



The use of milbemycin oxime in a prophylactic anthelmintic programme to protect puppies, raised in an endemic area, against infection with *Spirocerca lupi*

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ABSTRACT

Spirocerca lupi is primarily a parasite of dogs and other carnivores. Clinical signs of infection are regurgitation, vomiting, weight loss, coughing and dyspnoea. Sudden death can also occur due to a ruptured aortic aneurysm. In this study, the Eastern Cape Province of South Africa was identified as an area with a high prevalence of *S. lupi*. A subsequent investigation, to evaluate the efficacy of milbemycin oxime as a prophylactic agent for canine spirocercosis, involved 58 puppies that were raised in this area in accordance with local husbandry procedures. Approximately half of the puppies served as untreated controls. Puppies in the treatment group received milbemycin oxime (minimum dose of 0.5 mg/kg body weight) when they were between 2 and 6 weeks old. They then received five further treatments at approximately 28-day intervals. The treatment was orally administered in tablet form. After the sixth treatment, puppies from both the treated and control groups were euthanized and post-mortem examinations were performed. Twenty-four out of 27 dogs in the untreated control group had become infected by *S. lupi*. In comparison, only 19 out of 31 dogs in the treatment group had evidence of spirocercosis as demonstrated by aortic nodules. The prophylactic regimen reduced the severity of aortic lesions and prevented 86.5% of *S. lupi* from becoming established in the thoracic aorta. It also prevented 89.4% of *S. lupi* from becoming established in the oesophagus and significantly reduced the number of oesophageal nodules. Milbemycin oxime markedly reduced the level and severity of *S. lupi* infection in treated puppies raised in an endemic area of South Africa. It deserves further evaluation as a potential prophylactic treatment for spirocercosis.

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1. Introduction

Various surveys, conducted in the urban and peri-urban environments of South Africa, demonstrate that domestic dogs, and more particularly stray dogs, are infected with a variety of gastro-intestinal nematodes and cestodes (Ortlepp, 1934; Verster, 1979). More recent surveys have

yielded *Spirocerca lupi* and this nematode now appears to be an increasing cause of disease in dogs in several areas of the country. Minnaar and Krecek (2001) and Minnaar et al. (2002) reported a 13–14% prevalence of *S. lupi* when they surveyed two different areas of South Africa, and a questionnaire survey of 350 veterinary practices by Lobetti (2000) indicated that the incidence of *S. lupi* may actually be as high as 28% in the provinces of Gauteng and KwaZulu-Natal. DuToit et al. (2008) established a 13.5% incidence of infection in the dung beetle intermediate hosts in urban areas of Tshwane (Pretoria) Metropole, and

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our own unpublished observations indicated that Grahamstown and its surrounding environs in the Eastern Cape Province were likely to be endemically infected with *S. lupi*.

S. lupi is a spirurid nematode. It has a worldwide distribution, but is most prevalent in tropical and subtropical climates (Bailey, 1972; Van der Merwe et al., 2008). It is primarily a parasite of dogs, although other species such as felids and wild canids can also be infected. Infection occurs via the ingestion of third stage larvae (L₃) which are encysted in coprophagous beetle intermediate hosts or in various paratenic hosts (Sen and Anataraman, 1971). In the area where this study was conducted scarabaeid beetles, notably *Onthophagus* spp., are considered to be the most likely route of larval transmission. Once ingested the larvae excyst into the stomach, before migrating within the walls of the gastric and gastro-epiploic arteries to reach the caudal thoracic aorta in approximately 10 days (Bailey, 1972; Van der Merwe et al., 2008). There, the parasites remain and mature for up to 3 months before completing their final moult and migrating to the adjacent caudal oesophagus (Sen and Anataraman, 1971; Bailey, 1972; Van der Merwe et al., 2008). The migration and development of the parasite cause damage to the aortic and oesophageal walls: scarring, nodules and aneurysms may develop within the aorta and large nodules containing mature nematodes can protrude into the oesophageal lumen within 3–9 months post-infection (Bailey, 1963; Sen and Anataraman, 1971). Embryonated eggs are passed into the lumen of the oesophagus via fistulae in the oesophageal mucosa, and from there the eggs are passed into the surrounding environment via vomiting or defaecation (Bailey, 1972).

