

PRELIMINARY REPORT ON THE TRANSMISSION OF *PARAFILARIA BOVICOLA* IN SOUTH AFRICA

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ABSTRACT

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The filarial worm *Parafilaria bovicola* causes streaks of blood on the skins of live cattle and slimy bruise-like lesions on the subcutaneous surfaces of their carcasses. To determine the vectors of this worm a field survey was conducted in November 1972 and during the summer of 1973/74 on 6 farms in the Transvaal. A total of 10 093 flies was collected off cattle and examined for the infective 3rd stage *Parafilaria* larvae. Of the 12 fly species collected, *Musca lusoria*, *Musca domestica*, *Musca xanthomelas*, *Musca* n. sp., *Musca sorbens* and *Musca fasciata* were the commonest. Third stage larvae were found in 3 species all belonging to the subgenus *Eumusca*, viz. *M. lusoria*, *M. xanthomelas* and a new *Musca* species. The infection rate in these flies was usually less than 1% and most of the worms were recovered from the heads of female flies.

The same 3 species were successfully infected artificially in the laboratory, the infection rate ranging from 40,0%-53,8%. The measurements of larvae from these flies agreed closely with those of larvae recovered from field-collected flies.

Female *P. bovicola* worms perforate the skin of cattle and deposit their eggs and/or microfilariae into the blood which trickles down from the holes. Most of these bleeding marks were noticed between July and December 1973. As 3rd stage larvae were recovered from flies from August 1973 to February 1974 the main period for transmission is likely to be between July and February. Since the 3 flies suspected of being vectors feed mainly on eye secretions it is believed that most transmission takes place via the orbital route. Thereafter development of the worms in cattle to the adult stage will take approximately 7-10 months.

INTRODUCTION

The original description of the filarial worm *Parafilaria bovicola* by Tubangui (1934) was based on females collected from cattle in the Philippines. These worms were excised from small, bleeding nodules or swellings (Fig. 1 & 2) noted on the skins of the live animals by De Jesus (1934).

During the same year Gulati (1934) in India referred to a worm that he named *Filaria haemorrhagica* which was common in bullocks used for ploughing. He also noted the bleeding nodules containing filarial worms which, from his description, are obviously *P. bovicola*. In severe cases animals lost condition and it became difficult to keep them in work. Other records of *P. bovicola* in Indian cattle and water buffalo have been reviewed by Patnaik & Pande (1963).

Faure (1935) saw bleeding cattle in Tunisia and Morocco and on autopsy recovered filarial worms from haemorrhagic, gelatinous and oedematous foci in the subcutis corresponding in position with the bleeding spots on the skin. These worms were identified as *Setaria haemorrhagica*.

In 1949 *P. bovicola* was recorded in Rumania by Metianu and in Ruanda-Urundi by Fain & Deramée. Fain & Herin (1950) also noted the bloody gelatinous and oedematous areas on the subcutis which were associated with the adult worm. In 1955 they described how the female worm laid embryonated eggs into the blood dripping from the hole she had made through the skin. Using a magnifying glass and forceps they saw and collected females in the process of ovipositing.

P. bovicola infection has been reported from Canada in Charolais cattle recently imported from France but has apparently not become established there (Niilo, 1968; Webster & Wilkens, 1970).

Parafilariasis was recorded in South Africa for the first time in cattle from the northern Transvaal by Pienaar & Van den Heever in 1964. They recovered numerous worms from the subcutis of infected animals and noticed "areas of dirty greenish-yellow coloured

inflammatory oedema" resembling "partially healed superficial contusions". They drew attention to the possible economic importance of such carcass lesions due to disfigurement when they were trimmed off and to early spoilage, particularly where muscle tissue is exposed, and also because "such carcasses would not qualify as being free of blemish for export purposes." In 1971 the incidence of these lesions appeared to be increasing and a project was initiated at Onderstepoort to study the economic importance, incidence, distribution and transmission of this worm (Van den Heever, Nevill & Horton, 1973).

It is not known how *P. bovicola* is transmitted in nature. Fain & Herin (1955) suspected *Musca domestica* of being the vector and tried to prove this. They recovered 1st stage larvae from the gut of the fly after 3 days but had to discontinue this work following the death of their colony. Until the present study, no further work on the transmission of *P. bovicola* has been recorded.

