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## RESEARCH NOTE

# A RAPID METHOD FOR DIFFERENTIATING BETWEEN THE INFECTIVE LARVAE OF *OESOPHAGOSTOMUM COLUMBIANUM* AND *CHABERTIA OVINA*

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ABSTRACT

VAN WYK, J. A., 1977. A rapid method for differentiating between the infective larvae of *Oesophagostomum columbianum* and *Chabertia ovina*. *Onderstepoort Journal of Veterinary Research*, 44 (3), 197–200 (1977).

When freshly-collected, exsheathed infective larvae of *Oesophagostomum columbianum* and *Chabertia ovina* were frozen in liquid nitrogen and subsequently thawed, conspicuous vesicles appeared in the intestinal cells of the larvae. The mean number of vesicles, which differed in size according to the species was 20 for *O. columbianum* and 32 for *C. ovina*.

*Résumé*

*UNE MÉTHODE RAPIDE POUR DISTINGUER LES LARVES INFECTIEUSES DE* OESOPHAGOSTOMUM COLUMBIANUM *DE CELLES DE* CHABERTIA OVINA

*Lorsque des larves infectieuses, dépouillées de leur cuticule, de* Oesophagostomum columbianum *et* Chabertia ovina *sont recueillies, plongées immédiatement dans l'azote liquide et ensuite dégelées, des vésicules bien visibles apparaissent dans leurs cellules intestinales. On a vérifié que le nombre de ces vésicules, dont la grandeur varie suivant les espèces, était en moyenne de* 20 *pour* O. columbianum *et de* 32 *pour* C. ovina.

## INTRODUCTION

Differentiation of the infective larvae (L3) of *Oesophagostomum* spp. and *Chabertia ovina* is difficult and time-consuming (Eckert, 1960), as, up to the present, it involved either the counting of intestinal cells (Mönnig, 1931; Dikmans & Andrews, 1933) or a comparison of the total lengths of the larvae (Eckert, 1960). Morgan (1930) considered it impossible to differentiate the larvae and the reports of other workers are confusing. Mönnig (1931) reported 20–24 intestinal cells for L3 *O. columbianum* and 24 cells for *C. ovina,* and Dikmans & Andrews (1933) list 16 cells for *O. columbianum* and 32 cells for *C. ovina.* Eckert (1960) was of the opinion that only measurements of total length of the larvae were valid for differentiation of *O. venulosum* and *C. ovina* as "the morphological characteristics not established by measurement are often variable and therefore only conditionally usable".

This paper describes a simple and rapid technique for differentiating freshly collected L3 of *O. columbianum* and *C. ovina.*

## MATERIALS AND METHODS

Pure strains of nematodes are maintained in the laboratory as described by Reinecke (1973) and Van Wyk, Gerber & Van Aardt (1977).

Freshly collected L3 of the 2 species were treated separately as follows: The larvae were exsheathed in 0,16% sodium hypochlorite solution, washed with physiological saline on filter paper, frozen rapidly in the gas phase of liquid nitrogen and later thawed in water at 55 °C (Campbell, Blair & Egerton, 1972; Rose, 1973; Van Wyk *et al.,* 1977).

The thawed larvae were killed with iodine solution and examined under a compound microscope.

In addition, a count was made of the intestinal cells in ensheathed and exsheathed L3, lightly stained with Lugol's iodine. Only larvae with well-defined intestinal cells were examined.

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## RESULTS

Vesicles, which were so conspicuous that they could be counted quickly at 80–100× magnification, developed in the intestinal cells of frozen and thawed L3 (Fig. 1–4); of the larvae examined the vesicles of *O. columbianum* were larger than those of *C. ovina.*

The treated *O. columbianum* L3 contained a mean of 19,6 vesicles, with a range of 16–21 (Table 1), while the mean number of intestinal cells of the untreated larvae was 18,2 (range 17–20). Twenty-nine of 56 treated L3 (52%) each contained 20 vesicles (Table 2).

At 1 000× magnification it appeared that each large vesicle was contained within a separate intestinal cell (Fig. 3).

TABLE 1 Numbers of vesicles or intestinal cells in the various groups of larvae

| Larval treatment | Vesicles or intestinal cells per larva | | | No. of larvae examined |
|---|---|---|---|---|
| | Range | Mean | S.D.* | |
| *O. columbianum* | | | | |
| Frozen (vesicles).... | 16–22 | 19,6 | 1,1 | 56 |
| Fresh (intestinal cells) | 17–20 | 18,2 | 0,8 | 20 |
| *C. ovina* | | | | |
| Frozen (vesicles).... | 28–36 | 32,0 | 1,4 | 50 |
| Fresh (intestinal cells) | 30–33 | 31,5 | 0,8 | 35 |

* S.D.=Standard deviation

In *C. ovina,* 32,0 vesicles were counted per treated L3 (range 28–36) and 31,5 intestinal cells (range 30–33) per untreated larva (Table 1). Thirty-two of 50 treated L3 (64%) each contained 32 vesicles (Table 3).

