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ORIGINAL PAPER

Efficacy of a combination of imidacloprid 10%/moxidectin 2.5% spot-on (Advocate® for dogs) in the prevention of canine spirocercosis (*Spirocerca lupi*)

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Abstract The nematode *Spirocerca lupi* is a major canine parasite in warm regions of the world, classically causing parasitic nodules in the esophagus, aortic aneurysms, and spondylitis. This study evaluated the preventive efficacy of monthly treatment with imidacloprid 10%/moxidectin 2.5% spot-on (Advocate® for dogs) administered over a period of 9 months in young dogs naturally exposed to *S. lupi* on Réunion island. One hundred and twelve puppies, aged from 2.0 to 4.0 months and with a negative spirocerca fecal examination at inclusion, completed the study. They were randomly allocated to two groups. Group A puppies (n=58) received nine spot-on treatments with Advocate® at the minimum dose of 2.5 mg moxidectin/kg bw at monthly intervals. Control group B puppies (n=54) received no treatment for *S. lupi*. During the study, regular clinical and fecal examinations were performed, as was final upper gastrointestinal endoscopy. Endoscopy showed that 19 dogs from group B had spirocerca nodules, corresponding to a prevalence of 35.2% in dogs aged 12 to 14 months. In contrast, only one dog from group A had a nodule, corresponding to a preventive efficacy of 94.7% ($p<$ 0.0001). None of the 378 fecal examinations were positive for *spirocerca*. This study confirms a high prevalence of canine spirocercosis on Réunion and shows that infestation occurs in very young puppies. Furthermore, it demonstrates that monthly spot-on administration of a combination of imidacloprid 10%/moxidectin 2.5% (Advocate® for dogs) in puppies starting at the age of 2 to 4 months achieves effective and safe prevention of canine spirocercosis.

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Introduction

Spirocerca lupi (Nematoda: Spirurida, Thelaziidae) is a parasite of dogs that can also affect other animals, mainly carnivores such as foxes, wolves, coyotes, and wild Felidae. The life cycle is indirect, with coprophagous beetles acting as intermediate hosts. A large variety of Amphibia, reptiles, birds, and small mammals such as hedgehogs, mice, and rabbits can act as paratenic hosts (Bourdoiseau 2000).

The adult *S. lupi* occurs in a nodular mass in the wall of the esophagus. The female lays embryonated eggs that leave the nodule via an opercule and are shed in the host's feces. Eggs are ingested by the intermediate host, coprophagous beetles, and develop to the infective (L3) stage within 2 months. In the carnivore host, the infective larvae penetrate the gastric mucosa, and migrate within the walls of the gastric arteries to the thoracic aorta. About 3-months post-infection, the larvae leave the aorta and migrate to the esophagus where they provoke the development of granulomas as they mature to adults over the next 2 to 3 months (Fig. 1). The prepatent period is usually 4 to 5 months (Soulsby 1986; Bourdeau 1985; Bourdoiseau 2000).

The clinical signs associated with canine spirocercosis depend on the location of the lesions. The development of chronic esophagitis leads to vomiting and regurgitation, weakness, emaciation, loss of weight, anemia, or bleeding (Mazaki-Tovi et al. 2002). Other signs include sudden death due to the rupture of an aortic aneurysm (Rinas et al. 2009) or the rupture of the esophagus, and evolution of the nodules into tumors (Bourdoiseau 2000; Reche-Emonot et al. 2001).

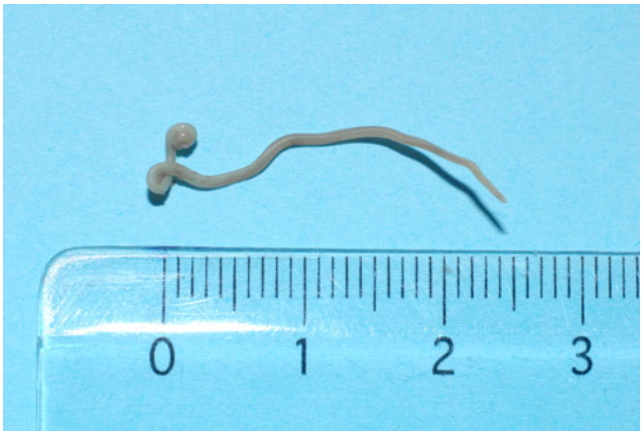

Fig. 1 Adult *Spiroceca lupi*

The lesions caused by *S. lupi* are mainly due to the migration of the larvae and adults in the tissues. The most frequent lesions are esophageal nodular masses and granulomas, aortic scars, and aneurysms (Fox et al. 1988). Spondylitis and spondylosis of the caudal thoracic vertebrae may be observed, as may the neoplastic transformation of granulomas into fibrosarcoma or osteosarcoma (Soulsby 1986; Johnson 1992; Ranen et al. 2004; Van der Merwe et al. 2008). Aberrant migration of *S. lupi* worms can occur, and the resulting nodules can affect various organs including the thoracic organs, the gastrointestinal tract, the urinary system, and the subcutaneous tissues (Mazaki-Tovi et al. 2002).

