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STUDIES ON *PARAFILARIA BOVICOLA* TUBANGUI, 1934. 2. CHEMOTHERAPY AND PATHOLOGY

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ABSTRACT

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The filaricidal effects of trichlorphon, arsenic trioxide, sodium antimony biscathecol disulphonate and nitroxynil against *Parafilaria bovicola* were the subject of this investigation. Levamisole hydrochloride was retested in a separate trial to assess the time required for healing after successful treatment.

A comparison of carcass lesions and the percentage of lesion area in untreated controls with those in treated animals reconfirmed the efficacy of levamisole hydrochloride, while nitroxynil gave more promising results. Both lesions and lesion area were reduced by 76% by the former and by 93% by the latter compound. The other drugs only slightly suppressed *P. bovicola* activity.

After treatment with levamisole hydrochloride, active lesions were still present for 1–4 weeks and visible lesions on slaughter for 8 weeks. Since the visible lesions during the 8 weeks varied from acute to chronic and as they disappeared 9 weeks post treatment, it is suggested that provision should be made for a healing period of at least 9 weeks.

Résumé

ETUDES SUR PARAFILARIA BOVICOLA *TUBANGUI*, 1934. 2. *CHIMIOTHERAPIE ET PATHOLOGIE*

Cette étude porte sur l'efficacité de trichlorphon, trioxide arsénical, disulphonate biscathecol antimonié d'azote et nitroxynil contre la filaire Parafilaria bovicola. *Pour déterminer la durée de la période de guérison à la suite d'un traitement efficace, l'hydrochloride de lévamisole était testée de nouveau dans un essai à part.*

Une comparaison des lèsions an niveau des carcasses et le pourcentage des zones lésées chez les contrôles non-traités avec ceux d'animaux traités, a confirmé l'efficacité de l'hydrochloride de lévamisole, tandis que la nitroxynil paraissait encore plus efficace. Il y a eu une diminution de 75% et du nombre de lésions et des zones lésées à la suite de l'emploi du premier et de 93% à la suite de l'emploi du dernier. Les autres vermifuges n'ont effectué qu'une suppression légère de l'activité de P. bovicola.

A la suite d'un traitement à l'hydrochloride de lévamisole des lésions actives ont persisté de 1 *à* 4 *semaines et celles qui étaient visibles à l'abattage pendant* 8 *semaines. Comme ces dermières peuvent être soit aiguës soit chroniques et comme leur disparition a lieu* 9 *semaines après le traitement, les auteurs estiment nécessaire de garder une période de guérison d'au moins* 9 *semaines.*

INTRODUCTION

During 1974–75, clinical observations and treatment of animals with natural infestations of *P. bovicola* were carried out by Viljoen (1976). These experiments were continued subsequently, with the results reported here.

In an earlier trial Viljoen used trichlorphon when lesions were already resolving but, as this could have masked the drug's effect, it was included again in the present study.

In addition, antimonial drugs were tested because various workers have stated that these preparations are curative (Gulati, 1934; Khajuria, 1966; Masillamony & Souri, 1959; Sahai, Singh & Srivastava, 1965 and Srivastava, Sinha, Singh & Sahai, 1972).

In the past, arsenical preparations were used routinely for tick control in cattle dips in tick-infested areas. By 1964, however, when *P. bovicola* infestations were first diagnosed in the Republic by Pienaar & Van den Heever, arsenical dips had largely been replaced by organophosphorus acaricides. As it seemed possible that arsenic trioxide may have had a suppressive effect on *P. bovicola* in the live animal, it was included in the present experiments.

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Viljoen (1976) showed that high doses of levamisole hydrochloride, which are lethal to *P. bovicola*, resulted in the recovery of the treated animals. This fact was not known, however, before the previous experiment, and the specimens collected from these animals were inadequate for studying the healing process and its duration. Levamisole was therefore used again in the present experiment and administered at 12 mg/kg on 4 successive days. Samples of all visible lesions taken when the animals were slaughtered at 2–3 weekly intervals were examined microscopically.

Included in the experiment was the effect of nitroxynil as a filaricide.

MATERIALS AND METHODS

Experimental work with cattle born during the period October–Desember 1974 was conducted at Zoutpan, a Government farm in the northern Transvaal. At the end of August 1975, 51 oxen were selected from the animals exhibiting lesions of *P. bovicola* and randomly subdivided into the following 6 groups:

(1) One group of 10 control animals.

