The efficacy of milbemycin oxime against pre-adult *Spirocerca lupi* in experimentally infected dogs

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ARTICLE INFO

ABSTRACT

The aim of this investigation was to determine the efficacy of milbemycin oxime in preventing the oesophageal encapsulation of *Spirocerca lupi*, following the experimental infection of dogs. Two studies were conducted which involved a total of 21 purpose-bred Beagles. Each dog was infected with approximately 40, third stage infective *S. lupi* larvae. The larvae were dissected from scarabaeid beetles that had been collected from areas endemic for spirocercosis. In the first study, milbemycin oxime (minimum dose 0.5 mg/kg body weight) was administered to seven dogs on day 30 post-infection. Seven other dogs served as untreated controls. In the second study, milbemycin oxime (also at a minimum dose of 0.5 mg/kg body weight) was administered to four of seven infected dogs on day 28 post-infection. Treatment was repeated at 14- or 28-day intervals. All of the dogs, from both studies, were euthanized 168 or 169 days after infection. All *S. lupi* were recovered, and lesions in the thoracic aorta and oesophagus were described and quantified. A single treatment with milbemycin oxime was 79.8% effective in preventing the establishment of *S. lupi* in the oesophagus. This treatment significantly ($p < 0.05$) reduced both the number of *S. lupi* within the oesophagus and the size of the oesophageal nodules. The efficacy of anthelmintic treatment was increased to 100% when repeat doses of milbemycin oxime were administered at 14- or 28-day intervals. These repeat treatments completely prevented the establishment of *S. lupi* within the oesophagus and thereby averted the development of oesophageal nodules. As expected, none of the treatment protocols reduced *S. lupi* related damage within the aorta because the administration of milbemycin oxime only began after the larvae had completed their first stage of migration.

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1. Introduction

Spirocerca lupi is primarily a parasite of the thoracic aorta and the oesophagus of domestic dogs (Bailey, 1972). The infection rate of canines is variable and appears to be related to their country of habitation along with their size, breed and behaviour. Generally, larger breeds of dog (especially hunting breeds) are more at risk than smaller dogs, and Labradors appear to be particularly susceptible to spirocercosis (Mylonakis et al., 2001; Mazaki-Tovi et al., 2002). Infection is often more prevalent in warmer climate countries and areas within South Africa are known to be endemically infected (Minnaar and Krecek, 2001; Minnaar et al., 2002; Du Toit et al., 2008; Van der Merwe et al., 2008).

The numerous clinical signs associated with spirocercosis are due to the migratory life cycle of the nematode. Canines become infected by ingesting larvae encysted within either the coprophagous beetle intermediate host or any of the various paratenic hosts (including small mammals, reptiles and birds). Once within the canine stomach,

the *S. lupi* larvae excyst and penetrate the stomach wall (Bailey, 1972). Over the next one to three weeks, the larvae migrate to the caudal aorta via the walls of the gastric arteries (Nazarova, 1964, cited by Bailey, 1972; Hu and Hoeppli, 1936, cited by Van der Merwe et al., 2008). Tissue scarring, fibrosis and aortic aneurysms occur as a result of this migration and emboli can also form. Interestingly, dogs often display no externally detectable signs at this stage of the infection but sudden death can occur if an aortic aneurysm ruptures (Ivoghli, 1977; Rinas et al., 2009).

It takes approximately 3 months, during which the larvae moult from the third to the fourth stage, before the mature larvae exit through the wall of the aorta and migrate to the submucosa of the caudal oesophagus. Here, their presence evokes oesophageal nodule development. Chronic and debilitating symptoms such as regurgitation, vomiting, respiratory distress and anaemia (Dvir et al., 2001; Mazaki-Tovi et al., 2002) are common and these become more marked as the nodules increase in size and number. The dogs become progressively emaciated due to their inability to swallow and the nodules can also become neoplastic (Ranen et al., 2004). Hypertrophic osteopathy is often associated with spirocercosis and aberrant migration can also cause damage to many other organs and systems, including the neurological system (Mazaki-Tovi et al., 2002; Du Plessis et al., 2007; Chai et al., 2008).