Valuable information has been obtained from studies on the transmission of *Parafilaria multipapillosa* of equines. Baumann (1946) thought *Musca* species were the intermediate hosts of this species. Subsequently Gnedina & Osipov (1960) showed that *Haematobia atripalpis* is a suitable intermediate host for the development of the larvae of this species in Russia and Osipov (1962) succeeded in transmitting the worm by feeding infected *H. atripalpis* on horses.

Webster & Wilkens (1970) were convinced that bleeding due to *P. bovicola* only commenced when cattle were exposed to sunlight and referred to the work on *P. multipapillosa* by Baumann (1946), who claimed that sunlight was necessary to stimulate the females to oviposit. The writer noted that most bleeding caused by *P. bovicola* occurs during the main daylight hours and was convinced that the vectors would be found among the flies feeding on these bleeding lesions. This report summarises the findings to date on the transmission of this parasite in South Africa.

MATERIALS AND METHODS

Examination of field-collected flies for infective P. bovicola larvae

Flies were collected on farms in 6 widely separated bushveld and lowveld regions of the north-western, northern, central and eastern Transvaal where parafilaria is enzootic. Their coordinates are as follows:

Mara Research Station..	23° 09' S	29° 34' E
Leamington.....	24° 01' S	27° 15' E
Doornpan.....	24° 02' S	27° 32' E
Mooiplaats.....	24° 53' S	30° 25' E
Syferfontein.....	25° 22' S	26° 15' E
Zoutpan Research Station	25° 24' S	28° 06' E

To collect flies, citrated blood was poured onto the backs of cattle held in a crush and the haematophagous flies attracted to it and its vicinity were collected with hand nets. In contrast to the catches near homesteads and permanent yards which were dominated by *M. domestica*, a variety of dung-breeding flies was collected out in the veld. Collections were therefore made in the veld except where crushes were unavailable.

Flies were held alive in small cages in a polystyrene foam cooler box and on return to the camp or laboratory were anaesthetized, pinned, identified to species and sexed. The flies were then removed from the pins, their heads and bodies squashed separately between two 76 × 26 mm glass slides and the preparation examined under the 25× magnification of a stereoscopic dissecting microscope with good transmitted light.

If present, 2nd and 3rd stage filarial larvae were placed in a drop of saline, fixed with heat and mounted in a small drop of 70% ethyl alcohol and 5% glycerine on an inverted cover slip over the hollow of a cavity slide (Nelson, 1959). The cover slip was sealed with clear nail varnish to prevent drying of the mountant.

Dr Anna Verster (Helminthology Section, Veterinary Research Institute, Onderstepoort) provisionally identified a set of 3rd stage larvae as *P. bovicola* by comparing them with the descriptions and drawings of 3rd stage larvae of *P. multipapillosa* by Gnedina & Osipov (1960). These larvae, recovered from flies at Mara in November, 1972, were used for reference purposes when tentatively identifying further field collections of 3rd stage larvae from flies. The tentative identifications were subsequently confirmed by comparison with 3rd stage larvae of *P. bovicola* recovered from flies artificially infected in the laboratory (see below).

Laboratory infection of flies with P. bovicola

Flies were collected off cattle at Zoutpan Research Station using the method described earlier. They were divided into 2 groups and kept in the laboratory at 27 °C and 50–60% R.H. in wire frame cages (20×20×20 cm) covered with mutton cloth. Prior to infection they were fed only sugar water but thereafter they were supplied with petri dishes containing sugar crystals, a dry food mixture of whole milk powder, brewer's yeast and cholesterol, and a water-soaked pad of cotton wool. Fresh citrated ox blood was given daily on a cotton wool pad.

A gravid *P. bovicola* female was collected from the subcutaneous fat surface of an ox carcass at the Pretoria abattoir and cut into ±2 mm lengths in a few drops of saline. Many hundreds of embryonated

eggs and some active microfilariae were liberated (Fig. 3). Approximately 1 ml citrated ox blood was mixed with these eggs and microfilariae. A Pasteur pipette was used to transfer small drops of this infected blood to the upper surface of fine gauze covering a 500 ml cardboard cup in which half the flies were held. They immediately started lapping up the blood through the gauze. Thereafter they were returned to their holding cage and treated in the same way as the other half which was used as a control group.

Dead flies were removed daily from the cages, identified and squashed to check for developing filarial worms. After 14 days the surviving flies in both groups were identified, squashed and 3rd stage filarial larvae were mounted on slides for comparison with 3rd stage worms recovered from field-collected flies (Fig. 4).

