Onderstepoort J. vet. Res., 49, 29–40 (1982)

STUDIES ON *PARAFILARIA BOVICOLA* TUBANGUI, 1934
III. PATHOLOGICAL CHANGES IN INFESTED CALVES

J. H. VILJOEN[1] and J. A. W. COETZER[2]

ABSTRACT

VILJOEN, J. H. & COETZER, J. A. W., 1982. Studies on *Parafilaria bovicola* Tubangui, 1934. III. Pathological changes in infested calves. *Onderstepoort Journal of Veterinary Research*, 49, 29–40 (1982).

More lesions were found in the carcass of an animal that has been naturally infested with *Parafilaria bovicola* than in one artificially infested with a single subcutaneous injection of infective larvae of this species. This may be because natural infestations are either more frequent or more successful. Similarities in the distribution of lesions in naturally and experimentally infested animals suggest that certain predilection sites may be used by the intermediate fly hosts. Subcutaneous areas infiltrated with eosinophils are more conspicuous during the first 20 days after infestation and during the patent phase of the life cycle of *P. bovicola*.

Yellowish discolorations caused by oedema are usually present in all lesions. When these are combined with eosinophil infiltrations, the lesions become yellowish-green. After the appearance of bleeding spots the green colour of lesions is dominated by the appearance of a brown pigment (haemosiderin) in numerous macrophages. The histopathological changes in the dermis, subcutis and superficial muscles bordering the affected areas are described.

INTRODUCTION

Both the macroscopic and the microscopic lesions caused by *Parafilaria bovicola* in the subcutis of infested animals were described by Pienaar & Van den Heever (1964). These lesions include parasitic granulomas and tracts associated with the movements of the worms. In their account of the pathological changes of lesions in animals successfully treated with anthelmintics after the appearance of bleeding spots, Viljoen & Boomker (1977) emphasized the changing nature of the cellular reactions during the healing process. Examples of these are the development of areolar connective tissue and the degree of mineralization seen in parasitic granulomas.

Nevill (1979) divided the macroscopic lesions in cattle into 3 categories: acute (light yellow), sub-acute (yellowish-brown to greenish) and chronic (with connective tissue covering the lesions). These lesions had been caused by adult worms in the animals, most of which had been slaughtered after the appearance of bleeding spots. There are no references in his report to lesions caused by the larval stages of *P. bovicola*, and few data on the microscopic changes that develop in the subcutis during the aging of the lesions caused by adult worms.

The aim of the present trial was therefore to compare the lesions found during the developmental period (prepatent period) with those found during patency, with the object of estimating the age of these lesions by their macroscopic and microscopic appearance.

MATERIALS AND METHODS

Experimental animals for pathological evaluation

A total of 39 crossbred Afrikaner cattle were slaughtered during the period 23 July 1974–27 November 1979 for studying the parasitic life cycle and pathogenesis of *P. bovicola*.

Thirty calves, 28 from the experimental farm at the Veterinary Research Institute, Onderstepoort, and 2 from the Highveld region, were infested artificially by subcutaneous injection of infective larvae of *P. bovicola* and then killed at various intervals up to 240 days after infestation to cover the mean developmental period of 250 days (Viljoen, 1976). Tissues from 19 of these calves were collected for histopathological examination to determine the changes associated with lesions of varying ages caused by the developing worms. In 13 of the calves the lesions were simultaneously evaluated for changes in colour or appearance.

A light infestation of *P. bovicola* was diagnosed in animals kept at the Onderstepoort experimental farm during October 1978, but no positive cases were seen in cattle at the Veterinary Research Institute itself either before or during the experimental period. In an attempt to obviate any possibility of natural infestations, all calves that had been artificially infested for periods of more than 3 days were transferred from the experimental farm to the Institute when they were only 1–3 days old, usually during the period March–August when fly populations and the percentage of infested flies were low (Nevill, 1975).

Suckling calves were hand-fed, weaned early, and kept in isolated but sunny paddocks from which they were transferred directly to the Onderstepoort abattoir for slaughter.

When infestations were carried out during the summer (September–February), the young calves transferred from the experimental farm were kept in insect-free stables at the Institute until slaughter.

Calves 2680 and 2678 were reared in the Highveld where *P. bovicola* has not been recorded. They were weaned and then transferred directly to the Institute where they were infested artificially.

The relevant data from the 9 control animals slaughtered in a previous trial (Viljoen, 1976) were used to evaluate the histopathological changes and macroscopic appearance of lesions up to 194 days after the appearance of the first bleeding spots. These calves originated from the Government Experimental farm at Zoutpan north of Pretoria and were born from 3 October 1973–8 November 1973. As soon as the 1st bleeding spots were noticed (from 6 June 1974–15 August 1974), the calves were transferred to isolated paddocks at Onderstepoort and then slaughtered for trial purposes from 23 July 1974–14 January 1975 (Viljoen, 1976).

Laboratory infestation of flies with P. bovicola

Laboratory-reared or field-collected *Musca lusoria* or *M. xanthomelas* were used as intermediate hosts. They were fed on drops of blood containing eggs and microfilariae of *P. bovicola* which were placed directly on top of the gauze of their holding cages (Nevill, 1979).

Infestations of experimental calves

Fourteen days after the flies had been infested they were squashed and the infective larvae were dissected out. These larvae were then transferred to Petri dishes containing a mixture of Eagle's solution and 30% inactivated bovine serum and stored for periods of up to 12 hours at 37 °C. The following antifungal and antibacterial

[1] State Veterinay Office, Swellendam 6740

[2] Section of Pathology, Veterinay Research Institute, Onderstepoort 0110

Received 9 November 1981—Editor

agents were added per mℓ of the medium: 2,5 mcg amphotericin B, 40 mcg tylosin, 100 units sodium benzylpenicillin, 200 mcg streptomycin sulphate, 100 units polymyxin B sulphate and 100 mcg neomycin sulphate. Prior to infestation the infective larvae were concentrated in 1 mℓ of the medium in a 2 mℓ plastic syringe held vertically point up. A 14 gauge needle was then fitted and the syringe was inverted. The syringe was kept in this position for at least 30 seconds to concentrate the larvae near its tip, after which, with the syringe still pointing down, the calf was infested by subcutaneous injection of the larvae into the left or right scapular region dorsal to the spina scapulae.

