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EVALUATION OF THE EFFICACY OF ANTHELMINTICS AGAINST PARAFILARIOSIS IN CATTLE

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ABSTRACT

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Parafilariosis was first described in South Africa in 1964, thereafter being discovered at numerous localities in the country. When it became obvious that *Parafilaria bovicola*, for which no treatment was known, caused considerable economic losses, trials involving a series of compounds were conducted to identify candidate remedies. This paper describes an anthelmintic test for evaluating the efficacy of compounds for registration for field use.

Recovery of *Parafilaria* worms is impractical for anthelmintic testing, and consequently the lesion sizes of treated and control groups of cattle are compared statistically, using appropriate statistical tests. The seasonal incidence of mature worm infection in cattle in South Africa is such that trials should commence after June and be completed before the end of January, allowing a lapse of 70 days between treatment and slaughter for resolution of the lesions. The presently available parafilaricidal compounds while of value for treating slaughter stock, when used alone will probably not be effective for control of infection in the field.

INTRODUCTION

Parafilariosis in South Africa was first reported by Pienaar & Van den Heever in 1964, although farmers had observed bleeding spots on cattle for at least 45 years prior to their report (Carmichael, 1989). The condition was initially found in cattle near Potgietersrus in northern Transvaal and thereafter in numerous localities throughout the country (Carmichael & Koster, 1978).

Although there was no evidence that the disease was spreading, the discoveries in new localities were interpreted by many as a spread of parafilariosis from what was regarded as the primary focus (Schafer, 1975; Anon., 1976). Although it is possible that there was an increase in the intensity of infection in the endemic areas, increased diagnosis probably resulted from increased awareness and vigilance (Carmichael, 1989).

Reports of an escalation of the disease caused considerable concern amoung the farming community in the country, especially because no anthelmintics had been shown to be effective against the parasite, and financial loses (loss of affected tissue, down-grading and condemnation of carcases) were considerable (Carmichael & Koster, 1978). Consequently, Viljoen (1976) and later Viljoen & Boomker (1977) screened compounds for parafilaricidal action and identified a few candidate remedies.

However, no standardized test was available for the evaluation of compounds for registration for field use. The test outlined in this paper was developed for this purpose.

THE ANTHELMINTIC TRIAL

1. Factors considered in determining the trial design

(a) *Selection of cattle for the trial*

(i) For the following practical reasons only naturally infected cattle can be used:

First, the natural method of *P. bovicola* transmission has not been determined, hence it is not possible at present to standardize a technique for artificial infections. Although Nevill (1979) has succeeded in establishing artificial infections through several routes, consistent results were not obtained and expensive laboratory support (maintenance of *Musca* colonies) was required.

Second, the prepatent period of *P. bovicola* is 7–10 months (Viljoen, 1976; Nevill, 1979) and the most severe lesions of parafilariosis are caused by adult worms (Carmichael & Koster, 1978). This means that artificially infected animals would need to be maintained for several months before a trial could commence.

(ii) Because the severity of parafilariosis varies between the sexes and ages of naturally-infected cattle (Carmichael, 1981), it is important to select a homogeneous group from a single herd that has been on the farm of origin for at least a year. This will reduce, as far as possible, variations between animals in lesion size which may be due to sex, age, breed, or degree of exposure to *P. bovicola*.

(b) *Quantification of the infection*

(i) *Bleeding spots (focal cutaneous haemorrhages).* A reduction in the number of bleeding spots on the animal was used as an indication of efficacy of candidate compounds in the search for effective parafilaricidal anthelmintics (Viljoen, 1976). Although valuable as corroborative evidence and as a means of screening compounds, a reduction in bleeding spots is insufficient for adequate evaluation and comparison of remedies. Lesions have been encountered in animals with no pre-slaughter evidence of bleeding spots and the spots on untreated infected animals show considerable short term variations in number and locality, probably due to subcutaneous worm migration (Viljoen, 1976; 1982).

(ii) *Worm recovery.* Total worm recoveries are impractical with the available methodology. Usually only a few worms are present (Carmichael, 1989), they are difficult to detect and may be widely distributed throughout the subcutaneous tissue, the intermuscular fascia and possibly other areas of the body. Accurate total *in situ* counts are therefore impossible with existing techniques.

