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Short communication

Efficacy of sarolaner on the treatment of myiasis caused by *Cochliomyia hominivorax* (Diptera: Calliphoridae) in dogs

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ABSTRACT

The aim of this study was to evaluate the larval expulsion, larvicidal effect, retention rate of dead larvae and overall efficacy of sarolaner on the treatment of myiasis caused by New World screwworm *Cochliomyia hominivorax* in naturally infested dogs. Eight Beagle dogs received a single dose of saroalner 40 mg, with dosage ranging from 2.7 mg/kg to 3.7 mg/kg. Evaluations occurred every 15 min in the first hour, every hour for up to 6 h, and after 24 h of treatment. At 24 h post-treatment, total wound cleaning was performed, including removal and counting of remaining live and dead larvae. The animals received antibiotic, analgesic and wound cleaning support until complete wound healing. The average expulsion of the larvae was 50.9 % occurring mainly after 4 h of treatment. The larvicidal effect was 70.6 % at 6 h after treatment and 100 % at 24 h. The mean retention rate of dead larvae of sarolaner was 33.9 %, The overall efficacy was 100 %, thus making sarolaner an excellent treatment option in myiasis caused by *C. hominivorax* larvae in dogs.

1. Introduction

The New World screwworm (NWS), *Cochliomyia hominivorax* (Diptera: Calliphoridae), causes primary myiasis in humans and warm-blooded animals in the American continent (Guimarães and Papavero, 1999). In companion animals, NWS myiasis is a debilitating disease, and can be fatal depending on time until diagnosis and treatment, level of infestation, and the site of infestation (Correia et al., 2010).

In the recent past, treatment of NWS in companion animals relied on the use of systemic macrocyclic lactones or topical products based on organophosphates or carbamates, most often using products not labelled for the intended use or animal species. Extrapolation of therapies used in farm animals to companion animals raises numerous points of concern as it exposes owners and pets to unnecessary risk of toxicity from organophosphates and/or ivermectin (Han et al., 2018). Current therapies for NWS myasis in dogs and cats are mainly based on oral products containing the neonicotinoid nitenpyram, along with appropriate management of the wound and support treatment (Cardoso and Ramadinha, 2007; Correia et al., 2010; Souza et al., 2010). More recently, new ectoparasiticide products based on spinosyns and isoxazolines have shown to be effective therapeutic options against myasis by the NWS and *Chrysomyia bezziana* in dogs (Oliveira et al., 2018; Han et al., 2017, 2018).

Sarolaner is a new isoxazoline, a potent new class of ectoparasiticide for companion animals. Efficacy of sarolaner has been demonstrated against various common ectoparasites of dogs, including ticks (*Rhipicephalus sanguineus*, *Amblyomma maculatum, Amblyomma cajennense*, *Ixodes scapularis,* and *Ixodes ricinus* (Six et al., 2016a; Scott et al., 2017)), mange mites (*Demodex* spp., and *Otodectes cynotis* (Six et al., 2016b)), and the cat flea, *Ctenocephalides felis* (Six et al., 2016c). The mechanism of action of the isoxazoline class of compounds is well documented. Isoxazolines exhibit antiparasitic activity through specific blockade of insect GABA- and glutamate-gated chloride channels (Garcia-Reynaga et al., 2013; Gassel et al., 2014; Ozoe et al., 2010).

The objective of this study was to evaluate the larval expulsion, larvicidal effect, retention rate of dead larvae and overall efficacy of sarolaner on the treatment of naturally acquired screwworm myiasis by *C. hominivorax* in dogs.

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2. Materials and methods

2.1. Overview

The study was conducted in the Laboratory of Experimental Chemotherapy in Veterinary Parasitology (LQEPV) from the Universidade Federal Rural do Rio de Janeiro (UFRRJ), Southeastern Brazil in accordance with Good Clinical Practices as described in VICH guideline GL9, Good Clinical Practice (EMEA, 2000). All protocols were reviewed and approved by the Institutional Animal Care and Use Committee (CEUA # 3646180918).