The prevalence of *S. lupi* is increasing in many areas of the world and a cost-effective, efficacious anthelmintic is required to help control this severe and debilitating disease. To enable its use as a prophylactic as well as a therapeutic option, such a product would have to demonstrate efficacy against both adult and larval stages of the nematode. Several anthelmintic products have been evaluated and demonstrate good efficacy against adult *S. lupi* which are established within the oesophagus (Reinecke et al., 1981; Berry, 2000; Lobetti, 2000; Mylonakis et al., 2008), but very few products are known to be efficacious against the larval forms situated in, or migrating from the aorta. Fortunately, milbemycin oxime may be such a compound: Kelly et al. (2008) demonstrated good anthelmintic efficacy when milbemycin oxime was used against naturally acquired adult *S. lupi* situated within the oesophagus, and our own natural infection study fully supported these findings (Kok et al., 2010). Additionally, the highly significant reduction in aortic larval burden observed in the treated dogs within our natural infection study, very effectively demonstrated the efficacy of milbemycin oxime against the larval stages of *S. lupi*. Unfortunately, due to the high level of repeat infection encountered by these dogs, the anthelmintic markedly reduced but did not completely eliminate the pathological changes within the aorta.

In order to appropriately refine the milbemycin oxime dosing regimens for such clinical situations, the specific activity of the anthelmintic against the pre-adult stages of *S. lupi* still needs to be confirmed. For this reason, the primary aim of this study was to target treatment to larvae developing within or migrating from the aorta, and to determine whether treatment at these stages prevented establishment of the nematode within the oesophagus. Experimental inoculations, similar to those described by Bailey (1972) and Lavy et al. (2003) were performed, so

that the time for treatment could be extrapolated from the precise time of infection. The successful infection of each dog was confirmed by a pathological assessment of the aorta. This provided an additional opportunity to analyse whether any of the treatment protocols also reduced or eliminated aortic damage and larval burden.

2. Materials and methods

This investigation consisted of two studies which were carried out in compliance with GCP. South African animal welfare regulations, as stipulated in the "National Code for Animal Use in Research, Education, Diagnosis and Testing of Drugs and Related Substances in South Africa" were followed and the protocols were submitted to the ClinVet Animal Ethics Committee (CAEC), the composition of which was in compliance with the National Code, for approval.

A total of 21 purpose-bred Beagles were used in the studies. Their ages ranged between 9 and 18 months at the time of experimental infection. They were randomised between study groups on the basis of bodyweight and gender so that all groups included both male and female animals, weighing between 9.1 and 16.7 kg at the time of first treatment. The dogs were individually identified, by means of implanted transponders, and were housed in pens situated inside brick and steel outdoor kennel units. Food and potable water were provided in stainless steel bowls. As the study facility is located in an area that is free of *S. lupi* infection, the risk of exposure to natural infection was minimal. However, as an extra precaution, faeces were removed from the pens with increased frequency, in order to avoid contact between the dogs and any migratory dung beetles. Because of the extended duration of the study, the majority of the dogs were kept as pairs within each pen. All of the dogs had access to exercise areas where they had close association with other Beagles.

To allow monitoring of the dogs for infection- or drug-associated adverse effects, all of the animals were housed separately for a period of at least 24 h after infection or treatment. They were also placed in individual pens on all days that faeces were to be collected for worm egg counts. A veterinarian was responsible for ensuring the health of the dogs and treated them for disorders other than those associated with *S. lupi* infection. The dogs were dipped regularly in flumethrin (Bayticol: Bayer Animal Health) in order to control ectoparasites. No anthelmintic, other than the investigational product, was administered during the study. During the course of the second study three dogs were diagnosed with ehrlichiosis/babesiosis. It was considered prudent to treat all of the dogs in this study either therapeutically or prophylactically with doxycycline, diminazine and imidocarb diproprionate. Three of the dogs had suspected relapses and were given a repeat course of medication.

2.1. Infection

All of the dogs used in both studies were experimentally infected with *S. lupi*. Infective third stage *S. lupi* larvae were dissected from naturally infected scarabaeid beetles (notably *Onthophagus* spp.) collected from dog faeces

in a locality endemic for spirocercosis. The excysted larvae were placed in a normal saline solution and used for infection within 24 h of excision from their intermediate hosts. On the day of infection, food was withheld from each dog for approximately 12 h. Approximately 30 min before infection, each dog was pre-medicated with acepromazine (0.25 mg/kg IM) to suppress the vomiting that frequently occurs directly after infection (Bailey, 1972; Lavy et al., 2003). Each dog received approximately 40 S. lupi infective larvae, suspended in 1–2 ml of normal saline. The larvae were administered directly into the stomach by means of a stomach tube, which was flushed through with approximately 10 ml of normal saline. The dogs were observed for approximately 1 h post-infection and any vomiting, or other adverse events that occurred, were recorded. Any vomit that was not re-ingested was examined microscopically to determine whether larvae were present.

