EXPERIMENTS WITH CERTAIN DIPTERA AS POSSIBLE TRANSMITTERS OF BOVINE ONCHOCERCIASIS.

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(With 16 Text-figures).

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Onchocerca gibsoni Cleland and Johnston.

A survey of the early work on bovine Onchocerciasis was published by one of us in 1911, while later work was again summarised in 1916 (Johnston 1911, 1916).

The probable origin of the parasite and its geographical distribution have been dealt with by Cleland and Johnston (1910) and by Gilruth and Sweet (1911, 1915). The anatomy of the worm, pathological effects and seat of infection have been fully treated by the above mentioned authors and also by Leiper and Breinl.

Nothing is yet definitely known regarding its life history. All efforts to find the embryos in the blood circulation have been unsuccessful, though Cleland showed that they may occur in the subcutaneous tissue. In post mortem examinations of infected cattle, they have been found in smears from the reflected skin or subcutaneous tissue of various parts of the brisket, legs and neck. Cleland also found living embryos in thickened areas under the skin of cattle. Breinl showed that embryos can penetrate the skin of the beast, though he himself regarded the result as perhaps pathological. Nicoll, though unable to confirm Breinl's work demonstrated that the larvæ were capable of migrating through the thick capsule of the nodule in considerable numbers.

The possibility of direct transmission has not been overlooked but it is considered highly improbable. Subcutaneous injection of larvæ, smearing them on the skin and drenching them to a calf in milk have been tried unsuccessfully by several investigators. Various theories have been put forward as to the intermediary host. The idea that it might be (1) a carnivorous animal (Gibson 1893), (2) a leech (Gilruth and Sweet, Breinl), (3) a crustacean (Cleland and Johnston, Breinl), has each been suggested and rejected with little attempt at investigation.* The theories that lice and flies are likely transmitters have received more attention. Gilruth and Sweet (1911) considered that the calf louse *Hæmatopinus vituli* L. presented the most hopeful possibility. Later, however, as a result of their experiments they were compelled to abandon the idea that either of the cattle lice, *H. vituli* or *H. eurysternus*, could act as a means of transmission of *O. gibsoni* (1912).

With regard to the fly theory, the most important work has been carried out by Cleland who examined numerous *Stomoxys calcitrans* after they had been allowed to feed on fresh nodules. Living Onchocerca embryos were found in one case on the third day after feeding (1914). Attempts were also made by the same author in conjunction with Dodd and McEachran (1917) to infect calves by exposing them to the attacks of certain insects. *Stomoxys calcitrans*, *Tabanids* and mosquitoes (*Culicelsa vigilax* and *Scutomyia atripes*) tested thus all gave negative results.

Breinl examined Tabanids, *Stomoxys calcitrans* and several species of mosquitoes fed over nodules, all with negative results (1913).

McEachran and Hill (1915) carried out work similar to Breinl's using *Stomoxys calcitrans*, *Lyperosia exigua*, several species of *Tabanus* and *Silvius* and also *Hæmatopinus tuberculatus* (buffalo louse), but were unable to shew any infection.

The two similar experiments carried out in Darwin by McEachran and Hill (1915) and by D'ckinson and Hill (1917) have not lent support to the fly theory (Hill and

*Miss M. Henry has apparently examined Cladocera as possible transmitters (P.R.S., N.S. Wales, 52, 1918, p. 463).

others, 1917). In both these experiments Victorian calves were imported; those allowed to run with the herd became infected, but those confined in an open pen with a concrete floor in close proximity to the paddock containing infected cattle remained unaffected, as also did those kept in a fly-proof pen.

More recently work has been carried out in New South Wales by Dr. Cleland and Miss Somerville (1919) who have made a "nodule survey" of certain parts of that State which shows that nodules are more common in parts of the State which have summer rains than in those where the rainy season occurs in the winter.*

Large numbers of Tabanids have been examined at Kendall, N.S.W. by trained assistants under the direction of Professors S. J. Johnston and J. B. Cleland. Out of several thousands of flies dissected, worms were found in three (Cleland 1918, p. 27), but so far an account of these parasites has not been published.

In 1914, Mr. Henry Tryon (Ann. Rep. Dept. Agric. Queensland 1914, p. 116) suggested the possibility that the larval worms (*Habronema* sp.) which infest the cattle fly, *Musca vetustissima*, might represent a stage in the life history of "*Spiroptera* (*Onchocerca*) *gibsoni*." Mr. Tryon informed us that he had some years previously mentioned the presence of these worms to several individuals interested in helminthology. We now know that the parasites in question are larval stages of *Habronema muscæ* and *H. megastoma*. Cleland stated in 1914 that Onchocerca larvæ were not found alive in the alimentary canal of *Musca vetustissima* 24 hours after ingestion.

A further attempt to follow out the life history of *O. gibsoni* was made during the period November, 1918, to January, 1920. Lines of work were indicated firstly by the prevalent idea that the intermediary was a Tabanid, and secondly by the suggestion of Dr. Bancroft that the intermediary was to be found among the non-blood-sucking flies living in association with cattle, *e.g.*, *M. fergusoni* (Johnston and Bancroft, 1919).

*Robles (Bull. Soc. Path. Exot. 12, 1919, p. 442) believes that certain species of *Simulium* are transmitters of *O. cæcutiens*, a human Onchocerca related to *O. volvulus*, and recently described by Brumpt (Bull. Soc. Path. Exot. 12, pp. 464-473).

The work of Breinl and Nicoll has shewn that the larvæ can penetrate both the capsule of the nodule and also the skin of the beast. It is also possible that the larvæ may travel in the lymphatics and reach a surface where the skin is tender, *e.g.*, round the lips and eyes. If this were the case, their ingestion by certain non-blood-sucking flies would be very probable, since such are commonly to be found round these mucous surfaces. Re-inoculation of the beast would occur by the escape of the larvæ from the fly, while the latter was feeding at a mucous surface.

The investigation centred on the one hand round *Tabanus circumdatus*, the commonest Tabanid of the district, and on the other around *Musca fergusoni* and, to a less extent, *M. vetustissima*, *M. terræreginæ*, and a small black *Fannia* sp.

The method of procedure was as follows :—

(1) Attempt to infect flies by feeding them on fresh nodules.

(2) Examination of wild flies.

Only the latter method yielded at all interesting results, the former being consistently negative. Although the investigation failed in its main object there has been collected a certain amount of information in regard to the helminth parasites of the flies dealt with.

Among the Tabanidæ of the Eidsvold district (Upper Burnett River, Queensland), specimens of the following species have been taken from time to time by Dr. T. L. Bancroft, some of the identifications being made by E. Austen, of the British Museum, and by F. H. Taylor, formerly of the Townsville Tropical Institute, but for most of them we are indebted to Dr. E. W. Ferguson, of the Bureau of Microbiology, Sydney, who has undertaken to work out the various species. There are at least 26 Tabanids, 19 belonging to *Tabanus* (12 named and 7 not fully identified), three to *Silvius*, three to *Pangonia* and one to *Erephopsis*.

