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RESEARCH COMMUNICATION

A COMPARISON OF FIXATIVES SUITABLE FOR SCANNING ELECTRON MICROSCOPY OF *HABRONEMA* SPP.

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ABSTRACT

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Members of the genus *Habronema* (Nematoda: Habronematidae) were preserved in 4 fixatives for examination with scanning electron microscopy (SEM). Tegumental features of the anterior and posterior extremities of these specimens were compared to evaluate the effect of the fixatives: modified Flemming's solution, glutaraldehyde (GA), Karnovsky's fixative and 70 % ethanol. Fixatives were assessed on the appearance of the tegument, evidence of any wrinkling, shrinkage or swelling and the degree of extension in the male tail. Seventy per cent ethanol gave the most satisfactory results.

INTRODUCTION

In the preparation of nematodes for scanning electron microscopy (SEM), artefacts such as shrinkage and distortion should be reduced to a minimum. The choice of a fixative is therefore critical. Fixatives considered suitable for the SEM of nematodes include paraformaldehyde, formal-acetic-acid (FAA) (Allison, Ubelaker, Webster & Riddle, 1972), formalin (Green, Stone, Turner & Clark, 1975), alcohol formaldehyde mixtures and glutaraldehyde (Lichtenfels, 1982). Good preservation implies that upon examination of the nematodes with SEM they resemble the *in vivo* condition.

It appears that many of the properties of certain agents used as SEM fixatives were based on experience obtained from histological observations and techniques used in the collection, fixation and long-term storage of helminth parasites for light microscopy (LM) and that these were applied to electron microscopy. This may well be valid for transmission electron microscopy (TEM), where the ultrastructural preservation of cellular contents are critical, whereas with SEM, especially for organisms with a rigid tegument, other criteria may apply when only the appearance of the surface morphology is of interest.

In this study members of the genus *Habronema* which parasitize the stomachs of equids (Lichtenfels, 1975; Scialdo-Krecek, 1984), were selected to compare the effect of 4 fixatives on the tegument of males.

MATERIALS AND METHODS

Live specimens collected from the stomachs of equids were immediately placed in normal saline (0,85 % NaCl) maintained for 10–15 min at 37 °C. The specimens were transferred to each of the 4 fixatives and processed further at room temperature. The fixatives were:

(a) A modified Flemming's solution, as described by Van Niekerk, Els & Krecek (1987),

(b) 2,5 % (v/v) glutaraldehyde (GA), pH 7,2, in 0,1 M sodium cacodylate buffer with an osmolality of 560 mOsmols,

(c) 70 % (v/v) ethanol at approximately 20 °C and

(d) Karnovsky's fixative containing 2 % (v/v) paraformaldehyde and 2 % (v/v) glutaraldehyde in 0,1 M sodium cacodylate buffer at pH 7,4 (Karnovsky, 1965) (osmolality about 2 000 mOsmols).

The specimens remained in the respective fixatives for 5 days before further processing. Subsequent to this initial fixation, specimens in the GA and Karnovsky's fixative were rinsed twice 0,1 M sodium cacodylate buffer for 15 min each, post-fixed with 1 % OsO_4 in 0,15 M sodium cacodylate buffer for 2 h and rinsed in 0,15 M buffer.

All the specimens were simultaneously dehydrated in 70 %, 80 %, 90 % and 100 % (twice) ethanol for 1 h each, critically point-dried with CO_2, mounted on stubs, carbon-coated in a vacuum evaporator and finally gold-palladium sputter coated. Specimens were examined and photographed with a JEOL JSM-35C scanning electron microscope operating between 8 and 12 kV.

Assessment of the effects of the fixatives was based on the appearance of the nematode tegument with particular regard to any collapse, wrinkling, shrinkage or swelling. Additionally, the extension of the male tail and visibility of the papillae were noted.

RESULTS

Scanning electron micrographs of the pseudolabia and papillae of the head region of *Habronema* spp. (Fig. 1A, B, C & D) and the pre- and postanal papillae of the ventral surface of the male tail (Fig. 2A, B, C & D) are compared. Important features for species identification within this genus include the number and position of the papillae on the ventral caudal surface of the male's posterior extremity (Lichtenfels, 1975; Yamaguti, 1961).

DISCUSSION

An important consideration in this study was the extension of the caudal ventral surface in the male. The fixative of choice in this regard was 70 % ethanol (Fig. 1C). Flemming's solution also extended the tail in a straight position, though some collapse was evident (Fig. 1A). The 2 remaining fixatives, however, caused curling of the male extremity (Fig. 1B & D) and were therefore inadequate for closer examination of the papillae.

