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THE DEVELOPMENT OF *PARAFILARIA BOVICOLA* IN *MUSCA XANTHOMELAS* AND *MUSCA LUSORIA**

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ABSTRACT

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Artificially infected adult flies were used in this study. In both *Musca* species, *P. bovicola* developed in the fat-body cells, mainly of the abdomen. Escape from the midgut and penetration of the fat-body cell was possibly achieved by use of the cephalic hook of the microfilaria. At 27 °C, development to the 3rd larval stage took 9 days, but maximum length was only reached after 11 days. Sharp increases in larval length took place on Days 6–7 and on Day 9. These sudden increases possibly indicate moults to the 2nd and 3rd larval stages respectively.

During larval development the fat-body cell increased markedly in size to form a thin-walled capsule around the larva. On reaching the 3rd stage, larvae escaped from the capsules and migrated to the head cavity and proboscis.

Résumé

LE DÉVELOPPEMENT DE PARAFILARIA BOVICOLA *CHEZ* MUSCA XANTHOMELAS *ET* MUSCA LUSORIA

Des mouches adultes, artificiellement infectées, furent utilisées dans cette étude. Chez les deux espèces de Musca, P. bovicola *se développa dans les cellules aux corps gras, surtout celle de l'abdomen. L'évasion de l'intestin moyen et la pénétration de la cellule aux cops gras fut vraisemblablement réalisée par l'utilisation du crochet céphalique de la microfilaire. A 27 °C le développement du troisième stade larvaire prit 9 jours, mais la longueur maximum fut seulement atteinte après 11 jours. Des augmentations accentuées dans la longueur larvaire prirent place aux jours 6–7 et au jour 9. Ces augmentations subites indiquent vraisemblablement des mues au deuxième et troisième stades larvaires respectivement.*

Pendant le développement larvaire la cellule aux corps gras s'accrut en dimension de manière marquée pour former une capsule à une paroi minse autour de la larve. Quand elles parviennent au troisième stade, les larves s'échappent des capsules et émigrent vers la cavité de la tête et du proboscis.

INTRODUCTION

Studies on the development of the immature stages of *Parafilaria bovicola* in its intermediate hosts have previously been limited to those of Sahai & Singh (1971), who recovered larvae 6–96 h old from the abdomens of a few *Musca vitripennis* which had earlier fed on a bleeding nodule. Because of the unavailability of infective flies this work was discontinued.

In Stavropol Province in Russia, Gnedina & Osipov (1960) studied the development of *Parafilaria multipapillosa* in *Haematobia atripalpis* which had been allowed to feed on skin lesions of naturally infected horses. At temperatures which varied from 20,0–36,5 °C, the infective stage was reached in 10–15 days. The larvae spent the first 5 days in the body cavity of the flies and only after 5–8 days did further development take place in the fat-body.

The present studies on *P. bovicola* in its intermediate hosts were aimed at determining the general appearance, size, developmental times and site of development of each larval stage. These studies were conducted simultaneously in 2 species of vector flies, *Musca lusoria* and *Musca xanthomelas*, to determine whether these species are equally suitable for larval development. A detailed morphological study of the different larval stages was not attempted.

MATERIALS AND METHODS

The method used to infect the flies was described by Nevill (1975). The *M. lusoria* used were of unknown age while the *M. xanthomelas* were approximately 4 days old. All flies originated from the Onderstepoort laboratory colonies and were maintained at 27 °C and 60% R.H. Following infection, flies of each species were dissected daily until at least 1 fly was found to contain immature larvae of *P. bovicola* on 11–12 successive days. A total of 52 *M. xanthomelas* and 40 *M. lusoria* were examined. Fewer *M. lusoria* were dissected as they were included for comparative purposes only. Most of the flies examined were females since it was thought that they would have larger fat-bodies and consequently be more suitable for worm development.