The clinical signs of spirocercosis are variable and depend upon the location and the severity of the lesions. Some dogs remain asymptomatic whereas others may die suddenly due to a ruptured aortic aneurysm (Ivoghli, 1977; Rinas et al., 2009). Unfortunately, most dogs suffer from a chronic and debilitating illness during which dysphagia, vomiting, regurgitation, weight loss and respiratory disease are common clinical signs (Dvir et al., 2001; Mazaki-Tovi et al., 2002). Aberrant parasite migration can cause problems in multiple areas of the body and infection can be complicated by neoplastic transformation, spondylitis, hypertrophic osteopathy and neurological dysfunction (Bailey, 1963; Brodey et al., 1977; DuPlessis et al., 2007; Harrus et al., 1996; Mazaki-Tovi et al., 2002; Ranen et al., 2004). Diagnosis of spirocercosis can be difficult due to the location of the parasites and the intermittent release of eggs (Chai et al., 2008). Special faecal flotation techniques (such as the modified sugar flotation protocol), radiography and oesophagoscopy tend to be the most reliable diagnostic procedures (Markovics and Medinski, 1996; Dvir et al., 2001; Mazaki-Tovi et al., 2002).

The treatment of spirocercosis is also problematical. Surgical resection is often impossible due to extensive and multiple lesions and there is currently no licenced product that kills both adult and larval stages of the parasite. Encouragingly, significant efficacy has been demonstrated with the use of macrocyclic lactones: oesophageal nodule resolution was achieved with the use of doramectin in two different protocols (Berry, 2000; Lavy et al., 2002) and Mylonakis et al. (2004) demonstrated an improvement in

the efficacy of subcutaneous ivermectin injections by combining it with an oral prednisolone regimen. Kelly et al. (2008) also demonstrated good efficacy and safety when milbemycin oxime was used in a preliminary study to treat six naturally infected dogs.

Whilst the investigation of treatment options continues, control measures to reduce the incidence of spirocercosis would significantly improve animal welfare. Exposure of dogs to larval forms of the parasite could be decreased by preventing hunting and scavenging and by removing faeces from the environment but this would require significant lifestyle alterations. Ideally a cost effective and efficacious prophylactic treatment option is required to curb the spread of *S. lupi* in endemic areas. The macrocyclic lactone, doramectin, has already demonstrated some prophylactic efficacy in experimentally infected dogs (Lavy et al., 2003), but there are concerns regarding its extra-label use, the mode of administration and possible breed specific toxicities (Yas-Natan et al., 2003). Milbemycin oxime is also a macrocyclic lactone but it is orally administered, has a good safety record and is already used for the control of other gastro-intestinal nematodes in dogs. This study therefore aimed to determine the prevalence of *S. lupi* infection in pups in the Grahamstown region and to subsequently investigate the efficacy of milbemycin oxime as a prophylactic treatment for canine spirocercosis in pups raised within this region.

2. Materials and methods

Both studies were carried out in accordance with the requirements of good scientific conduct. 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.

2.1. Prevalence study

Grahamstown and its immediate surroundings in the Eastern Cape Province were chosen as the locality for a pilot study because dogs from that region were frequently found to be heavily parasitized by several helminth species. Puppies, born to pregnant mongrel bitches originating from five households in this region, were raised by their dams and owners in accordance with local husbandry practices. They received routine canine vaccinations, a vaccination against rabies and a single spot-on formulation of permethrin. No other pharmaceutical products were administered during the study. When the youngest of the puppies reached approximately 6 months of age, they were transported to a laboratory near Bloemfontein (age range = 5 months and 27 days to 6 months and 23 days). They were euthanized 10 days later.

At necropsy, the oesophagus was removed from its anterior end up to the stomach. Thereafter, the thoracic aorta was removed from the heart up to at least the coeliac artery or slightly beyond. Portions of the brachial and brachiocephalic arteries, as well as the diaphragm,

were left attached to the aorta for orientation. An intensive macroscopic examination of the aorta and oesophagus was conducted and the size of any lesions attributable to *S. lupi*, and their positions relative to the diaphragm, were recorded. All nodules within the aorta and oesophagus were carefully dissected and visible nematodes were removed. The contents of the nodules were then washed over a sieve (aperture size 0.15 mm) to recover any remaining parasites. The nematodes recovered from the nodules and the contents of the sieve were preserved in 10% formalin before being identified and counted.

