

## THE EFFECT OF ARSENICAL DIPS ON *PARAFILARIA BOVICOLA* IN ARTIFICIALLY INFECTED CATTLE IN SOUTH AFRICA

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### ABSTRACT

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The possible adverse effect of arsenical tick control dips on *Parafilaria bovicola* infections was investigated in 48 artificially infected cattle. A treatment group of 24 cattle was dipped in a plunge dip containing 1600 ppm arsenic trioxide. A control group of the same size was dipped in an organophosphate dip containing a mixture of chlorfenvinphos and dioxathion.

Regular weekly to 3-weekly dipping had no effect initially on the prevalence of ovipositional blood spots of *P. bovicola* in either group. However, from 4 months after bleeding commenced there was a significant reduction in blood spots in the arsenic-dipped cattle and, on slaughter at 12-14 months after infection, the arsenic group had significantly fewer live worms and fewer carcass lesions.

Arsenic residues in muscle samples of treated cattle were 11.6 times higher than in the controls. It is proposed that arsenic residues in the sub-cutaneous muscle layers increase with repeated dipping until a level toxic to *P. bovicola* is finally reached. Older cattle would therefore be refractory to infection and their carcasses at slaughter would not be affected.

### INTRODUCTION

Although *Parafilaria bovicola* infections were recorded for the first time only in 1963 (Pienaar & Van den Heever, 1964), the bleeding spots on the skin of the live animal have been seen for many decades. The spontaneous bleeding in spring was thought by some farmers to be an indicator of vigorous growth (Nevill, 1980).

*Parafilaria* lesions on carcasses and their economic importance were recognized only from 1964 onwards (Pienaar & Van den Heever, 1964; Van den Heever, Nevill & Horton, 1973). Many explanations have been advanced to explain this sudden real or apparent increase in *Parafilaria* lesions in cattle marketed from the Bushveld. From the writer's own experience at the Pretoria abattoir from 1971 onwards it is clear that the problem had for many years been confused with bruising, so that the increase here was apparent and not real. However, records at the Cato Ridge abattoir in Natal show that in 1981/82 total carcass condemnations due to parafilariasis increased by 585 % over the previous year (Wallace, Weaver, Kretzmann & Payne, 1983).

The rapid increase in the number of cattle slaughtered in recent years, the greater movement by road from remote areas and the earlier age at which cattle are marketed are all factors which could contribute to an apparent increase in the number of infected animals. Another important factor which could have had a limiting effect on the prevalence of *Parafilaria* infections may have been the use of arsenic in dips against ticks, since dipping has always been an essential practice in the Bushveld. Arsenical dips, either alone or in combination with chlorinated hydrocarbon insecticides and later organo-phosphate compounds, have been used extensively from 1910 up until about 1967 (Bekker, 1960; Malan, 1975). Since arsenic can be absorbed through the skin (Clarke & Clarke, 1975), and since organic arsenic compounds have been used in the treatment of *Wuchereria bancrofti* in man (Hawking, 1955), there appeared to be a possibility that arsenical dips would control *P. bovicola* infections in cattle.

With this in mind, in October 1978 the writer examined cattle for *P. bovicola* blood spots on a farm in the N.W. Transvaal Bushveld, as arsenical dipping was still practised on this farm. As a control the cattle on the 4 surrounding 'non-arsenic' farms were also examined. The difference between the 'arsenic farm' and the other 4 was highly significant ( $P=0,01$ ), since only 1 % of the cattle dipped in arsenic showed blood spots. During a

repeat visit exactly 1 year later the same tendency was found on the 'arsenic farm' and on all but 1 of the other 4 farms. Two of these farms were still highly significantly different from the 'arsenic farm' ( $P=0,01$ ) while 1 farm only showed a significant difference ( $P=0,05$ ).

To further investigate the value of arsenical dips for the control of *P. bovicola*, a controlled experiment using artificially infected cattle was undertaken on the Onderstepoort farm.