(2) Four treatment groups of 9 animals each.

(3) One treatment group consisting of the 5 remaining animals.

To facilitate treatment and observations, all the animals were transferred to the experimental farm of the Veterinary Research Institute, Onderstepoort.

TABLE 1 Programme of treatment, 1975

Group No.	Animals treated	Remedy	Period of treatment	Dosage rate	No. of treatments	Method of treatment
1	9	Trichlorphon—50% m/v (Dylox Inject: Bayer Agrochem)	16–25 Sept.	15 mg/kg	4 (at 3-day intervals)	Intramuscular injection
2	9	Arsenic trioxide—80% m/v (Arsenical Dip: Agricura)	10 Sept.–5 Nov.	0,16% Dip	9 (at weekly intervals)	Dipping
3	9	Sodium antimony biscathecol disulphonate—13,5% m/v (Antimosan: Bayer Agrochem)	16 Sept.–1 Oct.	50 ml	3 (at weekly intervals)	Intramuscular injection
4	9	Nitroxynil—34% m/v (Trodax: May & Baker)	18–22 Sept.	20 mg/kg	2 (at 3-day intervals)	Subcutaneous injection
5	5	Levamisole hydrochloride—7,5% m/v (Ripercol-l: Ethnor)	15–18 Sept.	12 mg/kg	4 (daily)	Intramuscular injection

Treatments

As indicated in Table 1, 5 compounds were used. Healing was studied in the group of 5 animals treated with levamisole.

Apart from the group which was dipped weekly in arsenic trioxide until slaughter, all groups were treated during the second half of September 1975.

Clinical observations before, during and after treatment were carried out as for the previous trial (Viljoen, 1976).

Slaughter trials

The control animals were slaughtered during the period 6 November 1975–18 March 1976. During the examination of the carcasses, the number of lesions were recorded and the dimensions of each measured directly on the carcass. After skinning, the total skin surface of each animal was estimated in cm^2 by calculating the product of the length through the midline and the breadth at the widest part of the skin.

To differentiate between typical parafilarial lesions and bruised areas, smears were taken from all lesions and stained by the Giemsa method to detect the presence or absence of eosinophiles. In the final calculations only eosinophile positive areas were taken into account and lesions and lesion surfaces per animal in each group calculated.

With the exception of the animals in the levamisole group, which were slaughtered between 9 October and 9 December 1975 for the assessment of the period of healing, all animals in the treatment groups were slaughtered between 6 November and 11 December 1975. Carcass examinations were similar to those for the untreated controls. In the levamisole group, samples of all visible lesions were collected in 10% neutral buffered formalin. After being embedded in paraffin wax, sections of 3μ thickness were examined microscopically to determine the possible time of healing.

RESULTS

The seasonal incidence of bleeding points in control animals in the 1975–76 survey is shown in Fig. 1 and that of the animals receiving the various treatments in Fig. 2–4.

The percentage increase or decrease of visible bleeding points after treatment is compared in Table 2 with that of the control group.

TABLE 2 Effect of treatment on frequency of bleeding points observed

Group description or treatment	Bleeding points observed		Percentage increase or decrease
	During the 6 weeks before treatment	During the 6 weeks after treatment	
Untreated controls.....	62	175	+182
Trichlorphon.....	59	158	+168
Arsenic trioxide (dipping)	41	47	+14,6
Sodium antimony.....	40	30	−25
Nitroxynil.....	71	37	−48
Levamisole hydrochloride	33	8	−76

During the 6-week-period after treatment, nitroxynil reduced the number of bleeding points by 48% and levamisole by 76%. Sodium antimony reduced the bleeding points by only 25% during the same period.

Table 3 is a summary of the effects of each compound on the number of lesions and the eventual lesion area per carcass in each group. On a percentage basis levamisole and nitroxynil decreased lesion areas by 76% and 93% respectively. Smear examinations revealed that 93% of all visible lesions and 94% of the total lesion surface were positive for eosinophile infiltrations.

The reactions of individual animals after levamisole treatment are summarized in Table 4.

Bleeding points were observed for 1–4 weeks after treatment in all these animals and, 4–8 weeks after treatment, the carcasses of the first 3 animals slaughtered (W+1, W+2 and W+5) were still affected. However, 9–12 weeks after treatment, animals W+3 and W+4, slaughtered on 20 November and 9 December 1975 respectively, proved negative on carcass examination.