Helminths prepared for SEM are prone to some distortion, shrinkage or swelling. Although some initial cleaning of the nematodes is preferable, e.g. rinsing in warm saline to remove mucous and other debris, this could influence the effect of the subsequent fixation and the appearance of the tegument. An optimal collecting and cleaning system requires further investigation. In

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FIG. 1 Scanning electron micrographs of *en face* view of *Habronema* spp. after fixation in (A) modified Flemming's solution, (B) glutaraldehyde, (C) ethanol and (D) Karnovsky's fixative. Note lateral papilla (lp), submedian papilla (smp) and pseudolabia (p)

general, when material is freely available, it is possible to screen many specimens and select those with minimal defects. Problems arise, though, when only a restricted amount of material (i.e. 1 or 2 specimens of a new species) is available for SEM. In such a case it is essential to make the right choice of fixative and to use an acceptable procedure of processing to reduce artefacts, such as adequate dehydration times and careful critical point-drying techniques.

Several fixatives have been proposed for the preparation of parasitic nematodes for SEM. Lichtenfels (1982) use GA and OsO_4 for small nematodes, while Fagerholm & Lövdahl (1982) reported that initial fixation of live specimens in cold GA resulted in considerable shrinkage. In the present study, the fixative best suited for the preparation of *Habronema* for SEM and for species identification is unequivocally 70 % alcohol, followed by Flemming's solution. In addition, ethanol, particularly, has the advantage of being a standard laboratory reagent. The use of 70 % ethanol as an initial fixative is contrary to Horobin (1982) and Berland (1982), but the differences may be attributable to the different groups of nematodes or tissue which they studied. It should be noted that the extended male tail does not represent the natural condition, but this orientation is preferable for morphological studies.

Two fixatives in this study were suitable for the preservation of *Habronema* spp. for SEM. If the tegument of the male is to be studied, the first choice, which we recommend, is 70 % ethanol, and the second, a modified Flemming's solution.

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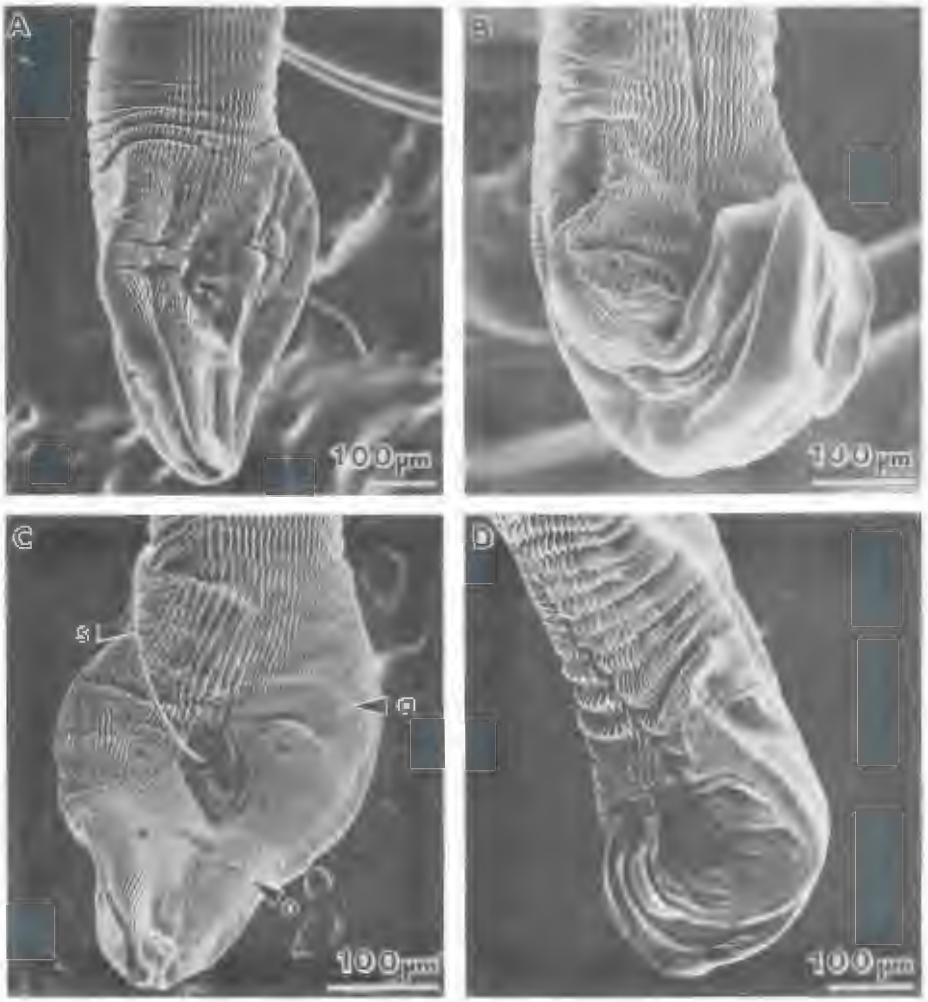

FIG. 2 Scanning electron micrographs of the male posterior extremities of *Habronema* spp. after fixation in (A) modified Flemming's solution, (B) glutaraldehyde, (C) ethanol and (D) Karnovsky's fixative. Note spicule (s), preanal papilla (a) and postanal papilla (o).

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