

## THE EXPERIMENTAL TRANSMISSION OF *PARAFILARIA BOVICOLA* TO CATTLE IN SOUTH AFRICA USING *MUSCA* SPECIES (SUBGENUS *EUMUSCA*) AS INTERMEDIATE HOSTS

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### ABSTRACT

NEVILL, E. M., 1979. The experimental transmission of *Parafilaria bovicola* to cattle in South Africa using *Musca* species (subgenus *Eumusca*) as intermediate hosts. *Onderstepoort Journal of Veterinary Research*, 46, 51-57 (1979).

In controlled experiments in an insect-free stable, cattle became infected with *Parafilaria bovicola* when *Musca lusoria*, infected with the larvae of this worm, were allowed to feed on a fresh skin incision, and when infective larvae were placed on fresh skin incisions, injected subcutaneously or into the jugular vein, or instilled into the eyes. The sites of blood spots caused by ovipositing *P. bovicola* females and the sites of carcass lesions were seldom close to the site of infection, an indication that the worms had migrated. The prepatent period of *P. bovicola* in 4 cattle which developed blood spots ranged from 242-319 days.

Neither of the infected cattle that were kept continuously in a shady stable showed blood spots, but 4 out of 7 infected cattle which spent some time in the sun bled. However, carcass lesions on shaded cattle were similar in appearance to those on cattle kept outdoors.

Infective larvae were stimulated to escape from the mouth-parts of infected *M. lusoria* and *Musca xanthomelas* s.s. when these were fed citrated ox blood warmed to 38-40 °C. No escape took place when the flies were fed warmed saline or warmed 15% sucrose solution.

### Résumé

#### TRANSMISSION EXPÉRIMENTALE DE PARAFILARIA BOVICOLA AU BÉTAIL EN AFRIQUE DU SUD PAR L'INTERMÉDIAIRE DE MOUCHES-HÔTES DU GENRE MUSCA (SOUS-GENRE EUMUSCA)

Au cours d'expériences contrôlées dans une étable exempte d'insectes, du bétail s'est trouvé infesté de *Parafilaria bovicola* lorsqu'on a laissé des *Musca lusoria*, elles-mêmes infestées de larves de ce ver, se nourrir sur une incision cutanée fraîche; et aussi lorsque des larves infestantes ont été soit placées sur des incisions cutanées fraîches, soit injectées sous la peau ou dans la veine jugulaire, soit encore instillées dans les yeux. Les taches de sang causées par l'oviposition des femelles de *P. bovicola* ainsi que les lésions de la carcasse étaient rarement localisées près de l'endroit de l'infestation, ce qui indique que les vers avaient migré. Chez 4 têtes de bétail où des taches de sang se sont formées, la période prépatente de *P. bovicola* s'est étendue de 242 à 319 jours.

Aucun des animaux que l'on avait gardés continuellement dans une étable ombragée n'a montré de taches de sang; mais, des sept qui ont passé quelque temps au soleil, quatre ont saigné. Toutefois les lésions de la carcasse sur le bétail tenu à l'ombre avaient la même apparence que celles observées sur le bétail laissé en plein air.

On a provoqué la sortie de larves infestantes des pièces buccales de *M. lusoria* et *Musca xanthomelas* s.s. en nourrissant ces mouches de sang de boeuf citraté, réchauffé à 38-40 °C. Il n'y a pas eu de sortie quand les insectes buvaient une solution saline chauffée ou une solution chauffée de sucrose à 15%.

### INTRODUCTION

In 1975 Nevill reviewed the world-wide importance and history of *Parafilaria bovicola* and described his studies to find the insect vectors in South Africa. In field surveys and by artificial infection in the laboratory he proved that development to the infective 3rd stage larva could take place in 3 *Musca* species, namely, *M. lusoria*, *M. xanthomelas* s.s. and an undescribed *Musca* species similar to *M. xanthomelas* (*M. xanthomelas* s.l.).

