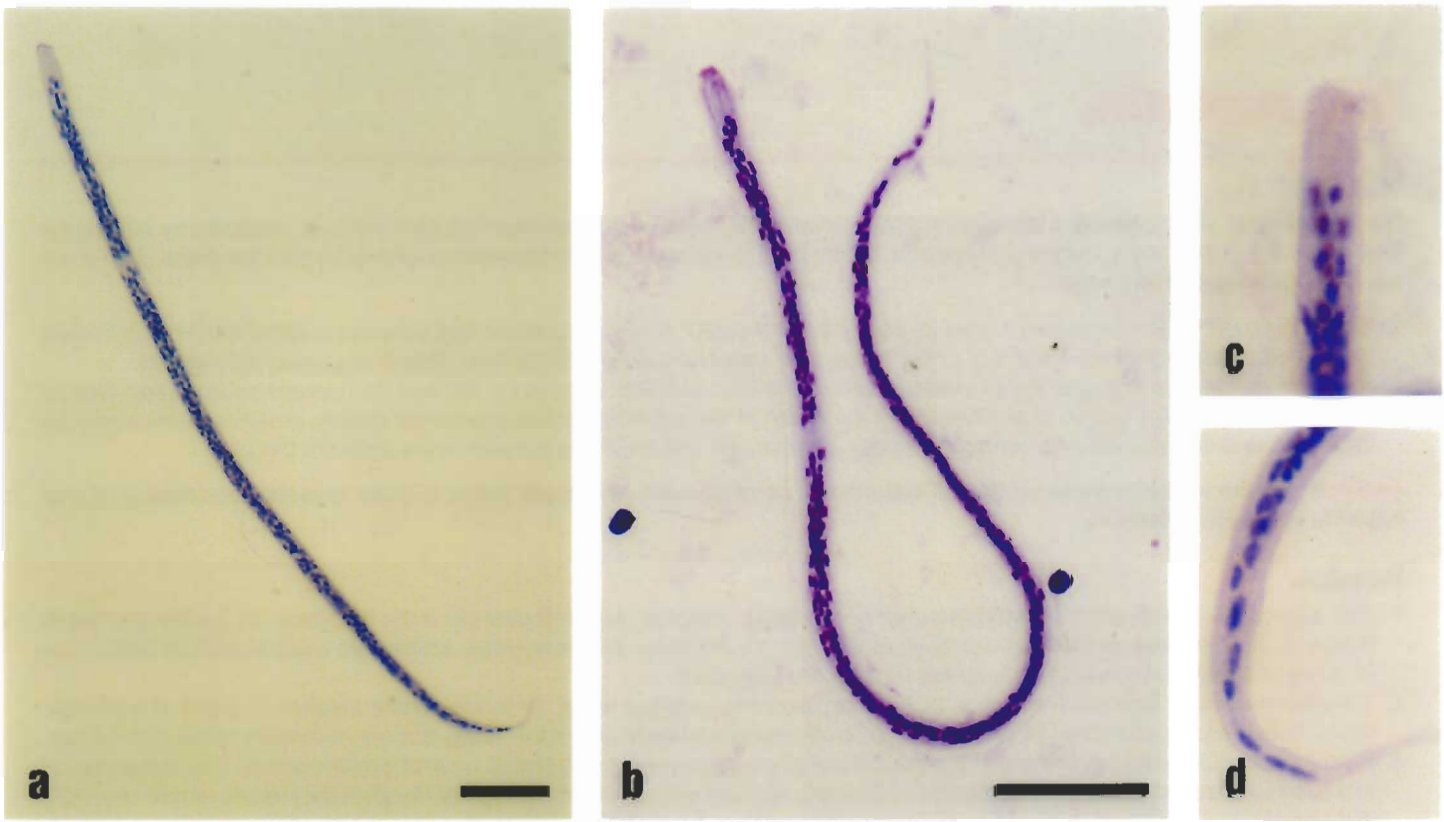