2.2. Prophylactic study

During September and October 2005, pregnant mongrel bitches were recruited from 23 households in the Grahamstown region. The bitches and resulting puppies remained within their original homes and were managed according to the customary husbandry procedures of their owners. They were housed mostly outside and were not fed a specified diet. Faeces were generally not removed. The puppies received a full physical examination and routine vaccinations at 6 and 9 weeks of age. Ectoparasite infestations and skin conditions were not recorded or treated as these were considered to be a natural part of the husbandry conditions at the field site.

At the time of the first prophylactic treatment, one puppy was selected at random from the first litter. Its ear was tattooed with a unique uneven number which assigned it to the treatment group. A second randomly selected puppy from the first litter then received an even numbered ear tattoo which assigned it to the control group. All pups in the first litter were alternately assigned to each study group before the same procedure was used to allocate the pups in the next and subsequent litters. This method ensured that the pups from each litter were evenly distributed between the treatment and control groups. The prevalence study had highlighted that not all puppies enrolled in the investigation would be available for follow-up at the end of the animal phase. Therefore, 114 pups were recruited for the prophylactic study. The requirement was to have approximately 30 puppies in each group at the end of the field trial phase.

Each puppy in the treatment group received milbemycin oxime (Milbemax[®]; Novartis Animal Health) (a tablet formulation containing praziquantel) at a minimum dose of 0.5 mg/kg body weight. The tablets were orally administered at approximately 28-day intervals on a total of six occasions. The first treatment took place when the puppies were between 14 and 43 days old. The puppies were weighed before each administration of the anthelmintic and their weights were used to calculate their individual dose. Individual body weights ranged between 0.5 kg and 2.25 kg at the time of the first treatment and between 2.5 kg and 12.45 kg at the time of the last treatment.

Upon completion of the field study phase, the pups were vaccinated against rabies before being transported to the ClinVet laboratory facility near Bloemfontein. The ages of the pups ranged from 162 days (23.1 weeks) to 192 days (27.4 weeks). At the laboratory facility, each dog was

placed in a separate pen comprising 6 m² of living space inside a kennel unit. Temperature and humidity were ambient, but the dogs were protected against rain. Pens were cleaned daily. At this time, only 58 of the original 114 animals were still available; 31 in the treated group and 27 untreated controls. Twenty-four of these were males and 34 were females. Identity tags attached to collars were fitted to each dog, and each pen was identified with a number corresponding to that on the dog's collar. A proprietary certified maintenance diet of dried pellets was provided once daily in stainless steel bowls. Potable water was available in stainless steel bowls and was replenished twice daily. During the period of stay in the ClinVet kennels, a physical examination of each dog was carried out.

Euthanasia and necropsy of all dogs took place between 9 and 14 days after transfer to the laboratory facility. A nominated veterinarian assigned new unique identification numbers to each dog at the time of euthanasia, thereby blinding the study to all personnel responsible for the necropsy procedures and parasitological assessments. The same necropsy procedures as described in the prevalence study were followed. For each animal, the size and number of nodules present in the aorta and oesophagus were recorded. In addition, the degree of damage to the aorta was recorded using a subjective scoring system; 0 = no lesions visible on the inner surface of the aorta to +++++ = large section of the aorta severely damaged with several aneurysms evident (Table 2 legend). All nematodes were recovered and counted as previously described.

In order to calculate the efficacy of the treatment program, the numbers of *S. lupi* recovered at necropsy were compared separately in the treated and the control groups.

The formula used was:

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

where N_1 = geometric mean (GM) number of *S. lupi* recovered at necropsy from either the aorta or oesophagus of those animals in the treated group. N_2 = GM number of *S. lupi* recovered at necropsy from either the aorta or oesophagus of those animals in the control group.

The same formula was used for the number of nodules.

Descriptive statistics were calculated for all parameters measured and efficacy was calculated from the geometric means of the worm counts or nodule counts. A two-sample *t*-test was used to compare the investigated parameters. In order to achieve normality of data, a log transformation was applied to some of the parameters. In addition, the Mann–Whitney *U*-test (non-parametric comparison) was also applied to the data. All calculations were performed using SAS[®], Version 8.2 software.

3. Results

3.1. Prevalence study

Of the twenty-eight puppies initially recruited for the study, 14 pups (five males and nine females) were available for necropsy. These dogs originated from five litters and were thus raised in five different households. At the time of necropsy, their weights ranged between 3.0 kg and 11.1 kg.

Table 1
Prevalence study: aortic nodules, oesophageal nodules and associated numbers of *Spirocerca lupi* in untreated dogs raised in an endemic region.

