THE LIFE CYCLE OF *OHbayashinema ERBAEVAE* (NEMATODA, HELIGMOSOMOIDEA, HELIGMOSOMIDAE) IN *OCHOTONA RUFESCENS RUFESCENS* (OCHOTONIDAE)

AUDEBERT F.*, CASSONE J.*, BACCAM D.*, KERBOEUF D.** & DURETTE-DESSET M.C.*

Summary :

The morphogenesis and the chronology of the life cycle of *Ohbayashinema erbaevae* Durette-Desset *et al.*, 2000, a parasite of *Ochotona daurica* from Buriatia were studied in detail in an experimental host, *Ochotona rufescens rufescens*. Worm-free pikas were each infected *per os* with *O. erbaevae* larvae and were killed at one day post infection (DPI 1) and every 12 hours from 1.5 to 8 days post infection. By DPI 1, all the larvae were exsheathed and in the small intestine. The third moult occurred in 2.5-3.0 days. The last moult occurred in 4.0-4.5 days. The prepatent period was eight days and the patent period lasted between two and 12 weeks. The distribution of *O. erbaevae* along the small intestine of the pikas was assessed. For each experiment, a morphological description of the different stages of the life cycle was provided. The morphogenesis and the chronology of the life cycle of *O. erbaevae* appear to be identical with those of two other genera of the family of the Heligmosomidae, *Heligmosomum* Railliet & Henry, 1909 and *Heligmosomoides* Hall, 1916. They confirm that the three genera belong to the same family. The presence of an abortive posterior genital branch in the female of *O. erbaevae*, which represents the posterior part of the genital primordium of the didelphic females, supports the systematic position of the genus *Ohbayashinema* between the didelphic genus *Citellinema* Hall, 1916 and the monodelphic genera *Heligmosomum* and *Heligmosomoides*.

KEY WORDS : *Ohbayashinema erbaevae*, Nematoda, Heligmosomoidea, morphogenesis, life cycle.

Résumé : CYCLE BIOLOGIQUE D'*OHbayashinema ERBAEVAE* (NEMATODA, HELIGMOSOMOIDEA HELIGMOSOMIDAE CHEZ *OCHOTONA RUFESCENS RUFESCENS* (OCHOTONIDAE)

La morphogénèse et la chronologie du cycle biologique d'*Ohbayashinema erbaevae* Durette-Desset *et al.*, 2000, parasite d'*Ochotona daurica* en Bouriatie sont étudiées en détail chez un hôte expérimental, *Ochotona rufescens rufescens*. Des pikas sains sont infestés par voie buccale avec des larves d'*O. erbaevae* et tués à un jour post-infestation (J1) et toutes les 12 heures de 36 heures à huit jours. À J1, toutes les larves sont dégainées et se trouvent dans l'intestin grêle. La troisième mue a lieu entre J2.5 et J3. La dernière mue a lieu entre J4 et J4.5. La période prépatente dure huit jours et la période patente de deux à 12 semaines. La distribution d'*O. erbaevae* tout au long de l'intestin grêle des lièvres siffleurs a été déterminée. Pour chaque expérience, une description morphologique des différents stades du cycle biologique est fournie. La morphogénèse et la chronologie du cycle biologique d'*O. erbaevae* apparaissent identiques à ceux de deux genres de la famille des Heligmosomidae, *Heligmosomum* Railliet & Henry, 1909 et *Heligmosomoides* Hall, 1916. Ces données confirment l'appartenance des trois genres à la même famille. La présence d'une branche génitale postérieure non fonctionnelle chez la femelle d'*O. erbaevae*, branche qui représente le primordium génital postérieur des femelles didelphes, conforte la position systématique du genre *Ohbayashinema* entre le genre didelphe *Citellinema* Hall, 1916 et les genres monodelphes *Heligmosomum* et *Heligmosomoides*.

MOTS CLÉS : *Ohbayashinema erbaevae*, Nematoda, Heligmosomoidea, morphogénèse, cycle biologique.

INTRODUCTION

In the family of the Heligmosomidae, the life cycles of four species have recently been studied by N'Zobadila *et al.* (1996a, b): *Heligmosomum mixtum* (Dujardin, 1845) and *Heligmosomoides laevis* (Dujardin, 1845) parasites of arvicoline rodents in the Palearctic region; *Heligmosomoides p. polygyrus* (Dujardin, 1845) a parasite of murine rodents in the Palearctic region

and *H. p. bakeri* Durette-Desset *et al.*, 1972, a parasite of murine and sigmodontine rodents in the USA. The latter species was also studied by Quinnell *et al.* (1991). All other papers dealing with *H. p. polygyrus* in fact deal with *H. p. bakeri*. The life cycle of *Heligmosomoides kurilensis kobayashii*, a parasite of *Apodemus speciosus* from Japan was studied by Asakawa (1987).

