

# Observations on the life-cycles and larval morphogenesis of, and transmission experiments with *Cooperioides hamiltoni* and *Cooperioides hepaticae* (Nematoda: Trichostrongyloidea) parasitic in impala, *Aepyceros melampus*

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The previously unknown life-cycles of *Cooperioides hamiltoni* and *Cooperioides hepaticae* parasitic in impala, are recorded. Observations on the morphology of the developmental stages of these two nematode species are described and their habitat as well as their similarities and differences are discussed. *C. hepaticae* was successfully transferred to domestic sheep. Attempts were also made to transmit *C. hamiltoni* to sheep.

Die nog onbekende lewensiklus van *Cooperioides hamiltoni* en *Cooperioides hepaticae*, wat parasities voorkom in rooibokke, word beskryf. Morfologiese ondersoek van die ontwikkelingsstadia van hierdie twee nematode-species word beskryf en hul habitat, ooreenkomste en verskille word bespreek. Skape is suksesvol met *C. hepaticae* besmet. Daar is ook probeer om *C. hamiltoni* eksperimenteel aan skape oor te dra.

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A two-year study (March 1973–75) on helminths of impala, *Aepyceros melampus* (Lichtenstein, 1912) in Natal (Anderson 1980), presented the opportunity to observe the hitherto unknown aspects of larval morphogenesis. Previous reports which emanated from the two-year study include the life cycle of *Pneumostromylus calcaratus* Mönnig, 1932, a lungworm of impala (Heinichen 1974; Heinichen Anderson 1976, 1982) and *Cooperia fuelleborni* (Anderson 1986). The aim of the present study is to report on the life cycle of *Cooperioides hamiltoni* Mönnig, 1932 and *Cooperioides hepaticae* Ortlepp, 1938 which includes attempts to transmit *C. hamiltoni* and *C. hepaticae* to domestic sheep.

## Materials and Methods

The studies were carried out on the Nyala Game Ranch of Mr. Scott-Barnes, situated 20 km north-west of Empangeni in Natal. Over a period of two years 46 impala were examined. To recover all the worms from the intestine, the contents were put into a modified Baermann apparatus and placed into Shone's waterbath. The method of recovery for the adult, 5th stage and L<sub>4</sub> (fourth-stage larvae) of *C. hamiltoni* from the ingesta and scrapings of the proximal part of the small intestine followed Heinichen (1973). The description by Mönnig (1932) was used to identify the adult and 5th stage *C. hamiltoni* recovered from the ingesta. *C. hamiltoni* L<sub>4</sub> recovered from the small intestinal wall scrapings were easily distinguished from the *Cooperia fuelleborni* larvae as described in the results below.

The recovery of adult, 5th stage, and L<sub>4</sub> *C. hepaticae* from the liver followed the method of Anderson (1982) whereas the identification of adult *C. hepaticae* was based on Ortlepp (1938).

Recovery of L<sub>3</sub> of both *C. hamiltoni* and *C. hepaticae*, followed the method for *C. fuelleborni* larvae (Anderson 1986). For the recovery of the L<sub>1</sub> and L<sub>2</sub> larvae the culture method of Whitlock (1956), modified by Reinecke (1961) was used. A small sample of the faeces from the culture was

removed by forceps after two to three days and examined under the microscope for L<sub>1</sub>. This procedure was repeated after seven to eight days to recover the L<sub>2</sub>. The larvae were removed, placed on a glass slide, heat-killed and examined in a drop of water.

The morphological measurements and drawings of all larval stages were carried out as published previously for *C. fuelleborni* (Anderson 1986).

Three lambs were each infected with 1 800 L<sub>3</sub> of *C. hepaticae* and killed eight, 10 and 21 days afterwards. The lamb examined 21 days after infection was also infected with *C. hamiltoni*, but only 1 000 L<sub>3</sub> were available. This work was carried out by Dr A. Verster (pers. comm.) at Onderstepoort.