In earlier exploratory infections and examinations, the haemocoele and fat-bodies of the abdomen and head, the malphigian tubules, thoracic muscles, internal genitalia and sections of the gut were examined under cover slips in insect saline (0,75% NaCl, 0,035% KCl, 0,021% $CaCl_2$) for developing larvae of *P. bovicola*. Except for a few larvae near the thoracic muscles most developing larvae were found in the haemocoele and fat-bodies of the abdomen and head. The present examinations were therefore restricted to the latter areas, except for the first few days when the stomach contents were also examined.

When dissecting flies the abdomen and head of the fly were placed on separate glass slides; the abdomen was opened ventrally in a drop of saline and the stomach transferred to a drop of water on another slide. To detect eggs or microfilariae in the ingested blood the stomach was ruptured and the blood mixed with the water until the red cells lysed. This material was then covered with a cover slip. The network of fat-body cells lining the underside of the abdominal tergites was mounted separately under a cover slip in Pampel's fluid (4 mℓ glacial acetic acid, 6 mℓ 40% formaldehyde, 15 mℓ 95% ethyl alcohol, 30 mℓ distilled water) or in glycerine, either with or without passing it through 70% ethyl alcohol +5% glycerine. Care was taken to spread the net of fat cells as thinly as possible for better visibility when examining the cell contents for developing larvae. The head was also dissected in a drop of saline.

After all large particles and/or organs from the dissected head or abdomen had been removed, cover slips were placed over the haemocoelic fluid and saline which remained on the slides and this was examined for developmental stages. At all times throughout the study developing larvae were common in these preparations.

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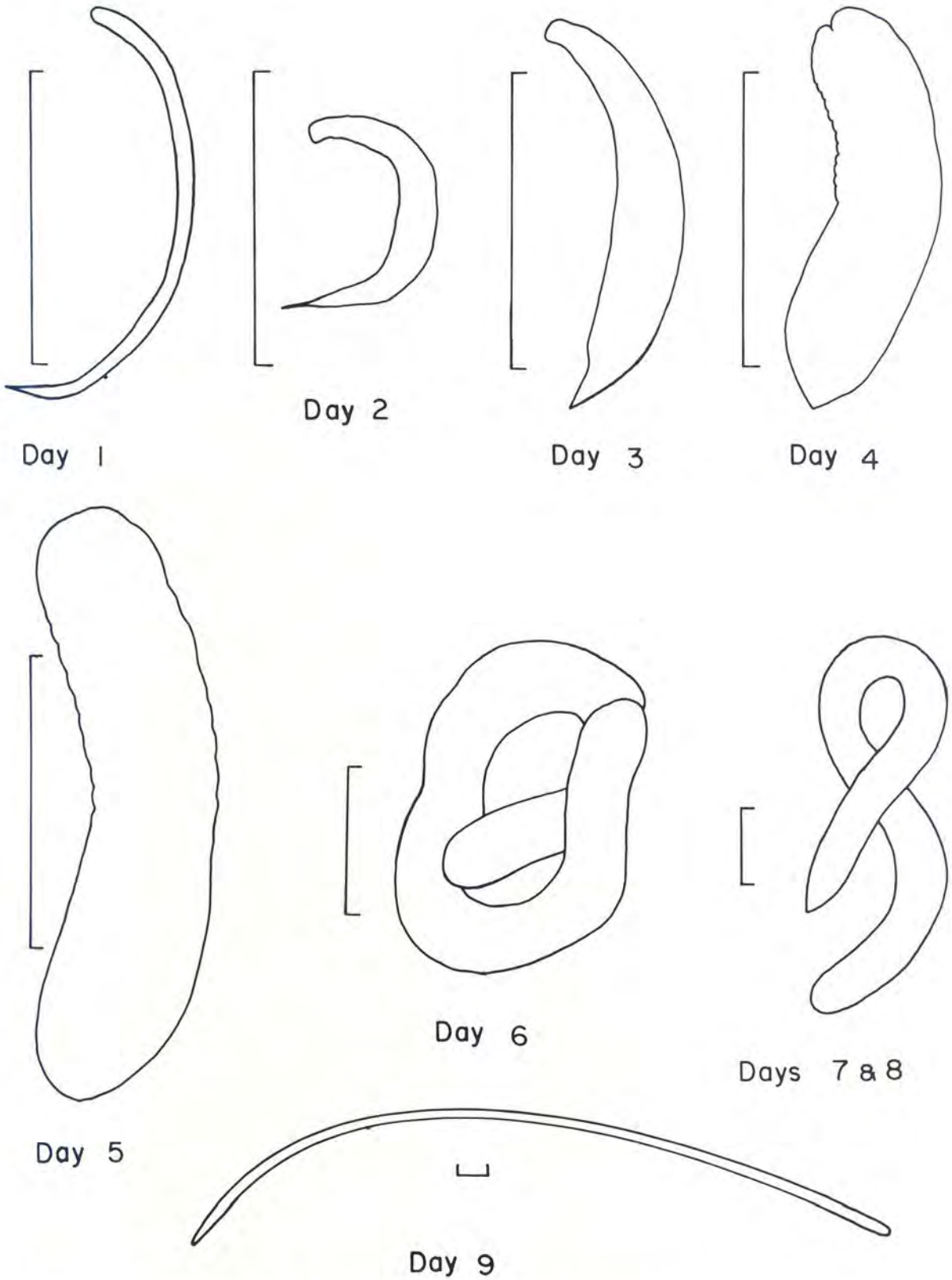

