Available online at www.sciencedirect.com
SCIENCE DIRECT®
Veterinary Parasitology 122 (2004) 287–292
veterinary parasitology
www.elsevier.com/locate/vetpar

Efficacy of a single milbemycin oxime administration in combination with praziquantel against experimentally induced heartworm (*Dirofilaria immitis*) infection in cats

Claudio Genchi [a,*], Robert Cody [b], Graziano Pengo [c], Gottfried Büscher [b], Daniela Cavalleri [b], Valeria Bucci [d], Pablo Junquera [e]

[a] *Department of Animal Pathology, Università degli Studi di Milano, Via Celoria 10, 20133 Milano, Italy*
[b] *Novartis Animal Health Inc., 4002 Basel, Switzerland*
[c] *Centro Veterinario Oriolo, 26012 Castelleone, Italy*
[d] *Novartis Salute Animale, 21040 Origgio, Italy*
[e] *Vetparcs GmbH, 8044 Zürich, Switzerland*

Received 22 December 2003; received in revised form 7 April 2004; accepted 21 April 2004

Abstract

The efficacy of a combination of milbemycin oxime and praziquantel in preventing the establishment of experimentally induced heartworm (*Dirofilaria immitis*) infection was investigated in a study involving 24 young domestic short-hair cats. The animals were inoculated with 50 infective larvae on day 0. Subsequently they were divided into two groups of 12 animals each. The animals in group 1 were treated once with medicated tablets containing 4 mg milbemycin (minimum dose 2 mg/kg body weight) and 10 mg praziquantel (MILBEMAX®) on day 30 after infection. Cats in group 2 received placebo tablets on the same day. On day 183 post-infection a blood sample was taken from each animal before euthanasia and necropsy. The blood samples were tested for the presence of microfilariae and the necropsied animals were examined for the presence of adult worms. Microfilariae were not found in any of the investigated cats. No heartworms were found in the animals in group 1 (treated with medicated tablets). Out of the 12 placebo-treated cats 1 was heartworm-free, whereas all the others were found to be infected with 1–3 adult heartworms.

Keywords: Milbemycin oxime; Heartworm; Cat; Efficacy

* Corresponding author. Tel.: +39 2 5031 8101; fax: +39 2 5031 8085.
E-mail address: claudio.genchi@unimi.it (C. Genchi).

0304-4017/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetpar.2004.04.011

1. Introduction

Heartworm infection is an unpredictable disease in cats. The clinical presentation includes no clinical signs, recurrent disease and chronic disease. Acute symptoms are possible and even a few heartworms (1–2 worms) may cause sudden death (Genchi et al., 1992; McCall et al., 1994; Dillon, 1998). The prevalence of the infection in cats is lower than in dogs living in the same area, but various studies have shown that in endemic areas in Italy (Genchi et al., 1992; Kramer and Genchi, 2002), Japan (Roncalli et al., 1998) and North America (Guerrero et al., 1992) up to 27% of cats may be infected. Since treatment of established heartworm infections carries serious risks for the affected animals, chemoprophylaxis is recommended for cats living in such high risk areas (Doiron et al., 2001).

Milbemycin oxime, a mixture of milbemycin A3 and A4 oximes, is a macrocyclic lactone which has been shown to be highly effective against canine heartworm (Grieve et al., 1991; Tagawa et al., 1993, 1994; Blagburn et al., 1995) as well as against various parasitic nematodes in dogs (Bowman et al., 1990, 1991a) and cats (Bowman et al., 1991b; Humbert-Droz et al., 2004). In previous experimental studies investigating the chemoprophylactic effect of milbemycin oxime against feline heartworm, repeated treatments at dose rates between 0.50 and 1.22 mg/kg body weight after 60 and 90 days after inoculation with *D. immitis* larvae, resulted in complete prevention (Stewart et al., 1992). However, after a single treatment at similar dose rates, one study (Fukase et al., 1996) reported complete prevention, while another did not (Stewart et al., 1992). The present study was carried out to confirm the efficacy of a single treatment of milbemycin oxime at a dose of 2–4 mg/kg body weight in combination with praziquantel as a feline heartworm preventative.