2.2. Experimental design and methods

Eight Beagle dogs from the Laboratory of Experimental Chemotherapy in Veterinary Parasitology of Universidade Federal Rural do Rio de Janeiro, four males and four females, naturally infested by *C. hominivorax* maggots between December 2018 (summer) and June 2019 (fall) were included in the study. Dogs ranged from 1 to 10 years of age and 10.7 kg–14.7 kg of weight. The lesions were distributed in different body areas: lumbar region near the tail (3), head (1), back (2), and posterior limb (2). After diagnosis of NWS myiasis by observation of maggots in wound, the dogs received sarolaner (Simparica® Zoetis) in a single dose orally, following the manufacturer's recommended dose for control of the cat flea and the brown dog tick in dogs. Dogs received sarolaner doses ranging from 2.7 to 3.7 mg/kg (Table 1). This product has no label claim for the treatment of NWS myiasis in dogs or other animal species. After treatment, the dogs were kept in individual kennels measuring 70 cm × 70 cm × 70 cm, with removable trays placed in the bottom to evaluate the spontaneous expulsion of live or dead larvae. Observations were done: 15 min, 30 min, 45 min, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, and 24 h post-treatment, when expelled larvae present on these trays were collected and quantified. After these observation periods, the remaining larvae were mechanically removed from the wound using surgical tweezers. Mechanically removed larvae were assessed for motility under a dissecting scope, and then counted as dead or alive. Dogs were sedated (Acepromazine 0.05 mg/kg e Meperidine 2.0 mg/kg, intramuscularly) to provide more comfort to the animal and to facilitate the removal of all existing larvae within the wound. Following larval removal, wounds were cleaned and treated using antiseptic (0.5 % clorhexidine solution and 1 % silver sulfadiazine cream). As support treatment, dogs were administered anti-inflammatory (meloxicam, 0.2 mg/kg, SC) and antibiotic (Pentabiótico Veterinário® penicillin 24.000 UI/kg, streptomycin and dihydrostreptomycin 10 mg/kg, SC). Dogs were clinically evaluated daily for general health conditions and healing status of the lesions. In this period the animals were kept in kennels of 6m^2 with solarium area and covered area. After complete wound healing the animals returned to the laboratory's maintenance kennel.

The criteria for evaluating the efficacy of sarolaner against *C. hominivorax* followed Oliveira et al. (2018). Briefly, we considered the overall efficacy (OEF) as: [(number of dead larvae expelled + number of live larvae expelled + number of dead larvae removed/total number of larvae)] × 100. Larval expulsion rate (LER) was calculated for each time point and for each dog using the formula [(number of dead larvae expelled + number of live larvae expelled/total number of larvae)] × 100. The larvicidal effect (LEF) was calculated for each time point and for each dog by the formula [(number of dead larvae expelled + number of dead larvae removed/total number of larvae)] × 100 and the retention rate of dead larvae (RDL) was calculated by the formula [(number of dead larvae mechanically removed / total number of larvae)] × 100.

3. Results and discussion

All maggots expelled or mechanically removed from the myiasis wounds were morphologically identified as *C. hominivorax* second and third instar larvae, according to Guimarães and Papavero (1999). A total of 1228 larvae were recovered, dead or alive, from the eight dogs, with an average of 153.1 ± 159.4 larvae per dog. The average larval count was higher than that observed in previous larvicidal efficacy studies against NWS in dogs, and numbers of larvae per animal were as variable as one of these studies (Correia et al., 2010; Oliveira et al., 2018).

The mean larval expulsion rate was 50.8 %, the mean larvicidal efficacy was 61.2 % and the mean overall efficacy was 100 % (Table 1). Results for the number of dead and live expelled larvae per animal by observation period, number of manually removed larvae dead or alive, percentages of expulsion, larvicidal effect and overall efficacy are present in Table 1. No adverse effects of drug treatment or any other procedures were observed throughout the study.

A single dose of sarolaner had an OEF of 100 %. The efficacy of a single-dose sarolaner protocol against NWS myasis was comparable to that obtained in a study by Correia et al. (2010), in which two doses of nitenpyram were required to achieve 100 % efficacy, and by Han et al (2018) in which animals were free of *C. bezziana* larvae within 8 h post-treatment after a single dose of nitenpyram. The efficacy of sarolaner was superior to that of a single dose of spinosad against NWS, which achieved an OEF of 80 % (Oliveira et al., 2018). Han et al (2018) had efficacy results with spinosad and afoxolaner similar to ours with sarolaner where 100 % of the larvae of *C. bezziana* were dead in 24 h. Afoxolaner, reached 100 % efficacy 24 h after treatment of myiasis caused by *C. bezziana* (Han et al., 2018), whereas Han et al. (2017) successfully treated *C. bezziana* infestation in three dogs using a combination of spinosad and milbemycin, within 8 h post-treatment.

