

## STUDIES ON *DICTYOCAULUS FILARIA*

### II. MIGRATION OF THE DEVELOPMENTAL STAGES IN LAMBS

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#### ABSTRACT

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Developmental stages of *Dictyocaulus filaria* (Rudolphi, 1809) were recovered from the right colic mesenteric lymph nodes of lambs 18 hours after infestation. The majority of the parasites remain in the lymph nodes until Day 6 and from Day 7 onwards migrate via the thoracic duct to the heart and pulmonary arteries. From Day 8 to Day 14 parasites occur in considerable numbers in the lung tissue. Although some worms may be present in the trachea and bronchi on Day 4, they are not present in large numbers prior to Day 10. From the 12th day the numbers in the lung tissue decrease while those free in the respiratory passages increase.

#### INTRODUCTION

In 1914 Romanovitch & Slavine successfully infested four lambs with *Dictyocaulus filaria* (Rudolphi, 1809) by feeding them with infective larvae. Subsequent work by Guberlet (1919), Hobmaier & Hobmaier (1929) and Kauzal (1933) confirmed that the life cycle of this parasite is direct.

Hobmaier & Hobmaier (1929) and Kauzal (1933) studied the migration of the parasites in their normal host, the sheep. Although their studies were based on a limited number of animals they showed that the larvae migrate to the lungs via the mesenteric lymph nodes. Other investigations on this migration were carried out in abnormal hosts: in guinea pigs by Kauzal (1933) and in rabbits and mice as well as guinea pigs by Soliman (1953).

#### MATERIALS AND METHODS

The experimental design is summarized in Table 1.

The methods used were the same as those described by Anderson & Verster (1971).

#### 1. Migration through the lymph nodes to the lungs

The following organs of Sheep 1, 2, 3, 4, 5, 6, 8, 9, 15, 16 and 17 were examined separately for developmental stages of *D. filaria*:

- (i) *Lymph nodes*: mandibular, parotid, lateral and medial retropharyngeal (atlantal and supratharyngeal), deep cervical, hepatic (atrial-hepatic), dorsal and ventral abomasal, cranial mesenteric (duodenal), right colic (anterior and posterior jejunal-ileal, caecal and colic) and caudal mediastinal (mediastinal).
- (ii) *Gastro-intestinal tract*: tongue, oesophagus, rumen, reticulum, omasum, abomasum, duodenum, jejunum, ileum, caecum, colon and rectum.
- (iii) *Respiratory system*: the trachea and bronchi were washed to remove the parasites and the following parts of lungs examined separately: cranial and caudal parts of the left cranial lobe (left apical and left cardiac), the right middle lobe (right cardiac)

TABLE 1 *Experimental design*

Sheep No.	Age of sheep (months)	Breed	No. infective larvae dosed	Age of larvae (days)	Age of infestation at autopsy
1	9	Merino	15 000	26	18 hours
2	46	Merino	10 000	10	1 Day
3	16	Merino	15 000	18	1 Day
4	13	*Dorper	15 000	11	2 Days
5	10	Merino	15 000	8	4 Days
6	44	Merino	10 000	10	5 Days
7	12	Merino	10 000	10-30	6 Days
8	7	Merino	15 000	18	8 Days
9	13	Dorper	15 000	11	9 Days
10	6	Merino	10 000	10-30	9 Days
11	12	Merino	10 000	10-30	10 Days
12	5	Merino	10 000	10-30	11 Days
13	12	Merino	10 000	10-30	12 Days
14	6	Merino	10 000	10-30	12 Days
15	10	Merino	15 000	26	14 Days
16	26	Merino	10 000	10	21 Days
17	4	Dorper	15 000	11	28 Days
18	4	Dorper	15 000	—	4 Days
19	6	Dorper	11 000	30-38	7 Days
20	5	Merino	15 000	9-13	14 Days

\*A Dorper is a Dorset Horn × Blackhead Persian sheep

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and the left and right caudal lobes (left and right diaphragmatic).