Histopathological examinations of samples from slaughtered animals in the levamisole group seem to confirm these observations. In the first 2 animals slaughtered (W+1 and W+2) lesions were more

acute than those in the 3rd animal slaughtered (W+5). Perivascular cell infiltrations (lymphocytes and eosinophils) were more marked while oedema and fibrin deposits were also seen. Migration tracks were clearly visible. In the tissue surrounding these tracks eosinophils and macrophages, the latter containing haemosiderin, were especially numerous and pronounced. The dead parasites seen in samples from animal W+2 appeared to be autolyzed with only moderate mineralization. They were enclosed by the typical granulomatous reaction observed by Pienaar & Van den Heever (1964) and a few giant cells were also seen adjacent to the thin connective tissue capsule.

In samples from animal W+5, slaughtered 8 weeks after treatment, the lesions were partly healed and difficult to see. The parasitic granulomas contained a pronounced areolar connective tissue layer with collagen more advanced in its development, while the enclosed parasites showed advanced autolysis and mineralization. In addition, cellular reactions were limited to a few scattered eosinophils and polymorphs. Animals slaughtered 9 and 12 weeks after treatment were free of lesions and healing was probably completed.

In the present survey the mean prepatent period for all experimental animals was 253,608±24,781 days. This period represents the mean interval between birth and the appearance of the first bleeding lesions.

TABLE 3 Effect of treatment on lesions per carcass observed and percentage carcass area affected

Group description or treatment	Animals slaughtered	Period of slaughter	Mean number of carcass lesions		Percentage increase or decrease (lesions)	Mean Subcutaneous area per animal (cm^2)	Mean lesion areas (cm^2)		Mean percentage affected areas		Percentage increase or decrease (lesion areas)
			Total	E+			Total	E+	Total	E+	
Untreated controls	10	6 Nov. 1975–18 March 1976	8,5	8,2	—	24 168	2 715	2 640	11,2	10,9	—
Trichlorphon.....	9	6 Nov.–11 Dec. 1975	6,3	6,2	−24	20 860	3 181	3 138	15,2	15,0	+38*
Sodium antimony	9	6 Nov.–11 Dec. 1975	5,4	5,2	−27	22 144	1 899	1 848	8,6	8,3	−24
Arsenic trioxide (Dipping)	9	6 Nov.–11 Dec. 1975	5,3	5,2	−27	22 750	1 554	1 542	6,8	6,7	−38
Levamisole hydrochloride	5	9 Oct. – 9 Dec. 1975	2,2	1,8	−78	21 950	725	568	3,3	2,6	−76
Nitroxynil.....	9	6 Nov. 1975–8 Jan. 1976	2,0	0,8	−90	21 500	390	181	1,8	0,8	−93

E+ =Lesions or lesion areas eosinophile positive
Total=Visible lesions or lesion areas
* =Mean increased because extensive lesions present in one animal

TABLE 4 Individual results after levamisole treatment and slaughter

Identification	Date treated	Bleeding points observed		Period between treatment and slaughter (weeks)	Date slaughtered	Visible carcass lesions		Histopathological classification of lesions
		During the 6 weeks before treatment	During the 6 weeks after treatment			Total	E+	
W+1	15–18 Sept. 1975	10	2**	4	9 Oct. 1975	4	4	Acute
W+2	15–18 Sept. 1975	3	1	6	30 Oct. 1975	5	5	Acute
W+5	15–18 Sept. 1975	5	1	8	11 Nov. 1975	2	1	Chronic*
W+3	15–18 Sept. 1975	9	1	9	20 Nov. 1975	0	0	—
W+4	15–18 Sept. 1975	6	3	12	9 Dec. 1975	0	0	—

E+ =Eosinophile positive lesions
Total=All visible lesions
* =Chronic changes
** =Animal slaughtered 4 weeks after treatment

