THE BIOLOGY OF HYMENOLEPIS MICROSTOMA (DUJARDIN)

 $\mathbf{B}\mathbf{y}$

J. L. HICKMAN

Department of Zoology, University of Tasmania

(With 7 figures in the Text)

ABSTRACT

The occurrence of cysticercoids of Hymenolepis microstoma (Dujardin) in Cestrinus punctatissimus Pascoe (Tenebrionidae) and the adult cestode in Mus musculus L. respectively, from the Domain, Hobart, Tasmania, is recorded. Five of the 14 mice and 36 of the 1,356 C. punctatissimus examined were found infected.

Both the adult and the larval stages of the cestode are described.

Experimentally infected white mice voided gravid proglottides 17 days after ingestion of the cysticercoids. The cestode grew 100 mm, in length in the first 16 days.

The oncosphere has a U-shaped penetration gland and is surrounded by four membranes.

INTRODUCTION

Whilst examining insects for cysticercoids of *Oochoristica vacuolata* Hickman, the larval stage of another cestode was found. This subsequently proved to be the cysticercoid of *Hymenolepis microstoma* (Dujardin 1845) hitherto unknown to occur in Tasmania or elsewhere in Australia.

The biology and distribution of *H. microstoma* has been described by Joyeux and Kobozieff (1927, 1928) and Dvorak *et al.* (1961). However, in their summary of the distribution of the parasite, the latter authors omitted to mention the occurrence of the adult cestode in the Belgian Congo (Mahon 1954)

In view of the account of Joyeux and Kobozieff, no attempt has been made in the present investigation to study the development of the cysticercoid.

MATERIALS AND METHODS

Cysticercoids identified as those of *Hymenolepis microstoma* were obtained from naturally infected specimens of the tenebrionid *Cestrinus punctatissimus* Pascoe collected from the Domain, Hobart.

Four white mice were raised under controlled conditions in the laboratory and used for experimental infection. Three of them were each fed with an infected specimen of the tenebrionid. The fourth mouse was made to swallow three cysticercoids from the haemocoel of an infected beetle by pipetting them down its throat. The faeces of the mice were then examined regularly for eggs of the cestode. Of the three mice which were fed with infected beetles, one was dissected and examined at the end of 16 days, the other two at the end of 22 days. The fourth mouse, which was fed with three cysticercoids, died at the end of 175 days and was then dissected and examined. A record was made of the location, number, maturity and size of the cestodes present.

During the years 1956 to 1959 a total of 1,356 *C. punctatissimus* were collected from the field and examined. The infection with cysticercoids of *H. microstoma* was recorded and the centres of high infection noted. Snap-traps were then set in these localities and 14 specimens of *Mus musculus* L. were caught. On dissection five were found infected with *H. microstoma*. The stomach content of the mice and the position, number, size and maturity of the cestodes present recorded.

On removal from the host the tapeworms were placed in water and allowed to relax. They were then fixed in Bouin's fluid. Three of the gravid worms from the experimentally infected mice and three from those naturally infected were stained in Ehrlich's haematoxylin and mounted. The scolices of two worms from the experimentally and two from the naturally infected mice were compressed and mounted without staining. The eggs from gravid segments were stained in an aqueous solution (1 : 10,000) of neutral red. Observations were made on both living and preserved cysticercoids. The latter were fixed in Bouin's fluid and mounted either unstained or after staining in alum carmine.

INFECTION OF THE PRIMARY HOSTS

On dissection the four white mice were all found infected. The degrees of infection were 2, 7, 8, 15. The mouse containing two worms had been fed with only three cysticercoids. The number of cysticercoids in the beetles fed to the other mice was not determined.

The 14 mice collected in the field were caught during the period 27/5/'59 to 5/6/'59. Five were found to be infected and these contained 1, 2, 5, 8 and 14 worms respectively. All the worms were gravid.