2.2. Study 1

Study 1 involved 14 of the 21 experimentally infected dogs. Milbemycin oxime was administered to seven of these dogs on a single occasion (corresponding to day 30 post-infection). The milbemycin oxime was supplied in a tablet formulation and was administered per os. The tablet contained 12.5 mg milbemycin oxime in combination with 125 mg praziquantel (Milbemax®; Novartis Animal Health Inc.). Animals were dosed according to the manufacturer's instructions, ensuring that the minimum dose was 0.5 mg milbemycin oxime/kg body weight. Dose calculations were based on body weights determined 1 or 2 days before treatment. All of the dogs weighed more than 5 kg so they all received tablets intended for medium to large breed dogs. The other seven dogs served as infected but untreated controls.

2.3. Study 2

Study 2 involved the seven remaining, experimentally infected dogs. Three of these dogs served as infected but untreated controls. The other four dogs received their first treatment with milbemycin oxime on day 28 post-infection. Two of these four dogs received a total of nine further treatments at 14-day intervals. The other two dogs received four additional treatments at 28-day intervals. Milbemycin oxime was administered in the same formulation, and the dosage was calculated in the same way, as described in Study 1. All of the dogs were of a sufficient weight to receive tablets intended for medium to large sized dogs.

A summary of the experimental design for Study 1 and Study 2 is shown in Table 1.

2.4. Assessment

In both studies, faecal collection commenced on day 128 post-infection and was repeated once weekly thereafter. The faeces were examined according to the methods of Markovics and Medinski (1996) to determine the presence of S. lupi eggs. All dogs were necropsied on day 168 or 169 post-infection. Prior to euthanasia, food was with-

held from each dog for 16–20 h. At necropsy, the whole oesophagus was removed. Thereafter, the thoracic aorta was extracted from the heart to at least the coeliac artery or slightly beyond. Parts of the brachial and brachiocephalic arteries and diaphragm were left attached to the aorta to assist with orientation. The wall and lumen of the thoracic aorta and oesophagus were thoroughly examined and any lesions were described and quantified. Quantification included measurement of the size and number of any nodules and determination of their position relative to the diaphragm. A descriptive comparison was made of the differences in the appearance of the aortic lesions between the treated and control groups of dogs. The following criteria were used:

0 = No lesions visible on the intima.
+ = Inflammatory/puckered lesions only just visible.
++ = Two to three lesions clearly visible, no aneurysms.
+++ = Several distinct inflammatory lesions; parasite migratory tracks often visible; commencement of aneurysm development.
++++ = Distinctly damaged area of aorta; all of the above evident.
+++++ = Aorta severely damaged over a large area; several aneurysms evident.

All nodules were opened and all visible worms were removed. The remaining contents of the nodules were then washed through a sieve (aperture size 0.15 mm), in order to recover any residual worms. All recovered worms were counted and preserved in 10% formalin.

The main numerical value for statistical analysis was the mean number of worms recovered from dogs in the treated group compared to the mean number of worms recovered from dogs in the control group. The numbers of worms recovered from the aorta were evaluated separately from those recovered from the oesophagus.

Efficacy was calculated using the following formula:

$$\%\text{Efficacy} = \frac{N_2 - N_1}{N_2} \times 100$$

where N_1 = geometric mean (GM) number of S. lupi recovered from treated dogs; N_2 = GM number of S. lupi recovered from untreated control dogs.

Percentage efficacy, as a measure of the effectiveness of treatment towards preventing the encapsulation of adult worms in the oesophagus, was the primary goal. The faecal worm egg counts of the treated group were also intended to be compared to those of the control group. Percentage reduction in these counts was to be a secondary goal. Data on the size and numbers of lesions were used descriptively.

Means, standard deviations, and medians were calculated for the number of worms recovered or lesions recorded. Because of the non-normality of the data, non-parametric comparisons were used; namely the Mann–Whitney U-test and a stratified variant thereof. Statistical significance was set at $p < 0.05$. Statistical analyses were conducted using SAS Version 9.1.3. Statistical analysis was not possible in Study 2, as too few animals were included in the study.