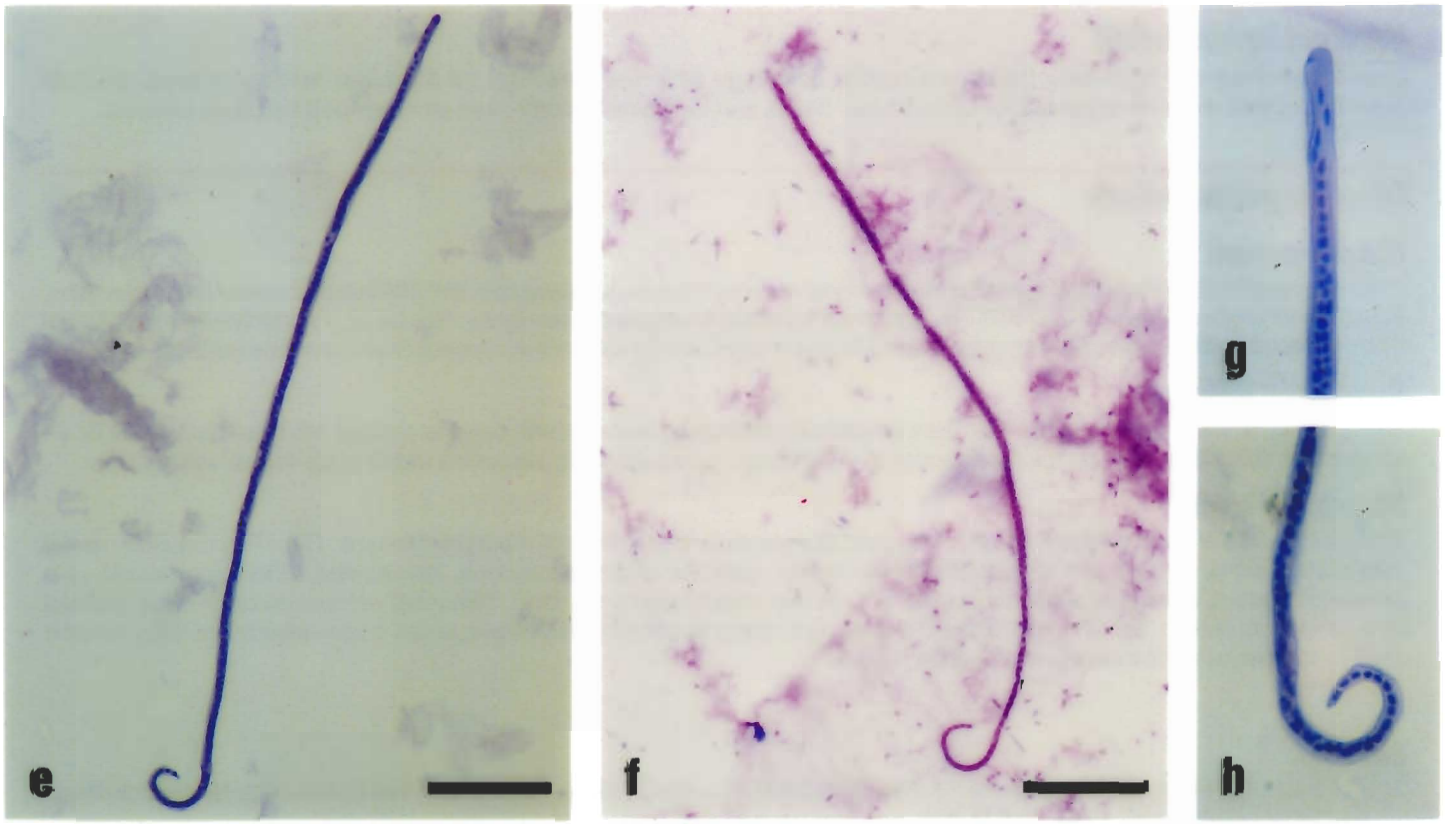
Note: All measuring bars = 30 µm