- (iv) *Body fluids*: blood from the inferior vena cava, portal and jugular vein; lymph from the thoracic duct; cerebrospinal, pericardial, thoracic and peritoneal fluids.
- (v) *Other organs*: liver, diaphragm, pancreas, spleen, cerebral hemispheres, cerebellum, spinal cord and mesentery.

In Sheep 7, 10, 11, 12, 13 and 14 the following lymph nodes were examined separately: hepatic, dorsal and ventral abomasal; duodenal; anterior jejuno-ileal; posterior jejuno-ileal; caecal; colic and mediastinal. The parts of the lungs were examined similarly to those of the other animals in this experiment. The techniques used for the recovery of the parasites from these animals were modified as follows:

- (a) The nylon gauze in the modified Baermann apparatus was replaced with glass fibre gauze with apertures 1,05 mm by 1,37 mm.
- (b) The lymph nodes were placed on a glass-fibre gauze sieve fixed between two perspex rings 10 cm in diameter and 2 cm high. This was immersed in normal saline 4 cm deep in a 2 l beaker, which was then placed in a waterbath at 40°C for 6 hours.
- (c) The parasites were extracted in a water bath, not in an incubator.
- (d) Developmental stages from the lung were extracted in 1,2 per cent saline in traps placed in a waterbath. The lung tissue was placed in fresh saline at 2-hourly intervals.

### 2. Migration through the thoracic duct

The thoracic duct of Sheep 18 was catheterized on Day 4 and lymph drained from it for 4 hours before autopsy. Sheep 19 was catheterized on Day 7 and lymph withdrawn from 170 to 174 hours and again from 190 to 192 hours after infestation. The containers into which the lymph drained were replaced every 30 minutes. The lymph yield from each of the twelve 30-minute periods was divided into three aliquots A, B and C and examined as follows:

*Aliquot A*: This was heated to 60°C and treated as described by Anderson & Verster (1971).

*Aliquot B*: The lymph was subjected to pepsin digestion for periods varying from 1,5 to 6 hours. Iodine solution and formalin were added. It was washed through a 400 mesh sieve and both the residue and the filtrate examined.

*Aliquot C*: Iodine solution was added and it was washed through a 400 mesh sieve; both the residue and the filtrate were examined.

### 3. Migration through the liver

On Day 14, 150 ml 5 per cent Evans-blue was administered intravenously to Sheep 20. Fifteen minutes later the sheep was electrocuted, the liver removed and perfused with normal saline to remove parasites present in its circulatory system.

## RESULTS

### 1. Migration through the lymph nodes to the lungs

The numbers of parasites recovered from the various organs at different periods after infestation, as well as the percentage recovery, are summarized in Table 2.

In Table 3 the number of parasites recovered from the various lymph nodes, the lung tissue and the trachea and bronchi is expressed as a percentage of the total number of worms recovered.

The distribution of the developmental stages in the mesenteric lymph nodes, lung tissue and in the trachea and bronchi are illustrated graphically in Fig. 1. Sheep 6, which was autopsied on Day 5, is not included in this graph as parasites from the duodenal-pancreatic lymph nodes were pooled with the anterior and posterior jejuno-ileal lymph nodes and those from the lung tissue with those from the trachea and bronchi. On Day 1, 9 and 12 two sheep were examined; in Fig. 1 the data for Day 1 represent the mean number of parasites recovered from Sheep 2 and 3, Day 9 those for Sheep 9 and 10 and Day 12 those for Sheep 13 and 14. There are no marked differences in the distribution of the parasites in Sheep 2 and 3 on Day 1 and in Sheep 13 and 14 on Day 12 (Table 3). However, in Sheep 9 and 10, which were autopsied on Day 9, the distribution of the parasites differs markedly. In Sheep 9 52,0 per cent of the parasites were in the mesenteric lymph nodes, 43,4 per cent in the lung tissue and 4,1 per cent in the trachea and bronchi, while in Sheep 10 the distribution was 92,5 per cent, 1,0 per cent and 6,5 per cent respectively in these organs. The total number of parasites recovered from these animals also differed markedly, viz. 581 from Sheep 9 and 3 686 from Sheep 10.