Erephopsis guttata Donovan
Pangonia auriflua Donovan
,, *bancrofti* Austin (*Erephopsis bancrofti* Taylor)
,, *concolor* Walker

**Silvius* *australis* Ricardo
* ,, *notatus* Ricardo
* ,, *psarophanes* Taylor
Tabanus *regis-georgii* Macquart
,, *cyaneus* Wied.
,, *circumdatus* Walker
,, *mastersi* Taylor
,, *doddi* Taylor
,, *parvicallosus* Ricardo
* ,, *duplonotatus* Ricardo
* ,, *oculatus* Ricardo
* ,, *rufinotatus* Bigot
* ,, *dubiosus* Ricardo
,, *eidsvoldensis* Taylor
* ,, *australicus* Taylor
* ,, *hackeri* Taylor
* ,, sp. near *laticallosus*, probably *T. batchelori* Taylor
* ,, sp.?
* ,, *Walteri* Taylor
* ,, sp. nov. Therioplectes group near *T. edentulus* Macq.
* ,, *pallipennis* Macq.

During the period November, 1918, to January, 1920, only the following species were taken, *T. circumdatus*, *T. dubiosus*, *T. cyaneus*. *T. mastersi* and *T. australicus*. The first named was the only species at all plentiful and then only during March and April, 1919. A few specimens were taken in May, 1919, after wnich no more were seen until October. During the later months of 1919, and the beginning of 1920, they remained extremely scarce, probably owing to the severe drought. *T. circumdatus* was found to be most plentiful in country thickly timbered with wattle and in the vicinity of scrubs even though several miles away from permanent water ; the two striped Tabanids, *T. mastersi* and *T. australicus* were usually taken along the river. Many attempts were made to discover the breeding habits of these Tabanids but the few larvæ discovered in the muddy sand at the edge of the river water have so far yielded only uncommon species of *Tabanus* and *Silvius*.

Captured Tabanids were kept in captivity and fed on nodules when the latter were obtainable. The flies were fed on honey and water, dates (when procurable) and raisins. They usually lived well on this food and one

*Those species marked thus were identified for us by Dr. E. W. Ferguson.

was kept alive for thirty days, so that blood is not necessary to sustain life, though it may be for the production of fertile ova. Although all the flies dissected were females, very few were found to contain ripe ova and then usually only one ripe ovum was seen in each ovary in spite of the very large number of follicles present.

ATTEMPTS TO INFECT TABANIDS ARTIFICIALLY.

1. On November 30th, 1918, seven *T. circumdatus* and five striped tabanids *T. mastersi* and *T. australicus*, were given access to fresh nodules. These flies were examined at intervals of from two to four days but no development had occurred.

2. On December 30th, 1918, 20 *T. circumdatus* were fed on a fresh nodule. These were dissected at intervals of from one to ten days but no development had taken place. In one a living larva was found in the intestine one day after feeding, but in all other cases when found they were dead.

3. On April 4th, 1919, fifteen *T. circumdatus* were fed on a nodule. These were examined at intervals of from one to eight days, but no development had occurred. In one examined one day after feeding there were numerous fairly large filarial embryos encysted in the fat body.

4. On May 16th, 1919, one *T. circumdatus* was fed on a nodule. It died next day but no live embryos were seen.

5. On January 8th, 1919, two *T. dubiosus* were fed on a worm nodule. On dissection after two and three days respectively, no embryos were seen.

Total 43 *T. circumdatus*.
5 Striped Tabanids (*T. mastersi* and *T. australicus*).
2 *T. dubiosus*.

Cleland, Dodd and McEachran (1917) failed to infect calves, using *Tabanus regis-georgii*, *Diatomineura inflata* and *Silvius* sp., also the mosquitoes *Culicelsa vigilax* and *Stegomyia atripes*.

Hill, McEachran and Dickinson did not detect larvæ in cattle ticks (*Boophilus australis*); *Lyperosia exigua*, *Stomoxys calcitrans*; *Tabanus mastersi* and *T. nigritarsus*; and the mosquitoes *Myzorhynchus bancrofti*, *Culicelsa*

vigilax, *Chrysoconops acer*, *Culex sitiens*, *Pseudoskusea basalis* and *Tæniorhynchus uniformis* (1917).

Result of efforts to infect *M. fergusoni*, *M. vetustissima*, *M. terræ-reginæ* and the small black *Fannia* with *Onchocerca gibsoni*.

The method followed was to allow the caged flies to suck the juices from a freshly cut live worm nodule obtained as soon as possible after the slaughter of the bullock.

The above species of flies which fed very eagerly on nodules were killed at varying intervals for dissection. In the case of *M. fergusoni* and *M. vetustissima* it was noticed that only a few flies out of each batch actually ingested the embryos in any quantity. Dead embryos were found in an undigested state in flies up to the 6th day after feeding. Living embryos were once found in a fly one day after feeding. In the case of the small black *Fannia*, on the other hand, embryos were ingested by the majority of flies in one batch experimented with, many remaining undigested, chiefly in the crop, for as long as 14 days after feeding. On another occasion undigested embryos were found in a fly of this species 16 days after feeding. In all cases, however, the embryos were dead In no case was there any suggestion of development of the Onchocerca embryos within the fly, and, as far as these experiments go, they do not indicate any of the above flies as carriers of the parasite. It must be remembered, however, that the method of allowing flies to feed on an opened nodule is totally unnatural, since the majority of the embryos obtained in this way are liberated from the uterus of the female by the act of cutting and may not be in a fit state to commence development in the intermediate host. In nature the embryos in their passage from the parent to the exterior—however this may be accomplished —may become, as it were, strengthened and fitted to begin their cycle in the invertebrate host.

Our results are given for convenience in tabular form. The number of flies dissected out of each batch, whether captured or bred in the laboratory, and the number of days which were allowed to elapse before dissection are indicated. Other parasites present are noted under "Remarks."

M. fergusoni.

No. of experiment.	No of flies.	Bred or Captured.	No. of days after feeding when dissected.	Remarks.
1	11	captured ..	2– 4 days	No Onchocerca embryos seen *Habronema* in one.
2	25	,, ...	2–10 days	Half digested Onchocerca embryos seen in one fly on 3rd day. *Agamospirura muscarum* in 5. *Habronema* in one.
3	12	bred cowdung ..	3 days	No Onchocerca seen.
4	21	16 captured .. 5 bred cowdung	3– 7 days	No Onchocerca seen. *Agamospirura muscarum* in one captured fly.
5	94	captured ..	1–15 days	No Onchocerca seen, *A. muscarum* seen in 2 flies dissected on 11th day.
6	54	,, ...	1– 8 days	A large number of dead but undigested Onchocerca embryos seen in intestine of several flies dissected 1–6 days after feeding. *Habronema* spp. present in 5 ; *A. muscarum* in one and *Agamonema fanniae* in one.
7	22	bred cowdung ..	3–14 days	Numerous Onchocerca embryos and eggs in intestine of one fly dissected on 3rd day ; one dead but intact seen in a fly on 6th day.
8	69	bred cowdung ..	1– 5 days	Living Onchocerca embryos were seen in intestine of a fly dissected after one day. Dead embryos were detected in two flies on 2nd and 3rd day.
9	5	captured ..	4 days	No Onchocerca seen. *A. muscarum* present in 3 of these flies.
Total	313	flies		No development noted in any.

