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Mémoire.

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THE FATE OF THE FILARIA *MONANEMA MARTINI* IN TWO RODENT HOSTS: RECOVERY RATE, MIGRATION, AND LOCALIZATION [1]

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SUMMARY

In *Meriones unguiculatus,* the recovery rate of 80 inoculated larvae was low (about 20 %) and irregular. In the natural host *Lemniscomys striatus,* the recovery rate was about 50 % with inoculated doses of 30, 80 or 400 L3, but slightly higher for 400 L3. This rate was constant from day 2 to month 8 post infection (p. i.). When 7-9 reinoculations were performed in one year, the recovery rate of the late inoculation was of only 14 %.

After subcutaneous inoculations, larvae penetrated into the peripheric lymphatic vessels from hour 6 p. i. and migrated to the lombar and mesenteric lymphnodes; this first migratory phase was achieved 5 days p. i. Later, the larvae migrated into the digestive tract lymphatic system. Filarial localization did not depend upon the L3 dose: half were found in the caecum and anterior colon (3 cm) wall, and half were distributed in the posterior colon, mesentery and small intestine. A small number (3-5 %) of the filariae were found in the pulmonary blood vessels, as a result of accidental migration by the thoracic canal. A similar phenomenon is known in the lymphatic filariae *Brugia* spp. in rodents and *Conispiculum flavescens* in a lizard. Several arguments suggest that the genus *Monanema* is fundamentally lymphatic.

Migrations and life of filariae in the lymphatic system seems to be more usual than it is generally admitted. In onchocerciasis, this may at least partially explain the lymphopathology of the inguinal region.

RÉSUMÉ : **La filaire *Monanema martini* chez deux rongeurs-hôtes : rendement, migration et localisation.**

Chez *Meriones unguiculatus,* le taux de développement des larves inoculées est faible (20 % en moyenne) et irrégulier. Chez l'hôte naturel *Lemmniscomys striatus,* quelle que soit la dose inoculée (30, 80 ou 400 L3), ce taux avoisine 50 % ; toutefois il est légèrement augmenté pour la dose de 400 L3. Le rendement est stable du 2e jour au 8e mois suivant l'inoculation. Sept à 9 réinoculations faites sur 1 an abaissent à 14 % le rendement du dernier inoculat. A la suite des inoculations sous-cutanées, les larves pénètrent dans les vaisseaux lymphatiques périphériques dès la 6e heure et migrent vers les lymphocentres lombaires et mésentériques ; cette première phase de migration s'achève en 5 jours. Les larves s'enfoncent par la suite progressivement dans le système lymphatique des parois du tube digestif. La répartition des filaires est stable quelle que soit la dose de L3 : la moitié vit dans la paroi du cæcum et des 3 cm antérieurs du côlon ; le reste se répartit entre le côlon postérieur, le mésentère et l'intestin grêle. 3-5 % des filaires sont dans les vaisseaux sanguins pulmonaires, à la suite de remontées accidentelles par le canal thoracique. Un phénomène analogue s'observe avec les filaires lymphatiques, *Brugia* spp. chez les rongeurs et *Conispiculum flavescens* chez un lézard. Plusieurs arguments suggèrent que le genre *Monanema* est fondamentalement lymphatique. Chez les filaires, les migrations et la vie dans le système lymphatique semblent plus générales qu'il n'est couramment admis. Elles pourraient, dans l'onchocercose, contribuer à expliquer la lymphopathologie de la région inguinale.

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INTRODUCTION

Monanema martini Bain, Bartlett & Petit, 1986 is a filaria with skin-dwelling microfilariae which cause in its rodent hosts cutaneous and ocular lesions resembling those of onchocerciasis (Vuong *et al.,* 1985 et 1986; Sakka, 1989).

This filaria realizes an interesting new tool: contrary to the usual models, such as bovine onchocerchiasis (Copeman, 1979) or *Onchocerca* spp. microfilariae inoculated into white mice (Bianco *et al.,* 1986), it allows one to study simul-

taneously the parasitological, immunological, and host-species factors, thus measuring their relative importance.

The biology of *M. martini* in the rodent is to date briefly described (Bain, 1989; Kläger *et al.,* 1989): the filaria is maintained in one of its natural hosts, the small prolific murid *Lemmniscomys striatus,* which regularly develops patent infection with 30 inoculated infective larvae; filariae are adult in 3 weeks post inoculation; they live in the lymphatic vessels of the large intestine (cæcum and anterior colon), rarely in the blood system (lungs and heart). Microfiladermia generally occurs from 50 days post-inoculation to one year with a peak density between 6-10 months p. i. in the ear-skin; the microfiladermia generally ends one year p. i. *Meriones unguiculatus* were also used as experimental hosts; 40 % of them developped microfiladermia when inoculated with 30 L3.