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Theoretically, all worms could be recovered by incubating the entire tissues of the animal in saline baths, but such a procedure would make trials prohibitively expensive, both in terms of labour and capital. Moreover, it would be impractical to confirm what percentage of the total worm burden had migrated from the tissues into the saline, without which the efficacy of the compound could not be calculated.

(iii) *Differences in* P. bovicola *lesion surface area.* This indirect method of determining the efficacy of a compound against *P. bovicola* is not ideal, because the factors influencing lesion area are unknown and it is difficult to define the perimeter of many lesions, let alone their depth. Nevertheless, it was selected as the most practical means of evaluating the effect of treatment. The assumption was made that if the surface area of *P. bovicola* lesions was significantly smaller in treated than in control animals, this was brought about by some direct effect of the anthelmintic on the parasite and hence on the lesion/s it induced, rather than by some direct effect upon the host that may have influenced its response to the parasite.

(c) *Diagnosis*

Parafilaria lesions may be confused with bruising (Pienaar & Van der Heever, 1964), and, because both conditions may occur simultaneously, it is imperative that the diagnosis of all lesions be confirmed.

Pienaar & Van den Heever (1964) described numerous eosinophils as a characteristic of *Parafilaria* lesions; these cells are readily demostrated in Giemsa-stained impression smears of the lesions (Van den Heever, Nevill & Horton, 1973) but are rarely found in smears made from bruised areas and unaffected subcutaneous tissue (Carmichael, 1989).

(d) *The time of the year when trials must be conducted in South Africa*

Because of the seasonal occurrence of parafilariosis, trials should commence after the beginning of June and be terminated before the end of January; thereafter there is a natural seasonal regression in the severtity of lesions (Carmichael & Koster, 1978) which may lead to underestimation of the true efficacy of the test compound.

(e) *Interval between treatment and slaughter*

Most lesions resolve by 9 weeks after treatment with an effective remedy (Viljoen & Boomker, 1977), but it is possible that new lesions may appear after 18 weeks (Soll, Carmichael, Chambers & Ziervogel, 1984). In addition, lesions may heal spontaneously if there is too long a delay before the animals are slaughtered, especially if treatment is given later than October. Therefore, it is recommended that the animals be slaughtered 10–12 weeks after treatment.

2. **The trial design**

(a) *Preliminary investigation*

Because of the cost of conducting a full efficacy trial, it is recommended that a preliminary indication of the efficacy of a candidate compound should be obtained before embarking on full-scale efficacy trials.

A decrease in the number of bleeding sports after treatment may be interpreted as a preliminary indication of possible efficacy of a candidate remedy. Bleeding spots on all animals in a herd should be enumerated daily for 7 days before treatment and subsequently at least weekly for a period of 42 days. Time and experimental animals are thus conserved. Comprehensive trials and slaughter are not warranted if the bleeding spots are not markedly reduced within the 6 weeks following treatment.

(b) *Interval between treatment and slaughter*

The trial should commence after the beginning of June and be terminated before the end of January, and 10–12 weeks should elapse between treatment and slaughter.

(c) *Diagnosis and selection of trial animals*

At least 10 treated and 10 control cattle must be used in the trials. However, in order to ensure adequate power in the statistical test, it is preferable to use 15–20 cattle in each of the treated and control groups. All the animals must be derived from the same herd, be from a highly endemic area, and, as far as possible, must be homogeneous in age, breed and sex. Selection is based on the presence of bleeding spots.

Haemorrhages from other causes can be differentiated by examining exuding blood or dried blood caked on the hair for the presence of the characteristic ova, which contain microfilariae (Nevill, 1975). The ova are, however, very small and often a very careful search must be made to demonstrate their presence.

The animals are ranked according to live mass and are blocked into replicates (homogeneous mass groups) according to the number of treatment groups (e.g. 3 cattle per replicate if 2 treatments and 1 control group are compared). From each block 1 animal is allocated to each experimental group using tables of random numbers. The experimental groups are in turn likewise assigned to the various treatments.

