STUDIES ON *FILAROIDES OSLERI* INFESTATION IN DOGS

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I. History

Osler (1877) investigated a pneumonic disease that had broken out amongst young foxhounds belonging to the Montreal Hunt Club in Canada. He stated, " at the time of the visit only one pup was ill, presenting symptoms of diminished airspace in the chest . . . On the following day it was found at autopsy that in addition to the pneumonia there were numerous small parasitic worms in the trachea and bronchial tubes ". Osler went on further to describe the symptoms and pathology noticed, noteworthy being the following:—

" Cough was not a prominent symptom, being absent in many cases. When present, it was short and husky, not the regular distemper cough. In the case brought to the infirmary the cough was well marked and was dry and short ". Eight cases were examined post mortem and in five of these Osler found, " irregular little masses of greyish-yellow colour, which on close inspection are found to be localised swellings of the membrane, containing small parasitic worms, the white bodies of which can be seen lying upon and partially embedded in these elevations. They are most abundant just at the bifurcation . . . Very few of the worms are found lying free on the mucous membrane; almost all of them are attached to the masses or buried in them ".

Osler had this to say about the origin of the epidemic:— " The origin of the epidemic must, I am afraid, like that of so many other diseases, remain obscure . . . There had been no change in the locality nor in the food . . . The disease broke out, too, during a spell of very severe weather when the food left in the pans froze quickly. The course of the epidemic was short, lasting six or seven weeks, a sufficient time, however, to destroy almost all the pups in the kennels ". Osler went on to call the parasite *Strongylus canis bronchialis* but when he asked Cobbold (1879) in London to identify the worms, the latter named them *Filaria osleri*.

Other reports of its occurrence in dogs soon followed that of Osler from Germany (Blumberg, 1882), India (Gaiger, 1909), United States of America (Milks, 1914), Great Britain (Hare, 1930; Ascoli, 1936), Australia (Keep, 1951) and Scotland (Urquhart, Jarrett & O'Sullivan, 1954). In South Africa the disease was simultaneously reported by Ortlepp (1945) and Steyn (1945). Apart from the domestic dog (*Canis familiaris*) one case was reported by Price (1928) in a coyote, *Canis latrans texensis*.

Hall (1921) proposed the creation of a new genus for this parasite namely *Oslerus* and *Filaria osleri* was then known as *Oslerus osleri* until Dougherty (1943) proposed its inclusion in the genus *Filaroides*—hence its accepted name *Filaroides osleri* (Cobbold, 1879).

II. Breeds of Dogs Affected

F. osleri has been reported in Foxhounds (Osler, 1877; Ogilvie, 1962), Beagles (Milks, 1914, 1915), mongrels (Olsen & Bracken, 1959), Cocker Spaniels (Ogilvie, 1962; Lauder, 1962), Greyhounds (Urquhart *et al.*, 1954), Otter Hounds (Urquhart *et al*, 1954), Miniature Poodles (Lauder, 1962; Dorrington, 1966), Dachshunds (Lauder, 1962), Bull Mastiffs (Ortlepp, 1945, 1963), Boxers (Dorrington, 1959), Pugs (Dorrington, 1959), Alsations (Dorrington, 1966) and Labradors (A. J. J Maree, private practitioner, Stellenbosch, 1966).

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III. SOUTH AFRICAN HISTORY

Ortlepp (1945) received three Bull Mastiffs at Onderstepoort for examination. He diagnosed *F. osleri* from swabs taken from the throat and confirmed this at autopsy. Simultaneously Steyn (1945) described two clinical cases referred to him in Johannesburg also involving Bull Mastiffs. Ortlepp (Onderstepoort, personal communication, 1963) was able to trace back the history of all infested cases subsequently discovered to an imported Bull Mastiff bitch even though many owners were loath to admit having the infestation among their breeding stock.

Malherbe (1954) reported that *F. osleri* was for all practical purposes an infestation confined to the Bull Mastiff breed in South Africa where it was assuming such serious proportions that this particular breed seemed doomed to extinction. Such was the accepted position until 1958 when Boxers were found to be suffering from the disease as well (Dorrington, 1959).

Mason (1958) (private practitioner, Grahamstown, personal communication) performed an autopsy on an adult Boxer dog " Duke ", that was reported to have strangled itself with its lead. He found well established *F. osleri* lesions.

The dam of Duke, Judy, was found to be free of *F. osleri* seven years later but another bitch, Biddy, of the same stud was known to have suffered from a chronic tracheo-bronchitis that had failed to respond to any therapy.

Biddy was unfortunately never examined for *F. osleri*, and was purchased from a breeder in the Western Cape Province whose stud was found subsequently also to be infested. Biddy's dam was imported in whelp from England and in view of subsequent observations, can be assumed quite conclusively to have introduced the disease to Boxers in South Africa. The possibility of cross infestation amongst adult dogs seems highly unlikely in view of findings discussed later in this paper. This would appear to contradict the case of Biddy and Judy, mentioned earlier, but it has been established that in this particular stud these two bitches were in close association at that time so that Biddy, although not infesting Judy, did in fact infest her puppies. The other possibility is that Judy was infested at that time, but when examined seven years later, had lost the infestation. This would agree with Pillers (1935) who stated that if infested dogs were kept in clean insect free loose boxes for four to five months the worms would die off and the nodules would disappear. However, this possibility is most unlikely in view of the life cycle studies mentioned later.

It has also been observed by the writer that mildly infested dogs, with one to three small nodules, remain infested for many years even if kept away from infested studs.

Apart from the brachycephalic breeds, e.g. Bull Mastiffs, Pugs and Boxers, *F. osleri* has also been found in French Poodles in South Africa (Dorrington, 1966), Alsations (Dorrington, 1966) and Labradors (Maree, *loc. cit.*), so that it can now no longer be claimed to be a breed-specific disease.

In conclusion, it can be stated that *F. osleri* has a world-wide distribution affecting many different breeds of dogs not necessarily confined to studs.

IV. Symptoms

Verminous bronchitis, as Osler (1877) originally called the disease caused by *F. osleri*, is characterized clinically by a sporadic yet persistent cough—a true chronic tracheo-bronchitic non-productive cough giving the impression of the causal agent being situated in the middle third of the respiratory tract. It has been observed by the writer that in most cases the dog at rest will show no signs of tracheitis, but if exercised or excited will usually develop a fit of coughing. The cough remains dry with eventual attempts at vomition—the latter delivering a white frothy mass of mucus.

The symptoms often depend on the severity of the infestation; however, grossly infested cases have been known to show little or no symptoms. This is true especially in adult dogs. In the case of younger dogs the disease becomes more pronounced so that even at rest forced expiration is seen. A ruptured mediastinum frequently follows with resultant emphysema. Emaciation develops in such advanced cases notwithstanding a fairly good appetite; the dogs prefer to sleep standing and adopt the typical air hunger position. In cases where young dogs are so affected death is inevitable. Mortality is not high in most cases, but depending on the severity of the infestation, it can reach 75 per cent in some litters, usually occurring between the ages of four to eight months.

The age at which clinical symptoms are first observed varies between two-and-a-half months to well over two years, the average age for the first appearance of symptoms being four to six months. The younger the dog when it shows the first signs of infestation, the poorer is its chance of survival. The converse need not necessarily apply, however, as two year-old dogs have suddenly developed the typical symptoms for the first time and shown rapid deterioration with death occurring a few months later. The reason for this apparent delay in the onset of symptoms remains obscure.

In infested studs marked variations in the symptoms and prognosis have been observed.

TABLE 1.—*Variations in symptoms of dogs infested with F. osleri*

Class	Infestation	Condition	Cough	Breathing at rest	Prognosis
1	Non-infested.	Good........	Absent......	Normal........	Excellent
2	Infested......	Good........	Absent......	Normal........	Good
3	Infested......	Good........	Sporadic.....	Normal........	Good
4	Infested......	Emaciated...	Persistent....	Normal........	Adults—fair Puppies—Hopeless
5	Infested......	Emaciated...	Absent......	Laboured, forced expiration	Hopeless

From Table 1 it will be seen that, as in Class 2, the infestation cannot necessarily be diagnosed on clinical grounds alone. Hence the futility of attempts at eradication on clinical grounds only is quite obvious.

V. Pathogenesis

The pathogenesis of *F. osleri* infestation depends to a large extent on the number of nodules, the age of the dog, the location of the nodules in the trachea or bronchi and the concurrent degree of infestation with other helminths.

Generally, the fewer and smaller the nodules the less severe the clinical symptoms will be, but some dogs have shown only mild symptoms and yet were grossly infested with one bronchus completely occluded and the other no more than 10 per cent patent. The degree of mechanical obstruction, therefore, need not necessarily correspond with the severity of the symptoms observed. This is an important fact to consider when examining a dog infested with *F. osleri*.

Similarly the writer has found that the younger the dog showing signs of the infestation, the more severe the symptoms are likely to be as the condition develops and the higher is the mortality. The reason for this is more fully discussed under susceptibility, but one aspect to be borne in mind here, is the anatomical peculiarity of the respiratory tract in young animals. The bronchial tubes of a young animal, compared with those of an adult, are much softer and less rigid. As a result of the irritating nodules in the trachea and bronchi of an infested puppy an increase in mucus formation can be expected. Because of the softer bronchi in young animals, this mucus is harder to expel and hence accumulates. This not only partially occludes or reduces the diameter of the lumen but destroys or renders ineffective, at least over an important area, the ciliary action so essential for expelling mucus. Such a young dog is, therefore, likely to develop " verminous pneumonia ". In fact this is the sequence of events frequently seen in young dogs and recorded in the literature (Osler, 1877; Steyn, 1945).

The writer has observed that where a few nodules are located in the trachea anterior to its bifurcation, it is likely that the dog will not show much clinically—refer Class 2 (Table 1) while the typical chronically affected animal is likely to have the nodules concentrated around the bifurcation of the trachea—Classes 3 and 4 in adults (Table 1). In Class 5 the nodules are situated not only in the trachea, but also at the bifurcation extending into the primary as well as the secondary intra-pulmonary bronchi. In such cases it is not uncommon to find a single large nodule completely obliterating the lumen of a bronchial branch, thereby rendering that lobe of the lung inactive. In other cases pedunculated nodules, located at the bifurcation, have been seen to cause only intermittent symptoms of dyspnoea as a result of their valve-like action.

It has been observed that the greater the concurrent infestation with other helminths, especially ascarids, the more deleterious is the effect of *F. osleri*.

As mentioned earlier, pneumonia and emaciation are frequent sequelae to infestation with *F. osleri*. The explanation for these sequelae is the mechanical obstruction due to the nodules and the accumulation of mucus in the bronchi. Moreover, this leads directly to inadequate gaseous exchange in the terminal alveoli with a resultant hypoxia and increase in the carbon dioxide level in the blood. Generalized cyanosis is, therefore, a symptom frequently observed in advanced cases. Such prolonged hypoxia and hypercapnia undoubtedly have a depressing effect on the vital organs, the liver and kidneys especially, thereby accounting for the advance in emaciation in spite of an adequate intake of quality food.

VI. SUSCEPTIBILITY

Since *F. osleri* has been diagnosed in many different breeds of dogs it can be stated that all members of *Canis familiaris* are equally susceptible. Furthermore, there is no difference in sex susceptibility as it has frequently been seen by the writer, in litter after litter from infested bitches, that both sexes are infested to the same degree. This fact was confirmed in the transmission experiments, discussed later under " life cycle ", where half of the total of each sex in a litter was infested while the other half, constituting the controls, remained uninfested. Similarly no difference in age susceptibility was found experimentally.

Under natural conditions, however, adult dogs seemed to be highly resistant to infestation, whereas young puppies especially under the age of six weeks, were nearly all susceptible. This difference may be due to the following factors:

1. *Anatomical*

Apart from the less rigid physical structure of the trachea and bronchi in younger animals already mentioned, histological differences in the trachea between puppies and adult dogs were observed:—

(a) The ciliated epithelial membrane of puppies has a noticeable paucity of goblet cells. This results in the presence of less mucus in the trachea of puppies than in older dogs. The expulsion of foreign matter or parasites would conceivably be more difficult and a parasite could attach itself more rapidly under such conditions (see Plates 1 and 2).

PLATE 1.—Ciliated epithelial membrane of trachea of newly born pup showing isolated goblet cells and no glandular tissue × 320.
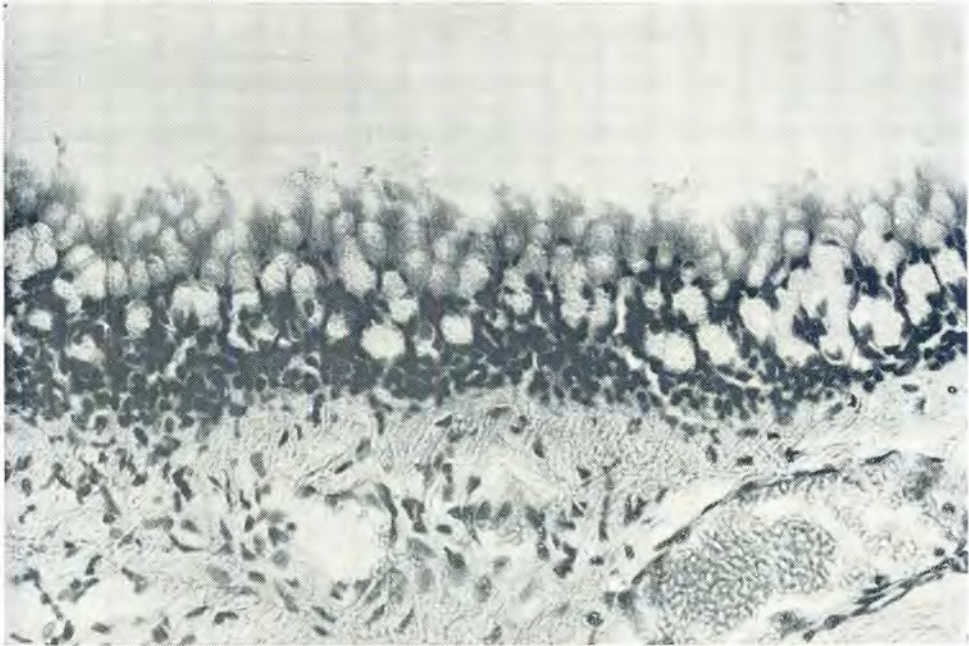
PLATE 2.—Ciliated epithelial membrane of trachea of adult dog showing numerous goblet cells × 312.