Gnedina & Osipov (1960) showed that *Parafilaria multipapillosa* of horses developed to the 3rd stage in a biting fly, *Haematobia atripalpis*. This fly, however, differs from the *Musca* vectors of *P. bovicola* in that it can also pierce the skin to feed. Osipov (1962) proved that *H. atripalpis* could become infected when it fed on egg-laden blood on the skin of infected horses and that it transmitted the infective larvae to uninfected horses when it bit its hosts. He was unable to infect horses by feeding them infected flies or by injecting infective larvae subcutaneously. Blood spots made by ovipositing female worms were first seen 281 and 387 days respectively after infected flies had fed on the 2 reacting horses. He suggested that after infection the worms migrated in the subcutaneous cellular tissue to nearly all regions of the body.

Webster & Wilkins (1970) noted that bleeding caused by *P. bovicola* commenced when cattle were released from stables after a 6-month quarantine

period. They believed that this observation supported the work on *P. multipapillosa* by Baumann (1946), who claimed that sunlight was necessary to stimulate the females to oviposit.

The aims of the present study were to infect cattle with 3rd stage *P. bovicola* larvae by various routes; to note the migration of the worms; to determine the developmental period from infection with the 3rd stage larvae to egg-laying by the adult worm; to note whether continuous shading would affect oviposition, and to determine whether infective larvae can escape from the proboscis of both *M. lusoria* and *M. xanthomelas* s.s.

### MATERIALS AND METHODS

#### Experimental animals

Details of the experimental design are summarized in Table 1. All infections were performed at the Veterinary Research Institute, Onderstepoort. Three cattle (9496, 9465, 9437), which had already spent 10-12 months in an insect-free stable, were used in a pilot experiment. The remaining 19 animals in the experiment were bought as weaners ( $\pm 8$  months old) on the highveld of the Orange Free State where winters are severe and no *Parafilaria* cases have been recorded.

The cattle were artificially infected and kept for at least 78 days in an insect-free stable. For each route of infection employed 1 animal was stabled for the duration of the experiment in an attempt to determine whether bleeding is dependent on sunlight and if

EXPERIMENTAL TRANSMISSION OF *PARAFILARIA BOVICOLA* USING *MUSCA* SPECIES

 TABLE 1 Transmission of *P. bovicola* to cattle—experimental design

Route of infection	Bovine	Age (months)	Date infected	No. of infective worms	Days cattle held	
					Insect-free stable	Paddock in sun
Intravenous.....	heifer 9496*.....	20	1974-04-05	33	161	528
Intravenous.....	ox 362.....	22	1975-08-26	36	301	0
Intravenous.....	ox 364.....	22	1975-08-22	50	186	117
Intravenous + eye.....	ox 354.....	14	1975-01-03 1975-03-06	8 3**	235 173	181 181
Eye.....	bull 9465*.....	18	1974-02-08 1974-02-21 1974-04-05	31** 3** 33	217 204 161	347 347 347
Eye.....	heifer 369.....	14	1975-01-02 1975-01-09 1975-02-06	50 5** 2**	302 295 267	109 109 109
Eye.....	ox 358.....	16	1975-03-06 1975-08-15 1975-08-22	3** 1** 29	474 312 305	0 0 0
Eye.....	ox 355.....	38	1976-12-23 1977-01-03	92 73	89 78	184 184
Eye.....	bull 359.....	38	1976-12-23 1977-01-03	98 69	89 78	184 184
Nostril.....	ox 356.....	19	1975-05-07 1975-08-22 1975-08-26	15** 42 44	412 305 301	0 0 0
Nostril.....	ox 365.....	14	1975-01-02 1975-02-06	52 17**	309 274	108 108
Sub-cutaneous.....	ox 371.....	14	1975-01-03 1975-02-21	133 4	307 258	109 109
Sub-cutaneous.....	ox 366.....	22	1975-08-22	36	186	117
Sub-cutaneous.....	ox 367.....	23	1975-09-22 1975-09-26	41 22	274 270	0 0
Incision.....	bull 9437*.....	20	1974-04-05	33	161	347
Incision.....	ox 357.....	22	1975-08-25	39	183	118
Incision.....	ox 353.....	22	1975-08-22 1975-10-21	40 50	308 248	0 0
Incision + infected <i>M. lusoria</i> .....	ox 370.....	22	1975-08-25 1975-10-21	—	183 126	118 118
Tabanids + infected flies.....	ox 361.....	24	1975-10-21	—	309	0
Tabanids + infected flies.....	ox 363.....	37	1976-12-07 1977-01-21	—	167 122	122 122
Control.....	ox 360.....	8	—	—	94	0
Control.....	ox 372.....	47	—	—	1 078	123

\* Pilot trial (the 3 cattle used were stabled for 10–12 months prior to attempts at infection).