M. vetustissima.

No. of experiment.	No. of flies.	Bred or Captured.	No. of days after feeding when dissected.	Remarks.
1	15	captured ..	1–13 days	No Onchocerca embryos seen *Habronema* spp. present in two.
2	20	captured ..	4– 6 days	Dead Onchocerca embryos seen in crop of one fly dissected on 4th day, *Habronema* present in two. *Agamospirura muscarum* in one.
3	26	bred cowdung ..	4 days	No Onchocerca present.
Total	61	flies		No development seen in any.

M. terræ-reginæ.

1	41	bred cowdung ..	14–26 days	No Onchocerca embryos detected.
Total	41	flies		No development noticed in any.

Black Fannia.

1	44	captured ..	3–14 days	Onchocerca embryos were found in 34; the embryos were recovered up to the 14th day in an undigested conditions, but in all cases quite motionless. They were usually found in the crop or intestine, but occasionally free in the abdomen, having in these cases probably been set free by the rupture of one or other of the above organs during dissection.
2	9	captured ..	2–18 days	Dead Onchocerca embryos were found in 3 flies dissected on 6th, 11th and 16th days.
3	24	captured ..	1–11 days	Dead Onchocerca embryos were found in one fly on the 2nd day.
4	2	captured ..	1 day	No Onchocerca detected.
Total	79	flies		No development observed in any.

Onchocerca bovis, Piettre.

We desire to mention the occurrence of a second species of *Onchocerca* in Australian cattle. Our attention was drawn to its presence by Mr. N. V. Brown who forwarded specimens from cattle slaughtered at Rockhampton. He stated that they occurred in a more or less tangled condition in the connective tissues between the ligamentum nuchæ, also in the stifle joint, and mentioned that the same kind of parasite was to be met with between the spleen and stomach. This situation is similar to that in which Stiles and Hassall found their undescribed *Filaria lienalis* which is now generally regarded as being an *Onchocerca*, and which we now suggest is probably a synonym of *O. bovis*.

The material was sent to us early in 1919 but was only cursorily examined at the time, being provisionally labelled as *O. gutturosa* Neumann, the female resembling that of the Algerian species.

The finding of many males while recently overhauling the material led to their re-examination. It was seen that they differed from the males of *O. gutturosa* but apparently resembled those of *O. bovis* Piettre, as far as available information allowed us to compare them. We have not been able to obtain Piettre's original paper (1912) in Brisbane, and have had to content ourselves with a translation of another of his articles (1916) on bovine Onchocerciasis in South America; and with the tabulated measurements contained in Dr. Sweet's excellent paper on "Onchocerciasis in Cattle, etc., in countries other than Australia" (1915, pp. 45-7).

The male papillary arrangement is of the *Onchocerca* type, there being on each side four closely set perianal papillæ, a post anal near the tail, and two caudal. The longer spicules measure from .180 to .215 mm. and the shorter .050 to .070 mm. The measurements in Dr. Sweet's table are .180 to .210 and .065 to .075 mm. respectively. The males lie free in a more or less loosely coiled manner while the females are more or less loosely entwined in the fibrous tissue. At least four males were obtained from the tissue which contained apparently a single female. The body markings of the latter are of the type figured by Neumann as occurring in *O. gutturosa*.

We are of opinion that the *Onchocerca* worms (male and female) which Cleland found in a loose coil near the hip joint of an ox in N.S. Wales (1914, p. 47 ; 1914, p.p. 137, 150), belonged not to *O. gibsoni*, but to the same species as that which we now identify as *O. bovis*. The situation is one of those mentioned by Piettre (1916), in which *O. bovis* may be met with.

Examination of Captured Flies for the Presence of Parasites.

We deem it of interest to give an account of the various parasites found in flies during our examinations. The species of *Habronema*, *H. muscæ*, *H. megastoma* and *H. microstoma* are omitted, since they have been dealt with in another paper (Johnston and Bancroft, 1920).

Examination of Tabanidæ.

Tabanus circumdatus was found to be infected with one species of microfilariæ. The total number of flies examined and the number infected were as follows :—

T. circumdatus					
Nov 10th, 1918, to Jan. 11th, 1919..	111	examined ..	4	infected	
March 25th—April 19th, 1919	305	,, ..	9	,,	
April–May, 1919 : ..	32	,, ..	3	,,	
October 11th, 1919—January 14th, 1920	17	,, ..	2	,,	
Total for *T. circumdatus*	465	,, ..	18	,,	or 3.8%
T. cyaneus—the blue Tabanid	1	,, ..	0	,,	
T. dubiosus—the black Tabanid	5	,, ..	0	,,	
T. mastersi } Striped *T. australicus* } Tabanids	30	,, ..	0	,,	
Total for all species	501	,,	18	,,	or 3.5%

Extent of infection. In three cases very few larvæ embryos (1-9) were found, but in all other cases the infection was extremely heavy. In eight instances only young encysted forms were seen, in the remaining 10 cases fully developed larvæ were present, small forms occurring also in some of them. In one case where an actual count of the number of embryos was made the fly was found to contain 234 fully developed and very active worms, 94 being contained in the head and proboscis, 116 in the thorax and 24 in the abdomen. Other instances would probably have yielded still larger figures had time been spent in

counting them. The enormous number of larvæ does not seem to inconvenience a fly greatly since the individual referred to above had lived for a fortnight in captivity in apparent good health prior to dissection.

Agamofilaria tabanicola n.sp.