(b) Less developed mixed glandular tissue (serous and mucous) is evident in the trachea of puppies when compared to that of adult dogs. As mentioned in (a) above, this results in a diminution in secretions, thereby facilitating the establishment of a parasite in this region (compare Plates 1 and 3).

2. Habitat

It is natural for a nursing bitch to lick and clean her newly born pups constantly, particularly during the first few weeks of life, whereafter she will still feed them but not pay as much attention to their hygiene. If a nursing bitch is infested with *F. osleri* and discharging eggs at that particular time, it is conceivable that she can pass numerous eggs directly on to her pups.

From the life cycle experiments discussed later it will be seen that this in fact does occur. Hence the greatly increased exposure of young puppies to infestation.

3. Natural Resistance

A non-infested adult dog kept in close contact with infested bitches for over a year developed one small nodule only. This would suggest a natural resistance.

In a transmission experiment with two adult dogs discussed later, the writer found, however, that they were as susceptible to infestation as puppies. This apparent natural resistance can, therefore, be ascribed only to the degree of exposure and to a lesser extent to anatomical differences.
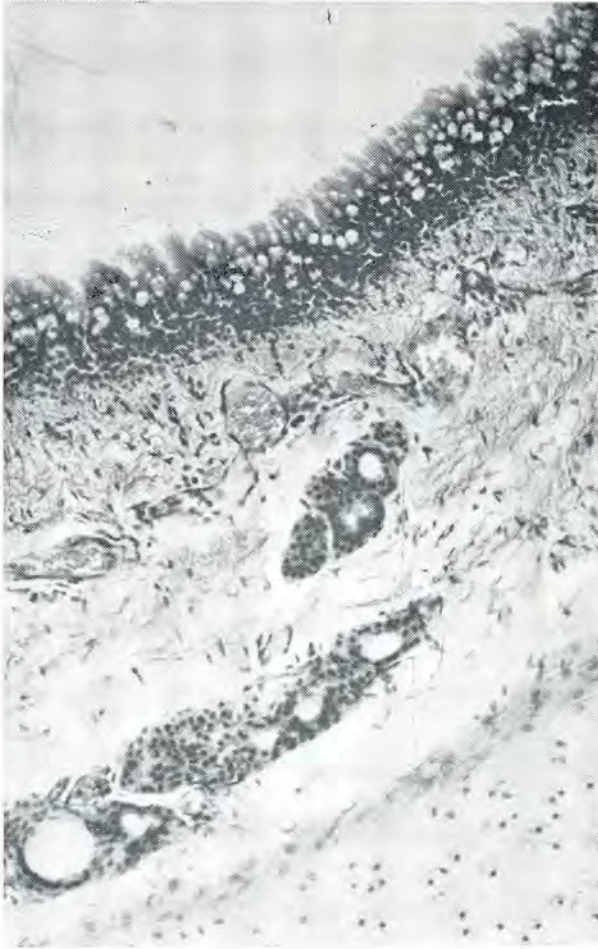
PLATE 3.—Tracheal epithelium of adult dog showing numerous goblet cells and mixed glandular tissue in submucosa × 285.

VII. LESIONS

Under natural conditions macroscopic lesions can be seen for the first time at the age of two-and-a-half months when using the illuminated bronchoscopic technique discussed later.

Under experimental conditions as detailed under the life cycle studies, a positive diagnosis can be made as early as two months. A negative diagnosis, however, should only be arrived at at the age of five to six months since there may only be a few small lesions in cases where infestations are minimal.

Most reports on *F. osleri* describe the lesion seen at autopsy (Osler, 1877; Ascoli, 1936; Urquhart *et al.*, 1954; Ortlepp, 1945; Steyn, 1945; Olsen & Bracken, 1959; Ogilvie, 1962; Mönnig, 1950). They are as follows:—

1. *Macroscopic lesions*

The lesions encountered post mortem depend largely upon the degree of infestation. Where only a few nodules are present, no abnormalities apart from the nodules will be seen in the lungs or any other organs. Where gross infestation is present, secondary changes are likely to appear such as emphysema, varying degrees of pneumonic changes in the lungs as well as cyanosis and general emaciation.

PLATE 4.—Trachea and bronchi of dog showing *F. osleri* papillomata × 1·5.

In a typical case of infestation one can expect thick frothy mucus in the trachea, bronchi or bronchioli. On the mucosal surface of the trachea and especially at the level of the bifurcation, one will find hyperaemic, raised, elongated and/or pedunculated nodules with their long axes usually corresponding to that of the trachea. They vary in size from miliary nodules to plaques measuring 2 cm long by 1 cm wide and 1 cm deep. In some cases they completely occlude secondary bronchi.

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These nodules have a pinkish grey colour and even though they sometimes appear granulomatous, their surface is smooth and transparent. Coiled masses of white worms embedded in the nodules are clearly visible from the surface with many of the posterior extremities of the female worms protruding into the lumen of the trachea. The nodules are soft and friable and are easily scraped or cut off from the tracheal lining. There is no ulceration of the mucosa and any pressure on the nodules will cause many more worms to protrude from them. In grossly infested cases the nodules extend from the bifurcation of the trachea into the primary bronchi (see Plate 4) and have on occasion been observed in the secondary bronchi.

When extirpated nodules are placed in normal saline or water, numerous filamentous worms will protrude from them within seconds. This phenomenon should facilitate the making of a rapid tentative diagnosis of *F. osleri* infestation.

PLATE 5.—Section of trachea of dog showing early development of *F. osleri* nodule × 162.

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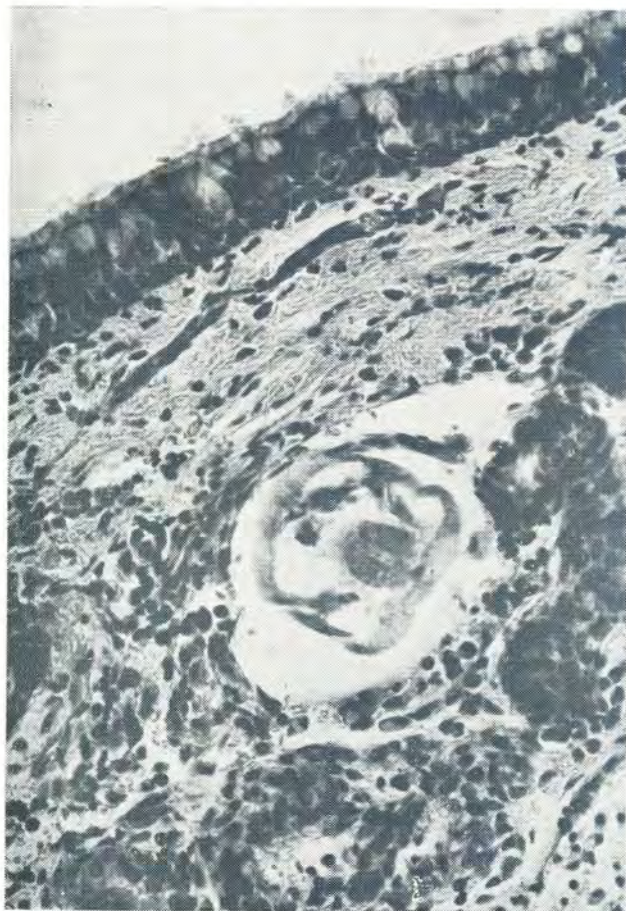
PLATE 6.—Intact tracheal mucosa of dog showing early development of *F. osleri* nodule with lymphocytic infiltration × 300.

PLATE 7.—Tracheal mucosa of dog showing early development of *F. osleri* nodules with plasma cell infiltration × 187.
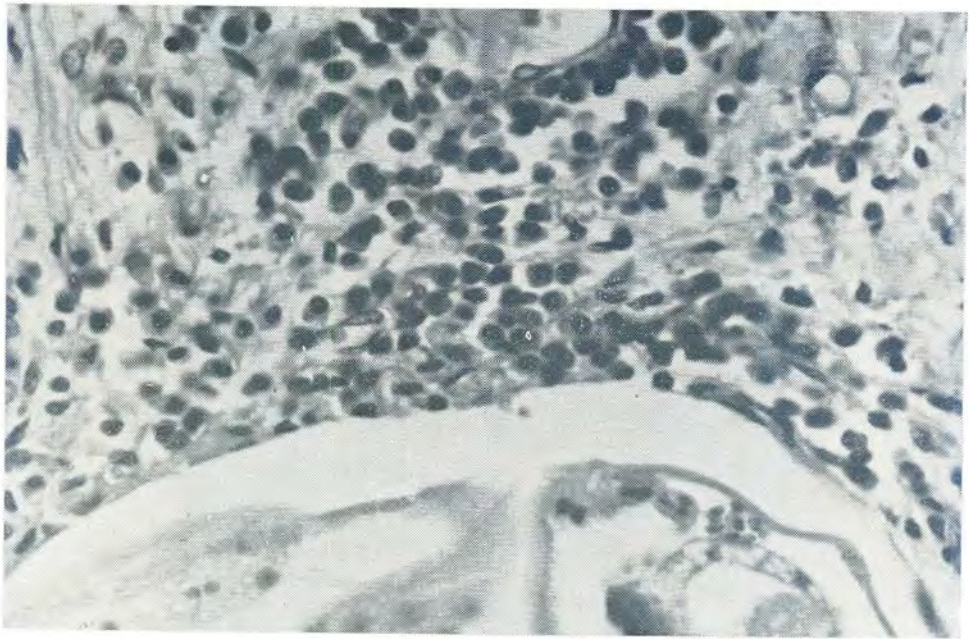
PLATE 8.—Early *F. osleri* nodule in trachea of dog showing plasma cell infiltration × 475.
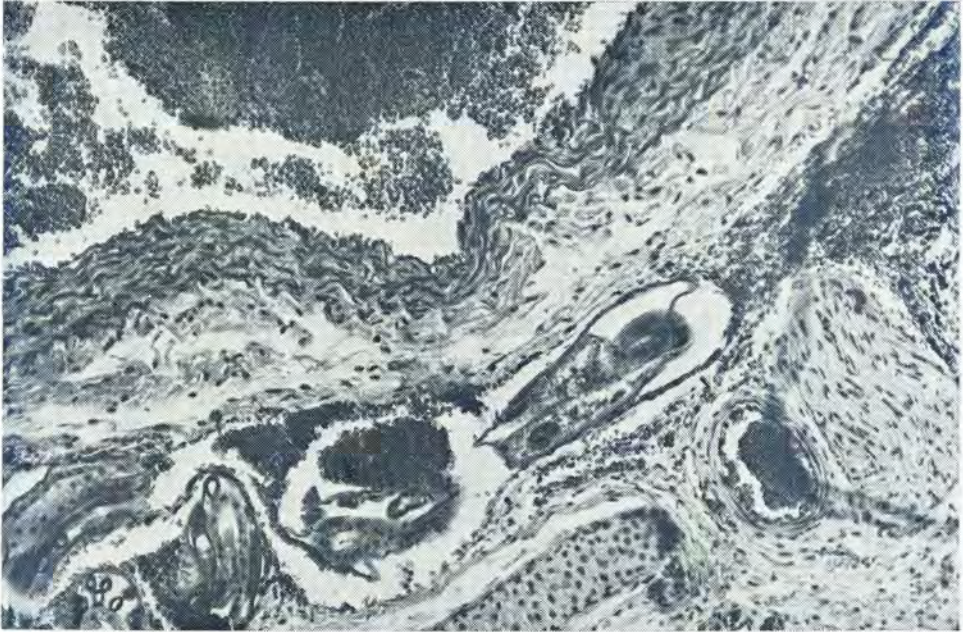
PLATE 9.—*F. osleri* larvae in peri-bronchial tissues × 315.

PLATE 10.—Trachea of dog with well developed *F. osleri* nodule showing worms in cross section × 125.