According to Durette-Desset (1974), the genus *Ohbayashinema* Durette-Desset, 1974, parasitic in Holarctic pikas, is morphologically intermediate between the genus *Citellinema* Dikmans, 1935, a parasite of terrestrial Sciuridae and the genera *Heligmosomoides* Hall, 1916 and *Heligmosomum* Railliet & Henry, 1909, mainly parasites of arvicoline rodents. The aim of this work is to compare the life cycles of *Ohbayashinema*, *Heligmosomoides* and *Heligmosomum* to test the systematic position of the genus *Ohbayashinema*.

* Laboratoire de Biologie parasitaire, Protistologie et Helminthologie et CNRS FR 63, Muséum national d'Histoire Naturelle, 61, rue de Buffon, 75231 Paris cedex 05, France.

** Equipe d'Helminthologie, PAP, INRA Tours, BP 1, 37380 Nouzilly, France.

Correspondence : M.C. Durette-Desset.

Tel.: 33 (0)1 40 79 35 09 – Fax: 33 (0)1 40 79 34 99.

e-mail: mcdd@cimrs1.mnhn.fr

MATERIALS AND METHODS

HOSTS

The life cycle of *Obbayashinema erbaevae* was studied in *Ochotona rufescens rufescens* (Gray, 1842). The pikas came from a laboratory colony (Laboratoire de pharmacologie et de toxicologie fondamentales CNRS-Toulouse) (Puget, 1973). Experimental infections were conducted in parasite-free males, two to three months old at the time of infection.

COPROCULTURES

Initial coprocultures were conducted in Buriatia from eggs found in the faeces of the natural host *Ochotona daurica* (Pallas, 1776) according to Brumpt's method (1922), modified by Durette-Desset & Cassone (1987). One hundred sheathed (L3) larvae were obtained and brought to the laboratory. Further coprocultures were performed using the same method for the maintenance of the strain and the larvae were stored at 4°C before use. The free-living stages used for the study of the morphogenesis were obtained from cultures on agar (Hubert & Kerboeuf, 1984).

EXPERIMENTAL INFECTION

The pikas were infected *per os*, (with three to eight weeks old infective larvae) by mean of a gastric canula.

a - Maintenance of the strain: the first two pikas were each infected with 50 infective larvae from *O. daurica*,

the natural host, the 13 others ones with 50, 100 or 200 infective larvae from the experimentally infected *O. rufescens* (Table I).

b - Chronology of the life cycle: fourteen pikas were each infected with 200 sheathed L3 from the experimental hosts except the pika examined at four days post-infection (DPI 4) which was infected with 50 sheathed L3. Pikas were euthanized by CO₂ inhalation, from one day post-infection (DPI 1) to eight days post-infection (DPI 8).

RECOVERY OF PARASITES

The free living stages were collected 12 hours post-hatching (HPH 12) and then every day from one day post-hatching (DPH 1) to DPH 5 (date of the appearance of the first infective larvae). The larvae were drawn alive, after being immobilized by heat between slide and coverglass.

For the recovery of parasitic stages, the small intestine (duodenum to ileo-caecal junction) of the pikas was removed immediately after death and divided into five segments, from the duodenum (SI 1) to the ileo-caecal junction (SI 5). The first segment corresponded with the duodenal loop; the last four segments were of equivalent length. The total of the worms per section were counted and fixed in boiling 70 % ethanol and preserved in it until required for examination.

CALCULATION OF THE PREPATENT AND THE PATENT PERIODS

Two pikas were used to calculate the prepatent period. They were infested by 200 sheathed L3. The eggs were

N ^{os}	Infective dose (L3)	Duration of coproculture (weeks)												Number of collected larvae
		1	2	3	4	5	6	7	8	9	10	11	12	
1	50	299	119	64	127	17	2							628
2	50	20	46	16	2	0								84
3	50	472	318	312	573	349	308	153	56	117	1			2,659
4	50	37	350	431	114	199	76	12	4					1,223
5	50	352	409	90	87	107	45	36	26	5				1,157
6	50	262	489	287	316	453	222	97	21	3				2,150
7	50	282	54	503	540	182	148	284	108	28	18	29	0	2,176
8	50	32	65	3	235	5	4							344
9	50	22	69	7	4	41	16	0						159
10	50	4	0	1										5
11	50	80	26	2										153
12	100	10	175	528	5									718
13	200	73	977	794	326	44	1							2,215
14	200	82	39	26	29	2								178
15	200	200	1,474	745	0									2,423