## Results

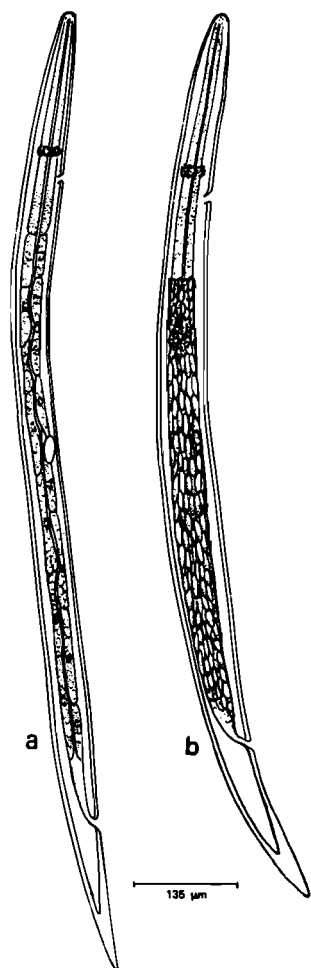
The measurements of L<sub>1</sub>, L<sub>2</sub> and L<sub>3</sub> are summarized in Table 1 and Figures 1a and 1b illustrate the L<sub>3</sub> of *C. hamiltoni* and *C. hepaticae*. L<sub>2</sub> was found to be longer than L<sub>1</sub>, whereas L<sub>3</sub> had the same length as L<sub>2</sub>. In a few L<sub>3</sub> of *C. hamiltoni* 15 intestinal cells were counted. The total length of *C. hamiltoni* is usually slightly longer than that of *C. hepaticae*, but otherwise show little differences in their morphology. All free-living larval stages of these two parasites often contained dark food granules (Figure 1b) and therefore the details of the intestinal cells and the genital primordia were not clear. The tail end of the L<sub>3</sub> of *C. hamiltoni* is slightly more pointed than that of *C. hepaticae*.

The development from egg to infective larva of nematode species with a direct life-cycle is similar for all species, viz. eggs are discharged from the host and the first two larval stages develop in the faeces. Infective L<sub>3</sub> larvae then migrate up the vegetation and enter the host orally, when grazing. A study was made of the free-living stages of *C. hamiltoni* and *C. hepaticae*. The L<sub>1</sub> and L<sub>2</sub> were found in faecal cultures after 5–7 and 8–10 days incubation at 26°C. The L<sub>3</sub> were collected from the side of the glass after

**Table 1** Dimensions ( $\mu\text{m}$ ) of preparasitic stages ( $L_1$ ,  $L_2$ ,  $L_3$ ) of *Cooperioides hamiltoni* and *Cooperioides hepaticae*\*

Dimensions	$L_1$				$L_2$				$L_3$			
	<i>Cooperioides hamiltoni</i> $n = 3$		<i>Cooperioides hepaticae</i> $n = 10$		<i>Cooperioides hamiltoni</i> $n = 6$		<i>Cooperioides hepaticae</i> $n = 10$		<i>Cooperioides hamiltoni</i> $n = 10$		<i>Cooperioides hepaticae</i> $n = 10$	
	Range	Mean	Range	Mean $\pm$ SD	Range	Mean	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD
Total length (mm)	567–648	615,67	540–685	623,90 $\pm$ 51,80	578–702	664,67	594–691	652,30 $\pm$ 36,80	594–702	633,40 $\pm$ 31,30	562–675	623,30 $\pm$ 41,30
Width												
At anterior end	9–11	10,00	9–11	10,30 $\pm$ 0,90	9–11	10,50	9–11	10,50 $\pm$ 0,70	7–10	8,70 $\pm$ 0,82	9–14	11,20 $\pm$ 1,80
Maximum	18–20	19,50	18–25	20,40 $\pm$ 2,80	19–24	21,67	20–27	20,90 $\pm$ 6,00	20–24	22,10 $\pm$ 0,99	18–27	22,90 $\pm$ 2,70
At anus	15–16	15,50	16–17	16,30 $\pm$ 2,60	16–17	16,50	13–17	14,80 $\pm$ 5,40	13–18	15,50 $\pm$ 1,65	12–17	14,60 $\pm$ 1,80
Length of oesophagus	96–101	99,00	92–146	112,30 $\pm$ 15,90	117–148	130,83	123–151	135,30 $\pm$ 9,50	130–162	143,00 $\pm$ 9,45	126–156	140,40 $\pm$ 8,90
Distance from excretory pore to anterior end	91–95	93,33	72–110	93,80 $\pm$ 9,00	98–116	102,50	91–136	104,50 $\pm$ 9,30	98–111	103,00 $\pm$ 3,97	72–120	101,80 $\pm$ 14,40
Distance from nerve ring to anterior end	82–89	85,33	74–109	90,10 $\pm$ 14,30	85–107	97,83	88–109	96,50 $\pm$ 10,00	82–102	92,80 $\pm$ 5,87	70–98	85,20 $\pm$ 8,69
Genital primordium												
Length	–	–	13	13,00**	9–13	10,67	8–13	10,40**	8–17	12,00***	7–13	10,10 $\pm$ 2,00
Width	–	–	7	7,00**	5–8	7,00	6–7	6,80**	4–7	5,40***	5–9	7,40 $\pm$ 1,40
Distance from genital primordium to tail	–	–	328–367	337,00**	221–247	234,00	250–313	287,00**	273–306	288,80***	252–549	298,40 $\pm$ 28,80
Distance from anus to tail (with sheath if present)	101–117	108,67	101–130	115,70 $\pm$ 11,30	100–121	113,83	91–129	114,30 $\pm$ 8,70	85–120	101,60 $\pm$ 11,78	98–143	108,80 $\pm$ 15,00
Distance from tail to sheath	–	–	–	–	–	–	–	–	34–65	45,50 $\pm$ 8,95	36–65	49,50 $\pm$ 8,82

\* All measurements given in  $\mu\text{m}$  unless stated otherwise; \*\* Only six worms measured; \*\*\* Only five worms measured.