In both experimentally and naturally infected mice the majority of the cestodes had their scolex attached in the bile duct. The strobila of the longer specimens extended into the duodenum. However, in one instance the posterior part of one worm was observed coiled up in the gall bladder. On another occasion, in a mouse infected with 14 tapeworms, an entire worm was found in the gall bladder. The worm, however, was dead and partly decomposed. Only once was a tapeworm found attached to the duodenum. As this tapeworm was immature and found in the experimentally infected mouse which was dissected only sixteen days after infection, it is probable that it may have failed to establish itself in the bile duct and would have eventually been voided.

The stomachs of the 14 mice collected in the field contained seeds (6), Araneae (6), Lepidoptera larvae (4), Hemiptera (1) and Formicidae (1) (frequency indicated in parentheses). None of the mice contained *C. punctatissimus*.

DESCRIPTION OF THE ADULT CESTODE

The specimens from the naturally infected *Mus musculus* were identical with those from experimentally infected mice. The following is an abridged description of the mature specimens.

Length of cestode, 94-190 mm. Width of strobila. Scolex (excluding rostellum)— 1.25-2.0 mm. length, 138-195 micra; width, 230-270 micra. Suckers—length, 64.4-90.0 micra; width, 55.2-87.0 micra. Rostellum-length, 46-67 micra; width, 46-49 micra. Rostellum sac—length, 103.5-161.0 micra; width, 103.5-138.0 micra. *Hooks*—24-26 arranged in a single crown. *Length of hooks*—from end of handle to point of blade 13.8-15.0 micra; from end of guard to point of blade 5.8-9.2 micra; from end of guard to end of handle 12.7-15.0 micra. Seqments—600-900; broader than long (mature 0.9 x 0.14 mm., gravid 1.53 x 0.24 (mm.). Genital pores unilateral and on the right, \frac{1}{3} of the length of the segment from the anterior margin. Excretory vessels—diameter of dorsal vessel, 5.8 micra; diameter of ventral vessel, 9.2 micra. Genital ducts—pass dorsal to the lateral nerve and both excretory vessels. Testes—number, 3, very rarely 5 or 6; position variable (see later); transversely oval, 115 x 103-127 x 115 micra (in mature segments). Cirrus—short, 46 micra long, surrounded at its base by gland cells. Cirrus pouch—elongated and reaching the lateral nerve and excretory vessels: 138 x 53-161 x 64 micra. Internal seminal vesicle—fills greater part of pouch; length, 115-138 micra. External seminal vesicle—elongated, expanded distally; length (approx.), 173 micra; maximum diameter, 69 micra. Ovary—situated slightly to the poral side of the middle of the segment; transversely elongated and with 2 to 3 lobes; length 69-80 micra; width, 230-299 micra. Vitellinegland—posterior to the middle of ovary, compact and transversely oval; length, 46-58 micra; width, 69-92 micra. A Vaginal sphincter is present at poral end of vagina. Seminal receptacle—elongated, expanded distally; length (approx.), 138 micra; maximum diameter, 83 micra. Uterusdorsal, sac-like. Eggs-oval, 64.4 x 60.0-90.0 x 60.0 micra; with 4 membranes (see later) enclosing the oncosphere; 2-3 polar filaments. *Oncosphere*—25.3 x 24.2-27.6 x 25.3 micra; provided with penetration glands. *Oncospheric hooks*—ventro-lateral hooks, stout, length 18.4 micra; dorso-lateral hooks narrow, length, 17.3 micra; median hooks narrow, length , 20.7 micra (Figs. 1, 2, 3, 4 and 5).



Fig. 1.-Rostellar hooks.

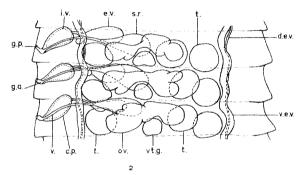


Fig. 2.—Ventral view of mature proglottides (uterus not shown). c.p., cirrus pouch; d.e.v., dorsal excretory vessel; e.v., external seminal vesicle; g.a., common genital atrium. g.p., common genital pore; i.v., internal seminal vesicle; ov., ovary; s.r., seminal receptacle; t., testis; v., vagina; v.e.v., ventral excretory vessel; vt.g., vitelline gland.