2. Materials and methods

Animals. Twelve male and 12 female domestic short-hair cats were purchased from a research colony. On arrival at the trial site (day 11) the male cats were aged between 8 and 9 weeks and weighed between 680 and 1020 g (mean 807 g) and the female cats were aged between 11 and 12 weeks and weighed between 980 and 1380 g (mean 1232 g). They had not been treated with any anthelmintics. The cats were housed in individual pens in a purpose-built animal rearing facility fitted with mosquito netting and air conditioning. Each pen measured 1 m × 1 m × 2 m ($l \times w \times h$), had a concrete floor, wire mesh sides and a metal resting plate. Each pen was provided with a litter box. The animals were fed twice a day with a commercial dried cat food and drinking water was supplied ad libitum. The individual animals were identified by tattoos in the pinnae bearing a unique identification. All animals were determined free from exposure to *D. immitis* as demonstrated by a negative antibody test (HESKA^{TM} Solo Step^{TM} Feline Heartworm, HESKA Co., USA). All the animals were vaccinated with leucorifelin—PHC (Merial) on days −5 and +16. The cats were observed once daily regarding their general health, behaviour, appetite and any adverse effects from the beginning of acclimatisation through to necropsy.

Treatments. On day 0, after 11 days of acclimatisation, each cat was inoculated with 50 infective *D. immitis* larvae suspended in 1 ml Hank's balanced salt solution. The larvae

were collected from *Aedes aegypti* mosquitoes (Liverpool strain, reared in the University of Milan) and prepared for inoculation according to McCall et al. (1981). Each animal was injected subcutaneously in the right lateral side of the neck. On day 29, all cats were allocated to the treatment groups after being weighed. Allocation to the treated and the placebo groups was done based on weight. The heaviest cats on day 29 (six males between 1.6 and 1.9 kg and six females between 1.8 and 2.0 kg) were allocated to group 1 to be treated with medicated tablets in order to ensure that the dose rate was as close as possible to the minimum recommended dose rate of 2 mg milbemycin oxime/kg body weight. The lightest animals (six males weighing 1.5 kg each and six females weighing between 1.7 and 1.8 kg) were allocated to group 2 to be treated with placebo tablets. On day 30 each animal in group 1 was treated with one tablet containing 4 mg milbemycin oxime and 10 mg praziquantel (MILBEMAX®; Novartis Animal Health Inc., Basel, Switzerland). The animals in group 2 received placebo tablets. The tablet was placed into the pharynx behind the tongue. In a few cats, swallowing was helped with the delivery of a small volume of water with a syringe. Each animal was observed in its cage for 1 h for successful intake of the tablet. The trial was blinded by separating the responsibilities for tablet administration and for the subsequent clinical and parasitological observations.

Assessment of efficacy. On day 183 (153 days after drug administration), all animals were necropsied. The cats were anaesthetised with an intramuscular injection of a mixture of acepromazine and ketamine followed by an intravenous injection of sodium pentobarbital. A blood sample was collected before administration of the pentobarbital. The pleural and peritoneal cavities were examined for the presence of heartworms and the cranial and caudal vena cava and azygous vein were ligated before removal of the heart and lungs. Precava, right atrium, right ventricle and pulmonary arteries (including the lobar arteries) were carefully dissected and examined for worms. Worms that were normal in mobility and appearance were considered alive. Worms that were non-motile, transparent, opaque or had a wrinkled cuticle were considered dead. The mean worm reduction achieved was calculated according to the formula $(1-W_m/W_p) \times 100$, with W_m being the geometric mean worm number in the group treated with medicated tablets, and W_p the same parameter in the placebo group. Blood samples collected before euthanasia were examined for microfilariae using a modified Knott test. Original worm counts and transformations were checked to establish if they satisfy the assumption of normal distribution using the Shapiro–Wilks test. The Mann–Whitney *U*-test was used to compare the worm counts in group 1 with those in group 2. For calculating geometric means, counts were increased by 1 before taking the mean, to allow zero values to be included.