### MATERIALS AND METHODS

#### Artificial infection of calves

Fifty bull calves, between 1 and 7 days old, and mostly Afrikaner crossbreeds, were infected artificially with 3rd stage *P. bovicola* larvae between 25 September 1979 and 20 November 1979. The calves were born and raised on the Onderstepoort farm which falls just within the enzootic area for *P. bovicola*.

The 3rd stage larvae were dissected out of colonized *Musca xanthomelas* which had been infected when 4-7 days old and incubated at 27 °C for 11-14 days (Nevill, 1975; 1979). From 106-174 larvae in 1 ml of Eagle's medium were injected downwards under the skin of the right shoulder of each calf. This method was described by Nevill (1979) and Viljoen & Coetzer (1982).

The infection of the flies and of the calves had to be synchronized so that the calves were infected at as young an age as possible. Eventually, after 11 separate infection attempts over a period of nearly 2 months, all the calves had been injected. At each infection attempt the available calves were allocated to each of 2 separate groups so that ultimately each group of 25 would have a comparable history.

The 2 groups were run on the same veld but were dipped to control ticks in 2 separate plunge dips. One of these contained 1600 ppm  $As_2O_3$  and the other 'Supamix'<sup>1</sup> (50,6 % chlorfenvinphos and 55,0 % dioxathion) diluted 1 in 2200 to act as control. From 3 September 1980 until the end of the trial, however, the 'Supamix' group, for convenience, were hand-sprayed.

The calves were treated 28-32 times at the following intervals (see also Tables 1 & 2):

26 September 1979	—	30 January 1980	Every 2-3 weeks
6 February 1980	—	26 March 1980	Weekly
16 April 1980	—	25 June 1980	Every 1-2 weeks

EFFECT OF ARSENICAL DIPS ON *PARAFILARIA BOVICOLA* IN ARTIFICIALLY INFECTED CATTLE IN SATABLE 1 Cattle artificially infected with *P. bovicola*, Onderstepoort, 1979/80—experimental design and blood spot analysis for group treated with 'Supamix'

Ox No.	Date infected (1979)	No. of infective larvae	No. of blood spots	Days 1st—last blood spot	Prepatent period (days)	No. of times treated
3564	8 October	138	15	127	226	31
3611	8 October	138	0	—	—	31
3613	12 October	135	105	155*	222	30
3617	12 October	135	9	64	306	30
3619	19 November	160	64	127	212	28
3649	12 October	135	5	106	264	30
3729	20 November	130	0	—	—	28
3736	15 October	140	4	43	303	30
3753	16 October	174	0	—	—	30
3759	20 November	130	40	71	267	30
3761	26 October	160	147	155*	208	29
3764	20 November	130	0	—	—	28
3790	20 November	130	10	71	267	28
3791	26 October	160	1	1	334	29
3795	20 November	130	28	85	253	28
3800	26 October	160	0	—	—	29
3804	29/30 October	253	0	—	—	29
3807	30 October	123	85	155*	204	29
3816	26 October	160	68	141	222	29
3820	19 November	160	0	—	—	28
3822	29 October	130	0	—	—	29
3871	19 November	160	16	127	212	28
3873	5 November	106	0	—	—	29
3880	20 November	130	37	120	218	28

\* 155 days is the entire period from first to last examination for blood spots

TABLE 2 Cattle artificially infected with *P. bovicola*, Onderstepoort, 1979/80—experimental design and blood spot analysis for 'arsenic'-dipped group