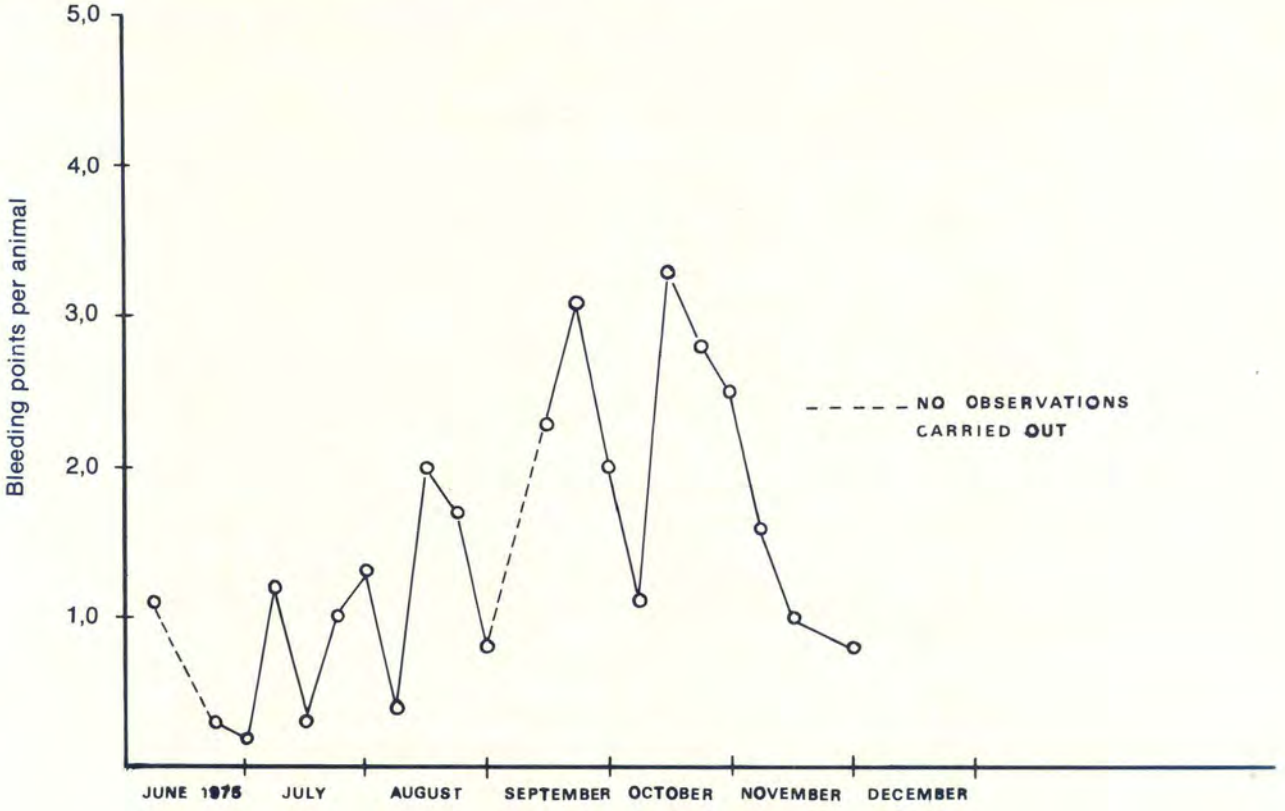

FIG. 1 Incidence of bleeding points in untreated control animals

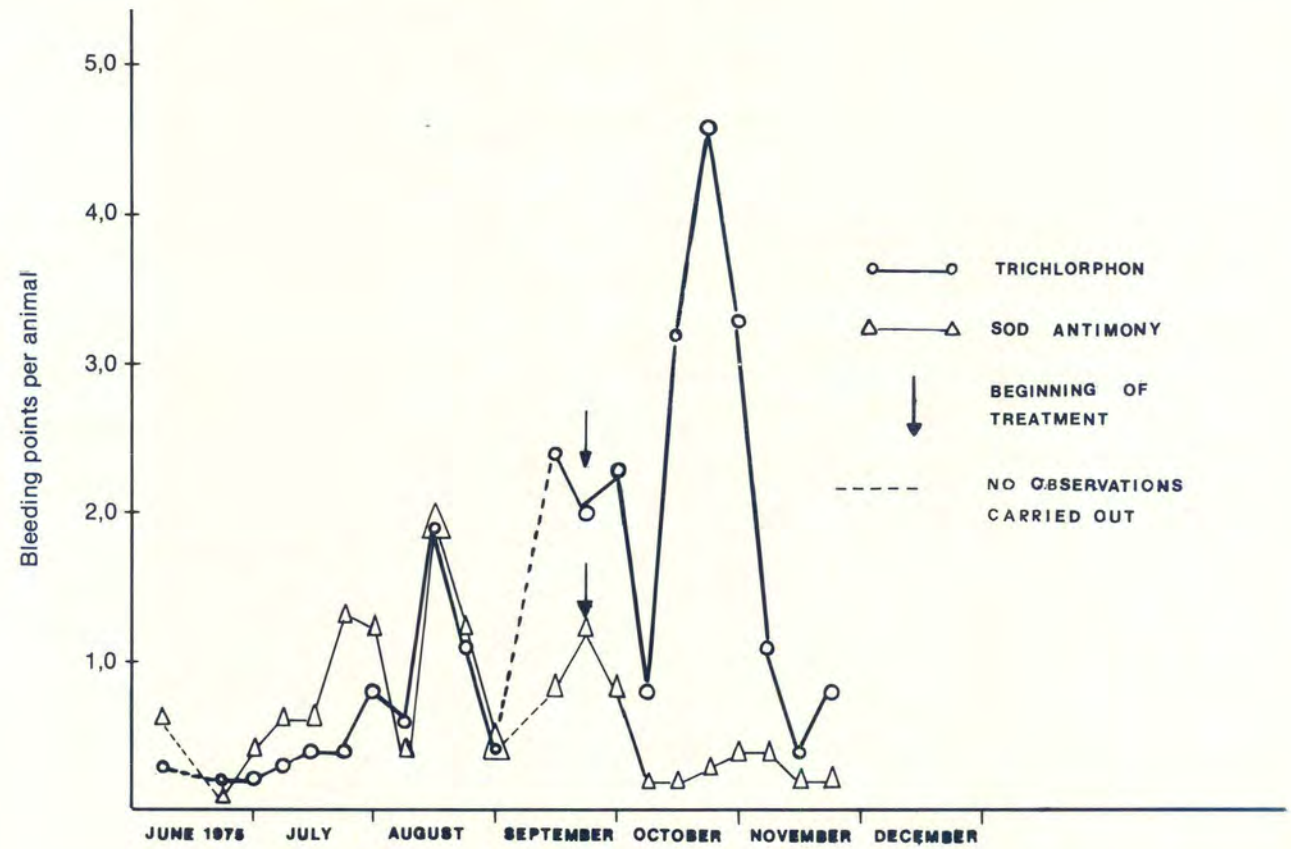

FIG. 2 Effect of treatment on bleeding points observed

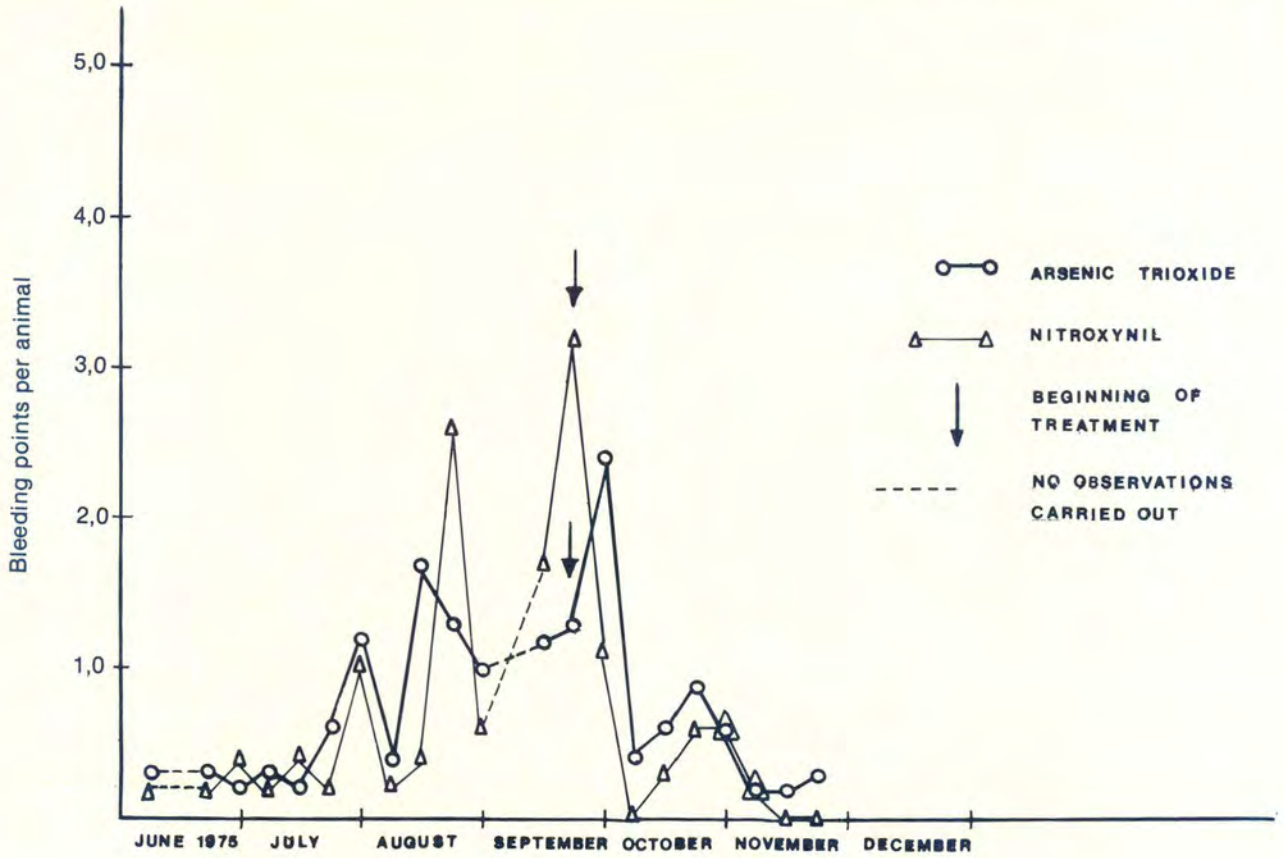

FIG. 3 Effect of treatment on bleeding points observed

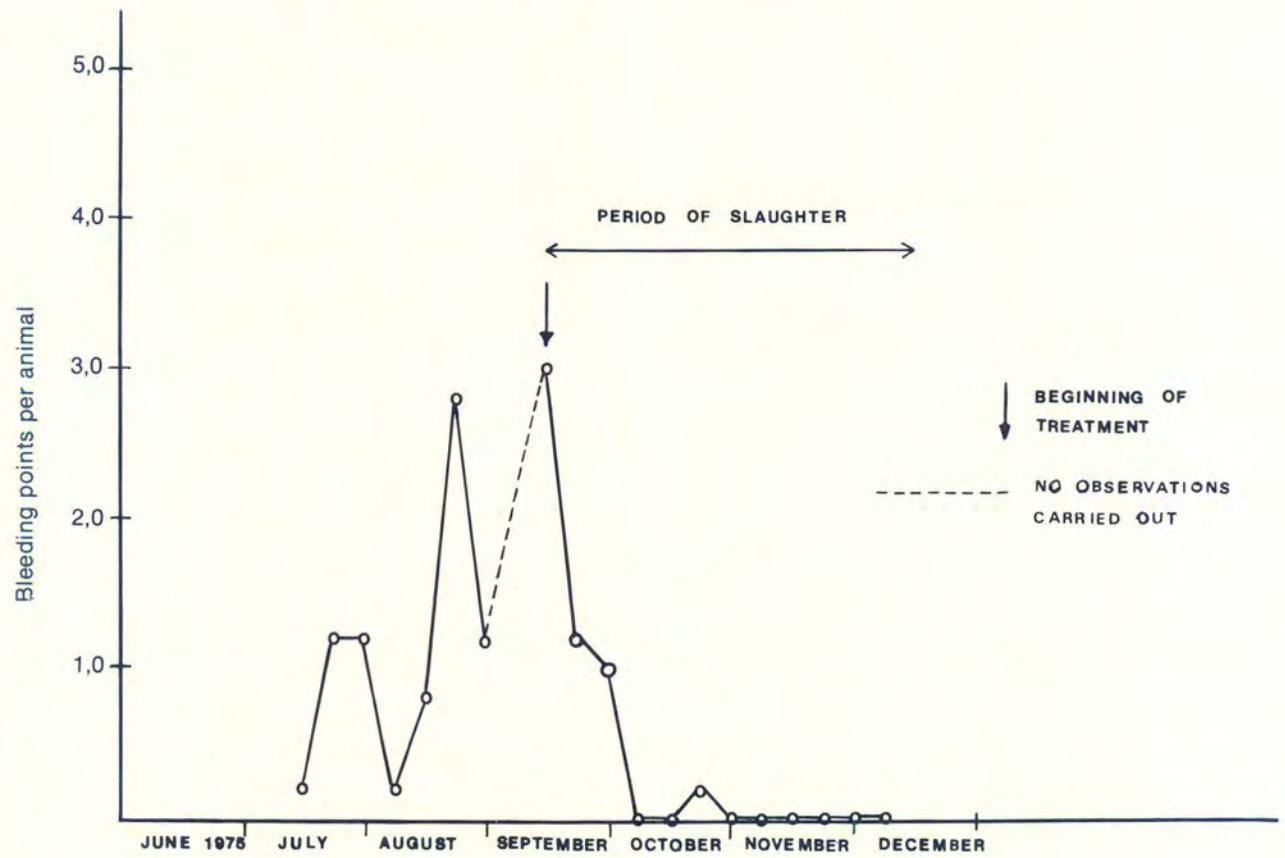

FIG. 4 Levamisole hydrochloride treatment and period of healing

Discussion

For the purposes of this experiment it was decided that the following 2 minimum requirements must be fulfilled before a drug may be regarded as having some effect:

(A) Bleeding points must be reduced by 50% or more in animals treated during September or early October before the natural decline in the bleeding incidence.

(B) Typical lesions and lesion area per carcass must be less than half of those found in the control group.

Only levamisole and nitroxynil fulfilled these requirements.