The study was conducted at the facilities of the Centro Veterinario Oriolo, 26012 Castelleone, Italy, according to the EU guidelines for Good Clinical Practice (European Commission Note for Guidance III/3767/92, Brussels, July 1994), to The Rules Governing Medicinal Products in the European Union (Guidelines Veterinary Medical Products, Brussels, 1999), to the WAAVP Guidelines for the Evaluation of Anthelmintics for Cats and Dogs (Jacobs et al., 1994) and to the recommendations of the Veterinary International Co-operation on Harmonisation (Efficacy of Anthelmintics: Specific Recommendations for Felines, VICH 2001, Guideline 20).

3. Results

Considering the weight of each animal at treatment, the mean dose rate of milbemycin oxime for the cats in group 1 was 2.3 mg/kg (2.1–2.5 mg/kg) for the male and 2.1 mg/kg (2.0–2.2 mg/kg) for the female cats. No microfilariae were found in the blood samples collected from both groups. No adult worms were found in the animals treated with medicated tablets. A total of 18 adult worms (13 males and 5 females) corresponding to a mean of 1.34 worms/animal (range 1–3 worms) were found in 11 animals (91.7%) treated with placebo tablets. No worms were found in one of the animals in the placebo-treated group. The difference between both groups was statistically significant ($P < 0.0004$).

No adverse reactions due to treatment were recorded during the study. Body weight and food consumption (data not shown) were unaffected by the different treatments. The only observed signs (slight trembling and laboured breathing immediately after drug administration) were recorded in both treated and placebo groups (one animal each), and disappeared within 1 h. No other signs of adverse reactions were recorded during the study.

4. Discussion

Eleven out of 12 cats in group 2 (placebo) became infected with adult worms. This confirms the infectivity of the heartworm strain used and the adequacy of the larval challenge and is consistent with infection rates in the control cats obtained by other authors, e.g. 83% by Stewart et al. (1992) and 88% by McTier et al. (2000). The low number of adult worms (1–3) found in the infected cats of the placebo-treated group is also comparable with the results of other studies with artificially induced infections (McTier et al., 2000) as well as with the findings in naturally infected cats (Genchi et al., 1992; Guerrero et al., 1992; Roncalli et al., 1998) and are known to be typical for feline heartworm infection (Dillon, 1998). The absence of microfilariae in the blood of both the control and the treated cats at necropsy is also in agreement with the findings of other studies (Genchi et al., 1992; Guerrero et al., 1992) and is known to be a frequent feature of heartworm infections in cats (McCall et al., 1994).

In the present study, a single treatment with medicated tablets containing 4 mg of milbemycin oxime completely prevented the establishment of artificially induced infections with *D. immitis* in cats. The dose rates achieved during the study ranged between 2.0 and 2.5 mg milbemycin oxime/kg body weight, well at the lower end of the recommended dose rate range of 2–4 mg/kg that results from the use recommendation of 1 tablet for animals between 1 and 2 kg body weight. The test medication also contained praziquantel to provide broad-spectrum anthelmintic efficacy against cestodes. This is known not to be active against *D. immitis* (Andrews et al., 1983), so that the observed efficacy can be attributed exclusively to the milbemycin oxime content of the tablets.