For the first 6 days 97,7 to 99,1 per cent of the parasites are in the right colic lymph nodes. With the exception of Sheep 10, the number in these lymph nodes decreases progressively from Day 8 to Day 14 and thereafter they disappear from these organs.

TABLE 2. Number of *D. filaria* recovered from the different organs

Sheep No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Age of infestation (days)	0,75	1	1	2	4	5	6	8	9	9	10	11	12	12	14	21	28
Abdominal cavity - Mesenteric lymph nodes . . . . .	1 345	409	934	2 674	7 879	988	3 922	1 012	305	3 410	969	107	143	47	20	0	0
Intestine . . . . .	12	13	2	3	0	1	—	0	0	—	—	—	—	—	0	0	0
Liver . . . . .	0	3	4	2	0	0	—	4	0	—	—	—	—	—	0	0	0
Thoracic cavity - Trachea & bronchi . . . . .	0	0	0	0	7	—	3	25	24	38	240	607	1 402	1 284	393	731	4 706
Lung tissue . . . . .	0	0	0	32	85	34	31	660	252	238	482	811	557	696	558	693	1 060
Other organs . . . . .	9	1	0	0	0	—	—	1	0	—	—	—	—	—	3	0	0
Total number <i>D. filaria</i> recovered . . . . .	1 366	426	940	2 711	7 971	1 023	3 956	1 702	581	3 686	1 691	1 525	2 102	2 027	974	1 424	5 766
% Recovery . . . . .	9,1	4,3	6,3	18,1	53,1	10,2	39,8	11,4	3,9	35,9	16,8	14,8	20,2	19,7	6,5	14,2	38,4



TABLE 3 *Number of D. filaria in different organs (expressed as a percentage of the total number recovered)*

Sheep No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Age of infestation (Days)	0,75	1	1	2	4	5	6	8	9	9	10	11	12	12	14	21	28
% age of worms recovered																	
Lymph nodes -																	
Atrial-hepatic, dorsal & ventral abomasal	0	0,2	0	0	0	0	0	0,9	0	0	0	0	0	0,2	0	0	0
Duodenal-Pancreatic	0,7		0,9	0,04	0,2		0	0,8	0,5	0,2	0	0,7	0	0,1	0	0	0
Anterior Jejuno-ileal	11,3	95,8	45,1	92,0	91,7	92,8	20,2	49,2	47,0	0,4	2,4	0,5	0,3	0,2	1,4	0	0
Posterior Jejuno-ileal	85,1		52,2	6,7	7,0		54,0	7,1	2,1	90,6	25,4	5,8	1,8	1,6	0	0	0
Caecal & Colic	1,3	0	1,2	0,07	0	3,8	24,9	1,4	2,9	1,3	29,5	0	4,7	0,2	0,6	0	0
Total in lymph nodes	98,4	96,0	99,4	98,7	98,9	96,6	99,1	59,4	52,5	92,5	57,3	7,0	6,8	2,3	2,1	0	0
Lungs -																	
Trachea & bronchi	0	0	0	0	0,1		0,1	1,5	4,1	1,0	14,2	39,8	66,7	63,3	40,3	51,3	81,6
Lung tissue	0	0	0	1,2	1,0	3,3	0,8	38,9	43,4	6,5	28,5	53,2	26,5	34,3	57,3	48,6	18,3

