THE FILARIA *LITOMOSOIDES GALIZAI* IN MITES; MICROFILARIAL DISTRIBUTION IN THE HOST AND REGULATION OF THE TRANSMISSION¹

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SUMMARY

The mites, *Bdellonyssus bacoti*, are engorged on rodents having 800 to 60,000 microfilariae/10 mm³ blood. Quantitation of *L. galizai* larval development shows that an additional blood meal improves development and that high microfilaremia do not result in a proportional increase in the number of infective larvae.

The first important stage of transmission regulation occurs during ingestion of microfilariae: the numbers of ingested microfilariae are lower than expected in cases of high microfilaremia. This phenomenon cannot be ascribed to the mite vector that engorges a constant blood meal whatever the level of microfilaremia. Contrarily, one finds that microfilarial density in the small peripheral blood vessels (blood drawn from incision of the dorsal skin) increases less than in large blood vessels (retro-orbital sinus). A

similar observation was reported by Dickerson *et al.* (1989) working with *Wuchereria bancrofti*. We assume that in both cases, the high microfilaremia cause the small blood vessels accessible to the vector to become saturated with parasites.

Although regulation during engorging is not the sole factor to monitor the infection in *B. bacoti* (another one operates during larval development of *L. galizai*), demonstrating its existence seems to us fundamental: it points out the concept that sub-ingestion, as well as over-ingestion, shows the inequalities of microfilarial densities in the host which seem to be dependent on mechanical factors such as the diameter of blood vessels and the size of microfilariae.

RÉSUMÉ : La filaire *Litomosoides galizai* chez l'acarien vecteur ; distribution des microfilaires chez l'hôte et régulation de la transmission.

Le vecteur, *Bdellonyssus bacoti*, est gorgé sur des rongeurs ayant 800 à 60.000 microfilaires/10 mm³. L'analyse quantitative du développement larvaire de *L. galizai* montre que celui-ci est meilleur quand le vecteur prend un repas de sang surnuméraire, et que les fortes microfilariémies n'entraînent pas une augmentation proportionnelle du nombre de larves infectantes.

Une première et importante phase de régulation s'effectue lors de l'ingestion des microfilaires : celles-ci sont sous-ingérées quand les microfilariémies sont fortes. Ce phénomène ne peut être imputé au vecteur, qui prend un volume de sang constant quelle que soit la microfilariémie. Par contre, on constate que la densité microfilarienne du sang périphérique, prélevé après incision de la peau du dos, augmente moins que celle du sang profond, prélevé au sinus

rétro-orbital. Un phénomène analogue est observé par Dickerson *et al.*, 1989 avec *Wuchereria bancrofti*. Nous supposons que, dans les deux cas, les fortes microfilariémies entraînent une saturation en parasites dans les petits vaisseaux accessibles au vecteur.

Bien que la régulation lors de l'ingestion ne soit pas la seule à contrôler l'infection chez *B. bacoti* (une autre a lieu durant le développement larvaire de *L. galizai*), sa mise en évidence nous paraît fondamentale ; elle appuie la notion que la sous-ingestion, de même que la sur-ingestion, reflètent l'existence d'inégalités des densités microfilariennes chez l'hôte, lesquelles paraissent être sous la dépendance de facteurs mécaniques : diamètres des vaisseaux du système circulatoire et dimensions des microfilaires.

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INTRODUCTION

Litomosoides galizai Bain, Petit, Diagne, 1989, a filaria of a South-American cricetid, can complete its development in laboratory mice (Diagne *et al.*, 1989; Bain *et al.*, 1990), and produce an unusually high microfilaremia in *Meriones unguiculatus*, this high microfilaremia is useful in quantitative analysis of development in the vector *Bdellonyssus bacoti* (Hirst, 1913). The present work investi-

gates optimum conditions for experimental transmission with respect to two main factors:

— the effect of an additional bloodmeal on filarial development which occurs principally in the interstitial tissue of the mite, which tissue increases its metabolic activity after feeding (cf. Diagne *et al.*, 1989);

— the relation between microfilaremia of the final host and number of infective larvae in the vector. This analysis demonstrates that the microfilaremia is not always a good predictor of the number of infective larvae. The reasons for this are investigated and discussed.