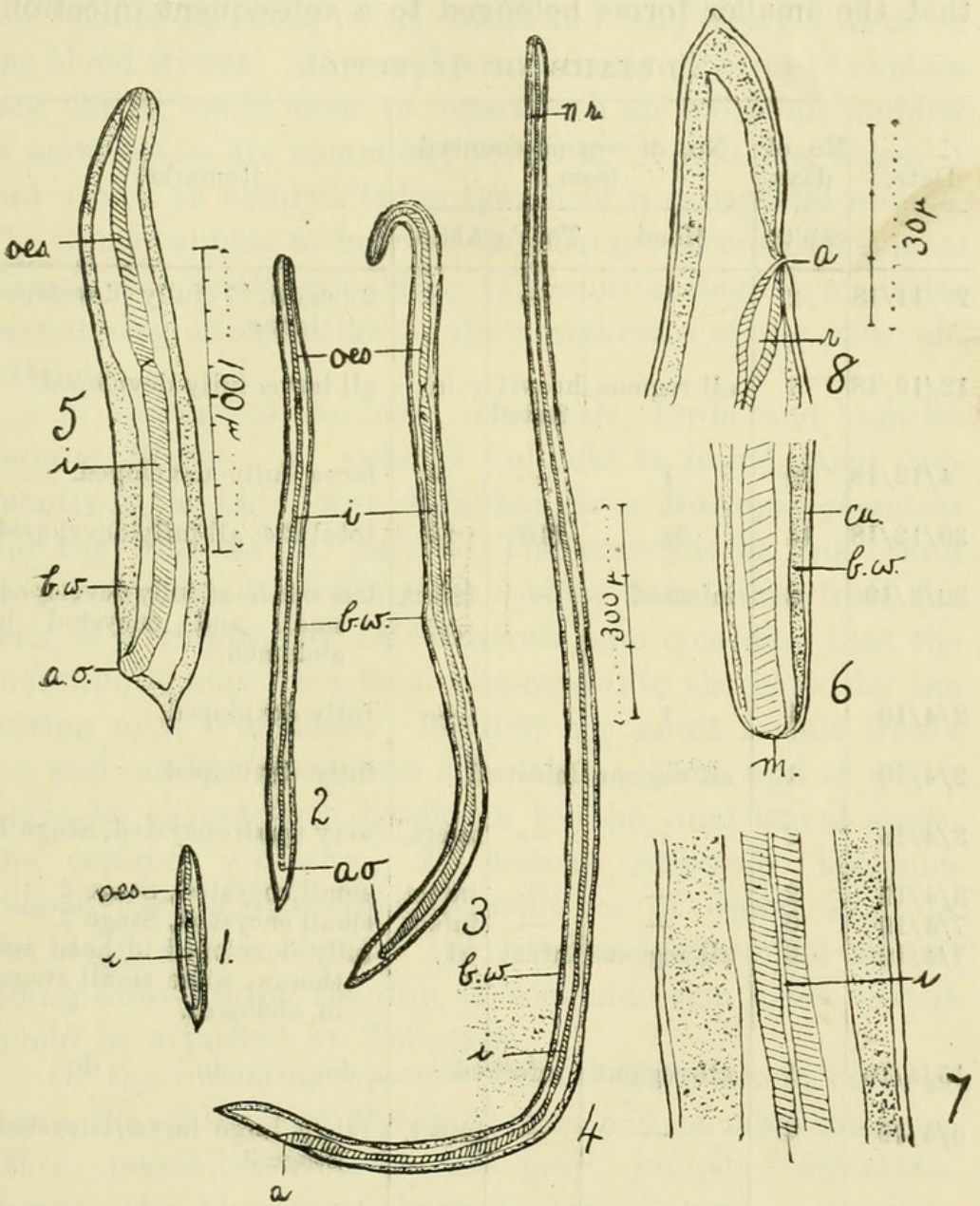
(Text-figures 1-8).

The microfilariæ taken up with the blood or lymph of their vertebrate host by the March fly leave the gut and encyst in the fatty tissue in the abdomen of the insect, the earliest stages found being in this region. Larvæ ranging in length from 150 μ to 1250 μ have been met with in this situation (figs. 1-3) The final stage in the fly has been found free in the abdomen, thorax, head and proboscis (fig. 4). The worms seem to be attracted to the proboscis and in a heavily infected fly they are seen to be congregated in and around the base of that organ. In one instance after the proboscis was severed from the head in normal saline, two larvæ emerged for about half their length from the apparently uninjured tip of the proboscis.

The youngest worms seen measured 150 μ-260 μ in length with a maximum width of 21-30 μ (fig. 5). There is a definite cuticle ; the œsophagus is clearly marked off from the intestine and the anus lies at about 25 μ from the pointed posterior end. The nerve ring lies at about 50 μ from the anterior end (stage 1).

As development proceeds (stages 2 and 3) there is a progressive lengthening but only a slight increase in width ; in fact the final stage is thinner at either end (though not in the mid region) both relatively and absolutely than in the earlier stages. In the final stage (stage 4) the worms measure from 1.93-2.4 mm. with a maximum width of 35 to 40 μ. The nerve ring lies at 115-120μ from the anterior end. The œsophago-intestinal unction, which is so well marked in the earlier forms, becomes gradually less distinct and cannot be made out in the final stage. The intestine is a long narrow tube and is somewhat dilated to form a rectum. The anus lies at about 40 μ from the tip of the tail which is bluntly rounded, though at the tip of the tail the cuticle is drawn out into a little point giving a characteristic appearance (fig. 8). There is a clear cuticle 1 μ in width, internal to which is the body wall from which

the intestine is separated by a space (fig. 7). The surface of the parasite is marked by very numerous transverse structures which are so low and closely arranged that they are recognisable only under the oil immersion. Hence the cuticle appears to be quite smooth.



Text-figures 1–4 .. Stages in growth of *Agamofilaria tabanicola* ; drawn to same scale shown beside fig. 4.
Text-figure 4 .. Fully developed larva of *A. tabanicola.*
Text-figure 5 .. Very young stage of *A. tabanicola*, drawn to adjacent scale.
Text-figures 6–8 .. Highly magnified views of fully developed larva of *A. tabanicola*, drawn to scale adjacent to fig. 8.
Text-figure 6 .. Head.
Text-figure 7 .. Portion of middle of body.
Text-figure 8 .. Tail.

EXPLANATION OF LETTERING.

a, anus; *a.o.*, anal operculum; b.w., body wall; *cu.*, cuticle; *gr.*, granules; *i.*, intestine; *i.r.*, rudiment of intestine; *m.*, mouth; *n.r.*, nerve ring; *oes.*, œsophagus; *p.*, papilla; *ph.*, pharynx.

Larvæ in different stages of development may be observed in the same fly. In some cases the discrepancy in sizes was so marked—large forms being found in the head and very small ones encysted in the fat body—as to suggest that the smaller forms belonged to a subsequent infection.

DETAILS OF INFECTION.