Dog I.D.	Thoracic aorta		Oesophagus						
	Number of nodules		Size (mm)		Number of <i>S. lupi</i>	Distance from diaphragm (mm)			
	Largest	Smallest	Largest	Smallest					
86	5	80	110	3 × 3	20 × 12	2	40	25 × 5	4
87	16	30	142	4 × 4	15 × 7	20	N/A	N/A	0
88	5	50	85	6 × 4	17 × 10	1	70	32 × 12	5
89	8	37	130	3 × 3	10 × 10	5	75	25 × 18	6
90	6	68	120	3 × 3	10 × 6	3	N/A	N/A	0
91	4	65	145	4 × 4	7 × 6	3	75	35 × 25	11
92	7	65	137	4 × 4	12 × 7	2	56 and 10	15 × 10	2
93	5	65	105	5 × 5	7 × 7	4	50	25 × 13	5
94	0	No evidence of infection				0	No evidence of infection	30 × 15	0
95	0	No evidence of infection				0	No evidence of infection		0
96	0	No evidence of infection				0	No evidence of infection		0
97	0	No evidence of infection				0	No evidence of infection		0
98	5	45	95	2 × 2	13 × 10	4	N/A	N/A	0
99	4	73	95	5 × 4	6 × 6	2	N/A	N/A	0

3.1.1. Necropsy findings

The necropsy findings are summarized in Table 1.

No pathological signs of infection by *S. lupi* were evident in four of the 14 pups. In those four animals, there was no evidence of pathological damage in either their thoracic aortae or oesophagii, and no *S. lupi* larvae or adults were recovered.

3.1.1.1. Aorta. *S. lupi* nodules were present in 10 of the 14 puppies. These nodules were mainly situated on or in the wall of the aorta, but occasional ones were found within the surrounding connective tissue. They varied in size from approximately 3 mm × 3 mm, up to 20 mm × 12 mm. They were evident from approximately 4 cm anterior to the diaphragm and were distributed throughout the length of the thoracic aorta. In some cases their distribution extended to the level of the heart. When incised, the nodules contained debris suggestive of previous haemorrhage and inflammation. Parasitic migratory tracts were occasionally seen on the aortic intima. In few cases, the aortic wall bulged outwards forming an aneurysm. Approximately half of the aortic nodules contained immature *S. lupi*, varying in length from approximately 3 to 5 cm. A total of 46 immature *S. lupi* were recovered.

3.1.1.2. Oesophagus. Six of the 10 puppies that demonstrated infections of the thoracic aorta, also showed evidence of *S. lupi* in the oesophagus. Five of these puppies had a single oesophageal nodule and the remaining pup had two. The nodules varied in size from approximately 15 mm × 10 mm up to 30 mm × 15 mm. They were mostly located between 4.0 cm and 7.5 cm anterior to the diaphragm, with one situated only 1 cm anterior to the diaphragm. The number of nematodes recovered from the nodules in the individual dogs tended to be between one and six, but 11 nematodes were recovered from a single nodule in one dog. A total of 33 *S. lupi* were recovered from the six puppies with oesophageal nodules. There was no evidence of any openings between the nodules and the lumen of the oesophagus. Four puppies had evidence of aortic infection and damage, but demonstrated no oesophageal nodules. One of these four pups had 16 nodules in the thoracic aorta from which 20 larvae were recovered.

3.2. Prophylactic study

Monthly visits were made to the field site in order to administer the investigational anthelmintic product and no serious medical conditions were seen amongst the recruited puppies. However, only 58 of the original 114 pups were still available for necropsy at the end of the animal phase. The remaining 58 puppies came from 20 litters and were therefore raised in 20 different households. The most pups available from any one litter were five (mean 2.9 ± 1.02). Thirty-one puppies remained in the treated group (20 females and 11 males) and 27 remained in the untreated control group (14 females and 13 males).

Table 2Prophylactic study: macroscopically visual damage to the aorta caused by *Spirocerca lupi* infection, in treated and control dogs raised in an endemic area.