For the purpose of identification the measurements of field-collected and laboratory-reared 3rd instar larvae were compared. The following measurements were made: Length, width of larva at end of oesophagus, length of oesophagus from cephalic end, distance of nerve ring from cephalic end, distance of anal opening from caudal end and depth of buccal cavity (Fig. 5 & 6).

RESULTS

Examination of field-collected flies for infective P. bovicola larvae

A list of the 10 093 flies collected from cattle at 6 collecting points in the Transvaal between November 1972 and April 1974 and the number of flies infected with *P. bovicola*, is given in Table 1.

There were 9 *Musca* species, which accounted for most of the flies. The remaining 165 flies belonged to the genera *Stomoxys*, *Haematobia* and *Morellia*.

M. lusoria was the most abundant species and was relatively plentiful at all collecting sites. *M. domestica* was 2nd in overall abundance and was extremely common at "Doornpan" in November 1973, after a period of good rains.

Flies listed as "*M. xanthomelas*" were 3rd in abundance but it is now known that 2 species are included under this name. In December 1973, K. P. N. Kleynhans (Plant Protection Research Institute, Pretoria, personal communication) pointed out that *M. xanthomelas* can easily be confused with an undescribed *Musca* species. Since this similarity was noted numerous specimens of this undescribed species have been recognized from the collection sites. Relatively few specimens belonging to the *M. xanthomelas* complex were collected at Mooiplaats.

Musca sorbens was normally scarce, except for a flush at "Doornpan" in September 1973 during an extremely hot, dry period. *Musca fasciata* was collected regularly in small numbers throughout the collecting area. *Musca crassirostris* was scarce despite reports that it was a pest during the previous hot, dry summer. *Musca lasiophthalma*, *Musca conducens*, *Haematobia* and *Morellia* species were rarely collected while *Stomoxys* was comparatively abundant.

Table 2 summarises the monthly fly collections and infection rates for the 3 *Musca* spp. from which the infective 3rd stage *P. bovicola* larvae were recovered.

TABLE 1 Flies collected on 6 farms in the Transvaal during a survey to determine the transmitter(s) of *P. bovicola*

Fly species	Mara		Zoutpan		Doornpan		Leamington		Mooiplaats		Syferfontein		Totals		No. of flies infected	% Flies infected
	November 1972 October 1973- April 1974	1 126 (14)** 727 217 (4) 25 221 90 145 9 4 0 5 0 2	July 1973- April 1974	1 958 (11) 352 262 (1) 115 88 62 131 16 2 3 9 3 0	September 1973 -March 1974	393 (1) 947 255 (1) 94 90 (1) 623 47 7 2 3 30 14 0	September 1973-March 1974	272 (3) 257 77 16 60 48 46 39 1 0 47 11 0	September 1973-April 1974	427 (4) 141 13 (1) 6 2 10 84 0 0 0 0 31 0 13	September 1973- November 1973	171 143 37 (1) — — 19 14 31 0 0 0 0 0	4 347 2 567 861 256 461 852 467 102 9 6 122 28 15	42		
<i>M. lusoria</i>																
<i>M. domestica</i>																
" <i>M. xanthomelas</i> "**																
<i>M. xanthomelas</i>																
<i>Musca</i> n. sp.....																
<i>M. sorbens</i>																
<i>M. fasciata</i>																
<i>M. crassirostris</i>																
<i>M. conducens</i>																
<i>M. lasiophthalma</i>																
<i>Stomoxys</i> spp.....																
<i>Haematobia</i> spp.....																
<i>Morellia</i> sp.....																
Totals.....	2 571	3 001	2 505	874	727	415	10 093	42	—							

* Flies identified as *M. xanthomelas* prior to December 1973 include some *Musca* n. sp.