The vesicles were not always evenly distributed within the intestinal cells. In some instances, they appeared to be subdivided, more than 1 occurring in a single cell (e.g. craniad in Fig. 1), and sometimes

giving it a granular appearance (e.g. craniad in Fig. 4). In other instances, vesicles from more than 1 cell appeared to have fused.

Well-demarcated vesicles occurred only in larvae which were freshly collected at the time of treatment. In older larvae, both treated and untreated, the intestinal cells were poorly defined or indistinguishable.

TABLE 2 Frequency distribution of the number of vesicles in 56 frozen and thawed *O. columbianum* infective larvae

| No. of vesicles per larva | Frequency in 56 larvae | |
|---|---|---|
| | Distinct* | Partly indistinct† |
| 16 | 0 | 1 |
| 17 | 0 | 0 |
| 18 | 8 | 3 |
| 19 | 0 | 7 |
| 20 | 24 | 5 |
| 21 | 1 | 6 |
| 22 | 0 | 1 |

* Intestinal cell structure intact. No subdivision or fusion of vesicles

† A few vesicles were either fused, subdivided or difficult to distinguish, while the rest were distinct

TABLE 3 Frequency distribution of the vesicle counts in 50 frozen and thawed *C. ovina* infective larvae

| No. of vesicles per larva | Frequency in 50 larvae | |
|---|---|---|
| | Distinct* | Partly indistinct* |
| 28 | 0 | 1 |
| 29 | 0 | 1 |
| 30 | 2 | 4 |
| 31 | 0 | 0 |
| 32 | 26 | 6 |
| 33 | 0 | 5 |
| 34 | 1 | 2 |
| 35 | 0 | 1 |
| 36 | 0 | 1 |

* See Table 2 for key

## Discussion

The vesicles which developed with this method were so conspicuous that L3 *O. columbianum* and *C. ovina* could be distinguished at a glance even at low magnification. Furthermore, because of the large difference in the numbers of vesicles in the 2 species, it was usually necessary to count the vesicles in only a few larvae to differentiate the species from one another.

Unfortunately, not all the larvae develop conspicuous vesicles, and thus it is not possible (as it is with intestinal cell counts) to identify every larva in a batch. As a result it will probably not be possible to do an accurate differential count of a mixture of the 2 species.

It is not known why the mean number of intestinal cells in *O. columbianum* (18) did not agree exactly with the mean number of vesicles (20). Possibly some of the cells were poorly defined and were therefore overlooked.

The nature of the vesicles, which appeared to have fluid contents, and their effect on the larvae were not determined. It is of interest, however, that most of the larvae containing vesicles after thawing were very active and many of them were able to develop and give rise to patent infestations in sheep. Furthermore, the phenomenon was encountered in a variety of both bovine and ovine nematode species (Van Wyk *et al.*, 1977).

The mean number of intestinal cells in untreated L3 of *O. columbianum* (18, Table 1) falls between the numbers determined by Dikmans & Andrews (1933) and Mönnig (1931). The disparity in the number of cells found by different workers is probably due to the fact that the cell walls are not well defined.

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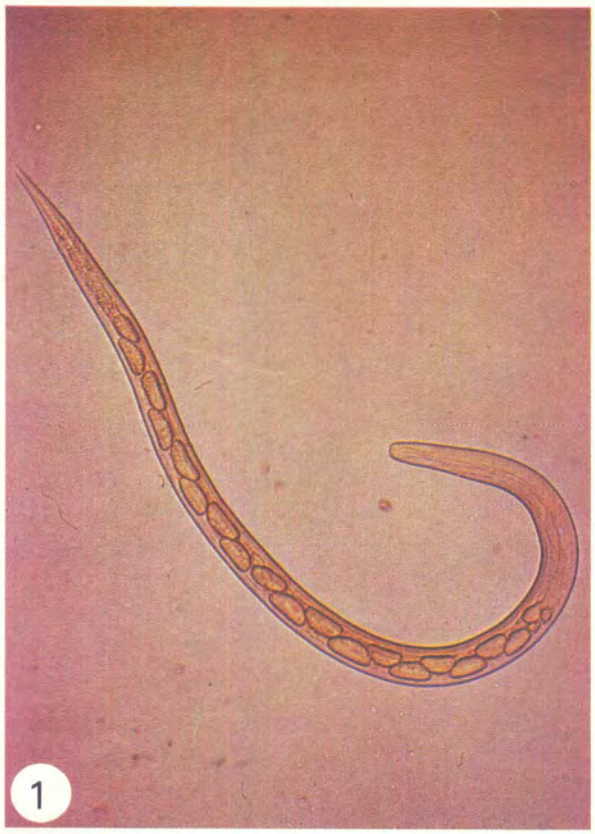