Lesion score summary				
Score	Number of animals to which score was allocated		Percentage of animals to which score was allocated	
	Control group	Treated group	Control group	Treated group
0	5	15	18.50	48.40
+	7	12	25.90	38.70
++	4	2	14.80	6.40
+++	5	2	18.50	6.40
++++	5	0	18.50	0
+++++	1	0	3.70	0

0=no lesions on inner surface of aorta. +=inflammatory/puckered lesions only just visible. ++=two or three lesions clearly visible; no aneurysms. +++=several distinct inflammatory lesions; parasitic migratory tracts often visible; aneurysms starting to develop. ++++=distinctly damaged aorta; all of the above evident; aneurysms more developed. +++++=worst case scenario; aorta severely damaged over a large area; several aneurysms evident.

3.2.1. Necropsy findings

3.2.1.1. Aorta. The extent of the damage caused by *S. lupi* to the wall of the thoracic aorta, as assessed by a visual scoring system, is summarized in Table 2. In the control group, 22 of the 27 puppies had lesions on the inner surface of the aorta. In 11 of these affected individuals, the lesions were scored at +++ or more, indicating serious damage to the wall of the aorta. In the treated group, 16 of the 31 puppies also showed signs of damage to the thoracic aorta, but only two of these animals received a score of +++ and none received higher scores.

In the untreated control group, 24 (88.9%) of the 27 puppies were infected with *S. lupi* as judged by the occurrence of nodules on the thoracic aorta. The number of nodules in individual dogs varied from 0 to 15 (total 169; arithmetic mean 6.3, standard deviation 4.1). Only 19 (61.3%) of the 31 dogs in the treated group showed evidence of aortic nodules and the number of nodules in the individual dogs tended to be lower (ranging from 0 to 9). The total number of aortic nodules (78) in the treated group was less than half that of the control group total, and the arithmetic mean was only 2.5 (standard deviation 2.8). *S. lupi* larvae were recovered from the aorta of all but one of the affected dogs in the untreated control group, whereas the aortic nodules in eight of the treated animals harboured no larvae. In the untreated control group, a total of 196 *S. lupi* (arithmetic mean 7.3, standard deviation 7.7) were recovered from the aortic nodules. The number of nematodes recovered from individual dogs tended to range from 0 to 19, although one pup harboured 34. In the treated group, only 31 *S. lupi* (arithmetic mean 1.0, standard deviation 1.5) were recovered from the aortic nodules and the number of larvae recovered from the individual dogs varied from one to four. The data on *S. lupi* associated aortic nodules and the number of nematodes recovered at necropsy is summarized in Table 3.

3.2.1.2. Oesophagus. In the control group ($n=27$) the *S. lupi* infection had spread to the oesophagus of 12 of the 24 puppies that had demonstrated infection of the thoracic aorta. The number of oesophageal nodules varied between one and four in these individual animals and *S. lupi* were recovered from nine of the 12 affected pups. In total, 43 *S. lupi* were recovered from the oesophageal nodules, with numbers varying from 1 to 13 in individual animals. In

the treated group ($n=31$), the *S. lupi* infection had spread to the oesophagus of only four of the 19 puppies that had demonstrated infection of the thoracic aorta. Only one oesophageal nodule was found in each of the four pups, and only one *S. lupi* was recovered from each of three of these dogs. No nematodes were discovered in the oesophageal nodule of the fourth pup. The data on the *S. lupi* associated nodules found in the oesophagus and the number of nematodes recovered from these nodules during necropsy, is summarized in Table 3.

3.2.1.3. Efficacy data. Percentage efficacy, based on geometric means, is also summarized in Table 3. Efficacy was calculated separately for each of the four parameters: number of aortic nodules, number of *S. lupi* in the thoracic aorta, number of oesophageal nodules and the number of *S. lupi* in the oesophagus. Although a treatment efficacy of only 68.1% was calculated in respect of the reduction in the number of aortic nodules, both the two-sample *t*-test and the Mann–Whitney *U*-test results indicated that the decrease was actually highly significant ($p=0.0004$). Likewise, a highly significant difference ($p<0.01$) was found between the group variables for both the number of *S. lupi* found within the thoracic aorta and the number of oesophageal nodules. The efficacy of treatment exceeded 80% in both cases. The highest effect of treatment (efficacy 89.4%) was seen in the reduction of *S. lupi* in the oesophagus, and both statistical tests indicated that this lowering of oesophageal infection was significant ($p=0.0160$).

4. Discussion

The prevalence study demonstrated that only a percentage of the originally recruited pups were available at the end of the 6-month field study phase. A similar reduction in the prophylactic study sample population was therefore predicted and this factor was taken into account when the pups were recruited into the study. Losses were attributed to various factors inherent to the study area, but none were thought to be related to infection with *S. lupi* or to the administration of the treatment product.