\*\* Infective worms from wild-caught flies. All other worms used were from flies infected in the laboratory

continuous shade affects the development and/or appearance of carcass lesions. Those animals that were to be exposed to sunlight were later moved to paddocks where they spent between 108 and 184 days, a period far shorter than the 250 day mean prepatent period (Viljoen, 1976). This ensured that the appearance of blood spots (Fig. 1) could be attributed to experimental and not to accidental natural infection.

Two uninfected oxen were kept as controls.

#### Laboratory infection of flies with *P. bovicola*

The method used to infect flies was similar to that described by Nevill (1975). Infective larvae were obtained in 1 of 3 ways. They were dissected either from the heads of naturally infected *M. lusoria*, *M. xanthomelas s.s.* and *M. xanthomelas s.l.* collected at Zoutpan Research Farm, approximately 45 km north of Pretoria, or from flies collected at Zoutpan and subsequently infected in the laboratory, or from a laboratory colony *M. xanthomelas s.s.* which were

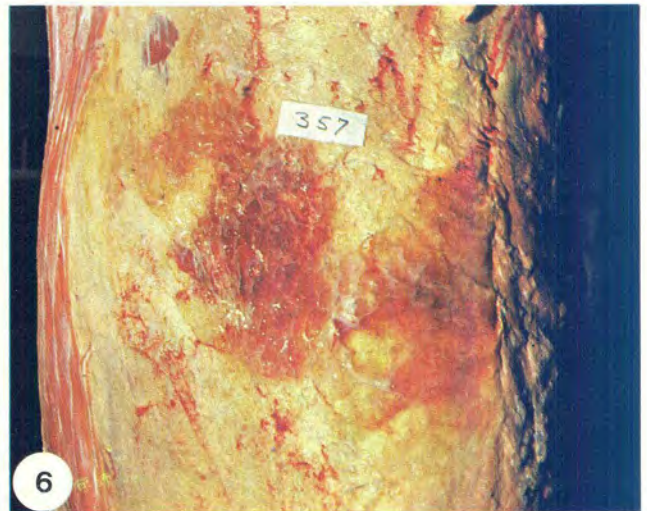
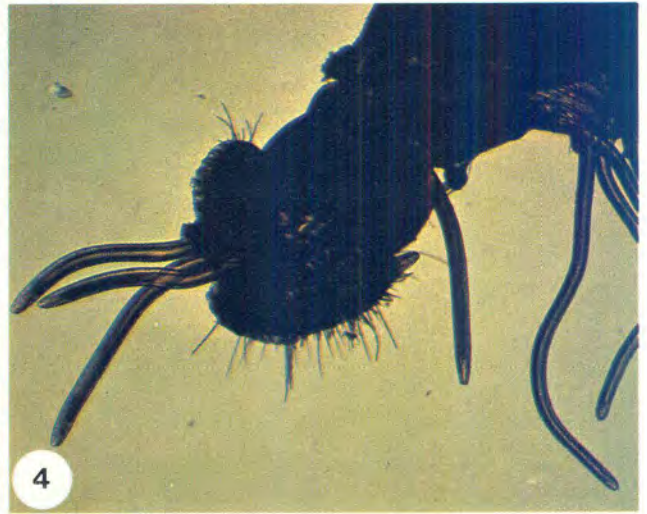


FIG. 1 Blood spot on calf caused by the egg-laying activity of *P. bovicola*  
 FIG. 2 *M. lusoria* ♀ (large fly) feeding on blood on an ox  
 FIG. 3 Hand net collection of vector flies coming to citrated blood on the backs of cattle  
 FIG. 4 Infective 3rd stage *P. bovicola* larvae in the proboscis of *M. lusoria*  
 FIG. 5 Sub-acute carcass lesions on ox 367 infected 270–274 days previously by subcutaneous injection of infective *P. bovicola* larvae  
 FIG. 6 Sub-acute carcass lesion on ox 357 infected 301 days previously by introducing infective *P. bovicola* larvae into fresh skin incisions

infected in the laboratory. Although a colony of *M. lusoria* was established, it did not flourish and there were seldom sufficient flies available for infection on any given date (Fig. 2, 3 & 4).