It may be advantageous to remove the cattle from the endemic area after treatment, as it has been shown in experimental primary infections that larvae can induce lesions during the prepatent period (Viljoen & Coetzer, 1982).

3. **The autopsy**

(a) *Examination on a "blind" basis*

It is important that the trial cattle be randomly slaughtered and that examination and sampling be done "blind". The person engaged in the examination should not be aware of which animals are treated ones or controls. In addition, it is recommended that lesion trimming be done on the same basis by a meat inspector who routinely trims such affected carcases.

It is therefore advisable to have the cattle slaughtered at a recognized abattoir. As treatment takes place 10–12 weeks before slaughter, there are usually no anthelmintic residues, and trimmed carcases can be marketed for human consumption, thus reducing costs.

(b) *Diagnosis*

Before the carcase is washed, 2 impression smears are made of each suspected lesion. At the same time, each lesion is numbered, so that those found to be eosinophil-negative on examination of the impression smears may be disregarded in determining the efficacy of the test compound.

(c) *Estimation of the surface area of the lesion*

After the impression smears are made, each lesion

is traced on plastic sheeting with a felt pen. It is often difficult to determine precisely the perimeter of some lesions, this being the main reason why the examination is done "blind".

Because lesions are usually asymmetrical, the lesion area should be calculated with the aid of graph paper or an image analyser etc., rather than estimated from measurements of length and breadth.

(d) *"Trimmings"*

After the lesion outlines have been traced, the carcase is trimmed as during routine meat inspection (see 3. Autopsy). The mass of tissue ("trimmings") removed on account of parafilariosis must be recorded separately for each carcase.

The lesion surface area is considered to be more appropriate and convenient for the calculation than the mass of tissue trimmed, for the following reasons:

(i) Under the conditions prevailing at abattoirs there is insufficent time for indeterminate (suspected) lesions to be confirmed *Parafilaria* positive or negative before meat inspection (trimming) begins.

(ii) The depth of the lesions can only be determined by removing the affected tissue, with the result that various amounts of unaffected tissue are removed in addition to the diseased parts.

(iii) Not all parafilarial lesions are removed at meat inspection (Carmichael, 1989).

4. **Statistical evaluation**

(a) *Lesion area*

If the body mass of the experimental animals is not related to the total lesion area in the control group, it is disadvantageous to conduct an analysis of paired data based on the blocking of the animals (into replicates) prior to random allocation to treatments, as outlined above. The reason for this is the reduction in degrees of freedom if a paired analysis is carried out.

In order to compensate for this problem, and to give the anthelmintic the benefit of any doubt, it is suggested that both a paired and an unpaired analysis be conducted and that the compound concerned be classed as effective, even if it qualifies in only 1 of the 2 analyses.

(i) *Unpaired analysis* (may also be used if the trial design, other than is suggested above, is unpaired and the numbers of treated and control cattle are unequal).

The test statistic is given by:

$$t = \frac{\bar{B}_L - \bar{K}_L}{\sqrt{\frac{S^2_{B_L}}{n_B} + \frac{S^2_{K_L}}{n_K}}}$$

Where $\bar{B}_L$ is the mean of the log lesion surface areas of the cattle in the treated group (Note that 1 is added to each lesion surface area to make allowance for zero areas, since log 0 does not exist and log 1=0; also, if more than 10 % of the treated animals have no lesions, $S^2_{B_L}$ is replaced by $S^2_{K_L}$ in the above formula);

$\bar{K}_L$ is the mean of the logs of the *reduced* lesion surface areas of the untreated (control) group; i.e. the mean of the logs of $K_* = K \cdot \frac{v}{100}$, where

K is the lesion surface area of the individual untreated (control) animals and

v varies according to the levels of efficacy required, e.g., v = 100 − 90 = 10 where the claim is to be that the remedy is more than 90 % effective, or v = 5 for 95 % and v = 1 for 99 % effective, etc;

S^2_B and S^2_K are the respective variances† of the B_L and K_L values;

n_B and n_K are the numbers of cattle in the treated and control groups, respectively. Degrees of freedom for the t-statistic are: $n_B + n_K - 2$.