**Figure 1** Infective larvae of: (a) *Cooperioides hamiltoni*; (b) *Cooperioides hepaticae*.

12–16 days of incubation.

Only the  $L_3$  of *C. hepaticae* were kept in water to determine their longevity. It was found that *C. hepaticae* remained alive in water for up to one month at room temperature.

The  $L_4$  of *C. hamiltoni* was found mainly in scrapings from the mucosa of the proximal part of the small intestine. The 5th stage and adult occurred in the ingesta of the proximal part of the small intestine.

The measurements of male and female  $L_4$  and 5th stage *C. hamiltoni* and *C. hepaticae* are listed in Tables 2 and 3 and their features illustrated in Figures 2–5. Only a limited number of  $L_4$  of the *Cooperioides* species were found and therefore no late  $L_4$  females were distinguished. In the  $L_4$  the anterior end of the oesophagus has two small rod-like structures, similar to, but smaller than those described by Douvres (1956) in *Ostertagia*. The  $L_4$  female of *C. hepaticae* has a rounder, blunt posterior end (Figure 3b) while *C. hamiltoni* has a small, thinner tip, (Figure 2b) similar to that of the adult. In both species, the males can also be distinguished from the female by a swollen posterior end (Figure 2c & 3c).

In the 5th stage the cephalic region of *C. hamiltoni* and *C. hepaticae* is conspicuously swollen, and bears 8–10 coarse annulations, as in the adult (Figure 4a & 5a). There are 10 longitudinal, cuticular ridges observed in the middle region of both *Cooperioides* species (Figure 4e & 5e).

In the 5th stage of *C. hepaticae* a peculiar indentation was observed just anterior to the excretory pore (Figure 5a). In the male this indentation was situated 237–324  $\mu\text{m}$  (304,60  $\pm$  13,85) and in the female 267–324  $\mu\text{m}$  (299,80  $\pm$  14,94)

**Table 2** Dimensions of the parasitic (L<sub>4</sub> and 5th) stages of male *Cooperioides hamiltoni* and *Cooperioides hepaticae*\*

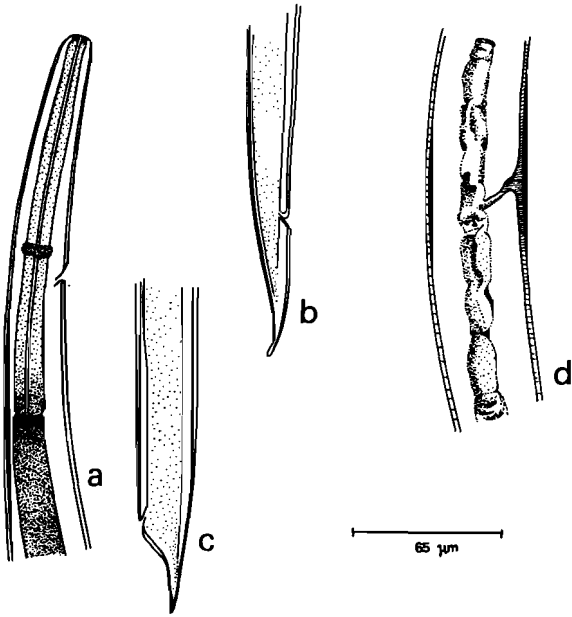
Dimensions	L <sub>4</sub>				5th			
	<i>Cooperioides hamiltoni</i>		<i>Cooperioides hepaticae</i>		<i>Cooperioides hamiltoni</i>		<i>Cooperioides hepaticae</i>	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Total length (mm)	0,80–0,93	0,86±0,05	1,19–1,66	1,38±0,18	4,56–6,94	5,40±0,77	5,29–6,50	5,91±0,33
Width								
At anterior end	12–13	12,40±0,52	14–16	14,80±1,03	41–49	45,60±3,06	38–46	40,20±2,74
Across oesophagus	20–29	23,40±2,67	26–29	26,70±1,25	57–108	77,40±16,57	54–81	61,30±8,30
Maximum	22–30	25,30±2,11	26–43	27,60±7,68	113–162	147,40±17,78	84–113	96,60±10,12
In front of bursa	–	–	–	–	113–162	147,40±17,78	84–113	96,60±10,12
At anus	20–26	22,50±2,12	17–34	26,20±6,58	–	–	–	–
Oesophagus								
Length	144–194	158,60±15,69	241–306	261,70±20,09	382–459	420,00±24,35	389–478	442,60±24,31
Width at the base	13–14	13,30±0,48	16–22	18,70±2,00	39–59	44,90±5,63	27–41	33,70±3,74
Distance from excretory pore to anterior end	96–117	106,50±6,47	139–195	160,60±17,60	230–349	280,79±40,27	319–373	350,7±19,05
Distance from nerve ring to anterior end	85–100	93,10±5,86	111–150	137,50±12,02	211–314	244,70±39,78	230–351	303,80±40,63
Genital primordium								
Length	28–91	56,14**	–	–	–	–	–	–
Width	4–8	6,57**	–	–	–	–	–	–
Distance from genital primordium to tail	241–332	279,86**	–	–	–	–	–	–
Distance from anus to tail	50–65	55,60±4,06	52–72	62,30±6,43	–	–	–	–
Length of spicules	–	–	–	–	200–221	210,40±7,73	181–216	198,00±11,32