** () = infected flies.

TABLE 2 The incidence of 3rd stage *P. bovicola* larvae in 3 *Musca* spp. collected at 6 sites in the Transvaal

Collection dates	Sites*			<i>M. lusoria</i>			" <i>M. xanthomelas</i> "**			<i>M. xanthomelas</i>			<i>Musca</i> n. sp.			Totals		
	No. of flies	No. infected	% infected	No. of flies	No. infected	% infected	No. of flies	No. infected	% infected	No. of flies	No. infected	% infected	No. of flies	No. infected	% infected	No. of flies	No. infected	% infected
November 1972.....	89	8	8,99	83	4	4,82	—	—	—	—	—	—	—	—	172	12	6,98	
July 1973.....	152	0	0	39	0	0	—	—	—	—	—	—	—	—	191	0	0	
August.....	174	0	0	73	1	1,37	—	—	—	—	—	—	—	—	247	1	0,40	
September.....	778	6	0,77	204	3	1,47	—	—	—	—	—	—	—	—	982	9	0,92	
October.....	476	3	0,63	99	0	0	—	—	—	—	—	—	—	—	575	3	0,52	
November.....	442	5	0,68	240	0	0	—	—	—	—	—	—	—	—	682	3	0,44	
December.....	520	5	0,96	101	0	0	—	—	—	—	—	—	—	—	645	5	0,76	
January 1974.....	470	4	0,85	22	0	0	—	—	—	—	—	—	—	—	621	4	0,64	
February.....	590	4	0,68	—	—	—	—	—	—	—	—	—	—	—	113	4	0,54	
March.....	288	0	0	—	—	—	—	—	—	—	—	—	—	—	175	1	0,57	
April.....	368	0	0	—	—	—	—	—	—	—	—	—	—	—	131	0	0	
Totals.....	4 347	33	0,76	861	8	0,93	256	0	0	461	1	0,22	5 925	42	0,71			

* Sites: M = Mara Research Station, Z = Zoutpan Research Station, L = Leamington, D = Doornpan, Mp = Mooiplaats, S = Syferfontein

** Flies identified as *M. xanthomelas* prior to January, 1974, include some *Musca* n.sp.

TABLE 3 Laboratory infection of *Musca* species with *P. bovicola* 22.3.1974-5.4.1974

Musca species	Fly group	No. of flies alive on:			No. of flies infected on Day 14	% live flies infected	3rd stage larvae in head		3rd stage larvae in abdomen		3rd stage larvae in head and/or abdomen*	
		Day 0	Day 14	Day 14			Total	Range	Total	Range	Total	Range
<i>M. lusoria</i>	Test.....	24	0	13	0	7	0	57	1	1	0	0
	Control.....	15	5	7	2	0	0	0	0	0	0	0
<i>M. xanthomelas</i>	Test.....	2	2	2	1	1	0	9	0	0	0	0
	Control.....	2	1	2	1	0	0	0	0	0	0	0
<i>Musca</i> n. sp.	Test.....	6	0	5	0	2	0	0	0	0	33	16-17
	Control.....	7	0	5	0	0	0	0	0	0	0	0
<i>M. sorbens</i>	Test.....	10	2	10	2	0	0	0	0	0	0	0
	Control.....	5	0	3	0	0	0	0	0	0	0	0
<i>M. domestica</i>	Test.....	2	1	2	0	0	0	0	0	0	0	0
	Control.....	8	1	1	1	0	0	0	0	0	0	0
Totals.....	Test.....	44	5	32	3	10	0	66	1	1	33	16-17
	Control.....	37	7	18	4	0	0	0	0	0	0	0

* The heads and abdomens of some flies were not examined separately

Third stage larvae were found in 33/4347 *M. lusoria*, 8/861 "*M. xanthomelas*" and 1/461 *Musca* n. sp. (see also Table 1). The infection rates for *M. lusoria* and "*M. xanthomelas*" in November 1972 were extremely high, 8.99% and 4.82% respectively, when compared with the 1973/74 summer season. This could be due either to seasonal differences or simply to the different collecting method in use in 1972, when flies were caught individually in test tubes directly off the cattle.

The infection rate of *M. lusoria* in 1973/74 was always less than 1%, infected flies being collected from September to February. The infection rate in "*M. xanthomelas*" was a little over 1% and was confined to August and September. Only 1 *Musca* n. sp. was infected (March, 1974). It is noteworthy that only female flies were infected.

The mean number of 3rd stage larvae recovered from field-collected flies was 4.7. The majority of these larvae were found in the heads of flies, up to 43 occurring in a single head.

Laboratory infection of flies with *P. bovicola*

The results of this experiment appear in Table 3. On Day 14 3rd stage *P. bovicola* larvae were recovered from the females of 3 of the 5 *Musca* species used, all belonging to the subgenus *Eumusca*, viz. *M. lusoria*, *M. xanthomelas* and the new *Musca* sp. No larvae were recovered from flies which died before Day 14.