The aim of this study was to analyze quantitatively the relationships between the inoculated infective larvae and the recovered filariae, as well as the migration route and localization of *M. martini.*

MATERIAL AND METHODS

The infective larvae of *M. martini* were obtained from amblyommids (Bain *et al.,* 1985) and inoculated subcutaneously into 3-12 weeks old rodents *(L. striatus* and *M. unguiculatus)* in a precise region of the body, the right side of lombar area; one animal was inoculated in the right axillar area *(table I).*

A — Doses of inoculated infective larvae and post-inoculation times of examination

1) L. striatus: these animals were inoculated once or several times.

a) Mono-inoculated group *(table I:* n° 1-38, L.s.): inoculated doses were 30, 80 or 400 infective larvae. Animals were dissected at 2-15 days p. i. (larval filarial stages), 21-240 days p. i. (adult filarial stage), and one year p. i. (old infections).

b) Multi-inoculated group *(table I:* n° 39-46, L.s. Z and X): inoculations were performed during one year, the total number of L3 varying from 120 to 160; the first inoculation consisted of 30 L3, the last one of 30 or 80 L3 (= challenge dose); between these two, the rodents received 5-6 inoculations of 10-30 L3 at 5 to 150 days intervals. All these animals had microfilariae. Necropsies were performed 9-15 days after the last inoculation (to allow recognition of the corresponding larvae), or later.

2) M. unguiculatus (table I: n° 47-52, M. u.): these animals received one dose of 80 L3 and were dissected at 5 to 30 days p. i.

B — Necropsies

The organs of the chloroformed rodents were dissected separately in a warmed medium (RPMI plus 20 % calf serum, at 28-30° C). The caecum, anterior colon (3 cms long and characterized by the presence of internal spirally coiled ridges), heart, lungs, liver, spleen, and kidneys were observed in each animal; skin and carcass were studied in animals necropsied at 2 to 15 days p. i.; the following organs were not examined in the first necropsied animals but were later included in the study *(table I):* posterior colon, small intestine, stomach, and mesentery. Each organ was examined twice at 12 hour intervals under the stereomicroscope.

Table I. — *Individual results of the dissected rodents inoculated with* M. martini: *number of recovered filariae in each organ and percentage of total recovery related to the number of inoculated L3 (F/L3). N° R: rodent; number 30 with ° indicates inoculation in the right axillar region instead of in the right lombar region; n : number of inoculations per rodent; Exp: experiment; L.s.: mono-inoculated* L. striatus; *L.s. Z: multi-inoculated* L. striatus *with result of the challenge dose; Ls X:* idem *with result of all the doses; M.u.:* M. unguiculatus; *n L3: number of inoculated infective larvae; D: number of post-inoculation days; Cae: caecum; a. C: anterior colon; p. C: posterior colon; In : small intestine; st: stomach; Mes: mesentery; Lu: lungs; He: heart; Sc: subcutaneous tissue (skin + carcass); nF: total number of filariae; F/L3: observed percentage (with 5 % added, when intestine is not dissected). Liver, spleen, kidneys never had filariae thus are not presented in the table. The O % recovery rate in the rodent n° 4 is interpreted as an experimental error and not included in the analysis.*

N°R	n	I	Exp	n L3	D	Cae	a C	p C	In	S t	Mes	Lu	He	S c	n F	F/L3
1	1		L. s.	30	2	0	0	/	/	/	-	0	0	3	/	/
2	1		L. s.	30	2	1	0	1	0	1	0	0	0	5	8	26,7
3	1		L. s.	30	5	0	2	0	9	0	-	0	0	0	11	36,7
4	1		L. s.	30	5	0	0	0	0	0	-	0	0	0	0	0
5	1		L. s.	30	5	1	0	0	0	0	11	0	0	0	12	40
6	1		L. s.	30	5	0	0	1	0	0	6	0	0	0	7	23,3
7	1		L. s.	30	15	5	8	1	0	0	2	0	0	0	16	53,3
8	1		L. s.	30	30	9	2	0	4	0	5	0	0	0	20	66,6
9	1		L. s.	30	30	6	13	0	/	/	-	0	0	0	/	69,6*
10	1		L. s.	30	60	2	8	4	0	0	-	0	0	/	14	46,7
11	1		L. s.	30	88	8	3	11	2	0	2	1	0	/	27	90
12	1		L. s.	30	150	5	7	1	/	/	-	0	0	/	/	46,7*
13	1		L. s.	30	180	7	5	0	/	/	-	0	0	/	/	44*
14	1		L. s.	80	2	0	0	/	/	/	-	0	0	17	/	/
15	1		L. s.	80	5	1	0	0	0	0	50	1	0	1	53	66,2
16	1		L. s.	80	5	0	6	/	/	/	-	0	0	0	/	/
17	1		L. s.	80	12	17	8	9	0	0	-	1	0	0	35	43,5
18	1		L. s.	80	15	0	23	/	/	/	-	0	0	0	/	/
19	1		L. s.	80	28	13	10	10	4	0	5	0	0	0	42	52,5
20	1		L. s.	80	51	7	11	7	2	0	8	1	0	0	36	45
21	1		L. s.	80	60	12	14	11	0	/	-	0	0	/	37	46,2
22	1		L. s.	80	180	8	3	18	/	/	-	2	0	/	/	42,8*
23	1		L. s.	80	180	7	0	21	/	/	-	5	0	/	/	45*
24	1		L. s.	80	180	6	10	11	/	/	-	1	0	/	/	38*
25	1		L. s.	80	180	18	12	8	/	/	-	0	0	/	/	52,5*
26	1		L. s.	400	2	10	5	6	6	2	14	3	6	119	171	42
27	1		L. s.	400	3	5	2	5	0	0	86	3	0	37	137	34,5
28	1		L. s.	400	4	15	0	5	0	0	135	2	0	40	197	49,2
29	1		L. s.	400	5	15	46	/	/	/	-	4	0	6	/	/
°30	1		L. s.	400	5	5	3	3	0	0	191	0	0	2	204	51
31	1		L. s.	400	5	136	12	21	8	3	-	0	0	31	212	53
32	1		L. s.	400	21	79	46	24	6	0	45	7	1	0	208	52
33	1		L. s.	400	72	59	8	39	25	0	18	25	3	0	178	44,5
34	1		L. s.	400	240	50	35	29	13	0	-	12	25	0	164	41
35	1		L. s.	60	420	3	1	11	0	0	7	2	0	0	24	40
36	1		L. s.	80	378	11	25	7	0	0	2	1	0	0	46	58
37	1		L. s.	80	372	3	8	9	1	0	2	0	0	0	23	29
38	1		L. s.	80	374	4	0	4	3	0	1	5	0	0	17	21
39a	7		Ls Z	80	9	0	0	1	0	0	4	1	0	0	9	30
40a	8		Ls Z	30	10	0	0	1	0	0	6	0	0	0	1	3
41a	9		Ls Z	80	11	2	0	1	1	0	8	0	0	0	7	23
42a	9		Ls Z	30	13	0	0	0	0	0	0	1	0	0	6	8
43a	9		Ls Z	30	20	0	2	1	0	0	5	1	0	0	12	15
39b	6		Ls X	161	455	1	5	18	5	0	4	5	0	0	38	24
40b	7		Ls X	148	410	12	10	16	4	0	2	0	0	0	44	30
41b	8		Ls X	161	475	1	5	18	5	0	4	5	0	0	38	24
42b	8		Ls X	125	335	11	2	13	4	0	16	1	0	0	47	38
43b	8		Ls X	124	385	10	6	8	2	0	3	1	0	0	30	24
44	8		Ls X	160	270	15	27	12	0	1	5	2	0	0	62	39
45	7		Ls X	133	365	9	9	11	0	0	18	3	0	0	50	38
46	7		Ls X	145	372	4	0	3	2	0	6	2	0	0	17	12
47	1		M.u.	80	5	1	1	0	0	0	6	0	0	0	8	10
48	1		M.u.	80	5	0	0	0	0	1	9	0	0	1	11	14
49	1		M.u.	80	14	2	0	2	0	0	26	0	0	0	30	38
50	1		M.u.	80	15	0	0	0	0	0	1	0	1	0	2	3
51	1		M.u.	80	21	13	0	2	0	0	3	0	0	0	18	23
52	1		M.u.	80	30	8	5	7	1	0	6	2	0	0	29	36

