Onderstepoort J. vet. Res., 51, 115–118 (1984)

THE LONGEVITY OF ADULT *PARAFILARIA BOVICOLA* AND THE PERSISTENCE OF THEIR ASSOCIATED CARCASS LESIONS IN CATTLE IN SOUTH AFRICA

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ABSTRACT

NEVILL, E. M. & VILJOEN, J. H., 1984. The longevity of adult *Parafilaria bovicola* and the persistence of their associated carcass lesions in cattle in South Africa. *Onderstepoort Journal of Veterinary Research*, 51, 115–118 (1984).

The slaughter of naturally-infected heifers and oxen at regular intervals after the first *P. bovicola* ovipositional blood spots appeared revealed that no female worms with embryonated eggs could be found after 259 days, no live worms after 372 days, and no carcass lesions after 519 days. In one bull, however, blood spots and carcass lesions persisted throughout 2 seasons. With the possible exception of bulls, therefore, annual reinfection of cattle appears to be necessary for the continuation of the transmission cycle of *P. bovicola*.

INTRODUCTION

The minimum prepatent period of *Parafilaria bovicola* in 2 artificially-infected cattle was 242 days (Nevill, 1979). In a herd of 353 naturally-infected cattle, the shortest period from birth to 1st blood spot ranged from 191 to 279 days for 81,8 % of the oxen and 47,1 % of the heifers (Nevill, 1984). It remained to be determined how long females can continue to lay eggs, how long they can survive after egg laying has stopped, and the period which must elapse before the carcass lesions which they create have disappeared. A study of blood spot incidence can then be used to determine how long an infected animal will act as a source of infective material and the time required for infected cattle which are introduced to uninfected areas, to lose their infection and carcass lesions.

Nelson (1964) stated that most filarial worms continue to produce microfilariae for long periods. He cited the work of Nelson & Grounds (1958), who found that more than 50 % of the people at Kodera in Kenya were still infected with *Onchocerca volvulus* 11 years after the vector *Simulium neavei* had been eliminated.

Horses infected with *Parafilaria multipapillosa* have frequently been sold to countries free of this parasite, and workers in these countries have subsequently noted the period over which bleeding took place. Thus Railliet & Moussu (1892) in France stated that bleeding in horses from Hungary lasted for 3 or 4 years. Andersson, Jalkanen & Nurmio (1976) in Finland observed bleeding in horses imported from near Rostov and possibly Stavropolis in south-eastern Russia. All the horses bled during the first 2 summers and 1 possibly during the third summer, but most bleeding occurred during the first summer. Gibson, Pepin & Pinsent (1964) recorded bleeding caused by *P. multipapillosa* infection in a 5-year-old mare imported into Britain from Russia. It was found to be bleeding on arrival and it bled again the following year.

In experimental transmission of *P. multipapillosa*, Osipov (1962) found that horses infected in July 1960 bled from April–September 1961 and again during April 1962, which is in agreement with observations on natural infections with this species of *Parafilaria*.

There is no reference in the literature as to how long *P. bovicola* survives other than the observation by Webster & Wilkins (1970) in Canada that Charolais cattle imported from France showed *P. bovicola* bleeding lesions either during the 6-month quarantine period or within the first year after importation.

To investigate this aspect of the life-cycle of *P. bovicola* in South Africa, the recurrence of blood spots in some naturally-infected cattle was recorded regularly for up to 3 consecutive summers before slaughter, while other infected animals were kept for various periods in insect-free stables prior to slaughter and carcass appraisal for lesions and worms.

MATERIALS AND METHODS

Preliminary observations on blood spot recurrence

In preliminary experiments, which started in 1972, the blood spots seen on each of 3 approximately 8-year old culled Hereford Bulls, sent from Mara Research Station near Louis Trichardt, for slaughter at Onderstepoort, were recorded daily on individual outline diagrams over periods of 15–27 months. The blood spots on an ox and a heifer (both 15 months old) from 'Mara' were likewise recorded at Onderstepoort over 27 months. One of the bulls and the ox spent most of this period in an insect-free stable, while the remaining animals remained outside for the full period.