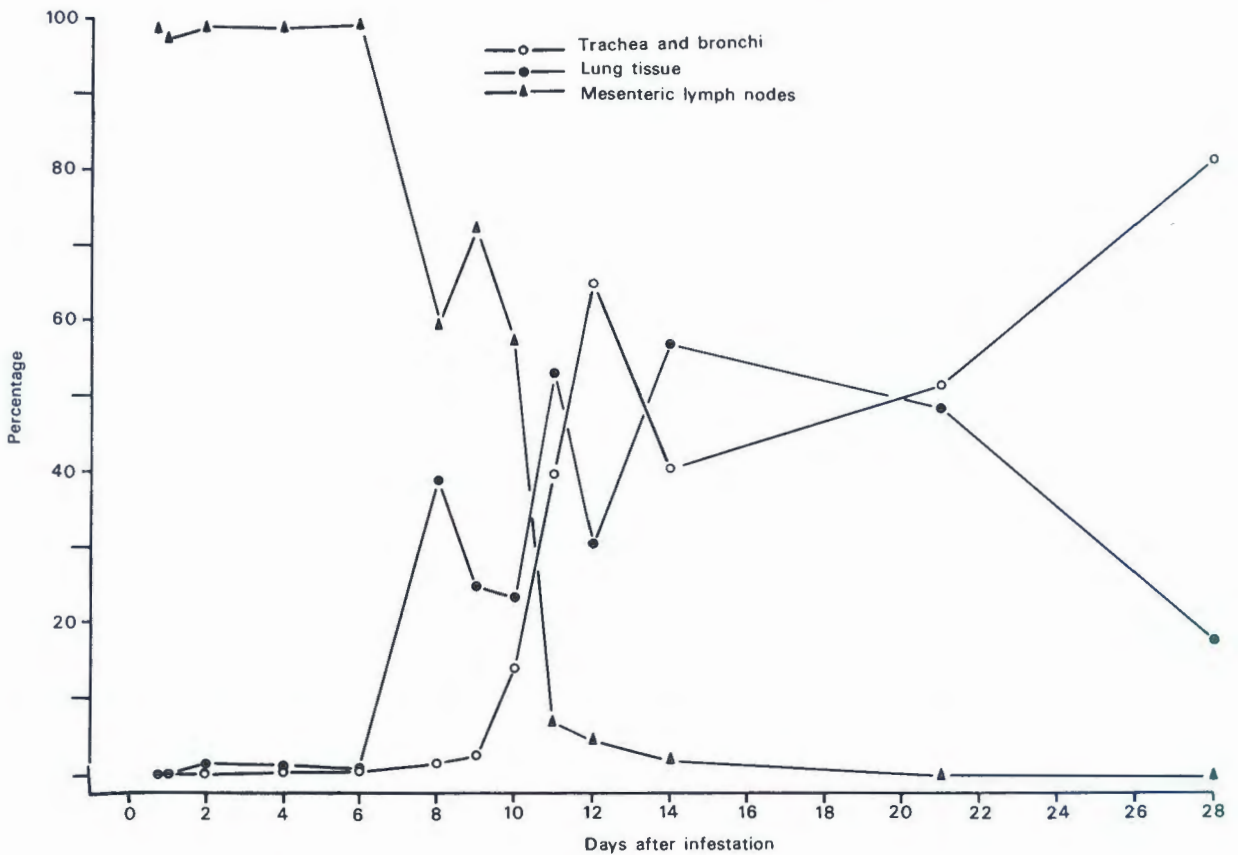


FIG. 1 The migration of *D. filaria* expressed as a percentage of the worms recovered from the mesenteric lymph nodes, the lung tissue and the trachea and bronchi

A single parasite was recovered from the pooled mandibular, parotid and lateral and medial retropharyngeal lymph nodes of Sheep 2. The mediastinal lymph nodes of Sheep 7 and 14 contained one and three parasites respectively.

A small number of parasites (1,2 per cent) are present in the lung tissue on Day 2. Thereafter the number in-

creases progressively to reach a peak of 57,3 per cent on Day 14 and then decreases again to 18,3 per cent on Day 28.

Small numbers of parasites are present in the trachea and bronchi from Day 4 onwards. By Day 11, however, this number has increased to 39,8 per cent and on Day 28 to 81,6 per cent.