The flies were held at 27 °C and  $\pm 60\%$  R.H. in a cage 300 mm  $\times$  240 mm  $\times$  200 mm covered with plastic gauze and were infected by drops of blood containing eggs of *P. bovicola* placed on the gauze top. Prior to infection, blood, milk powder and dung were withheld from the flies for 22 h, sugar for 6 h and water for 2 h. After infection, they received a daily diet of non-refined brown sugar, milk powder, fresh cow-dung, citrated ox blood (0.3% sodium citrate) and water.

#### *Infection of experimental cattle*

(i) Infective 3rd stage larvae dissected from the heads of vector flies (Fig. 4) were held before use for up to 2 h at room temperature in a petri dish containing 0.85% saline.

For infections of the eye, nostril and fresh skin incisions, the larvae were counted, then concentrated in  $\pm 1$  ml 0.85% saline and drawn into a Pasteur pipette. The experimental cattle were held in a crush in an insect-free stable and larvae were instilled inside the eye-lids, into the nostrils or into fresh incisions made with a scalpel on the dorsal aspect of the loins.

(ii) For subcutaneous and intravenous infections the larvae were held in  $\pm 1$  ml saline in a syringe held vertically point down, to concentrate them near the tip of the syringe. To infect intravenously, a 14 gauge needle was inserted into the jugular vein, the syringe attached, some blood drawn up and then the contents injected. A similar method was used for subcutaneous infections except that the worms were injected downwards under the skin of the neck or loins immediately the needle was attached to the syringe. After withdrawal of the needle fresh saline was taken up to check if any worms remained in the syringe and if so these were also injected.

(iii) Where starved, infected *M. lusoria* were allowed to feed on fresh scalpel incisions on the dorsal loins, they were confined in an open-bottomed cage over the wounds. When they had fed, the bottom of the cage was closed and they were dissected to recover infective larvae from their heads and to determine whether any fresh blood was present in their stomachs, which would indicate that they had fed. A control group of unfed, infected flies from the same batch was also dissected to determine the percentage infected and the mean number of worms per fly head. If these were much higher than in the fed group, then there was good reason to believe that larvae had left the test flies and entered the wounds. If not, the infection was repeated.

(iv) Transmission of *P. bovicola* could possibly occur when vector flies feed on wounds caused by tabanid bites. To test this theory tabanid flies were collected near a water-course at Zoutpan. They came to rest on a large piece of hessian draped over a slowly moving microbus, from which they were collected individually in glass test tubes, transferred to a cloth cage and given a sugar-water pad. The cage was placed in a styrofoam cooler box kept cool by means of a freezer pack and transported to the laboratory. Most of the tabanids belonged to the genus *Haematopota*, but species of *Tabanus*, *Atylotus* and *Chrysops* were also collected and used in the experiments.

At 27 °C and  $\pm 60\%$  R.H. the tabanids lived for 15 days or longer when fed brown sugar crystals and water. In an attempt to infect oxen via tabanid

feeding wounds, starved tabanids and *Musca* vectors that were thought to be infected were placed together in open-bottomed cages on the dorsal surface of the loins of experimental oxen. Most of the tabanids fed, thus creating wounds. Since these infection attempts were not entirely satisfactory, they were discontinued after 1 or 2 months.

#### *Clinical diagnosis of parafilaria*

For the duration of the experiment the cattle were examined almost daily for the presence of blood spots (Fig. 1). When these were present, the date and position of the spots were recorded on an outline diagram of an ox and the blood examined as follows for the presence of worm eggs:

A sample of hair with either dry or wet blood was clipped off and placed in a few millilitres of water in a small petri dish to lyse the blood cells. After about 15 min the hair was removed and the dish contents examined for embryonated eggs, using a dissecting microscope at 50 or more magnifications with good substage lighting. When the sample was positive, isolated small embryonated eggs were found lying on the bottom of the dish.