MATERIAL AND METHODS

1 — Care and infection of mites: as previously described (Diagne *et al.*, 1989), experiments were performed with females of *B. bacoti*, maintained at 28° C. Usually the rodent was left one night on sawdust containing mites; but in experiments performed to count the number of ingested microfilariae, the rodent was on the sawdust only 15 mn.

In mass transmission experiments, fed mites were counted and maintained in Erlenmeyer flasks in groups of 100 to 300; 12 days later, samples of 20 to 50 mites were dissected; in each experiment, rate of mite mortality was calculated; if it was abnormal ($\geq 30\%$) data were rejected. In some experiments, mites were maintained in small tubes, in groups of 20 and dissected individually. All dissections were done in RPMI with 20 % of newborn calf serum.

2 — Vertebrate hosts: jirds (*Meriones unguiculatus*) and laboratory mice (*Mus musculus*) were used. Mice were inoculated 3 to 5 months before exposure to vectors and had 940 to 3,208 microfilariae/10 mm³. Jirds were inoculated 2 to 14 months before exposure to vectors: at ≤ 5 months, one group had 2,254 to 60,296 microfilariae/10 mm³ and another at ≥ 8 months, had 814 to 12,840 microfilariae/10 mm³; a third group in between these times had 1,967 to 41,076 microfilariae/10 mm³.

3 — Measurement of microfilarial densities: the microfilaremia (MfS) was determined in 10 mm³ of blood punctured at the retro-orbital sinus of the rodent, with a calibrated capillary tube; this was done before each engorgement of mites. In some cases, blood samples were also taken from dorsal skin by incision with a razor blade. Microfilariae were counted on Giemsa stained blood smears.

4 — Counts of ingested microfilariae: fed mites were immediately opened on a slide and gently smeared in a drop of medium; smears were Giemsa stained.

5 — Weighing mites: unfed and fed mites were weighed; before fed ones were weighed, they were held in small tubes filled with filter paper, for 30 minutes to eliminate excess fluid following the bloodmeal.

6 — Statistical tests used are given in the text.

I — EFFECT OF AN ADDITIONAL BLOODMEAL

Three experiments were performed; each consisted of two groups of mites from the same colony and fed on two jirds, one with low and the other with high microfilaremiae (table I, experiments 1A-B, 2C-D, 3E-F). After the bloodmeal, each group was divided into two samples of 20 mites, of which only one was allowed to take an additional bloodmeal 5 days p. i. The twelve mite samples were kept in small tubes to control the mite blood intake and mortality. Mites were dissected individually on day 11 p. i., by which time the majority of the larvae have reached

TABLE I. — *Litomosoides galizai* development in mites without or with an additional blood-meal 5 days p. i.: comparison between survival rates of mites, mean number of larvae per mite, percentage of second stage larvae among total number of larvae. Dissection performed at day 11 p. i.; MfS = number of microfilariae in 10 mm³ of blood from retro-orbital sinus; L2 = second stage larva; L3 = infective larva; $\pm$ standard deviation; tests of probability (P): χ^2 (Chi-square), z value.

Exp.	1		2		3	
Mf S	A	B	C	D	E	F
	5028	54058	3609	41076	4100	60296
0 Meal	85.2	95.3	90	91.3	100	98.5
1 Meal	92.7	92.9	93	89	91.6	91.6
Signif.(X ²)	P>0.2	P>0.5	P>0.5	P>0.5	0.02<P<0.05	P>0.1
Percentage of mites survival						
0 Meal	5.1 $\pm$ 4.1	3.7 $\pm$ 4.5	5.4 $\pm$ 5.2	2.7 $\pm$ 5.3	3.4 $\pm$ 5.4	2.5 $\pm$ 3.1
1 Meal	6.7 $\pm$ 5.4	4.3 $\pm$ 4.5	7.2 $\pm$ 7.8	6.5 $\pm$ 11.6	4.2 $\pm$ 5.6	3.2 $\pm$ 4.8
Signif.(z value)	P<0.09	P#0.52	P#0.3	P#0.10	P<0.62	P#0.40
Larval mean number(L2+L3) per mite						
0 Meal	6.4	3.3	12.9	3.5	7.8	4.0
1 Meal	2.3	0.6	1.5	2.3	0.8	2.4
Signif.(X ²)	P<0.05	P>0.05	P<0.001	P>0.5	P<0.01	P<0.01
Percentage of L2						

the infective stage (Diagne *et al.*, 1989). In table I it is shown that:

— survival rates of mites were generally not significantly different between samples with or without an additional bloodmeal ($P > 0.05$; test χ^2);

— mean numbers of larvae (L2 + L3) per mite were higher in the six samples which had engorged a second time but the differences were not significant ($P > 0.05$; z value);

— the proportion of L2 among larvae was significantly lower in 4 of the 6 samples which had a second meal ($P < 0.05$; test χ^2 with Yate's correction). Retarded larvae were generally old second stages.

In conclusion, the effect of an additional bloodmeal on *L. galizai* development was only slight but nevertheless positive: more larvae rapidly reached the infective stage and mite mortality was not increased. In the later experiments, an additional bloodmeal was given to the infected mites on day 5 p. i.

II — INCREASING MICROFILAREMIA

AND NUMBER OF INFECTIVE LARVAE; EVIDENCE OF LIMITATION

Thirty-three experiments were conducted with mites infected on mice or jirds with 814 to 60,296 microfilariae/10 mm³.

The results are presented in figure 1 and table II. The maximum number of infective larvae observed was 8.5 L3 per mite. Up to about 5,000 microfilariae/10 mm³, the mean number L3/mite varied greatly but was positively correlated with microfilaremia. At higher microfilaremiae, the mean number of L3/mite was less variable and did not increase with increasing microfilaremia.

III — PHASES OF LIMITATION

Experiments were performed to define the phases of limitation.

A — Mean number of ingested microfilariae; variation in « ingestion efficiency »

On dissection, most microfilariae found in the digestive caeca of mites were more or less disrupted, making it difficult to count them. In contrast, the microfilarial sheaths, were generally intact, or their large anterior and sharp posterior extremities were easy

L3/A

FIG. 1. — Relationship between mean number of infective stages per mite (L3/A) and microfilaremia. MfS = number of microfilariae in 10 mm³ of blood from the retro-orbital sinus; white squares: experiments with mice; black squares: experiments with jirds.

TABLE II. — Relationship between microfilaremia and mean number of infective larvae per mite (L3/A). Additional blood meal given; dissection performed at day 12 p. i. or day 11 p. i. (*); in this case, the mean larval number = L2 + L3; u1 and m1 etc. = bloodmeals respectively on jird or mouse; MfS = microfilaremia from ocular sinus, as in Table I.

Hosts	Mf S	L3/A	Hosts	Mf S	L3/A
u1	814	1.13	u8	2920	2.97
m1	940	1.38	m11	3208	2.22
m2	1246	0.91	u9	3609*	7.2
m3	1332	0.25	u10	4100*	4.2
m4	1377	0.21	u11	4317	4.35
u2	1524	1.21	u12	5028*	6.7
m5	1704	2.75	u13	5089	7.5
m6	1774	2.34	u14	5876	7.5
u3	1964	6.13	u15	11680	6.07
u4	2254	1.87	u16	12028	4.88
m7	2463	0.83	u17	12840	3.07
u5	2484	8.5	u18	17248	7.73
m8	2549	0.46	u19	41076*	6.5
u6	2718	1.22	u20	47424	4.95
u7	2720	1.4	u21	54058*	4.3
m9	2776	0.8	u22	60296*	3.2
m10	2829	4.82			

to distinguish. Thus counts of ingested microfilariae were based on the number of sheaths.

Eight experiments were conducted on low or high microfilaremia (table III). Data were analysed using Spearman's rank test.

— The mean number of ingested microfilariae varied directly with microfilaremia ($p = 0.542$; $N = 79$; $P \leq 0.01$).

— The « ingestion efficiency », *i. e.* the ratio of ingested microfilariae to microfilaremia, decreased with increasing microfilaremia

TABLE III. — Relationship between microfilaremia and number of ingested microfilariae. MfS = microfilaremia from ocular sinus, as in Table I; nA = number of dissected mites; IMf/A = mean number of ingested microfilariae per mite with range; mg = weight of one hundred fed mites; $\pm$ standard deviation.