These larvae were used for the maintenance of the strain and calculation of the patent period. The first two pikas (N^{os} 1 and 2) were each infested with 50 infective larvae from the natural host, the 13 others with 50 (N^{os} 3 to 11), 100 (N^o 12) or 200 (N^{os} 13 to 15) infective larvae from the experimental host.

Table I. – Chronology of exodus of *Obbayashinema erbaevae* infective larvae from coprocultures.

collected by Raynaud's method (1970). The patent period was calculated from the 15 pikas used for the maintenance of the strain (Table I).

The raw data are deposited in the Laboratoire de Biologie Parasitaire, Protistologie, Helminthologie, under the number N18052 and the parasites are deposited in the Helminthological Collections of the Muséum National d'Histoire Naturelle, Paris N 727.

DISTRIBUTION OF THE PARASITE

In order to homogenize the results, only pikas infected with 200 (L3) were used, derived from the 14 pikas utilised for the study of the chronology of the development (prepatent period included). Three pikas were utilised for the maintenance of the strain.

The data were pooled into three populations: A, larvae and immature worms; B, adults during the prepatent period and C, adults during the patent period. Regressions of the numbers of parasites on the lengths of intestines were calculated for the three populations of worms using Excel software.

Symbols used to designate the different stages were: (L3), 3rd stage larvae sheathed in the L2 cuticle; (L4), 4th stage larvae sheathed in L3 cuticle; (Im.), immature worm sheathed in the L4 cuticle. A stage is considered as sheathed when, at the very least, its tail is visible inside the cuticle of the previous stage. HPH: hour post-hatching; DPH: day post-hatching; DPI: day post-infection.

RESULTS

MORPHOGENESIS

Free living stages: L1, L2, (L3) (Figs 1-3)

L1, L2 and (L3) are morphologically very similar. Twenty intestinal cells are present in each stage. The pharynx is well differentiated, being of similar size in L1 and L2, slightly shorter in (L3). L1 and L2

have a rhabditoid oesophagus, in (L3) it is stronglyloid. In all three stages, the length of the oesophagus is about the third of the body length. The excretory pore was observed only in (L3), situated between the nerve ring and the end of the oesophagus (Fig. 2). In the first two stages, the genital primordium is of similar size (10 µm) and occurs at a similar position, slightly below mid-body level (at 45 % from the caudal extremity). For the third stage, the genital primordium is larger (18 µm) and is situated at 41 % from the caudal extremity. L2 is differentiated from L1 only by its width and (L3) is differentiated from L2 by the size increase of approximately 10 %.

Measurements are given in Table II.

Parasitic stages: L3, L4 and Im (Figs 4-17)

Determination of sex: by DPI 1, the majority of the larvae L3 have a simple, more or less oval genital primordium and the pharynx is well differentiated (Figs 4, 5). The sex of L3 is not distinguishable. The sex of L4 is easily distinguishable by DPI 2.5: enlargement of male tail (Figs 9, 10); male anlage a fourth longer on average than female anlage (ratio between the length of the anlage and the length of the body being 21.2 % in the male and 9.8 % in the female) (Table III).

Identification of the stages: L4 is differentiated from L3 by the following points: lack of pharynx (Fig. 6); lateral alae, bifid in L3 (Fig. 7), synlophe in L4 with three ventral ridges orientated from right to left and two tiny ridges, one in front of right lateral field, one in front of left lateral field, orientated perpendicularly to body surface (Fig. 8); sharp increase in both length of larvae and anlagen (Table III). The immature worms are differentiated from L4 by the appearance of a cephalic vesicle and a synlophe (see Durette-Desset *et al.*, 2000) and by the total differentiation of each part of the anlage. In relation to the adults, the immature males have non sclerotized spicules and the immature females have no eggs in the uterus.