The high percentage infection in these flies (40.0%–52.8%) as well as the complete absence of *P. bovicola* in the control flies, is strong evidence that they were successfully infected artificially. The species infected agree with the results of the field surveys (Tables 1 & 2).

Again most larvae were recovered from the heads, especially in *M. lusoria*, where only 1 was found in the abdomen. In the new *Musca* sp., however, 3rd stage larvae were found in the head and abdomen and, in 1 specimen, also in the thorax. A total of 100 worms was recovered from 10 flies, a maximum of 21 worms being recovered from 1 fly.

Identification of 3rd Stage larvae of *P. bovicola*

Live 3rd stage larvae are sluggish and inclined to coil in saline whereas *Thelazia* larvae which are similar, make sinuous swimming movements. They have a large conspicuous greenish-brown spot near the anal opening and a finely striated surface, in contrast to the marked serrations on *Thelazia* larvae. These characters enable one to recognize the live larvae and distinguish them from other 3rd stage larvae that may be found in flies. Heat-killed larvae nearly always lose the dark anal spot but they can be differentiated quickly from other larvae by the shape of their anterior and posterior ends, their finely striated surface and their size (Fig. 5–6).

A comparison of the measurements of field-collected and laboratory-reared 3rd stage *P. bovicola* larvae and those of *P. multipapillosa*, as recorded by Gnedina & Osipov (1960) is given in Table 4.

The measurements of laboratory and field specimens agreed closely for 4 of the 5 criteria. Field specimens were, however, generally longer than their laboratory counterparts, though the ranges of the 2 groups

overlapped. The greater mean length of the field specimens can possibly be ascribed to the fact that there were usually fewer larvae per fly. This latter explanation is supported by the fact that the 43 larvae recovered from the head of a heavily infected field-collected fly were stunted, their lengths ranging from 2.2–2.9 mm.

The measurements of the 2 *Parafilaria* spp. were similar, with *P. multipapillosa* being slightly smaller except for the anal opening which is situated at the same distance from the caudal end in both species.

The general agreement of the measurements in Table 4 thus confirms the earlier tentative identification of 3rd stage *P. bovicola* larvae in field-collected flies.

DISCUSSION

The infective 3rd stage larvae of *P. bovicola* have been recovered from both field-collected and laboratory-infected females of 3 dung-breeding *Musca* species, i.e. *M. lusoria*, *M. xanthomelas* and a new species closely resembling *M. xanthomelas*. All 3 species belong to the subgenus *Eumusca*. This relationship is particularly interesting as it suggests the presence of a common factor enabling these flies to become infected and development of the larvae to proceed to the infective stage. This factor may be either physiological or associated with their feeding habits.

Although larval development can take place in these flies their ability to transmit the infective worms has still to be proved. Osipov (1962) has shown that the developing larvae of *P. multipapillosa* migrate under the skin after their transmission to horses by *H. atripalpis*. It is therefore likely that the larvae of *P. bovicola* also migrate from their point of entry to the various sites on the body where bleeding eventually occurs. Since the 3 fly species found carrying *P. bovicola* were observed to feed mainly on the lachrymal secretions of cattle there is a strong possibility that the majority of infective larvae enter the final host via the orbital route. These flies also feed on the nose, wounds, abscesses (Nevill, unpublished observations, 1974), and on the sites of punctures made by biting flies (Patton, 1932). Transmission could therefore also be associated with periods of biting fly abundance. The different possible routes of infection are being investigated.

At Zoutpan Research Station more than 25% of a herd of 40 1-year-old heifers regularly had *Parafilaria* bleeding spots between July 1973 and the end of December 1973. From January to April 1974 this figure dropped rapidly to 10% and less. Of the flies collected during the summer season of 1973/74 the infection rate with *P. bovicola* was highest between August 1973 and February 1974 (Table 2). This period coincides roughly with the period when bleeding spots are commonest and must be taken to be the main period for *P. bovicola* transmission.

Calves born during this period will be exposed to infected flies and are likely to become infected almost immediately. The first bleeding spots on these calves occur between 7 and 10 months later. This can therefore be regarded as the approximate period required for female *P. bovicola* to develop to maturity in cattle. It agrees closely with the 281 days required by *P. multipapillosa* in horses (Osipov, 1962).