If more than 10 % of the treated group has no lesions and $S^2_{B_L}$ is replaced by $S^2_{K_L}$, the degrees of freedom reduces to $n_K - 1$.

Look up the 5 % t-value (or the required confidence level if other than 5 %) in Student's t-table (one-tailed test) only if the calculated t-stastistic is negative in value. If the statistic is positive, the treatment is not significantly effective at the required level. Use the negative of the t-value found in the table. If the calculated t-statistic is smaller than the tabled 5 % value, for example, the anthelmintic is significantly effective at the required level of efficacy (according to the choice of v).

An example of the application of the above formula to theoretical trial data is shown in Table 1 that depicts an anthelmintic that is more than 95 % effective at a 95 % confidence level ($P < 0,05$).

(ii) *Paired analysis* (equal numbers of cattle in the treated and control groups). An example of the application of this test appears in Table 2.

The test statistic is given by:

$$t = \frac{\bar{D}_L}{\sqrt{\frac{S^2_{D_L}}{n_B}}}$$

Where $\bar{D}_L$ is the mean of the logs of the differences B–K*, i.e. the mean of $D_L = \log (B–K_*)$ where $K_* = \frac{v}{100} \cdot K$, the *reduced* lesion surface areas of the individual control cattle; with

K the lesion surface areas of the individual control cattle and B the lesion surface areas of the individual cattle in the treated group;

v varies according to the levels of efficacy required, as in the example for unpaired analysis;

$S^2_{D_L}$ is the variance of D_L values;

and n_B is the number of treated or control cattle;

Look up the t-values as for the unpaired observations at $n_B - 1$ degrees of freedom.

(b) *Mass of trimmings*

The masses of tissue trimmed from the treated and control cattle may be compared statistically with the same techniques described for lesion surface area.

DISCUSSION

The lesion size has been expressed as a percentage of the body area in some anthelmintic evaluations (Viljoen & Boomker, 1977; Wellington, 1978). This procedure, however, entails more labour at the abattoir and there is no indication that it is a more accu-

† Variance equals the square of the standard deviation.

TABLE 1 Theoretical example: unpaired analysis (>95 % efficacy level)

Animal No.	Total lesion area (cm^2)		$K \cdot \frac{5}{100}$	Log K*	Log (B + 1)
	Treated cattle (B)	Untreated (K)	(K*)	(K_L)	(B_L)
1	20	3 491	174,6	2,242	1,322
2	0	1 677	83,9	1,924	0
3	0	2 252	112,6	2,052	0
4	0	1 792	89,6	1,952	0
5	604	1 685	84,3	1,926	2,781
6	0	6 271	313,6	2,496	0
7	0	2 761	138,1	2,140	0
8	0	5 074	253,7	2,404	0
9	80	1 523	76,2	1,882	1,903
10	0	4 076	203,8	2,309	0
11	20				1,322
Arithmetic mean	•	•	•	$\bar{K}_L = 2,1328$	$\bar{B}_L = 0,6662$
Variance	•	•	•	$S^2_{K_L} = 0,0486$	$S^2_{B_L} = 0,09969$

$$t = \frac{\bar{B}_L - \bar{K}_L}{\sqrt{\frac{S^2_{K_L}}{n_B} + \frac{S^2_{K_L}}{n_K}}}$$

(since more than 10 % of treated cattle have no lesions)

$$= \frac{0,6662 - 2,1328}{\sqrt{\frac{0,0486}{11} + \frac{0,0486}{10}}}$$

$$= -15,23 \; (P < 0,05)$$

TABLE 2 Theoretical example: paired analysis # (> 95 % efficacy level)