	Day post hatching-stage		
	1 L1	2-4 L2	5-9 (L3)
Number of larvae	31	28	17
Total length	0.352 ± 0.024	0.397 ± 0.045	0.432 ± 0.030
Maximum width	0.023 ± 0.002	0.025 ± 0.004	0.024 ± 0.003
Length of pharynx	0.013 ± 0.001	0.013 ± 0.002	0.008 ± 0.001
Distance from nerve ring to anterior end	0.076 ± 0.007	0.080 ± 0.010	0.090 ± 0.010
Distance from excretory pore to anterior end	—	—	0.113 ± 0.012
Length of oesophagus	0.122 ± 0.008	0.130 ± 0.009	0.159 ± 0.007
Distance from genital primordium to posterior end	0.160 ± 0.015	0.181 ± 0.021	0.147 ± 0.023
Length of tail	0.071 ± 0.005	0.072 ± 0.007	0.036 ± 0.006

Mean of measurements ± S.D. L1: larva 1; L2: larva 2; (L3): larva 3 sheathed in the L2 cuticle.

Table II. – Measurements (mm) of free-living stages of *Obbayashinema erbaevae*.

Figs 1-11. – Free-living and parasitic stages of *Obbayashinema erbaevae* Durette-Desset *et al.*, 2000. 1-3. Free-living stages: 1. L1 by DPH 1; 2. (L3) by DPH 4; 3. (L3), head, by DPH 4. 4-11. Parasitic stages. 4-6. Anterior extremities: 4. L3, left lateral view, by DPI 2; 5. L3, head, lateral view, by DPI 2. 6. L4, male, left lateral view, by DPI 3. 7-8. Transverse sections at mid-body. 7. L3 by DPI 1. 8. (L4), male, by DPI 2.5. 9-11. Development of the genital anlage of the male. 9. L4, right lateral view, by DPI 2.5; 10. L4, tail, right lateral view by DPI 2.5; 11. (Im.), tail, left lateral view, by DPI 4.5.

Legend: DPH: days post hatching; DPI: days post infection; (L3): L3 sheathed in the cuticle of L2; (L4): L4 sheathed in the cuticle of L3; (Im.): immature worm sheathed in the cuticle of L4.

Abbreviations: D: dorsal, R: right, L2 S: sheath of L2; br: bursal rays; sv: seminal vesicle (B); t: testis (C); vd: vas deferens (A).

Scale lines: 1, 2: 80 µm; 3, 5: 10 µm; 4: 60 µm; 6: 40 µm; 7: 20 µm; 8: 30 µm; 9: 20 µm; 9A, 9B, 9C: 50 µm; 10, 11: 70 µm.

In addition to the appearance of the cephalic vesicle and the synloph, the main changes concern the size and the formation of the anlage during development

SIZE

By DPI 1, L3 is the same size as free living (L3); by DPI 2.5, L4 is about three times bigger than L3. By DPI 4.5, the immature worm is about three times bigger than L4 (Table II).

MALE ANLAGE AND FORMATION OF CAUDAL BURSA (Figs 9-11)

From DPI 2.5 to DPI 3, the differentiation of the sexual organs is visible: testis (former anterior part of anlage), maturation zone and vas deferens (former posterior part of anlage) (Fig. 9); at the level of the anus, a cavity has formed in the place of the future caudal bursa (Fig. 10). By DPI 4, in sheathed immature worms, the bursal rays begin to differentiate (Fig. 11).

FEMALE ANLAGE AND FORMATION OF THE OVEJECTOR (Figs 12-17)

By DPI 2, in the less well developed L4, the female anlage migrates backwards and elongates (Fig. 12). By DPI 2.5, the development of the anlage is very rapid, particularly in the caudal direction (Figs 13, 14). The cuticle, situated in front of the anlage, from ventral side, is differentiated into a duct (the future *vagina vera*) which divides the anlage into two unequal parts, the posterior one being the shorter (Fig. 14). During the course of organogenesis, this part develops poorly. It is abortive and remains linked to the anterior part (Figs 15, 16, 17). It is still present in the adult. The anterior part is divided into three sections which represent the future ovary, uterus and ovejector (Fig. 14). By DPI 4.5, in sheathed immature worm, the three parts of the anlage are well differentiated and the three parts of the ovejector (vestibule, sphincter and infundibulum) are distinguishable (Fig. 17). Measurements are given in Table III.

CHRONOLOGY OF THE LIFE CYCLE

Free living stages

The eggs hatched within 15 h and 30 min at 24°C. L1 were present until DPH 1. The first moults were observed 24 h after the appearance of L1. L2 were present from DPH 2 to 4. The second moult appeared by DPH 3 and most larvae had moulted between DPH 4 and 5 and the last ones by DPH 9 (Table II).