The diagnosis of spirocercosis usually consists in the detection of the characteristic eggs by fecal flotation (Markovics and Medinski 1996; Bourdoiseau 2000), although sensitivity is low due to intermittent egg shedding. Endoscopy is a reliable examination to detect the esophageal and gastric *S. lupi* nodules (Reche-Emonot et al. 2001). Additional examination includes radiographs to detect the nodules or resulting tumors in affected organs (i.e., esophagus, thoracic vertebrae etc.; Mazaki-Tovi et al. 2002).

S. lupi is a cosmopolitan parasite which affects canines mainly in tropical and subtropical areas. Clinical cases or observations in domestic dogs have been reported in a large number of countries including South Africa (Van der Merwe et al. 2008), Israel (Ranen et al. 2004, 2008; Dvir et al. 2008), Argentina (Rinas et al. 2009), Brazil (Tudury et al. 1995), Kenya (Murray 1968), Iran (Naem 2004), India (Chandrasekar et al. 1995) and the southern US (Dixon and McCue 1967). Spirocercosis is observed very occasionally in France (Bourdeau 1985), but it is a major canine disease on Réunion (Indian Ocean). An epidemiological and clinical study performed on Réunion showed a clinical prevalence of 4.8% (120 confirmed clinical cases out of 2,498 examined dogs), and an asymptomatic prevalence of 27.5% (14 positive coproscopic examinations out of 51 examined asymptomatic dogs; Reche-Emonot et al. 2001).

Of the anthelmintic drugs which have been tested to treat spirocercosis, macrocyclic lactones are the most interesting. In the majority of study protocols, the drug was administered subcutaneously. Ivermectin has been reported to be ineffective against *S. lupi* (Last and Smith 2007). However, ivermectin (1,000 μg/kg body weight (bw)) in combination with nitroxynil (10 mg/kg bw) administered subcutaneously was reported to be successful in treating infected dogs on Réunion in 81.6% of cases. A clinical cure was achieved in animals following both endoscopic evaluation and fecal flotation (Reche-Emonot et al. 2001). Mylonakis et al. (2004) reported that two doses of ivermectin (600 mcg/kg bw, s.c., 2-weeks apart) in combination with oral prednisolone (0.5 mg/kg bw twice a day for 2 weeks followed by dose tapering) promoted nodular regression and suppressed fecal egg shedding for up to 2 months following the last treatment.

Doramectin has produced positive results in both spontaneous and experimental cases. Berry (2000) demonstrated that 200 μg/kg bw of doramectin injected s.c. at 14-day intervals for three subsequent treatments was effective in treating spirocercosis in 5/7 infected animals. Doramectin was again shown to be effective by Lavy et al. (2002) using an alternative dosing regimen of 400 μg/kg bw injected s.c. every 14 days for six treatments, followed by monthly dosing until resolution of the parasitic nodule. Reduction of eggs shedding was observed in the feces as little as 3–10 days after the first treatment.

Kelly et al. (2008) reported that milbemycin oxime (11.5 mg p.o. on days 0, 7 and 28, and then monthly) stopped the shedding of *S. lupi* eggs after 3–44 days in six treated dogs and promoted nodular regression.

There are few reports on the prevention of spirocercosis. The prophylactic effect of doramectin was investigated by Lavy et al. (2003) in experimentally infested dogs. Five dogs were injected subcutaneously with doramectin (400 μg/kg bw on three occasions 30–days apart) and then inoculated with 40 infectious *S. lupi* larvae (L3) 1 month after the last doramectin treatment. Although doramectin did not entirely prevent canine spirocercosis, it reduced the clinical signs associated with infection and delayed and reduced egg shedding.

