Research Note

Diagnosis of *Strongyloides stercoralis* by acid-fast staining

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Abstract

Faecal smears of patients and *Strongyloides stercoralis* obtained from dogs were acid-fast stained with Kinyoun and Auramine O staining procedures. Both larval (rhabditiform and filariform) and adult parasites were effectively stained by these methods. Acid-fast staining can serve as a useful procedure for diagnosing strongyloidiasis.

Strongyloides stercoralis is an intestinal nematode with a worldwide distribution, and is indigenous to south-eastern regions of the United States, including Kentucky, Tennessee, Florida and southern Appalachia (Liu & Weller, 1993). In an immune competent host, infection of *S. stercoralis* typically elicits only mild gastrointestinal symptoms. Conversely, in an immune compromised patient, parasites reproduce by autoinfection leading to a hyperinfection syndrome, producing pulmonary infection that may present as asthma, chronic bronchitis, haemoptysis, eosinophilia and pulmonary infiltrates (Woodring *et al*., 1996).

The diagnosis of strongyloidiasis is usually accomplished by the detection of larvae in the stool via Lugol's iodine stain (Liu & Weller, 1993). *Strongyloides stercoralis* may also be identified in wet preparations of sputum, bronchoalveolar lavages, bronchial washings and brushings, lung biopsies, or examination of pleural fluids; via Gram's or Papanicolaou stain (Smith *et al*., 1985). An ELISA for detecting the serum IgG against a crude extract of *S. stercoralis* is available, but shows cross-reactivity with several helminths (Grove, 1996). In the present study, we report the usefulness of acid-fast staining of sputum and other respiratory material as well as faecal smears with Auramine O and Kinyoun, for the detection of larval and adult stages of *S. stercoralis*.

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Stool samples containing rhabditiform larvae of *S. stercoralis* were obtained from patients, admitted to the James H. Quillen Veteran Affairs Medical Center, Johnson City, Tennessee, and processed using the formalin–ethyl acetate sedimentation procedure (Garcia & Bruckner, 1993). A thin smear of concentrated sediment was prepared on glass slides, air dried and heat fixed. *Strongyloides stercoralis* filariform larvae and adults were harvested from faecal charcoal cultures of a dog infected with the parasite in Dr Gerhard Schad's laboratory (University of Pennsylvania) as described (Schad *et al*., 1984). Larvae and adults of *S. stercoralis* were re-suspended in 0.2% bovine serum albumin (Sigma Chemical Co., Missouri) and placed on slides previously treated with rabbit coagulase plasma (Remel, Inc., Kansas), to help obtain adhesion of parasites to the slide. The slides were air dried and heat fixed. Slides were then stained using Auramine O fluorochrome (Becton Dickinson Microbiology Systems, New Jersey) and Kinyoun (Difco Laboratories, Michigan) acid-fast stains, as described (Garcia & Bruckner, 1993). Air-dried unstained slides were also used to determine autofluorescence. Slides were examined using a Zeiss ultraviolet microscope, equipped with BP 450-490 exciter and LP 520 barrier filters, respectively.

Microscopic examination of human faecal smears, prepared using Auramine O, showed characteristic orange-yellow fluorescence of *S. stercoralis* rhabditiform larvae under ultraviolet light (fig. 1A). Unstained larvae exhibited minimal autofluorescence (data not shown).