PLATE 11.—Section of mesenteric lymph node of a dog showing *F. osleri* larvae in trabeculae and medulla × 125.
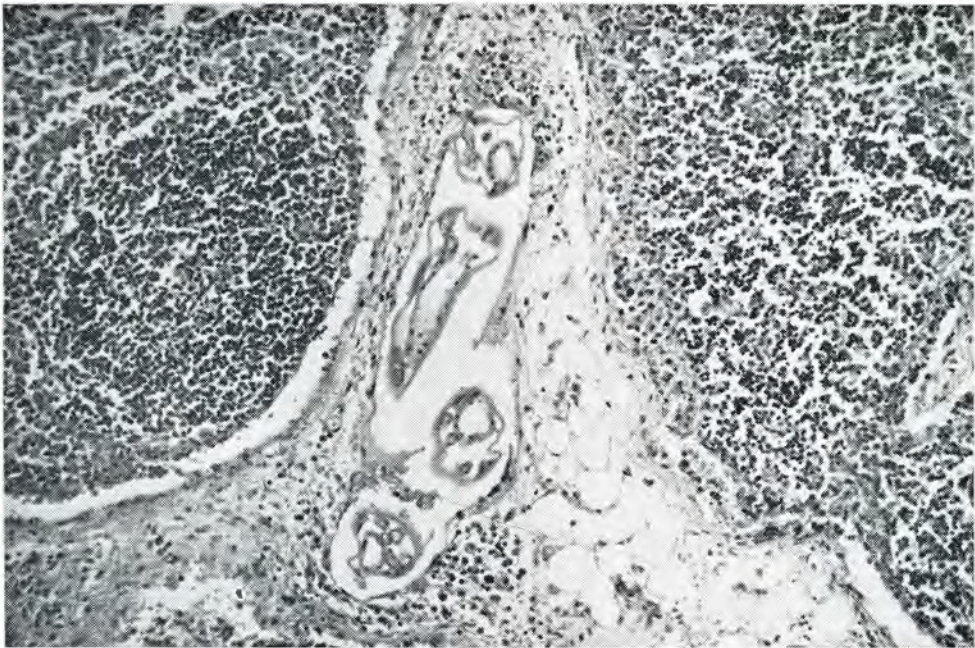
PLATE 12.—Section of mesenteric lymph node of dog showing *F. osleri* larvae in trabeculae × 310

PLATE 13.—Section of mesenteric lymph node of dog showing *F. osleri* larva in medulla × 310

2. *Microscopic lesions*

Histopathologically a typical nodule reveals the following structure:

A granulomatous-like lesion lying in and below the mucosa of the trachea or bronchus and supported by collagenous fibres with an infiltration of plasma cells and lymphocytes. The ciliated epithelium overlying the nodules is intact. On section the large nodules contain cross-sections of coiled up masses of adult worms whereas the miliary nodules contain only immature worms. These worms lie in the tissue spaces and lymphatics both above and below the muscularis mucosae. The spaces are not lined with epithelium but are surrounded by a thin fibrous wall consisting of compressed collagen and elastic fibres. No eosinophiles are present. Petechial haemorrhages are present in the tracheal wall and larvae are visible in the eggs within the female nematodes (see Plates 5 to 10 inclusive).

Normal serous glands are present in the tracheal wall and do not contain any worms. There is generally little foreign body or acute inflammatory reaction to be seen.

Immature worms are not found in the general bloodstream but occur in the lymph nodes of the mesentery, trachea and bronchus. Parasites occur most frequently in the capsule and trabeculae of the lymph nodes lying loose in the lymph vessels. An infiltration of plasma cells and lymphocytes with an increase in connective tissue formation as well as hyperaemia with haemorrhage occur. Where found in the medulla of a lymph node, the parasites cause little cellular reaction. Petechial haemorrhages in the surrounding lymphoid tissue are visible with an infiltration of a few macrophages (Plates 11, 12 and 13).

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PLATE 14.—*F. osleri* embryonated eggs in uteri of female worm

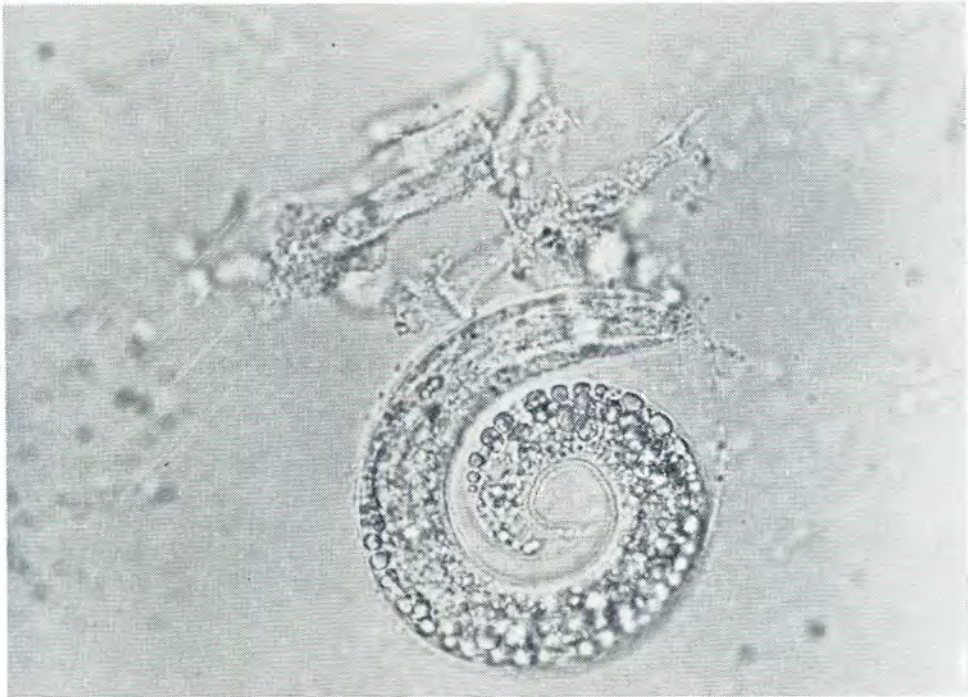PLATE 15.—*F. osleri* egg in early stage showing thin-walled membrane

PLATE 16.—*F. osleri* egg fully embryonated

VIII. Morphology of the Parasites

Filaroides osleri is described as a slender filiform nematode (Ortlepp, 1945; Olsen & Bracken, 1959; Dietrich, 1962; Mönnig, 1950). The average ratio of males to females in a nodule is 2·5: 1 (Anna L. de V. Pienaar, Stellenbosch, personal communication, 1963).

1. *Adult male:* These are about 6 mm in length; they have a simple buccal cavity without lips. A cuticular collar is present 12 to 18 μ behind the anterior extremity. There is a clearly defined rhabditiform oesophagus 213 to 252 μ in length. The spicules 99 to 112 μ long, lead to the cloaca 18 to 30 μ from the posterior extremity.

2. *Adult female:* The female is thicker and longer than the male and measures 10 to 13·5 mm in length. The anterior extremity is similar to that of the male but slightly larger. The two uteri extend anteriorly to the oseophageal-intestinal junction. The anus and vulva open close together in a cleft at the posterior extremity. The female worms are ovoviviparous but do not lay eggs continuously (Plate 14).

3. *Eggs:* The egg is oval, 90 microns long and 60 microns wide, with an outer membrane 1 micron thick. The egg, which is fully embryonated when laid, is provided with a thin, pliable membrane instead of a shell; the shape of the egg thus varies with the movements of the larva which is active within the egg at 37°C (Plates 15 and 16).

4. *Larvae:* Newly hatched larvae measure 232 to 266 μ in length. The oesophagus is not clearly visible and is about one quarter of the total length of the worm. The tail is pointed and clearly shows a characteristic kink.

IX. Diagnosis

The following methods of diagnosis have been suggested (Ortlepp, 1945; Steyn, 1945; Mönnig, 1950; Malherbe, 1951; Dorrington, 1964).

1. *Clinical examination:* The characteristic cough of the patient when exercised or excited after a period of rest can suggest *Filaroides* infestation but is unreliable since not all infested dogs cough. A suspicious cough requires further investigation.

2. *Swab examination:* Microscopic examination of swabs taken from the pharyngeal/laryngeal area may show the typical embryonated eggs, but frequently yields negative results in cases known to be infested (Malherbe, 1951; Dorrington, 1964). This may be attributed to the dispersal of eggs in the mass of mucus present by the time they reach the buccal cavity thus making them difficult to find. Furthermore, female worms do not lay eggs continuously. Several months may pass before eggs can be recovered from an infested dog by this method (Lauder, 1962).

3. *Faecal examination:* When using the flotation method the typical embryonated eggs may be found in some cases but for reasons already mentioned under the previous heading, the method is similarly unreliable. A negative faecal examination cannot be considered conclusive (Ascoli, 1936; Lauder, 1962; Dorrington, 1964).

4. *Roentgen examination:* In well established cases a lateral X-ray of the thoracic cavity will show the nodules at the bifurcation of the trachea (Lauder, 1962; Dorrington, 1959). In the opinion of the writer this method of diagnosis is unreliable, however, because small nodules particularly where only a few are present as in both recent and well established cases, can easily be missed by this technique.

5. *Intratracheal swab examination:* This method, as described by Malherbe (1951), although more likely to yield positive results than those mentioned previously, is unreliable because of the intermittent egg-laying habits of the females.

All five methods have speed, economy and simplicity as their main advantage but are scientifically unreliable.

6. *Bronchoscopic examination:* Direct intratracheal examination with a distally illuminated bronchoscope is the only reliable diagnostic method (Urquhart *et al.*, 1954; Ogilvie, 1962; Dorrington, 1963) and will reveal the pinkish nodules in the region of the tracheal bifurcation. Where examinations are made on animals under six months of age the writer has found it advisable, in addition, to examine microscopically some tracheal mucus for the typical eggs, since in the very early stages the incompletely developed nodules may not be easily visible to the inexperienced eye. Lesions as small as 1·0 mm can be seen with a bronchoscope and in doubtful cases can be removed with biopsy forceps and examined for the typical nematodes.

Thorough exploration of the trachea and the primary and secondary bronchi is necessary because, although lesions occur mainly at the bifurcation of the trachea, nodules may be present deep in the intrapulmonary bronchi.

X. DIFFERENTIAL DIAGNOSIS

In the diagnosis of *F. osleri* infestation the following conditions also require careful consideration:

i. Infestations of the upper respiratory tract, such as tonsillitis, pharyngitis and laryngitis. Direct examination of these areas will reveal the inflammatory reactions.

ii. Infestations of the middle respiratory tract, such as tracheitis and bronchitis. In these instances a correct diagnosis can only be made on the history and a bronchoscopic examination.

iii. Infestation of the lower respiratory tract, e.g. pneumonia. In these cases a deeper cough accompanied by a high fever, is evident. Ausculation of the lung area will confirm the presence of pneumonia.

iv. Tracheal or laryngeal stenosis. Bronchoscopic examination will reveal the site of the stenosis.

v. Foreign body obstruction. This condition has a rapid onset. Bronchoscopic examination will reveal the obstruction.

vi. Pulmonary carcinomatosis. This is a chronic condition mostly seen in aged dogs and confirmed by Roentgen examination.

vii. Cardiac failure of aged dogs. Auscultation with a stethoscope will differentiate this condition from *F. osleri* infestation.

XI. The Life Cycle of *Filaroides osleri*

Historical

Several theories have been advanced regarding the life cycle of *F. osleri*. Osler (1877) suggested the inhalation of dried embryos lodging at the bifurcation of the trachea as being the most likely method of infestation, i.e. infestation through the air rather than via the blood. Urquhart *et al.* (1954) thought that the life cycle was direct with the intrapulmonary route of migration occurring via the lymph stream. They arrived at these conclusions after a single transmission experiment in which approximately 300 larvae collected from the faeces of infested dogs and kept in a moist atmosphere for seven days, were fed to a six-week old puppy. Sixty days after dosing, the pup was killed but showed no macroscopic lesions. Microscopic examination revealed a worm in cross-section lying exterior to the tracheal cartilage, and they concluded that this was probably a *F. osleri* worm.

Dubnitskii (1955) according to Olsen & Bracken (1959) showed that the slug (*Agriolimax reticulatus*) was the intermediate host of the lungworm (*Filaroides martis*) of mink and polecat. Olsen & Bracken (1959) were of the opinion that if the life cycle of closely related species such as the lungworm (*Filaroides falciformes*) of the badger, where slugs (*Deroceras agrestis, Capea hortensis, C. nemoralis*), and the land snail (*Fruticicola hispida*) act as the intermediate hosts, were considered, it seemed likely that some invertebrate may act as an intermediate host for *F. osleri*.

Ortlepp (1945) was of the opinion that, analogous to closely related nematodes, an intermediate host was probably necessary.

More recently Dorrington (1965) claimed, as a result of two successful experiments, that the life cycle was direct, not requiring the presence of any invertebrate intermediate host.

Other attempts have been made to transmit the infestation. Taylor (1936) fed 50 slugs, one snail, one ground beetle, three click beetles, one dipterous larva, one ground caterpillar, 19 wood lice and four earthworms, all collected from infested premises, to four puppies. When these were killed, seven months later, no signs of infestation were seen.

Oldham (1954) fed several hundred larvae which had been kept alive for ten days, to a dog which was killed four months later and autopsied. No evidence of *Filaroides* infestation was seen.

Ortlepp, (1945) attempted feeding embryonated eggs to puppies housed in wire netting cages. These were slaughtered months later without any visible signs of infestation.

Experimental

Materials and Methods

Local History: The pedigreed Boxers of a well known breeder in the Cape Province were found to be infested with *F. osleri* and were placed at the disposal of the writer for study. By 1960 the whole stud was infested—this comprised stud bitches and their pups of varying ages. Complaints about coughing pups from this breeder were persistent. A stage was reached where future breeding seemed hopeless as one litter after another eventually died out or had to be destroyed. Upon careful investigation it was discovered, however, that not every litter was infested nor every pup of individual litters.

When this stud was first established in 1958 the hygiene was not of a particularly high standard, but subsequent improvement of the stud during the ensuing years resulted in the hygienic conditions being brought to a state of excellence; all the dogs were kept on concrete which was hosed thoroughly each day. Part of the kennels was under cover and provided with warm, well protected sleeping quarters while the remainder consisted of fenced off open runs with concrete floors. Wire netting separated the individual kennels but direct contact between dogs in adjacent kennels was still possible. The run-off water from each kennel was piped to French drains. No open through drains from one kennel to another existed.

During the winter months the ground surrounding the French drains became moist, and was covered with a luxuriant growth of grass in which snails and slugs were found. There were also flies, fleas and mosquitoes but no active breeding grounds of these arthropods existed at any time. The rations fed to the dogs were adequate and balanced.

Notwithstanding the excellent hygienic conditions prevailing at this stud, the incidence of infestation remained high so that a stage was reached in 1961 when all breeding activity ceased due to strong public pressure and the lack of success in raising litters.

In view of the reports on the possibility of intermediate vectors, whelping bitches were isolated within the confines of the owner's house.