The intermediate host species used, the number of infective larvae injected into the calves and the developmental period in days in each calf before necropsy was carried out, are summarized in Table 1. With the exception of Calves 2680 and 2678, which were subcutaneously infested in the left scapular region, all the calves were infested on the right side. Calf 2880 was originally infested in the right scapular region on 25 July 1978 for an infestation period of 184 days. Three days prior to necropsy this calf was reinfested in the left scapular region to provide data for a 3-day infestation period.

Carcass examinations

Calves with artificial infestations were slaughtered during the period 27 April 1977–27 November 1979.

Tissue smears from affected areas, stained with either Giemsa (Anon., 1968) or the Diff-Quick stain*, were examined for the presence or absence of eosinophils and a thorough evaluation was made of the colour and appearance of lesions. The numbers and positions of eosinophil-positive lesions were recorded in the 5 areas outlined by Viljoen (1976), i.e. head, neck and dewlap; withers, shoulders, upper forelimbs and pectoral region; back and loins; ribs and flanks, and hindquarters and upper legs.

Samples collected

Samples of affected skin and subcutis were collected for histopathological examinations in 10% buffered formalin from the most conspicuous lesions and thereafter routinely processed and embedded in paraffin wax. Sections 4–6 μm thick were cut and strained with haematoxylin and eosin (HE). The Perl's reaction and Schmorl's special staining techniques (Pearse, 1961) were applied to some of these sections.

In the control group of 9 animals, carcasses were examined as outlined by Viljoen (1976). Although samples were collected for histopathological examinations and the colour and appearance of lesions evaluated, these data were held over for the present paper.

RESULTS

Worms recovered

Worms were recovered from all artificially-infested calves, indicating that all infestations had successfully been carried out (Table 1).

Number and distribution of lesions in artificially-infested animals

The number and distribution of areas positive for eosinophils on the carcasses of 19 calves slaughtered 3–240 days after infestation, before bleeding spots appeared, are illustrated in Fig. 1–20. In these figures the overlap of lesions into adjacent areas is also shown.

Lesions on the carcasses of animals 2880, 2812, 2680 and 2811 (Day 3+–Day 13+) were still limited to the area of the injection or its immediate vicinity (calves 2880 and 2680 were infested in the left scapular region). During this period a gradual enlargement of lesions was also quite obvious (Fig. 1–4).

From the 3rd week (Day 20+) lesions became more scattered and separate lesions were noticed on the right side of the carcass (Fig. 5).

On Day 31+ lesions bordered the hindquarters of both the left and right sides of the carcass (Fig. 6). On Day 63+ they were noted on the hindquarters of the opposite sides of the carcass (Fig. 8). From Day 63+ until the end of the observation period (Day 240+) lesions were frequently indistinct or absent at the site of injection (Fig. 8–20).

Visibility of lesions

Lesions were very pronounced for the first 31 days, but thereafter were ill-defined and located with difficulty from Day 34+–Day 112+. Subsequently, from Day 116+–Day 240+, lesions again became increasingly visible (see Fig. 1–20 for the illustration of visibility).

Number, distribution and visibility of lesions in natural infestations

Data on the number and distribution of eosinophil-positive lesion in naturally infested animals which were slaughtered after the appearance of bleeding spots are illustrated in Fig. 21–28.

In all these animals there were more lesions than in artificially infested cattle, and well-defined borders made the carcass lesions clearly visible. Although lesions were present in all the different regions, most were concentrated in or adjacent to the scapular region.

Macroscopic pathology

The macroscopic appearance of carcass lesions in animals slaughtered before and after the appearance of bleeding spots in an artificially and naturally infested group of animals is indicated in Tables 2 & 3 respectively.

The macroscopic features of lesions of animals slaughtered more or less at the same time were so similar that some of these results are omitted from Table 2. Calf 1787, however, was included to illustrate an infestation period of 135+ days. This calf was injected with 76 larvae in the right scapula and at necropsy 4 eosinophil positive lesions were found. Calf 03, slaughtered 160 days after the appearance of bleeding spots, was added to the list of those recorded in Table 3. This calf was literally covered with 15 poorly demarcated lesions of different sizes.

Lesions evaluated during the first 3 weeks (Day 3+–Day 20+) after artificial infestation are comparable with those present during the period 47–194 days after the appearance of bleeding spots in the naturally infested group (Tables 2 & 3). They were distinctly oedematous, occasionally haemorrhagic, gelatinous or even slimy, with congested blood vessels. During the developmental period, 0–240 days after artifical infestation, lesions were more yellow or yellowish-green (Fig. 29) than the marked yellowish-brown or brown discoloration of older lesions 47–194 days after the appearance of bleeding spots (Fig. 30). From Day 31+–Day 240+ after artificial infestation lesions became less oedematous or gelatinous and the congested or haemorrhagic appearance was virtually absent. The yellowish-green or diffuse green discolorations were still present, however, especially during the period 112+–240+ days after subcutaneous infestation, and seemed to be pathognomonic for infestations at this stage.

Histopathology

The most prominent histopathological lesions observed both before and after the appearance of bleeding spots were in the subcutaneous tissues of the infested animals.