#### *Carcass examination for parafilaria*

In most cases the experimental cattle were slaughtered 9–10 months after infection and their carcasses carefully examined for *Parafilaria* lesions (Fig. 5 & 6) and for dead or living worms.

Although lesions may be confused with bruising, they are usually wet, slimy or jelly-like, and contain large numbers of eosinophils. They were placed in the following categories: acute (light yellow); sub-acute (yellow-brown to greenish) (Fig. 5 & 6) and chronic (no obvious lesion but a well-developed covering of connective tissue and slight surface sliminess) (Table 2).

The shape, size and description of each lesion were recorded on the same outline diagram of an ox used for recording bleeding points. For easier comparisons and evaluation the sites of infection, blood spots and lesions were later transferred to a diagrammatic outline of an ox hide (Fig. 7).

#### *Stimulus for infective larvae to escape from the head of a fly*

Tests were conducted to determine the stimulus required to activate 3rd stage infective larvae to escape from the mouth-parts of infected flies (Fig. 4).

Infected, hungry *M. lusoria* were placed in two 250 ml cardboard cups covered with fine gauze. A glass test tube, 75 mm long and 10 mm in diameter filled with citrated ox blood, was fitted into a hole in the bottom of each cup with the top 3 mm projecting into the cup. One tube was kept at room temperature ( $\pm 22$  °C) while the protruding part of the tube below the 2nd cup was heated in a waterbath to 38–40 °C.

After the flies had fed on the blood they were dissected and the number of 3rd stage larvae in their heads was recorded. The blood in the tubes was mixed with water in a large petri dish to lyse the red blood cells, and 3rd stage larvae, when present, were removed and counted.

In later tests both infected *M. lusoria* and *M. xanthomelas s.s.* were fed blood at 38–40 °C to note whether infective worms are capable of leaving the heads of both fly species (Table 4).

To investigate the significance of warmth, infected flies were also fed 0.85% saline or 15% sucrose solution heated to 38–40 °C.