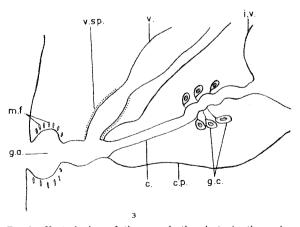


Fig. 3.—Ventral view of the reproductive ducts in the region of the genital atrium in a compressed semi-gravid proglottis. Vagina displaced from its position ventral to the cirrus pouch. c., cirrus; c.p., cirrus pouch; g.a., common genital atrium; g.c., gland cells. i.v., internal seminal vesicle; m.f., muscle fibres around atrium; v., vagina; v.sp., vaginal sphincter.

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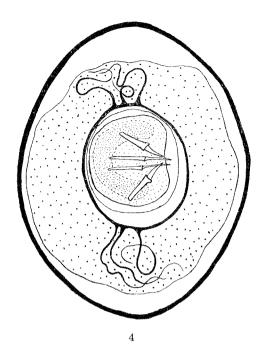


Fig. 4.--Egg from a gravid proglettis.

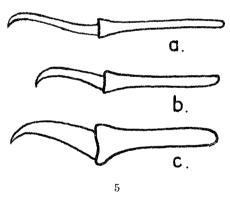


Fig. 5.—Hooks of the oncosphere. a. median hook, b. dorso-lateral hook, c. ventro-lateral hook.

As indicated above some variations in the number and arrangement of the testes were observed. Thus in a strobila in which the testes were evident in 377 of the proglottides, there were three testes in each 374 segments, five in each of two and six in one. In 318 of the segments containing three testes, two testes were situated to the left of the vitelling gland (i.e., aporal) and one on the right (poral); in 53 segments the three testes were aporal; in the remaining 3 segments one testis was aporal and two poral. One of the 2 segments containing five testes had three on the left and two on the right, the other had two on the left and three on the right. In the segment containing six testes, three were on the right and three on the left.

From the outside inwards, the investments of the oncosphere are as follows:—(1) an outer rather rigid oval shaped membrane; (2) a thin flexible membrane closely applied to the inner side of the first investment; (3) a granular fluid; (4) a thick rigid oval shaped membrane, the embryphore, 34.5×30.0 – 37×30 micra, from the poles of which arise 2 to 3 filaments that project into the fluid filled space; (5) a thin membrane closely surrounding the oncosphere (Fig. 4).

GROWTH IN THE LENGTH OF THE ADULT CESTODE

Eggs and partly disintegrated proglottides were found in the faeces of experimentally infected mice after the sixteenth day following infection. The seven cestodes from the mouse dissected and examined on the sixteenth day measured 17, 33, 40, 85, 87, 92 and 112 mm. long respectively. Three chains of segments were also found in the duodenum indicating that shedding had commenced. Eight cestodes from one mouse and 15 from another examined on the twenty-second day varied in length from 50 to 127 mm. The two cestodes from the mouse fed with 3 cysticercoids and examined 175 days afterwards measured 160 mm. in length. It appears that the cestode commences to shed proglottides when it is about 100 mm. long.

INFECTION OF THE INTERMEDIATE HOST

The gravid proglottides of *H. microstoma* are shed from the strobila as chains of segments. These partly disintegrate as they pass through the intestines and are no longer active when voided with the faeces of the host. As a result of the breaking up of the proglottides the eggs become dispersed through faecal material. To become infected, the tenebrionid must feed either on the faeces containing the eggs of the cestode or on some vegetation contaminated with the eggs after disintegration of the faecal pellets.

TABLE 1

Degree of infection of Cestrinus punctatissimus with cysticercoids of Hymenolepis microstoma.