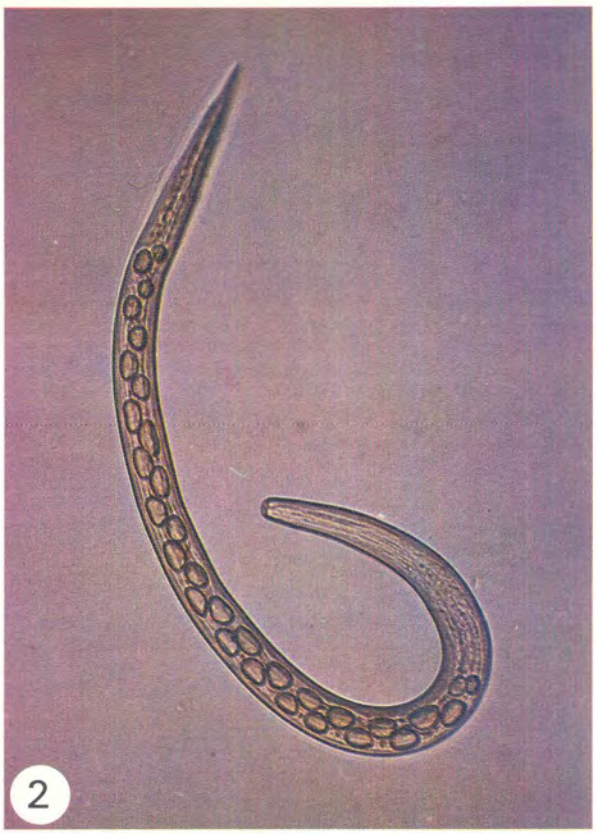


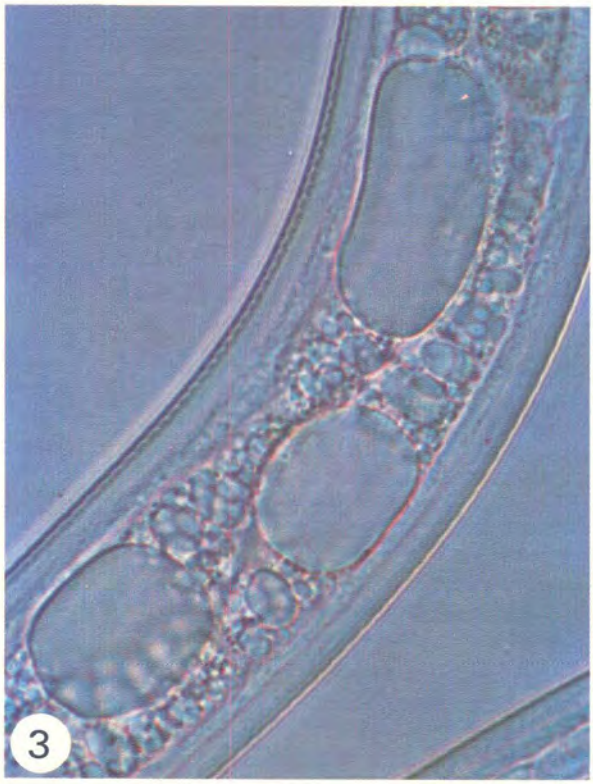


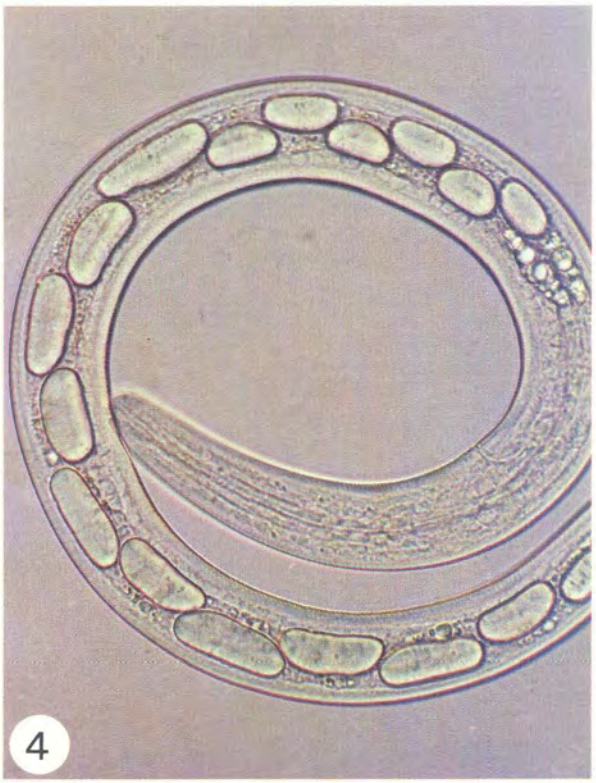


FIG. 1 *O. columbianum* infective larva with 21 vesicles (×275)
FIG. 2 *C. ovina* infective larva with 32 vesicles (×300)
FIG. 3 *O. columbianum* infective larva showing distribution of vesicles within the intestinal cells (×2 000)
FIG. 4 *O. columbianum* infective larva showing subdivision of the vesicles craniad (×750)