THE COLLECTION AND IDENTIFICATION OF FIRST STAGE LARVAE OF BOVINE GASTROINTESTINAL NEMATODES: MODIFICATION OF THE WHITLOCK TECHNIQUE (1959)[1]

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SINOPSE.- Rodrigues M.L.A. & Honer M.R. 1984. [**Obtenção e identificação de larvas de primeiro estágio (L_1) de nematódeos gastrintestinais de bovinos: Modificação da Técnica de Whitlock (1959).**] The collection and identification of first stage larvae of bovine gastrointestinal nematodes: Modification of the Whitlock technique (1959). *Pesquisa Veterinária Brasileira 5 (1): 1-3.* Inst. Biologia, Univ. Fed. Rural do Rio de Janeiro, Km 47, Seropédica, RJ 23460, Brazil.

Com o objetivo de diminuir o tempo entre amostragem e o diagnóstico das infecções por nematódeos gastrintestinais de bovinos, modificou-se a técnica de Whitlock (1959). A mudança básica consistiu da não utilização da bomba de pressão negativa, para a retirada do líquido, na obtenção de ovos e do filtro de Buchner para a filtração da suspensão e na substituição do frasco usado por Whitlock (1959) por outro adaptado por nós. Essas modificações tornaram o processo menos oneroso e facilitaram seu uso na rotina laboratorial, dando bons resultados devido à sua fácil execução. As larvas de primeiro estágio (L_1) podem ser identificadas em um período de tempo menor (24 horas) do que o utilizado para a larva infectante (7 dias). Culturas puras e as larvas, assim obtidas, foram consideradas como larvas de referência para comprovar a identificação das larvas de culturas mistas provenientes dos ovos coletados nas fezes. Trabalhou-se com *Haemonchus contortus* de ovinos, *Haemonchus placei, Cooperia* spp., *Trichostrongylus spp.* e *Oesophagostomum radiatum,* de bovinos, apresentando-se medidas e descrição das características morfológicas.

TERMOS DE INDEXAÇÃO: Larva de primeiro estágio (L_1), nematódeo gastrintestinal, técnica diagnóstico, bovino, ovino.

ABSTRACT.- Modifications were made of the Whitlock (1959) technique for the collection and identification of first stage larvae (L_1) of gastrintestinal nematodes of sheep, to diagnose similar infection in cattle.

Simplifying the original technique by ommiting the water pump, special collecting tube and filtration, L_1 could be collected within 24 hours. Identification is possible with the same degree of facility as for bovine L_3 or sheep L_1. Measurements and descriptions are given of the L_1 *Haemonchus contortus* (ovine), *H. placei, Trichostrongylus spp.,* and *Oesophagostomum radiatum* (bovine).

INDEX TERMS: First stage larvae (L_1), gastrointestinal nematodes, diagnostic technique, bovine, ovine.

INTRODUCTION

The routine diagnosis of nematode infections in cattle usually involves the egg-counting technique of Gordon & Whitlock (1939) and larval cultures, since the eggs cannot be readily identified in routine laboratory work.

Whitlock (1959) described a technique for the recovery and identification of the first stage larvae (L_1) of sheep nematodes. The advantage of this tecnique is the speed with which a diagnosis can be made, since L_1 can be obtained within 24 hours. No attempts have been made to apply this technique to bovine infections, nor has it been widely used in the diagnosis of infections in sheep, although recommended by Gordon (1967).

A study has been made of the original technique (Whitlock 1959), with a view to its simplification and adaptation to the rapid diagnosis of bovine nematode infections.