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TABLE 4 Recovery of parasites from the various parts of the lung

Sheep No.	1	3	4	5	7	8	9	10	11	12	13	14	15	16	17
Age of infestation	0,75	1	2	4	6	8	9	9	10	11	12	12	14	21	28
Part of lung															
Trachea & bronchi . . . . .	0	0	0	0,09	0,08	1,5	4,1	1,0	14,2	39,8	66,7	63,3	40,3	51,3	81,6
Left lung															
Cranial lobe															
Cranial part . . . . .	0	0	0,2	0,1	0	0,8	3,6	0,6	0,2	1,9	0,3	1,1	3,0	1,3	2,2
Caudal part . . . . .	0	0	0,3	0,1	0,03	4,9	9,3	0,9	1,5	3,9	1,9	3,1	8,7	5,4	2,0
Caudal lobe . . . . .	0	0	0	0,1	0,3	12,7	8,1	1,6	11,7	19,6	5,8	11,8	16,5	3,4	2,3
Right lung															
Cranial lobe . . . . .	0	0	0,2	0,2	0,03	3,6	11,4	0,2	1,2	3,1	0,3	2,1	3,0	2,5	5,9
Middle lobe . . . . .	0	0	0,2	0,1	0,1	6,6	1,0	1,2	3,3	7,8	3,2	6,0	7,5	5,9	2,3
Caudal lobe . . . . .	0	0	0,4	0,4	0,4	10,3	10,0	2,0	10,6	16,9	15,0	10,3	18,6	30,1	3,6

In Table 4 the number of parasites recovered from the different parts of the lungs is expressed as a percentage of the total number recovered. With the exception of Sheep 16 and 17, the majority occur in the two caudal lobes probably because these are larger than the other lobes of the lung. In ten sheep the left caudal lobe constitutes 32,9 per cent and the right 38,5 per cent of the total weight of the lungs. The right caudal lobe is larger than the left one and this possibly accounts for the fact that it contained more parasites in the majority of the sheep.

Small numbers of parasites were recovered from various parts of the alimentary canal and from other organs which are not listed in Table 2. These parasites may have originated either from the lumen or from the wall of the intestine because the ingesta was not removed from the gut before it was cut into pieces and placed in the Baermann apparatus. A single parasite was recovered from the duodenum of Sheep 6. The jejunum of Sheep 1 and 2 contained 12 and 10 parasites respectively, while the ileum of Sheep 2 contained three and that of Sheep 3 and 4, one each. Single parasites were recovered from the caecum of Sheep 4 and from the colon of Sheep 4 and 5. The mesentery of Sheep 1 contained nine parasites. A single parasite was recovered from the diaphragm of Sheep 8 and three were present in the thoracic fluid of Sheep 16.

2. Migration through the thoracic duct

The lymph present in the thoracic duct of Sheep 1, 2, 3, 4, 5, 6, 8, 9, 15, 16 and 17 did not contain any larvae.

Lymph was drained for hourly intervals for a total

period of 4 hours from the thoracic duct of Sheep 18 on Day 4. During the first 3 hours 210 ml, 215 ml and 205 ml lymph was collected but the tube became blocked during the last hour when 95 ml only was drained. This lymph was negative for parasites and although the mesenteric lymph nodes contained parasites their number was not determined. There were 106 larvae in the lung tissue.

Lymph collection from the catheterized thoracic duct of Sheep 19 commenced 170 hours after infestation when a total of 582 ml was collected over 4 hours. Sixteen hours later a further 181 ml lymph was collected over 2 hours.

The lymph from each 30 minute period was divided into three aliquots A, B and C. The amount of lymph withdrawn in each period, and the number of parasites recovered in the different aliquots, are summarized in Table 5. The greatest number of parasites, viz. 21, was recovered from Aliquot A, which was heated to 60°C prior to fixation. Aliquot B, which was subjected to digestion with pepsin/HCl, did not contain any helminths. Ten parasites were found in the residue of aliquot C, which was treated with iodine solution prior to sieving it.

As these aliquots probably contained approximately the same number of parasites, it may be assumed that about 60 parasites would have entered the circulatory system in the 6 hours during which lymph was collected.

3. Migration through the liver

Developmental stages were recovered in small numbers from the livers of Sheep 2, 3, 4 and 8 (Table 2).