Infested bitches were kept in the rooms inside the house from the time of whelping until weaning. Bitches never came into contact with other dogs or possible intermediate vectors with the exception of the odd flea. The litters reared in this manner proved to be no different to those reared in the kennels outside. From one such litter pups were sold at six weeks of age and removed from the premises permanently yet were later found to be as badly infested as those left behind. It was clear that the infestation in these particular cases had been acquired by the age of six weeks. In yet another litter one pup was sold at four weeks of age and simultaneously removed from the premises only to show well established lesions four months later.

These observations suggested the presence of an intermediate host to be unlikely. Subsequent experiments confirmed this supposition.

Housing of Experimental Dogs

Because the possibility of the presence of an intermediate host could not be entirely excluded, all dogs used for experimental purposes were housed under the following conditions:

Ten kennels were built with wire netting partitions; five of these kennels were completely enclosed with mosquito-proof screen wire so that no flies, mosquitoes, snails, slugs, etc. could possibly enter them. In all the initial experiments described hereafter, the dogs were housed under these fly-proof conditions so as to exclude the possibility of any intermediate hosts playing a role. Individual external drains were incorporated—these discharged into a series of septic tanks.

All the experimental dogs were vaccinated at ten weeks of age against distemper, infectious hepatitis and leptospirosis and dosed for *Toxocara canis* prior to any experimentation.

Intra-Uterine Transmission

Experiment 1 (a) Intra-uterine transmission

Materials and Methods

An infested Boxer bitch " Haydie " whelped on 23 July 1963. Five pups were born. Two pups were removed immediately before being licked by the mother, and transferred to an uninfested Irish terrier bitch that had whelped on 18 July 1963. Simultaneously two of the Irish terrier pups were transferred to the infested Boxer bitch. No difficulty was experienced in persuading the Boxer bitch to accept the Irish terrier pups since this breed willingly suckles other puppies. Prior to transferring the Boxer pups to the Irish terrier, they were well rubbed down with whatever discharges were available from the Irish terrier to avoid their being rejected. Fortunately the Irish terrier accepted these pups willingly.

The Irish terrier bitch thus had two Boxer pups and one Irish terrier pup whereas the Boxer bitch had three Boxer and two Irish terrier pups. Each bitch was housed individually under the fly-proof conditions mentioned earlier.

Results

Pups reared by Boxer bitch: On 20 January 1964 the three Boxer pups and the two Irish terrier pups were examined by using the illuminated bronchoscopic technique and all were found to have become infested. They were subsequently destroyed.

Pups raised by Irish terrier: On 18 October 1963 one Boxer pup died of gastro-enteritis. At autopsy no signs of infestation were visible. On 7 November 1963 the other Boxer pup was examined by means of a bronchoscope with negative results. On 18 November 1963 the Irish terrier pup was also examined with negative results.

Experiment 1 (b) Intra-uterine transmission

In this experiment an Alsatian bitch, examined and found free of infestation, whelped almost simultaneously with two Boxer bitches, Jane and Cherry—both these stud bitches were infested with *F. osleri*. Jane's puppies were delivered by Caesarean section. The Alsatian foster-mothered a total of eight pups taken from these two Boxers. The pups were removed from the Boxer bitches before any licking had taken place. Some of these puppies were examined at three-and-a-half months of age and found free of infestation and subsequently sold, while the others were kept until six to seven months old and also found to be free of infestation. Moreover one bitch pup was kept for stud purposes and at the age of one year, on examination was found to be still free of infestation.

From these experiments it is clear that if pups are removed immediately from infested bitches at the time of parturition and reared by uninfested foster bitches, such pups will remain free of infestation.

It may be concluded, therefore, that no intra-uterine transmission takes place in *F. osleri* infestation.

The transmission of F. osleri

From the experience gained in the previous experiment as well as from observations made under the isolation conditions already mentioned, it became evident that direct transmission experiments were now indicated. Prior to these experiments being initiated, however, possible arthropod hosts found in and around the premises were examined.

Possible arthropod hosts examined

The following arthropods were examined: Flies (*Musca domestica*), mosquitoes (*Culex* spp.) and snails.

The method adopted was to dissect out the gut and examine its entire contents under a microscope. No larvae were seen.

Experiment 2. Direct transmission of F. osleri

Materials and Methods

1. *Collection of worm eggs:* Using a 50 cm biopsy forceps and a 40 cm illuminated bronchoscope, papillomata were removed on alternate days from the trachea of infested donors under thiopentone sodium anaesthesia. Usually two to three papillomata of 1 cm in diameter were adequate for the purpose of this experiment. These nodules were kept in normal saline at room temperature and the number of eggs per ml, laid by the female worms within the nodules, determined by microscopic counts of the saline suspension. The quantity of normal saline in which the nodules were kept depended on the number of puppies to be dosed. As a rule the volume was kept as small as possible (between 5 and 10 ml) so that newly born puppies were not dosed with too great a volume at any one time. The method used to determine the number of embryonated eggs per ml was the following: The number of times the loop of a platinum wire had to be immersed into normal saline to remove one ml precisely, was predetermined and found to be 40. The loop of the platinum wire was then immersed in the well shaken nodule containing saline solution and its contents placed on a microscope slide and examined under the low power magnification of a microscope. Only embryonated eggs were counted. The number multiplied by 40 gave the total number of eggs per ml of the suspensions. Egg counts were repeatedly checked and the results were found to be constant so that the number of eggs dosed was uniform. However, if the suspension was kept for 12 hours or more for re-use later, another egg count was advisable since more eggs were released by the females during storage. In such cases further dilution of the suspension with normal saline was necessary so as to maintain a constant egg count per ml.

In all the experiments described an endeavour was made to dose not less than 1 ml of egg-containing saline suspension at a time. This ensured an accurate dosage rate at all times. Where a smaller volume was dosed, the margin of error could be significant because of factors such as the residual suspension in the dosing catheter and syringe, possible wastage and sedimentation of the eggs between the time of shaking the suspension and the actual dosing. This collection and preservation technique was used in all subsequent experiments discussed below.

2. *Infestation:* A mongrel Alsatian type bitch was kept under fly and insect-proof conditions until she whelped on 20 September 1964. There were four pups of each sex. She was examined with a bronchoscope and found to be free of infestation.

On 21 September 1964 all four bitch puppies were dosed intra-oesophageally with an average of 48 eggs of *F. osleri* harvested as already described. This was repeated daily for 14 consecutive days, the last dosing taking place on 4 October 1964. The four male puppies were not infested and acted as controls.

The method used to dose the puppies was as follows: A hypodermic syringe and needle attached to a snugly fitting length of 2 mm diameter urinary catheter was used. A predetermined volume of the well shaken suspension of *F. osleri* eggs in normal saline was drawn into the syringe. While gently squeezing open the jaws of the puppy, the pliable catheter was inserted deep down the oesophagus preferably as near to its abdominal demarcation as possible, and the suspension slowly expressed. To avoid inserting the catheter into the trachea its point was always directed to the left of the pharynx of the pup.

All eight puppies were reared together by their mother and were kept under insect-proof conditions.

Results

On 23 January 1965 when the puppies were four months old, they were examined with a bronchoscope with the following results:

i. All four male pups showed no signs of infestation.

ii. Three bitch puppies were grossly infested with *F. osleri* nodules and yielded numerous eggs on subsequent microscopic examination of the tracheal mucus simultaneously removed.

iii. A positive diagnosis in the fourth bitch pup was not made. At that stage the author had little experience in critically assessing an early case, but on reflection and with the experience subsequently gained, this bitch pup was undoubtedly infested.

In this successful transmission experiment, each pup received approximately 700 eggs over a period of 14 days. Half of this total number of eggs was dosed 24 hours after collection whereas the other half was harvested and was dosed immediately.

The experiment showed that *F. osleri* could be transmitted directly to new born puppies by dosing them daily for two weeks, and constituted the first successful transmission experiment recorded.

Experiment 3. *Direct transmission with weekly dosing*

In an endeavour to develop a more practical method of dosing, a further experiment was undertaken with a pregnant crossbred Corgi bitch. She was examined for *F. osleri* and found to be free of infestation.

Materials and Methods

Whelping took place on 19 October 1964 and five pups were produced. Three bitch puppies were dosed intra-oesophageally with approximately 100 *F. osleri* eggs each, seven days after birth and thereafter twice at weekly intervals. Each puppy thus received a total of about 300 eggs over a period of three weeks. Two male puppies were left as uninfested controls. The bitch and her whole litter of five pups were kept under the same insect-proof conditions mentioned earlier.

Results

On 20 February 1965, four months later, the three bitch puppies were examined; two had definite *F. osleri* lesions—these were small and few in number—while the third was regarded as suspicious; histological sections of the tracheal mucosa of this pup later showed definite infestation. The two male puppies remained free of infestation.

The results obtained in this experiment showed that the infestation could be transmitted successfully to newly born pups by dosing at weekly intervals, but that 100 eggs would appear to be the minimum dosage per week to ensure positive results. This and subsequent experiments have shown an apparent direct correlation between the total number of eggs dosed and the degree of infestation produced.

Experiment 4. Sex susceptibility

Although under the conditions prevailing at the stud no evidence was found of any difference in sex susceptibility, a separate experiment was conducted to confirm this.

Materials and Methods

A pregnant Alsatian bitch was examined for *F. osleri* and found to be free of infestation. She whelped on 28 February 1965, producing three male and four female puppies. Dosing commenced one day later on 1 March 1965 and ended on 13 March 1965.

Two male pups and two female pups were dosed intra-oesophageally with 50 eggs each on alternate days over a period of 14 days. One male and two female puppies of this litter remained undosed and were kept as controls. To avoid any possible confusion the tails of the controls were amputated.

Results

On 27 June 1965, one long-tailed male puppy was examined and found to have become infested to an average degree. Subsequent bronchoscopic examinations of the other long-tailed puppies on 10 July 1965, showed them all to be infested, one of them severely. The short-tailed controls remained uninfested.

Results obtained from this experiment served to confirm the observations that under natural conditions both sexes are equally susceptible to infestation.

Experiment 5. Cross-infestation

Although a separate experiment was not conducted to show that there is no cross-infestation amongst individual pups in a litter, sufficient evidence was obtained from the foregoing experiments to prove this fact conclusively.

In Experiments 2, 3 and 4 a total of 11 puppies was dosed with eggs over different periods while a total of nine undosed puppies of the same litters remained in contact with those dosed and acted as controls. Conclusive positive results were obtained in 10 of the 11 dosed pups, mostly at three to four months after dosing, whereas all the nine controls remained negative; these controls were only considered negative six months after dosing. Dosed and undosed puppies of each litter were housed together until at least four months of age. Thereafter the undosed controls were segregated.

The fact that none of the controls picked up infestation from its litter companions is considered sufficient evidence to prove that cross-infestation does not occur amongst puppies. As will be mentioned later, sexually mature egg-laying female worms are evident long before puppies reach the age of four months. Susceptibility among adult dogs will be discussed below.

Experiment 6. Intratracheal transmission

An attempt at transmitting the infestation by depositing the eggs or larvae directly into the trachea of new born pups was also undertaken. Practical difficulties encountered, however, led to this experiment being abandoned.

The following factors made further experimentation unreliable and any results that were obtained of no conclusive value.

i. For comparative purposes, newly born puppies had to be used. In such puppies of even the larger breeds, the epiglottis is impossible to locate because of the relatively large and short tongue. Dosing intratracheally was, therefore, impossible.

ii. The lumen of the trachea is very small and the cartillage rings easily compressible so that the introduction of a needle into the lumen from without proved most difficult.

iii. The coughing reflex was the most serious difficulty encountered. In humans, anaesthetising the trachea is advocated but because of the factors mentioned in (ii), this was not possible. Any coughing whatsoever could result in the subsequent swallowing of the eggs previously deposited in the trachea, thereby rendering the whole experiment unreliable and of no value.

iv. Foreign body pneumonia especially at this particular age was a very real problem.

v. General anaesthesia by inhalation was contemplated as this could facilitate the deposition of the eggs in the trachea but was abandoned either because of the danger of subsequent foreign body pneumonia or of coughing after recovery. The subsequent effect of the volatile anaesthetic on the deposited eggs was also a factor to be considered.

vi. Concentration of the saline suspension was possible but the volume required for practical purposes remained irritant and resulted in coughing.

vii. Furthermore, desiccation of the eggs or larvae resulted in loss of mobility and possible viability thereby precluding reliable conclusions being made.

Experiment 7. Adult transmission

Materials and Methods

Two adult dogs weighing 8 Kg and 20 Kg respectively were examined for *F. osleri* infestation with negative results.

Each dog was dosed with approximately 150 eggs intra-oesophageally and seven days later a similar number dosed, each dog receiving at least 300 eggs. In order to ensure that the eggs were actually deposited deep in the oesophagus the dogs were on both occasions anaesthetised with thiopentone sodium.

To ensure deposition of the whole volume of the egg-containing normal saline suspension deep down the oesophagus the dosing technique was modified as follows:

A urinary catheter of 2 mm diameter was passed down the lumen of a 50 cm long metal catheter of approximately 9 mm diameter to its distal end; these were passed down the oesophagus together to its gastric end and using the inner catheter, the egg-containing saline suspension was deposited intra-oesophageally. A small volume of air was passed through the catheter subsequently to ensure that none of the suspension remained in the catheter.

Results

When examined two months later, both dogs were infested. The smaller dog had one nodule 50 mm in diameter with several small nodules at the tracheal bifurcation, while the larger dog had only one definite nodule of a few mm in diameter and a few smaller lesions, probably those of *F. osleri* in the early stages. No eggs were present in the larger dog.

Discussion

The results of this experiment indicate conclusively that dogs of all ages are equally susceptible to *F. osleri* provided the eggs are swallowed. Under natural conditions, as discussed under susceptibility, the degree of exposure to infestation would appear to play the decisive role.

