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IDENTIFICATION OF INFECTIVE DICTYOCAULUS VIVIPARUS LARVAE ISOLATED FROM HERBAGE BY THE BILE-AGAR TECHNIQUE*

By

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ISKANDER, A. R. and R. J. JØRGENSEN: *Identification of infective Dictyocaulus viviparus larvae isolated from herbage by the bile-agar technique.* Acta vet. scand. 1980, *21*, 330—335. — A morphological description was given of the infective stage of the cattle lungworm Dictyocaulus viviparus. The larvae were stained with iodine and examined under an ordinary microscope after isolation with the bile-agar technique used for processing herbage samples. Under these conditions the internal structures appeared of limited value to the identification. The larvae were characterized by their measurements as well as by their shape and profile. A comparison between larvae isolated from the field and larvae harvested from faecal cultures revealed that field larvae were smaller and more uniform in size. Of the reported characteristics the typical profile, the comparatively small size (range 305—400 μm), and the retaining of iodine stain were found most useful in routine recognition of D. viviparus infective larvae in isolates from herbage samples containing free living nematode species as well as infective stages of parasitic species.

Dictyocaulus viviparus; infective larva; identification; bile-agar technique.

The isolation and identification of infective Dictyocaulus viviparus larvae form an important part of studies of the epidemiology of bovine parasitic bronchitis. In such studies the recognition of a single infective larva may be decisive.

Several papers have been published on the morphology of the infective larvae of the common nematode parasites of sheep (*Morgan* 1930, *Mönnig* 1931, *Dikmans & Andrews* 1933). *Keith* (1953), *Corticelli & Lai* (1964), *Henriksen* (1972) and *Borgsteede*

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& Hendriks (1974) reported on the identification of L_3 of the common bovine gastrointestinal strongyles. Although a morphological description of D. viviparus pre-parasitic stages cultured in vitro from eggs was given by *Daubney* (1920), there appears to be no reports describing infective D. viviparus larvae isolated from pasture samples. The purpose of the present investigations was therefore to describe such infective larvae as they appear under the microscope.

The described larvae were all processed by the bile-agar technique (*Jørgensen* 1975), since this technique has proved valuable in field studies carried out in Denmark (*Jørgensen* 1980) and elsewhere (*Duncan et al.* 1979).

MATERIAL AND METHODS

Pasture samples were collected from an experimental field contaminated by grazing calves suffering from patent verminous bronchitis. The samples were picked by hand from grass growing 5—10 cm from the faecal pats, placed in plastic bags and afterwards washed in an apparatus similar to a cement mixer. Sediments from the washings were collected and processed according to the technique described by *Jørgensen* (1975) whereby the sediment is embedded in bile-agar and incubated overnight. Only viable larvae are recovered, stained with iodine and the surroundings decolourized with thiosulphate. Fourty larvae isolated in this way were examined. By way of comparison, 40 larvae obtained from the laboratories of Allen & Hanburys, England, were also examined. These larvae had been cultured to the infective stage on faeces in the laboratory before being harvested and stored in water. Measurements of the larvae were obtained by the use of a standard microscopic scale. The silhouettes were drawn from paper prints of microphotos.

RESULTS

Visible internal structures of the isolated larvae were found to be few and inconsistent. The presence and position of the excretory pore and of a genital primordium was observed in some but not in all larvae. No other internal structures were observed except granules which were either absent or were variable in numbers. In less than 1 % of the larvae the internal parts were found to be partly loosened and contracted from the cuticle.

This artifact resulted in a "boiled" appearance. All larvae retained the iodine stain for at least 30 min. The intensity of their colour was either darker or it was comparable to that of trichostrongyle larvae present in the same pasture samples.

The profile of the infective stage of D. viviparus in shown in Fig. 1. As can be seen, the larvae are found to be uncoiled to

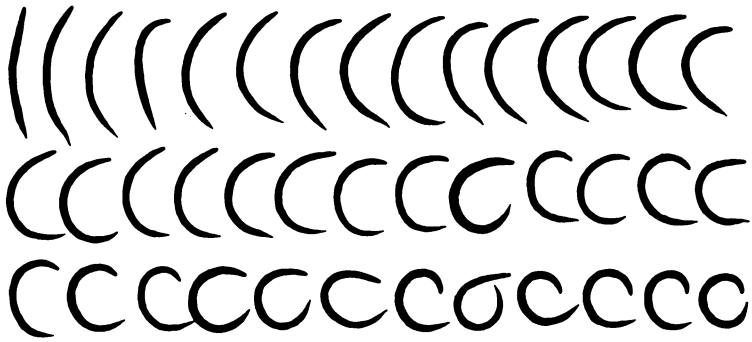

Figure 1. Profiles of 40 infective Dictyocaulus viviparus larvae isolated from pasture samples. The larvae are drawn as silhouettes from microphotos.

various degrees, ranging from a c-shape to an almost straight appearance. Table 1 shows the measurements of specimens isolated from pasture samples as well as of specimens originating from faecal cultures. The frequency distributions of these measurements are shown in Fig. 2.