Table 1
Experimental design of studies to determine the efficacy of milbemycin oxime against experimental infections of *Spirocerca lupi*.

Day	Activity	
	Study 1	Study 2
−7 to −1	Acclimatization	Acclimatization
0	Experimental infection of dogs	Experimental infection of dogs
+27	Randomization	Randomization
+28	N/A	Allocation to groups. Treat dogs in treatment groups
+30	Allocation to groups. Treat dogs in treatment group	N/A
+42 to +154	N/A	Treat dogs in treatment groups either at 14-day or 28-day intervals
+128 to +163	Faecal worm egg counts at 7-day intervals	Faecal worm egg counts at 7-day intervals
+168 and +169	Necropsy	Necropsy

N/A = not applicable.

3. Results

Fourteen of the 21 dogs vomited within the first hour of infection. Each of the affected individuals vomited between one and three times. The first episode of vomiting occurred as quickly as 9 min post-infection, but most of the affected dogs vomited between 20 and 60 min after administration of the larvae. The maximum number of larvae recovered from one sample was three, but in the majority of samples, larvae were completely absent.

3.1. Establishment of infection

In Study 1, the mean total number of *S. lupi* recovered from the untreated control dogs was 15.9 and in Study 2, the mean total number of worms recovered was 17.3. This represented successful mean establishments of 39.8% and 43.3% of the 40 third stage larvae used to infect each dog. All dogs, in both studies, demonstrated aortic lesions and nodules consistent with infection by *S. lupi*. Fig. 1a–f depicts some examples of the damage to the aorta and the damage scores that were assigned.

3.2. Study 1

A summary of the data is shown in Tables 2a and 2b.
Analysis of faecal egg count data was not possible because *S. lupi* eggs were not recovered from any of the faecal samples taken from the dogs in this study. The degree of damage to the thoracic aorta of dogs in the control and treated groups was similar, and no statistically significant differences were found between any of the parameters measured or the damage scores assigned. Slightly more worms were recovered from the aortae of the treatment group compared with the control group, but the mean values did not differ significantly. In contrast, a single treatment with milbemycin oxime, significantly ($p < 0.05$) reduced the number of worms recovered from the oesophagus, with a total of only 29 worms being recovered from the treated dogs compared to a total of 99 worms recovered from the control group. The efficacy of milbemycin oxime against oesophageal *S. lupi*, based on geometric means, was calculated as 79.8%. In this single treatment study there was no significant difference between the total number of oesophageal nodules detected in the treatment or the control group. However, the average oesophageal nodule size was significantly ($p < 0.05$) smaller in the treatment group.

3.3. Study 2

A summary of the data is shown in Table 3.
Analysis of faecal egg counts was not possible as *S. lupi* eggs were only recovered from two dogs on a single occasion (corresponding to day 163 post-infection). Both of these dogs belonged to the untreated control group and at necropsy these individuals harboured 19 and 24 worms within their oesophageal nodules. As in Study 1, no significant differences were found between the control and either of the treatment groups, when the criteria for assessing aortic damage were compared. However, there was a complete absence of *S. lupi* larvae in the aortae of the treated dogs, whereas three *S. lupi* larvae were recovered from the aorta of the untreated control dogs. Likewise, no *S. lupi* adults were recovered from the oesophagus of the treated dogs, whereas a total of 49 were recovered from the three untreated control dogs. Both repeat treatment protocols prevented establishment of *S. lupi* in the oesophagus, as demonstrated by a complete absence of oesophageal nodules in all treated dogs. In contrast, each dog from the control group had either one or two oesophageal lesions.