* C.A. Milch (Pty) Ltd, P.O. Box 143, Krugersdorp, 1741

TABLE 1 The laboratory infestation of calves with infective larvae of *P. bovicola* for recovery of developing worms and the evaluation of pathological changes

Calf No.	Source of intermediate hosts		No. of infective larvae injected	Total No. of worms recovered	Days from experimental infestation to slaughter
	Laboratory reared	Field collected			
2880***	Mx* & Ml**	Mx & Ml	150	49	3
2812	– – –	Mx & Ml	120	6	7
2680	Mx & Ml	Mx & Ml	148	6	9
2811	– – –	Mx & Ml	100	12	13
2678	Mx & Ml	Mx & Ml	148	4	20
3762	Mx – –	– – –	174	16	31
3147	Mx & Ml	Mx & Ml	130	8	34
3144	Mx & Ml	Mx & Ml	129	20	63
2535	– – –	Mx & Ml	100	5	63
3616	Mx – –	– – –	170	5	63
2547	– – –	Mx & Ml	102	10	78
3143	Mx & Ml	Mx & Ml	130	26	91
3140	Mx & Ml	Mx & Ml	130	19	112
2851	– – –	Mx & Ml	98	21	116
1787	– – –	– – Ml	76	7	135
1815	Mx – –	– – –	99	3	154
1721	– – –	Ml – –	77	7	178
2880	Mx – –	– – –	100	10	184
1718	– – –	Ml – –	75	9	195
2856	– – –	Mx & Ml	118	21	217
1750	– – –	– – Ml	75	7	240

Mx*=*Musca xanthomelas*
Ml**=*Musca lusoria*
***=Calf No. 2880 infested twice, 184 and 3 days prior to necropsy

TABLE 2 The macroscopic appearance of lesions in experimentally infested calves slaughtered during the developmental period of *P. bovicola*

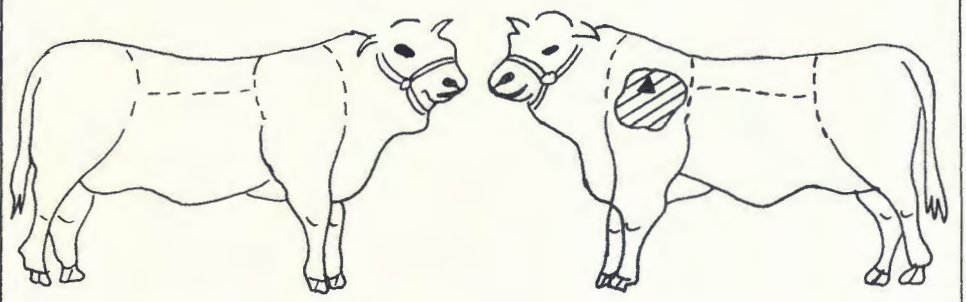
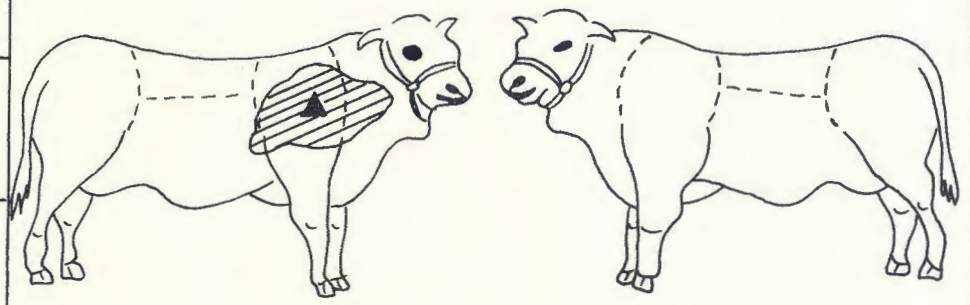
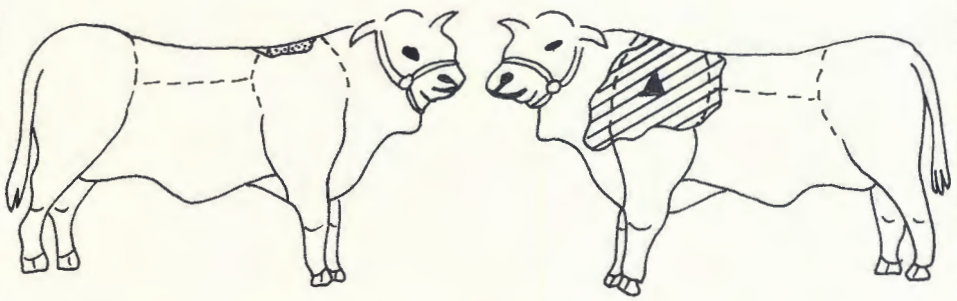
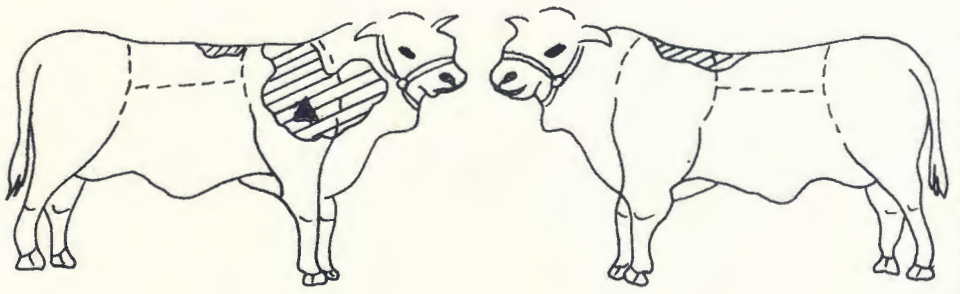
Calf No.	Days from experimental infestation to slaughter	Oedematous or gelatinous	Congested or haemorrhagic	Yellowish-green	Yellowish-brown
2880*	3	++	++	++	–
2680	9	++	++	++	+
2678	20	++	++	+	–
3762	31	+	–	+	–
3147	34	+	–	+	–
3616	63	+	+	–	–
3143	91	+	+	–	–
3140	112	+	–	+	–
1787	135	+	–	+	+
1815	154	+	+	++	–
1721	178	+	–	+	–
2880	184	+	–	+	++
1718	195	+	–	+	–
1750	240	+	++	+	–

–Absent or barely visible
+Moderate
++Pronounced
*Calf No. 2880 infested twice, 184 and 3 days prior to necropsy

TABLE 3 The macroscopic appearance of lesions in naturally infested calves slaughtered 47–194 days after the first appearance of bleeding spots caused by *P. bovicola* adults

Calf No.	Days after the first appearance of bleeding spots	Oedematous or gelatinous	Congested or haemorrhagic	Yellowish-green	Yellowish-brown
01	47	+	++	+	++
06	83	++	+	–	++
09	97	+	+	–	+
010	111	+	+	–	++
05	125	++	++	–	+
03	160	+	–	+	++
07	166	++	–	+	++
02	181	++	++	–	++
04	194	++	++	–	++