XII. The Mode of Transmission

Before embarking on further transmission experiments the writer considered it advisable to study the eggs and first stage larvae of *F. osleri*.

1. *Hatchability of the embryonated eggs*

Hatchability of the eggs depends to a large extent on the degree of development within the egg. The larvae within the eggs lying in the uteri prior to being laid are actively motile. Once the eggs are laid, and kept in normal saline at room temperature, the majority of larvae hatch within 24 hours.

2. *Viability of the larvae*

Motility of the larvae was regarded as an indication of viability. The following observations were made:—

i. When kept at 37°C in an incubator on 1 per cent agar plates, the larvae remained alive for six days.

ii. When kept at room temperature, the larvae remained motile for nine days.

iii. Larvae kept on 1 per cent agar plates at 37°C suddenly became motile between the end of the fifth day and the beginning of the sixth day.

As will be mentioned later, motility *in vivo* apparently takes place much earlier.

3. *Experiment 8. First migration determination using agar plates*

The purpose of this experiment was to establish the possible route taken by the larvae after dosing intra-oesophageally.

Materials and Methods

Each organ removed was examined for the presence of *F. osleri* larvae in two different ways, viz.:—

i. One per cent agar plates kept at 37°C were used for possible migration of the larvae away from the organ specimen—bacterial contaminants following the route of migration, it was considered, would readily show up any larval movement if this occurred.

ii. Warm tap water for migration observation.

Eight puppies were born on 30 June 1965. At noon on 1 July 1965 each was dosed intra-oesophageally with 200 eggs. At noon on 2 July 1965 seven of these puppies were dosed again with 150 eggs each, the eighth puppy was killed and the following organs aseptically removed: trachea, lung, thymus, blood, aorta, heart, diaphragm, liver, gall bladder, spleen, duodenum, stomach, mesenterium, kidneys and mesenteric lymph nodes. Portions of each of these organs were placed on agar plates and in warm water.

Results

Six hours after collecting these organs the agar plates and warm water specimens were examined for any larval movement. None was noticed. Twenty-four hours after collection, the preparations were re-examined as well as 48 hours afterwards. All these examinations failed to show up any larval movement. Bacterial contamination prevented further examination of these specimens.

On 3 July 1965, six of these puppies were again dosed with 200 eggs as before; the seventh pup was killed and the various organs treated as in the case of the eighth pup. No larval movement was observed.

Subsequent dosing with amounts varying between 150 and 200 eggs, and slaughtering took place only on alternate days. The sixth and fifth pups similarly examined also failed to show any larval migration from any organs.

This experiment was abandoned without yielding any information on the possible migratory route of *F. osleri*.

4. *Experiment 9. Second migration determination*

A modified Baermann technique as demonstrated by P. J. S. Anderson (Onderstepoort, personal communication, 1965) was used for this experiment. In addition, massive doses of eggs were used so as to improve the chances of recovering larvae.

Materials and methods

Two glass jars of different diameters were used—the inverted mouth of one just fitting into the mouth of the other. A fine mesh nylon material (apertures 250 microns) separated the two jars. Macerated organs in normal saline at 37°C were placed in the top jar and inverted over the bottom jar that had been filled with normal saline at 37°C previously so as to allow no air space between the two jars.

Three puppies born on 6 September, were dosed with 80 eggs each on 9 September. This was repeated on 14 September, and again on 18 September they were each dosed with approximately 1,000 eggs. On 19 September they again received 1,000 eggs each. Three days later (22 September 1965) at 12.45 hours one puppy was slaughtered and at 16.00 hours the following specimens were removed aseptically, portions of which were placed in formalin for histological examination, while the remainder were utilized for the Baermann tests: the lungs, trachea, thymus, heart, bronchial lymph node, diaphragm, liver, periportal lymph node, spleen, mesenteric lymp nodes, mesenterium, stomach, gall bladder, duodenum, colon, kidneys, intercostal muscles, pancreas, muscle, spinal cord, cerebellum, right cerebrum.

Each organ, placed separately in Baermann jars, was incubated at 37°C for 28 hours and thereafter kept at room temperature, care being taken to replenish whatever saline solution had evaporated so as not to break the continuity of the fluids that were separated by the nylon mesh. Periodic sampling of the sediment in each lower jar was undertaken and at the end of the test, the contents of each of the lower jars were centrifuged at 2,000 r.p.m. for ten minutes, the supernatant fluid examined microscopically for larvae, decanted and the residual material again centrifuged. The supernatant fluid and sediment of each lower jar were again thoroughly examined for larvae.

TABLE 2.—F. osleri *larvae recovered three days after dosing with* 2,000 *eggs*

Organ	Time in the Baermann apparatus			
	5 *hours*	17 *hours*	28 *hours*	40 *hours*
Right lung...........	Nil	One	One	Few
Left lung............	Nil	Nil	Nil	One

PLATE 17.—*F. osleri* larva recovered from lung. Expt. 9 × 275.
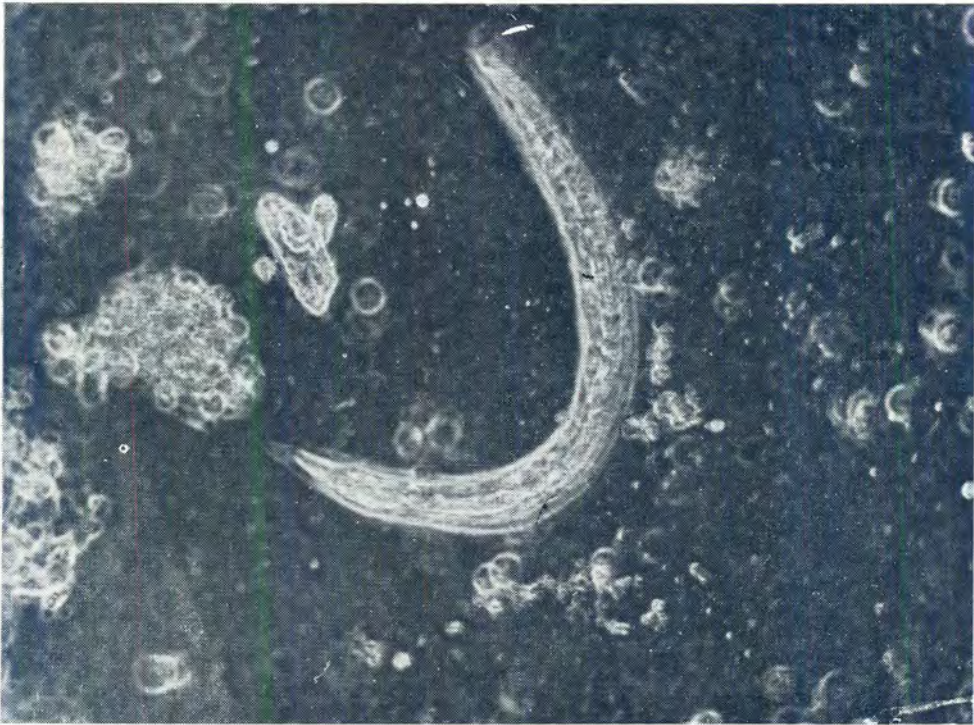
PLATE 18.—*F. osleri* larva recovered from lung (Expt. 9). Phase contrast × 242.

PLATE 19.—*F. osleri* larva recovered from lung (Expt. 9) showing pointed tail.   Phase contrast

Results

A. Worms recovered:

Larvae were recovered from the lungs only and the results are summarized in Table 2.

This experiment showed that—

(i) very few larvae were recovered notwithstanding the large number of larvae dosed;

(ii) the larvae migrated rapidly to the lungs after ingestion since no other organs yielded any larvae, and

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(iii) a period of over 24 hours elapsed before the larvae migrated from the tissue in the Baermann apparatus (see Plates 17, 18 and 19).

The two remaining puppies unfortunately were killed and eaten by their foster mother while this experiment was in progress.

B. Histological examinations:

The histopathological changes observed in this experiment are described in Experiment 10 below.

5. *Experiment* 10.   *Third migration determination*

In this experiment an attempt was made to establish the route taken by the larvae for the first three days after infestation before they reached the lungs.

Materials and methods

Seven puppies born on 24 October 1965, were dosed on 26 October 1965 with approximately 200 eggs each and were individually killed at specified times after the dosing (see Table 3).   Methods of worm recovery have been described in Experiment 9.   The organs collected for examination were: the liver, stomach, small intestine, mesenteric lymph nodes, right and left lung.

As much blood as possible was aspirated from the right chambers of the still beating heart immediately after slaughter.   The cells were haemolyzed by adding water to the blood immediately after collection and subsequently centrifuged as described previously.   The supernatant fluid and sediment were examined for possible larvae.

Results

A. Worms recovered:

The number of larvae recovered is summarized in Table 3.

TABLE 3.—*Larvae recovered at autopsy*

Puppy	Hours after dosing	Liver	Blood	Stomach	Small intestine	Mesenteric lymph nodes	Left lung	Right lung
1................	4	0	0	1	0	0	0	0
2................	8	0	0	1	1	0	0	0
3................	12	0	0	1	1	6	0	0
4................	24	0	0	0	0	3	0	0
5................	48	0	0	0	0	0	0	0
6................	60	0	0	0	0	0	0	0
7................	84	0	0	0	0	0	0	1

The following conclusions may be drawn from the results presented in Table 3:—

(i) The larvae pass rapidly from the stomach to the small intestine from where they as rapidly enter the lymphatic system of the mesenterium.

(ii) Within 12 hours of dosing, the highest concentrations of larvae are found in the mesenteric lymph nodes.

(iii) At 24 hours larval numbers had decreased, probably indicating that migration via the lymphatic system was proceeding.

(iv) The failure to demonstrate larvae in the lungs until the 84th hour when a single larva only was found in the right lung is probably due to faulty technique and is somewhat at variance with the results obtained in the second migration experiment (see Table 2).

The failure to recover any larvae in the blood collected from the right chambers of the heart may have been due to the following factors:—

1. With approximately only 1 per cent of larvae being recovered after dosing, a large volume of blood would have had to be collected at a specific moment before larvae could be expected to be found. This is impossible in small puppies especially after opening the thoracic cavity for aspiration of blood from the right chamber only—the heart rapidly ceases to beat because of shock, anoxia and pulmonary collapse.

2. Even though blood is aspirated from the right chamber of the heart, the lymph from the ductus thoracicus is greatly diluted by the blood from other parts of the body. The chances of locating larvae under these circumstances are remote.

Liver: The failure to find any larvae in the liver specimens (see histology) may be accounted for as follows:—

During incubation of the liver specimens, large numbers of hepatic cells pass through the nylon sieve and after centrifugation collect in the sediment. This accumulation of cells makes the examination for the possible presence of larvae most difficult. A single larva under these circumstances could easily be missed.

B. Histological results

Serial sections were made of each organ listed, stained with haematoxylin-eosin and examined microscopically.

In Experiment 10 histopathological changes were observed in the mesenteric lymph nodes and liver only, while in Experiment 9 the changes were confined to the lungs. No changes were observed in any other organs. The following are the changes observed:

i. *Mesenteric lymph nodes:* These lesions have already been described in Chapter VII and illustrated in Plates 11, 12 and 13.

ii. *Liver:* A marked periportal infiltration of leucocytes (polymorphs, lymphocytes, plasma cells) with a similar increase periportally of connective tissue, the absence of eosinophiles as in the lymph nodes, and numerous granulomata occur in the vicinity of the portal areas consisting mainly of macrophages, epithelioids and polymorphonuclear leucocytes. In isolated granulomata parasitic remnants of *F. osleri* larvae may occur (Plate 20). Various stages of vascular changes are seen repeatedly, namely, perivascular leucocytic infiltrations, mainly polymorphs. In some karyorrhexis with necrosis of the vascular wall is visible (Plate 21).
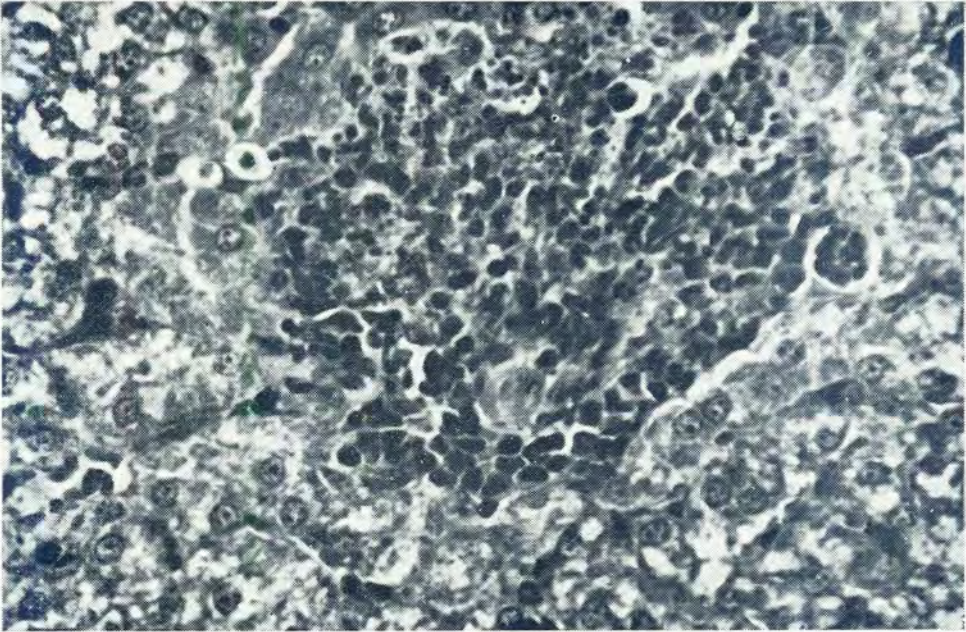
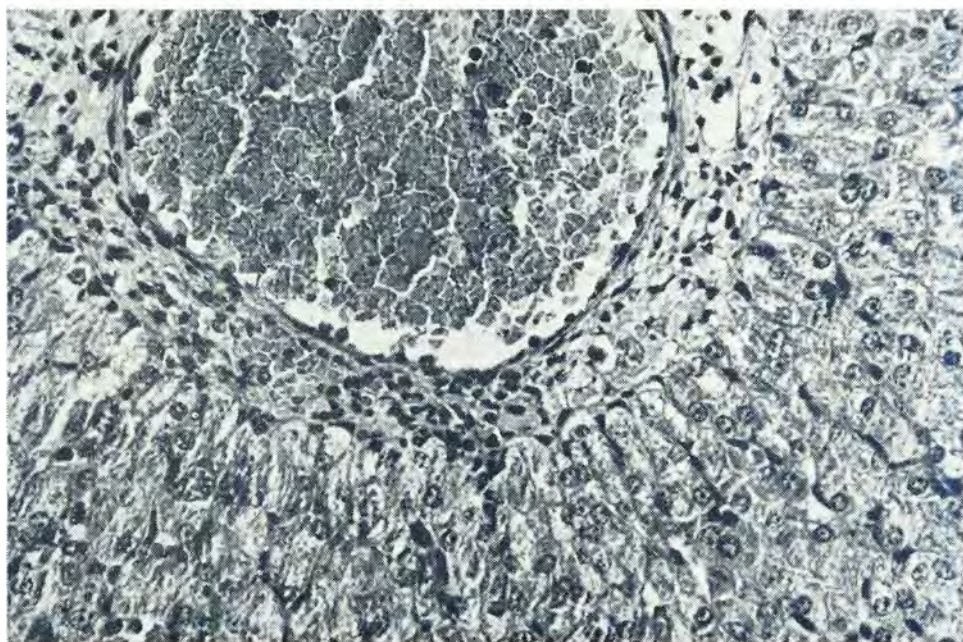PLATE 20.—Expt. 10. Section of liver of dog showing granuloma with cross section of *F. osleri* larva × 475