At the end of these periods the cattle were slaughtered and their carcasses examined for lesions and worms.

Comparison of P. bovicola *survival and carcass lesion persistence in heifers, oxen and old bulls*

The Research Stations of 'Zoutpan' and 'Towoomba' 30 km NNW and 90 km NNE of Onderstepoort respectively, were visited weekly between 8 June 1977 and 29 July 1977, and recently weaned heifers and oxen, approximately 8 months old, were examined for blood spots containing embryonated *P. bovicola* eggs produced by females ovipositing for the first time. Twelve infected heifers and 12 infected oxen were selected and transferred to insect-free stables at Onderstepoort, 10 of the oxen on 11 July 1977, and 2 oxen and 12 heifers on 9 August 1977.

Five culled bulls, all older than 5 years, were obtained from 'Mara' on 22 July 1977 and kept in an open paddock at Onderstepoort until blood spots containing embryonated eggs were seen. The bulls were then transferred to an insect-free stable. Although 12 old bulls were required for this experiment, it was not possible to obtain so many.

To serve as a control, so that the results obtained from similar groups slaughtered at progressively longer intervals thereafter could be compared, 2 heifers and 2 oxen of the 'Zoutpan'/'Towoomba' group were slaughtered at Onderstepoort 2–3 months after arrival during the period that *P. bovicola* females were actively laying eggs (October 1977). Similar numbers of the remaining animals were slaughtered at intervals of approximately 4 months until no worms and few lesions could be detected. Thereafter, the intervals between slaughter were reduced to a few weeks until all had been slaughtered.

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Received 27 February 1984—Editor

TABLE 1 The seasonal recurrence of blood spots caused by *Parafilaria bovicola* in naturally-infected cattle from 'Mara' kept at the Veterinary Research Institute, Onderstepoort

Sex of bovine and identity No.	Date of arrival at Onderstepoort	Where held	No. of blood spots			Date slaughtered	Days from arrival to slaughter	Carcass lesions
			Summer 1971/72	Summer 1972/73	Summer 1973/74			
Bull 8967	4 Feb 1972	Stabled from June 1973	55	77	16	2 May 1974	810	Chronic, discoloured
Bull 8962	4 Feb 1972	Outside	13	0		7 May 1973	450	Many lesions + eosinophils
Bull 8971	4 Feb 1972	Outside	17	0		7 May 1973	450	Lesions + eosinophils + 1 live ♀
Ox 8941	23 Jan 1972	Stabled from 25 Feb 1972	20	0	0	2 May 1974	810	Acute, small
Heifer 8939	23 Jan 1972	Outside	2	0		7 May 1973	450	No lesions or eosinophils

Because the number of bulls available was limited, the initial slaughter date in October 1977 and the winter slaughter date in June 1978 were omitted, and usually only 1 bull was slaughtered at a time.

At slaughter, the size, shape and appearance of *Parafilaria* lesions and the position of both dead and living worms were indicated on an outline diagram of an ox. Where doubt existed as to the nature of a lesion, as was often the case during the second summer, smears were made, stained with Giemsa in the usual manner and checked for the presence of unusually high numbers of eosinophils. If these were present, the lesion was assumed to have been caused by *P. bovicola*.

To facilitate the comparison of lesions in animals slaughtered at different times, the lesion area as indicated on the outline diagram of an ox was roughly calculated and expressed as a percentage of the total body area.

The 3 lesion categories, namely acute, sub-acute and chronic as defined by Nevill (1979), were again used in all the experiments.

The worms collected off the carcasses were sexed and examined for embryonated eggs by dissection in 0,85 % saline.