Ox No.	Date infected (1979)	No. of infective larvae	No. of blood spots	Days 1st—last blood spot	Prepatent period (days)	No. of times dipped
3599	16 October	174	0	—	—	30
3604	12 October	135	42	155*	222	30
3610	15 October	140	15	120	247	30
3614	19 November	160	10	64	240	28
3615	25 September	117	7	43	309	32
3618	19 November	160	0	—	—	28
3622	8 October	138	0	—	—	31
3668	8 October	138	2	8	310	31
3669	12 October	135	16	113	250	30
3715	8 October	138	1	1	261	31
3733	20 November	130	0	—	—	28
3734	15 October	140	0	—	—	30
3735	20 November	130	30	127	197	28
3745	26 October	160	3	36	299	29
3746	12 October	135	0	—	—	30
3747	20 November	130	1	1	299	28
3756	16 October	174	0	—	—	30
3758	29 October	130	102	155*	205	29
3793	26 October	160	2	127	313	29
3797	29 October	130	73	141	219	29
3801	26 October	160	1	1	208	29
3810	29 October	130	1	1	296	29
3872	19 November	160	117	155*	184	28
3882	20 November	130	7	50	267	28

\* 155 days is the entire period from first to last examination for blood spots

July 1980 — August 1980 No dipping  
 3 September 1980 — 22 October 1980 Weekly

*Ovipositional blood spot evaluations*

Approximately 8 months after the 1st calves had been infected, examinations of the animals in both groups for blood spots produced by ovipositing *P. bovicola* females were started. The animals were examined on dipping days, just prior to being dipped, using the methods described by Nevill (1984) to locate, record and verify blood spots. This would provide proof that the calves had been successfully infected and could perhaps indicate if arsenic was adversely affecting the worms. Useful information on the prepatent period and the period of ovipositional activity of *P. bovicola* could also be gained.

*Evaluation of carcasses for lesions and worms*

Nine animals from each group were slaughtered from 5–16 days after the last dipping. To allow time for possi-

ble changes in the appearance of the lesions between the 2 groups due to lesion ageing and resorption, the remaining animals were slaughtered approximately 36 days later, 41–54 days after the last dipping. Because 1 calf died, the size of the groups was reduced to 24 each.

The following 2 categories of lesions were recognized (Nevill, 1979):

1. Subacute: yellow-brown to greenish and usually slimy or jelly-like;
2. Chronic: areas of slight superficial sliminess.

The shape, size and position of these lesions were indicated on carcass outline diagrams for each half carcass. Muscle, skin and liver specimens were taken and frozen for later arsenic residue determinations. Unfortunately only 8 muscle specimens were analysed later.

The surface muscle layers were then removed in strips, placed in buckets to which 0.85 % saline was

TABLE 3 Cattle artificially infected with *P. bovicola*, Onderstepoort, 1979/80—carcass appraisal for group treated with 'Supamix'

Ox No.	Days infection to slaughter	Worms recovered				% carcass lesion area	
		Embryonated ♀♀	Other ♀♀	♂♂	Total	Sub-acute	Chronic
3564	429	1	3	1	5	2,5	56,6
3611	394	—	7	—	7	4,0	64,4
3613	385	4	1	4	9	26,8	32,2
3617	426	1	3	1	5	0,5	46,5
3619	379	2	—	—	2	3,6	78,3
3649	389	1	2	2	5	0,5	57,8
3729	352	—	1	1	2	2,7	45,9
3736	380	1	4	—	5	3,1	3,4
3753	388	—	3	4	7	9,2	72,5
3759	379	1	—	—	1	8,6	51,1
3761	370	2	1	3	6	15,3	32,8
3764	388	—	4	—	4	0	49,4
3790	386	2	2	3	7	1,0	60,2
3791	367	—	1	—	1	10,0	10,1
3795	380	1	5	2	8	3,4	11,9
3800	416	—	3	2	5	0	50,3
3804	416	—	3	2	5	5,4	68,5
3807	370	2	2	—	4	2,8	61,0
3816	403	5	—	—	5	1,9	62,3
3820	396	—	2	1	3	0	65,0
3822	420	—	4	2	6	2,2	50,8
3871	385	—	6	—	6	0,4	70,2
3873	403	—	1	2	3	0	20,5
3880	380	2	—	1	3	12,0	57,9
Totals		25	58	31	114	4,83	49,15
Means							