Parasitic stages

The development of larvae in the digestive tract of the host is indicated in Table IV.

Day post infection	Stage	Number of larvae	Sex	Total length	Maximum width	Length of oesophagus	Distance from excretory pore to anterior end	Length of tail	Length of genital anlage	G/L	Distance from vulvar opening to poserior end
1	L3	4	?	0.433 ± 0.026	0.021 ± 0.001	0.155 ± 0.007	0.101 ± 0.008	0.043 ± 0.005	0.018 ± 0.005	—	—
2	L3	4	?	0.671 ± 0.142	0.024 ± 0.005	0.181 ± 0.028	0.116 ± 0.027	0.041 ± 0.011	0.026 ± 0.009	—	—
2.5	L3 (L4)	5	?	0.949 ± 0.219	0.039 ± 0.010	0.302 ± 0.087	0.210 ± 0.055	0.043 ± 0.008	0.034 ± 0.018	—	—
		7	M	1.497 ± 0.207	0.038 ± 0.004	0.315 ± 0.037	0.190 ± 0.019	0.039 ± 0.011	0.393 ± 0.148	21.2 %	—
3	L4 & (Im)	9	F	1.608 ± 0.292	0.040 ± 0.004	0.314 ± 0.035	0.194 ± 0.027	0.054 ± 0.009	0.287 ± 0.085	9.8 %	0.160 ± 0.014
		4	M	1.643 ± 0.687	0.040 ± 0.007	0.356 ± 0.049	0.218 ± 0.045	0.040 ± 0.007	0.385 ± 0.162	—	—
4	L4 & (Im)	5	F	1.998 ± 0.352	0.037 ± 0.004	0.328 ± 0.038	0.203 ± 0.029	0.063 ± 0.003	0.195 ± 0.116	—	0.165 ± 0.011
		4	M	2.880 ± 0.374	0.050 ± 0.008	0.390 ± 0.045	0.242 ± 0.026	0.045 ± 0.006	1.242 ± 0.476	—	—
4.5	(Im) & Im	2	F	2.750 ± 0.806	0.050 ± 0.000	0.385 ± 0.021	0.240 ± 0.014	0.060 ± 0.000	0.670 ± 0.382	—	0.173 ± 0.016
		10	M	4.831 ± 0.926	0.081 ± 0.013	0.530 ± 0.111	0.348 ± 0.075	—	3.455 ± 1.431	—	—
		10	F	5.186 ± 0.542	0.073 ± 0.013	0.521 ± 0.056	0.346 ± 0.060	0.070 ± 0.007	2.291 ± 0.551	—	0.198 ± 0.038
6	Ad	10	M	6.661 ± 0.854	0.085 ± 0.013	0.601 ± 0.038	0.384 ± 0.025	—	6.094 ± 0.836	—	—
		10	F	7.469 ± 1.039	0.093 ± 0.010	0.665 ± 0.064	0.424 ± 0.036	0.063 ± 0.010	6.158 ± 1.050	—	0.291 ± 0.023

Mean of measurements ± S.D. L3: larva 3; (L4): larva 4 sheathed in L3 cuticle; L4: larva 4; (Im): sheathed immature stage; Im: immature worm; Ad: adult worm; F: female; M: male; G/L: The ratio in percent from the distance between the genital hemi-primordium and the posterior extremity of the body (G) on the length of the body (L).

Table III. — Measurements (mm) of the parasitic stages of *Obhayashinema erbaevae* from experimentally-infected pikas.

Figs 12-17. – Development of the genital organs of the female of *Obbayashinema erbaevae* Durette-Desset *et al.*, 2000. 12. L3, genital primordium, left lateral view, by DPI 2. 13. L4, genital anlage, left lateral view, by DPI 2,5. 14. L4, posterior extremity, left lateral view, by DPI 2,5. 15-17. (Im.), posterior extremities, right lateral view by DPI 4,5.

Legend: DPI: days post infection; (Im.): immature worm sheathed in the cuticle of L4.

Abbreviations: apb: abortive posterior branch; i: infundibulum; ov: ovary; s: sphincter; u: uterus; v: vulva; ve: vestibule.

Scales lines: 12: 60 μ m; 13: 50 μ m; 14: 40 μ m; 15-17: 70 μ m.