Date.	No. of days in cap'ty.	No. of worms counted from			Remarks.
		Head.	Thor'x	Abdo.	
26/11/18	2	1	—	8	2 small, 7 fully developed larvæ.
12/12/18	7	all regions	heavily infested		all larvæ fully developed
4/12/18	30	1	—	—	larva fully developed.
30/12/18	14	94	116	24	total 234, all fully developed
29/3/19	2	infected	—	infect.	larvæ in head fully developed small and encysted in abdomen.
2/4/19	1	1	—	—	fully developed.
2/4/19	1	all regions	infected		fully developed.
3/4/19	2	—	—	infect.	very small encysted, Stage 1.
3/4/19	2	—	—	infect.	small encysted, Stage 2.
7/4/19	6	—	—	infect.	small encysted, Stage 2.
7/4/19	6	all regions	infected		fully developed in head and thorax, some small stages in abdomen.
15/4/19	1	all regions	infected.		do. do. do.
5/4/19	4	—	—	infect.	fairly large larvæ, encysted, Stage 3.
—	—	—	—	infect.	very small larvæ, encysted, infection not heavy, Stage 1
—	—	—	—	infect.	small, encysted, heavy infection, Stage 2.
—	—	—	—	infect.	do. do. Stage 2.
15/10/19	7	all regions	heavily infected.		fully developed.
9/1/20	1	—	—	infect.	Heavy infection, numbers fully developed, but most were small and encysted.

It is almost certain that these microfilariæ do not represent the intermediate stage of *O. gibsoni*. The smoothness of the cuticle is different from what one would expect in the final larval stage of such a markedly corrugated worm as *O. gibsoni*. Again, judging by the post mortem findings of others only a few Onchocerca embryos occur in any one spot of the body of the beast and very rarely if at all in the blood stream. It would be extremely difficult to explain how the fly could come to ingest such an enormous number of embryos as are commonly found in a single fly, unless it had access to embryo laden lymph in a superficial nodule. The worms appear to have all the characteristics of a typical *Filaria* and in all probability represent a stage in a species parasitising one or other of the vertebrates of the Eidsvold district.

A number of the local mammals, birds and reptiles harbour filariæ. *F. websteri* Cobbold is found fairly frequently in the knee joint of the kangaroo *Macropus giganteus* and the whiptail *M. parryi*. The embryos of this filaria have only once been found in a blood film (taken from the neck) of a whiptail by Dr. Bancroft who considers that the microfilariæ may have been liberated into the blood by the cutting of a lympahtic. Besides, the adult female filaria has well marked transverse annulations, some sign of which might be expected to be shewn by the final larval stage. The common opossum, *Trichosurus vulpecula*, harbours *Filaria trichosuri* Breinl, the embryos of which occur in the blood stream, but as the host is a nocturnal animal hiding away during the day, it does not seem likely that it should be attacked by Tabanids.

Of the numerous species of local birds which harbour filaria, the soldier bird, *Myzantha garrula* is the most common. Other possibilities are :—the grey jumper, *Struthidea cinerea* ; the blue eared honey eater, *Entomyza cyanotis* ; the babbler, *Pomatostomus frivolus* ; the mutton bird, *Corcorax melanorhamphus* ; the blue jay, *Coracina robusta* ; the magpie, *Gymnorhina tibicen* ; the chip-chip, *Pardalotus melanocephalus* ; the mopoke, *Podargus strigoides* ; the nightjar, *Aegotheles novæhollandiæ* ; the crow, *Corvus coronoides* ; the darter, *Plotus novæhollandiæ* ; the black cormorant, *Phalacrocorax sulcirostris* ; and a number of others (see Johnston, 1916).

Among the reptiles the "goanna" *Varanus varius* is commonly infested with a large filaria, but the embryos have not been found in the blood stream. The jew lizard, *Amphibolurus barbatus*, harbours a small filaria, the embryos of which occur abundantly in the blood of some individuals. It has been proved, however, by Dr. T. L. Bancroft, that *Culex fatigans*, the common house mosquito, can act as a host for this latter filaria; consequently its normal host is almost certain to be one of the native mosquitoes. The water dragon, *Physignathus lesueurii*, is parasitised by *F. physignathi* Johnston.

It had been intended during the latter months of 1919 to try to infect Tabanids by feeding them on the infected blood of several of the commoner species of birds and on the fluid surrounding the knee joints of kangaroos parasitised by *F. websteri*, but the extreme scarcity of the flies prevented the work from being proceeded with. This seasonal scarcity of Tabanids has been recently referred to by Ferguson and Henry (P.L.S., N.S.W., 1919, p. 829).

Examination of Muscids, etc.

Examination of wild flies of the species *M. fergusoni* Jnstn. and Bancr., *M. vetustissima* Walker, *M. terræ-reginæ* Jnstn. and Bancr., and the little black Fannia *Homalomyia* sp., all, with the exception of *M. terræ-reginæ* Jnstn. and Bancr., common on stock at certain times in the year, revealed the fact that local flies are parasitised by four distinct kinds of nematodes, namely: *Habronema muscæ*, *H. megastoma*, *Agamospirura muscarum* and *Agamonema fanniæ*. *M. fergusoni* and *M. vetustissima* harbour all four kinds; *M. terræ-reginæ* only the first three as far as is known; and the Fannia only the last named.

M. fergusoni.

Total number of flies examined	1176		
Number infected with *Habronema* spp.	26	=	22%
Number infected with *Agamospirura muscarum*	81	=	68%
Number infected with *Agamonema fanniæ* ..	5	=	0.4%

M. vetustissima.

Total number of flies examined	280		
Number infected with *Habronema* spp. ..	14	=	5%
Number infected with *Agamospirura muscarum*	4	=	1.4%
Number infected with *Agamonema fanniæ* ..	1	=	0.3%

M. terræ-reginæ.

Total number of flies examined	21		
Number infected with *Habronema* spp. ..	1	=	5%
Number infected with *Agamospirura muscarum*	1	=	5%

Fannia (*Homalomyia*) sp.

Total number of flies examined	259		
Number infected with *Agamonema fanniæ* ..	4	=	1.5%

Details of Infection.

M. fergusoni.

Date.	Number Examined	No. parasitised by		
		Habronema spp	*Agamospirura muscarum*	*Agamonema fanniæ.*
Nov. 17th—Dec. 7th, 1918	54	4	1	—
Dec. 8th—Jan. 9th, 1919	61	1	6	2
March 23rd—April 16th	87	1	3	—
May 9th—May 31st ..	201	3	5	1
June 1st—June 24th ..	147	6	2	—
June 25th—Sept. 13th ..	278	10	9	2
Sept. 15th—Sept. 29th ..	127	—	24	—
Sept. 30th—Oct. 9th ..	134	—	19	—
Oct. 14th—Dec. 3rd ..	67	—	10	—
Jan. 3rd—Jan. 12th, 1920	20	1	2	—
Totals ..	1176	26	81	5

M. vetustissima.

Nov. 19th—Dec. 6th, 1918	33	—	—	1
Dec. 12th—Jan. 9th, 1919	26	6	—	—
Mar. 19th—April 16th ..	13	1	—	—
May 9th—Sept. 13th ..	36	3	—	—
Sept. 15th—Jan. 12th, 1920	172	4	4	—
Totals ..	280	14	4	1

M. terræ-reginæ.

Mar. 25th, 1919—Jan. 21st 1920	21	1	1	—

Fannia, sp.