TABLE 5 Number of *D. filaria* recovered from the lymph of sheep 19

Period of Collection (Hours after infestation)	Amount of lymph collected (ml)	Number of <i>D. filaria</i>			
		Aliquots			Total
		A	B	C	
170,0 - 170,5 . . . . .	125	0	0	0	0
170,5 - 171,0 . . . . .	80	2	0	0	2
171,0 - 171,5 . . . . .	86	3	0	2	5
171,5 - 172,0 . . . . .	68	3	0	0	3
172,0 - 172,5 . . . . .	66	0	0	0	0
172,5 - 173,0 . . . . .	53	4	0	0	4
173,0 - 173,5 . . . . .	58	1	0	0	1
173,5 - 174,0 . . . . .	46	0	0	0	0
190,0 - 190,5 . . . . .	53	0	0	2	2
190,5 - 191,0 . . . . .	44	5	0	2	7
191,0 - 191,5 . . . . .	20	1	0	2	3
191,5 - 192,0 . . . . .	64	2	0	2	4
<b>TOTAL . . . . .</b>	<b>763</b>	<b>21</b>	<b>0</b>	<b>10</b>	<b>31</b>



A 5 per cent solution of Evans-blue (150 ml) was administered intravenously to Sheep 20 quarter of an hour before autopsy. This amount was, however, excessive and as a result the tissues were too heavily stained to distinguish tracks which the parasites may have made during migration.

No parasites were recovered from the blood collected by perfusion of the liver with saline. A total of 3 335 parasites was recovered from this animal; 3 271 (98,1 per cent) from the mesenteric lymph nodes and 64 (1,9 per cent) from the lung tissue. There were no parasites in the lymph contained in the thoracic duct.

#### DISCUSSION

Although Romanovitch & Slavine (1914) and Guberlet (1919) infested lambs with *D. filaria* they did not follow their migratory route from the intestine to the lungs.

Hobmaier & Hobmaier (1929) showed that a part of the developmental cycle of *D. filaria* occurs in the mesenteric lymph nodes. They infested a sheep on three successive occasions and then autopsied it when the parasites were 4, 5 and 10 days old. Immature males and females as well as larvae that were moulting were present in the mesenteric lymph nodes and immature adults in the lungs. The liver of this animal contained three larvae.

According to Kauzal (1933), larvae of *D. filaria* are present in the mesenteric lymph nodes of lambs 24 hours after infestation. In guinea pigs and mice these parasites also reach the lymph nodes within 24 hours; they remain in these nodes up to Day 7 in guinea pigs and Day 10 in mice but their number decreases as the infestation ages (Soliman, 1953). This study showed that the majority of the parasites reach the mesenteric lymph nodes within 18 hours (Sheep 1) and do not leave these organs until after the 6th day (Table 3). On Day 8 there is a marked decrease in the number of parasites in the lymph nodes (Sheep 8), while the number in the lung tissue increases simultaneously. The mesenteric lymph nodes of Sheep 10 and Sheep 20, which were autopsied on Day 9 and Day 14, contained large numbers of parasites; it is not known why migration to the lungs was delayed in these two animals.

Kauzal (1933) found that *D. filaria* is present in the lung tissue of lambs on Day 5 and in guinea pigs on Day 8. Soliman (1953) records them in the lung tissue of guinea pigs and mice on Day 3 and in rabbits on Day 5. In this study 32 larvae were found in the lung tissue 2 days after infestation (Sheep 4). In a study on the morphogenesis of *D. filaria* a single parasite was recovered from the lung tissue of an animal autopsied 1 day after infestation (Verster, Collins & Anderson, 1971).

In guinea pigs, parasites pass from the lung tissue to the trachea and bronchi on Day 7 (Soliman, 1953). In lambs the migration from the lung tissue to the bronchi commences on Day 4 (Sheep 5: Tables 2 & 3).