Onchocerca volvulus microfilariae from skin snips in haematoxylin (a, c, d) and Giemsa stains (b). This microfilaria is large and has no sheath, a long head space (c) and, typically, a flexed tail (d). The column of body nuclei is only moderately compact. The most important diagnostic feature is that ***O. volvulus*** is found in the skin and only rarely in the blood.



Mansonella streptocerca microfilariae from skin snips in haematoxylin (e,g,h) and Giemsa stains (f). ***M. streptocerca*** is readily distinguished from ***O. volvulus*** by its very slender shape and "crooked" tail (e, f, h). Note that the column of nuclei starts in the anterior extremity as a single row of 10–12 (or more) nuclei (g) and extends to the end of the tail (e,f,h).

Tissue examination

Skin snips

The microfilariae of *Onchocerca volvulus* and *Mansonella streptocerca* that reside in the skin are best detected by looking for their presence in skin snips. Intensity of infection is reflected in the numbers of microfilariae emerging from the snips. Skin snips are obtained in one of two ways:

1. Skin snips can be standardized in both size and weight through the use of sclerocorneal punches of either the Holth or Walser type. These instruments take snips of uniform diameter (approximately 2.3–2.5 mm). This is the preferred method.
2. A needle can be used to raise the skin and a razor blade to cut off the raised area; forceps and curved scissors can also be used. Such skin snips vary in size, shape, and the depth of the cut. When snips are cut too deeply, small capillaries may be lacerated and the snip may be contaminated by microfilariae that might be present in the patient's blood.

Caution: It is of the utmost importance that all instruments used for each patient are sterile in order to avoid transmission of viral hepatitis B and HIV infections.

Procedure

1. Skin snips should be taken from selected sites on the body. In Africa, the preferred site is the iliac crest; in Central and South America, the iliac crest or the scapular area; and in Yemen, the lower calf. In surveys, ideally two snips should be taken from all three of these sites on each side of the body of the individual.
2. Transfer skin snips from each site to a drop of normal saline, distilled water, or tissue culture medium in a well of a 96-well, flat-bottom, tissue culture tray; or place snips on a microscope slide in one of the fluids. It is not necessary to tease the snips.
3. Examine after 30 minutes to 3 hours. (Tissue culture trays may be covered with plastic wrap or similar material, and slides placed in a covered Petri dish, to retard evaporation.) If the wells or slides are negative for microfilariae, allow the snips to remain overnight in an incubator at 37 °C or at room temperature and examine them again. If microfilariae are present they will be apparent in the fluid. The morphological features of *O. volvulus* and *M. streptocerca* are so distinct that differentiation of microfilariae is quite easy.
4. To make permanent preparations of microfilariae, remove the skin snips, transfer the fluid to a slide if necessary, and allow the fluid to evaporate. When the slide is thoroughly dry, fix the microfilariae in methanol and stain with Giemsa or haematoxylin stain.

Urine and hydrocele fluid

Pour 15 ml of urine or hydrocele fluid into a conical centrifuge tube and centrifuge for 5 minutes at 350*g* or more. Pour off supernatant and examine sediment for microfilariae. Slides can be stained and/or fixed as described for blood samples.

Other diagnostic methods

Microhaematocrit

Originally used for diagnosis of trypanosomiasis, the microhaematocrit procedure is equally useful for the diagnosis of filarial infections, especially when the numbers of microfilariae present are too small for efficient detection by thick blood films. Only a small amount of blood is needed, so that one or two drops obtained by finger-prick can be used when venepuncture cannot be performed (*2*).

Quantitative buffy coat

The utilization of the quantitative buffy coat tube (microhaematocrit tube recoated with acridine orange) has been reported to be an acceptable rapid diagnostic test for the detection of microfilariae, with a sensitivity equivalent to that of the thick blood film (*3*).

Microfilariae counts

Accurate counts of microfilariae can be made from stained thick blood films of measured volume. Counting requires careful systematic scanning of the blood film with the low-power objective of the microscope. The stained slides can be kept as a permanent record. Equally reliable counts can be made from membrane filters which, if mounted with a coverslip, can be retained as a permanent record. Some investigators prefer using a counting-chamber technique, which is very reliable but does not lend itself to species identification or permanence (*4*).

References

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