MATERIALS AND METHODS

We have retained the three phases described by Whitlock (1959), to assist in the description of the modifications and adaptations made in this laboratory:

a) *recovery of eggs from faeces.* In this phase of the technique the processing of faeces follows the description of Whitlock (1959), with two important exceptions: the negative pressure pump, used to empty the flatsided medicine bottles containing the layer of eggs on upper surface, has been eliminated, due to an observed loss of eggs and the difficulties of applications encountered in field conditions. The bottles are emptied from a nearly horizontal position manually, after removing the cap and taking care that the flow of sugar solution is relatively slow and

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does not disrupt the layer of eggs. In addition, the Buckner filter was not used because of a loss of eggs in the filter paper. After careful removal of the detritus in the lower part of the bottle with a weak jet of water the eggs are washed directly into the culture receptacle.

b) *culture of eggs to the first larval stage (L_1).* Whitlock (1959) used a specially constructed tube with a cottonwool plug and iodised insert to kill larvae at the same stage of development. This tube has been replaced by small Petri dishes ($\phi = 5$ cm), and later excavated slides in which the washed suspension of eggs is poured. After closing, the eggs are cultured in an incubator at 37°C when ± 80% develop within approximately 18 hours and 100% in 24 hours.

c) *Identification of the first stage larvae.* Adult females of *Haemonchus placei, Cooperia spp.* and *Oesophagostomum radiatum* were dissected and the eggs incubated as above to obtain L_1, which were then heat-fixed, stained and measured to serve as reference larvae for those obtained in mixed cattle faeces. In this case, faecal cultures were also made according to the technique of Roberts & O'Sullivan (1950) and the infective larvae obtained identified using the key of Keith (1953). The infective and reference larvae were then compared with L_1 cultures from animals with mixed infections, to confirm identification.

Staining of larvae

After heat-fixation of the L_1 in Petri-dishes or excavated slides, cottonblue lactophenol 0,01% was used to stain them, as an aid in studying their finer morphology, but it should be emphasized that this is not essential for their identification.

Measurements of larvae

One hundred larvae of *H. contortus* (ovine), *H. placei* (bovine), *Cooperia spp.* and *Oesophagostomum radiatum* (bovine) were measured with the aid of a scale projected by the camera lucida of a WILD M20 microscope in phase contrast, which was also used for drawing and photographing the larvae. Larvae of *Trichostrongylus spp.* are described but not listed in the table due to the paucity of material available.