\* All measurements are given in µm unless stated otherwise; \*\* Only seven worms measured.

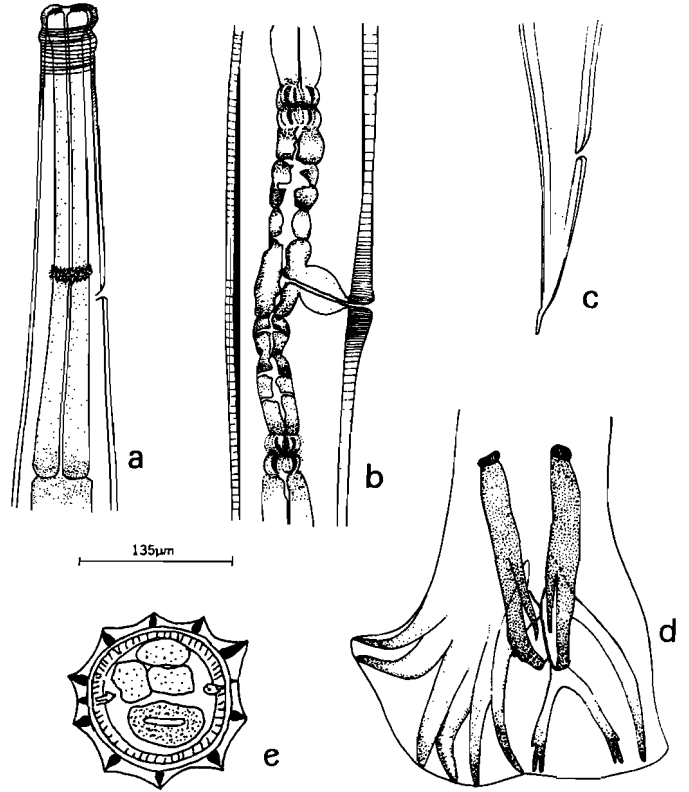
**Table 3** Dimensions of the parasitic (L<sub>4</sub> and 5th) stages of female *Cooperioides hamiltoni* and *Cooperioides hepaticae*\*

Dimensions	L <sub>4</sub>				5th			
	<i>Cooperioides hamiltoni</i>		<i>Cooperioides hepaticae</i>		<i>Cooperioides hamiltoni</i>		<i>Cooperioides hepaticae</i>	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Total length (mm)	0,77–1,19	0,89±0,13	1,17–1,86	1,44±0,21	5,87–7,67	6,91±0,63	5,59–6,80	6,32±0,35
Width								
At anterior end	10–13	12,40±0,97	14–20	16,00±1,89	32–54	46,30±7,07	43–51	45,50±3,21
Across oesophagus	20–26	23,80±2,30	25–34	27,30±2,91	32–86	65,80±14,91	57–65	60,20±4,32
Maximum	21–30	25,00±3,02	18–39	26,50±7,93	86–167	131,10±28,13	81–108	94,40±10,30
At vulva	–	–	–	–	86–167	131,10±28,13	81–108	94,40±10,30
At anus	14–18	15,50±1,43	14–27	17,90±4,38	30–52	40,40±6,19	32–38	35,70±2,98
Oesophagus								
Length	159–197	173,80±10,71	238–293	260,80±20,31	378–464	434,50±29,02	427–497	464,60±20,11
Width at base	12–14	13,30±0,67	13–20	17,70±2,21	35–118	54,40±24,01	32–41	34,50±3,24
Distance from excretory pore to anterior end	91–134	114,20±12,12	133–176	154,10±13,99	254–358	299,40±26,92	287–383	337,30±28,69
Distance from nerve ring to anterior end	91–107	96,20±8,28	117–166	142,20±14,67	208–301	253,50±31,55	236–361	306,50±35,04
Genital primordium								
Length	8–130	62,00**	7–130	84,83***	–	–	–	–
Width	5–14	9,25**	3–13	7,50***	–	–	–	–
Distance from genital primordium to tail	150–351	230,75**	163–260	205,33***	–	–	–	–
Length of ovijectors	–	–	–	–	297–459	362,00±48,81	335–410	376,30±25,02
Distance from vulva opening to tail	–	–	–	–	918–1836	1432,60±256,85	807–994	944,30±54,48
Distance from anus to tail	46–72	58,20±8,01	65–98	73,90±10,90	122–167	142,30±16,42	108–146	131,00±11,60