FIG. 1 Diagrammatic illustration of the shape and size of *Parafilaria bovicola* larvae at daily intervals during their development in vector flies maintained at 27 °C. The scale for each larva equals 100 μm.

Examination of slides for developmental stages of *P. bovicola* was done under a compound microscope, 400 magnifications commonly being used for the early stages and 40 magnifications for the 3rd stage. An eyepiece micrometer was not used to measure the larvae as the majority were coiled or curved. Instead, the outlines of many of the larvae were traced with the aid of a drawing tube. A stage micrometer was used to indicate exact size on these drawings and the larval length was measured by means of a cotton thread laid along the long axis of the larva in the drawing.

A ratio (mf) was used to indicate relative changes in length and was determined by dividing the length of a larva by the length of a microfilaria. For example, 5,00 mf indicates a larva 5 times longer than a microfilaria.

Results

Fig. 1, 2 & 3 illustrate the shapes, sizes and appearance of all ages of developing larvae seen during these studies, while Tables 1 & 2 give the location in the flies plus the mean lengths, widths and ranges on successive days following infection of the larvae that were measured.

Development of P. bovicola *in* M. xanthomelas

Twenty-five microfilariae from a gravid *P. bovicola* and 176 larvae of different ages from 32 female and 7 male flies were measured (Table 1) and their location and their appearance noted. The length of the larvae was also compared with the length of the microfilariae. A summary of their daily changes in size, shape and location in the fly follows.

One day after infection, *P. bovicola* larvae were recovered from blood in the stomach and from the, abdominal haemocoele, and 1 larva shaped like an S was seen lying inside a fat-body cell (Fig. 2C). Their appearance and measurements were similar to those of microfilariae [Fig. 1 (Day 1) & 2A; Table 1] and a distinct cephalic hook could be discerned under high magnification (Fig. 2B).

By Day 2, however, only the odd microfilaria-like larva was found (still in the stomach), but numerous larvae, now twice as wide as before (mean 12 μm) and beginning to assume a sausage shape, could be seen in the cells of the fat-body of the abdomen, with some free in the haemocoele (Fig. 1 & 2D).