Number cysticercoic speciment C. punctat	ds p	er					c.	Number of specimens of punctatissimus infected
1				 		 		4
2				 		 		3
3								2
4	,			 		 		0
5								1
6				 		 		1
			• • • • •	 		 		į.
7				 	• • • •	 		_
8				 		 		
9				 		 		2
11				 		 		1
13				 		 		1
17								ī
24								0
38	* * * * *				• • • •			-
	• • • • •	• • • •						1
42				 		 		Ţ
50				 		 		1
86				 		 		1
93				 		 		1
98				 		 		1

Thirty-six (2.65%) of the 1,356 Cestrinus punctatissimus dissected and examined were found infected with cysticercoids of H. microstoma. The monthly percentage infection is shown in figure 7. Nearly 39% of the infected specimens were collected during August. The degree of infection ranged from 1 to 98 (see Table 1).

The cysticercoids were free in the haemocoel of the insect and generally among the muscles in the metathorax and the organs in the abdomen. However, on one occasion they were also found among the muscles in the head.

DESCRIPTION OF THE CYSTICERCOID

The body of the fully developed cysticercoid is divisible into an anterior almost spherical cyst enclosing the scolex and a posterior spherical or elongated solid cercomere (Fig. 6).

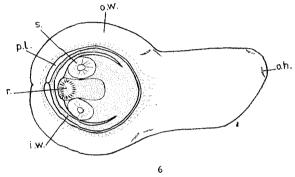


Fig. 6.—Cysticercoid. i.w., inner wall; o.h., oncosphere hook; o.w., outer wall; p.l., primative lacuna; r., rostellum. s., sucker.

The overall length of the cysticercoid varies from 0.3 to 7.7 mm.; the diameter of the cyst from 150 to 225 micra and the size of the cercomere from 0.15-7.4 mm. long and 0.15-0.49 mm. wide.

With few exceptions, cysticercoids with long "tails" were found only in beetles infected with few (9 or less) larvae. Those from heavily infected beetles almost invariably had a short cercomere (i.e., less than 0.5 mm.).

The cyst has an outer and inner wall separated by the nearly obliterated primitive lacuna. The outer wall consists of (1) a cuticle continuous with that covering the cercomere, (2) a cellular layer generally about 11.5 micra thick and continuous with the subcuticular layer of the cercomere, and (3) a layer (approximately 9 micra thick) of muscle fibres which are continuous with the circular and longitudinal fibres in the cercome. The inner wall is thin and encloses the scolex. It is cellular and contains numerous large granules. Posteriorly the wall merges with the base of the scolex.

The external opening of the canal formed when the scolex was withdrawn into the cyst is closed. It is indicated externally only by a slight depression in the outer and inner walls. The inner part of the canal is retained as the cavity in front of the scolex enclosed by the inner wall.

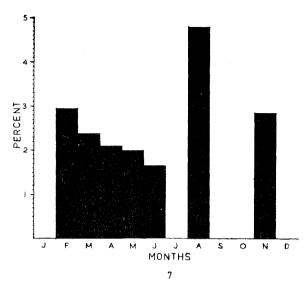


Fig. 7.—The monthly percentages of the total number of Cestrinus punctatissimus collected from 1956 to 1959 and infected with Hymenolepis microstoma.

DISCUSSION

Only six species of cestodes, which in their adult stage normally inhabit the bile duct of their host, have so far been recorded, namely, Hymenolepis microstoma, Progamotaenia festiva (Rud.), Thysanosoma actiniodes Dies., Stilesia hepatica Wolff., Crossotaenia baeri Mahon and Wyominia tetoni Scott. Joyeux and Kobozieff (1928) in their description of H. microstoma state that the scolices may be attached in the duodenum or bile duct. However, Dvorak et. al. (1961) found this cestode attached in the duodenum only twice in the hundreds of infected mice which they examined. Likewise I found attached outside the bile duct only one of the 62 tapeworms from 9 mice found to be infected.