–Absent or barely visible
+Moderate
++Pronounced

Calf No. 2880 Injection site: *L. scapula* Days after infestation: 3	FIG. 1  See Fig. 17 for lesions 184 days of age
Calf No. 2812 Injection site: *R. scapula* Days after infestation: 7	FIG. 2 
Calf No. 2680 Injection site: *L. scapula* Days after infestation: 9	FIG. 3 
Calf No. 2811 Injection site: *R. scapula* Days after infestation: 13	FIG. 4 

▲ Pathological samples collected
▨ Distinct
▩ Indistinct

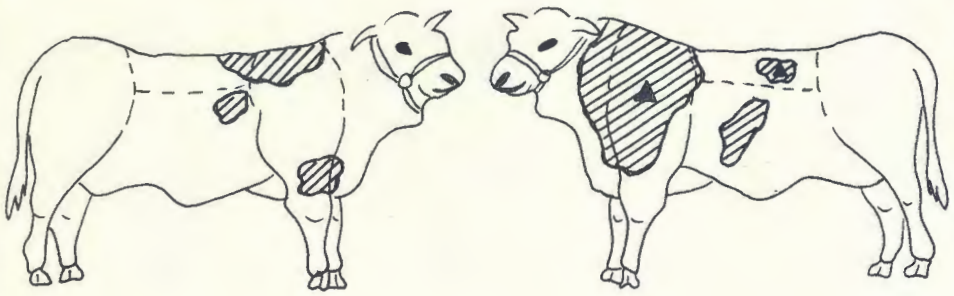
FIG. 1–20 The number and distribution of carcass lesions positive for eosinophils in calves 3–240 days after subcutaneous artificial infestation with infective larvae of *P. bovicola*