4. Discussion

Despite receiving a premedication, over half of the dogs vomited following experimental infection with the *S. lupi* larvae but fortunately this had no significant effect on the establishment of infection. Mean establishment rates were 39.8% and 43.3% in the control animals of Study 1 and Study 2, respectively. These rates were slightly lower than the 47% achieved by Bailey (1972), but they still represented a remarkably high level of infection given the circuitous migratory lifecycle of the nematode and the lower infective dose used in our studies. Unfortunately, even though appropriate flotation techniques were used, the analysis of faecal egg counts was not possible in either study, as insufficient *S. lupi* positive faecal samples were obtained. The detection of *S. lupi* eggs in faeces is notoriously unreliable (Markovics and Medinski, 1996; Dvir et al., 2001; Mazaki-Tovi et al., 2002) and it is possible that the times of faecal collection did not correspond with the intervals when *S. lupi* eggs were being intermittently released. It is also possible that our studies were too short in duration to make full use of this diagnostic technique, as Lavy et al. (2003) found that egg-laying remained low until 166 days post-infection and increased most steadily from 178 days onwards.
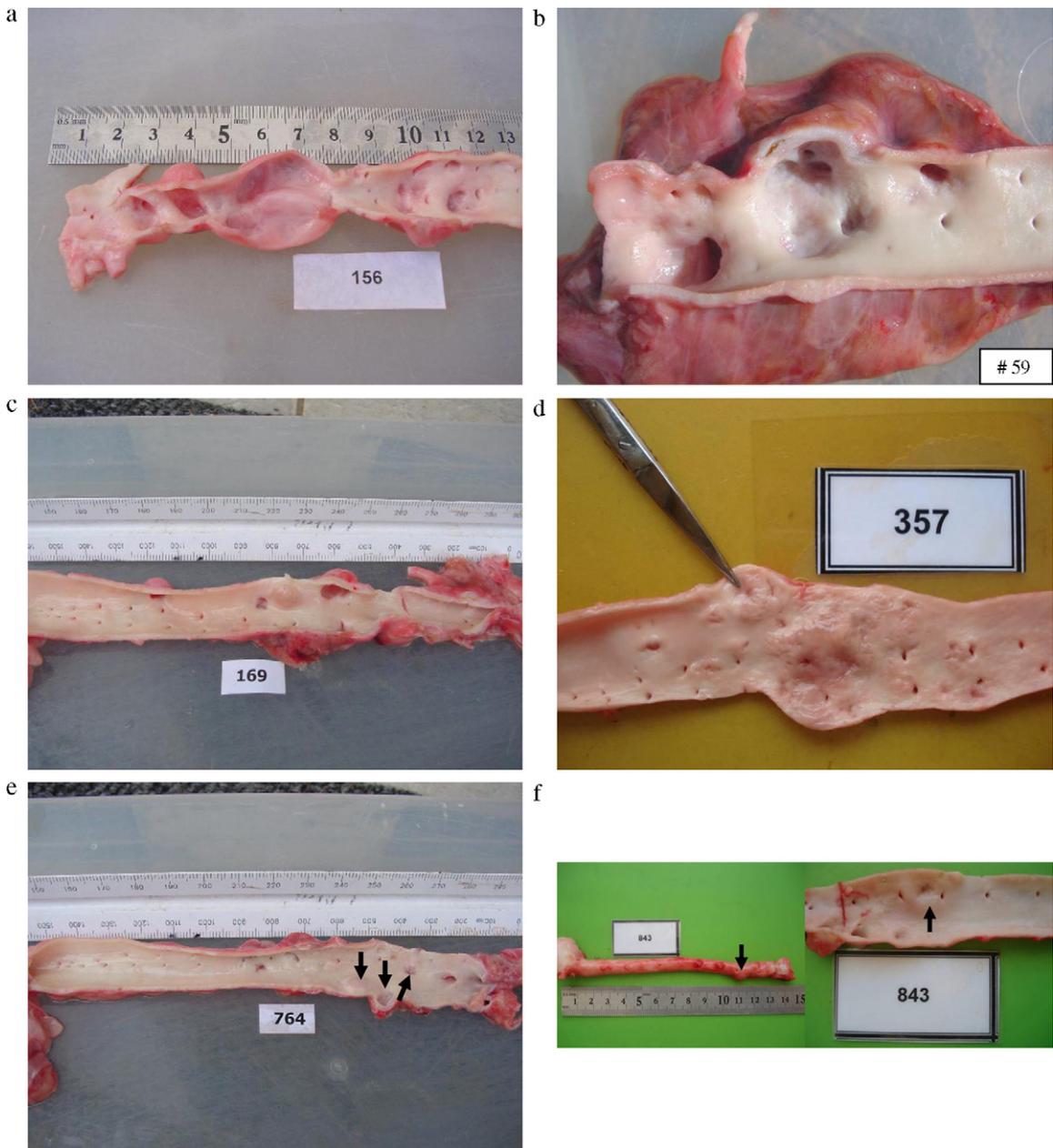
Fig. 1. (a) Thoracic aorta opened from the approximate position of the diaphragm on the left. Group: untreated control. Damage score +++++. (b) Approximately 2.5 cm of the aorta, bordering onto the diaphragm, contains strongly expanded aneurisms. Group: untreated control. Damage score ++++. (c) Aneurisms starting to develop in the region of the thoracic aorta bordering on the diaphragm. Group: untreated control. Damage score +++. (d) Area of "puckered" lesions approximately 5 cm from the diaphragm. Group: Milbemax® treated at 28-day intervals. Damage score +++. (e) Thoracic aorta opened from the approximate position of the diaphragm on the right. Some lesions are indicated by arrows. Group: Milbemax® treated, single dose. Damage score ++. (f) Thoracic aorta shown unopened on the left. On the right the opened part shown is close to the approximate position of the diaphragm. Arrows indicate the position of the most prominent lesion. Group: Milbemax® treated at 14-day intervals. Damage score +.