PLATE 21.—Expt. 10. Section of liver of dog showing perivascular infiltration of leucocytes × 320

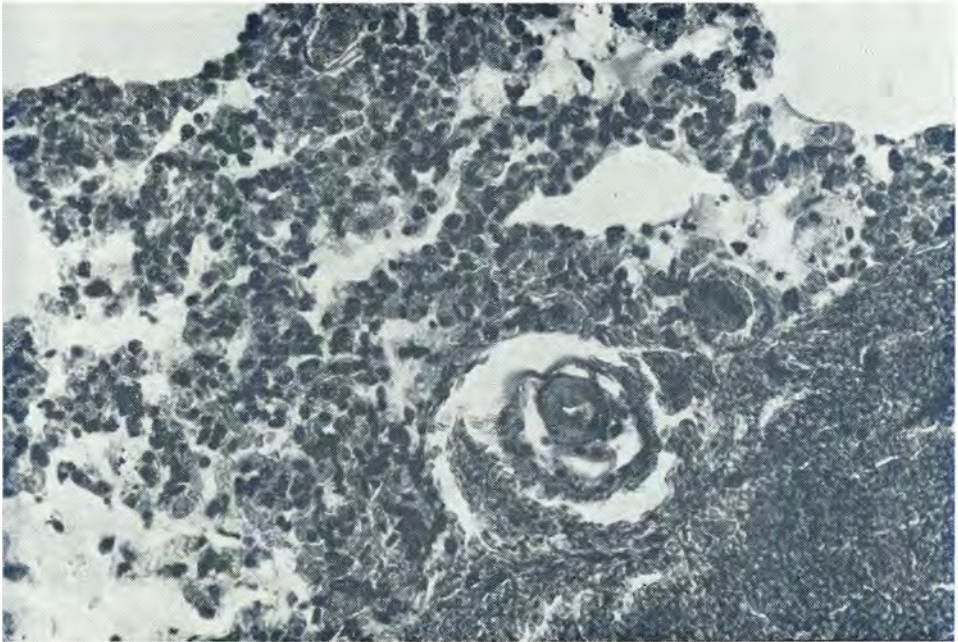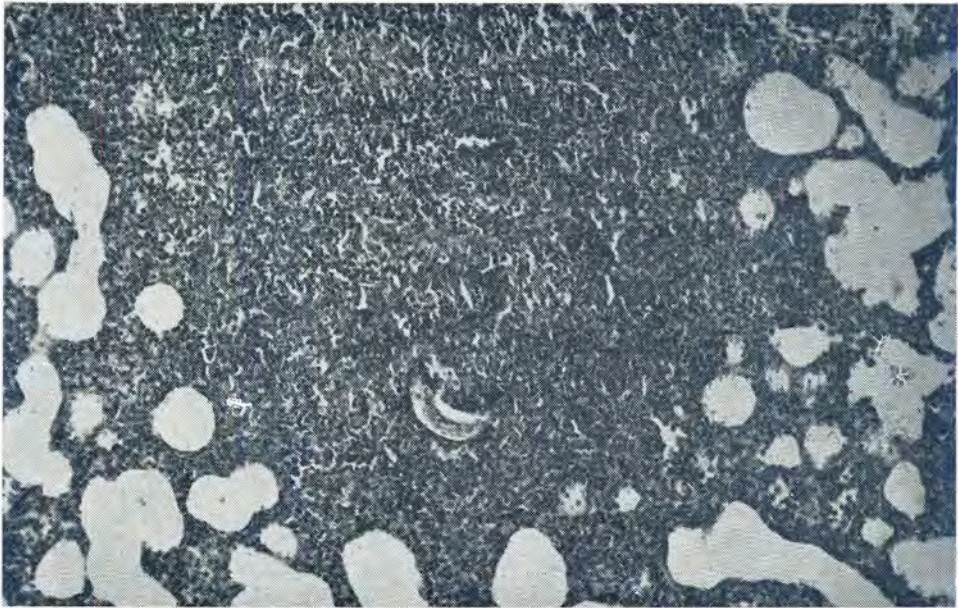PLATE 22.—Cross section of larva of *F. osleri* in lung alveolus of dog. Early lesion × 320

PLATE 23.—Cross section of larva of *F. osleri* in granuloma in lung of dog × 130

PLATE 24.—Expt. 10. *F. osleri* larva recovered from mesenteric lymph node (probably male)× 310

iii. *Lungs:* In the early lesions there are recent haemorrhages with an infiltration mainly of macrophages and a few polymorphs together with a collapse of the surrounding lung tissue. Cross sections of *F. osleri* larvae are sometimes visible in these areas (Plate 22).

Granulomata develop where larvae fail to reach the alveoli. These consist of polymorph infiltration with no evidence of eosinophiles. Karyorrhexis is present with the surrounding lung tissue showing emphysema and oedema (Plate 23).

Vasculitis, bronchopneumonia and prominent fibrosis of the lung are evident in some older lesions.

6. *The morphology of the migrating larvae*

A. *Lymphatic stage*

The morphological differences between the migrating larvae recovered from the lymphatic nodes of the mesentery and the first stage larvae hatched from the eggs are so great as to justify the former being identified as second stage larvae. This is based on the following changes seen:

i. The length of the larvae recovered varied between 300 and 400 μ. A difference in size amongst those recovered from the lymph nodes was observed—a sex differentiation could possibly have accounted for this phenomenon (males 300 μ and females 400 μ) (see Plates 24 and 25).

ii. A clearly defined coiled oesophagus, occupying one sixth of the total length of the larva, is visible. It appears to end in a terminal bulb (Plates 26 and 27).

iii. A corrugated cuticle in the region of the neck is also visible (Plates 26 and 28).

iv. The posterior extremity of this larva is elongated and narrow; approximately one sixth of the total length, and terminates in a well defined sharp point (Plate 29).

The morphology of a newly hatched larva has been described in Chapter 8.

B. *Pulmonary stage*

Once again there is a pronounced difference in appearance of these larvae—the third stage larvae.

i. The length is approximately 320 μ. The larvae are thicker and more compact (Plates 18 and 19).

ii. The coiled oesophagus is no longer visible but a narrower cervical area is to be seen (Plate 19).

iii. The corrugated cuticle was not in evidence.

iv. The tail is shortened, still terminating in the sharp point (Plate 19).

7. *Experiment* 11. *Fourth migration determination*

Materials and methods

Four puppies born on 24 October 1965 were dosed with approximately 1,200 eggs each on 3 November 1965. Three were slaughtered 12 hours, 24 hours and 48 hours respectively after dosing and the same organs as in the third migration experiment examined by means of the Baermann apparatus. The fourth puppy was destroyed ten weeks later for histological examination.

Results

A. Worms recovered

No larvae were recovered due to putrefaction of the specimens which was well advanced within 12 hours of slaughter.

B. Histological findings

All the specimens of the first three puppies unfortunately were discarded. The histological examination of the fourth puppy ten weeks after dosing showed well established lesions developing in the trachea (Plate 10).

Conclusions: The migrating larvae present in the organs probably disintegrated during the putrefactive process. Since subsequent histological examination ten weeks after dosing showed typical lesions, it can, however, be concluded from this experiment that a single dose of just over 1,000 eggs is sufficient to establish the infestation in puppies.

8. *Experiment* 12. *Fifth migration determination*

Materials and methods

Three puppies born on 31 October 1965, were dosed on 10 November 1965 with about 3,000 eggs each, harvested 12 hours earlier; two were slaughtered 12 hours and 24 hours respectively after dosing. The third puppy was slaughtered seven weeks after dosing and the trachea, lungs and bronchial lymph nodes examined histologically.

Results

A. Worms recovered: Once again no larvae were recovered from the organs examined.

B. Histological findings: Typical histopathological changes, as already described in Experiment 10 were observed in the liver of the puppy slaughtered 12 hours after dosing. Parasites were seen in the granulomata (Plate 20).

No changes were evident in the lungs or mesenteric lymph nodes of the two puppies slaughtered 12 to 24 hours after dosing.

The trachea and bronchial lymph nodes of the third puppy slaughtered seven weeks later, showed well established lesions of *F. osleri* (Plate 7).

PLATE 25.—*F. osleri* larva recovered from mesenteric lymph node (probably female) × 255
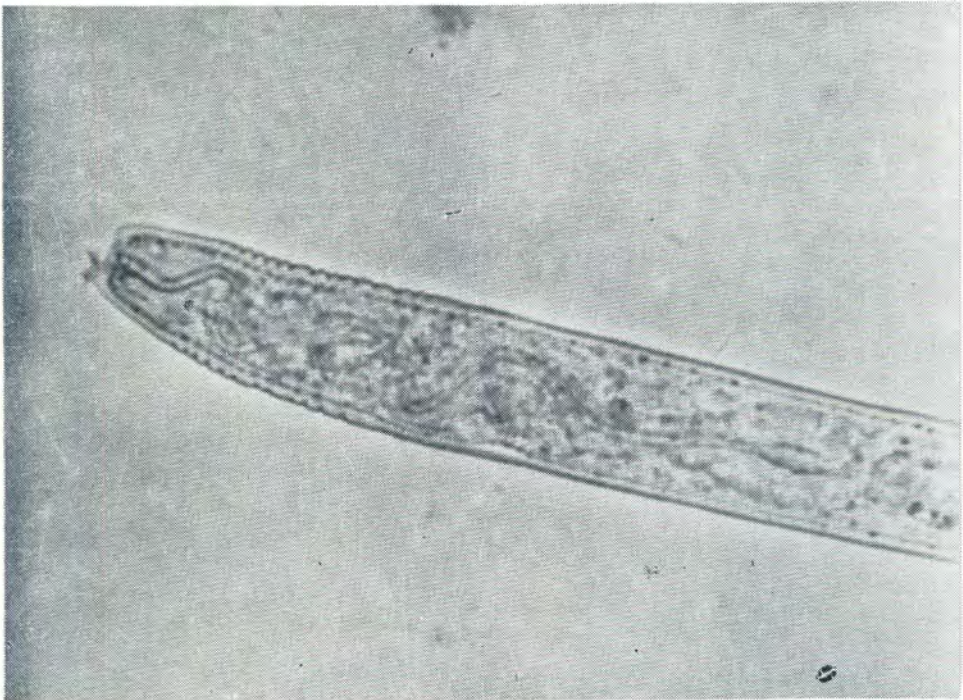
PLATE 26.—Expt. 10. Larva of *F. osleri* recovered from mesenteric lymph nodes showing corrugated cuticle in cervical region
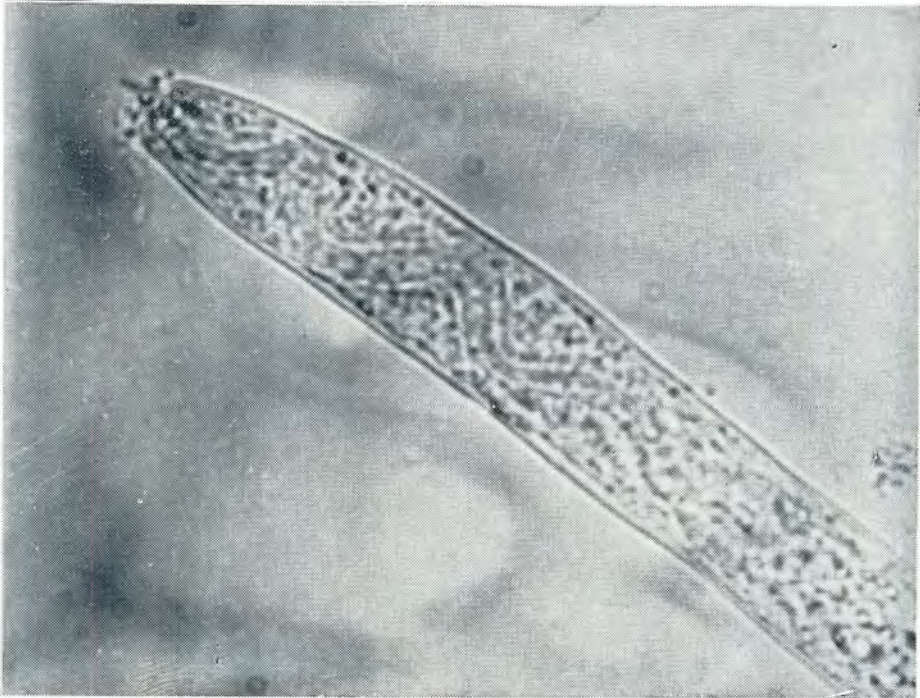
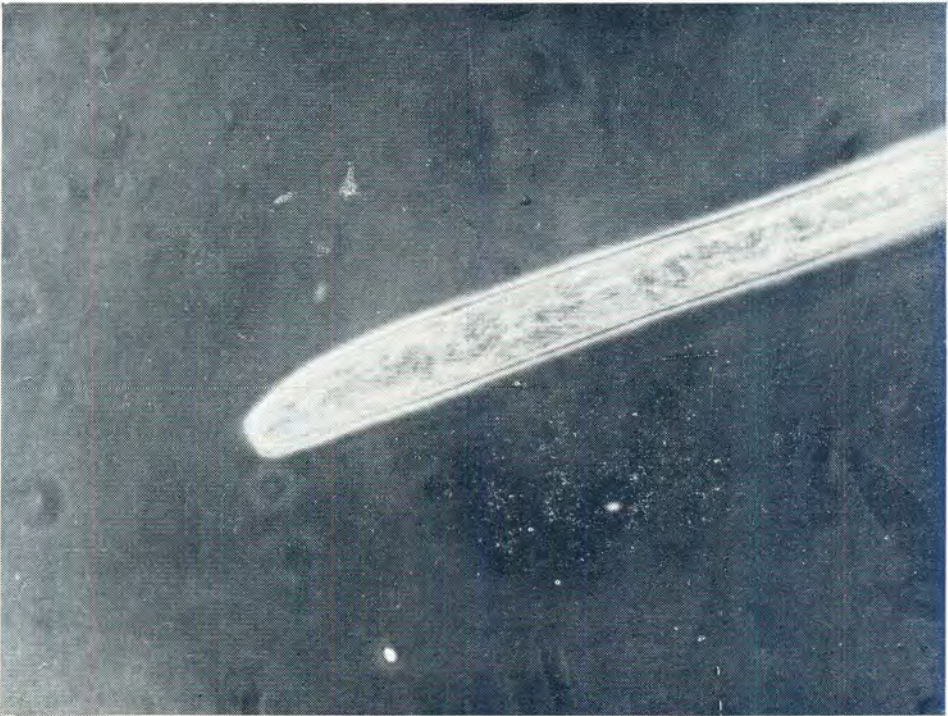PLATE 27.—Expt. 10. Larva of *F. osleri* recovered from mesenteric lymph nodes showing coiled oesophagus