C — Statistical analysis

Analysis was performed with the statistical package STAT-ITCF (1988), using either classical methods (analysis of variance: Anova, chi-squared) or multivariate analysis (principal components analysis, multiple correspondance analysis).

RESULTS

Table I gives for each rodent the number of inoculated L3 and of parasites in each organ, the total worm burden, and the percentage of infective larvae which were recovered.

1 — Recovery rates of inoculated larvae *(tables II and III)*

— *The mono-inoculated* L. striatus :

The results were based upon rodents totally dissected as well as those in which the stomach and small intestine were not observed; this was possible because the stomach is generally not parasitized and in the small intestine the rate of parasitism is usually low (around 5 % of the inoculated larvae); thus, in these cases the observed rates were corrected by adding 5 %.

Table II. — *Mean recovery rate of inoculated larvae in mono-inoculated* L. striatus *during larval stages (D: 2-15) and adult stage (D: 21-240) (abbreviations in table I).*

N° R	n L3	D	n R	F/L3
2-7	30	2-15	6	36±14,7
8-13	30	21-240	6	60,6±19
Σ	30	2-240	12	45,3±14,9
15,17	80	2-15	2	54,8±144,5
19-25	80	21 à 240	7	46±4,7
Σ	80	2-240	9	47,9±6,3
26-28,30,31	400	2-15	5	45,9±9,46
32-34	400	21 à 240	3	45,8±10,3
Σ	400	2-240	8	45,9±5,9

Table III. — *Mean recovery rate of inoculated larvae in the different groups of* L. striatus *and in mono-inoculated* M. unguiculatus *(abbreviations in table I).*

Exp	D	n I	n L3	n R	F/L3
L.s.	≤ 240	1	30-400	29	46,3±5,9
L.s.	≥365	1	60-80	4	36,8±25,1
Ls Z	≤ 240	7-9	30-80	5	14±13,6
M.u.	≤ 240	1	80	6	20,4±14,9

Anovas were done on the percentage of recovered larvae with the three infective doses and the different periods of post-inoculation examination. The data obtained later than 374 days p. i. were not included as they were much lower than those recorded before 240 days.

For the *L. striatus* necropsied between 2-240 days p. i. there was no significant effect of doses and period (at $p \leq 0.05$), even when interaction between the two parameters was taken into account. The mean recovery rate was 46 %.

One year p. i., this rate was reduced to 36 %. The range of variation was particularly wide: 21 and 28 % in the two animals with decreasing microfiladermia, 40 % and 57,7 % in the two others with stable microfiladermia.

— *The multi-inoculated* L. striatus :

The rates of development in this group were established on the last inoculated dose and during the larval stages; thus they were compared to those of the mono-inoculated *L. striatus* necropsied between days 2 to 15 p. i. These two groups showed a disparity ($F = 9,10$ at $p \leq 0.05$).

The mean recovery rate of the 7-9 th inoculation was reduced to 14 % and showed wide individual variations.

— *The mono-inoculated* M. unguiculatus :

This group was also necropsied early; it showed a disparity with the equivalent mono-inoculated *L. striatus* ($L = 5,99$ at $p \leq 0.05$). The mean recovery rate was 20 % with wide individual variations.