In Study 1, after receiving a single dose of milbemycin oxime at 30 days post-infection, the dogs in the treatment group harboured significantly ($p < 0.05$) fewer oesophageal *S. lupi* than the dogs in the untreated control group. In addition, the single treatment protocol significantly ($p < 0.05$) reduced the size of the oesophageal nodules in the treated animals, even though a similar reduction in the total number of nodules was not achieved. As milbemycin oxime

(Milbemax®: Novartis Animal Health Inc.) has a mean residence time of approximately 5–6 days in fasted dogs (Jung et al., 2002), these findings strongly indicate that this anthelmintic compound is efficacious against the early (L3) larval stages of *S. lupi*. *S. lupi* larvae only reach the aorta 1–3 weeks post-infection and develop there for approximately 3 months before migrating to the oesophagus and becoming adults (Nazarova, 1964, cited by Bailey,

Table 2a
Numbers of aortic and oesophageal nodules observed in experimentally infected dogs that were untreated or treated once with milbemycin oxime.

Treated dogs			Untreated dogs		
Dog identification number	Number of nodules		Dog identification number	Number of nodules	
	Thoracic aorta	Oesophagus		Thoracic aorta	Oesophagus
ID 1	1	0	ID 8	6	4
ID 2	6	2	ID 9	8	1
ID 3	3	2	ID 10	6	2
ID 4	2	4	ID 11	3	2
ID 5	4	1	ID 12	4	1
ID 6	4	1	ID 13	2	4
ID 7	3	0	ID 14	7	3
AM	3.3	1.4	AM	5.1	2.4
SD	1.6	1.4	SD	2.2	1.3
GM	2.9	1.1	GM	4.7	2.1

AM, arithmetic mean; SD, standard deviation; GM, geometric mean.

Table 2b
Numbers of *Spirocerca lupi* recovered from experimentally infected dogs that were untreated or treated once with milbemycin oxime.

Treated dogs			Untreated dogs		
Dog identification number	Number of worms		Dog identification number	Number of worms	
	Thoracic aorta	Oesophagus		Thoracic aorta	Oesophagus
ID 1	2	0	ID 8	3	20
ID 2	2	6	ID 9	2	9
ID 3	5	4	ID 10	1	11
ID 4	3	3	ID 11	0	19
ID 5	2	10	ID 12	1	14
ID 6	1	6	ID 13	1	13
ID 7	4	0	ID 14	4	13
AM	2.7	4.1	AM	1.7	14.1
SD	1.4	3.6	SD	1.4	4.0
GM	2.5	2.8	GM	1.4	13.7
Efficacy	None	79.8%			

AM, arithmetic mean; SD, standard deviation; GM, geometric mean.

1972; Hu and Hoeppli, 1936, cited by Van der Merwe et al., 2008). The activity of the single dose of milbemycin oxime would only have corresponded to the early stages of larval establishment within the aorta, as the anthelmintic would have been cleared from the host system well before the majority of larvae began their next stages of maturation and subsequent migration. Study 1 therefore showed that milbemycin oxime can successfully reduce nematode establishment within the oesophagus

(efficacy = 79.8%) when its use is targeted against the early pre-adult stages of the nematode.