Pikas Nos	Infective dose	DPI	Total worms	Success rate	Parasitic stages					
					L3	(L4)	L4	(Im)	Im	A
1	200	1	3	1,5	3 100 %					
2	200	1,5	2	1,0	2 100 %					
3	200	2	2	1,0	2 100 %					
4	200	2,5	24	12,0	5 21 %	19 79 %				
5	200	3	8	4,0			7 88 %	1 12 %		
6	200	3,5	2	1,0			0 0 %	2 100 %		
7	50	4	6	3,0			1 17 %	5 83 %		
8	200	4,5	76	38,0				12 16 %	64 84 %	
9	200	5	56	28,0				14 25 %	42 75 %	
10	200	5,5	2	1,0						2 100 %
11	200	6	62	31,0						62 100 %
12	200	6,5	48	24,0						48 100 %
13	200	7	20	10,0						20 100 %
14	200	7,5	51	26,0						51 100 %
15	200	8	11	6,0						11 100 %

DPI: Day post infection; L3: larva 3; (L4): larva 4 sheathed in the L3 cuticle; L4: larva 4; (Im): immature worm sheathed in the L4 cuticle; Im: immature worm; A: adult worm. On the right hand side of the table, the number in bold print represents the number of worms of a given stage, the number underneath representing the ratio of this stage in relation to the whole round population.

Table IV. – Kinetics of development of *Obbayashinema erbaevae* in experimental host *Ochotona rufescens rufescens* during the prepatent period.

No larvae were recovered from the liver, the heart or the lungs. At DPI 1, the L3 were exsheathed and in the small intestine. Some penetrated the mucosa. By DPI 2.5, most of the larvae were (L4), the third moult occurring between DPI 2.5 and 3. The L4 were visible until DPI 4. By DPI 3, the first sheathed immature worms appeared. They were visible until DPI 5. The last moult occurred between DPI 4 and 4.5. The immature worms became sexual adults by DPI 5.5. The prepatent period lasted eight days (Table IV). The patent period lasted between two and 12 weeks (Table I).

DISTRIBUTION OF WORMS ALONG THE SMALL INTESTINE DURING THE PREPATENT AND PATENT PERIODS

Regression equations of the number of worms on the length of the intestine from the pylorus were calculated for the three parasite populations: (A) larval (L3, L4) and immature (Im.), (B) DPI 6 to DPI 8 adults (prepatent period) and (C) DPI 9 to DPI 34 adults (patent period). We found that the most appropriate curves of the distribution of the parasites along the small intestine were different according to the developmental stages. The most

significant regression curves were third-order polynomial ones for prepatent worms [populations (A) and (B)] while there was a power fitting regression curve for the adult worm population (C). Highly significant regression coefficients were found for all three curves ($P < 0.01$). These curves showed a peak for the larval population (A) in the third segment of the small intestine. When the parasites began to mature, they migrated anteriorly. Nearly 90 % of the worm population (B) were found in the second segment of the small intestine. After the first eggs were produced, the worm population (C) migrated to the first segment (duodenal loop) and the majority of the worms were found in the duodenum.

DISCUSSION

MORPHOGENESIS

The morphogenesis of *Obbayashinema erbaevae* is similar to that of two other genera of the family of the Heligmosomidae, *Heligmosomum*

Railliet & Henry, 1909 and *Heligmosomoides* Hall, 1916 studied by N'Zobadila *et al.* (1996a).

The main features of these genera are as follows: the free living stages have 20 intestinal cells, the L3 and L4 possess no cephalic vesicle, unlike the adults, and the L3 has two lateral bifid alae.

The synlophe of the L4 of *Heligmosomoides laevis*, *Heligmosomum mixtum* and *O. erbaevae*, possesses three ventral ridges orientated from right to left and two tiny ridges, one in front of the right lateral field, the other in front of the left lateral field, orientated perpendicularly to the body surface. The synlophe of the L4 of *Heligmosomoides p. polygyrus*, possesses six ventral ridges orientated from right to left. All the adult worms have a synlophe which possesses an axis orientated from right to left (N'Zobadila *et al.*, 1996a; Durette-Desset *et al.*, 2000).

The tails of the L3 and L4 have a single sharp point. The tails of female adults of Heligmosomidae are rounded with a caudal tip. (N'Zobadila *et al.*, 1996a; Durette-Desset *et al.*, 2000).

