

IDENTIFICATION OF FIRST STAGE LARVAE L₁ OF BOVINE NEMATODES¹R.K. REINECKE² and NORAH D. REINECKE

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A obtenção de larvas de primeiro estágio no período de 18 horas da coleta de fezes possibilitou a identificação dos principais gêneros de nematódeos que comumente parasitam bovinos. Morfologicamente variaram como segue: — Larvas curtas (média de comprimento de 235-363 µm): *Strongyloides* — o esfago ocupa aproximadamente 30% do comprimento total da larva, cauda sem bainha (em todas as outras larvas o esfago é curto e a cauda da bainha presente); *Dictyocaulus* — forma de parênteses ou encurvada, células intestinais negras, bainha da cauda 15 µm; *Trichostrongylus* — larva ligeiramente curva, bainha da cauda 31 µm, triangular. Larvas de comprimento médio (366-376 µm): *Haemonchus* — cauda da larva em forma de cunha, bainha da cauda fina, filamentososa, curva ou enrolada; *Cooperia* — bainha da cauda em forma cônica, extremidade anterior termina com 2 corpos refringentes. Comprimento da larva entre 437-472 µm e largura 22-23 µm ao nível do bulbo esofágico; *Ostertagia* — células intestinais semi-transparentes de coloração acinzentada ou prateada; *Bunostomum* — células intestinais escuras, marrons ou negras, cauda da bainha ligeiramente curva ou reta em linha com o eixo longo, bulbo esofágico pode ser proeminente; *Oesophagostomum* — larva muito longa (414 µm), bainha da cauda longa (93 µm) curva ou enrolada.

TERMOS DE INDEXAÇÃO: Larvas primeiro estágio, nematódeos, bovinos.

ABSTRACT.- The recovery of 1st stage larvae within 18h of collecting cattle faeces has made it possible to diagnose the common genera of cattle nematode parasites. Morphologically they vary as follows: — Short larvae (mean length 235-363 µm) are: *Strongyloides* — oesophagus 30% total length, no tail sheath (In all other larvae the oesophagus is short and tail sheath, i.e. extension of sheath of the tail beyond the tail tip, is present); *Dictyocaulus* — parenthesis shaped, intestinal cells black, tail sheath 15 µm; *Trichostrongylus* — straight slightly curved, tail sheath 31 µm, triangular. Medium length larvae (366-376 µm): *Haemonchus* — caudal end wedge shaped, tail sheath fine filamentous curved or coiled; *Cooperia* — tail sheath conical, anterior end 2 refringent bodies. Long larvae (437-472 µm and width 22-23 µm at the level of the oesophageal bulb): *Ostertagia* — intestinal cells semi-transparent grey or silver coloured; dark brown or black *Bunostomum* — tail sheath slightly curved or straight in line with the long axis, oesophageal bulb may be prominent; *Oesophagostomum* — very long (414 µm), tail sheath long (93 µm) curved or coiled.

INDEX TERMS: First stage larvae, cattle nematodes.

INTRODUCTION

The recovery of first stage larvae from 15 ml (L₁ 15 ml⁻¹) cattle faeces in clean water within 18 h of collection has

been described by Reinecke & Fonseca (1992). Whitlock (1959) described the recovery and identification of L₁ of sheep nematodes and Rodrigues & Honer (1985) identified L₁ of *Cooperia* spp (> 90% *C. punctata*), *Haemonchus* spp (*H. placei* and *H. contortus*), *Oesophagostomum radiatum* and *Trichostrongylus* spp. The latter authors stated that the shape and form of the tail of L₁ was more important for identification than the length of the tail, i.e. anus to tip of the tail.

First stage larvae of 2 genera and 6 nematode species of cattle are described in the present paper.

MATERIALS AND METHODS

First stage larvae of *Dictyocaulus viviparus* were recovered from gravid females chopped fine with scissors, sieved through cotton gauze, heat killed at 60°C and fixed in 10% formalin.