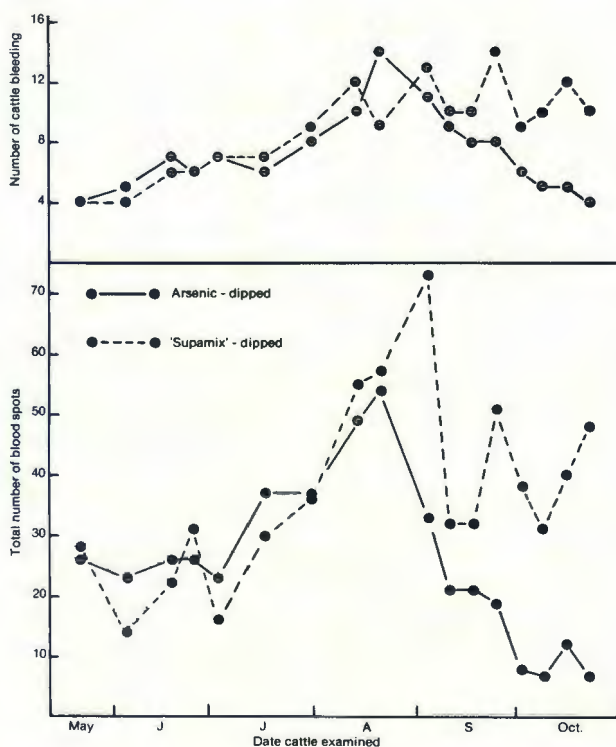


FIG. 1 Comparison of the prevalence and number of ovipositional blood spots of *Parafilaria bovicola* in 2 groups of 24 cattle each. One group was dipped regularly in 1600 ppm  $As_2O_3$  the other in 'Supamix' (chlorfenvinphos & dioxathion)—Onderstepoort 1980.

added, and heated in a water-bath to 40 °C, according to the method described by Viljoen (1982). After 3–4 hours the tissue was removed and the saline filtered and examined for *P. bovicola*. These were separated into males and females. After dissection in saline the females were further grouped into those containing embryonated eggs and those without.

#### Analysis of results

An image analyser<sup>1</sup> was used to measure carcass lesion areas on the outline diagrams and these were expressed as a percentage of the entire carcass area.

#### RESULTS

##### Ovipositional blood spots

The number of cattle in the 2 groups showing blood spots during the period they were being examined is indicated in Fig. 1. Seventeen cattle in the 'arsenic' group bled, compared with 15 in the 'Supamix' group. In the 'arsenic' group the number of cattle bleeding at a single examination reached a peak of 14 on 20 August 1980, but thereafter declined steadily to 4 on the last day (22 October). The 'Supamix' group showed a similar build-up, but fluctuated between 9 and 14 until the last day. Although the  $\chi^2$  test for 2 × 2 contingency tables showed that no significant differences existed between the 2 groups at any 1 sampling date, the overall tendency for change after 3 September is evident from Fig. 1.

The total number of blood spots for each animal in each of the 2 groups is recorded in Tables 1 & 2. The 'Supamix' group had an overall total of 634 spots compared with 428 for the 'arsenic' group. With the  $\chi^2$  test this difference is significant at the 1% level.

A comparison of the total number of blood spots for each group at each visit is also shown in Fig. 1. The tendency is the same as for the number of cattle bleeding in that after 3 September 1980 many fewer blood spots were recorded in the 'arsenic' group. The  $\chi^2$  test showed that these differences were statistically different (5% level) on 3 September and again from 24 September up until the end of the trial. When the total number of blood spots recorded in each group before 3 September was compared with the total number from 3 September on-

<sup>1</sup> Kontron Messgeräte MOP-10

EFFECT OF ARSENICAL DIPS ON *PARAFILARIA BOVICOLA* IN ARTIFICIALLY INFECTED CATTLE IN SATABLE 4 Cattle artificially infected with *P. bovicola*, Onderstepoort, 1979/80—carcass appraisal for 'arsenic'-dipped group