2 — Filarial migration and localization *(fig. 1, 2 and 3)*

— *Description based on the 400 L3 inoculated* L. striatus :

At day 2 p. i., 70 % of the recovered filariae were harvested from the soaking carcass and skin, the remaining (30 %) from the digestive tract (stomach to posterior colon), mesentery, heart, and lungs. At days 3-5, the larvae accumulated in the mesentery with a peak at day 5; they were localized in the lymphatic vessels and nodes of lombar, cranial mesenteric and caudal mesenteric lymphocenters (placed respectively between the kidneys, at the level of the caecal curve, and near the rectum); several times, groups of larvae (up to 40) were extracted from these nodes or their afferent vessels). The animals inoculated in the axillar region and necropsied 5 days p. i. showed a similar mesenteric concentration of the larvae.

At day 21 p. i., fewer filariae were present in the mesentery (21 %); on the contrary, 74 % of them were in the

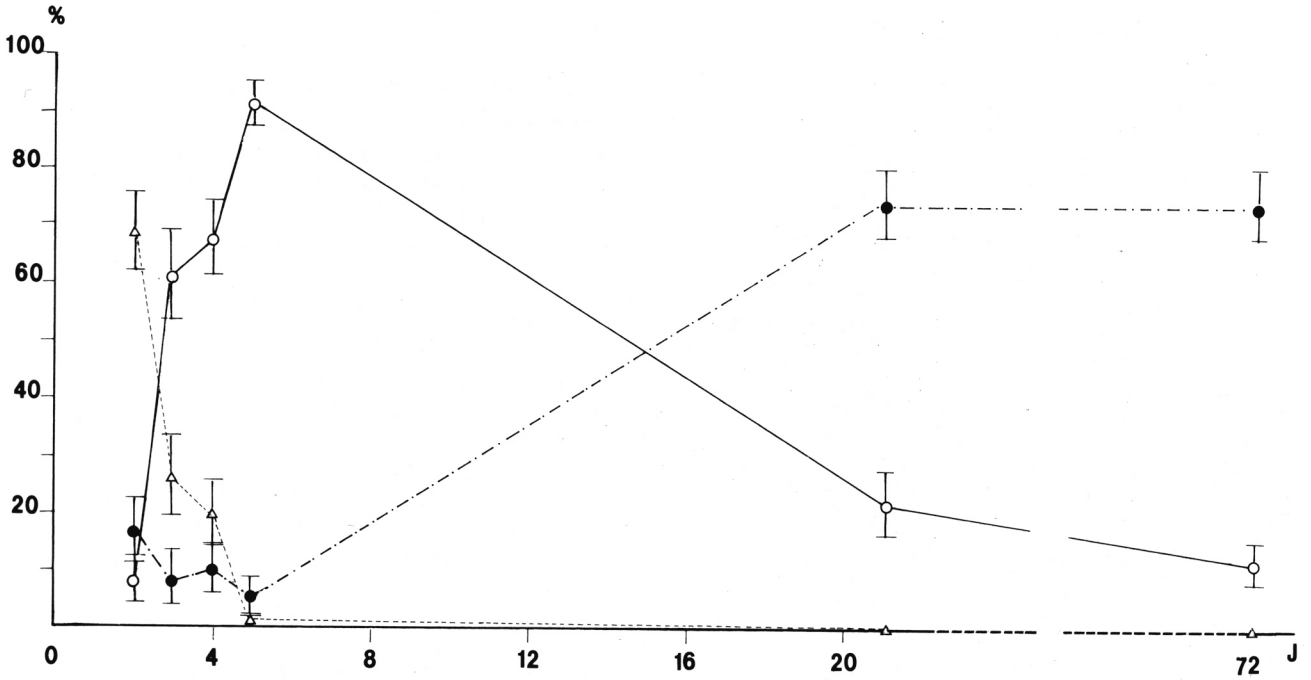

FIG. 1. — *M. martini* in 400 L3 inoculated *L. striatus:* chronological evolution of the filarial distribution in subcutaneous tissue (△-------), mesentery (○——) and digestive wall (●—.—.). Abscissa: p. i. days; ordinate: percentage of filariae in each organ related to the total worm burden.