Nov. 14th–23rd, 1918 ..	13	—	—	—
Mar. 29th—April 16th ..	51	—	—	—
May 9th—Aug. 6th ..	91	1 (?)*	—	3
Aug. 7th—Sept. 25th ..	104	—	—	1
Totals ..	259	1 (?)	—	4

*(?) *Habronema* embryo from egg, probably recently ingested by fly as no development had occurred.

Habronema spp.

The infection of *M. fergusoni*, *M. vetustissima* and *M. terræ-reginæ* by *Habronema muscæ* and *H. megastoma* has been fully dealt with in another paper.

Agamospirura muscarum n. sp.

(1) *M. fergusoni.* A few specimens of this fly were found infected from time to time up till September, 1919. From that time up till January 1920, when the work was concluded, the percentage of infected specimens rose considerably, while at the same time the percentage of *M. fergusoni* infected with *Habronema* fell to almost nil. Flies from all localities around Eidsvold township showed similarly high infection, whether collected along the river or in dry scrub country. Out of 81 cases of infection, the number of worms ranged from 1 to 12 with an average of 3.

The head alone was infected	13 times
The proboscis alone	6 times
The head and proboscis together	5 times
The thorax alone	5 times
The abdomen alone	26 times
The head (including proboscis) and abdomen	9 times
The head (including proboscis) and thorax	10 times
The thorax and abdomen	3 times
The head (including proboscis), thorax and abdomen ..	4 times

The small stages were found in rather thick yellowish cysts among the viscera in the abdomen; fully developed worms were also seen still encysted, but this stage was more commonly found free in abdomen, thorax or head. The tendency of the mature larva appears to be to migrate through the thorax to the head and proboscis. In many cases where fully developed worms were found in the head or thorax an equal number of the large, rather characteristic looking, empty cysts could be found in the abdomen. In several instances similar cysts were noticed in the abdomen but no worms could be found in any part of the body of the fly. It is almost certain that these flies were originally infected, the worms having escaped (as *Habronema* has been proved capable of doing) from the tip of the proboscis of the fly, while the latter was feeding on a wet surface.

M. vetustissima. An infected specimen was first met with in September, 1919. Of four cases of infection, the

number of worms ranged from 1 to 16, with an average of 8. The abdomen alone was infected twice ; the head, proboscis and abdomen once ; while in the fly containing 16 worms—the heaviest infection met with in any fly—all regions of the body were infected, three parasites being present in the head, four in the proboscis, three in the thorax and six in the abdomen.

M. terræ-reginæ. A single infected specimen of this species was met with on March, 1919, when a fly containing four worms in the proboscis was dissected.

In every case of infection in the three species dealt with, the parasitised subject was a female fly. Many more females were dissected than males, since among flies captured on stock the former sex predominates. However, the number of males dissected was quite large enough to warrant the expectation that an occasional infected specimen would be met with, at any rate during the latter part of 1919, when the percentage of infected *M. fergusoni* remained high. This, however, was not borne out by experience, although Habronemic infection occurred quite frequently among males. Two sets of figures giving record of the sex of flies (*M. fergusoni*) will illustrate this point. Out of 102 (seven males and 95 females) dissected during May and June, 1919, three males and one female were infected with *Habronema* spp. and four females with *Agamospirura muscarum*). During the last three and a-half months of 1919 out of 238 flies (21 males and 217 females) 31 females were infected with *Agamospirura muscarum*, no other nematodes being met with.

Description of Agamospirura muscarum n sp.

(Text-figures 9-15).

The early stages are found encysted in the abdomen of the fly. The smallest embryo met with measured 610 μ in length by 50 to 60 μ in breadth (fig. 9-13). The mouth led into a shallow pharynx 8 μ in depth, followed by a short thick œsophagus, the distance of its base from the oral opening being 120 μ. A nerve ring surrounded the œsophagus at a distance of 65 μ from the mouth. The intestine was a long straight tube leading into a rather large rectum 75 μ in length. An anal operculum was present.

The tail was drawn out into a point situated 60 μ from the anus. At this stage the transverse annulations were only faintly marked. A gradual increase in size takes place (figs. 10, 11), the fully developed worm measuring from 2.8 to 3.4 mm. in length. This stage (figs. 12, 14, 15) is usually found in the head and proboscis. The mouth is surrounded by several small papillae and leads into the pharynx which now measures 25 μ in depth by 15 μ in width (fig. 14). The œsophagus gradually increases in width from 20 μ to 35 μ at the œsophageo-intestinal junction which is situated 230 μ from the mouth. The nerve ring encircles the œsophagus at a distance of 160 μ from the anterior end. The long straight intestine leads into the rectum, which is 180 μ in length by 40 μ in the widest part. The anus, now open, is situated 85 μ from the tip of the tail. Three small papillæ are present on the tail, one terminal and two situated on either side a short distance in front of it. The worm is thick anteriorly tapering off towards the posterior end. The breadth at the base of the œsophagus is 95 μ, and at the anus 45 μ. At this stage the transverse annulations are well marked in the anterior portion of the worm, being most prominent about the junction of the œsophagus and intestine. They are situated from 5 to 7 μ apart. Towards the posterior end they become fainter and cannot always be made out in the anal region. In one specimen the annulations were rounded off, giving a different effect from that shown in fig. 14. In other respects the worm was a typical *Agamospirura muscarum*.

The characters of the parasite suggest that it represents the larval stage of one of the *Spiruroidea*, perhaps of the *Spiruridæ*, hence the larval collective generic designation. The specific name is given owing to the comparative frequency of the occurrence of the worm in many local Muscidæ.