RESULTS

Preliminary observations on blood spot recurrence

These observations are summarized in Table 1. Four of the 5 cattle used in the preliminary experiments bled only during the summer of 1971/72, soon after their arrival at Onderstepoort. However, even though no blood spots were seen on these animals in the summer of 1972/73, at the end of this summer the 2 bulls that were slaughtered had many lesions and 1 of them a live female worm. The heifer was negative. The ox, which was slaughtered a year later, had a few small acute lesions.

The remaining bull (No. 8967) bled for 3 consecutive summers, the first 2 of which it spent outside. At slaughter much discoloured slimy connective tissue covered its carcass surface but no worms were present.

Comparison of P. bovicola *survival and carcass lesion persistence in heifers, oxen and old bulls*

The lesion areas, lesion type and worms recovered are summarized in Table 2.

Heifers. Sixty-six to 96 days after the 1st blood spot was seen, carcass lesions covered a mean of 31,5 % of the body surface. After 208–238 days the lesion area was slightly greater (37,3 %,) but it had started to decrease (15,3 %) before the slaughterings that took place after 321–351 days. After 468 days only very small lesions (1,9 %) remained, and all had disappeared by 483 days after the appearance of the 1st blood spot.

Live female worms containing embryonated eggs were recovered from carcasses up to 96 days after the 1st blood spots were seen, and live females without embryonated eggs up to 351 days later, but no living or dead worms were found at slaughter after 460 days.

Oxen. The size of carcass lesions was marginally larger and the rate at which they disappeared slightly slower than those seen in heifers, all lesions having disappeared within 519 days of the 1st blood spot being seen.

Live female worms containing embryonated eggs were recovered 259 days after the 1st blood spots were seen and live females without embryonated eggs up to 372 days later, but no worms at all were found at slaughter after 504 days.

Old bulls. Two of the 3 old bulls slaughtered 480 days after their arrival at Onderstepoort, still showed subacute and chronic lesions and a live male worm was recovered from 1 of these 2 bulls.

DISCUSSION

The heifers and oxen used in these studies were born about October 1976. Nevill (1980) showed that most infective vector flies are to be found from August to March each year. For the purpose of understanding the results of the present trials it is assumed that the 1st blood spots seen in calves in 1977 were the result of infections immediately after birth in about October 1976. Thereafter these calves could have been reinfected at any time up to the end of March 1977, a period of approximately 150 days. In the discussion which follows, all periods observed in the trials have been adjusted to allow for this variation in the time of possible natural infection.

Contrary to some of the findings of various workers on *P. multipapillosa* (Railliet & Moussu, 1892; Andersson *et al.*, 1976; Gibson *et al.*, 1964, and Osipov, 1962), the present observations suggest that survival of a *P. bovicola* infection in a naturally-infected heifer or ox, is limited to approximately 201–372 days from the appearance of the first blood spots. The egg-laying period of female worms is at least 100 days shorter.

In heifers and oxen, the lesion area decreased from 37 % or more to between 15 % and 18 % about 350 days after the 1st blood spot was seen, but it took 483 days to disappear completely in heifers and 519 days in oxen. This long period possibly includes the 150-day developmental period mentioned earlier. The remaining 333–369 days would then include the egg-laying period, the post egg-laying period until death of the worm, and a

TABLE 2 The survival of *Parafilaria bovicola* and the persistence of carcass lesions in naturally infected heifers, oxen and old bulls kept in an insect-free stable