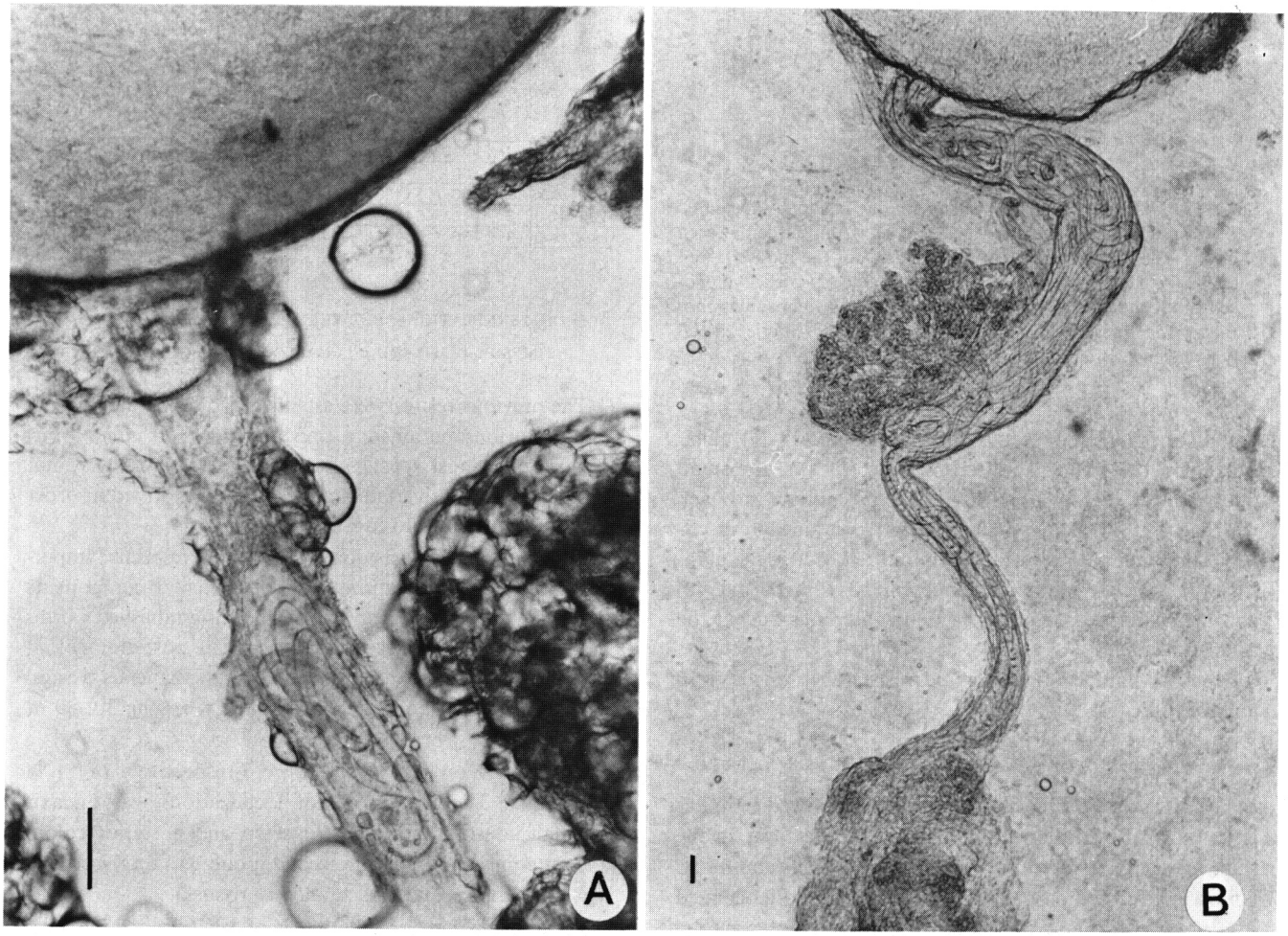

FIG. 2. — *M. martini* in *L. striatus;* A: larva in lymphatic afferent vessel of a mesenteric lymphnode, 4 days p. i.; B: adult filaria in similar localization, in a multi-inoculated animal (bar = 100 μm).

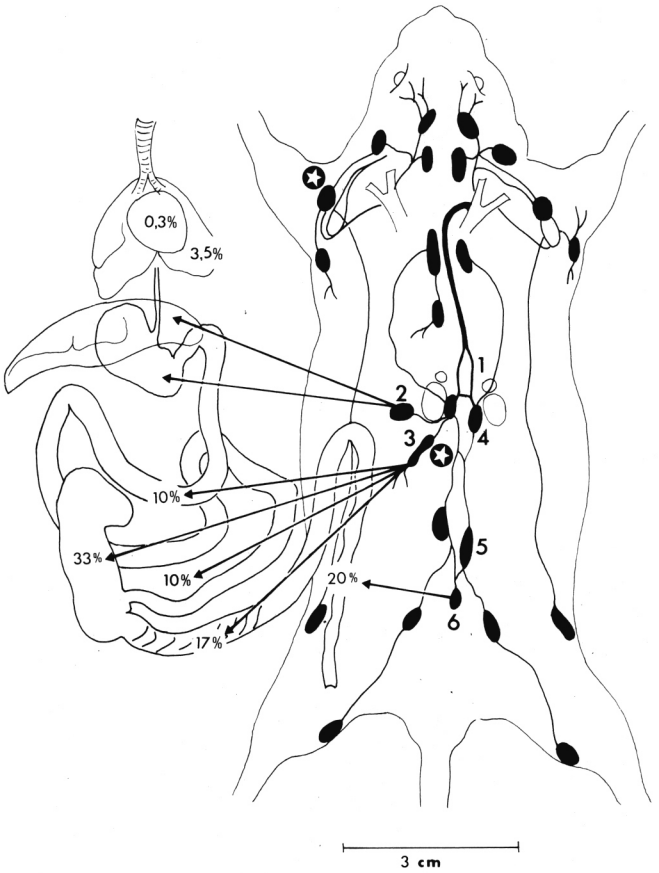

FIG. 3. — Distribution of *M. martini* filariae in mono-inoculated *L. striatus* expressed in percentages of the worm burden in each organ. The lymphatic system is after Cuq for the mouse (1966, fig. 4). *: inoculated area; 1: Pecquet cisterna; 2: coelic lymphocenter; 3: mesenteric cranial lymphocenter; 4: lombar lymphocenter; 5: internal iliac lymphocenter; 6: mesenteric caudal lymphocenter (sacral lymphocenter).

digestive wall, predominantly in the caecum (38 %), anterior colon (22 %), and posterior colon (11 %); the small intestine was slightly parasitized (2,8 %) and the stomach was negative; the lungs and the heart contained respectively 3,3 % and 0,5 % of the worm burden. Later, the parasitism decreased slightly in the mesentery (10 % at day 72) and increased to a mean of 10 % in the lungs and in the small intestine.