TABLE 1 The growth and sites of development of *Parafilaria bovicola* in artificially infected *Musca xanthomelas* maintained at 27 °C and 60% R.H. and a comparison with the length of newly hatched microfilariae (measurements in μm)

Days after infection	No. of infected flies examined ♀♀	♂♂	Site of larvae in fly*	No. of larvae measured	Mean length (range)	Mean width (range)	mf = Larval length / Microfilarial length
0			Microfilariae from eggs	25	173 (161–189)	6 (5–7)	1,00
1	6	1	gut; abd fat; abd haem.	24	148 (105–186)	6 (4–8)	0,86
2	2	0	abd fat	2	145 (143–148)	12 (—)	0,84
3	1	1	abd fat; head haem.	8	117 (74–161)	24 (15–28)	0,68
4	3	0	abd fat; abd cap. forming	8	162 (121–189)	37 (23–53)	0,94
5	2	1	abd fat; abd cap.; abd haem.	34	228 (154–329)	48 (28–57)	1,32
6	2	2	abd fat; abd cap.; abd haem.	14	608 (361–994)	41 (32–52)	3,51
			abd haem.	1	1 229 (–)	65 (—)	7,10
7	2	1	abd fat; abd cap.; abd haem.; head fat; head cap.; head haem.	6	1 279 (1 058–1 613)	66 (52–97)	7,39
8	1	0	abd haem.	9	508 (303–794)	61 (45–103)	2,94
			abd haem.; abd cap.	4	1 163 (1 071–1 355)	74 (71–77)	6,72
9	3	0	abd haem.	4	673 (355–984)	63 (59–71)	3,89
			abd haem.	4	1 225 (1 033–1 426)	67 (59–71)	7,08
			abd haem.; abd cap.	11	2 505 (2 049–2 934)	60 (56–68)	14,48
10	2	1	abd haem.	1	200 (—)	39 (—)	1,16
			abd fat; abd haem.	4	689 (574–839)	62 (52–87)	3,98
			abd haem.; head. haem.	10	2 810 (1 623–3 328)	62 (47–79)	16,24
11	3	0	abd haem.; head fat.	2	566 (436–697)	63 (61–65)	3,27
			abd haem.; head haem.	9	3 454 (3 066–3 853)	60 (50–74)	19,97
12	5	0	head haem.	21	3 087 (1 934–3 721)	58 (47–65)	17,84
Totals	32	7		201			

* Abbreviations used:
abd fat =abdominal fat-body cells
abd haem. =abdominal haemocoele
abd cap. =abdominal fat capsule
head fat =head fat-body cells
head haem. =head haemocoele
head cap. =head fat capsule