Pairs of cattle	Total lesion area (cm^2)		$K \cdot \frac{5}{100}$	Difference	Log difference i.e. log (B−K*)
	Treated cattle (B)	Untreated cattle (K)	(K*)	(B−K*)	(D_L)
1	20	3 491	174,6	−154,6	−2,19
2	0	1 677	83,9	− 83,9	−1,92
3	0	2 252	112,6	−112,6	−2,05
4	0	1 792	89,6	− 89,6	−1,95
5	604	1 685	84,3	+519,7	+2,72
6	0	6 271	313,6	−313,6	−2,50
7	0	2 761	138,1	−138,1	−2,14
8	0	5 074	253,7	−253,7	−2,40
9	80	1 523	76,2	+ 3,8	+0,58
10	20	4 076	203,8	−183,8	−2,26
Arithmetic mean	•	•	•	•	$\bar{D}_L = -1,41$
Variance	•	•	•	•	$S^2_{D_L} = 2,89$

$$t = \frac{\bar{D}_L}{\sqrt{\frac{S^2_{D_L}}{n_B}}}$$

$$= \frac{-1,41}{\sqrt{\frac{2,89}{10}}}$$

$$= -2,62 \; (P < 0,05)$$

For the purpose of this example one treated animal was deliberately discarded to illustrate equal groups (compare with Table 1)

rate index of infection than the lesion size *per se*. It seems probable that in a relatively homogeneous group of cattle of common origin, the size of the lesions will be related to the degree of infection and the size and metabolic activity of the worms and will not be influenced by the animal's body surface area. In any case, the possible influence of animal size is at least partly overcome by "blocking" the trial cattle in homogeneous groups by mass before random allocation (within mass groups) to the treatments.

Most abattoirs maintain records of *Parafilaria* infection which are reflected on the sale returns to the farmer; consequently anything less than complete anthelmintic efficacy is brought to the farmer's attention, who often regards the presence of residual infection or of unresolved lesions as complete failure of the treatment. This is understandable because a comparison with untreated control cattle is not made in these instances. The unfortunate sequel is that *only* highly effective remedies are acceptable for registration, otherwise both the registering authorities and the commercial companies marketing the anthelmintics are inundated with complaints of apparent inefficacy. Largely for this reason, the minimal requirement for a compound to be registered as an "effective" parafilaricidal in South Africa is at least a 90 % reduction (with a 95 % confidence level) in the total lesion area of treated cattle relative to untreated controls. At present this is the only efficacy category, no provision having been made for a compound that is markedly more effective. Nevertheless, by adjusting the statistical test, a "highly effective" category may be created at any higher level of efficacy.

Although both ivermectin† and nitroxynil†† (the only compounds registered in South Africa for treating parafilariosis) reduce lesion area by more than 90 % 70 days after treatment, bleeding spots still occasionally occur in treated animals (Viljoen, 1976; Viljoen & Boomker, 1977; Wellington, 1978; Wellington & Van Schalkwyk, 1982). For this reason, and because some lesions remain at slaughter, complaints have been received from consumers (J.A.v.W., unpublished observations, 1983).

Because of the long prepatent period of *P. bovicola*, bleeding spots observed after treatment are probably due to the survival of small numbers of worms rather than to reinfection. Some post-mortem pathology is therefore to be expected in cases where efficacy is less than 100 %.

Lesions found in treated animals at slaughter may, however, have another origin. Viljoen & Coetzer (1982) have shown that in single primary artificial infections, *P. bovicola* can induce lesions during the prepatent period. They concluded that: "Lesions on the carcase are more prominent immediately after parasitic invasion (Day 3+ – Day 20+) and again after adult female *P. bovicola* worms penetrate the skin".

It is unknown what contribution immature worms may make to lesions in naturally infected cattle, but if lesions due to reinfection are to be avoided, it is advisable to remove cattle in anthelmintic trials from the endemic region between treatment and slaughter.

A treatment-slaughter interval of 10-12 weeks has been selected, based on the work or Viljoen & Boomker (1977), Wellington (1978), Viljoen & Coetzer (1982), Swan, Soll, Carmichael & Schröder (1983) and Soll *et al.* (1984). A period of 50 days is insufficient for an adequate resolution of lesions, even after successful treatment (Soll *et al.*, 1984). Furthermore, unless a product is effective against all developmental stages of *P. bovicola*, a resumption of lesions may be expected at some time (as yet undetermined) after the recommended 10–12-week interval. Using artificial infections, Viljoen & Coetzer (1983) showed that: "Lesions were very pronounced for the first 31 days, but thereafter were ill-defined and located with difficulty from Day [34-112 after infection]. Subsequently, from Day [116-240], lesions again became increasingly visible . . .".