Mf S	nA	IMf/A	IMf	IMf/MfS	mg
			range		
3360	9	192 $\pm$ 128	52-439	0.0571	23.5
3609	8	139 $\pm$ 32	90-187	0.0385	26.2
4514	10	252 $\pm$ 127	98-472	0.0558	-
5028	7	183 $\pm$ 75	96-322	0.0364	-
28774	18	554 $\pm$ 344	142-1520	0.0193	24.8
35084	9	395 $\pm$ 191	134-647	0.0113	-
41076	9	324 $\pm$ 187	115-701	0.0079	24.3
54058	9	553 $\pm$ 213	112-775	0.0102	-

IMf/MfS

FIG. 2. — Relationship between the ratio ingested microfilariae/microfilaremia (IMf/MfS) and microfilaremia. MfS = number of microfilariae in 10 mm³ of blood from the retro-orbital sinus; IMf = number of ingested microfilariae.

($p = 0.745$; $N = 79$; $P \leq 0.01$; fig. 2). Using the transformation into neperian logarithm, the relation was $\text{Log. (IMf/MfS)} = -0.61 \text{ Log. MfS} + 1.908$ ($r = 0.60$; $P = 0.00$).

B — Recovery rate of ingested microfilariae

In 4 experiments, 2 using hosts with low and two using hosts with high microfilaremia, mean number of ingested microfilariae and recovery rate of L3 were compared with microfilaremia. In hosts with low microfilaremia there was a mean of 161 microfilariae ingested and 4.28 % recovery rate, whereas in hosts with high microfilaremia a mean of 438 microfilariae were ingested with a recovery rate of 1.23 % (table IV). The nature of the variability in the number of L3/mite makes it impossible to perform any statistical test, parametric or non parametric, on these values.

Thus, the level of infection in mites is affected by two factors: the number of microfilariae ingested and the ability of ingested microfilariae to develop to the infective stage in the vector (table IV).

IV — SEARCH FOR THE FACTORS CONTROLLING THE « INGESTION EFFICIENCY »

A — Mite blood intake related to microfilaremia

Weights of mites engorged on infected and uninfected jirds were

TABLE IV. — Variation in recovery rate of *L. galizai* in mites with low and high microfilaremia. In each case, two experiments are given and their means are calculated (Mean 1 and 2); R2/1 represents the ratios of these means. MfS = number of microfilariae in 10 mm³ of blood from the retro-orbital sinus; e.IMf = expected mean number of ingested microfilariae per mite (0.34 mm³ of ingested blood); IMf = actual mean number of ingested microfilariae per mite; L3 = mean infective larvae per mite; L3/IMf/100 = percentage of ingested microfilariae reaching the L3 stage; $\pm$ standard deviation.

	Mf S	e. IMf	IMf	L3	L3/IMf x100
	3609	123	139 $\pm$ 32	7.2 $\pm$ 7.8	5.17
	5028	171	183 $\pm$ 75	6.7 $\pm$ 5.4	3.66
Mean1	4318	147	161 $\pm$ 57	6.9 $\pm$ 6.5	4.28
	41076	1397	324 $\pm$ 187	6.5 $\pm$ 11.6	2
	54058	1838	553 $\pm$ 213	4.3 $\pm$ 4.5	0.77
Mean2	47567	1617	438 $\pm$ 228	5.4 $\pm$ 8.7	1.23
R 2/1	11	11	2.72	0.78	0.28

compared in order to assess if the mite blood intake could be influenced by density of microfilariae in the host.

— Five samples of 50 engorged uninfected mites weighed 11.5-13.2-12-12.6 and 12.4 mg, with a mean value of 12.3 mg/50 mites and a variance of 0.408.

— Four samples of 100 engorged mites on jirds with microfilaremia varying from 3,360 to 54,058 microfilariae/10 mm³ weighed 23.5-26.2-24.8-24.3 mg (table III), with a mean value of 12.3 mg/50 mites and a variance of 0.644.