Calf No. 2678

Injection site: *L. scapula*

Days after infestation: 20

FIG. 5

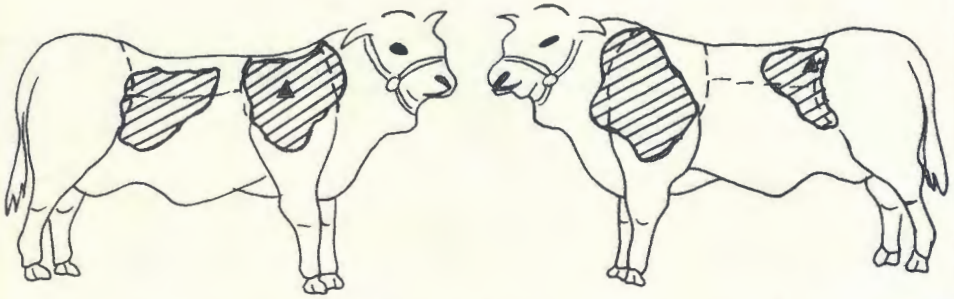


Calf No. 3762

Injection site: *R. scapula*

Days after infestation: 31

FIG. 6

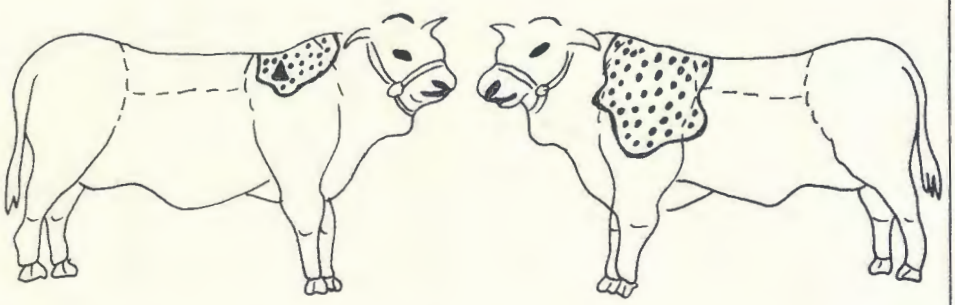


Calf No. 3147

Injection site: *R. scapula*

Days after infestation: 34

FIG. 7

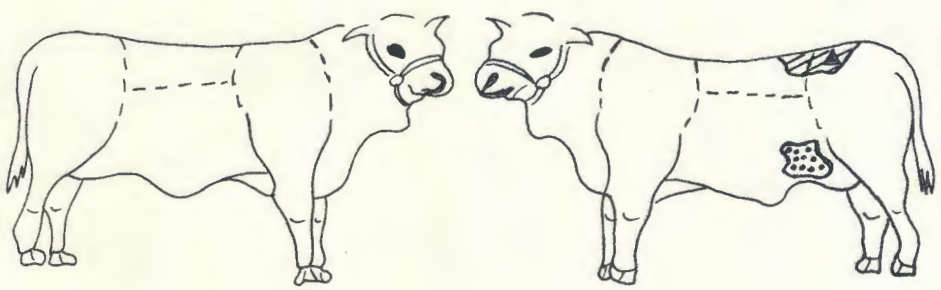


Calf No. 3144

Injection site: *R. scapula*

Days after infestation: 63

FIG. 8



▲ Pathological samples collected

▨ Distinct

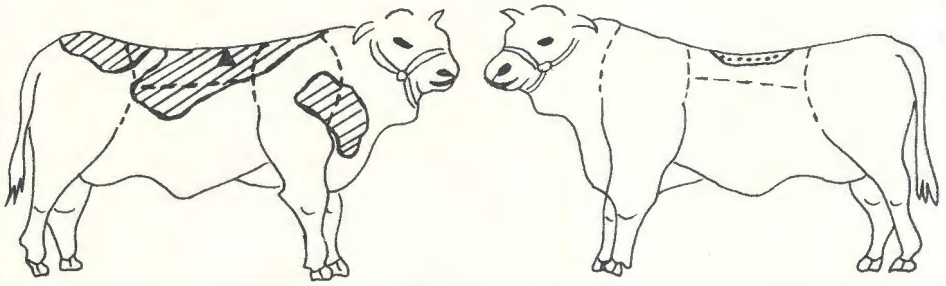
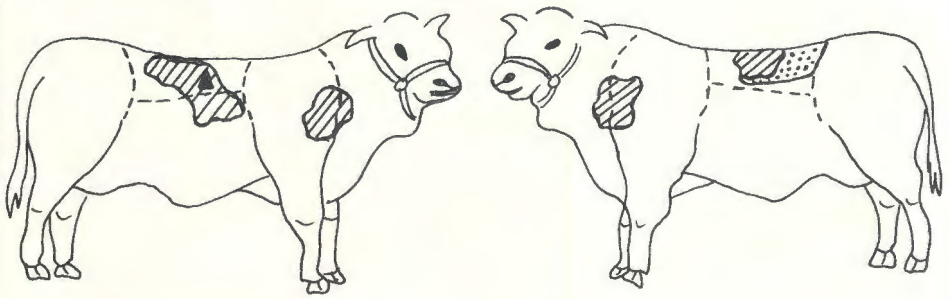
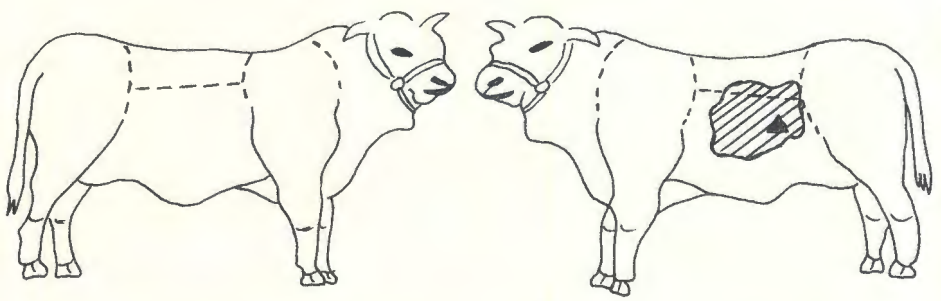
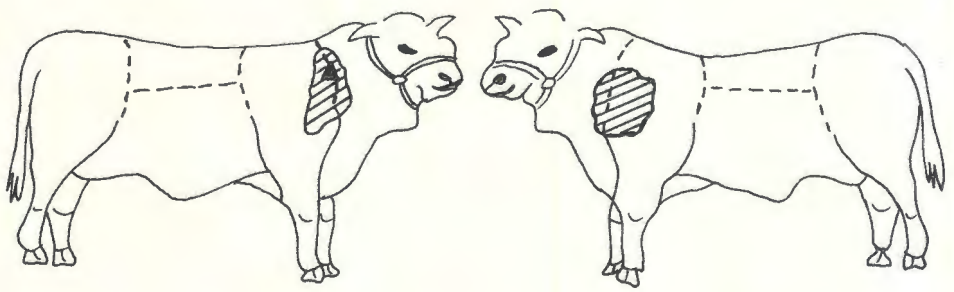
▩ Indistinct

Calf No. 2535 Injection site: *R. scapula* Days after infestation: 63	FIG. 9
Calf No. 3616 Injection site: *R. scapula* Days after infestation: 63	FIG. 10
Calf No. 2547 Injection site: *R. scapula* Days after infestation: 78	FIG. 11
Calf No. 3143 Injection site: *R. scapula* Days after infestation: 91	FIG. 12

▲ Pathological samples collected
▨ Distinct
▩ Indistinct

Calf No. 3140 Injection site: *R. scapula* Days after infestation: 112	FIG. 13
Calf No. 2851 Injection site: *R. scapula* Days after infestation: 116	FIG. 14
Calf No. 1815 Injection site: *R. scapula* Days after infestation: 154	FIG. 15
Calf No. 1721 Injection site: *R. scapula* Days after infestation: 178	FIG. 16

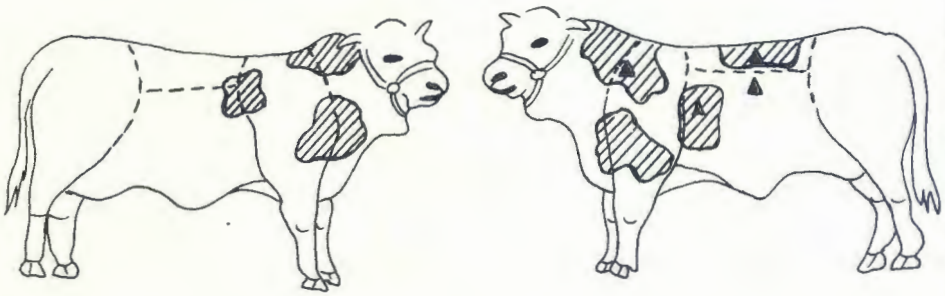
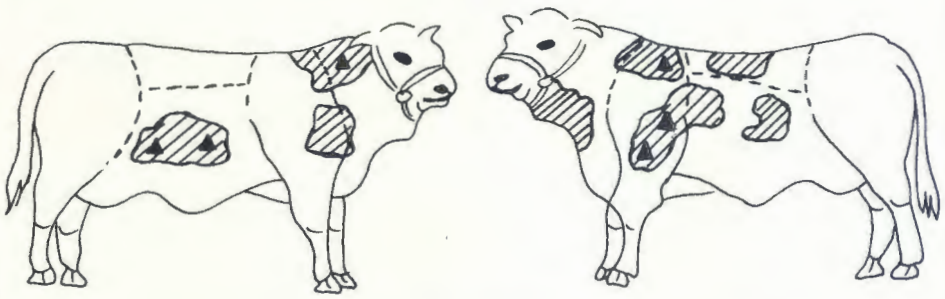
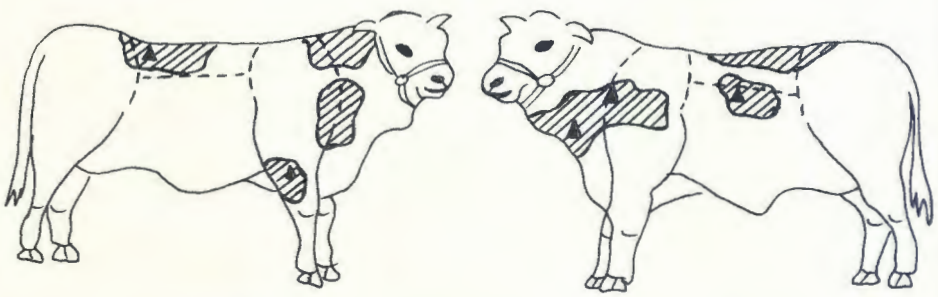
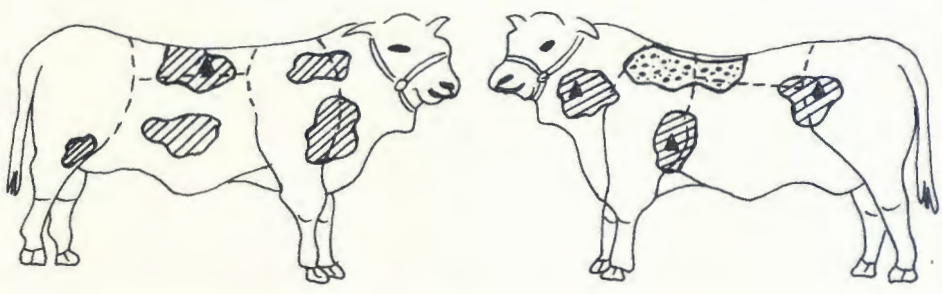
▲ Pathological samples collected
▨ Distinct
▩ Indistinct