Molts III and IV first occured respectively on days 10 and 21 p. i.

— *Comparison between the mono-inoculated* L. striatus :

The 30 and 80 L3 inoculated *L. striatus* showed a similar chronological evolution of the filarial localizations in the skin, mesentery, wall of the digestive tract, lungs and heart. Anovas performed on the three groups of mono-inoculated *L. striatus* and the adult filarial caecal data, for example, showed that the rate of parasitism was not dose dependant ($F = 1,31 < 9,55$).

In the three groups, about half of the recovered adult filariae were in the wall of the caecum and anterior colon; the others were mainly shared between posterior colon and mesentery and, at a low level, the small intestine *(fig. 3)*. Pulmonary parasitism was low, around 3 % during the first 8 months but it increased to 7 % in the one year old animals. Heart parasitism was extremely low (0,3 %).

— *Comparison with* M. unguiculatus *and the multi-inoculated* L. striatus :

The known data of *M. unguiculatus* (days 2 to 30 p. i.) and of the multi-inoculated *L. striatus* (days 9 to 20 p. i.) fit with the others *(table I)* except the pulmonary filarial rate which was particularly high for these young animals: 7 and 9 % respectively.

— *Sex-ratio*

This was studied in *L. striatus :* male and female worms were distributed similarly in each organ *(table IV)*.

TABLE IV — Monanema martini: *Male and female distribution in* Lemniscomys striatus. *For each organ: on left the male mean number, on right the female mean number. *: results based on some animals of the batch (abbreviations in table I).*

Exp	nL3	n R	Cae	a C	p C	In	Mes	Lu
L.s.	30	6	20 / 17	22 / 16	10 / 6	3 / 2 *	3 / 3 *	1 / 0
L.s.	80	7	36 / 35	30 / 30	48 / 38	5 / 1 *	4 / 9 *	4 / 6
L.s.	400	2	45 / 64	20 / 23	34 / 34	16 /13	11 / 7	17 / 20
Ls X	140	8	36 / 38	31 / 28	55 / 29	7 / 8	19 / 26	24 / 16

3 — INTERRELATIONS BETWEEN INOCULATED DOSES, TIMES P. I., FILARIAL LOCALIZATIONS, AND HOST SPECIES

The previous results were established on separate analysis of the various parameters (inoculated doses, times p. i., localizations, host species). All these parameters were not independant. Multivariate analyses were performed in order to control the results of Anovas.

a) A first analysis was done to assess the respective importance of each localization. Thus a principal components analysis was performed on the number of established worms in several organs (caecum, anterior and posterior colon, small intestine, mesentery, lungs, and subcutaneous tissues) for the 33 mono-inoculated *L. striatus* receiving 30, 80 or 400 L3.

The inertia on axes 1 and 2 were respectively of 51 % and 18 %. The first group of localizations was on axis 1 (small intestine and lungs, caecum and posterior colon, and anterior colon) and the second group was on the second axis (mesentery and subcutaneous tissues).

It was clear that for each batch of *L. striatus* three localizations were enough to describe the migration: caecum (Ca), anterior colon (a C) and mesentery (Mes).

b) The aim of the second analysis was to determine whether the type of infection (inoculated doses -DO-, single infection -Ls- or reinfection -LsZ), the period p. i. at which necropsies were made (PI), or host species (Mu, Ls) might be related to the percentage of recovered larvae (IS) and to the localization of worms. A multiple correspondance analysis was performed on the results of 43 necropsies of *L. striatus* and on those of *M. unguiculatus.* The active parameters were the percentages of worms established in one organ (caecum, anterior colon, mesentery) at a specified period and the rate of establishment of the parasite in all the organs. The values for each parameter were dispatched into four classes of equal or nearly equal numbers of individuals. The supplementary variables were hosts *(L. striatus, M. unguiculatus)* and types of infection.

Inertias on axes 1 and 2 were respectively of 22 and 15 %. The significant classes of the studied parameters are illustrated in fig. 4 as are all supplementary variables. It can be concluded from this figure that: — the significant parameters (over 4 % relative contribution per class of each parameters) on axis 1 were worms in the caecum (0-2 % and 33-72 % of worm burden), worms in the anterior colon (0 and 22-60 % of worm burden) and those in the mesentery (over 0-8 and 63-96 %). Axis 1 represents increasing success of infection (IS1 to IS4).

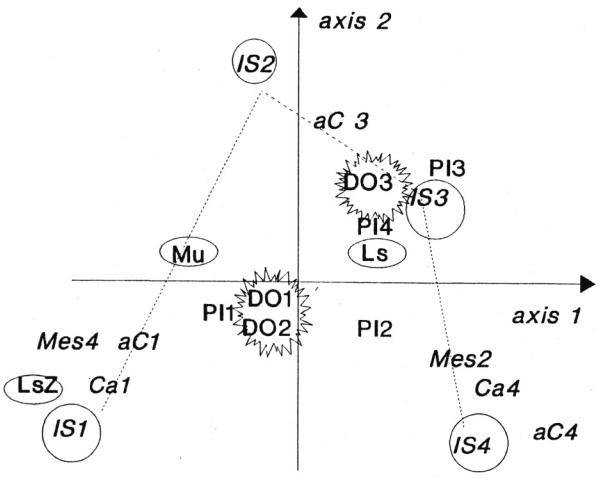

FIG. 4. — Localizations of filariae according to hosts, inoculated doses and days post-infection (Multiple correspondence analysis).