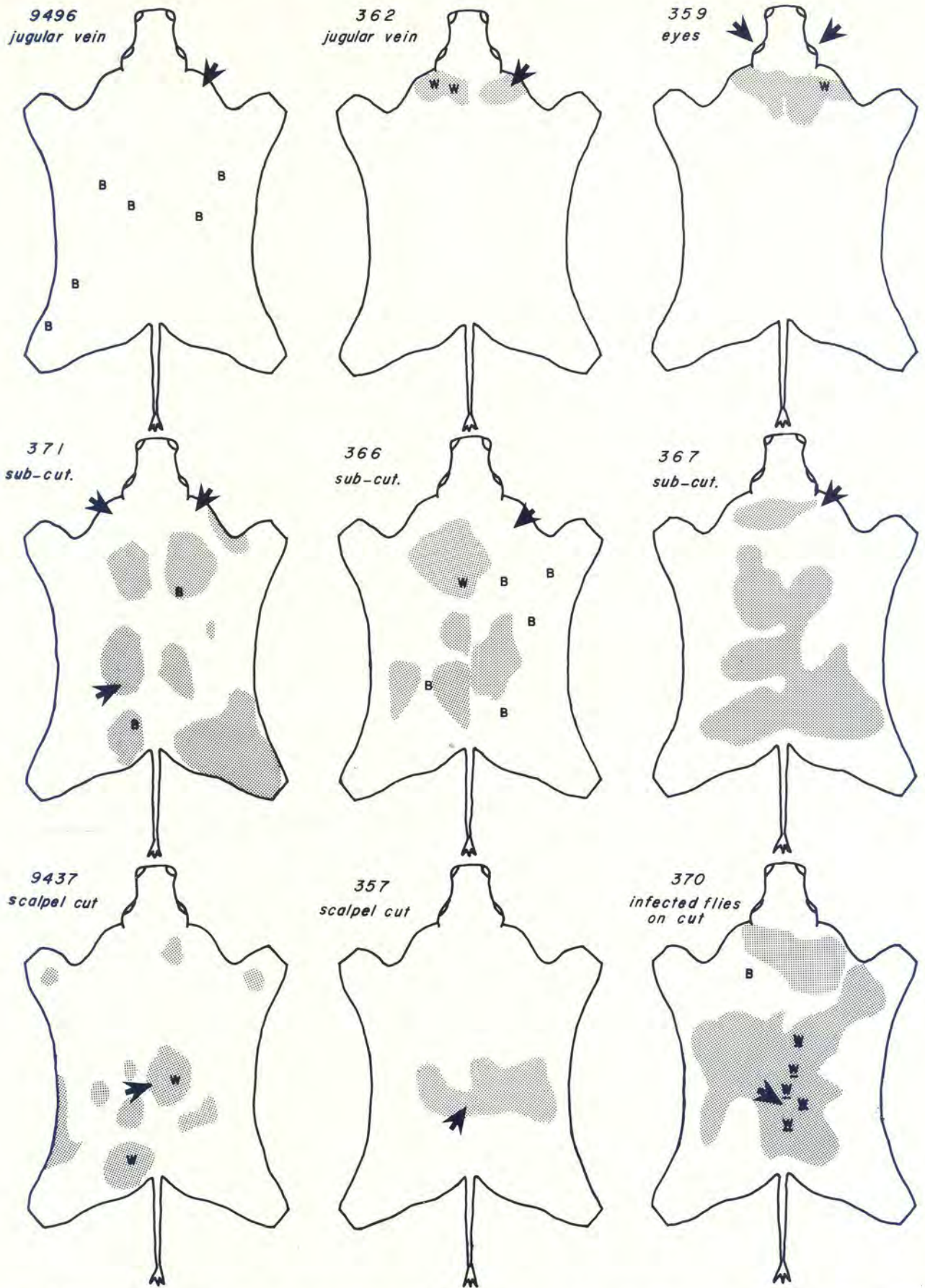


FIG. 7 Diagram of hides of experimental cattle infected with *P. bovicola* 3rd stage larvae via different routes to indicate sites of infection (arrows), blood spots (B), live worms in sub-cutis (W), dead worms (W) and carcass lesions (shaded areas)

## RESULTS AND DISCUSSION

Details of successful experimental transmissions are given in Table 2 and the sites of infection, lesions and adult worms are indicated diagrammatically in Fig. 7.

*Infection of experimental cattle*

Cattle were successfully infected via the following routes:

- (i) intravenous (jugular vein)..... 2/3 positive
- (ii) eye..... 1/5 positive
- (iii) subcutaneous (Fig. 5)..... 3/3 positive
- (iv) skin incision (cut) (Fig. 6)..... 2/3 positive
- (v) cut + infected *M. lusoria*..... 1/1 positive

Owing to the absence of both bleeding points and worms and the presence of doubtful changes on the carcass surface, the final result of infection attempts in 3 experimental cattle was uncertain. The carcass of ox 364, infected via the jugular vein, had a dull bruise-like appearance and many petechiae over the whole surface. This may have been due to repeated battering by other oxen with which it was kept. Ox 354, infected with small numbers of infective larvae through the eye and intravenously, appeared to have chronic carcass lesions covering the entire dorsal half of the body. The lesion area was vague but contained a greater number of capillaries and exhibited some superficial sliminess. Ox 358, infected via the eye, had a small yellow lesion about 100 mm in diameter on the right rib area, which was positive for eosinophils. This seemed to suggest parafilaria, so a further 2 cattle were infected via the eye, one of which became positive.

The comparative difficulty in infecting the experimental cattle via the eyes may be due to the technique used and even to the age of the animals. A high percentage of calves (90% at Zoutpan) became infected within 3 months or so after birth and it is difficult to believe that this all took place via wounds. The numerous vector flies present around cattle in the veld usually feed around the eyes and muzzle and a more plausible explanation is that they were infected via the eyes.

Attempts to infect oxen via tabanid bites by allowing the tabanids and *Musca* vectors to feed simultaneously, were unsuccessful. Although the *Musca* spp. sometimes fed on these wounds, the flies either had difficulty in feeding through long hair, or, as often happened, the ox reacted violently to being bitten and disturbed the cage of flies, so that flies escaped or were damaged, or else the flies were later found to come from a group in which very few

were infected. It was extremely difficult to perfect this technique even after many attempts, including one in which the animal was anaesthetized. Despite these unsatisfactory results there appears to be every possibility of infection being transmitted via tabanid bites since, in another test, infected *M. lusoria* transmitted *P. bovicola* to an ox (No. 370) after feeding on a fresh cut.