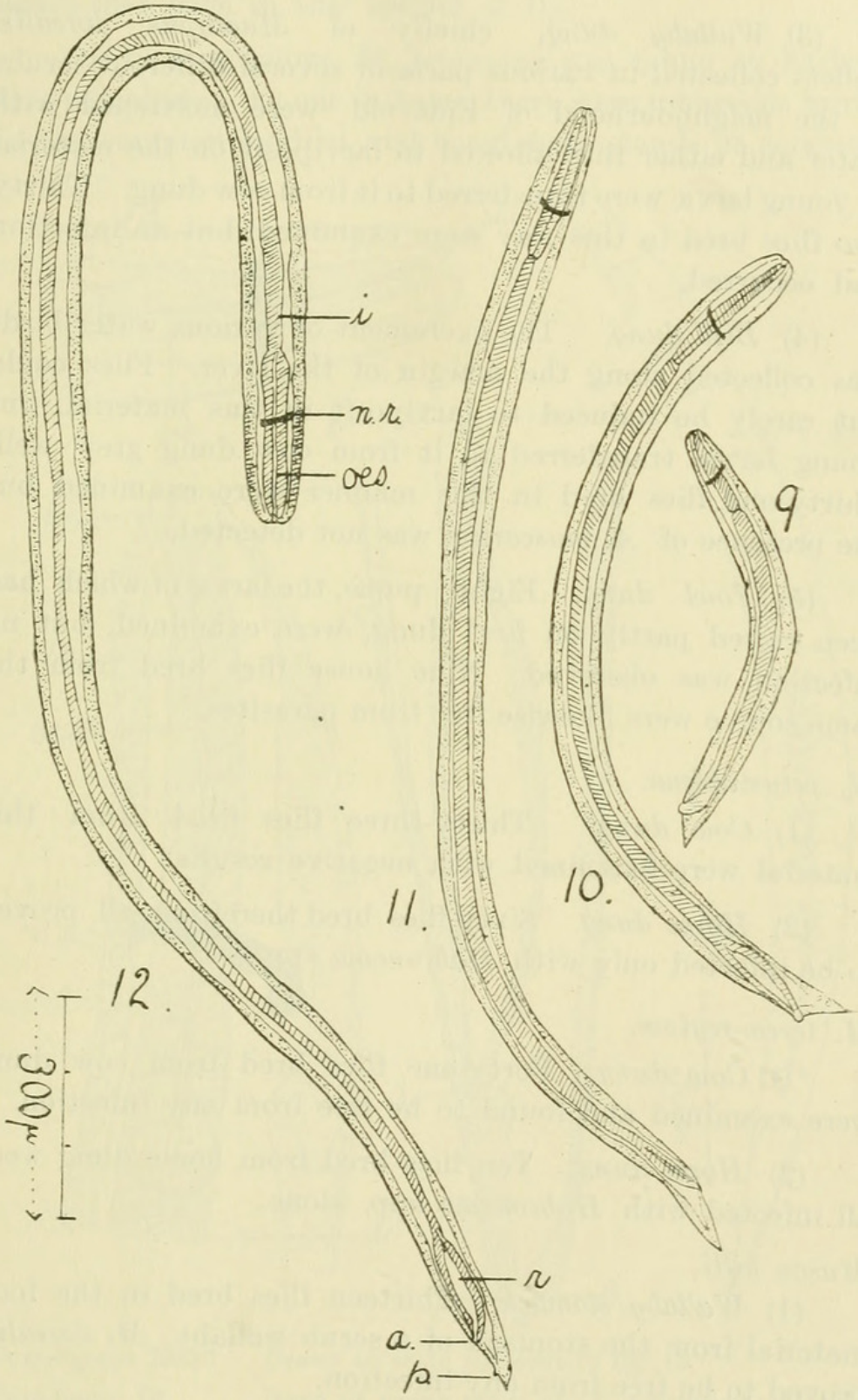
Attempts were made to trace the origin of the nematode by breeding out flies from various materials.

The results were as follows.

M. fergusoni.

(1) *Cow dung.* Two hundred and seventy-eight specimens (including a few pupæ) bred from cow dung from

various localities were examined, but none were found to harbour *Agamospirura muscarum.*



Text-figures 9–12 Stages in growth of *Agamospirura muscarum*, drawn to scale shewn beside fig. 12.

Text-figure 12 .. Fully developed larva.

(2) *Horse dung.* Of fifty eight (including a few pupæ) bred from horse dung from various sources, fifty-five

were infected with *Habronema* spp. but *A. muscarum* was not found.

(3) *Wallaby dung,* chiefly of *Macropus dorsalis.* Pellets collected in various parts of several different scrubs in the neighbourhood of Eidsvold, were moistened with water and either flies allowed to larviposit on the material or young larvæ were transferred to it from cow dung. Thirty two flies bred in this way were examined, but no infection had occurred.

(4) *Bird dung.* The excrement of various water birds was collected along the margin of the river. Flies could but rarely be induced to larviposit in this material, but young larvæ transferred to it from cow dung grew well. Thirty-one flies bred in this manner were examined but the presence of *A. muscarum* was not detected.

(5) *Fowl dung.* Eight pupæ, the larvæ of which had been raised partly on fowl dung, were examined, but no infection was observed. Nine house flies bred from the same source were likewise free from parasites.

M. vetustissima.

(1) *Cow dung.* Thirty-three flies bred from this material were examined with negative results.

(2) *Horse dung.* Sixty-flies bred therefrom all proved to be infected only with *Habronema* spp.

M. terræ-reginæ.

(1) *Cow dung.* Forty-one flies bred from cow dung were examined and found to be free from any infection.

(2) *Horse dung.* Ten flies bred from horse dung were all infected with *Habronema* spp. alone.

Musca hilli.

(1) *Wallaby stomach.* Thirteen flies bred in the food material from the stomach of a scrub wallaby, *M. dorsalis,* proved to be free from any infection.

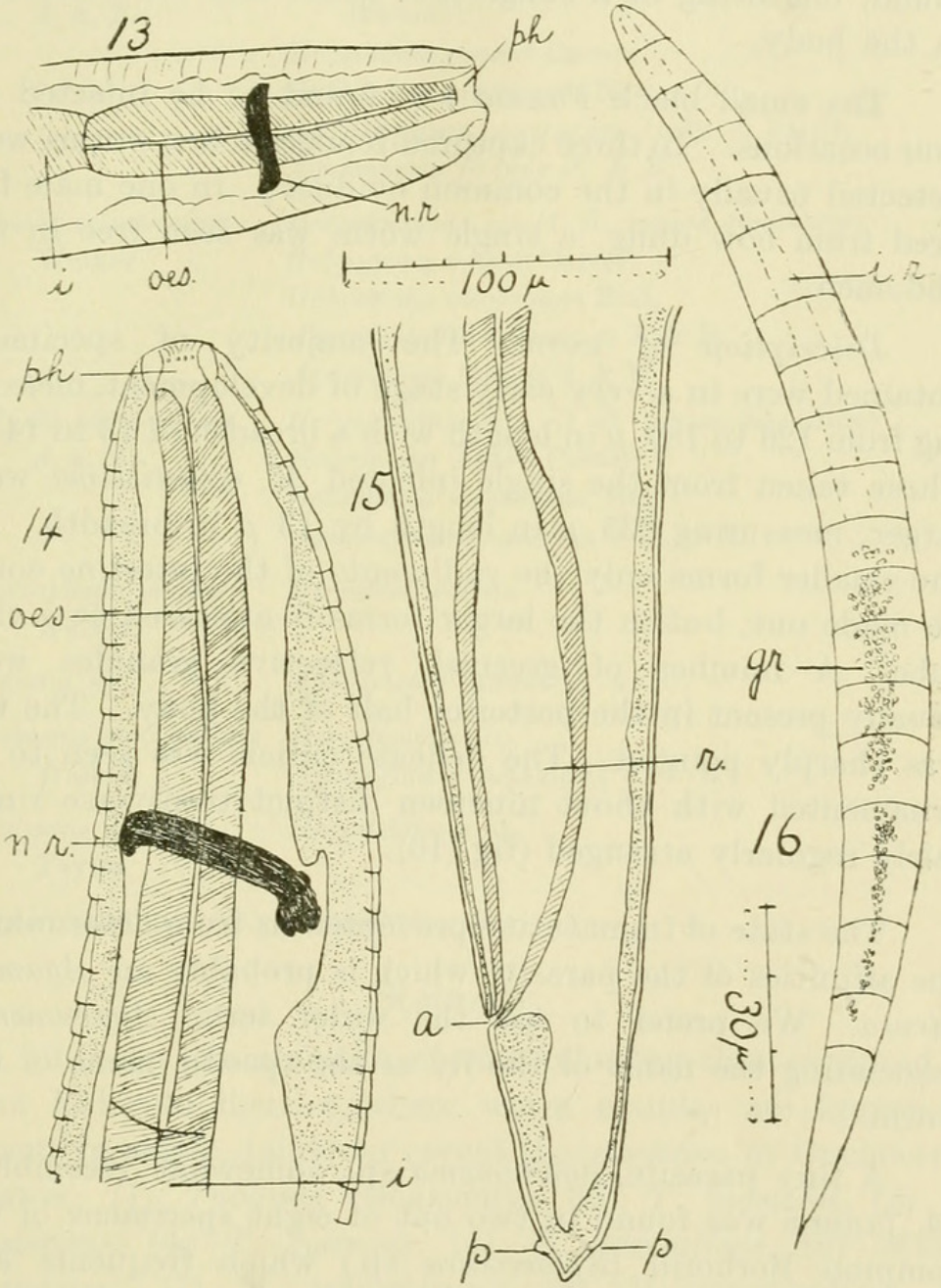
Agamonema fanniæ n. sp.