— Active variables (when significant):

Percentage of worms in caecum: Cae1: 0-2; Cae4: 33-72; percentage of worms in anterior colon: a.C.1: 0; a.C.3 11-22; a.C.4: 22-60; percentage of worms in mesentery: Mes2: > 0-8; Mes4: 63-96.

Infection success (larval dose/recorvered worms at necropsy × 100): IS1: 1-23; IS2: 23-41; IS3: 41-49; IS4: 49-90.

— Supplementary variables :

Hosts: Ls: mono-inoculated *Lemniscomys striatus;* LsZ: pluri-inoculated *Lemniscomys striatus;* Mu: mono-inoculated *Meriones unguiculatus.*

Doses: DO1: 30 L3/DO2: 80 L3; DO3: 400 L3

Days post-infection: PI1: < 15 days; PI2: 15-60 days; PI3: 61-240 days; PI4: > 240 days.

These analyses confirmed that *L. striatus* infected once was a more suitable host than the same species infected 7-9 times or *M. unguiculatus (fig. 4).* They also showed a slight difference between the 30-80 L3 mono-inoculated *L. striatus* and the 400 L3 inoculated ones, these last associated with high success of infection (IS3).

The higher success IS4 was not related to any infection parameter and is likely due to host factors.

DISCUSSION

1 — Quantitative relations between inoculated larvae and developed filariae

Numerous studies on the relations between the number of inoculated infective larvae and filariae recovered in the host were done on the various filarial models, particularly after repeated inoculations, in order to study the protective immunity or immune unresponsiveness (see the critical review of Philipp *et al.,* 1988). Reinoculations increase the total worm burden (up to one thousand worms were recovered in the example of *Litomosoides sigmodontis, in* Bertram, 1966); when the developmental rate of the challenge inoculate is studied, it appears generally reduced compared to that of a single dose (Kowalski & Ash, 1975; Ewert & Bosworth, 1975; Lucius *et al.,* 1986) or enhanced (Klei *et al.,* 1980).

With *M. martini — L. striatus,* a wide range of L3 doses — 30, 80, 400 — have been used in single inoculations. The mean recovery rate does not vary much from 50 %; but slightly higher results of infection were obtained with 400 L3 doses, as shown by multivariate analyses. The recovery rate is already established at day 2 p. i. and remains stable for at least 8 months, contrary to the *Brugia* models, which show a mortality during the larval and young adult stages (Schacher & Sahyoun, 1967; Ewert, 1971; Kowalski & Ash, 1975). The repeated inoculations of *M. martini,* which have been irregularly performed during one year on animals with positive microfiladermia, have reduced the recovery rate of the challenge dose to 14 %.

M. martini in *M. unguiculatus,* inoculated with 80 L3, has an irregular recovery rate and a lower one than in *L. striatus;* this is comparable to that observed with *M. globulosa* in the same host (Bianco *et al.,* 1983).

2 — Migration route

The histological data (Vuong *et al.,* in press) suggest that the L3 which rapidly enter the lymphatic vessels of the sub-cutaneous and muscular tissues in the inoculated area are those which will not be destroyed by the inflam-

matory defence reaction. These lymphatic larvae, wherever the inoculation has occurred (lombar or right axillar regions), reach the abdominal lymphocenters. This phase generally lasts 3 to 5 days but a small proportion of larvae migrate very rapidly as some are found in all the organs at day 2 p. i.

From the abdominal lymphnodes, the larvae are dispatched to the lymphatic vessels of the digestive wall by migrating against the lymph flow *(fig. 3)*. The caecum, anterior colon and small intestine depend upon the same nodes (the cranial mesenteric lymphocenter, *in* Cuq, 1966) but are not similarly parasitized: the two first organs are heavily and quickly infected (maximum rate at day 21 p. i.); the small intestine is less infected and later (arrival of filariae prolonged after 21 days). The posterior colon is infected as quickly as the caecum — anterior colon, suggesting that its larvae are dispatched by another center, the mesenteric caudal lymphocenter.

Early lymphatic migrations are known in *Brugia* spp. (see references *in* Denham & McGreevy, 1977), in *Wuchereria bancrofti* in cat (Ramachandran & Sivanandam, 1970), and in the lymphatic filaria of lizard, *Conispiculum flavescens* (Castellani & Willey, 1905) carefully studied by Menon *et al.* (1944).

In the non lymphatic filariae, the migrating larvae are said to be in the sub-cutaneous and inter-muscular tissues for *Dirofilaria immitis* (cf. Kume and Itagaki, 1955), and *Pelecitus spp., sensu* Bartlett *et* Greiner, 1986 (Spratt, 1972; Bartlett, 1984); but for *Litomosoides sigmodontis (= L. carinii)* a lymphatic larval phase has been demonstrated (Wenk, 1967).

In this last case, the lymphatic phase is rapid*; but Wenk's findings showed that the lymphatic migratory phase is not exclusively a behaviour of lymphatic filariae.

The *Dirofilaria* and *Pelecitus* data should also be explained by lymphatic migrations: their long stay in the muscular tissues is similar to the two weeks stay of *C. flavescens* in the muscular lymphatic vessels.