Larvae of *D. filaria* have been recovered from the liver of lambs (Hobmaier & Hobmaier, 1929) and of guinea pigs on Day 2 (Kauzal, 1933) and on Day 20 (Soliman, 1953). In this study from 2 to 4 parasites were found in the livers of Sheep 2, 3, 4 and 8, which were autopsied on Day 1, 2 and 8.

Intravenous injections of Evans-blue were used by Yokogawa, Yoshimura, Sano, Okura & Tsuji (1962) to investigate the migratory route of *Paragonimus westermani* (Kerbert, 1878) in cats. This stain concentrates in the migratory tracts made by the parasites and facili-

tates their recovery from the various tissues. Prior to autopsy 150 ml of a 5 per cent solution was administered to Sheep 20 but this amount proved to be excessive and migratory tracts could not be distinguished in the heavily stained tissues. No parasites were found in blood collected by perfusion of the liver of this animal.

The fact that there were no parasites in the liver of Sheep 20 does not prove conclusively that *D. filaria* does not migrate to the lungs via the blood stream and the liver. This animal, like Sheep 15, was autopsied on Day 14 when the majority of parasites had already reached the lungs. In Sheep 20, however, migration was delayed; 98,1 per cent of the parasites were in the mesenteric lymph nodes and 1,9 per cent in the lung tissue. The failure of the parasites to migrate to the lungs cannot be related to the age of the animal (5 months) as seven of the other animals varied from 4 to 7 months in age. The age of the infective larvae used to infest this animal (9 to 13 days) was similar to that used to infest the majority of the other animals. As in the case of Sheep 10, this is probably due to individual idiosyncrasy.

The presence of small numbers of parasites in the livers of some animals is probably due to aberrant migration of individual parasites and does not prove that they normally pass through this organ during migration. If passage through the liver is part of the normal migratory route, this organ should contain greater numbers of parasites than are usually found in it. Moreover, although Hobmaier & Hobmaier (1929) recovered three parasites from the liver of a lamb only one showed slight movements and the other two were dead.

As 21 parasites were recovered from one third of the lymph drained from the thoracic duct of Sheep 19, it may be assumed that at least 60 parasites were present in the lymph drained from this animal on Day 7. Although the number of larvae recovered from this lymph is negligible their presence in it is proof that the parasites enter the circulatory system via the thoracic duct.

This study shows the migratory route of *D. filaria* in sheep to be as follows: Within 18 hours after infestation the majority of the larvae have left the lumen of the small intestine and have entered the right colic mesenteric lymph nodes, particularly those which drain the lymphatic ducts from the jejunum and ileum. The majority of the parasites remain in the lymph nodes until Day 6 and from Day 7 onwards migrate via the thoracic duct to the anterior vena cava and thence to the heart and pulmonary arteries. From Day 8 to Day 14 developmental stages occur in considerable numbers in the lung tissue. Although some parasites may be present in the trachea and bronchi as early as Day 4, they are not present in large numbers prior to Day 10, when 14,2 per cent are free in the respiratory passages. From the 12th day the number of parasites in the lung tissue decreases, that in the trachea and bronchi increases and on Day 28 81,6 per cent are in the trachea and bronchi while 18,3 per cent are in the lung tissue.

*D. viviparus* follows a similar migratory route. According to Poynter, Jones, Nelson, Peacock, Robinson, Silverman & Terry (1960), larvae are present in the lungs of calves 1 to 3 days, and of guinea pigs 1 to 2 days after infestation; Douvres & Lucker (1958) record *D. viviparus* from the lungs of guinea pigs 18 hours after infestation. Developmental stages of *D. viviparus* have not been recovered from the liver of experimentally infested animals but Poynter *et al.* (1960) recovered them from the heart of a guinea pig 9 days after infestation.

SUMMARY

Developmental stages of *D. filaria* are present in the mesenteric lymph nodes of lambs 18 hours after infestation. They develop in the lymph nodes until Day 6 and from Day 7 onwards migrate via the thoracic duct to the heart and pulmonary vessels. From Day 8 to Day 14 there are considerable numbers of parasites in the lung tissue. The number free in the respiratory passages increases from Day 10 onwards.

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