Despite the positive results obtained with the reported treatment regimens with doramectin and ivermectin against *S. lupi*, the drugs are used off-label and have to be injected, which is inconvenient, time-consuming, and expensive for owners. It was therefore interesting to investigate the efficacy of a macrocyclic lactone (moxidectin) available in a more user-friendly spot-on formulation which is approved for use in dogs for a number of other internal parasitic infections.

The purpose of the study was to evaluate the preventive effects of monthly treatment with imidacloprid 10%/moxidectin 2.5% spot-on (Advocate®) administered over a period of 9 months in comparison to an untreated control

group, based on clinical and parasitological parameters in young dogs naturally exposed to *S. lupi* on Réunion island.

Materials and methods

Study design

The study was designed as a multicentre, randomized, controlled, blinded (endoscopy and fecal examinations were blinded) clinical field study conducted in three veterinary practices located on Réunion island (Indian Ocean) in accordance with Good Clinical Practice (VICH GL9 (GCP), Step 7) as adopted by the CVMP in June 2000. The effect of treatment was compared to a negative control group (untreated).

The study protocol was based on ten visits at 4-week intervals for the treated group, and four visits for the dogs in the untreated group (V1=inclusion, V2=8th month, V3=9th month, V4=10th month/termination). The main efficacy criterion was the non-occurrence of infection as confirmed by fecal and endoscopic examination at month 10 (end of the study).

Study animals

In view of the purpose of the study (evaluation of the preventive efficacy of the combination imidacloprid 10%/moxidectin 2.5% spot-on), the prepatent period of the parasite (4 to 5 months) and the significant prevalence of the parasite on Réunion, only very young dogs (from 2 to 4 months of age) were included in order to make sure that they were free of spirocerca infection at the time of inclusion.

One hundred and twelve (112) puppies naturally exposed to spirocerca infection completed the study during the period from July 2007 to March 2009. The study animals consisted of 56 males and 56 females of various breeds. At the time of inclusion, the age of the puppies ranged from 2.0 to 4.0 months (median, 3 months).

The puppies were randomly allocated to two groups. Group A puppies (n=58) received nine spot-on treatments with Advocate® (imidacloprid 10%/moxidectin 2.5% spot-on) at monthly intervals. They consisted of 27 males and 31 females, aged from 2.0 to 4.0 months (mean, 2.8 months). Group B puppies (n=54) received no treatment for *S. lupi* and consisted of 29 males and 25 females, aged from 2.0 to 4.0 months (mean, 2.9 months).

The main data describing the puppy population are shown in Table 1.

Treatment

In group A, animals were treated with imidacloprid 10%/moxidectin 2.5% spot-on (Advocate® for dogs; Bayer Animal Health GmbH, Leverkusen, Germany) at the minimum recommended dose of 2.5 mg moxidectin/kg bw s.c. (equivalent to 1 mL Advocate®/10 kg bw). Given that Advocate® for dogs is marketed in pre-dosed pipettes, the administered dose of moxidectin ranged from 2.5 to 6.25 mg/kg bw as indicated in Table 2.

Clinical examinations

Clinical examinations were performed at each visit (i.e. ten times for group A and four times for group B). The following clinical parameters were evaluated: general behavior, appetite,

Table 1 Description of the study animals

		Group A (treated)	Group B (untreated)
Total population		n=58	n=54
Sex	Male	n=27	n=29
	Female	n=31	n=25
Age (in months)	Minimum	2.0	2.0
	Maximum	4.0	4.0
	Mean	2.8	2.9
Length of coat	Short	n=32	n=34
	Average	n=20	n=13
	Long	n=6	n=7
Altitude of the household (m)	Minimum	0.0	0.0
	Maximum	1,400	1,400
	Mean	389.9	386.9
Total number of dogs in the household	Minimum	1.0	1.0
	Maximum	6.0	10.0
	Mean	1.8	2.0

Table 2 Applied dose of Advocate® for dogs

Weight of the dog [kg]	Size of the pipette	Volume of the pipette [ml]	Imidacloprid [mg/kg bw]	Moxidectin [mg/kg bw]
≤4 kg	Advocate for small dogs	0.4	minimum 10	minimum 2.5
4–≤10 kg	Advocate for medium dogs	1.0	10–25	2.5–6.25
10–≤25 kg	Advocate for large dogs	2.5	10–25	2.5–6.25
25–≤40 kg	Advocate for very large dogs	4.0	10–16	2.5–4
>40 kg	Appropriate combination of pipettes			

respiratory tract, cardiovascular system, gastrointestinal tract, musculo-skeletal system, skin, and other clinical signs.