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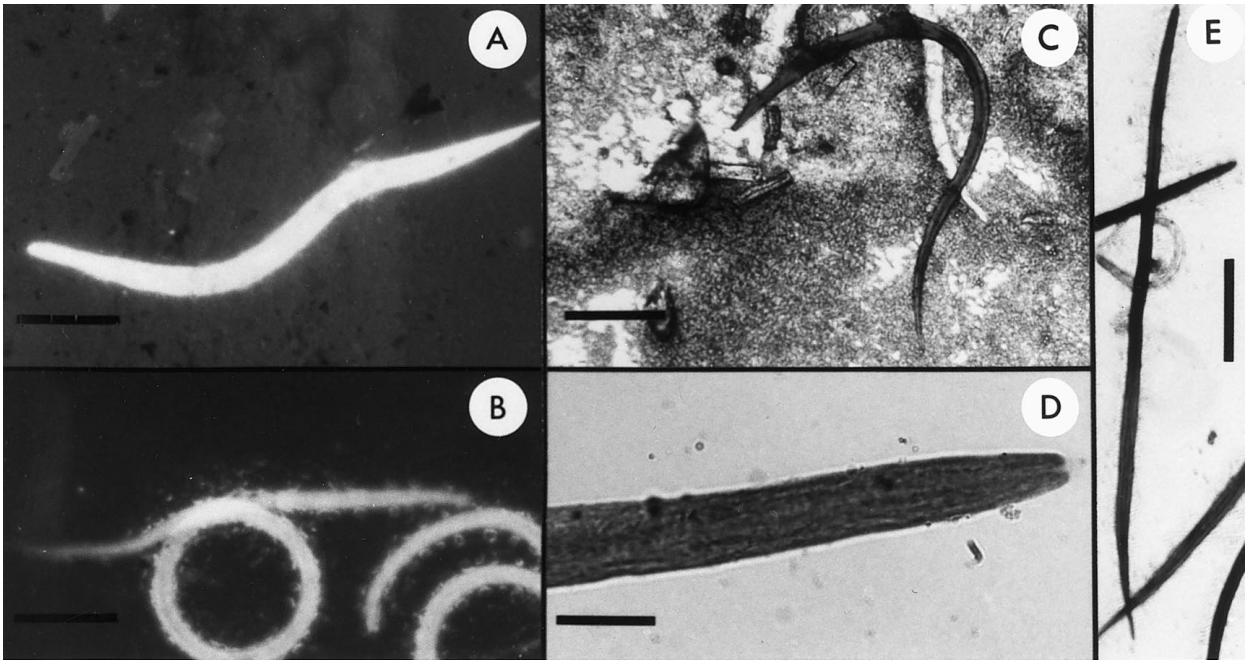

Fig. 1. Auramine O and Kinyoun acid-fast staining of different stages of *Strongyloides stercoralis*. (A) Human faecal smear stained with Auramine O, showing orange-yellow fluorescence of rhabditiform larva under ultraviolet light; scale bar represents 50 μm. (B) Adult female parasite showing orange-yellow fluorescence after Auramine O staining; scale bar represents 250 μm. (C) Human faecal smear showing a rhabditiform larva, stained in characteristic pink colour with Kinyoun acid-fast stain; scale bar represents 50 μm. (D) Anterior portion of a rhabditiform larva, stained with Kinyoun acid-fast stain, showing staining of the oesophagus; scale bar represents 25 μm. (E) Staining of filariform larva with Kinyoun acid-fast stain; scale bar represents 100 μm.

Adult females of *S. stercoralis*, obtained from cultures of dog faeces, also showed similar fluorescence with Auramine O staining (fig. 1B). Both rhabditiform larvae from stool (fig. 1A and C) and cultured filariform larvae (fig. 1E) were also stained distinctly with the Kinyoun acid-fast stain.

Although acid-fast staining using Auramine O and Kinyoun methods have been extensively used in the detection of *Mycobacteria*, several non-mycobacterial organisms also show varying degrees of acid-fastness with these stains, including *Rhodococcus*, *Nocardia*, *Legionella* and oocysts of *Cryptosporidium*, *Isospora* and spores of other microsporidia (Murray *et al*., 1995). However, this is the first report showing acid-fastness of a nematode parasite. In summary, routine acid-fast staining of sputum and other respiratory tract secretions, such as bronchial washings, may also serve as a useful screening procedure for the detection of *S. stercoralis*. Also, routine acid-fast staining of stools for *Cryptosporidium* and *Isospora* may also reveal *S. stercoralis* acid-fast larvae, if present.

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