The adult female of *O. erbaevae* possesses an abortive posterior branch to the genital system (Durette-Desset *et al.*, 2000). The female of *O. abei* has been described by Fukumoto & Ohbayashi (1986), but the authors did not notice the genital abortive branch. Therefore, we think it may be present in all species of the genus. In the other females of the Heligmosomidae, this posterior branch has disappeared in the adult worms. As was already demonstrated by Durette-Desset & Cassone (1987) in the study of the development in the genera *Neobeligionella* by Durette-Desset, 1970 (Heligmonellidae) and in that of *Heligmosomum* and *Heligmo-*

somoides by N'Zobadila *et al.*, (1996a), in the monodelphic genera, the anlage of (L4) is differentiated into two unequal parts, the posterior one being the shorter. During the course of the development, the latter does not develop any further. In the genus *Neobeligionella*, this part is separated from the anterior branch in the L4 and has disappeared in the immature worm. In *Heligmosomoides* and *Heligmosomum*, it remained attached until the sheathed immature stage and has disappeared in the adult. In *Ohbayashinema*, the posterior branch, although abortive, is still present in the adult. This character supports the systematic position of the genus *Ohbayashinema* between the didelphic genus *Citellinema* Hall, 1916, parasitic in Nearctic ground squirrels and the monodelphic genera *Heligmosomum* and *Heligmosomoides*, parasitic mainly in Holarctic arvicolid rodents.

CHRONOLOGY OF THE LIFE CYCLE

The life cycles of the parasite of the pikas and those of the voles and the field mice, studied by N'Zobadila *et al.* (1996 b) are similar, particularly in the rapidity of their development (length of the prepatent period respectively eight days in *Ohbayashinema*, nine to 12 in *Heligmosomoides* spp. and 13 to 14 in *Heligmosomum*).

The main characteristics of the known life cycles of the Heligmosomidae are the following:

- 1) The free living period lasts three to six days at 24°C.
- 2) The infective larvae are sheathed.
- 3) Infection occurs by the oral route; infection by the cutaneous route has not been attempted.
- 4) All the larvae exsheath in the stomach during the first few hours after infection.

Fig. 18. – Distribution of *Ohbayashinema erbaevae* Durette-Desset *et al.*, 2000 along the small intestine of pikas according to the developmental stages.

The small intestine is divided into five segments: the first segment corresponds to the duodenal loop, segments 2 to 5 are of equivalent length. The x axis corresponds to the different intestinal segments from the pylorus to the caecum; the y axis corresponds to the number of worms.

- Population A: L3, L4, Im. are described by a third order polynomial regression $y = 0.11x^3 - 4.21x^2 + 39.67x - 22.52$ ($r = 0.956$; $P < 0.01$)
- Population B: prepatent adults are described by a third order polynomial regression $y = 0.07x^3 - 2.5x^2 + 18.55x + 34.44$ ($r = 0.998$; $P < 0.01$)
- Population C: Patent adults are described by a power regression with $y = 10.98x^{-0.9395}$ ($r = 0.980$; $P < 0.01$).

5) The larvae are present mainly in the first quarter of the intestine for *Obbayashinema* and from the first half to the entire intestine in other species of Heligmosomidae. 6) The duration of moult 3 is variable but rapid: 12 hours in *Obbayashinema*, one day in *Heligmosomum*, one-two days in *Heligmosomoides*. 7) The duration of moult 4 is variable but rapid: two days in *Obbayashinema*, one day in *Heligmosomum* and *Heligmosomoides*.

DISTRIBUTION OF THE PARASITE (Fig. 18)

The highly significant and different regression curves of the distribution of the worms over the length of the small intestine demonstrates that movements occur during the development of *O. erbaevae* in the pika. Parasites moved progressively towards the pylorus. These distributions correspond with those found in natural infections. The seven pikas caught in Buriatia in 1997 (Durette-Desset *et al.*, 2000) were all parasitised with 2-22 worms ($X = 8$). In six pikas out of seven, the entire population of worms was made up of adults only and was located in the duodenal loop, immediately posterior to the pylorus. The last pika was parasitised by one adult male, four L4 in the second section of the small intestine and two L4 in the third section of the small intestine. This distribution confirms the migration of the worms to the duodenum during the prepatent period.

ACKNOWLEDGEMENTS

We wish to thank Dr A. Puget (Laboratoire de pharmacologie et de toxicologie fondamentale, CNRS-Toulouse-France) for providing the experimental hosts and N. Dognea who collected the infective larvae. We thank also Dr M. Erbaeva (Buryat Geological Institute, Siberian Department, Russian Academy of Sciences, Ulan-Ude, Buriatia) who organised our trip.