The fallacy of judging filaricidal efficacy on the decline of bleeding points only was already demonstrated in the previous survey (Viljoen, 1976). This is further emphasized by the results summarized in Table 2 as well as by the results given in Fig. 2, 3 and 4, from which levamisole appeared to be more effective than nitroxynil.

The number of lesions per carcass is a reasonably accurate method of assessing efficacy but can be misleading in the case of either very small or large lesions. The group treated with trichlorphon had fewer lesions per carcass than the control group but the actual lesion surfaces were considerably greater than those of the latter group (Table 3).

The results of this trial suggest that, in addition to levamisole, nitroxynil can also be used to drastically reduce or eliminate lesions in *P. bovicola*-infested animals. Even at the high total dosage rate of 40 mg/kg, the cost of treatment with nitroxynil is not excessive. This drug, being registered for use at 10 mg/kg only, would imply the administration of 4 separate injections and possibly a longer period between treatment and slaughter for human consumption. The therapeutic level of nitroxynil is at this stage still unknown. If the dosage rate of 40 mg/kg were to be reduced, both the number of injections and length of the withdrawal period could be correspondingly decreased.

The treatment with trichlorphon was ineffective even at the high dosage rate employed, and antimony and arsenic only had a mildly suppressive effect.

In animals treated with levamisole, the effect on *P. bovicola* was not immediate and active lesions were still observed 1–4 weeks post treatment. Following treatment, healing apparently occurred during the greater part of the first 8 weeks; therefore animals should not be slaughtered until at least 9 weeks after treatment.

The mean prepatent period of $253,608 \pm 24,781$ days recorded in the present survey compares closely with the $250,517 \pm 26,006$ days on the 1974–75 trial (Viljoen, 1976), the difference being non-significant at $P<0,01$. The seasonal pattern in the incidence of bleeding points was also the same in both seasons, with peak values in September and October. During this period the transmission of *P. bovicola* infestations should be at their higest levels.

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References

GULATI, R. L., 1934. Filaria haemorrhagica in cattle and its treatment. *Indian Veterinary Journal*, 11, 32–34.

KHAJURIA, R. R., 1966. Incidence and control of parafilariasis in bovines in Jammu province. *Indian Veterinary Journal*, 43, 749–750.

MASILLAMONY, R. & SOURI, B. M., 1959. A case of, filariasis in bovines. *Madras Veterinary College Annual*, 17, 30–33.

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.

SAHAI, B. N., SINGH, S. P. & SRIVASTAVA, V. K., 1965. Chemotherapy of parafilariasis in bovines. *Indian Veterinary Journal*, 42, 881–883.

SRIVASTAVA, V. K., SINHA, B. K., SINGH, S. P. & SHAI, B. N., 1972. Chemotherapy of *Parafilaria bovicola* infection in cattle. *Orissa Veterinary Journal*, 7, 151–153.

VILJOEN, J. H., 1976. Studies on *Parafilaria bovicola* (Tubangui, 1934). 1. Clinical observations and chemotherapy. *Journal of the South African Veterinary Association*, 47, 161–169.

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