Ox No.	Days infection to slaughter	Worms recovered				% carcass lesion area	
		Embryonated ♀♀	Other ♀♀	♂♂	Total	Sub-acute	Chronic
3599	386	—	2	—	2	0,6	23,6
3604	417	3	—	—	3	0	24,3
3610	416	1	1	1	3	0	4,1
3614	385	1	1	2	4	0	43,8
3615	440	—	—	—	0	0,7	24,9
3618	353	1	1	1	3	4,5	41,7
3622	396	1	1	—	2	0,6	62,0
3668	430	1	3	1	5	1,3	48,7
3669	418	—	—	—	0	1,9	60,0
3715	387	1	1	2	4	13,6	41,5
3733	391	1	2	1	4	0	0
3734	427	—	2	1	3	0	59,3
3735	379	1	2	1	4	11,0	40,3
3745	412	—	1	—	1	0	27,7
3746	434	—	3	—	3	0	65,0
3747	342	4	3	—	7	1,8	3,2
3756	433	—	2	—	2	1,2	49,1
3758	368	1	—	1	2	0	20,8
3793	375	—	3	—	3	2,7	57,7
3797	371	5	—	2	7	3,9	49,8
3801	413	—	1	—	1	0	39,2
3810	410	—	—	—	0	7,4	33,7
3872	346	1	—	3	4	0	5,5
3882	386	—	1	—	1	3,6	37,2
Totals		22	30	16	68		
Means						2,28	35,96

TABLE 5 A comparison of *P. bovicola* carcass lesion areas and worm numbers in 'Supamix'-dipped and 'arsenic'-dipped groups of cattle slaughtered at various periods after artificial infection—Onderstepoort 1980

Cattle groups based on period from infection to slaughter	Mean lesion area per carcass (%)				No. of worms	
	Sub-acute lesions		Chronic lesions		'Supamix'	'Arsenic'
	'Supamix'	'Arsenic'	'Supamix'	'Arsenic'		
352-386 days	7,48	3,48	42,92	30,43	54	45
No. of cattle	12	8	12	11	12	12
388-429 days	2,18	1,09	55,38	41,50	60	23
No. of cattle	8	6	12	12	12	9
All 24 cattle	4,83	2,28	49,15	35,96	114	68
Total cattle with lesions/worms	20	14	24	23	24	21
Total No. of cattle infected	24	24	24	24	24	24

wards ( $\chi^2$  test), the difference between the 2 groups during the 2nd half of the trial was found to be highly significant (1% level).

The periods during which individual cattle bled are given in Tables 1 & 2. Of the 32 cattle that bled, 6 continued to bleed throughout the full 155-day period during which they were examined, while 15 bled for 4 months or longer.

#### Prepatent period

The periods from artificial infection to the first recorded blood spot are given in Tables 1 & 2. It must be remembered, however, that cattle were not examined for blood spots prior to 21 May 1980, so some spots may have appeared earlier. The shortest period recorded was 184 days and the longest 334 days. The means for the 2 groups were 247,9 and 254,5 days respectively. These differences were not significant (t-test). A frequency distribution of the periods for all 35 bleeders showed a fairly even distribution between the shortest and longest periods recorded.

#### Carcass lesions

The lesion areas for individual cattle are given in Tables 3 & 4. Because of variations in the ages of the infections in the slaughtered animals it was not plausible to analyse the results statistically. Instead the cattle were grouped as indicated in Table 5, so that the lesion areas in infections of different ages could be compared with each other as well as between the 2 treatments.

All 24 cattle in each treatment group were infected with *P. bovicola*, since lesions and/or worms were present in all (Tables 1, 2, 3 & 4). Consequently, treatment groups could reasonably be compared directly with each other, and the lesion areas on the 12 calves with the 'youngest' infection could reasonably be compared with those in the 12 calves with the 'oldest' infection.