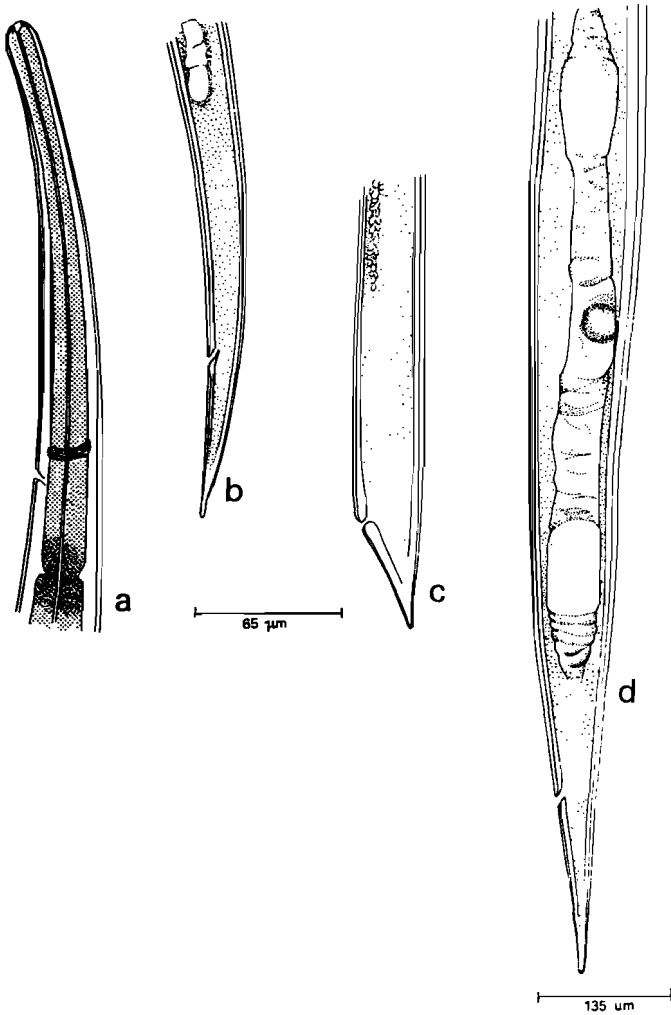
\* All measurements are given in µm unless stated otherwise; \*\* Only four worms measured; \*\*\* Only six worms measured.



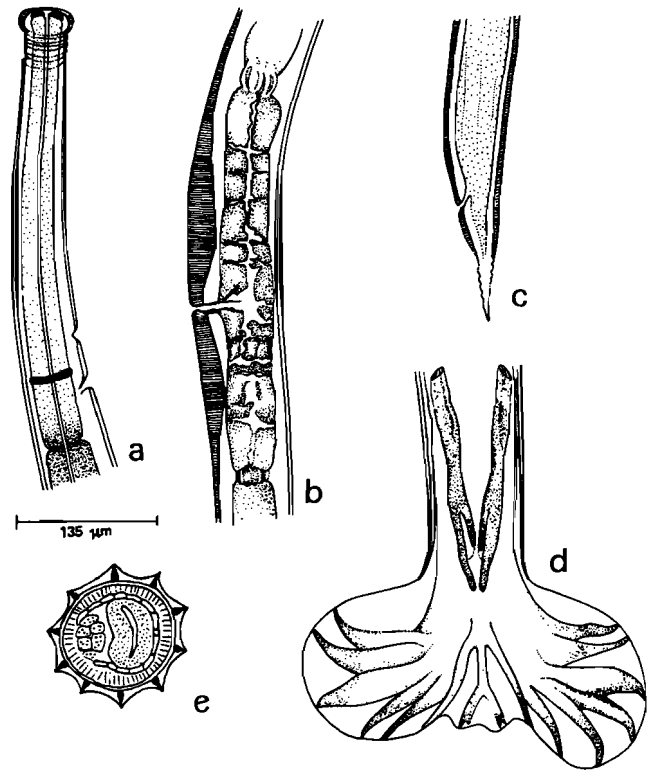
**Figure 2** L4 of *Cooperioides hamiltoni*: (a) Anterior end of female, (b) Posterior end of female, (c) Posterior end of male, (d) Vulvar region of late L4 female.



**Figure 4** 5th stage of *Cooperioides hamiltoni*: (a) Anterior end of female; (b) Vulvar region of female; (c) Posterior end of female; (d) Posterior end of male; (e) Cross section of male in the midbody region.