Pathological examinations verified that none of the milbemycin oxime treatment protocols prevented or reduced aortic damage. This was unsurprising given that all of the *S. lupi* larvae would have already reached the aorta by the time the anthelmintic was administered. Interestingly though, no actual larvae were recovered from the aortae of any of the dogs receiving repeat anthelmintic treatments in

Table 3
Numbers of aortic and oesophageal nodules observed and *Spirocerca lupi* recovered from experimentally infected dogs treated repeatedly with milbemycin oxime.

Dog ID	Thoracic aorta		Oesophagus	
	No. of nodules	No. of worms	No. of worms	No. of nodules
Treated at 14-day intervals				
ID 15	3	0	0	0
ID 16	2	0	0	0
Treated at 28-day intervals				
ID 17	8	0	0	0
ID 18	6	0	0	0
Untreated controls				
ID 19	10	0	19	1
ID 20	8	0	24	2
ID 21	6	3	6	2

Study 2, whereas three larvae were present within the aorta of one of the control dogs from this study. Aortic larvae were also isolated from the majority of control and single treatment animals at an equivalent time point in Study 1. It is possible, that the aortic larvae present in the repeat treatment group of dogs had already matured and started their migration to the oesophagus. However, as these observations were not replicated in the control group, it is also possible that milbemycin oxime administered at 14- or 28-day intervals, is highly effective in preventing the survival of S. lupi within the aorta. This would be another example of product efficacy against aortic larvae and would support the findings of our natural infection study (Kok et al., 2010). However, due to the low numbers of animals included in Study 2, assessment of a larger study population is required before this observation can be confirmed.

In addition to the absence of aortic larvae described above, no pre-adult or adult S. lupi were recovered from the oesophagus of any of the dogs receiving repeat treatment with milbemycin oxime in Study 2. There was also an absence of oesophageal nodules in these animals whereas both oesophageal nematodes and nodules were detected in all dogs in the control group. It could be suggested that these results only confirm the findings of Kelly et al. (2008), who administered repeat doses of milbemycin oxime to dogs naturally infected with S. lupi and demonstrated oesophageal nodule resolution within 95–186 days. However, due to the use of experimental inoculations, our studies actually assessed the efficacy of the anthelmintic against the developmental and migratory stages of S. lupi, rather than its activity against established adults. In fact, treatment was timed to correspond with the development of larvae within the aortae or the migratory phase of the mature L4 larvae towards the oesophagus. The combined results of Study 2 therefore show that milbemycin oxime demonstrates 100% efficacy against one or more of the pre-adult developmental stages of S. lupi and that the early use of the anthelmintic can completely prevent the establishment and encapsulation of S. lupi within the oesophagus of the host.

The results of these two experimental studies are very encouraging, but further investigations are required before the use of milbemycin oxime can be fully recommended for the treatment and prevention of spirocercosis. Ideally, efficacy results similar to those achieved in the repeat treatment study would have to be reproduced in a greater number of animals, under field conditions. An additional experimental infection study, designed to determine whether earlier, repeat doses of milbemycin oxime can prevent, reduce or eliminate the establishment of S. lupi within the aorta, would also be of benefit. Such a study would help to clarify whether milbemycin oxime is fully efficacious against the L3 larvae and whether a prophylactic regimen can be expected to prevent the development of aortic aneurysms and other early pathological changes associated with spirocercosis. However, even without these extra studies, our experimental inoculation trials have clearly demonstrated the efficacy of repeat doses of milbemycin oxime against pre-adult S. lupi. Improvements can be made to future milbemycin oxime dosing regimens based on the knowledge derived from these experimental studies

and previous natural infection trials. It is hoped that such refined protocols will eventually translate into effective prophylactic and therapeutic regimens which can be used in the control of S. lupi.

5. Conclusion

Milbemycin oxime, administered at repeat intervals of 2 or 4 weeks, demonstrated excellent efficacy against pre-adult S. lupi. It completely prevented the establishment and encapsulation of S. lupi in the oesophagus of experimentally infected dogs. This elimination of helminth related oesophageal pathology should prevent many of the debilitating clinical signs associated with spirocercosis if the results can be reproduced under natural conditions. Additional refinements to the treatment regimen are likely to improve the effect of the anthelmintic at the aortic level. Milbemycin oxime therefore warrants further investigation as a potential prophylactic and therapeutic anthelmintic option for the control of spirocercosis.

Conflict of interest

This study was funded by Novartis Animal Health, Basel, Switzerland. Two authors are current employees of Novartis Animal Health and assisted with the study design and the review of the manuscript.

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