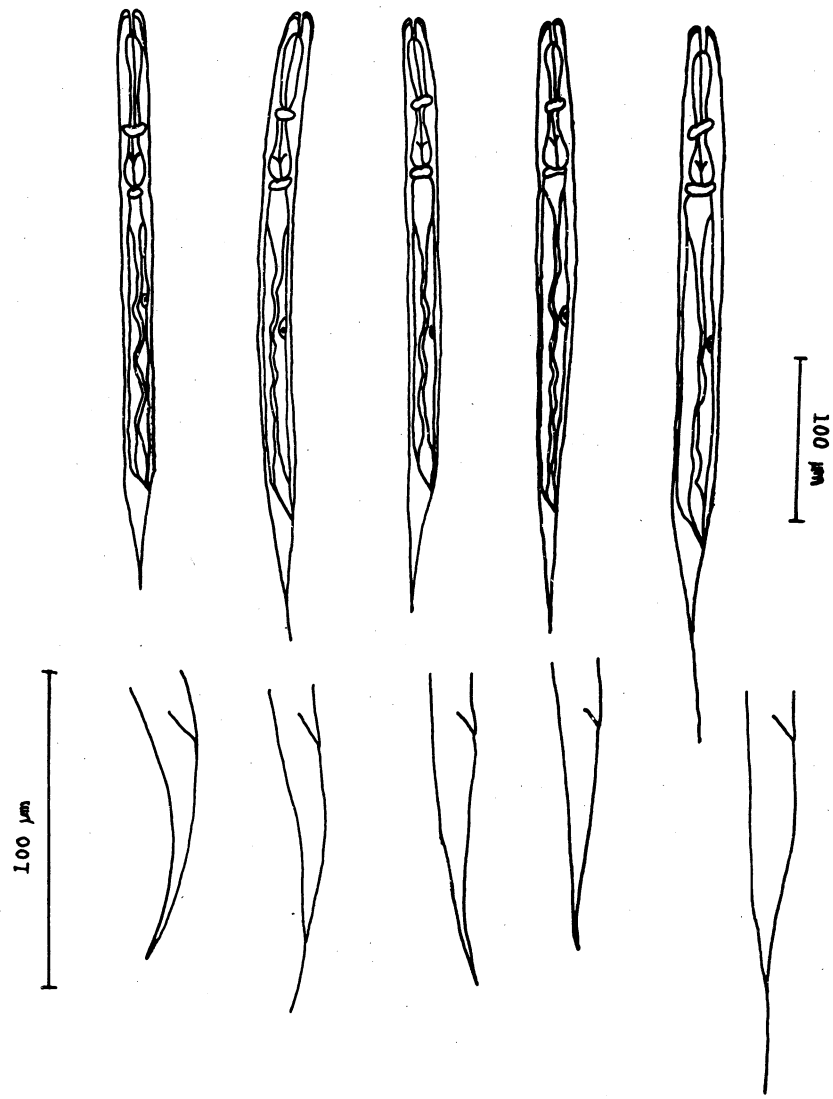

Fig. 1. *General morphology and enlarged tails of first stage larvae of (Left to right)* Trichostrongylus spp.; Haemonchus placei; H. contortus; Cooperia spp. *and* Oesophagostomum radiatum.

The measurements included here are: a) total length b) largest width and c) distance anus opening to the point of tail. Measurements are in μm with means ($\overline{X}$) and standard deviations (s) for each.

RESULTS

The results are presented in Figure 1. All the larvae examined showed basic characteristics which differ little from those found in sheep and described by Whitlock (1959). There is a small buccal cavity clearly open (in contrast to L_3), a rhabditiform oesophagus encircled by a conspicuous nerve-ring at level of the isthmus, the intestine is well-developed, with a sinuous lumen and poorly differentiated cells, the anus is oblique and ventral. In the majority of L_1 studied the genital primordium could be clearly seen. It is the form of the tail of the L_1 which is most characteristic, as it in the case of infective larvae and measuremets do not, in themselves, serve to differentiate the various genera. For *H. placei* the frequency distribution of measurements of the anus-tail is bimodal, as noted by Whitlock (1959), being slightly more accentuated in our data. It should be noted that Whitlock (1959) maintained *H. placei* in sheep.

The larvae of *Cooperia spp.* were chiefly *Cooperia punctata* (90% adults). We have not yet been able to differentiate between species, however, since pure strain donor animals were not available.

Morphology of the tail

The identification of the L_1 studied here is made principally on the basis of the morphology of the tail (Fig. 1).

a) *Trichostrongylus spp.* These larvae, although present in small numbers only, are readily identified by their short triangular and relatively short distance anus to tip of tail (60-65 μm).

b) *Haemonchus contortus.* The elongate tail filament is set out at a slight angle in all specimens studied. *H. contortus* L_1 are significantly shorter than those of *H. placei.* Mean total length 304.35 μm ± 24.65; width 19.82 μm ± 0,62; distance anus to tip of tail 60.25 μm ± 5.40.

c) *Haemonchus placei.* Similar morphologically to *H. contortus,* but larger. The finely elongate tip of the tail continues the long axis of the body. Total length 369.33 μm ± 24.65; width 19.32 μm ± 0.56, distance anus to tip of tail 74.86 μm ± 6.78.

d) *Cooperia spp.* The L_1 are characterized by very marked cuticular striations, by the presence of refringent bodies anteriorly and a conical tip to the tail which gradually terminates in a fine point. Total length 350.20 μm ± 21.50; width 19.58 μm ± 0.53, distance anus to tip of tail 70.85 μm ± 5.50.

e) *Oesophagostomum radiatum.* These larvae contain many granules giving them a dark appearance, have a long fine filamentous tip to the tail, and were the largest of those studied in cattle, not only in overall length but also in the distance anus to the tip of tail. Total length 401.20 μm ± 40.27; width 21.35 μm ± 0.90; distance anus to tip of tail 101.17 μm ± 14.96.

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