STUDIES ON PARAFILARIA BOVICOLA TUBANGUI, 1934.

2. CHEMOTHERAPY AND PATHOLOGY

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ABSTRACT


The filaricidal effects of trichlorphon, arsenic trioxide, sodium antimony biscalhocol 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. bovico/a 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 arsenical, disulphonate biscalhocol antimonin d'azote et nitroxynil contre le filaire Parafilaria bovicola. Pour déterminer la durée de la période de guérison à la suite d'un traitement efficace, l'hydrochloride de levamisole était testée de nouveau dans un essai à part.

Une comparaison des lesions an niveau des carcasses et le pourcentage des zones lésées chez les animaux non-traités avec ceux d'animaux traités, a confirmé l'efficacité de l'hydrochloride de levamisole, tandis que la nitroxynil paraissait encore plus efficace. Il y a eu une diminution de 75% et du nombre de lesions 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 levamisole des lesions actives ont persisté de 1 à 4 semaines et celles qui étaient visibles à l'abattage pendant 8 semaines. Comme ces dernières 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; Masilamony & Souri, 1959; Sahai, Singh & Srivastava, 1966 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.

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-December 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.
STUDIES ON PARAFILARIA BOVICOLA. TUBANGUI, 1934

TABLE 1 Programme of treatment, 1975

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Animals treated</th>
<th>Remedy</th>
<th>Period of treatment</th>
<th>Dosage rate</th>
<th>No. of treatments</th>
<th>Method of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9</td>
<td>Trichlorphon—50% m/v (Dylox Inject: Bayer Agrochem)</td>
<td>16-25 Sept.</td>
<td>15 mg/kg</td>
<td>4 (at 3-day intervals)</td>
<td>Intramuscular injection</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>Arsenic trioxide—80% m/v (Arsenic Dip: Agricura)</td>
<td>10 Sept.—5 Nov.</td>
<td>0,16% Dip</td>
<td>9 (at weekly intervals)</td>
<td>Dipping</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>Sodium antimony bismuth sulphate—13.5% m/v (Antimons: Bayer Agrochem)</td>
<td>16 Sept.—1 Oct.</td>
<td>50 ml</td>
<td>3 (at weekly intervals)</td>
<td>Intramuscular injection</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>Nitroxynil—34% m/v (Trofax: May &amp; Baker)</td>
<td>18-22 Sept.</td>
<td>20 mg/kg</td>
<td>2 (3-day intervals)</td>
<td>Subcutaneous injection</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>Levamisole hydrochloride—7.5% m/v (Ripercol:1; Ethnor)</td>
<td>15-18 Sept.</td>
<td>12 mg/kg</td>
<td>4 (daily)</td>
<td>Intramuscular injection</td>
</tr>
</tbody>
</table>

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 cm² 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.

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.

TABLE 2 Effect of treatment on frequency of bleeding points observed

<table>
<thead>
<tr>
<th>Group description or treatment</th>
<th>Bleeding points observed</th>
<th>Percentage increase or decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated controls..............</td>
<td>62 175</td>
<td>+182</td>
</tr>
<tr>
<td>Trichlorphon................. 59</td>
<td>158 55</td>
<td>+168</td>
</tr>
<tr>
<td>Arsenic trioxide (dipping)</td>
<td>41 47</td>
<td>+14.6</td>
</tr>
<tr>
<td>Sodium antimony...............</td>
<td>40 30</td>
<td>-25</td>
</tr>
<tr>
<td>Nitroxynil....................</td>
<td>71 37</td>
<td>-48</td>
</tr>
<tr>
<td>Levamisole hydrochloride......</td>
<td>33 8</td>
<td>-76</td>
</tr>
</tbody>
</table>

During 1-4 weeks after treatment in all these animals and, 4-8 weeks after treatment, the carcases 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.

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

<table>
<thead>
<tr>
<th>Group description or treatment</th>
<th>Animals slaughtered</th>
<th>Period of slaughter</th>
<th>Mean number of carcass lesions</th>
<th>Percentage increase or decrease (lesions)</th>
<th>Mean Subcutaneous area per animal (cm²)</th>
<th>Mean lesion areas (cm²)</th>
<th>Mean percentage affected areas</th>
<th>Percentage increase or decrease (lesion areas)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated controls</td>
<td>10</td>
<td>6 Nov. 1975-18 March 1976</td>
<td>8,5</td>
<td>8,2</td>
<td>24 168</td>
<td>2 715</td>
<td>11,2</td>
<td>10,9</td>
</tr>
<tr>
<td>Trichlorphon...</td>
<td>9</td>
<td>6 Nov.-11 Dec. 1975</td>
<td>6,3</td>
<td>6,2</td>
<td>-24</td>
<td>20 860</td>
<td>3 181</td>
<td>15,2</td>
</tr>
<tr>
<td>Sodium antimony</td>
<td>9</td>
<td>6 Nov.-11 Dec. 1975</td>
<td>5,4</td>
<td>5,2</td>
<td>-27</td>
<td>22 144</td>
<td>1 899</td>
<td>8,6</td>
</tr>
<tr>
<td>Arsenic trioxide (Dipping)</td>
<td>9</td>
<td>6 Nov.-11 Dec. 1975</td>
<td>5,3</td>
<td>5,2</td>
<td>-27</td>
<td>22 720</td>
<td>1 554</td>
<td>6,8</td>
</tr>
<tr>
<td>Levamisole hydrochloride</td>
<td>5</td>
<td>9 Oct.-9 Dec. 1975</td>
<td>2,2</td>
<td>1,8</td>
<td>-78</td>
<td>21 950</td>
<td>725</td>
<td>3,3</td>
</tr>
<tr>
<td>Nitroxynil...</td>
<td>9</td>
<td>6 Nov. 1975-8 Jan. 1976</td>
<td>2,0</td>
<td>0,8</td>
<td>-90</td>
<td>21 500</td>
<td>390</td>
<td>1,8</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Identification</th>
<th>Date treated</th>
<th>Bleeding points observed</th>
<th>Period between treatment and slaughter (weeks)</th>
<th>Date slaughtered</th>
<th>Visible carcass lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>During the 6 weeks before treatment</td>
<td>During the 6 weeks after treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W+5</td>
<td>15-18 Sept. 1975</td>
<td>5</td>
<td>1</td>
<td>11 Nov. 1975</td>
<td>Chronic*</td>
</tr>
<tr>
<td>W+3</td>
<td>15-18 Sept. 1975</td>
<td>9</td>
<td>1</td>
<td>20 Nov. 1975</td>
<td>Acute</td>
</tr>
</tbody>
</table>

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
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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 ± 24,781 days recorded in the present survey compares closely with the 250,517 ± 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 highest levels.

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REFERENCES


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