In earlier studies of the efficacy of milbemycin oxime as a heartworm preventative in cats, the administered dose rate was lower than in the present investigation. In one study, six consecutive treatments at 0.68–1.22 mg/kg were administered at 30 day intervals starting on day 9 after inoculation and achieved complete prevention (Stewart et al., 1992). In a second study, a single treatment with milbemycin oxime at 0.50–0.99 mg/kg administered

30 or 60 days after inoculation gave an incomplete protection, whereas two consecutive treatments at the same dose rate administered 60 and 90 days after inoculation provided full control (Stewart et al., 1992). In a third study, complete prevention was reported after a single treatment at 0.5 mg/kg administered 30 days after inoculation (Fukase et al., 1996). All these studies were performed at dose rates that correspond roughly to the minimum recommended dose for heartworm prevention in dogs, i.e. 0.5 mg/kg body weight. More recent investigations have shown that after oral administration cats need to receive a higher dose than dogs in order to achieve similar blood levels of milbemycin oxime (Cody, unpublished results). This likely explains why complete prevention was not achieved after a single treatment in the second study previously mentioned. As a consequence of this pharmacological behaviour in cats, the milbemycin oxime content of the tablets used in this study for cats of up to 2 kg body weight was raised to 4 mg. These tablets result in dose rates of 2–4 mg/kg body weight for cats weighing 1–2 kg and are currently approved for use in cats in Europe and other countries. The need for a higher dose in cats than in dogs is also known for orally administered ivermectin, another macrocyclic lactone used for heartworm prevention, for which the minimum recommended dose rates for use on dogs and cats are about 6 and 25 mg/kg body weight, respectively.

The high efficacy achieved in the three previously reported studies at less than half the dose rate currently recommended for use in cats provides evidence for a large confidence margin in the effectiveness of heartworm prevention using milbemycin oxime at 2 mg/kg body weight, thus compensating for slight errors in the administration of the product by the cat owners (e.g. underestimating the animals weight or slight delays in the 30 day interval administration schedule).

Acknowledgements

The authors are grateful to M. Longo and R.V. Facchini from Centro Veterinario Oriolo, Castelleone, for assistance during the clinical and laboratory work as well as to G. Strehlau from Novartis Animal Health, Basel, Switzerland, for his contribution to the statistical analysis.

References

Andrews, P., Thomas, H., Pohlke, R., Seubert, J., 1983. Praziquantel. Med. Res. Rev. 3, 147–200.

Blagburn, B.L., Hendrix, C.M., Vaughan, J.L., Lindsay, D.S., Tebbit, G.L., 1995. Efficacy of a chewable formulation of milbemycin oxime against preadult *Dirofilaria immitis* and adult *Ancylostoma caninum* in naturally and experimentally infected dogs. In: Soll, M.D. (Ed.), Proceedings of the Heartworm Symposium'95. American Heartworm Society, Batavia, IL, pp. 171–175.

Bowman, D.D., Johnson, R.C., Hepler, D.I., 1990. Effects of milbemycin oxime on adult hookworms in dogs with naturally acquired infections. Am. J. Vet. Res. 51, 487–490.

Bowman, D.D., Lin, D.S., Johnson, R.C., Hepler, D.I., 1991a. Effects of milbemycin oxime on adult *Ancylostoma caninum* and *Uncinaria stenocephala* in dogs with experimentally induced infections. Am. J. Vet. Res. 52, 64–67.

Bowman, D.D., Johnson, R.D., Fogelson, M., Hepler, D.I., 1991b. Effects of milbemycin oxime on adult ascarids (*Toxocara cati*) in cats with naturally acquired infections. In: Proceedings of the 36th Annual Meeting of the American Association of Veterinary Parasitologists, Abstract no. 16.

Dillon, R., 1998. Clinical significance of feline heartworm disease. Vet. Clin. North Am.: Small An. Pract. 28, 1547–1565.

Doiron, D.W., Dowimg, R., Longhofer, S.L., Nelson, C.T., Rubin, S.B., McCall, J.W., Knight, D.H., Seward, R.L., 2001. 2002 guidelines for the diagnosis, prevention and management of heartworm (*Dirofilaria immitis*) infection in cats. In: Seward, R.L., Knight, D.H. (Eds.), Proceedings of the Recent Advances in Heartworm Disease: Symposium'01. American Heartworm Society, Batavia, IL, pp. 267–273.