Fecal examinations

Fecal examinations were carried out for both A and B groups on four occasions: at V1 (inclusion visit) and at the last three visits (corresponding to V8, V9, and V10 for group A and V2, V3, and V4 for group B). Fecal samples were examined using a magnesium sulfate (MgSO4, density 1.28) flotation method and the number of *S. lupi* eggs per gram of feces was recorded for each sample (Thienpont et al. 1979).

The fecal examinations were blinded, so the laboratory did not know which group the evaluated samples originated from.

Endoscopy

Upper gastrointestinal (esophagus and stomach) endoscopy was performed on each dog during the last visit, corresponding with the 10th month of the study follow-up, by a clinician other than the one who examined the dog during the study (blinded endoscopy). Endoscopies were performed using mainly Optomed® flexible four-way directional tip (7.9 mm) endoscopes. For endoscopy, the dogs were anesthetized intravenously with ketamine (Clorketam® 1000, Vetoquinol, 5 mg/kg bw i.v.) and xylazine (Rompun®, Bayer, 1 mg/kg i.v.) in some cases followed by a volatile anesthetic such as isoflurane. The location (esophagus or stomach), size, number, and type of nodules (simple, operculated, or ulcerated nodule, tumor) were recorded as described by Reche-Emonot (Reche-Emonot et al. 2001).

Calculation of preventive efficacy

The effect of nine monthly treatments on the prevention of canine spirocercosis was calculated according to the following formula:

$$\%\,\text{Efficacy} = (\text{Number positive dogs(controlgroup)} - \text{Number positive dogs(treatment group)}) \times 100/\text{Number positive dogs(control group)}$$

Statistical analysis

The analysis was performed with the validated program Testimate Version 6.4 from IDV Gauting (validation of software, hardware, and user according to FDA 21 CFR Part 11) and Report Version 6.6 from IDV Gauting. All evaluation steps were documented in full and in-process controls were performed and also documented (time stamps, audit trail).

The primary criteria "non-occurrence of infection as confirmed by parasitological or clinical (endoscopic) examination at month 10 (end of study)" was assessed using the 2×2 table test (Fisher's exact test) for treatment group A vs. group B (untreated control group).

The hypothesis for the superiority test was: H0: pR−pT≤ 0.05 and H1: pR−pT>0.05 (pR: prevalence in reference group, pT: prevalence in treatment group A). The multiple-level alpha for the experiment was defined as a one-sided alpha=0.10.

The medical relevance of the differences between the groups was quantified using the Mann–Whitney superiority measure (MW) with its one-sided 90.0% confidence interval as the corresponding effect size. The efficacy analysis was performed using the PP population (per protocol).

All secondary criteria (efficacy and safety) were analyzed descriptively, using non-parametric tests as well if appropriate. Descriptive statistics were performed for all criteria: for continuous data: valid n; absolute values, change from baseline, percentage change from baseline; average, mean, and standard deviation, minimum, lower quartile, median, upper quartile, maximum per group; for category data: absolute and percentage values per group.

Results

Fecal examination

During the study, 196 fecal samples were collected and analyzed according to the protocol in group A (treated), and 182 in group B (untreated). In comparison with the expected collection of samples (i.e., four fecal samples per dog during the study), 36 fecal samples were missing in group A, and 34 in group B.

No *S. lupi* eggs were found in any of the 378 samples analyzed.

Endoscopy

Endoscopy was performed 10 months after inclusion in the 112 dogs and showed that 20 dogs were infested with *S. lupi*. The presence of nodules was observed in 19 dogs out of 54 (35.2%) in group B (untreated) and in only one dog out of 58 (1.7%) in group A (treated). The presence of spirocerca nodules in the treated group is statistically significantly lower ($p<0.0001$) than in the untreated group. This demonstrates the high preventive efficacy of the combination of imidacloprid 10%/moxidectin 2.5% spot-on against natural spirocerca infestation.

The location of the observed nodules was mainly esophageal (19 cases out of 20). Only one dog in group B had one stomach nodule, but no esophageal nodule.

Out of the 20 infested cases, simple nodules were observed in 11 dogs, operculated nodules in 13 dogs (Fig. 2), and ulcerated nodules in only two dogs. No tumoral form was observed. The size of the esophageal simple nodules ranged from 0.5 to 1.5 cm (average 0.9 cm). The range was 1.0 to 2.0 cm (average 1.4 cm) for operculated nodules, and 0.5 to 2.0 cm (average 1.25 cm) for ulcerated nodules.