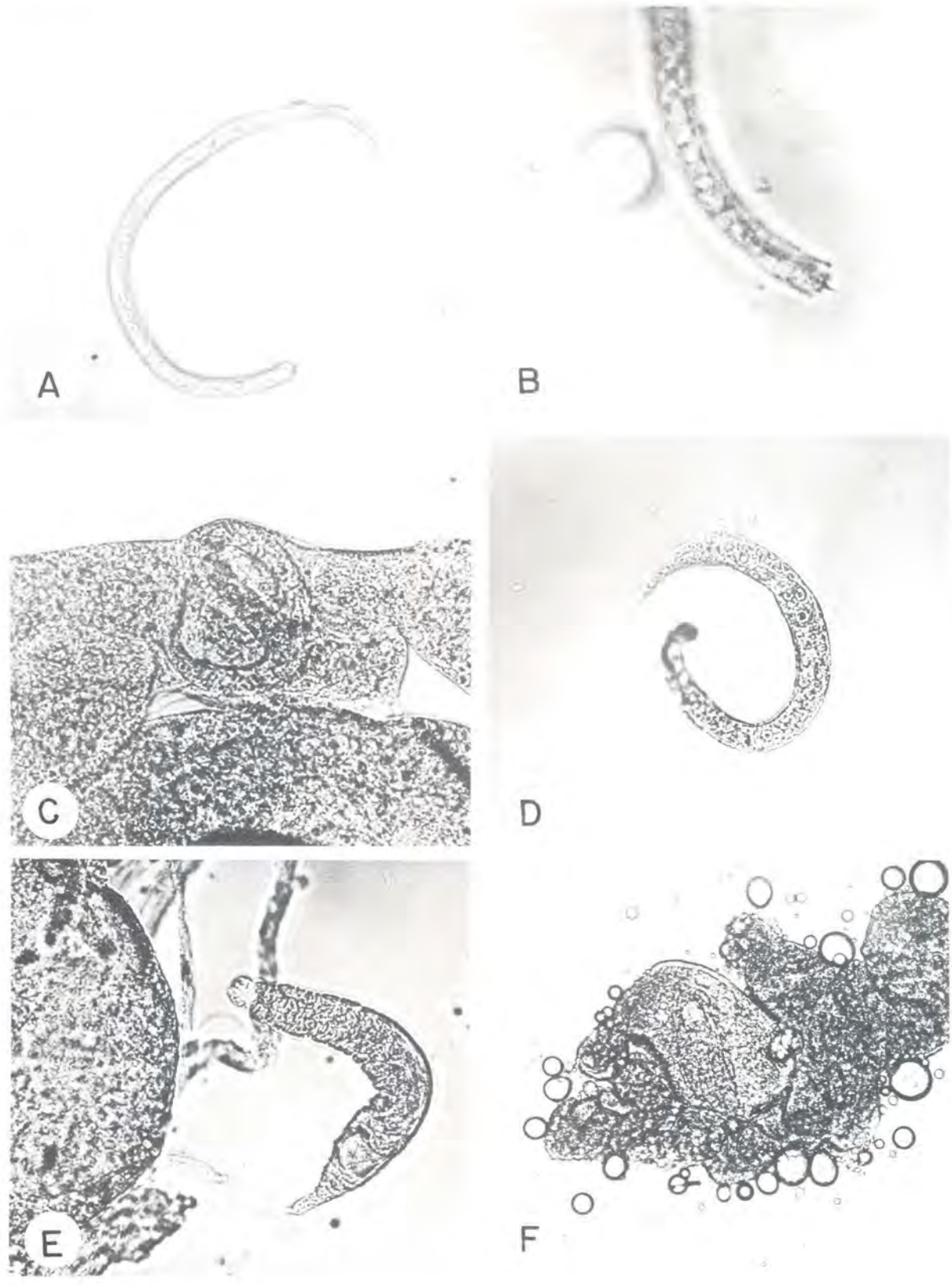

FIG. 2 Developmental stages of *Parafilaria bovicola*

A—Day 0; microfilaria of *P. bovicola* (×600)

B—Day 0; anterior extremity of microfilaria recovered from the midgut of an artificially infected *Musca* vector, showing the cephalic hook (×1 000)

C—Day 1; 1st stage larva in abdominal fat-body cell of vector fly (×400)

D—Day 2; 1st stage larva in abdominal haemocoele of vector fly (×600)

E—Day 3; 1st stage larva in abdominal haemocoele of vector fly (×550)

F—Day 3; 1st stage larva in enlarged fat-body cell in abdomen of vector fly (×150)

Three days after infection the larvae had contracted to their shortest length of only 117 μm (0,68 mf) but had increased in width to 24 μm. They were again found in the abdominal fat-body, where they occupied enlarged cells (Fig. 2F), and the abdominal haemocoele and were also free in the head (Fig. 1 & 2E).

At Day 4 the larval length had increased to 0,94 mf (162 μm) and the width to 37 μm (Fig. 1 & 3A). Capsules surrounding the larvae in the fat-body could be clearly seen. The process of capsule formation is discussed later.

By Day 5 the larval length of 228 μm (1,32 mf) was for the first time greater than that of the microfilariae; the width was 48 μm. The sausage shape appearance first noticed on Day 2 was still obvious (Fig. 1). Capsules were commonly seen (Fig. 3B).

On Day 6 there was little change in the width of most larvae but they had shown a rapid increase in length to 3,51 mf (608 μm) (Fig. 1 & 3C). One larva had even reached a length of 1 229 μm (7,10 mf) and a width of 65 μm.

Seven to 8 days after infection many coiled larvae in the range 1 058–1 613 μm (approximately 7 mf) could be clearly seen inside the capsules formed in the fat-body of both the abdomen and sometimes also the head (Fig. 1 & 3D, E). Their mean width of 66–74 μm was the greatest it would ever be. Many shorter larvae, similar to those seen on Day 6, were still present.