Calf No. 2880 Injection site: *R. scapula* Days after infestation: 184	FIG. 17  See Fig. 1 for lesion 3 days of age
Calf No. 1718 Injection site: *R. scapula* Days after infestation: 195	FIG. 18 
Calf No. 2856 Injection site: *R. scapula* Days after infestation: 217	FIG. 19 
Calf No. 1750 Injection site: *R. scapula* Days after infestation: 240	FIG. 20 

▲ Pathological samples collected

▨ Distinct

▩ Indistinct

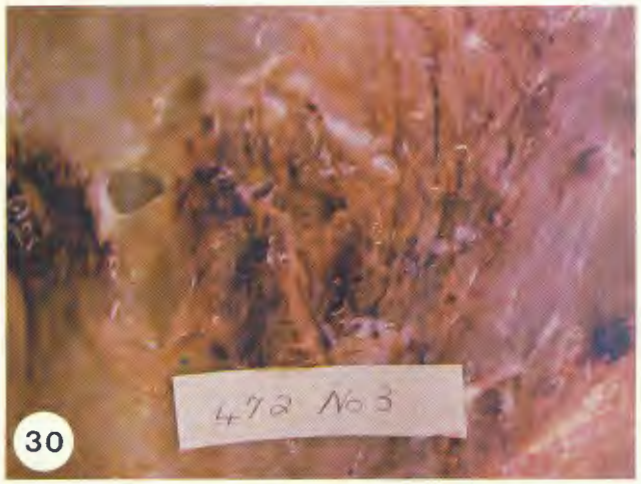
Calf No. 01 Date first bleeding spot observed: 6/6/74 Date of slaughter: 23/7/74 Period clinically positive: 47 days	FIG. 21 
Calf No. 06 Date first bleeding spot observed: 25/7/74 Date of slaughter: 16/10/74 Period clinically positive: 83 days	FIG. 22 
Calf No. 09 Date first bleeding spot observed: 8/8/74 Date of slaughter: 13/11/74 Period clinically positive: 97 days	FIG. 23 
Calf No. 010 Date first bleeding spot observed: 15/8/74 Date of slaughter: 4/12/74 Period clinically positive: 111 days	FIG. 24 

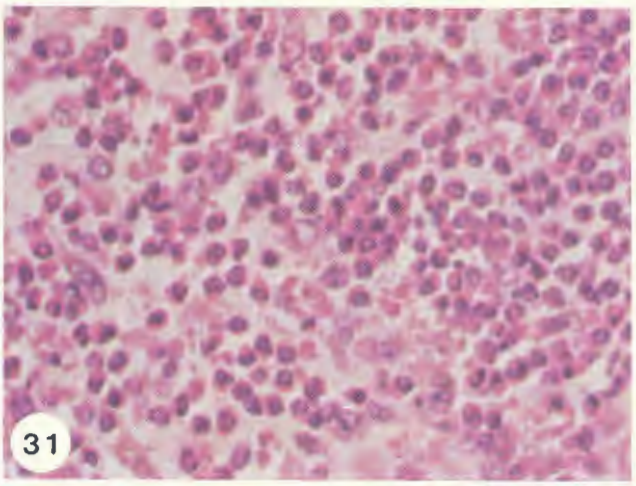
▲ Pathological samples collected
▨ Distinct
▩ Indistinct

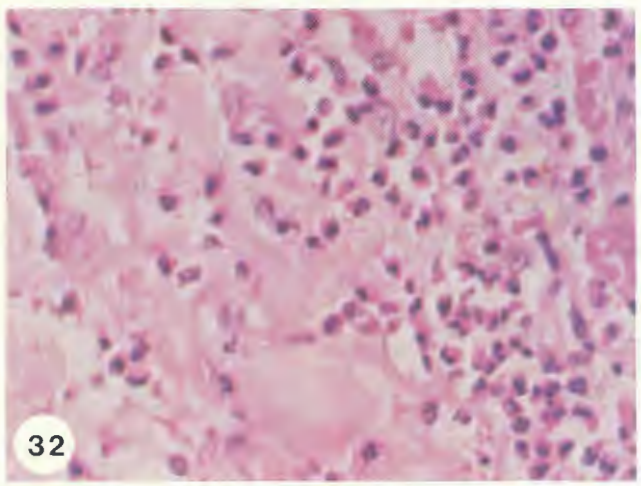
FIG. 21–28 The number and distribution of carcass lesions positive for eosinophil in cattle naturally infested with *P. bovicola* and slaughtered 47–194 days after the first appearance of bleeding spots