REFERENCES

- ASAKAWA M. Genus *Heligmosomoides* Hall, 1916 (Heligmosomidae: Nematoda) from the Japanese wood mice, *Apodemus* spp. III. The life cycle of *Heligmosomoides kurlensis kobayashii* (Nadtochii, 1966) in ICR mice and preliminary experimental infection to jirds. *Journal of the College of Dairying*, 1987, 12, 131-140.
- BRUMPT E. Précis de Parasitologie. (3^{ème} édition), Paris, Masson et Cie. 1922, 605 p.
- DURETTE-DESSET M.C. *Obbayashinema ochotoni* n. gen., n. sp. (Nematoda, Trichostrongyloidea) parasite d'un Lagomorpe du Népal; intérêt phylétique de ce genre. *Bulletin du Muséum National d'Histoire Naturelle*, Paris, 3^{ème} série, Zoologie 144, 1974, 216, 419-424.
- DURETTE-DESSET M.C. & CASSONE J. Sur deux Nématodes Trichostrongyloïdes parasites d'un Muridé africain. II. Chronologie des cycles, description des stades larvaires et des immatures. *Annales de Parasitologie Humaine et Comparée*, 1987, 62, 133-158.
- DURETTE-DESSET M.C., KINSELLA J.M. & FORRESTER D.J. Arguments en faveur de la double origine des Nématodes néarctiques du genre *Heligmosomoides* Hall, 1916. *Annales de Parasitologie Humaine et Comparée*, 1972, 47, 365-382.
- DURETTE-DESSET M.C., GANZORIG S., AUDEBERT F. & KAMIYA M. *Obbayashinema erbaevae* n. sp. (Nematoda, Trichostrongylina, Heligmosomoidea) a parasite of *Ochotona daurica* (Ochotonidae, Lagomorpha) from Buriatia. *Zoosystema*, 2000, 22, 667-676.
- FUKUMOTO S.I., KAMIYA M. & OHBAYASHI M. *Obbayashinema abei* sp. n. (Nematoda: Heligmosomidae) from the Northern pika, *Ochotona hyperborea* Pallas, in Hokkaido. *Japanese Journal of Veterinary Sciences*, 1986, 34, 233-240.
- HALL M.C. Nematode parasites of mammals of the orders Rodentia, Lagomorpha and Hyracoidea. *Proceedings of the United States National Museum*, 1916, 50, 1-258.
- HUBERT J. & KERBOEUF D. A new method for culture of larvae used in diagnosis of ruminant gastrointestinal strongylosis: comparison with fecal cultures. *Canadian Journal of Comparative Medicine*, 1984, 48, 63-71.
- N'ZOBADILA G., BOYER J. & DURETTE-DESSET M.C. Morphogénèse d'*Heligmosomoides polygyrus polygyrus* (Dujardin, 1845) (Trichostrongylina-Heligmosomoidea) chez *Apodemus flavicollis* en France. Comparaison avec les espèces proches : *Heligmosomoides laevis* (Dujardin, 1845) et *Heligmosomum mixtum* Schulz, 1954, parasites d'Arvicolidae. *Bulletin du Muséum National d'Histoire Naturelle*, Paris, 4^{ème} série, section A, 1996a, 18, 367-385.
- N'ZOBADILA G., BOYER J., VUONG P.N. & DURETTE-DESSET M.C. Chronologie du cycle et étude des pseudo-kystes d'*Heligmosomoides polygyrus polygyrus* (Dujardin, 1845) (Trichostrongylina-Heligmosomoidea) chez *Apodemus flavicollis* en France. Comparaison avec les espèces proches. *Parasite*, 1996b, 3, 237-246.
- PUGET A. Étude anatomique, physiologique et biochimique de l'Ochotone afgan (*Ochotona rufescens rufescens*) en vue de son utilisation comme animal de laboratoire. Thèse de Doctorat de l'Université de Toulouse, 1973, 572 p.
- QUINNELL R.J., BEHNKE J.M. & KEYMER A.E. Host specificity of and cross-immunity between two strains of *Heligmosomoides polygyrus*. *Parasitology*, 1991, 102, 419-427.
- RAILLIET A. & HENRY A. Sur la classification des Strongylidae et Metastrongylidae. *Comptes Rendus de la Société Biologique*, 1909, 66, 85-88.
- RAYNAUD J.P. Étude de l'efficacité d'une technique de coproscopie quantitative pour le diagnostic de routine et le contrôle des infestations parasitaires des bovins, ovins, équins et porcins. *Annales de Parasitologie Humaine et Comparée*, 1970, 45, 321-342.

Reçu le 10 mars 2001

Accepté le 14 juin 2001