Table 1. Measurements of 40 infective Dictyocaulus viviparus larvae isolated from pasture (pasture larvae) and of 40 D. viviparus larvae harvested from faecal cultures (culture larvae).

	Total length (μm)	Maximum width (μm)	Ratio: Length/width	Distance from tail of larvae to tail of sheath
Pasture larvae				
range	305—400	15—19	18—26	3—10
mean	343	16	21.5	6
s	22.5	1.0	1.5	1.9
Culture larvae				
range	350—450	16—27	15—25	3—15
mean	390	20	20.0	6
s	25.5	2.9	2.7	2.4

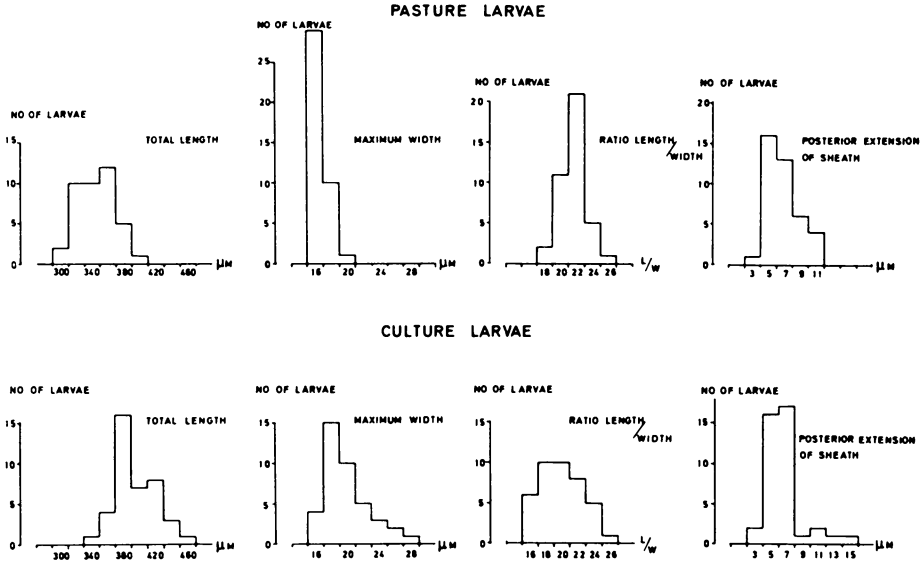

Figure 2. Frequency distribution of total length, maximum width, ratio length/width, as well as extension of sheath beyond end of larvae. Each histogram includes measurements of 40 larvae.

DISCUSSION

Differentiation between infective lungworm and trichostrongyle larvae on one hand and the bulk of the free-living nematode species present in herbage samples on the other is greatly facilitated by the slow versus rapid diffusion rate of iodine through the cuticle of the two groups of nematodes. The differentiation between D. viviparus infective larvae and the infective larvae of trichostrongyles may be based on the results of the present investigations together with the previously mentioned reports on bovine trichostrongyle larvae.

The differences found in the present investigations between the two populations of lungworm larvae are interesting. A possible explanation to the more uniform appearance and the smaller size of the field larvae is that this population is more selected through their natural translation from the faecal pats to the surrounding herbage. The characteristic variations in shape as shown in Fig. 1 together with the uniform size of the iodine-stained D. viviparus larvae have proved most important in the recognition of such larvae during screening of pasture isolates.

Larvae with detached and contracted internal structures are likely to have died during incubation. However, such larvae are equally well recognized since the low diffusion rate of iodine through the cuticle is retained and since the cuticle is unaltered in size and shape.

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SAMMENDRAG

Identifikation af infektive Dictyocaulus viviparus larver isoleret fra græsprøver ved hjælp af galdeagar-teknikken.

Der gives en morfologisk beskrivelse af det infektive stadium af kvægets lungeorm Dictyocaulus viviparus. Larverne blev farvet med jod, hvorefter de blev underkastet mikroskopisk undersøgelse efter at være isoleret med galdeagar-teknikken til undersøgelse af græsprøver. Larvernes indre struktur viste sig af begrænset værdi til identifikation under disse forhold. Der blev givet en karakteristik af larverne ved hjælp af deres mål, form og profil. En sammenligning mellem larver isoleret fra henholdsvis mark og fæceskulturer viste, at larver fra græsmarker var mindre og mere ensartede i størrelse. Af de nævnte karakteristika viste den typiske profil, deres relativt lille størrelse (305—400 μm), samt deres bibeholdelse af jodfarven sig mest anvendelige til screening for infektive D. viviparus larver af isolater fra græsprøver indeholdende både fritlevende nematodearter og infektive stadier af parasitære arter.

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