Variances of the two series of measures did not significantly differ (Fisher-Snedecor test, $F_4^3 = 6.59$ at 5 %), and Student's *t*-test showed that weights of mites fed on uninfected or infected jirds were not significantly different ($P < 0.05$).

The mean blood intake per mite, established on the weights of 100 unfed and 50 fed mites, was 0.20 mg.

B — Local microfilarial densities

Possible differences in microfilarial densities measured in the retro-orbital sinus and in other parts of the body on which mites feed were investigated. The area studied was the dorsal skin (B); by bleeding, one to five small irregular volumes of blood (1 to 5 mm³) were collected, giving total volumes of 2 to 15 mm³ (V); microfilarial densities were expressed for a constant volume of 10 mm³.

Seven jirds with 2,821 to 25,656 microfilariae/10 mm³ were used (table V, fig. 3). Data were analysed by Spearman's rank test.

— When microfilaremia increased in the ocular sinus (MfS), microfilarial densities increased also in the dorsal skin blood (MfB) ($p = 0.818$; $N = 12$; $P < 0.01$).

— The ratio MfB/MfS decreased significantly ($p = -0.678$; $N = 12$; $0.01 < P < 0.05$).

C — Comparison of variations in « ingestion efficiency » and dorsal microfilarial density

To compare precisely these data, the ratio MfB/MfS is corrected by a multiplying factor, so that the two regression lines:

— « ingestion efficiency » vs microfilaremia (fig. 2);

— dorsal skin microfilaremia/microfilaremia vs microfilaremia (fig. 3) intersect at the level of our mean « ingestion efficiency » data.

The multiplying factor is the volume of blood ingested by a mite; the calculated value is 0.34 mg.

After correction, the two slopes appear of similar size of order, which means that the « ingestion efficiency » may be related to the local microfilarial density. Nevertheless the slope of the corrected MfB/MfS vs microfilaremia line is slightly lower than that of the « ingestion efficiency » line (fig. 4).

TABLE V. — Microfilarial densities in 10 mm³ of blood from two different host regions, the retro-orbital sinus (MfS or S), and the dorsal skin (MfB or B) with the ratio (B/S). Seven jirds used (u); V = real volume of blood taken up by the experimenter at the back; n = number of data.

	Mf S	V	Mf B	V	B/S	n
u1	2821	10	3416	5	1.21	1
u2	4418	10	6301	10	1.42	2
u3	5582	10	6145	4.5	1.1	1
u4	6448	10	7111	4.5	1.1	2
u5	6929	10	7863	14	1.13	2
u6	17577	10	19288	15	1.09	2
u7	25656	10	20188	6.5	0.79	2

Fig. 3. — Relationship between the ratio microfilarial density from the dorsal skin/microfilaremia (MfB/MfS) and microfilaremia. MfS and MfB = number of microfilariae in 10 mm³ of blood from respectively ocular sinus and dorsal skin.

Fig. 4. — Comparison of the 2 regression lines: corrected ratio MfB/MfS as a function of MfS (y' ; continuous line —) and ratio IMf/MfS as a function of MfS (y ; broken line -----). IMf = mean number of ingested microfilariae per mite; MfS and MfB = number of microfilariae in 10 mm³ of blood from respectively ocular sinus and dorsal skin.

DISCUSSION

Significant regulation of filarial load occurs during the ingestion of microfilariae and during the filarial development. This second phase of regulation is a common fact, due to metabolic competition, and it is not discussed here. In contrast, the first phase was unexpected and it is our main concern.

The two other factors acting on the *L. galizai* development, not depending upon the microfilaremia, *i. e.* additional bloodmeal and early destruction of ingested microfilariae, are last discussed.

A — REGULATION BY NUMBER OF INGESTED MICROFILARIAE; BIOLOGICAL INTERPRETATION

The « ingestion efficiency », *i. e.* number of ingested microfilariae/microfilaremia, decreases with high microfilaremia (*fig. 2*). The mite blood intake seems constant and cannot explain this phenomenon. But we have seen that the microfilarial density at the dorsal skin (MfB) is not proportional to the microfilaremia (MfS): the ratio MfB/MfS vs microfilaremia decreases when the microfilaremia increases. The fact that, after correction, the two lines are close, shows that this factor may explain the decrease in « ingestion efficiency ».