**Figure 3** L4 of *Cooperioides hepaticae*: (a) Anterior end of female; (b) Posterior end of female (early L4); (c) Posterior end of male; (d) Posterior end and vulvar region of female (later L4).



**Figure 5** 5th stage of *Cooperioides hepaticae*: (a) Anterior end of female; (b) Vulvar region of female; (c) Posterior end of female; (d) Posterior end of male; (e) Cross section of male in the midbody region.

from the anterior end. It was seen in all 5th stage specimens, but not in adults. Here, however, a small duct, similar to the excretory pore, was observed approximately 100  $\mu\text{m}$  in front of the latter. In the adult male, this canal was situated 355–436  $\mu\text{m}$  ( $388,10 \pm 33,50$ ) and in the female 377–433  $\mu\text{m}$  ( $406,90 \pm 24,86$ ) from the anterior end. In a few worms, which were sufficiently cleared, a cell was visible at the end of the canal.

Measurements of the adult *C. hepaticae* are listed in Table 4 and, in Table 5 they are compared with those of *C. antidorca* (Mönnig 1931), *C. hamiltoni* (Mönnig 1933), *C. hepaticae* (Ortlepp 1938) and *C. kenyensis* (Daubney 1933).

The L<sub>4</sub> of *C. hepaticae* was obtained from an experimentally infected sheep. These L<sub>4</sub> were mainly in the mucosa of the proximal part of the small intestine. The 5th stage was found in the distal part of the small intestine of the impala. It seems that the larvae pass to the distal part of the intestine during their development, but during the 5th stage migrate anteriorly to enter the bile ducts where they develop into adult worms. Figure 6 shows the liver of a heavily infected impala. The gallbladder is swollen, since parasites have blocked the ends of the small bile ducts. These contained numerous adult *C. hepaticae*.

Of the three lambs with L<sub>3</sub> of *C. hepaticae* only one killed 21 days after infection, contained many L<sub>4</sub> and 5th stage worms. These were found in mucosal scrapings and the ingesta of the small intestine.

An attempt to transmit *C. hamiltoni* to sheep was unsuccessful. The larval dosage was perhaps inadequate. Attempts to obtain infective larvae from faecal cultures of *C. hamiltoni* were not as successful as those for *C. hepaticae*. The reason for this is unknown.

## Discussion

Very little research has been carried out previously on any of the larval stages of *Cooperioides*. There is little difference between the L<sub>3</sub> of *Cooperia fuelleborni* (Anderson 1986), *Cooperioides hamiltoni* and *C. hepaticae* and it would therefore be very difficult to distinguish between these species in a faecal culture. *C. fuelleborni* L<sub>4</sub>, however, is easily differentiated from that of the two *Cooperioides* species, since it is coiled into one circle and is also longer than the L<sub>4</sub> of *Cooperioides*. L<sub>4</sub> and 5th stage *C. fuelleborni* are found in the ingesta (Anderson 1986) whereas those larvae of *C. hamiltoni* are mainly found in scrapings of the mucosa of the proximal part of the small intestine.

In most nematode species, the development of the L<sub>4</sub> female can be subdivided into early and late stages. The late L<sub>4</sub> of *C. hamiltoni* however, was not recovered. The early L<sub>4</sub> of *C. hamiltoni* and *C. hepaticae* can be compared to the phases 3–5 and the late L<sub>4</sub> of *C. hepaticae* to phases 6–9 of *Ostertagia circumcincta* as described by Denham (1969). Also phases 10–11 correspond to the 5th stage female as

**Table 4** Dimensions of adult *Cooperioides hepaticae* \*

Dimensions	Male		Female	
	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD
Total length (mm)	11,61–14,59	13,19 $\pm$ 0,90	14,28–19,56	18,06 $\pm$ 0,52
Width				
At anterior end	46–55	50,36–3,18	54–68	62,40 $\pm$ 4,35
Across oesophagus	95–135	115,30 $\pm$ 12,94	116–143	126,40 $\pm$ 12,08
Maximum	200–270	224,84 $\pm$ 21,60	247–362	309,90 $\pm$ 36,82
At vulva	–	–	230–378	302,40 $\pm$ 55,35
In front of bursa	160–227	207,44 $\pm$ 19,70	–	–
At anus	–	–	35–59	50,00 $\pm$ 7,70
Oesophagus				
Length	545–648	612,58 $\pm$ 30,69	632–745	697,60 $\pm$ 36,25
Width at the base	43–68	56,21 $\pm$ 7,85	46–68	59,90 $\pm$ 7,14
Distance from excretory pore to anterior end	423–543	505,46 $\pm$ 40,31	444–675	525,40 $\pm$ 70,01
Distance from nerve ring to anterior end	360–485	426,90 $\pm$ 44,24	289–490	394,60 $\pm$ 57,90
Eggs				
Length	–	–	46–76	62,10 $\pm$ 9,02
Width	–	–	33–43	39,30 $\pm$ 3,53
Length of ovjectors	–	–	378–470	411,20 $\pm$ 32,11
Distance from vulva opening to tail (mm)	–	–	2,08–3,13	2,62 $\pm$ 0,38
Distance from anus to tail	–	–	95–189	142,80 $\pm$ 30,34
Length of spicules	203–232	223,16 $\pm$ 9,10	–	–
Dorsal spur of spicule	48–59	49,11 $\pm$ 15,82	–	–
Bursa				
Lateral lobe	314–449	373,20 $\pm$ 60,96	–	–
Dorsal lobe	125–135	131,90 $\pm$ 3,28	–	–