Endoscopy showed that infested dogs had mainly one nodule. Only four dogs from group B had two nodules, and two dogs had three nodules. The main findings of the endoscopic examination are presented in Table 3.

Calculation of efficacy

Based on the absence of nodules in the esophagus, monthly treatments with imidacloprid 10%/moxidectin 2.5% spot-on (Advocate®) administered over a period of 9 months to young dogs aged 2–4 months achieved a protection rate of 98.3% ($p<0.0001$) against spirocerca infection. None of the treated dogs (group A) had parasitic nodules in the stomach. Since the situation was very similar in the group B dogs (untreated), where only one dog out of 54 had a stomach nodule, no statistical difference between the groups could be established for this parameter. Comparison with the group B dogs (untreated) with esophageal or stomach nodules ($n=19$ out of 54) shows a preventive efficacy rate of 94.7% and establishes the statistical superiority of group A over group B (Fisher's exact test, $p<0.0001$).

Clinical findings

Only two dogs from group B with a positive endoscopy showed some of the classical spirocercosis signs: during the last visit (V4) one dog showed vomiting and reduced appetite and another dog exhibited vomiting. Among the endoscopy-negative dogs, only one showed vomiting on the day of the last visit (V10), all other clinical parameters being normal. During the study, none of the group A (treated) dogs showed any adverse event linked to administration of the treatment, thus confirming the good tolerance of the product.

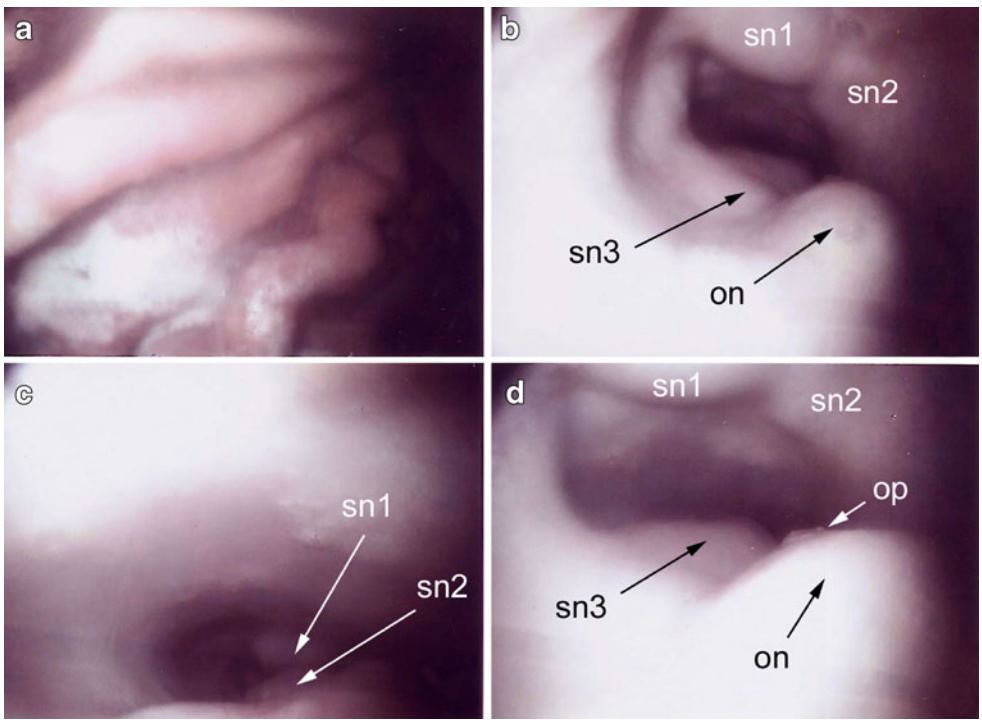

Fig. 2 Spirocerca nodules observed by endoscopy in dog COZ43 from the untreated control group. **a**: No nodule was observed in the stomach part. **b**: Four esophageal nodules could be observed: three simple nodules (*sn1*, *sn2*, *sn3*) and one operculated nodule (on). **c**: View showing two simple nodules (*sn1*, *sn2*). **d**: View showing the three simple nodules (*sn1*, *sn2*, *sn3*) and the opercule (*op*) of the operculated nodule (*on*)

Table 3 Results of the endoscopic examination (n=112 dogs)