By Day 9 there had been a second rapid increase in larval length to 14,48 mf (2 505 μm), nearly twice the length of larvae measured the previous 2 days. Width, however, had decreased to approximately 60 μm and this did not alter again during the following 3 days to the termination of the study (Fig. 1 & 3F). Many of these larvae were free in the abdomen and head, although some could still be seen inside capsules. They showed the characters of 3rd stage infective larvae as described by Nevill (1975). Larvae of the types seen on Day 6 and Days 7 and 8 were still present.

Ten and 11 days after infection most larvae were in the 3rd stage and free in the haemocoele of the head and abdomen. None were confined to capsules. Their lengths had now increased to 2 810 μm (Day 10) and a final 3 454 μm (i.e. 19,97 mf) on Day 11. Some Day-6-type larvae could still be found in the fat and haemocoele of the abdomen and in the head fat.

On the 12th day the head contents only were examined and numerous 3rd stage larvae were present. Their length depended on the number per head (Nevill, 1975).

Development of P. bovicola *in* M. lusoria

A total of 25 microfilariae from a gravid *P. bovicola* and 42 larvae of different ages from 18 female *M. lusoria* were measured (Table 2). The length of the larvae was also compared with the length of the microfilariae. The smaller numbers involved were, however, sufficient for comparison with larvae recovered from *M. xanthomelas* to determine whether any major differences exist between the 2 fly species in their suitability for larval development of *P. bovicola*.

TABLE 2 The growth and sites of development of *Parafilaria bovicola* in artificially infected *Musca lusoria* maintained at 27 °C and 60% R.H. and a comparison with the length of newly hatched microfilariae (measurements in μm)

Days after infection	No. of infected flies examined ♀♀	♂♂	Site of larvae in fly*	No. of larvae measured	Mean length (range)	Mean width (range)	mf = Larval length / Microfilarial length
0			Microfilariae from eggs	25	173 (161–189)	6 (5–7)	1,00
1	2	0	gut; abd haem.	7	151 (136–177)	5 (4–7)	0,87
2	2	0	gut	2	168 (153–184)	4 (3–4)	0,97
			abd fat; abd haem.	6	113 (84–161)	14 (10–20)	0,65
3	1	0	abd haem.	1	167 (—)	3 (—)	0,97
4	2	0	abd fat; abd cap.; abd haem.	3	167 (133–226)	37 (33–44)	0,97
5	2	0	abd fat; abd cap.; abd haem.	4	378 (300–477)	47 (42–55)	2,18
6	2	0	abd fat; abd cap.; abd haem.	4	510 (403–661)	50 (48–54)	2,95
7	2	0	abd fat; abd cap.; abd haem.	1	1 316 (—)	48 (—)	7,61
9	2	0	abd fat; abd cap.; abd haem.; head haem.	6	2 423 (1 677–3 230)	56 (52–59)	14,01
10	2	0	head haem.	1	897 (—)	77 (—)	5,18
			abd. haem.; head haem.	6	3 189 (2 639–3 705)	58 (56–59)	18,43
11	1	0	head haem.	1	3 361 (—)	59 (—)	19,43
Totals	18	0		67			