Adults of *H. microstoma* have been recorded from the following mammals:— *Mus musculus* L., *Rattus norvegicus* Erxl., *R. rattus* L. *Mastomys coucha* Smith, *M. coucha ugandae* Wint., *Arvicanthis abyssinicus* Rüpp, *Meriones shawi* Rozet, *Microtus agrestis* L., *Sigmodon hispidus* Say and Ord, *Apodemus* sp., *Promomys* sp., *Dendromus* sp. and *Leggada* sp. see Joyeux and Baer 1936, Mahon 1954, Yamaguti 1959. In addition, Dvorak *et al.* (1961) succeeded in infecting *Mesocricetus auratus* Waterhouse, but found that the cestode had an adverse physiological effect upon this host. The same authors were unable to infect rats with *H. microstoma*.

In Table 2 data on the characteristic features of *H. microstoma* as given by several authors are listed along with data for the specimens from Tasmania. The characteristics of the Tasmanian material are seen to agree essentially with those given by other workers on *Hymenolepis microstoma*. Previous descriptions, however, make no mention of the presence of a penetration gland

in the oncosphere, gland cells at the base of the cirrus and a vaginal sphincter. Moreover, other authors have recognised only two or three membranes around the oncosphere, whereas I observed four. Recently Voge and Berntzen (1961) found that the oncosphere of the related species, H. diminuta, is surrounded by five membranes. It therefore seems likely that a further membrane may yet be found surrounding the oncosphere of H. microstoma.

microstoma they were using. However, they succeeded in infecting Tribolium confusum, T. casteneum and Oryzaephilus surinamensis. As a result of the present investigation the tenebrionid Cestrinus punctatissimus may be added to the list of natural intermediate hosts of this tapeworm.

ACKNOWLEDGMENT

I wish to thank Dr. P. B. Carne of the C.S.I.R.O. for help in identifying the tenebrionid, Cestrinus punctatissimus.

Table 2 Data on characteristic features of H. microstoma. Measurements except where indicated are in micra.

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Feature	Luhe 1910	Joyeux and Kobozieff 1928	Mahon	1 1954 (b)	Dvorak et. al. 1961	Present work
Total length (mm.)	160 2.1 450 —	80-350 2 200 100 60	90 2 274-328 — 59-68	187-288 58-79 86-108	100-280	94-190 1.25-2.0 230-270 46-69 55-90
number	30 11 —	27 15 5 27 x 10	18-25 11-15 —	19-24 10-14 —	23-28 15 — —	24-26 13.8-15.0 5.8 9.2
length width Testes Ovary		135 50 170 x 120 —	144-180 54-58 	100-198 40-65 —	70-130 300 x 55	138-161 53-64 115-127 230-299 x 69-80
Outer membrane of egg: diameter	90 x 82	90 x 80	61-72		75-90	64.4-90.0 x 60.0
Embryophore: diameter	41	40	-			34.5-37.0
Number of polar filaments Oncosphere: diameter	32	2 30	36-40	31-43	3-5	x 30.0 2-3 25.3 x 24.2- 27.6 x 25.3
Embryonic hooks: length Cysticercoid (diameter of		17-20	_			17.3-20.7
anterior cyst-like region)		170-200		*****	-	150-225

(a) * from Arvicanthis & Mastomys; (b) * from Mus & Meriones.

In the present study, the rate at which the cestode was found to grow in its definitive host (100 mm. in 16 days) is comparable with that found by Joyeux and Kobozieff (1928) and Dvorak et al. (1961)

Cysticercoids of *H. microstoma* have been recorded (see Joyeux and Kobozieff (1928), Joyeux and Baer (1936) and Dollfus (1950) from the following insects—:

Coleoptera:

Tenebrionidae—Tenebrio molitor, T. obscurus, Cerandia cornutus.

Geotrupidae-Geotrupes sylvaticus.

Trogositidae—Trogosita mauritanica.

Orthoptera: Dolichopoda linderi. Dermaptera: Anisolabis annulipes.

Aphaniptera: Ceratophyllus fasciatus.

Dyorak et al. (1961) found that Tenebrio molitor was refractory to infection with the strain of H.

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