PLATE 28.—Expt. 10. Larva of *F. osleri* recovered from mesenteric lymph nodes. Anterior extremity. Phase contrast
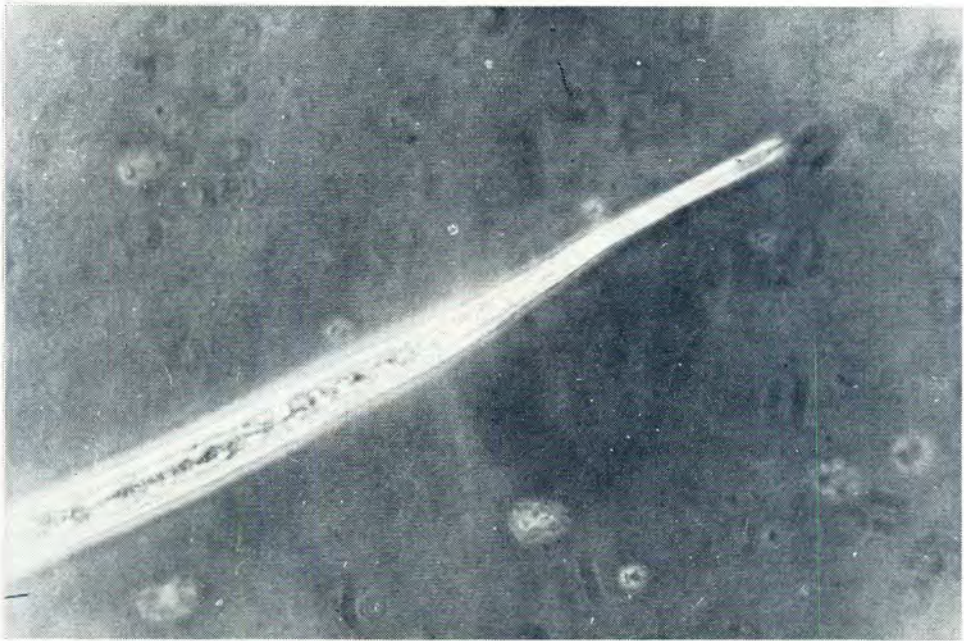
PLATE 29.—Expt. 10. Posterior portion of larva of *F. osleri* recovered from mesenteric lymph nodes showing sharp tail

Discussion: Bearing in mind that approximately 3,000 eggs were dosed in this experiment, the failure to demonstrate any larvae with the Baermann apparatus is disappointing. It is conceivable that larvae penetrating the intestinal mucosa and destined for the lymphatic vessels, have a reasonable chance of accidentally penetrating the venous capillaries and subsequently being carried to the liver via the portal system. From the histological observations, it appears that migration through the liver does occur but that the presence of the larvae there is of a transitory nature only. In the case of the lymph nodes a similar phenomenon occurs.

Another factor possibly governing the success or failure of the Baermann technique is the fact that no matter how aseptically the organs removed are handled, some bacterial contamination is inevitable. This, together with autolytic changes in the cells, may affect the activity of the larvae thus accounting fot their poor recovery.

XIII. Conclusions regarding the Life Cycle

The following conclusions regarding the life cycle of *F. osleri* may be made:—

i. The infestation is transmitted directly, without an intermediate host.

ii. The infestation of the host under natural conditions is acquired at a very early age.

iii. There is no evidence of intra-uterine transmission in the bitch.

iv. Dogs and bitches of any age are equally susceptible (Experiments 4 and 7) The fact that adult dogs can be infested experimentally but rarely, if ever, acquire the infestation naturally, appears to be due to their habits preventing them from coming into contact with or ingesting viable eggs in sufficient numbers to set up infestation.

v. A single dose of between 1,200 and 3,000 eggs, weekly doses for two or three weeks, or dosing on alternate days for two weeks, are all sufficient to transmit the infestation successfully.

vi. Within eight hours of infestation, the larvae penetrate the intestinal wall to appear in their largest numbers in the lymphatic nodes of the mesentery 12 hours after ingestion. At the same time, where penetration into the venous system has occurred, the larvae are present in the liver (Plate 20).

It must be assumed that from the lymph nodes the larvae pass via the ductus thoracicus to the anterior vena cava and thence into the right auricle. If caught up in the portal system, they reach the right auricle by way of the posterior vena cava. The larvae are then conveyed by the pulmonary artery to the lung capillaries. These they perforate and emerge into the alveoli from where they migrate to the bronchioli, bronchi and eventually reach their predilection site—the trachea at its bifurcation. At this site the necessary biochemical, physiological and anatomical conditions are apparently present so as to enable the larvae to establish themselves. Ten weeks later mature egg-laying female worms can be expected to be found.

XIV. TREATMENT

1. *History*

Lapage (1956) was of the opinion that no effective treatment is possible. Symptomatic treatment was recommended by Mönnig (1950) as being the rational method with the knowledge available at that time. Pillers (1935) claimed that a rest of four to five months in a clean insect-free loose-box would free a dog of the infestation. This statement is, however, incorrect as, in all of the many observations made during the present study, it has been found that a reasonably well infested dog never loses the infestation, regardless of the best hygiene and feeding. Mechanical scraping of the nodules intratracheally was attempted and eventually abandoned by Lauder (1962).

Ascoli (1946) claimed good results with the injection of 1 ml of 5 per cent phenol intratracheally. He injected the warm solution directly into the trachea just below the larynx and repeated the treatment one week later increasing the dose to 2 ml of a 10 per cent solution. The symptoms disappeared but in some cases returned a few months later. Two of the seven cases he treated died from resultant foreign body pneumonia, and from all accounts none was ever cured of the disease.

Steyn (1945) tried phenothiazine by the intratracheal route without success—he gave no details regarding dosage nor of the intervals between treatments.

Lithium antimony thiomalate (anthiomaline M & B) was reported by Forsyth (1954) and Malherbe (1965) to have beneficial effects. The former advocated 1 to 2 ml anthiomaline intramuscularly at three day intervals, three times while the latter found weekly intravenous injections of 5 ml for nine to twelve consecutive weeks adequate. Both these workers reported poor results when diethylcarbamazine (hetrazan) was used. Malherbe (1954) claims to have cured over two dozen cases with the use of either anthiomaline alone or in combination with hetrazan. He states on the one hand that the diagnosis and degree of progress of healing was assessed by the bronchial swab method (Malherbe, 1951). On the other hand he also states that dogs were periodically examined with a bronchoscope and in a few cases the final examination was carried out at autopsy. Since the bronchial swab method has been shown to be unreliable and as the actual number of dogs examined with a bronchoscope or autopsied is not stated, the actual efficacy of anthiomaline remains obscure. This conclusion is supported by further investigations made with both these chemotherapeutic agents which are reported below.

Improvement of clinical symptoms was obtained by Dorrington (1959) when a cyanacethydrazide (Dictycide I.C.I.) was injected subcutaneously at a rate of 10 mg/Kg body weight for three consecutive days. The cases remained positive, however.

Dietrich (1962) reported a successful treatment when using thiacetarsamide (Caparsolate Sodium, Abbotts) intravenously at the rate of 1 ml per Kg body weight for 21 days. This was confirmed by Dorrington (1963) when out of a total of ten infested dogs treated over varying periods, three were free of infestation two months after the completion of the 21 day course; shorter more intensive courses proved unsatisfactory.

Although some drugs appeared to be effective in some cases, it is doubtful whether any of them will cure all cases of *F. osleri* infestation.

2. *Experiment* 13. 21 *Day thiacetarsamide treatment*

Further investigation, following the reports of Dietrich (1962) and Dorrington (1963), was deemed necessary to test the efficacy of this drug more carefully.

Materials and methods

Five infested dogs were examined to assess the degree of infestation. Each dog was injected intravenously with 1 ml of thiacetarsamide (Caparsolate Sodium) per 5 Kg body weight for 21 consecutive days and examined with the aid of a broncho-scope six weeks after completion of the treatment.

Results

The results are given in Table 4.

TABLE 4.—*Results of* 21 *day treatment with caparsolate sodium at* 1 *ml/*5 *Kg*

Dog	Bronchoscope examination	Degrees of infestation	Re-examined 6 weeks later
Baron.............	5 July 1962.........	Average.............	No nodules
Cara..............	15 September 1962...	Mild................	Few small nodules
Candy.............	15 September 1962...	Average.............	No nodules
Tammy............	15 September 1962...	Average.............	Two loose nodules
Sugar.............	15 September 1962...	Average.............	No nodules

Discussion

Subsequent examinations were made on the dogs mentioned in Table 4 but no further improvement in their degree of infestation was found. Notwithstanding the fact that the drug could not be relied upon to effect complete cures in all cases, it had definite advantages in that it was safe, free from any side effects, easy to administer and reasonably effective (60 per cent).

3. *Experiment* 14. *Intratracheal surgery*

Although Lauder (1962) had previously abandoned transthoracic surgery, an attempt was made to remove the nodules via the trachea by means of a long biopsy forceps.

Materials and methods

Surgical technique: Infested dogs are anaesthetised with thiopentone sodium and a mouth gag applied. The dog is placed in a lateral position with its head fully extended and an illuminated bronchoscope is passed down the trachea as far as its bifurcation.

A good electric suction pump with suitable connections to a metal catheter and glass reservoir is used to clear the trachea and bronchoscope of blood during the course of the operation. Frequent flushing of the catheter with water to keep the suction line clear is necessary. A 50 cm biopsy forceps with cupped, cutting, crocodile jaws is inserted down the bronchoscope and the visible papillomata individually grasped and removed. After thoroughly clearing the trachea of all visible

papillomata, the bronchoscope is inserted into each bronchus and all visible nodules similarly removed. Frequent suction to remove blood, small papillomata, mucus and other debris, is essential so as to ensure a clear view of the affected area.

In some severely affected cases the time factor does not permit each nodule to be removed separately, especially where one bronchus is already completely occluded while the other is only partially patent. Any haemorrhage at this site in such cases will cause immediate asphyxiation. This is solved by rubbing off the nodules with a metal catheter and removing them by suction to create a larger functioning lumen in the affected bronchus as speedily as possible. Great care must be taken not to perforate the trachea as the resultant pneumothorax would prove fatal. Once the bronchus is reasonably patent the remaining nodules can be manually removed with a biopsy forceps. Thorough removal by suction of all blood and debris prior to the final withdrawal of the bronchoscope is essential. Post operative haemorrhage is of no consequence.

By using this technique no difficulty should be experienced in removing nodules 2 mm or more in size. Notwithstanding extensive experience with this surgical procedure 45 to 60 minutes is necessary to clear a badly infested case of all the *F. osleri* nodules.

Infested dogs: Three infested dogs were used initially—two were heavily infested and showed laboured stenotic respiration while the third had an average infestation.