3 — Pulmonary parasitism

A small proportion of *M. martini* has an abnormal migration route: when at the level of the lombar and mesenteric lymphocenters, they enter the thoracic channel; they arrive into the blood circulation by the jonction with the anterior vena cava. The rate of pulmonary parasitism is 3 % at day 21 p. i.; it slighty increases later, which means that these "migratory accidents" occur also during the adult stage of the filaria. The pulmonary parasitism seems more common in the multi-inoculated *L. striatus* and in the *M. unguiculatus:* respectively 9 and 7 % (at days 12 and 30).

The importance of the thoracic channel as a filarial migratory route is emphasized by the cardiac and pulmonary localizations of *Brugia* spp. in surrogate hosts (first shown by Zaini *et al.,* 1962 and Ahmed, 1966), by the mediastinal localizations of *C. flavescens* (Menon *et al.,* 1944) and by the study of Wenk on *L. sigmodontis.*

M. globulosa (Muller & Nelson, 1975) and *M. nilotica* El Bihari, Hussein & Muller, 1977 are known as pulmonary blood parasites but the larval migration in *M. globulosa* (Bianco *et al.,* 1983) suggests that the adult distribution should be more similar to that of *M. martini (table V). M. marmottae* (Webster, 1967), initially described as a parasite of the liver, in fact lives in the lymphatic vessels of the bile duct (Ko, 1972). These data suggest that the genus *Monanema* is fundamentally lymphatic.

Lymphatic life might be more common than usually expected: for example, the adults of *Mansonella (Esslingeria) vanhoofi* (Peel & Chardome, 1946), a species from the chimpanzee close to *M. (E.) perstans,* are prooved to be in the lymphatic vessels, at least when they parasite the liver (Rodhain, 1955); the "dermic" microfilariae live in lymphatic capillaries (Vuong *et al.,* 1985).

Table V — *Comparative localization of* M. globulosa *(data from Bianco* et al., *1983) and* M. martini *(own data) according to days p. i. Parasitism in every organ is expressed by % of the total number of recovered worms. For* M. martini, *the worms from the gut wall are substracted to this total number. glo M:* Monanema globulosa *in* Meriones unguiculatus; *mar M:* M. martini *in* M. unguiculatus *inoculated with 80 L3; mar L:* M. martini *in* L. striatus *inoculated with 400 L3.*

Exp	Org	D 5	D 10-15	D 18-30	D 70-105
glo. M	Sc	19	32	0	0
mar. M	Sc	4,5	0	0	0
mar. L	Sc	0,9	0	0	0
glo. M	Mes	61	41	100	0
mar. M	Mes	93,7	96,4	82	/
mar. L	Mes	69,9	/	85	38,3
glo. M	Lu	0	18	0	100
mar. M	Lu	0	0	18,2	/
mar. L	Lu	1	/	13,2	53,2
glo. M	He	0	9	0	0
mar. M	He	0	3,6	0	/
mar. L	He	1	/	1,9	6,4

CONCLUSION

In the model *Monanema martini-Lemniscomys striatus,* the recovery rate of inoculated larvae is high (46 %), grossly

* *Similar example is given by a non filarial nematode, the trichostrongylid Nippostrongylus brasiliensis,* with larvae penetrating also into the host by the cutaneous route: the lymphatic migration occurs during the first 18 hours and although the species was commonly maintened in the laboratory, this fact was overlooked forty years, until the study of Gharib (1953), later confirmed by the same author (1961 a, b). For the trematode *Schistosoma mansoni,* a short lymphatic phase was also demonstrated (Standen, 1953); in this case, it should occurs only in a proportion of the larvae (Wilson *et al.,* 1990).

constant from 30 L3 to 400 L3 but in fact slightly higher with the 400 L3 dose. The recovery rate is stable from day 2 to 8 month p. i. Reinoculations lower the recovery rate to 14 %. In *M. unguiculatus* the recovery rate is irregular; the mean is 20 %.

The *M. martini* data, which show that this filaria is localized in the lymphatic vessels of the intestine, in addition to analysis of previous papers on *Monanema* (Ko, 1972; Bianco *et al.*, 1983) suggest that the biology of this genus is fundamentally lymphatic. Some accidental migrations may occur into the lungs and heart via the thoracic channel route. Similar "accidents" are known in *Bruggia* spp. in rodent hosts and in the lymphatic lizard filaria *Conispiculum flavescens*. It might exist in humans, giving a possible explanation of the tropical eosinophilic pulmonary disease.

A migration through the lymphatic system and the lymphatic dwelling life might be more frequent than expected in the filariae as suggested by several scattered examples: a short lymphatic larval phase occurs in *L. sigmodontis* (cf. Wenk, 1967); *Mansonella (E.) vanhoofi* in the chimpanzee stays in the lymphatic vessels, at least when it parasitizes the liver (Rodhain, 1955); "dermic" microfilariae are in the lymphatic capillaries (Vuong *et al.*, 1985).

In human onchocerciasis, the parasite biology remains poorly known (Campbell, *in* Filariasis, 1987). The importance of the lymphatic pathology, especially developed in the inguinal area and explained by the destruction of microfilariae, should also be induced by larval lymphatic migrations from the legs to the upper part of the body. The usual location of the adult *Onchocerca* on the periostic surface, which is surrounded by the lymphatic spaces, also sustains the hypothesis of the *O. volvulus* lymphatic biology.

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