Two data are apparently in contradiction with this conclusion.

a — The small difference observed between the two slopes. This shows that the decrease of the ratio dorsal skin microfilaremia/microfilaremia is more accentuated in blood ingested by mites than in our dorsal skin blood samples.

b — The discrepancy between the calculated volume of blood ingested by mite and the value obtained by weighing the mites, *i. e.* 0.34 and 0.20 mg respectively. This seems essentially to be due to an important loss of fluid by mites after their blood meal, as do all haematophagous arthropods. It is interesting to note that the size of this estimated volume lost, which may appear important (38 % of the total volume ingested), fits well with the experimental data obtained by Boorman (1960) and Briegel *et al.* (1979) on *Aedes aegypti*.

In the related species, *L. sigmodontis* Chandler, 1932, no regulation of the infective larval load by number of ingested microfilariae has been described, although many data concern the relation between the microfilarial density of the rodent and the number of ingested microfilariae (Bertram *et al.*, 1946; Bertram, 1949; Freer, 1953; Tanaka *et al.*, 1963). This seems to be due to different experimental conditions which are less appropriate to this subject: mites collected after 24 h of contact with the cotton-rat, rodent not immobilized and disturbing the mite bloodmeal, range of used microfilaremiae not enough wide etc.

No early regulation as found in *L. galizai*/*B. bacoti* has

been demonstrated with other filariae. But data given by Dickerson *et al.* (1989) on *Wuchereria bancrofti*, concerning the microfilarial densities in the capillary and venous bloods, are interesting: the authors find that the ratio microfilaremia in capillary/microfilaremia in venous blood decreases with increasing venous microfilaremia.

Ingestion of microfilariae by the vector, and its relation to microfilaremia, is a complex phenomenon. Previous experiments on appropriate models (a host infected with 4 filarial species, inert microspheres of different diameters inoculated to a rodent, cf. Petit, 1985) indicate that this phase is dependant upon the following factors:

- parasite density varies according to the different diameters of blood vessel;

- blood taken by the vector or investigator is a mixture of blood from vessels of small, medium and large diameters, of which the relative proportion varies according to the organ studied and the puncture method (vector or investigator).

The *L. galizai* data (*tables III and IV; fig. 2 and 3*) may be interpreted with these notions.

- With low microfilaremia, microfilariae are almost equally distributed in the different vessels: the ratio actual/expected mean numbers of ingested microfilariae equals 1.09 (for an estimated blood volume of 0.34 mg).

- With high microfilaremia, the small peripheral vessels become saturated; this results in an apparent sub-ingestion: the ratio actual/expected mean numbers of ingested microfilariae equals 0.27. The fact that the range of ingested microfilariae is smaller than with low microfilaremia (*table III, fig. 2*) strengthens this notion of saturation. As mites probably engorge on smaller vessels than those bled by us, it is not surprising that the phenomenon of microfilarial saturation is more accentuated in the vector than in our blood puncture (*fig. 4*).

A similar microfilarial saturation of the small peripheral vessels may be invoked to explain the data on the distribution of *W. bancrofti* microfilariae.

B — OTHER FACTORS ACTING ON THE *L. galizai* DEVELOPMENT

The second phase of regulation which occurs during larval development has not been especially studied and is not discussed. Attention is given to two other factors which affect the mite parasitism but which are not dependant upon the level of microfilaremia.

The first factor is the destruction of ingested microfilariae when they arrive in the digestive caeca; 80 to 90 % are disrupted. Freer (1953) made the same observation with *L. sigmodontis*. Gorirossi (1950) described epipharyngeal and hypostomal teeth in *B. bacoti*; their presence explains this early destruction of microfilariae. Similar events are also

described in some other filaria-vector pairs (Coluzzi and Trabucchi, 1968; Bain *et al.*, 1974; Omar and Garms, 1975; McGreevy *et al.*, 1978; Buse and Kulhow, 1979).