4.1. Prevalence study

During this study, 10 out of 14 pups raised in Grahamstown and its environs, became infected with *S. lupi*. This

Table 3

Prophylactic study: mean numbers of lesions and *Spirocerca lupi* in the aortae and oesophagii of treated and control dogs. The efficacy of treatment is shown as a percentage.

Parameter	Group	Number of dogs	Arithmetic		Geometric		Min	Median	Max	Efficacy (%)
			Mean	S.D.	Mean	S.D.				
Thoracic aorta; nodules	Treated	31	2.5	2.8	1.5	2.1	0	2	9	68.1
	Control	27	6.3	4.1	4.7	4.6	0	7	15	
Thoracic aorta; <i>S. lupi</i>	Treated	31	1.0	1.5	0.6	1.0	0	0	4	86.5
	Control	27	7.3	7.7	4.4	5.4	0	5	34	
Oesophagus; nodules	Treated	31	0.1	0.3	0.1	0.3	0	0	1	80.8
	Control	27	0.7	1.0	0.5	0.7	0	0	4	
Oesophagus; <i>S. lupi</i>	Treated	31	0.1	0.3	0.1	0.2	0	0	1	89.4
	Control	27	1.6	3.3	0.7	1.4	0	0	13	

S.D. = standard deviation.

corresponds to a prevalence of 71.4%. All of the infected pups demonstrated pathological changes within their aortae and six out of the 10 reached the later stages of spirocercosis as attested by the presence of oesophageal nodules (Sen and Anataraman, 1971; Bailey, 1972). This variability in the distribution of lesions in the individual animals can be attributed to the limited duration of the study and the natural variability in the time, and therefore the resultant stage, of infection. It appears that at the end of a 6-month period, the majority of pups raised within the study area, harbour *S. lupi* in the transitory phase between aortic maturation and early infection of the oesophagus. Indeed, no fistulae were evident in the oesophageal nodules of the six pups, implying that the encysted female nematodes may be immature and non-egg laying, and thereby increasing the likelihood that the oesophageal infection was still in the early stages. All of the infected pups would probably have developed pathological signs of oesophageal infection if the study period had been extended. This is because the level of aortic infection tended to be high and several aortic nodules contained debris but no nematodes, indicating that the inhabitants may have already started their migration to the oesophageal wall.

Only four of the pups had no evidence of infection within either the thoracic aorta or the oesophagus. These pups were litter-mates and had been raised in the same household. In order to protect the puppies during his absence at work, the owner confined them to a cage during the day and early evening. They were also restricted to his enclosed premises at night. This would have minimized the exposure that the puppies would have had to the intermediate hosts, whereas the other puppies in the study were allowed considerably more freedom of movement and would have been exposed to the risk of infection with *S. lupi* from an early age. Although this finding confirmed that future studies would have to account for individual household factors affecting the chance of infection of each dog, it also verified that the selection procedure would produce a study sample that was representative of the dog population as a whole, especially if the number of households included within the study was increased.

This initial study confirmed a high prevalence of *S. lupi* in Grahamstown and in its immediate surroundings in the Eastern Cape Province. It also showed that puppies, raised in accordance with local husbandry practices in these endemic areas, would have a significant chance

of becoming infected with *S. lupi* by the time they were 6 months of age. In comparison, circumstantial evidence suggested that if exposure to intermediate and paratenic hosts is restricted by caging or residential boundaries, puppies may avoid infection with *S. lupi*. In those puppies that did become infected, the signs of spirocercosis were easily detected at necropsy; first in the thoracic aorta and later in the adjacent oesophagus.

4.2. Prophylactic study

Although the prophylactic milbemycin oxime regimen used in this study did not completely eliminate aortic damage, it did reduce the overall percentage of aortic infection by *S. lupi* from nearly 90% in the control group to only 61% in the treated group. In addition, the highly significant reduction in both aortic larval burden and aortic nodules, translated into a marked decrease in the aortic pathological score of treated animals. Importantly, only two out of 31 treated pups demonstrated grade +++ aortic lesions and none of the animals within this group had lesions of a higher grade. In comparison, over 40% of the control animals had lesions scored at +++ or more. Prophylactic milbemycin oxime may therefore prevent the formation of large aortic aneurysms and thereby prevent death from their subsequent rupture, but longer duration studies would have to be conducted to confirm this. Interestingly, eight of the dogs from the treated group harboured no larvae within any of their aortic nodules, whereas aortic larvae were recovered from all but one of the 24 infected control pups. The prophylactic study findings indicate an efficacy of milbemycin oxime against immature *S. lupi* larvae and possibly against the larvae encysted within aortic nodules. Aortic pathology may therefore be further reduced, or even eliminated, if the prophylactic protocol is adjusted to account for the rapid migration of *S. lupi* from the stomach to the aorta.