Adult females of *Bunostomum phlebotomum*, *Haemonchus* spp (*H. placei*, *H. contortus* and *H. similis*) and *Oesophagostomum radiatum*, were collected alive at necropsy and placed in warm saline where they laid eggs. Within 4-6 h at least 20 (and many more *Haemonchus*) females were removed, cut into small pieces with a sharp scissors, sieved through cotton gauze and eggs allowed to sediment in tap water and pooled with the same genus present in saline. They were incubated at 36°C in water, in flat-sided medicine bottles for 18-20h or longer. When > 75% of eggs had hatched, the larvae were heat killed and fixed in 10% formalin.

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It is essential to preserve L₁ in 10% formalin or a mixture of a suitable bactericide and fungicide. The microorganisms either destroy L₁ or distort their morphology.

Eggs of *Cooperia* spp (mainly *C. punctata*), *Strongyloides papillosus*, *Trichostrongylus axei* and *Ostertagia ostertagi* were recovered from faeces of cattle with mixed infections, incubated, heat killed and fixed in formalin described by Reinecke & Fonseca (1992). To confirm the genera present faeces from the same animal(s) were mixed with helminthologically free dry baked cattle faeces, incubated at 30°C for 6-8 days and infective larvae identified microscopically.

A drop of L₁ suspension was pipetted on to a glass slide, a cover slip placed on the suspension and L₁ examined with the aid of a compound microscope³ and measured with an ocular micrometre⁴.

The following were measured: total length, maximum width at the bulb of the oesophagus, length of the oesophagus (anterior end to end of the bulb) and the extension of the tail sheath beyond the tip of the tail of larvae hereafter referred to as the tail sheath. Measurement of 5-14 specimens were recorded in microns (µm), the mean (x) and standard deviation (s.d.) estimated.

³ Leitz Dialux 20 EB 10x oculars; objectives 6,3x, 16x, 25x and 40x.

⁴ Leitz Wetzlar ocular 10x.

Table 1. Dimensions (µm) of first stage larvae of cattle nematodes. Short larvae mean length 235-362 µm

	<i>Dictyocaulus viviparus</i>		<i>Strongyloides papillosus</i>		<i>Trichostrongylus axei</i>		
	Whitlock (1959)		Whitlock (1959)		Rodrigues & Honer (1985)		
Total length							
n ^a	11	14	205	11	200	-	
x ^b	363	235	216	362	342	-	
r ^c	294 -	216 -	172 -	345 -	289 -	-	
	423	276	252	385	387	-	
sd ^d	34	13,7	13,5	14,4	17,6	-	
Width							
n	9	14	-	9	50	-	
x	14	14	-	17	19	-	
r	11 -	11 -	-	16 -	16 -	-	
	17	20	-	18	22	-	
sd	2,3	2,5	-	0,83	1,6	-	
Oesophagus: length from the anterior end to the bulb							
n	7	14	-	8	-	-	
x	89	72	-	105	-	-	
r	67 -	67 -	-	101 -	-	-	
	117	87	-	108	-	-	
sd	21,5	8,2	-	2,9	-	-	
Tail sheath extension of the tail sheath beyond the tip of the tail							
n	8	-	-	10	-	-	
x	15	-	-	31	-	-	
r	11 -	-	-	21 -	-	-	
	19	-	-	37	-	-	
sd	2,3	-	-	6,3	-	-	
Anus to the tip of the tail							
n	-	-	-	-	50	-	
x	-	-	-	-	57	-	
r	-	-	-	-	52 -	60 -	
	-	-	-	-	64	65	
sd	-	-	-	-	2,9	-	

a N^o measured; b mean; c range; d standard deviation.

RESULTS

First stage larvae of the following 6 species and 2 genera were identified:

- Bunostomum phlebotomum*,
- Cooperia* spp (*C. punctata* predominantly, less *C. pectinata*),
- Dictyocaulus viviparus*,
- Haemonchus* spp (*H. placei*, *H. contortus* and *H. similis* in descending order of prevalence),
- Oesophagostomum radiatum*,
- Ostertagia ostertagi*,
- Strongyloides papillosus*,
- Trichostrongylus axei*.

Hereafter the L₁ will be referred to by their generic names.