The second factor is the additional bloodmeal; its effect appears positive for the *L. galizai* development. This is not the general rule. With the pair *Brelinia booliati*-*A. togoi*, for example, additional blood intakes have no favorable effect (Petit *et al.*, 1979, b); this filaria develops in the adipose tissue; a bloodmeal stimulates the ergastoplasmic growth and proteinic synthesis in fat cells (Behan *et al.*, 1978) but not in the parasitized syncytium. In the contrary, with *Molinema dessetae*-*Aedes aegypti*, the infective rate of mosquitoes is multiplied by 10, with 3 additional bloodmeals; in this case the adipose parasitized syncytium is stimulated as are normal fat cells; this stimulation and the additional food benefit the filaria (Petit and Spitalier-Kaveh, 1979, a). There are analogies between insect adipose tissue and mite interstitial tissue and a similar explanation can be proposed for *L. galizai* is improved transmission with one additional bloodmeal.

CONCLUSION

A basic problem in the epidemiology of filariasis is to establish the relationship between the microfilarial density in the host and the quantity of microfilariae ingested by the vector. The interest of the model which has been analysed there is that it gives a wide range of comparable data, obtained with low or very high microfilaræmia.

The main event shown is the existence of an early regulation by the number of ingested microfilariae. This number itself corresponds to the local density of the microfilariae in the host.

L. galizai in *Meriones unguiculatus* shows a peculiar relationship between microfilaræmia measured at the retro-orbital sinus and microfilarial density measured by bleeding the dorsal skin: this latter is lower when the microfilaræmia is high, because small vessels become saturated. The same explanation may apply to *W. bancrofti* microfilariae which show a similar relationship between capillary and venous microfilaræmia (Dickerson *et al.*, 1989).

Differences between deep and peripheral blood appear to cause variations in vector « ingestion efficiency ». Sub-ingestion is the result of local densities of microfilariae in the host, which depend upon mechanical factors such as vessel diameters and sizes of microfilariae (Petit, 1985).

Variation of the « ingestion efficiency » is not the sole fact of the blood microfilariae. In human onchocerciasis, a strong sub-ingestion is observed a few days after ivermectin treatment, which emphasizes the drug effect (Prod'hon *et al.*, 1987). It was suggested that surviving microfilariae might have an unusual distribution in the skin, making them less accessible than usual to the simulids, an hypothesis confirmed in a recent study (Jurgens and Schulz-Key, 1990).