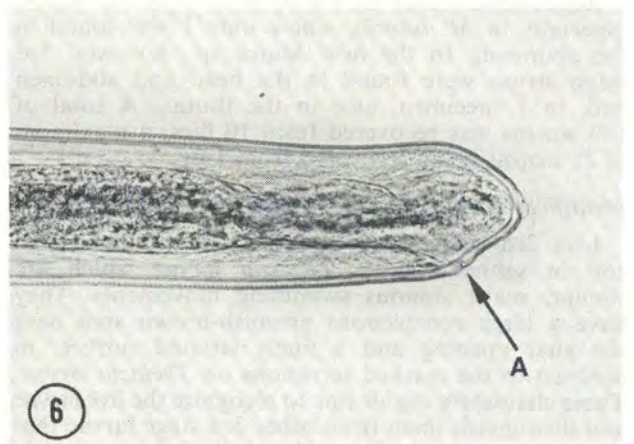
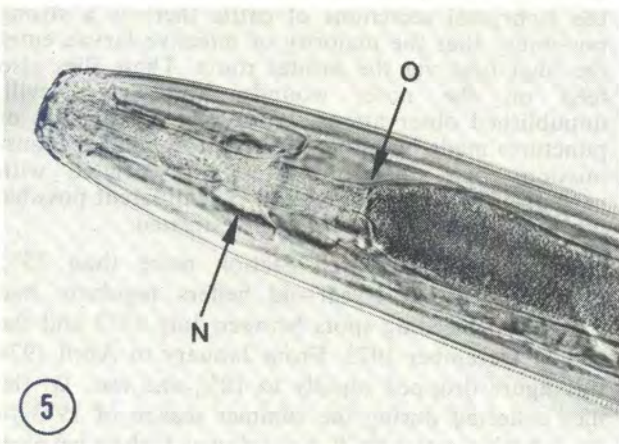
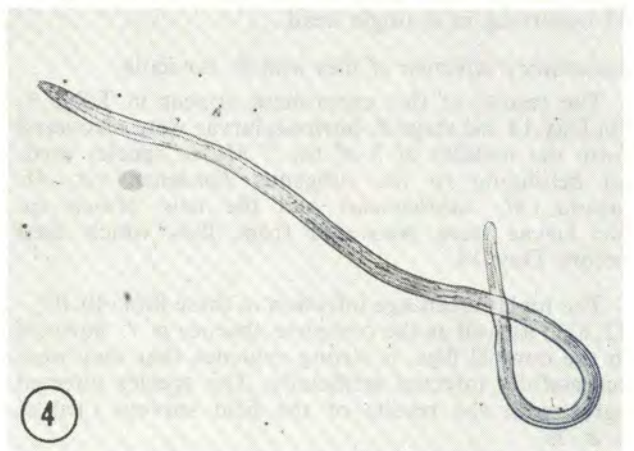
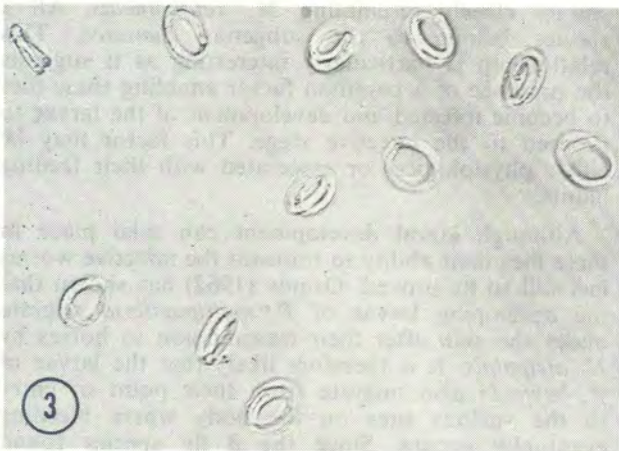
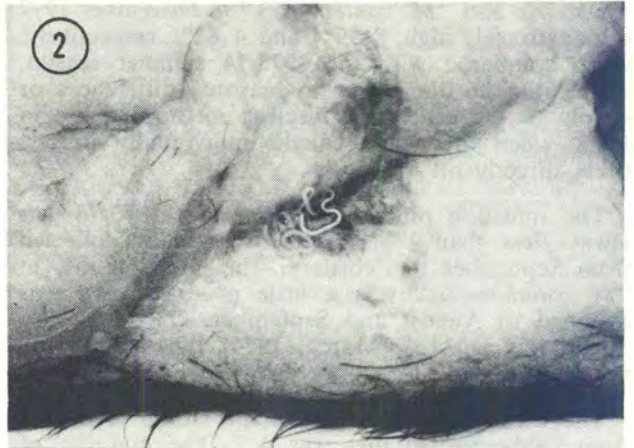
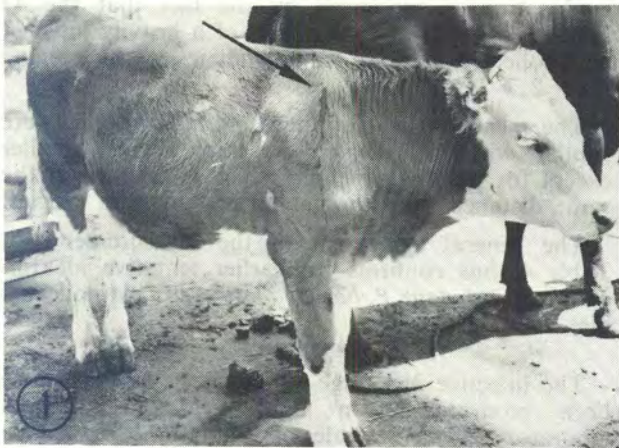