Fukase, T., McCall, J.W., Akihama, S., 1996. Evaluation of milbemycin oxime as a prophylactic agent for heartworm infection in domestic cats. Abstracts of the VII European Multicolloquium of Parasitology, Parma, September 2–6, Abstract D3 10, 232 pp.

Genchi, C., Guerrero, J., Di Sacco, B., Formaggini, L., 1992. Prevalence of *Dirofilaria immitis* in Italian cats. In: Soll, M.D. (Ed.), Proceedings of the Heartworm Symposium'92. American Heartworm Society, Batavia, IL, pp. 97–102.

Grieve, R.B., Frank, G.R., Stewart, V.A., Parsons, J.C., Belasco, D.L., Hepler, D.I., 1991. Chemoprophylactic effects of milbemycin oxime against larvae of *Dirofilaria immitis* during prepatent development. Am. J. Vet. Res. 52, 2040–2042.

Guerrero, J., McCall, J.W., Dzimianski, M.T., McTier, T.L., Holmes, R.A., Newcomb, K.H., 1992. Prevalence of *Dirofilaria immitis* infection in cats from the southeastern United States. In: Soll, M.D. (Ed.), Proceedings of the Heartworm Symposium'92. American Heartworm Society, Batavia, IL, pp. 91–95.

Humbert-Droz, E., Büscher, G., Cavalleri, D., Junquera, P., 2004. Efficacy of milbemycin oxime against *Ancylostoma tubaeforme* (fourth stage larvae and adults) in artificially infected cats. Vet. Rec. 154, 140–143.

Jacobs, D.E., Arakawa, A., Courtney, C.H., Gemmell, M.A., McCall, J.W., Myers, G.H., Vanparijs, O., 1994. World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the efficacy of anthelmintics for dogs and cats. Vet. Parasitol. 52, 179–202.

Kramer, L., Genchi, C., 2002. Feline heartworm infection: serological survey of asymptomatic cats living in northern Italy. Vet. Parasitol. 104, 43–50.

McCall, J.W., Calvert, C.A., Rawlings, C.A., 1994. Heartworm infection in cats: a life-threatening disease. Vet. Med. 89, 639–647.

McCall, J.W., Lindemann, B.A., Porter, C.A., 1981. Prophylactic activity of avermectins against experimentally induced *Dirofilaria immitis* infections in dogs. In: Otto, G.F. (Ed.), Proceedings of the Heartworm Symposium'80. Edwardsville, KS. Veterinary Medicine Publishing Co., pp. 126–130.

McTier, T.L., Shanks, D.J., Watson, P., McCall, J.W., Genchi, C., Six, R.H., Thomas, C.A., Dickin, S.K., Pengo, G., Rowan, T.G., Jernigan, A.D., 2000. Prevention of experimentally induced heartworm (*Dirofilaria immitis*) infections in dogs and cats with a single topical application of selamectin. Vet. Parasitol. 91, 259–269.

Roncalli, R.A., Yamane, Y., Nagata, T., 1998. Prevalence of *Dirofilaria immitis* in cats in Japan. Vet. Parasitol. 75, 81–89.

Stewart, V.A., Blagburn, B.L., Hendrix, C.M., Hepler, D.I., Grieve, R.B., 1992. Milbemycin oxime as an effective preventative of heartworm (*Dirofilaria immitis*) infection in cats. In: Soll, M.D. (Ed.), Proceedings of the Heartworm Symposium'92. American Heartworm Society, Batavia, IL, pp. 127–131.

Tagawa, M., Hara, Y., Ejima, H., Hayashi, Y., Kusano, K., 1994. Prophylactic efficacy of milbemycin oxime against multiple infection of dogs with *Dirofilaria immitis*. J. Vet. Med. Sci. 56, 779–780.

Tagawa, M., Okano, S., Hayashi, Y., Kusano, K., 1993. Prophylactic effect of milbemycin oxime against *Dirofilaria immitis* infection in dogs: optimum dose and administration time. J. Vet. Med. Sci. 55, 693–694.