Measurements are tabulated in Table 1, 2 and 3 and illustrated in Fig. 1 and 2. Larvae were identified on the total length, and length, width, shape and form of the tail tip and other characteristics described below. Larval dimensions by Sprent (1946). Whitlock (1959) and Rodrigues & Honer (1985) are also listed in Table 1, 2 and 3.

Table 2. Dimensions (µm) of first stage larvae of cattle nematodes. Medium larvae mean length 366-376 µm

	<i>Cooperia, C. punctata and C. pectinata</i>				<i>Haemonchus</i>				
	Whitlock (1959)		Rodrigues & Honer (1985)		Whitlock (1959)		Rodrigues & Honer (1985)		
	H.c. ^a		H.p. ^b		H.c.		H.p.		
Total length									
n	9	50	100	10	171	190	100	100	
x	376	330	350	366	345	363	304	369	
r	310 -	301 -	-	322 -	307 -	307 -	-	-	
	418	357	-	414	387	418	-	-	
sd	33,8	13,9	21,5	30,3	18,1	19,6	24,6	24,6	
Width									
n	7	50	100	10	50	50	100	100	
x	19	17	20	17	19	19	20	19	
r	17 -	16 -	-	16 -	17 -	17 -	-	-	
	23	19	-	21	23	23	-	-	
sd	2,3	0,9	0,53	4,2	1,2	1,4	0,62	0,56	
Oesophagus									
n	6	-	-	10	-	-	-	-	
x	107	-	-	102	-	-	-	-	
r	106 -	-	-	90 -	-	-	-	-	
	113	-	-	120	-	-	-	-	
sd	4,4	-	-	11,1	-	-	-	-	
Tail sheath extension of tail sheath beyond the tip of the tail									
n	7	-	-	10	-	-	-	-	
x	57	-	-	49	-	-	-	-	
r	55 -	-	-	41 -	-	-	-	-	
	60	-	-	69	-	-	-	-	
sd	1,8	-	-	19,2	-	-	-	-	
Anus to the tip of the tail									
n	-	50	100	-	50	50	100	100	
x	-	77	71	-	75	92	60	75	
r	-	68 -	-	-	70 -	81 -	-	-	
	-	87	-	-	84	101	-	-	
sd	-	4,3	5,5	-	3,2	5,5	5,4	6,78	

a *H. contortus*; b *H. placei*.

Table 3. Dimensions (µm) of first stage larvae of cattle nematodes. Long larvae mean length 379-472 µm

	<i>Bunostomum phlebotomum</i>	<i>Ostertagia ostertagi</i>	<i>Oesophagostomum radiatum</i>		
	Sprent (1946)		Rodrigues & Honer (1985)		
	Total length				
n	10	-	10	8	100
x	472	-	379	437	401
r	420 -	350 -	328 -	414 -	-
	517	450	460	471	-
sd	28,6	-	35,7	17,7	40,27
	Width				
n	9	-	11	7	100
x	23	-	22	22	21
r	16 -	-	19 -	18 -	-
	25	-	25	24	-
sd	2,9	-	1,73	2,4	0,90
	<i>Oesophagus</i>				
n	5	-	10	7	-
x	109	95	111	108	-
r	104 -	-	100 -	92 -	-
	120	-	129	115	-
sd	7,1	-	9,47	8,4	-
	Tail sheath-extension of tail sheath beyond the tip of the tail				
n	6	-	10	7	-
x	42	-	48	93	-
r	38 -	-	37 -	81 -	-
	55	-	62	101	-
sd	10,7	-	11,2	8,4	-
	Anus to the tip of the tail				
n	-	-	-	-	100
x	-	100	-	-	101
r	-	-	-	-	-
sd	-	-	-	-	14,96

Short larvae (Mean length less than 363 µm):

(i) *Strongyloides* (Fig. 1A): *Oesophagus* 30% of the length of the larva, fine 14 µm wide, mean length 235 µm and *no tail sheath*. Live larvae were very active.

All other larvae had a short oesophagus and a tail sheath which varied in length and shape. Whitlock (1959) and Rodrigues & Honer (1985) measured the length of the entire tail, i.e. anus to tip of the tail sheath. This will always be longer than the tail sheath described in the present paper.