FIG. 1 Blood streak on shoulder of 10-month-old ox caused by *Parafilaria bovicola* female below the skin
 FIG. 2 Gravid female *P. bovicola* (± 50 mm long) in subcutis of ox directly below blood streak
 FIG. 3 Embryonated eggs of *P. bovicola*. $\times 200$
 FIG. 4 Third stage infective *P. bovicola* larva (± 4 mm long) from the head of a fly
 FIG. 5 Anterior end of 3rd stage *P. bovicola* larva. $\times 320$. N = nerve ring, O = end of oesophagus
 FIG. 6 Posterior end of 3rd stage *P. bovicola* larva. $\times 320$. A = anal opening

*TABLE 4 Measurements of 3rd stage larvae of 2 *Parafilaria* species in mm

Criteria measured	<i>P. bovicola</i>			<i>P. multipapillosa</i>
	No. of specimens	Mean	Range	(Gnedina & Osipov, 1960)
Length.....	Lab.*.....	14	2,950	1,670-2,670
	Field**.....	27	3,544	
Width at end of oesophagus.....	Lab.....	14	0,059	0,033-0,050
	Field.....	26	0,060	
Length of oesophagus.....	Lab.....	12	0,113	0,088-0,096
	Field.....	26	0,113	
Distance of nerve ring from cephalic end..	Lab.....	11	0,060	0,040-0,050
	Field.....	23	0,060	
Distance of anal opening from caudal end..	Lab.....	8	0,021	0,021-0,025
	Field.....	16	0,021	
Depth of buccal cavity.....	Lab.....	0	—	—
	Field.....	9	0,008	

* 3rd stage larvae recovered from flies infected artificially in the laboratory.

** 3rd stage larvae recovered from field-collected flies.

Gnedina & Osipov (1960) and Osipov (1962) showed that *P. multipapillosa* could develop in and be transmitted by *H. atripalpis*. However, very few *Haematobia* spp. or their close relatives were encountered during the 1973-74 summer season in those parts of the Transvaal where *P. bovicola* is enzootic. *M. crassirostris* and *M. conducens*, both of which possess prestomal teeth and are able to scratch the skin and draw blood, were also scarce. Theoretically *Haematobia* spp. and these 2 species of *Musca* would all be ideal transmitters of *P. bovicola* since they would be able to lap up infected blood and later infect animals through their own feeding wounds.

The 1973/74 summer season was an unusually wet one and this may account for the comparative absence of these flies and for good collections of *Stomoxys*. It has already been noted that *M. crassirostris* was a pest during the previous hot dry summer so it is quite possible that other fly species could have been similarly adversely affected by the abnormally wet season. Further collections may confirm this observation.

The exact distribution of *P. bovicola* in southern Africa has still to be determined. Since the greater part of its life is spent inside a warm-blooded animal it is unlikely that this stage of the life-cycle will be affected by changes in climate. The factors that will limit its distribution will be climatic ones directly affecting the survival and/or incidence of the vectors.

An understanding of the biology of the fly vectors may suggest measures which can be used to reduce their number sufficiently to reduce or eliminate transmission.

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