\* All measurements are given in  $\mu\text{m}$  unless stated otherwise.

**Table 5** Comparison of measurements \* of adult *Cooperioides* species from various hosts

Dimension	<i>Cooperioides antidorca</i> (Mönnig, 1931) Daubney 1933		<i>Cooperioides hamiltoni</i>		<i>Cooperioides hepaticae</i>		<i>Cooperioides hepaticae</i>		<i>Cooperioides kenyensis</i> Daubney 1933 **	
	Springbok Mönnig (1931)		Impala Mönnig (1933)		Impala Ortlepp (1938)		Impala These results		Sheep Daubney (1933)	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Total length (mm)	6,2–7,0	7,7–9,7	5,7–6,4	7,2–8,2	11–13	15–17	11,6–14,6	14,3–19,6	4,5–6,0	7,2–9,0
Width										
At anterior end	47	47	–	–	–	–	46–55	54–68	40–55	40–55
Maximum	113	106–121	–	–	215	290	200–270	247–362	140	150
At vulva	–	–	–	140–150	–	–	–	230–378	–	–
In front of bursa	–	–	120–140	–	–	–	160–227	–	–	–
Distance of cervical papillae from anterior end	320	330–380	absent	absent	absent	absent	absent	absent	present	(not measured)
Oesophagus										
Length	350–400	460	–	–	625–640	720–754	545–648	632–745	500–650	500–650
Width at the base	–	–	–	–	–	64	43–68	46–68	–	–
Distance of excretory pore from anterior end	290	270–340	–	–	425	523–551	423–543	444–675	–	–
Distance of nerve ring from anterior end	230	250	–	–	355	448–476	360–485	289–490	–	–
Eggs										
Length	–	–	–	670	–	No eggs found	–	46–76	–	75
Width	–	–	–	370	–	No eggs found	–	33–43	–	40
Length of ovijectors	–	–	–	320–370	–	638	–	378–470	–	275–300
Distance from vulva opening to tail (mm)	–	1,60–2,03	–	1,32–1,60	–	0,25–0,26	–	2,08–3,13	–	1,00–1,90
Distance from anus to tail	–	159	–	160–200	–	160–175	–	95–189	–	150–190
Tip on tail	–	–	–	19–34	–	–	–	–	–	–
Length of spicules	192–196	–	200–230	–	172–203	–	203–232	–	200–210	–
Spur of spicule	–	–	–	–	50	–	48–59	–	–	–
Bursa										
Lateral lobe	–	–	–	–	320	–	314–449	–	–	–
Dorsal lobe	–	–	–	–	145	–	125–135	–	300–330	–
Number of lateral lines	10	10	12	12	10	10	10	10	12	12
Location	Small intestine		Small intestine		Liver		Liver		Abomasum	

\* All measurements are given in  $\mu\text{m}$  unless stated otherwise.

\*\* Gibbons (1978) synonymized *Cooperioides kenyensis* with the type species, *Cooperioides hamiltoni*.

described by Denham (1969). No such clear subdivisions are possible in the males. In both species the L<sub>4</sub> males can easily be distinguished from the females by a swollen posterior end (Figure 2c & 3c). The 5th stage of both *Cooperioides* species already showed the features of their adult forms, the only differences being that they were shorter, the male spicules not fully sclerotized and the females contained no eggs.

There are two very small, round structures on the anterior end of both L<sub>4</sub> *Cooperioides* species (Figure 2a & 3a). Keith (1967) described this structure in *Cooperia pectinata* as the 'provisional buccal capsule'.

A lens-like structure, situated on the ventral surface just anterior to the excretory pore, has been described by Goodey (1951) in the potato tuber nematode *Dictylenchus destructor*. Thorne, 1945. Goodey (1951) suggested that this structure be called a 'Hemizonid' and found that it was mainly confined to the members of the superfamily Tylen-

choidea Chitwood and Chitwood, 1937 (see Thorne 1949). Goodey (1951) found no apparent connection between this structure and the adjacent excretory pore. The biconvex structure observed in the 5th stage *C. hepaticae* and the cell and related canal seen in the adult, could possibly correspond with the 'Hemizonid'.