(ii) *Trichostrongylus* (Fig. 1B and 2B): Mean length 362 µm, curved or straight, intestinal cells light grey, tail sheath mean length 31 µm, pencil point or elongated triangle.

(iii) *Dictyocaulus* (Fig. 1F and 2F): Mean length 363 µm, intestinal cells dark brown or black. The mean length of the tail sheath was only 15 µm, the tail tip usually withdrawn. These larvae were usually curved, i.e. parenthesis shaped and live L₁ were very sluggish.

Medium length larvae (mean length 366-376 µm):

(i) *Haemonchus* (Fig. 1D and 2D): Mean length 366 µm and tail sheath 49 µm. The mean width was 17 µm at the oesophageal bulb. From the anus posteriorly larvae narrow to the tail tip giving the body a wedge shape caudally. The tail sheath was very fine filamentous usually curved or even coiled. Whitlock (1959) and Rodrigues & Honer (1985) differentiated between *H. contortus* and *H. placei* (Table 1). *H. contortus* tail was set at a slight angle to the body while *H. placei* was a finely elongated tip which continued along the long axis of the body (Rodrigues & Honer 1985).

(ii) *Cooperia* spp (Fig. 1C and 2C): Mean length 375 µm tail sheath 57 µm with a conical tip as described by Rodrigues &

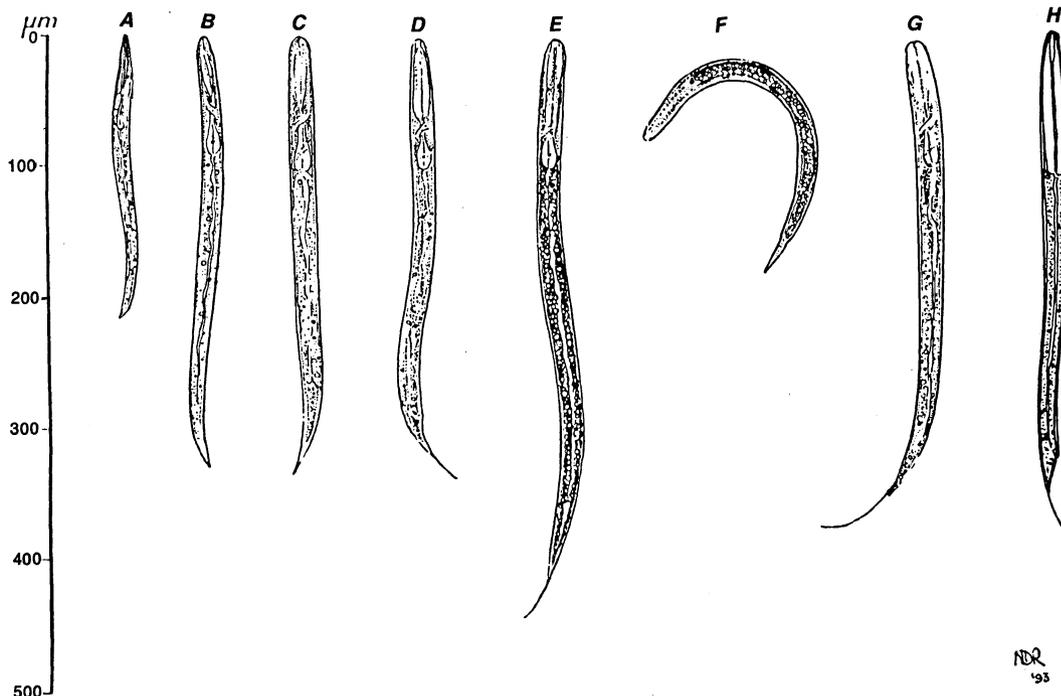


Fig. 1. A - *Strongyloides papillosus*; B - *Trichostrongylus axei*; C - *Cooperia* spp; D - *Haemonchus placei*; E - *Bunostomum phlebotomum*; F - *Dictyocaulus viviparus*; G - *Oesophagostomum radiatum*; H - *Ostertagia ostertagi*.