The persistence of bleeding spots on some treated cattle, together with a lack of data on the biology of the intermediate hosts, probably makes attempts to control the disease with anthelmintics alone economically unjustifiable. This has been borne out in a recent study (Nevill, 1984). On the other hand, in highly endemic regions it seems very likely that treatment of cattle is justified to reduce losses at slaughter 10–12 weeks later.

The financial benefit from mass treatment of slaughter stock will obviously depend upon the prevalence of infection in the herd. Data generated on selected infected trial animals should not be referred to a field situation, where, according to Carmichael & Koster (1978), substantial numbers of animals may not be infected at the time of treatment.

Condemnation of carcases because of parafilariosis has increased at at least one major abattoir in the country during recent years (Wallace, Weaver, Kretzman & Payne, 1983). This may be due to an increased severity of infection, or to more strict inspection criteria, but whatever the cause, the economic benefits of treating slaughter stock with anthelmintics may become increasingly important.

There is no evidence on which to base a recommendation for treatment of cattle before removal from endemic areas to "non-endemic" areas. The available data indicate that parafilariosis already occurs in all areas of South Africa which are ecologically suitable for transmission (Carmichael, 1989).

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REFERENCES

ANON., 1976. Annual Report. Department of Agricultural Technical Services, 1975–76. p. 18. Pretoria, South Africa.

CHARMICHAEL, I. H., 1981. Influence of age, sex and breed on naturally-acuired bovine parafilariosis. *South African Jounal of Science*, 77, 571–572.

CARMICHAEL, I. H. 1989. Studies on parafilariosis of cattle in southern Africa. D.V.Sc thesis. University of Pretoria, South Africa.

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.

NEVILL, E. M. 1984. Progress with the control of *Parafilaria*. *Onderstepoort Newsletter*, 2, 17–18.

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.

† Ivomec (MSD)

†† Trodax (Maybaker)

SCHAFER, F., 1975. Beef industry faces major crisis. *Farmer's Weekly*, Jan. 22, 1975, pp. 8–10.

SOLL, M. D., CARMICHAEL, I. H., CHAMBERS, P. G. & ZIERVOGEL, A., 1984. The influence of pre-slaughter treatment with invermectin on *Parafilaria bovicola* infestation in cattle in Zimbabwe. *Zimbabwe Veterinary Journal*, 15, 18–23.

SWAN, G. E., SOLL, M. D., CARMICHAEL, I. H. & SCHRÖDER, J., 1983. Efficacy of ivermectin against *Parafilaria bovicola*. *Veterinary Record*, 113, p. 260.

VAN DEN HEEVER, L. W., NEVILL, E. & HORTON, B. G. W., 1973. Bovine parafilariasis. *Journal of the South African Veterinary Medical Association*, 44, 333–334.

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.

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.

VILJOEN, J. H., 1982. The parasitic life cycle of *Parafilaria bovicola* and its pathogenesis in cattle. D.V.Sc. thesis. University of Pretoria, South Africa.

VILJOEN, J. H. & COETZER, J. A. W., 1982. Studies on *Parafilaria bovicola* Tubangui, 1934. 3. Pathological changes in infested calves. *Onderstepoort Journal of Veterinary Research*, 49, 29–40.

WALLACE, H. G., WEAVER, D. B., KRETZMAN, P. M. & PAYNE, J. R., 1983. Bovine parafilariasis: Condemnations at the Cato Ridge abattoir. *Journal of the South African Veterinary Association*, 54, 123–125.

WELLINGTON, A. C., 1978. The effect of nitroxynil on *Parafilaria bovicola* infestations in cattle. *Journal of the South African Veterinary Association*, 49, 131–132.

WELLINGTON, A. C. & VAN SCHALKWYK, L., 1982. The effect of a single injection of nitroxynil at 20 mg/kg live mass in the treatment of *Parafilaria bovicola* infestations in cattle. *Journal of the South African Veterinary Association*, 53, 91–94.

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