	Group A (treated; n=58 dogs)		Group B (untreated; n=54 dogs)	
Number of dogs with	Esophagus	Stomach	Esophagus	Stomach
No nodule	57 (98.3%)	58 (100%)	36 (66.7%)	53 (98.1%)
At least one nodule	1 (1.7%)	0 (0%)	18 (33.3%)	1 (1.8%)
Simple nodule(s)	1 (1.7%)	0	9 (16.7%)	1 (1.8%)
Operculated nodule(s)	0	0	13 (24.1%)	0
Ulcerated nodule(s)	0	0	2 (3.7%)	0
Tumor(s)	0	0	0	0

Discussion

The purpose of the study was to evaluate the preventive efficacy of monthly treatment with imidacloprid 10%/moxidectin 2.5% spot-on (Advocate®) administered over a period of 9 months in young dogs naturally exposed to *S. lupi* on Réunion Island. The parameters evaluated during the study were the presence of eggs of *S. lupi* in the feces and the endoscopic detection of gastroesophageal nodules, as well as the possible occurrence of typical clinical signs.

Many studies have shown that macrocyclic lactones (ivermectin, doramectin) used subcutaneously are effective in the curative treatment of *S. lupi*. (Mylonakis et al. 2004; Lavy et al. 2002; Reche-Emonot et al. 2001). The preventive efficacy of doramectin administered subcutaneously was evaluated in one experimental study (Lavy et al. 2003), but this showed that efficacy was not complete (2/5 treated dogs still showed radiographic changes). In the current study, the preventive efficacy of moxidectin was tested as a spot-on formulation (Advocate®), which makes regular administration easier for the pet owner. At the end of the study, 57/58 dogs from group A (98.3%) were spirocerca-free against only 35/54 dogs from group B (64.8%): this result confirms the high degree of preventive efficacy (94.7%, $p<0.0001$) of the tested formulation.

Spot-on application requires the treated animal to not be bathed during the 24 h after treatment in order to make sure that the active ingredient (moxidectin) can penetrate through the skin. During the study, one dog from group A lived close to a river and had very regular baths, meaning that the 24-h bath-free period post treatment was not observed due to lack of owner compliance. As a consequence, this was the only "treated" dog to developed *S. lupi* infection.

Endoscopy showed that 19/54 dogs from the untreated group (Group B) had spirocerca nodules, which corresponds to a prevalence of 35.2% in puppies aged 12 to 14 months. This high level of infestation in young dogs correlates with the results of the epidemiological study performed on Réunion by Reche-Emonot (Reche-Emonot et al. 2001), where two different analyses were performed. First, on the basis of coproscopy and/or endoscopy, 120 dogs aged less than 6 months to 13 years (out of 2,498 clinical examinations) were found to be infested, corresponding to a global clinical prevalence of 4.8%. However, the infestation clearly peaked in young animals (from 1 to 3.5 years), with 59 infested young dogs out of 120. Furthermore, in a parallel evaluation, 14 out of 51 fecal examinations of healthy dogs were spirocerca-positive, corresponding to a prevalence of 27.5%. The fact that, in our study, more than one third of the puppies from group B were infested before the age of 14 months justifies preventive measures being taken from a very young age.

Typical clinical signs of spirocercosis usually appear when the evolution of the nodules has led to chronic esophagitis. During this study, the experimental dogs were still young at the end of the follow-up (at most 14 months old). The fact that typical clinical signs such as vomiting or loss of appetite were observed in only two of the 19 infected dogs from the untreated group was therefore not unexpected.

None of the 378 coproscopic examinations were positive for spirocerca eggs, even those from infested dogs confirmed by endoscopy. Thirteen dogs out of 20 had one or more operculated nodules, from which the females could have shed eggs. These negative fecal findings could be related to the irregular shedding of eggs by the parasite, or to a possible lack of sensitivity of flotation with MgSO4, since spirocerca eggs are small (11–15 μm×30–40 μm) and the quantity of eggs shed can be limited at this relatively early stage of infection.

Conclusion

Monthly spot-on administration of a combination of imidacloprid 10%/moxidectin 2.5% (Advocate®) to puppies at the age of 2 to 4 months for a period of 9 months was shown to be effective and well tolerated for the prevention of canine spirocercosis. Considering the high prevalence of the infestation (35.2%) observed at the end of the follow-up in untreated study animals, regular preventive treatment of puppies living on Réunion may be recommended to avoid more severe clinical disease and potential fatalities when the infection progresses with increasing age of the dogs.

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