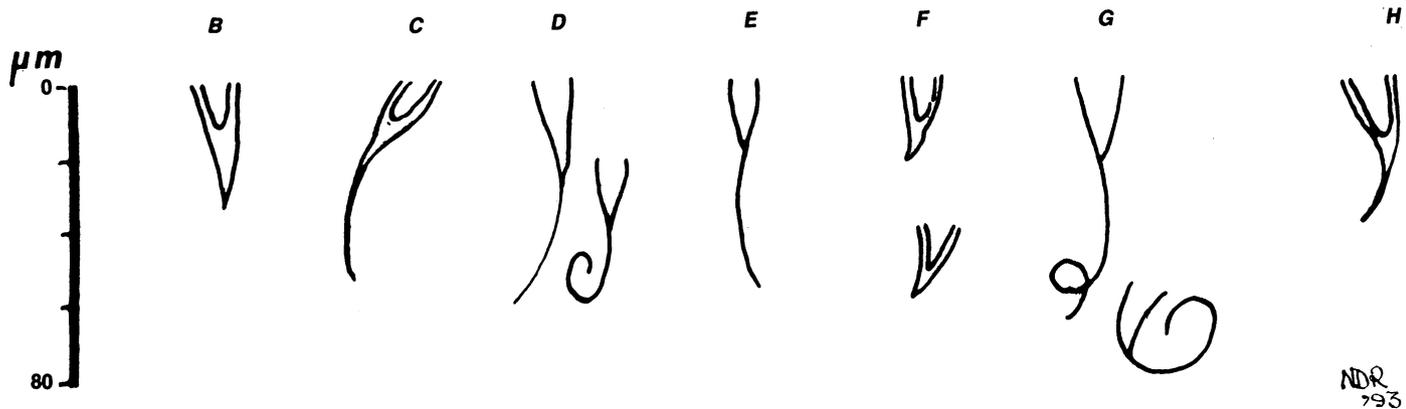


Fig. 2 Tail sheaths of first stage larvae of cattle nematodes: B - *T. axei*; C - *Cooperia* spp; D - *Haemonchus*; E - *B. phlebotomum*; F - *D. viviparus*; G - *O. radiatum*; H - *O. ostertagi*.

Honer (1985). Anteriorly on either side of the mouth were 2 refringent bodies, i.e. the anterior end glowed when examined microscopically. The present author was unable to confirm Rodrigues & Honer (1985) observation that there were: "very marked cuticular striations".

Long larvae (mean length 379-472 μm and broad 22-23 μm at the bulb of the oesophagus):

(i) *Ostertagia* (Fig. 1H and 2H): This wide stout larvae had clear intestinal cells a mean length of 379 μm and tail sheath 48 μm long; usually curved which ended in a blunt tip. Whitlock (1959) citing Threlkeld (1946) gave a total length ranging from 300-500 μm . In many respects the buccal capsule resembled the 3rd parasitic stage of *O. ostertagi* described by Douvres (1956). The mouth had a medial tubular buccal capsule starting with 2 cuticular thickenings anteriorly continuing with thin parallel walls and ending in a funnel shape posteriorly. *Oesophagostomum* had a similar mouth but there are no anterior cuticular thickenings.

(ii) *Bunostomum* (Fig. 1E and 2E): These larvae were usually straight or slightly curved with black or dark brown intestinal cells and the oesophageal bulb was often prominent. The mean length was 472 μm and tail sheath 42 μm and either in line with the body axis or slightly curved.

(iii) *Oesophagostomum* (Fig. 1G and 2G): The mean length was 437 μm and tail sheath 93 μm . The tail sheath was fine either curved, coiled or even watch-spring shaped. The mouth wall consisted of 2 lines opening anteriorly and intestinal cells were dark but not as dark as *Bunostomum* or *Dictyocaulus*.

DISCUSSION

The main advantage of differentiating L₁ to the level of genus is that a diagnosis can be made 18-20h after the specimens are collected. In the McMaster faecal egg counting technique e.p.g., egg counts were done using a mass of 4g of faeces, and if negative, the specimen was discarded. Using Visser filters 15 ml of faeces were used, eggs hatched in clean water at 37-40°C in 18h and L₁ diagnosed the following day (Reinecke & Fonseca 1992), not a week later, before third stage larvae were harvested from coprocultures to make a generic diagnosis with standard egg counts.

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