Cattle could not be infected via the nostrils.

The 2 control oxen were negative.

*The effect of continuous shading on the development and oviposition of P. bovicola*

The results of this aspect of the study are summarized in Table 2. Seven of the 9 positive cattle spent from 109–347 days in exposed paddocks, but blood spots were seen in only 4 of these. The remaining 2 positive cattle spent the entire experimental period in the insect-free stable. Blood spots were never seen on them during this period but on slaughter they were found to have typical sub-acute lesions and a female worm on 1 carcass contained embryonated eggs. This shows that normal development to the gravid female can take place in a shaded stable. It is possible that oviposition did take place under these conditions and that small blood spots under the hair went unnoticed.

The conclusion of Baumann (1946) and Webster & Wilkins (1970) regarding the importance of sunlight is supported by the present study, which includes 4 years of field observations, when bleeding spots were often recorded during the hottest part of the day. Also the proven vectors are only active in the day and this suggests that heat and/or light are necessary to stimulate the female *Parafilaria* to oviposit.

*Worm movement from site of infection*

In Fig. 7 the site of infection, bleeding points and carcass lesions are indicated for the 9 infected cattle. In many cases, particularly those infected subcutaneously (371, 366, 367) and heifer 9496 infected via the jugular vein, bleeding points and lesions were scattered over the body, often some distance from the site of infection. However, in all 3 cattle infected via a cut, namely, 9437, 357 and 370 lesions were also present at the infection site. At some stage, therefore, worm migration may take place, and this is in agreement with the finding by Osipov (1962) with *P. multipapillosa*. He was, however, unable to infect horses subcutaneously, though this was the most successful route for *P. bovicola*.

TABLE 2 Experimental infection of cattle with *P. bovicola*—the successful routes of infection and the effect of continuous shading on the development of blood spots and carcass lesions

Bovine	Route of infection	No. of days in sun	Blood spots	Days from infection to slaughter	Carcass lesions	Worms in lesions
9496.....	Intravenous.....	>341	+		(Not slaughtered)	
362.....	Intravenous.....	0	—	301	sub-acute	1♂ 1♀ (eggs)
359.....	Eye.....	184	—	262–273	sub-acute	1♀ (no eggs)
371.....	sub-cutaneous.....	109	+	367–416	chronic	0
366.....	sub-cutaneous.....	117	+	303	sub-acute	1♀ (eggs)
367.....	sub-cutaneous.....	0	—	270–274	sub-acute	0
9437.....	Incision.....	347	—	508	sub-acute	2♀♀ (eggs)
357.....	Incision.....	118	—	301	sub-acute	0
370.....	Incision + infected <i>M. lusoria</i>	118	+	244–301	sub-acute	5 (encapsulated)

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The successful infection via the jugular vein indicates that 3rd stage larvae may migrate via the blood stream and also that they can escape from the circulatory system to reach the subcutis.

*Prepatent period and time of infection*

The date of infection and the period to the appearance of the first blood spots are shown in Table 3.

The first blood spots appeared after 242–270 or possibly 319 days, depending on which infection was responsible for the first blood spot in ox 371. This can be assumed to be the prepatent period for *P. bovicola* and supports the 250 day mean prepatent period which Viljoen (1976) calculated for a group of 60 calves assumed to have been infected at birth. Osipov's (1962) 2 positive experimental infections in horses with *P. multipapillosa* bled after 281 and 387 days, a period similar to that found for *P. bovicola*.

Since *P. bovicola* blood spots are seasonal, it was thought that this might affect the length of the prepatent period if an animal were infected outside this period. The results of the experimental infections, however, show an almost identical prepatent period of 242 and 243 days for 2 animals infected in April and August respectively (Table 3).

*Laboratory infection of flies with P. bovicola*

In the laboratory a high percentage of male vector flies became infected, but no infected males were found amongst the field-collected flies (Nevill, unpublished data). Since, however, males accounted for only 8% of the vector flies collected from cattle over a 4-year period at Zoutpan (Nevill, unpublished data), their chances of becoming infected were apparently relatively small.