(Text.-fig 16).

This tiny active parasite was first met with in a specimen of *M. vetustissima* dissected in December, 1918. The worms

were free in the abdomen when noticed, but may have been liberated by the rupture of the genital ducts. It was not again met with in this species of fly.

On five occasions *M. fergusoni* was found to harbour this nematode. In one instance there were numerous worms in the common oviduct and conglobate glands or accessory



Text-figures 13–15 Drawn to scale adjacent to fig. 15.

Text-figure 13 .. Head of young stage of *A. muscarum*, shewn in fig. 9.

Text-figure 14 .. Head of fully developed larva of *A. muscarum*.

Text-figure 15 .. Tail of same.

Text-figure 16 .. *Agamonema fanniæ* entire specimen highly magnified; drawn to adjacent scale.

copulatory vesicles. In all other cases the flies were lightly infected, the worms being located in similar parts of the body. In one fly, which had been bred from cow dung and had been confined in a cage with captured specimens of *M. vetustissima* and the black *Fannia*, and had had access to cow and horse dung for larviposition, two worms were found, one being in a conglobate gland and the other free in the body.

The small black *Fannia* was found to be infected on four occasions. In three captured females a few worms were detected usually in the common oviduct; in one male fly, bred from cow dung, a single worm was seen free in the abdomen.

Description of worm. The majority of specimens obtained were in a very early stage of development, measuring from 126 to 180 μ in length with a breadth of 13 to 14 μ. Those taken from the single infected *M. vetustissima* were larger, measuring 235 μ in length by 13 μ in breadth. In the smaller forms only the rudiments of the intestine could be made out, but in the larger forms it appeared as a thin tube. A number of greenish refractive granules were usually present in the posterior half of the body. The tail was sharply pointed. The delicate cuticle was seen to be ornamented with about nineteen distinct transverse rings, fairly regularly arranged (fig. 16).

The state of immaturity prevented us from determining the affinities of the parasite which is probably an *Agamospirura*. We prefer to use the wider term *Agamonema*, associating the name of the fly as the specific name of the worm.

A tiny parasite, *Agamonema* sp., somewhat resembling *A. fanniæ* was found in two out of eight specimens of the common Borborid (*Sphærocera* sp) which frequents and breeds in horse dung in Brisbane. Two worms occurred in one and one in the other.

Herpetomonas (*Leptomonas*) spp.

Flagellates resembling *H. muscæ-domesticæ* Burnett, were found at Eidsvold in the intestine of *Musca fergusoni*, *M. vetustissima* and *M. terræ-reginæ*, while members of the

same genus occurred also in *Tabanus circumdatus* and *T. mastersi.* They were found in Brisbane in *Sarcophaga misera.*

The following is a list of parasites referred to in this paper as occurring in the Diptera examined :—

Musca fergusoni J. & B. ..	*Herpetomonas* sp. (? *H. muscæ-domesticæ* Burnett).
	Habronema muscæ Carter.
	Habronema megastoma Rud.
	Agamospirura muscarum Jnstn. & Bancr.
	Agamonema fanniæ J. & B.
Musca vetustissima Walker	*Herpetomonas* sp. (? *H. muscæ-domesticæ*)
	Habronema muscæ Carter.
	Habronema megastoma Rud.
	Agamospirura muscarum J. & B.
	Agamonema fanniæ J. & B.
Musca terræ-reginæ J. & B.	*Herpetomonas* sp. (? *H. muscæ-domesticæ*)
	Habronema muscæ Carter.
	Habronema megastoma Rud.
	Agamospirura muscarum J. & B.
Sarcophaga misera Walker.	*Herpetomonas* sp.
Fannia sp... ..	*Agamonema fanniæ* J. & B.
Tabanus circumdatus Walker.	*Herpetomonas* sp.
	Agamofilaria tabanicola J. & B.
Tabanus mastersi Taylor.	*Herpetomonas* sp.
Sphærocera sp. ..	*Agamonema* sp.

Summary.

1. An examination of the following flies captured in the Eidsvold district where worm nodules are known to occur in cattle, failed to reveal the presence of Onchocerca larvæ: (1) *Tabanus circumdatus* (2) *T. mastersi*, (3) *T. dubiosus*, (4) *T. cyaneus*, (5) *T. australicus*, (6) *Musca fergusoni*, (7) *M. vetustissima*, (8) *M. terræ-reginæ*, (9) *Fannia* sp.

(2) The Tabanids, *T. circumdatus*, *T. dubiosus*, *T. mastersi*, and *T. australicus*, as well as the three above named species of *Musca* and *Fannia* sp. failed to become infected with larvæ when fed on freshly cut worm nodules.

3. A second species of *Onchocerca* (*O. bovis* Pietttre) infests Australian cattle.

4. *Tabanus circumdatus* is commonly parasitised by a filarial larva (*Agamofilaria tabanicola*); while the three Muscids, in addition to harbouring larval *Hab onema muscæ* and *H. megastoma*, are infested by certain other larval nematodes. A species of *Fannia* commonly associated with cattle, also harbours a larval nematode.

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