* Abbreviations as in Table 1

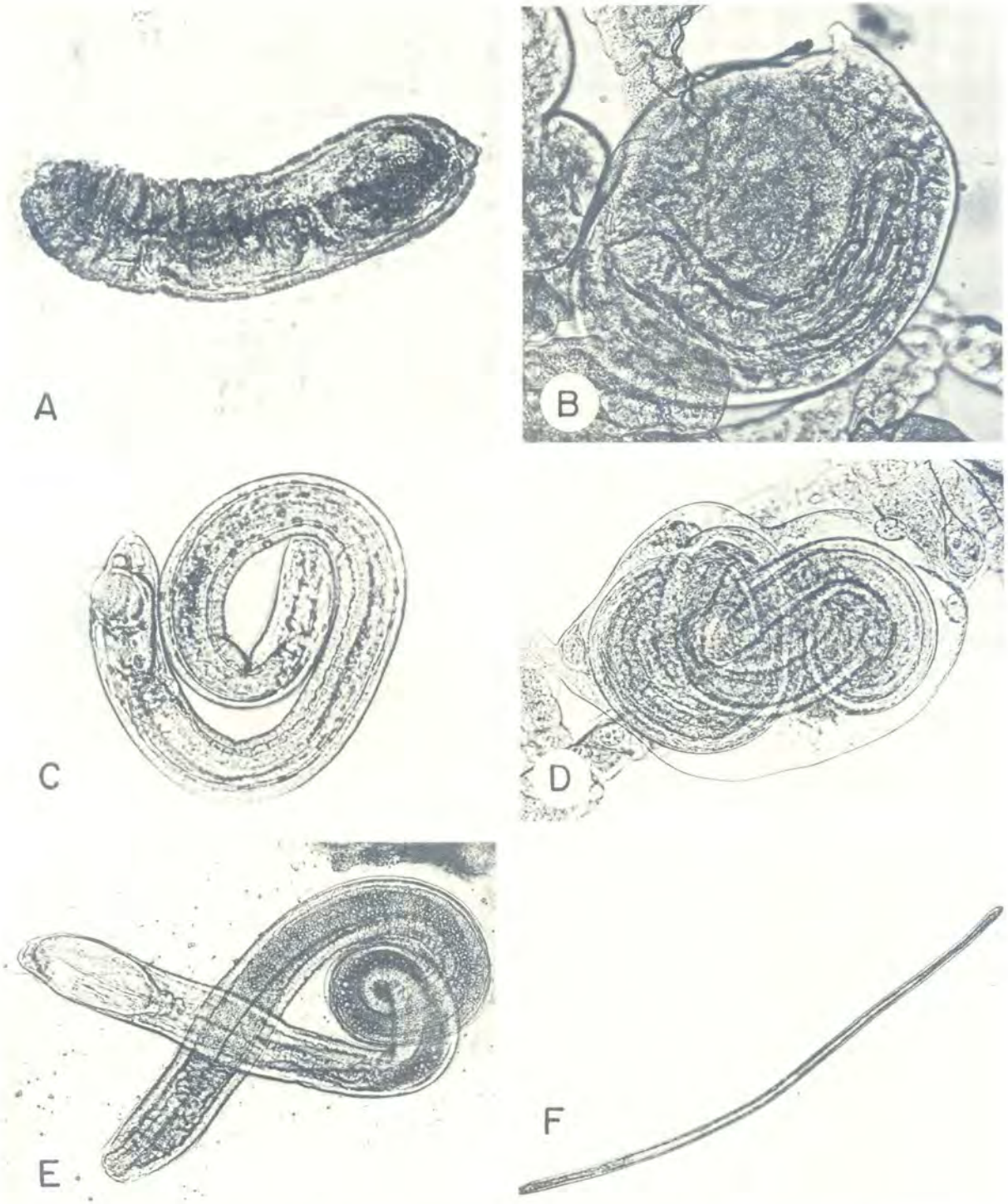

FIG. 3 Developmental stages of *Parafilaria bovicola*

A—Day 4; 1st stage larva in abdominal haemocoele of an artificially infected *Musca* vector (×530)
B—Day 5; 1st stage larva in capsule in abdominal fat-body of vector fly (×280)
C—Day 6; 1st stage larva in abdominal haemocoele of vector fly (×230)
D—Day 7; 2nd stage larva in capsule in abdominal fat-body of vector fly (×100)
E—Day 7; 2nd stage larva in abdominal haemocoele of vector fly (×180)
F—Day 11; infective 3rd stage larva from head of vector fly (×30)

The general development was similar in both fly species, the 3rd stage appearing in both after 9 days and increasing to a maximum length of over 19 mf by 11 days. The following minor differences were noticed:

(i) The shortest mean length of 113 μm (0,65 mf) was recorded after 2 days in *M. lusoria* as opposed to 3 days in *M. xanthomelas*.