Results

The results are summarized in Table 5.

TABLE 5.—*Intratracheal surgery for* F. osleri

Dog	Date of surgery	Surgical result	Re-examination
Dr. Botha's dog....	9 February 1963.....	Died (overdose of anaesthetic)	—
Herholdt's dog.....	9 February 1963.....	Died (asphyxia)......	—
Haydie............	2 March 1963........	Successful............	Small nodules 3 weeks and 5 weeks later

Discussion

Surgical removal alone is not a successful method of treatment but does relieve the respiratory distress.

Important practical points for consideration when undertaking this surgical technique should be borne in mind. These are:—

(i) Thiopentone sodium must be used very carefully. The use of a long-acting barbiturate for anaesthesia such as pentobarbital sodium would be highly dangerous in advanced cases of *F. osleri*. Light anaesthesia by giving repeated injections of 1 ml at a time to suppress any coughing and just sufficient to enable the surgeon to work satisfactorily, is far safer than larger doses of anaesthesia at longer intervals. There are two reasons for these dogs being poor surgical risks: firstly, the animals are usually debilitated, and secondly they have been on a high carbon dioxide level for a prolonged period of time. In such cases the anaesthetic requirements are far less than for those healthy dogs of equal weight. This fact was responsible for the loss of the first dog mentioned in Table 5.

(ii) In contrast to human practice, no topical anaesthetic should be instilled into the trachea pre-operatively. This procedure would normally be most welcome since light sedation only would then be necessary as would be the requirements for bronchograms. In cases already showing respiratory distress 0·5 ml of a topical anaesthetic injected intratracheally is just sufficient to completely occlude both bronchi resulting in irreversible asphyxia. This fact was responsible for the loss of the second dog mentioned in Table 5.

(iii) At first it was thought that the use of atropine would seem rational in ensuring a dry respiratory tract. The opposite was, however, found to be desirable because a moist mucous membrane facilitated coughing up and removal of any residual blood. A strong cough reflex is most desirable port-operatively.

(iv) Where a degree of cyanosis develops or, as is often encountered in severe cases where long and frequent suction has to be applied to clear the operative field, it is advisable to give pure oxygen via a Magill cuffed endotracheal tube, inserted into the bronchoscope with the cuff subsequently inflated, to replace accumulated CO_2 in the terminal bronchioles. Prolonged suction interferes with the gaseous exchange with a resultant increase of CO_2 in the alveoli.

(v) During recovery from the anaesthetic the dog should be positioned by elevating the thorax and abdomen with the head down to ensure natural drainage from the trachea. No ensuing pneumonia was ever encountered.

(vi) The table should be elevated appreciably so that when the surgeon is seated his eye level corresponds with that of the tube of the bronchoscope. Sufficient assistance should be available to attend to the anaesthesia, etc.

4. *Other chemotherapeutic agents tested:*

A variety of drugs was used in further attempts to discover a more efficient anthelmintic for *F. osleri.*

TABLE 6.—*Chemotherapeutic agents used and results obtained in the therapy of F. osleri*

Drug	Dose	No. of cases	Duration	Therapeutic effect
* Helmox..........	1 gr/Kg *per os*..	5	3 Consecutive days & repeated if necessary	Nil
Franocide..........	1 ml/5 Kg i.m...	5	3 Days repeated if necessary	Nil
Nankor............	2 gm/5 Kg *per os*	1	6 Times on alternate days	Nil
Triostam..........	1 mg/20 Kg i.v..	4	6 Days and 12 days....	Reduction but not curative
Thiabenzole in Houndmeal	0·25% per lb....	1	6 Weeks..............	Nil
Thiabenzole........	100 mg/Kg *per os*	2	Twice a week for 3 weeks	Nil
Mintic.............	1 fl oz/25 Kg *per os*	2	Twice a week for 3 weeks	Nil

* *Footnotes—*

Helmox (I.C.I.) cyanacethydrazide and phenothiazine
Franocide (Burroughs Wellcome) diethylcarbamazine
Nankor (Nicholas) Ronnel
Triostam (Burroughs Wellcome) sodium antimonygluconate
Thibenzole (Merck, Sharp and Dohme) Thiabendazole
Mintic (I.C.I.) 2 β (methoxyethyl) pyridine

In Table 6 a list of these anthelmintics together with their dosage rates, their duration and therapeutic effect is presented.

Results

Only triostam appeared to have any effect against *F. osleri* but even this drug appeared to be less effective than caparsolate.

5. *Surgery followed by chemotherapy*

From the foregoing experiments encouraging results were obtained with caparsolate, or triostam alone and also with intratracheal surgery alone. Nevertheless neither was completely satisfactory in ensuring 100 per cent efficacy in a large number of cases.

It appeared that the possibility existed that if these two promising methods were combined a highly effective method of treatment could be evolved. In the following experiment, surgery was performed followed immediately by chemotherapy.

Experiment 15. *Surgery followed by caparsolate or triostam therapy*

Materials and methods

Intratracheal surgery was successfully performed, as described under Experiment 14, on three infested dogs. Two were given a short intensive treatment thereafter while the third received triostam immediately after surgery.

TABLE 7.—*Intratracheal surgery followed by caparsolate or triostam*

Dog..............	Jester	Spot	Kerry
Infestation..........	Average	Average	Severe
Date of surgery....	23 March 1963.......	23 March 1963.......	23 March 1963
Drug used.........	Caparsolate..........	Caparsolate..........	Triostam
Dose..............	1 ml/5 Kg i.v. 3 times daily for 5 days	1 ml/5 Kg i.v. 3 times daily for 5 days	1 ml/20 Kg i.v. daily for 6 days
Examination 13 July 1963	No nodules..........	No nodules..........	Few small nodules
Re-examination 28 Nov. 1963	Small nodules........	Small nodules........	Small nodules

Results

The results, given in Table 7, indicate that surgery followed by a short intensive course of caparsolate (this dosage rate was two-thirds of the fatal dose) apparently cured two dogs. This was, however, only of temporary duration. Triostam proved unsatisfactory and was abandoned.

6. *Chemotherapy followed by surgery*

Since the results obtained in Experiment 15 were encouraging but not completely effective it was decided to reverse the procedure adopted and treat for a longer period with a safer dose of caparsolate.

Experiment 16. *Caparsolate therapy followed by intratracheal surgery*

Materials and methods

Initially three dogs with an average infestation were used. Each was dosed with 1 ml per 5 Kg caparsolate intravenously daily for 21 consecutive days. Six weeks after completing the therapy the dogs were examined bronchoscopically and simultaneously whatever nodules were visible were surgically removed as described in Experiment 14. Six weeks and again three months after surgery, they were examined bronchoscopically and the results obtained are given in Table 8.

TABLE 8.—*Caparsolate therapy followed by intratracheal surgery*

Dog	Examination 6 weeks after therapy	Examination 6 weeks after surgery	Examination 3 months after surgery
Candy............	Few nodules.........	No nodules..........	No nodules
Sugar.............	Few nodules.........	No nodules..........	No nodules
Tammy...........	2 small nodules......	No nodules..........	No nodules

Results

The results clearly indicate the success of a 21 day caparsolate therapy followed by intratracheal surgery. These three dogs were re-examined 6, 12 and 15 months later and both the bronchoscopic examinations and microscopic examinations of tracheal mucus removed at the time of the examination, showed no evidence whatsoever of *F. osleri* infestation.

Following the success of this experiment, eight infested dogs were similarly treated with equally gratifying results. It is evident that the combined therapeutic and surgical procedures as described provide an effective cure for *F. osleri* in dogs.

The following observations were made during the course of this therapy:—

(i) The papillomata assume a greyish firmer appearance a few weeks after the completion of the caparsolate treatment.

(ii) The papillomata disappear when once the nematodes are destroyed and this fact can be used to evaluate the efficacy of any treatment.

(iii) Contrary to the poor results mentioned, where regrowth of papillomata occurred following surgical removal alone, those papillomata observed and removed subsequent to a 21 day course of caparsolate therapy, will not develop again even though, at the time of removal, they may appear pink and active. It was noticed in some of the cases treated in this fashion that two to three weeks after surgical removal, papillomata were still present but if these were removed, subsequent examination showed the dog to be completely free of infestation. Incomplete original surgical removal was probably the reason for this condition. The effect of the arsenical compound on these nematodes appeared to persist for quite some time after the cessation of the treatment.

In some cases where dyspnoea was very severe, it was found expedient to remove a few of the largest papillomata that were severely obstructing the bronchi at first and then to commence treatment with caparsolate. Care must be taken with this

procedure as outlined in Experiment 14. In this way a great deal of respiratory distress was immediately relieved, and the dog was in a much better condition to undergo further surgical treatment at a later stage. In many cases it was found that if these large papillomata were not immediately removed the dog succumbed before it had benefited from the subsequent anthelmintic treatment.

7. *Conclusions regarding the treatment*

The following conclusions regarding the treatment of *F. osleri* in dogs may be made:—

(i) Remove any large papillomata surgically.

(ii) An intravenous injection of 1 ml per 5 Kg body weight of caparsolate sodium for 21 days followed six weeks later by intratracheal surgical removal of any remaining papillomata. This surgical removal may have to be repeated some weeks later.

(iii) Once a dog has been cleared by this method, it will remain permanently free of infestation.

(iv) Such treated dogs may still sporadically show the typical cough but this is no doubt due to cicatricial lesions. In many carefully observed cases such lesions are still discernible bronchoscopically many months after disappearance of all signs of papillomata.

XV. Notes on the Classification of *Filaroides osleri*

According to Dougherty (1943) the genus *Filaroides* van Beneden, 1858, should resort under the superfamily Metastrongyloidea Lane, 1917, not only because of the marked reduction in the typical strongyline bursa (common also to the superfamilies Filaroidea Weiland 1858 and Spiruroidea Railliet and Henry 1915 to which it was allocated incorrectly in the past) but also for the following reasons:—

(i) The *Filaroides* spp. occur in terrestrial mammals,

(ii) they also occur in carnivores and

(iii) they employ terrestrial gastropods as intermediate hosts—e.g. *F. martis* of mink requires the slug *Agriolimax reticulatus* as its intermediate host (Dubnitskii, 1955).

Yamaguti (1961) outlines the characteristics of the subfamily Filaroidinae as follows: " Mouth simple, surrounded by six low papillae. No buccal capsule, oesophagus claviform. Male: Posterior extremity blunt; no definite bursa, a small number of simple adanal and postanal papillae; spicules equal, of simple tubular type; gubernaculum present. Female: Tail blunt—conical with a pair of small lateral papillae at the tip and another pair just behind anus. Vulva near anus. Uteri parallel. Viviparous ". Yamaguti (1961) in his classification of the order Rhabdiasidea, describes the parasitic generation of the genus *Strongyloides* as follows: " Body slender attenuated anteriorly. Mouth with indefinite lips; buccal cavity practically absent. Oesophagus cylindrical, long without posterior bulb. Tail short, conical. Vulva in posterior third of body opening directly into uterine branches which are opposed. Ovaries reflexed. Oviparous; eggs embryonated at deposition ".

On the one hand, it has been shown *inter alia* that *F. osleri* does not require terrestrial gastropods as intermediate hosts nor are the female worms viviparous (both Filaroidinae characteristics), while on the other hand the characteristics of the parasitic forms of the Strongyloididae are in many ways very similar to those of *F. osleri* (direct life cycle, female worms oviparous with embryonated eggs at the time of laying).

It would appear, therefore, that in the light of these observations, the classification of the *F. osleri* justifies further investigation.

XVI. Prophylaxis

To prevent the introduction of *F. osleri* into a kennel, it is advisable to have all adult stud dogs, especially bitches, examined bronchoscopically and certified free of this infestation prior to introduction. Where young puppies are sold, a veterinary certificate that the bitch is free of the infestation should be provided. If possible, all members of the breeding kennel should be certified free of infestation.

Once a bitch has been found to be infested, the spread to her subsequent off-spring can be prevented either by removing all the puppies at the time of birth to certified uninfested foster bitches or they may be handreared.

Well fed adult dogs that are housed in warm dry kennels and kept free of other intestinal parasites are more likely to withstand *F. osleri* infestations.

XVII. Summary

1. The early history of *F. osleri* together with its world-wide distribution is discussed.

2. The various breeds of dogs affected are listed showing that most breeds have been affected at some stage.

3. The South African history is traced showing how some breeds and studs were infested.

4. The various symptoms found with infestation by *F. osleri* are enumerated.

5. The factors influencing the pathogenesis are mentioned and discussed.

6. All breeds of dogs of all ages are equally susceptible. Reasons for the seemingly greater susceptibility of the newly born puppies are advanced.

7. Both the macroscopic and microscopic lesions of *F. osleri* infestation are described. Mention is also made of the morphology of the parasite.

8. Diagnostic methods are dealt with and only the reliability of examination with a bronchoscope advocated.

9. The differential diagnosis of the condition and possible pitfalls in diagnosis are discussed.

10. Observations on the life cycle indicates this to be direct with no evidence of intra-uterine transmission.

11. Both sexes of the host animal are equally susceptible.

12. Infestation of the host is acquired at a very early age under natural conditions, the larvae being transported to the predilection site via the lymphatic system. Migration experiments are discussed in detail as is the morphology of the migrating larvae.

13. Histopathological changes observed in the lymph nodes, liver and lungs are described, the absence of eosinophiles especially being noteworthy.

14. Various chemotherapeutic agents are evaluated together with a description of a surgical technique for the removal of nodules.

15. A completely successful treatment consisting of a course of caparsolate sodium followed by intratracheal surgery is described and discussued.

16. The need for the possible re-classification of *F. osleri* in the light of the evidence advanced is suggested.

17. Prophylactic measures are listed.

XVIII. ACKNOWLEDGEMENTS

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