Ortlepp (1938) gave measurements of 13 males and nine females of *C. hepaticae* collected from an impala liver. Table 5 shows that the measurements of *C. hepaticae* recovered in this investigation are similar to those of Ortlepp (1938). Ortlepp (1938), however, gave the length for the distance of the vulva openings to tail as varying from 250–260  $\mu\text{m}$ , but in these investigations this distance ranged from 2,1–3,1 mm ( $2,6 \pm 0,38$ ). The females examined by Ortlepp (1938) did not contain eggs in the uterus and he therefore suggested that the liver is not their normal location. Gibbons (1978), however, also gives measurements of *C. hepaticae* eggs from females found in the impa-

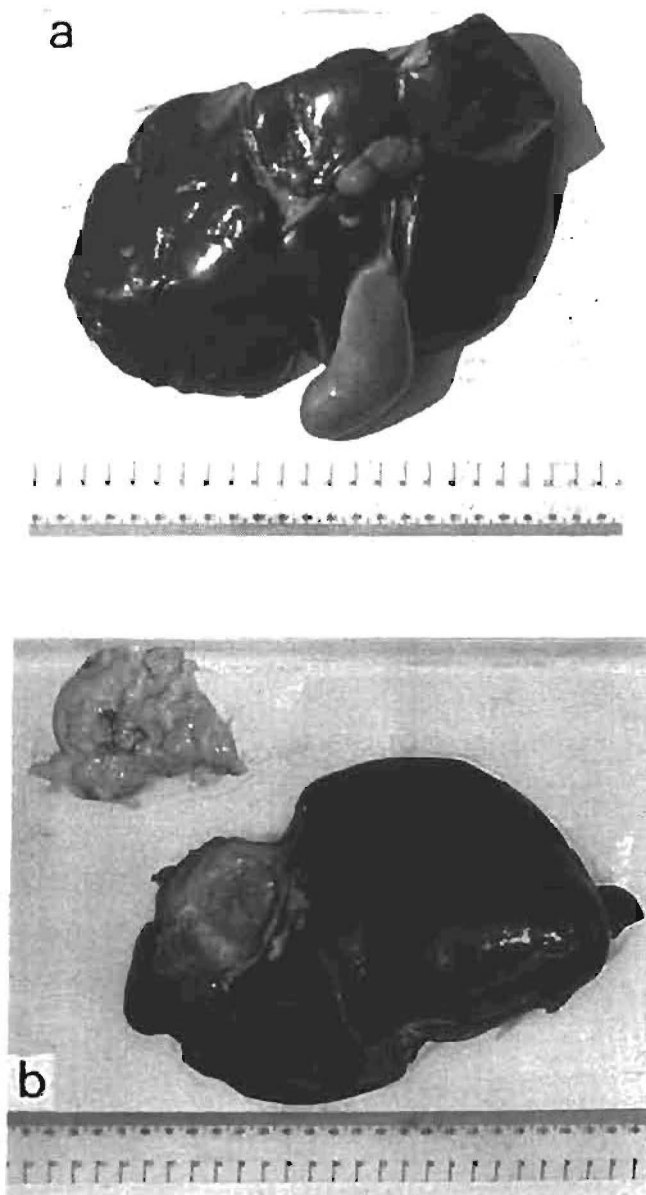


Figure 6 Impala liver heavily infected with *Cooperioides hepaticae*: (a) gallbladder enlarged as a result of the blocked bile ducts filled with adult *Cooperioides hepaticae*; (b) enlarged cyst filled with adult *Cooperioides hepaticae*.

la liver. In the present investigation *C. hepaticae* was found in the liver of 40 out of 46 impala, while only a few adult worms were recovered from the small intestine. Moreover, large numbers of eggs were present in the females, thus proving that the liver is in fact their normal habitat and that reproduction takes place in the bile ducts. From these 40 animals six young impala had a very large infection, including one with an enlarged cyst and gallbladder (Figure 6).

*C. kenyensis* from the abomasum of sheep in Kenya (Daubney 1933) is the smallest *Cooperioides* species (Table 5). *C. hamiltoni* from impala and *C. antidorca* from the springbok, both occurring in the small intestine, closely resemble *C. kenyensis*. *C. hepaticae* from the liver of impala, however, is twice the length of the other two species. *C. kenyensis* is now regarded as a synonym for *C. hamiltoni* (Gibbons (1978)). According to Gibbons (1978) *C. hamiltoni* is now the type species for the genus *Cooperioides*.

*C. hepaticae* and *C. hamiltoni* have not been found to occur naturally in sheep, goat or cattle. Horak (1978) transmitted *C. hamiltoni* successfully to sheep and goat, but could not transmit *C. hepaticae*. These results show that *C. hepaticae* can also be successfully transmitted to sheep.

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