Date slaughtered	Days from 1st blood spot to slaughter	Identity No.	Live worms from carcasses			No. of dead encapsulated worms	Type of carcass lesion			Presence of eosinophils **	% body area covered by lesions	Mean % lesion area per group
			♂♂	♀♀ Embryonated eggs			Acute	Subacute	Chronic			
				Present	Absent							
Heifers												
3 Oct 1977	66	2211		3				+			43,4	31,5
3 Oct 1977	96	2218		2	1			+			19,6	
22 Feb 1978	208	2206					+	+		+	54,4	37,3
22 Feb 1978	238	2214						+	+	+	20,1	
15 Jun 1978	321	2212			1			+	+		18,7	15,3
15 Jun 1978	351	2219			1	1		+			11,9	
1 Nov 1978	460	2215						+		+	2,6	1,9
1 Nov 1978	468	2207						+		+	1,2	
22 Nov 1978	483	2217								−	0	0
22 Nov 1978	490	2216								−	0	
30 Nov 1978	511	2208								−	0	0
30 Nov 1978	511	2209								−	0	
Oxen												
3 Oct 1977	74	2205	1	1				+			71,7	51,0
3 Oct 1977	75	2220		2				+			30,2	
22 Feb 1978	238	2083						+		+	16,4	36,8
22 Feb 1978	259	2087		2		2		+	+		57,2	
15 Jun 1978	351	2080			1	2		+	+		36,1	18,1
15 Jun 1978	372	2081			1					−	0	
1 Nov 1978	504	2082						+		+	6,8	5,6
1 Nov 1978	504	2089						+		+	4,3	
22 Nov 1978	511	2088						+		+	0,4	0,2
22 Nov 1978	525	2084								−	0	
30 Nov 1978	519	2085								−	0	0
30 Nov 1978	519	2086								−	0	
Old bulls												
22 Feb 1978	210*	1880			8				+		20,0	20,0
1 Nov 1978	450*	2193								−	0	0
22 Nov 1978	480*	2189	1					+	+	+	28,0	14,0
22 Nov 1978	480*	2191								−	0	
30 Nov 1978	480*	2192				1		+	+	+	10,5	10,5

* Days from arrival to slaughter

** No smears were made if worms were recovered

\+ Positive

− Negative

period for lesion resorption. Viljoen & Boomker (1977) found that lesion resorption took at least 63 days after successful treatment. After cattle have been moved to an uninfected district, a 17-month waiting period before slaughter should ensure that the carcasses of these animals are free of lesions at slaughter.

The evidence accumulated to date on worm survival in old bulls is more difficult to evaluate. Four of the 6 bulls slaughtered 450–480 days (15–16 months) after arrival at Onderstepoort showed lesions and/or the presence of live worms, while 2 had no lesions. Although lesions in heifers and oxen were present up to 468 days and 511 days respectively after removal to an uninfected area, no worms survived beyond 372 days.

The preliminary observations on the recurrence of blood spots in old bulls, however, showed that in 1 of the 3 (No. 8967), bleeding persisted for 3 seasons. It is likely that this bull was reinfected at 'Mara' in the summer of 1971/72 before being sent to Onderstepoort in February 1972, and that the blood spots observed in the summer of 1972/73 were a result of this infection. The blood spots seen in the summer of 1973/74 were then caused either by renewed egg-laying by worms which survived from the previous summer or by reinfection at Onderstepoort. The latter explanation is possible, but unlikely, since only 2 cases of natural transmission have been recorded so far at the Veterinary Institute, Onderstepoort, both in the summer of 1980/81 (Verster, 1981 personal communication). The survival and egg-laying activity of adult female *P. bovicola* for 2 consecutive summers in bulls must therefore be regarded as a possibility.

The foregoing studies made use of naturally-infected culled bulls from 'Mara'. However, in a survey at 'Mara' (Nevill, 1984), bulls of all ages were shown to have more blood spots than any other cattle and breeding cows the fewest. This difference and the greater longevity of *P. bovicola* in bulls, are thought to be linked with the levels of the sex hormones.

The results of this study suggest that, except perhaps in bulls, annual reinfection in spring to summer is necessary for the continuation of the transmission cycle of *P. bovicola*. Reinfection may only be necessary every 2nd year in bulls.

ACKNOWLEDGEMENTS

We wish to thank Dr G. Marincowitz for making calves from Towoomba and Zoutpan Research Stations available for this study, Dr F. Ludemann for experimental cattle from Mara Research Station, Miss I. H. A. Schatz for technical help and Prof. I. G. Horak for his helpful criticism of the manuscript.

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