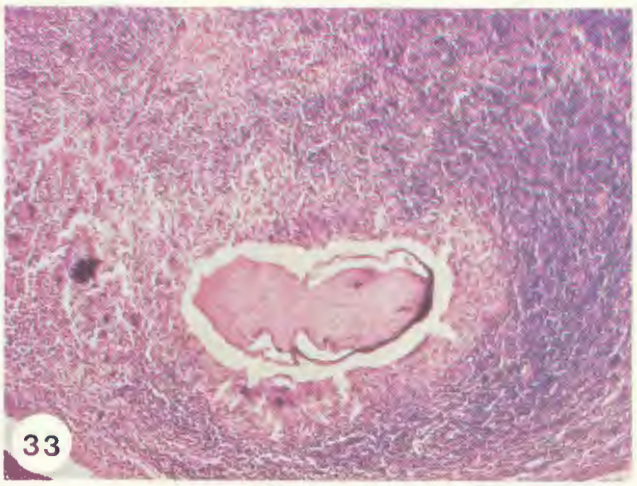
Calf No. 05 Date first bleeding spot observed: 11/7/74 Date of slaughter: 13/11/74 Period clinically positive: 125 days	FIG. 25
Calf No. 07 Date first bleeding spot observed: 1/8/74 Date of slaughter: 14/1/75 Period clinically positive: 166 days	FIG. 26
Calf No. 02 Date first bleeding spot observed: 6/6/74 Date of slaughter: 4/12/74 Period clinically positive: 181 days	FIG. 27
Calf No. 04 Date first bleeding spot observed: 4/7/74 Date of slaughter: 14/1/75 Period clinically positive: 194 days	FIG. 28

▲ Pathological samples collected
▨ Distinct









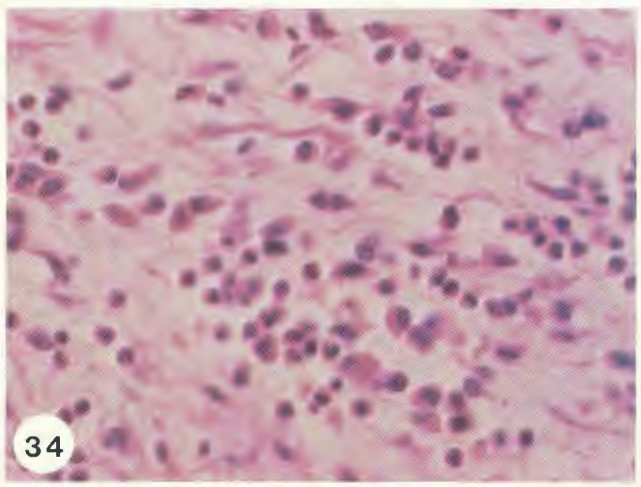


FIG. 29 A localized oedematous gelatinous discoloured lesion of the left scapular region 9 days after infestation (Calf No. 2680)

FIG. 30 Note gelatinous appearance of a yellowish-brown lesion in the subcutis of Calf No. 010, slaughtered 111 days after the appearance of the first bleeding spots

FIG. 31 Pronounced eosinophil infiltrations accompanied by some macrophages and lymphocytes in the subcutaneous tissues of Calf No. 2880, slaughtered 3 days after experimental infestation: HE × 200

FIG. 32 Note oedema and cellular infiltrate in the subcutis of Calf No. 2678, slaughtered 20 days after experimental infestation: HE × 200

FIG. 33 Remnants of a *P. bovicola* worm in a parasitic granuloma of Calf No. 01, slaughtered 47 days after the appearance of the first bleeding spots: HE × 75

FIG. 34 Pigment-laden macrophages in the subcutaneous tissues of Calf No. 05, slaughtered 125 days after the appearance of the first bleeding spots: HE × 500

The severity of the lesions and the type and degree of cellular infiltration at different sites on the same animal varied from animal to animal and also at different intervals after infestation.

The most striking microscopic lesion seen during the developmental period (before the appearance of bleeding spots) was the marked infiltration of eosinophils into the subcutis (Fig. 31). Three days after infestation this infiltration was discernible at the site of the experimental inoculation with *P. bovicola*. The eosinophils, which were distributed throughout the subcutaneous tissues, were frequently concentrated around the blood vessels. This infiltrate was invariably accompanied by a moderate subcutaneous oedema (Fig. 32). As seen in the subcutis of Calf No. 2678 (Fig. 32) slaughtered 20 days after infestation, many of the lymphatics were dilated with lymph and contained eosinophils, lymphocytes, neutrophils and macrophages.

The macrophage and lymphocytic infiltration present in the subcutis 3 days after infection became more pronounced with the increase in the age of the lesions. Plasma cells and a mild to moderate subcutaneous fibroplasia were likewise seen in older lesions. The degenerative and necrotic collagen fibres which were frequently seen in focal lesions gave the impression of parasitic migratory tracts. This was occasionally associated with typical parasitic granulomas (Fig. 33).

The dermal reaction was much milder in nature and was limited to a moderate perivascular eosinophil, lymphocyte and macrophage infiltration of the blood vessels in the *pars reticularis*.

Some of the fibres of the superficial muscles bordering on the inflamed areas in the subcutis were affected and showed hyalin degeneration and necrosis, with vacuolation, lysis and evidence of mineralization. These changes in the muscles, seen in many animals, were accompanied by a moderate eosinophilic, lymphocytic and macrophage infiltration. Evidence of regeneration was noticed in some of the affected fibres. The regional lymph nodes draining the infected areas were invariably stimulated and the reactive changes in the prescapular and prefemoral lymph nodes in 2 animals 9 days and 63 days after artificial infestation were very similar.

These changes were characterized by prominent primary and secondary follicles with reactive germinal centres, moderate reticuloendothelial cell proliferation, oedema and varying numbers of eosinophils in the cortex, medulla sinuses and capsule as well as in the periglandular connective tissue. A few pigment-laden macrophages containing haemosiderin and lipofuscin were also noted in the lymph nodes.

Once the worms were adult (after the appearance of bleeding spots), the histopathological lesions corresponded to those described above, except that pigment-laden macrophages (Fig. 34) were present in large numbers and the subcutis was more heavily infiltrated with lymphocytes, some plasma cells and mast cells.

There also appeared to be a notable increase in collagen fibres and a mesenchymal type of connective tissue in the subcutis.