*Sub-acute lesions:* Only 14 cattle in the 'arsenic' group showed lesions compared with 20 in the 'Supamix' group. The 'arsenic' group always had approximately half as many lesions as the 'Supamix' group. The cattle with the oldest infections had markedly fewer lesions of this type, no matter what the treatment. Also only 14 of the 24 cattle in the older group had sub-acute lesions compared with 20 in the younger group (Table 5).

*Chronic lesions:* The numbers of cattle in each group with this type of lesion were comparable, and nearly all were positive. In general, the 'arsenic' group had slightly fewer lesions, but their areas were still large. In both groups the lesion areas increased in the older group (Table 5).

#### Worms recovered

The number of worms in the various categories recovered from the carcasses of 48 cattle are recorded in Tables 3, 4 & 5. An analysis of these results using  $\chi^2$  tests showed that, except in the case of embryonated females, there were significantly fewer worms in each category in 'arsenic'-dipped cattle (5% level).

TABLE 6 Residues of arsenic trioxide in the subcutaneous muscle tissue of dipped cattle, Onderstepoort 1979/80

Ox No.	Dipped in	$\mu\text{g/g As}_2\text{O}_3$ (dry basis)
3613	'Supamix'	0,14
3736	'Supamix'	0,04
3761	'Supamix'	0,18
3807	'Supamix'	0,08
Mean	—	0,11
3715	'arsenic'	1,20
3758	'arsenic'	1,00
3797	'arsenic'	1,60
3872	'arsenic'	1,30
Mean	—	1,28

In addition, it can be seen from Table 5 that the number of worms recovered in the arsenic-dipped cattle diminished rapidly in the older animals, but not so in the 'Supamix' group where it increased slightly.

#### Arsenic residues

Only 8 subcutaneous muscle tissue samples were analysed for residues of arsenic trioxide. The results appear in Table 6.

Much greater (11,59 $\times$ ) amounts of  $\text{As}_2\text{O}_3$  were found in the arsenic-dipped cattle.

#### DISCUSSION

Repeated dipping in arsenic definitely has an adverse effect on *P. bovicola* and this could help to explain why this parasite went unrecorded for so long in South Africa. The present trial shows that, although initially there was no obvious adverse effect of arsenic on the worms in infected cattle, this became more apparent as time went by. In the arsenic group the number of cattle bleeding declined with time, as did the number of blood spots and the number of worms found in the carcasses. During the same period, in the 'Supamix' group, however, the number of cattle bleeding and the number of blood spots recorded did not decrease markedly with the passage of time, while the number of worms recovered from carcasses actually increased slightly.

It seems that it is necessary for the arsenic present in the surface muscle layers, where *P. bovicola* is found, to build up to a toxic level before it will adversely affect the worms present. It is clear from the residue determinations that there is a sharp increase in arsenic levels compared with those in the control group.

During the period when arsenic dips were commonly in use, the slaughter age of cattle was approximately 3 years and older. This would mean that a large proportion of the cattle on a farm should have built up a high enough level of arsenic to render them immune to *P. bovicola* infection. The annual calf crop, although susceptible to infection, would have served only to maintain the life-cycle and, as these young animals were not marketed, the infection would have had no economic significance. Alternatively the arsenic dip may have a direct toxic action on the ovipositing *P. bovicola* females at the time of dipping. If this were so, however, there should also have been a marked difference between the 2 treatment groups in the beginning of the blood spot season.

The large number of artificially-infected cattle provided the opportunity to collect more information on the biology of *P. bovicola*. In previous work (Nevill, 1984) the prepatent period was shown to range from 191–279 days in 81,8% of field-infected cattle. The present artificial infections support those findings, the minimum prepatent period being reduced to 184 days with the maximum 334 days. The even 'spread' between these 2 extremes suggests that the rate of development of individual worms varies. This could also account for the period of up to 155 days over which blood spots were recorded from individual animals, although the latter may be the actual ovipositional period of activity for this worm.

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