(ii) A microfilaria-like larva was still recovered from the abdominal haemocoele of *M. lusoria* after 3 days as opposed to 1 day in *M. xanthomelas*.

(iii) After 5 days, larvae in *M. lusoria* (378 μm) were longer than their counterparts in *M. xanthomelas* (228 μm), but on Day 6 larvae in *M. xanthomelas* (608 μm) were longer than those in *M. lusoria* (510 μm).

Discussion

In their study of development during the first 96 h after artificial infection of flies, Sahai & Singh (1971) made no mention of larvae in the fat-body and recorded developmental stages only in the abdomen. Gnedina & Osipov (1960) were more specific in the case of *P. multipapillosa* and stated that the larvae left the haemocoele and entered the fat-body after 5–8 days. This, however, seems unlikely, for the larvae they recovered at this stage were 1 220 μm long and would have been far too large to enter or fit into a normal fat cell.

The more logical stage of entry into cells of the fat-body would be during the active period of the microfilariae when a cephalic or cutting hook is present. Of this hook Nelson (1964) states: 'This seems to be an important structure which enables the microfilariae to escape from the gut into the haemocoele, and it is probably used to penetrate the cell membrane of the thoracic muscles or fat-body, or whichever tissue serves as the final site for development.'

During the present studies this hook could be seen under high magnifications in the microfilariae recovered from the stomach and body cavity. Careful examination of fat-bodies from the abdomens of numerous flies was eventually rewarded with the discovery of a microfilaria coiled in the form of an S inside a cell, 24 h after infection. Thereafter numerous early 'sausage stages' were seen developing inside cells.

From the 3rd day after infection cell enlargement could be discerned, followed within 1 or 2 days by clearly visible capsule formation around developing larvae.

The process of capsule formation in *Habronema muscae* infections of *Musca domestica* was described in 1922 by Roubaud & Descazeaux (cited by Poinar, 1969) as follows: 'As the nematode developed, the fat cell hypertrophied with marked thickening of the cellular membrane. The nucleus also enlarged and eventually disappeared while fat globules transformed into a homogeneous hyaline fluid that bathed the parasite. Finally the stretched cellular membrane formed a type of capsule over the nematode; often this structure became detached, falling into the body cavity of the host. Enclosed nematodes absorbed the capsule's contents. Then the membranous covering broke, and the third (or late second stage) juveniles were liberated into the haemocoele.'

In the present studies, larvae in all stages of development were found lying free in the haemocoele. Since all stages were also observed inside the cells of the fat-body, it is likely that they were freed from the fat-body during dissection, as suggested by Lavoipierre (1958).

The appearance of *P. bovicola* larvae undergoes many changes during the developmental period in the intermediate hosts. Sudden substantial increases in larval length possibly indicate that a moult to the next stage has taken place. Initially, the microfilaria-like 1st stage larva shortens until by Day 2 or 3 it is approximately 0,65 the length of a microfilaria but 2–4 times as wide. This is the beginning of the so-called 'sausage stage'. Larvae continue to increase in both width and length and retain their sausage shape until Day 5 or 6.

Between Days 6 and 7, however, larvae suddenly increase to approximately 7 mf, (about 1 300 μm), that is, nearly twice their previous length, and take on an elongated worm-like shape which is retained until Day 9. This no doubt represents the period of development of the 2nd stage.

Nine days after infection larval length again rapidly increases to approximately 14 mf (about 2 500 μm) and characteristics of 3rd stage larvae also appear. Thus, the 3 larval stages in the fly can apparently be differentiated on measurements alone.

In these studies both *M. xanthomelas* and *M. lusoria* have been shown to be equally suitable for development of the immature stages of *P. bovicola*. These fly species should therefore be good vectors, since Nevill (1979) showed that the 3rd stage larvae could escape from both via their mouth-parts when they fed on ox-blood warmed to 40 °C.

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