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REFERENCES

- Bain O., Durette-Desset M. C., de Léon R. : Onchocercose au Guatemala : l'ingestion des microfilaires par *Simulium ochraceum* et leur passage dans l'hémocèle de ce vecteur. *Ann. Parasitol. Hum. Comp.*, 1974, 49, 467-487.
- Bain O., Petit G., Diagne M. : Étude de quelques *Litomosoides* parasites de rongeurs; conséquences taxonomiques. *Ann. Parasitol. Hum. Comp.*, 1989, 64, 268-289.
- Bain O., Diagne M., Petit G., Taylor D. W. : *Litomosoides galizai*, a potential mouse model for the study of immunity against filarial parasites. *The British Soc. Parasit.*, Spring meeting, 4-6 th April 1990, Univ. Aberdeen, Abstr. n° 93, p. 52.
- Behan M., Hagedorn H. H. : Ultrastructural changes in fat body of adult female *Aedes aegypti* in relationship to vitellogenin synthesis. *Cell. Tiss. Res.*, 1978, 186, 499-506.
- Bertram D. S., Unsworth K., Gordon R. M. : The biology and maintenance of *Liponyssus bacoti* Hirst, 1913, and an investigation into its role as a vector of *Litomosoides carinii* to cotton rats and white rats, together with some observations on the infection in the white rats. *Ann. Trop. Med. Parasit.*, 1946, 40, 228-255.
- Bertram D. S. : Studies on the transmission of cotton rat filariasis. I : The variability of the intensities of infection in the individuals of the vector, *Liponyssus bacoti*, its causation and its bearing on the problem of quantitative transmission. *Ann. Trop. Med. Parasitol.*, 1949, 43, 313-332.
- Boorman J. P. T. : Observations on the feeding habits of mosquito *Aedes (Stegomyia) aegypti* (L.); the loss of fluid after a blood meal and the amount of blood taken during feeding. *Ann. Trop. Med. Parasitol.*, 1960, 54, 8-14.
- Briegleb H., Lea A. O., Klowden M. J. : Hemoglobinometry as a method for measuring blood meal sizes of mosquitoes (Diptera: Culicidae). *J. Med. Entomol.*, 1979, 15, 235-238.
- Buse E., Kulhow F. : Rasterelektronenoptische studien zur morphologie und bedeutung der vorderdarmbewehrung verschiedener stechmückenarten für die aufnahme von mikrofilarien. *Tropenmed. Parasit.*, 1979, 30, 446-454.
- Coluzzi M., Trabucchi R. : Importanza dell'armatura buccofaringea in *Anopheles* e *Culex* in relazione alle infezioni con *Dirofilaria Parassitologia*, 1968, 10, 47-59.
- Diagne M., Petit G., Bain O. : Maintien d'une filaire chez la souris. *C. R. Acad. Sci.*, Paris, 1989, 309, Série III, 25-28.
- Diagne M., Petit G., Seureau C., Bain O. : Développement de la filaire *Litomosoides galizai* chez l'acarien vecteur. *Ann. Parasitol. Hum. Comp.*, 1989, 64, 478-488.
- Dickerson J. W., Eberhard M. L., Lammie P. J., Roberts J. M. : Further evidence of a skewed distribution of microfilariae in capillary blood. *Trop. Med. Parasit.*, 1989, 40, 472-473.
- Freer P. M. : Observations on the early fate of the microfilariae of *L. carinii* (Travassos, 1919), filarial parasite of the cotton rat, after their ingestion by the vector, *B. bacoti* (Hirst, 1913). *Ann. Trop. Med. Parasit.*, 1953, 47, 13-25.
- Gorirossi F. E. : The mouth-parts of the adult female tropical rat mite, *Bdellonyssus bacoti* (Hirst, 1913) Fonseca, 1941 [= *Liponyssus bacoti* (Hirst)], with observations on the feeding mechanism. *J. Parasit.*, 1950, 36, 301-318.
- Jurgens S., Schulz-Key H. : Effect of ivermectin on the vertical distribution of *Onchocerca volvulus* microfilariae in the skin. *Trop. Med., Parasit.*, 1990, 41, 165-168.

- McGreevy P. B., Bryan J. H., Oothuman P., Kolstrup N. : The lethal effects of the cibarial and pharyngeal armatures of mosquitoes on microfilariae. *Trans. Roy. Soc. Trop. Med. Hyg.*, 1978, 72, 361-378.
- Omar M. S., Garms R. : The fate and migration of microfilariae of a Guatemalan strain of *Onchocerca volvulus* in *Simulium ochraceum* and *S. metallicum*, and the role of the buccopharyngeal armature in the destruction of microfilariae. *Tropenmed. Parasit.*, 1975, 26, 183-190.
- Petit G., Spitalier-Kaveh H. : La Filiaire *Dipetalonema dessetae* chez *Aedes aegypti*; étude du tissu adipeux parasité. *Ann. Parasitol. Hum. Comp.*, 1979a, 54, 81-92.
- Petit G., Spitalier-Kaveh H. : La Filiaire *Breinlia booliati* dans le tissu adipeux d'*Aedes togoi*, comparaison avec le couple *Dipetalonema dessetae*-*A. aegypti*. *Ann. Parasitol. Hum. Comp.*, 1979b, 54, 653-663.
- Petit G. : Ingestion des hématozoaires par le vecteur : Analyse de quatre filaires parasites d'un Saïmiri. *Ann. Parasitol. Hum. Comp.*, 1985, 60, 247-297 et 455-497.
- Prod'hon J., Lardeux F., Bain O., Hebrard G., Prud'hom J. M. : Ivermectine et modalités de la réduction de l'infection des Simulies dans un foyer forestier d'onchocercose humaine. *Ann. Parasitol. Hum. Comp.*, 1987, 62, 590-598.
- Tanaka H., Chiba T., Tanaka H. : Quantitative observations on the ingestion of the microfilariae of the cotton rat filaria, *Litomosoides carinii*, by its intermediate host, *Ornithonyssus bacoti* *Jap. J. Parasitol.*, 1963, 12, 191-195.