For the development of healthy infective larvae it is essential that their fly hosts receive a comprehensive diet. Earlier infection attempts, using male and female flies fed on sugar and water alone, resulted in a nil or low infection rate and infective larvae that were small,

weak and difficult to handle without injury. Thus the diet of the male fly in the veld will also influence its ability to act as a vector.

Gnedina & Osipov (1960), working with *P. multipapillosa*, fed experimentally-infected *H. atripalpis* on horse blood every 24 h, with sugar syrup in between, but failed to recover infective larvae from male flies. This could have been accidental but may possibly be connected with the diet or feeding habits of the male of this fly.

Harris, Miller & Frazar (1974), who studied the feeding activity of *Haematobia irritans*, found that this species fed more than 24 times a day and digested the contents of the midgut within 1–2 h. Females took 1.5 times as many blood meals as males, and the female feeding time per day was 1.7 times longer than that of the male.

*Stimulus for infective larvae to escape from the head of a fly*

Infective larvae escaped from the mouth-parts of both *M. lusoria* and *M. xanthomelas s.s.* when these fed on citrated ox blood warmed to 38–40 °C (Table 4), but never into blood at room temperature ( $\pm 22$  °C). In *M. lusoria* up to 65.1% of the worms present in the heads escaped into the blood compared with 41.7% in *M. xanthomelas s.s.*

The results are important since they show that in both species worm development can take place and the infective larvae are physically capable of leaving the mouth-parts and entering the definitive host.

The exact nature of the stimulus causing larval escape is unknown. Body heat would appear to be important but it is not the only factor, since worms did not escape into warm 15% sucrose solution or warm 0.85% saline when the flies were fed on these. Possibly certain proteins must also be present in the meal to stimulate larvae to escape.

TABLE 3 Experimental infection of cattle with *P. bovicola*—time of infection and prepatent period as indicated by the appearance of blood spots

Bovine	Date infected	No. of infective larvae	No. of blood spots	Days to first and last blood spots
9496.....	1974-04-05	33	6	242–341
371.....	1975-01-03	133	2	{ 319–364 270–315
	1975-02-21	4		
366.....	1975-08-22	36	5	243–291
370.....	1975-08-25	unknown (fly-transmitted)	1	{ 262 205
	1975-10-21	unknown (fly-transmitted)		

TABLE 4 The active escape of third stage *P. bovicola* larvae from the heads of infected flies into citrated ox blood kept at 38–40 °C

<i>Musca</i> spp.	No. of flies	No. of larvae		Available larvae escaping into blood (%)
		In blood	In fly heads	
<i>M. lusoria</i> .....	12	27	31	46.6
	13	153	82	65.1
<i>M. xanthomelas s.s.</i> .....	21	9	22	29.0
	54	123	172	41.7

## CONCLUSION

The foregoing experiments have proved that the infective stage of *P. bovicola* is transmitted to cattle when infected *M. lusoria* and possibly *M. xanthomelas s.s.* and *M. xanthomelas s.l.* feed on wounds. Fresh wounds on the skin of cattle can be caused in various ways, including bites by various tabanids and blood spots produced by ovipositing *Parafilaria* females. It was also possible to infect cattle by instilling 3rd stage larvae into the fluid surrounding the eye.

To reduce or eliminate transmission it is necessary to control the vectors or kill the worms in the cattle. Control of Tabanidae might also reduce the likelihood of transmission. Unfortunately highly effective insecticides with a long residual effect against flies are lacking, and no effective practical means of controlling Tabanidae is available locally. Parafilariasis is also only 1 of the many problems which face the cattle rancher, so regular practices aimed at fly control would be unpractical unless they were combined with existing tick control programmes.

The only practical solution appears to be the control of 1 or more of the developmental stages of *P. bovicola* in cattle. Viljoen & Boomker (1977) found that levamisole hydrochloride and nitroxylin reduced lesion areas by 76% and 93% respectively. Since nitroxylin has recently been registered for use against *Parafilaria* in South Africa, its strategic use should make it possible to interrupt transmission so that no worms reach sexual maturity.

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