Similar levels of variability were observed between the levels of oesophageal infection in individual animals as were noted in the prevalence study. Variations in the levels of infection of an individual can be attributed to natural variability in the time and level of exposure of each dog to infection. However, as the prevalence study showed, these differences can also be exacerbated by the animal husbandry procedures within each household because these affect the prevalence of infection in the immediate vicinity

of the home and the degree of access that each puppy has to the intermediate hosts. It is important to note that the prophylactic study initially included 20 different residences within the field trial phase. This inclusion of a large number of households ensured that the sample population was an excellent representation of the residential dog population as a whole, whilst minimizing the effect of individual management procedures on the resulting data. In addition, the study design guaranteed that representatives of each litter, and therefore animals exposed to similar housing conditions, were present in both the treatment and the control groups; thereby producing a similar distribution of infection stages within each study group.

Due to the long maturation phase within the aorta, the level of oesophageal infection in both study groups was generally lower than the level of aortic infection. However, the marked effect of prophylactic treatment was still apparent: only four pups from the treated group showed signs of oesophageal infection compared to 12 pups from the control group, and significantly only three *S. lupi* were recovered from the treated group compared to a total of 43 *S. lupi* recovered from the control group. In addition, a highly significant ($p=0.005$) effect of prophylaxis was demonstrated by a reduction in oesophageal nodules. It is important to note from a welfare perspective that many of the common clinical signs associated with spirocercosis are associated with large or numerous oesophageal nodules, and in this study, the efficacy of prophylactic milbemycin oxime was over 80% in reducing the number of oesophageal nodules and nearly 90% in lowering the oesophageal nematode burden. By delaying the *S. lupi* larval migratory phase or by its direct anthelmintic action on the larval and/or adult stages of the nematode, this study indicates that prophylactic treatment with milbemycin oxime significantly reduces the level of oesophageal infection and pathology and can therefore be expected to ameliorate many of the clinical signs associated with spirocercosis.

Other studies have demonstrated very good anthelmintic efficacy against *S. lupi* (Berry, 2000; Lavy et al., 2002) but significantly they did not include an assessment of aortic infection. Their dogs may have contained similar aortic lesions to those found in this study as affected animals are often asymptomatic. Lavy et al. (2003) reported a reduction in infection and clinical signs, similar to our own study, when they prophylactically treated five beagle dogs with doramectin. However, in the doramectin study, the dogs were experimentally infected and housed in an enclosed kennel, thereby preventing any exposure to intermediate hosts and avoiding re-infection. Our prophylactic study is the first to be performed within an endemic area and to demonstrate efficacy in the face of repeat challenge. It is also likely that the benefit of prophylaxis demonstrated in our study could be increased if treatment was initiated alongside improved animal husbandry and environmental control.

Milbemycin oxime is already licenced for the treatment of other nematodes in dogs, it is easily administered in the form of oral tablets and it is relatively cost effective. Kelly et al. (2008) previously demonstrated the efficacy of milbemycin oxime against adult forms of *S. lupi* when milbemycin oxime (0.5 mg/kg body weight) eliminated

the nematode from the oesophagus of naturally infected dogs. In addition to supporting these findings, our prophylactic study indicates an efficacy of the treatment product against immature *S. lupi* larvae. Milbemycin oxime therefore deserves further consideration and evaluation as a means of controlling spirocercosis within an endemic area. In particular, efforts should be made to establish an effective dosing regimen which targets the immature *S. lupi* larvae migrating to the aorta, thereby further reducing or eliminating pathological changes within the aorta and correspondingly controlling oesophageal infection.

5. Conclusion

S. lupi is endemic in Grahamstown and its surroundings in the Eastern Cape Province of South Africa. Puppies raised in this endemic area, who received prophylactic treatment with milbemycin oxime (0.5 mg/kg body weight) at monthly intervals, showed a significant reduction in the level and severity of infection with *S. lupi*.

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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