Green discolorations, particularly before the appearance of bleeding spots, were associated with massive infiltrations of eosinophils. In older lesions (after the appearance of bleeding spots) the green colour was dominated by different shades of brown owing to massive accumulations of haemosiderin in the macrophages. All the lesions were slightly yellow, most probably because of oedematous fluid in the tissues.

Live *P. bovicola* larvae and/or adults were found on all the carcasses of the animals listed in Tables 1, 2 & 3. Mean worm burdens per animal were 12,9 for animals slaughtered before the appearance of bleeding spots (artificially infested animals listed in Table 1) and 6,2 for those subsequently slaughtered (naturally infested). In 42% of the lesions from which histopathological sections were prepared live immature and/or adult *P. bovicola* worms were collected and the persistence of inflammatory reactions (oedema, congestion and haemorrhage) was therefore quite logical.

DISCUSSION

It was suggested by Baumann (1946) that exposure to sunlight was essential for *Parafilaria multipapillosa* females to penetrate the skin of the host animal before ovipositing. This conclusion was supported by the work on quarantine cattle by Webster & Wilkins (1970), and by Nevill (1979) during the experimental transmission of *P. bovicola* to cattle at Onderstepoort. With the exception of calves that had been artificially infested during the summer months and then kept in insect-free stables until slaughter to exclude possible reinfestation, the animals in the present trial were exposed as far as possible to direct sunlight.

The experiments reported in this paper show that natural infestations give rise to more lesions than those resulting from a single experimental infestation (8,0 and 2,7 lesions per infection in naturally and experimentally infested groups respectively). This is due either to repeated infestations or higher infectivity of larvae transmitted by the intermediate host(s) in the field.

The similarity in the distribution of lesions in the 2 experimental groups suggests that there are certain predelection areas for infestation by intermediate fly hosts. Most of the lesions occur in the forequarters, particularly the scapular region, and it is reasonable to assume that in the field infestations of cattle take place through wounds in the region at which the intermediate host(s) concentrate.

The initial enlargement of lesions, the appearance of separate lesions, and their presence in remote areas, especially on the opposite side of the carcass, must be associated with a process of subcutaneous migration. This comfirms the observation of Osipov (1962) on adult infestations of *P. multipapillosa* in horses and was suspected with *P. bovicola* by Viljoen (1976), who noticed that more than 50% of spots are only observed once in the same site.

Lesions on the carcass are more prominent immediately after parasitic invasion (Day 3+–Day 20+) and again after adult female *P. bovicola* worms penetrate the skin. During both periods pronounced inflammatory reactions are to be expected. A collagenase-like enzyme, similar to that described for the cercariae of *Schistosoma mansoni* and larvae of *Strongyloides ratti* (Lewert & Lee, 1956), could be responsible for both the local tissue reaction by invading larvae and the ease with which adult females of *P. bovicola* penetrate the skin.

These observations and the colour changes, which are more yellowish-green during the developmental period and yellowish-brown to brown once the worms are adult, are supported by the histological changes observed before and after the appearance of bleeding spots. Reactions were not confined to the subcutis; a mild dermal reaction was also notice while some of the fibres of the superficial muscles bordering the inflamed areas in the subcutis showed degenerative and regenerative changes. This observation is contrary to the original description by Pienaar & Van den Heever (1964), who did not find any dermal or muscular changes.

Visual changes observed with the aging of lesions can be used to determine their approximate age. These observations should, however, be confirmed microscopically.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to: Dr E. M. Nevill and his staff, Messrs G. van der Westhuizen, M. du Toit and D. G. de Klerk, for valuable assistance; Mr J. L. de B. van der Merwe and technicians for the general preparation and staining of all the histopathological sections; Miss W. Collins for the preparation of graphs and illustrations; Mr A. M. du Bruyn and staff for the photography, and Mr J. F. de Lange for his helpful cooperation at the experimental farm of the Veterinary Research Institute, Onderstepoort.

REFERENCES

ANON., 1968. Armed Forces Institute of Pathology, Washington. Manual of histologic staining methods. 3rd ed. New York, Toronto, London, Sydney: McGraw Hill.

BAUMANN, R., 1946. Beobachtungen beim parasitären Sommerbluten der Pferde. *Wiener Tierärztliche Monatsschrift,* 33, 52–55.

LEWERT, R. M. & LEE, C. L., 1956. Quantitative studies of the collagenase-like enzymes of cercariae of *Schistosoma mansoni* and the larvae of *Strongyloides ratti*. *Journal of Infectious Diseases,* 99, 1–14.

NEVILL, E. M., 1975. Preliminary report on the transmission of *Parafilaria bovicola* in South Africa. *Onderstepoort Journal of Veterinary Research,* 42, 41–48.

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.

OSIPOV, A. N., 1962. The development of *Parafilaria* in the final host. (In Russian). *Tezisy Dokladov Nauchnoi Konferentsii Vsesoyuznogo Obshchestva Gelmintologov AN SSSR,* Part 1, 129–131.

PEARSE, A. G. E., 1961. Histochemistry theoretical and applied. 2nd ed. London: J. & A. Churchill.

PIENAAR, J. G. & VAN DEN HEEVER, L. W., 1964. *Parafilaria bovicola* Tubangui, 1934, in cattle in the Republic of South Africa. *Journal of the South African Veterinary Medical Association,* 35, 181–184.

VILJOEN, J. H., 1976. Studies on *Parafilaria bovicola* (Tubangui, 1934). I. Clinical observations and chemotherapy. *Journal of the South African Veterinary Association,* 47, 161–169.

VILJOEN, J. H. & BOOMKER, J. D. F., 1977. Studies on *Parafilaria bovicola* Tubangui, 1934. 2. Chemotherapy and pathology. *Onderstepoort Journal of Veterinary Research,* 44, 107–112.

WEBSTER, W. A. & WILKINS, D. B., 1970. The recovery of *Parafilaria bovicola* Tubangui 1934 from an imported Charolais bull. *Canadian Veterinary Journal,* 11, 13–14.

Printed by and obtainable from the Government Printer, Bosman Street, Private Bag X85, Pretoria, 0001