The LER was 50.8 %, ranging from 26 % to 79.6 %, much lower than observed by Oliveira et al. (2018) mean 73.3 % and Correia et al. (2010) 92.5 %. The number of larvae that fell spontaneously from the lesion and counted as alive or dead on the trays (EL) per animal was: 39, 43, 76, 58, 186, 16, 54 and 68, for animals 1, 2, 3, 4, 5, 6, 7, and 8, respectively. The peak of larval expulsion from the lesions occurred 4 h after treatment. This peak in EL may be associated with the time when sarolaner begins to kill fleas which is 3 − 4 hours post-treatment (Fig. 1) (Woods and McTier, 2018).

The LEF mean of sarolaner was 61.2 % in the first 6 h of treatment, and 100 % at 24 h after treatment. All mechanically removed larvae in this period were dead. Afoxolaner also showed 100 % larvicidal efficacy at 24 h after the treatment in *C. bezziana* infestations in dogs (Han et al., 2018). The mean RDL of sarolaner was 33.9 %, which reinforces the importance of post-treatment debridement to complete the wound cleaning, and to prevent secondary bacterial infection, and re-oviposition, and potential re-infestation by *C. hominivorax*.

Overall, the absence of live larvae in the myiasis wound is the desirable clinical outcome, regardless if these larvae were expelled or died within the wound (Oliveira et al., 2018). This is due to the fact that the absence of live larvae ceases the worsening of the lesions and further injury, tissue inflammation, and secretion production, which may attract more female flies to oviposit (Correia et al., 2010; Han et al., 2017, 2018).Insecticidal products that induces rapid larvicidal effect and promote larvae expulsion are preferred options for the treatment of primary myiasis in dogs as it allows a quick restoration of quality of life but also facilitates complete removal of larvae from the host.

4. Conclusion

Sarolaner was effective in the treatment of naturally acquired myiasis caused by *C. hominivorax* in dogs. In cases of myiasis with concomitant infestation by other ectoparasites, sarolaner is the best treatment option, given its broad spectrum against ticks, fleas, and mites.

Table 1
Number of *Cochliomyia hominivorax* larvae after treatment with simparic 2.0 mg / kg in naturally infested dogs.

Dose (mg/kg) Observation time	Number of Cochliomyia hominivoraxlarvae per dog																		
	Dog 1		Dog 2		Dog 3		Dog 4		Dog 5		Dog 6		Dog 7		Dog 8		Total	Arithmetic mean	Geometric mean
	(3.7 mg/kg)		(3.3 mg/kg)		(3.1 mg/kg)		(3.5 mg/kg)		(2.7 mg/kg)		(2.8 mg/kg)		(3.1 mg/kg)		(3.2 mg/kg)				
	LL	DL	LL	DL	LL	DL	LL	DL	LL	DL	LL	DL	LL	DL	LL	DL			
15 min	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	–	–
30 min	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	–	–
45 min	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	3	–	–
60 min	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	–	–
120 min	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	–	–
180 min	0	0	15	1	8	0	8	0	0	0	0	0	0	0	3	0	35	–	–
240 min	1	0	6	0	39	0	29	0	72	11	0	0	25	0	12	0	195	–	–
300 min	10	1	1	1	17	1	6	0	64	0	0	0	13	0	26	0	140	–	–
360 min	12	3	0	0	5	0	3	0	12	0	13	0	8	0	13	0	59	–	–
1440 min	0	12	0	19	0	3	0	2	0	27	0	3	0	8	0	14	88	–	–
EL	23	16	22	21	72	4	47	11	148	38	13	3	46	0	54	0	518	–	–
MRL	0	20	0	11	0	62	0	29	0	343	0	25	0	131	0	89	710	–	–
Total	59		54		138		87		529		41		177		143		1228	–	–
LER (%)	66.1		79.6		55.1		66.7		35.2		39		26		38.6		–	50.8	31,8
LEF (%)	61		59.3		47.8		46		72		68.3		74		61.4		–	61.2	62,1
RDL (%)	33.9		20,4		44,9		33,3		64,8		60,9		74,0		62,2		–	33,9	49,6
OEF (%)	100		100		100		100		100		100		100		100		–	100	100

EL: expelled larvae; MRL: mechanically removed larvae; Total per animal; LER: larval expulsion rate; LEF: larvicidal efficacy; RDL: retention rate of dead larvae, OEF: overall efficacy, LL: Live larvae, DL: dead larvae

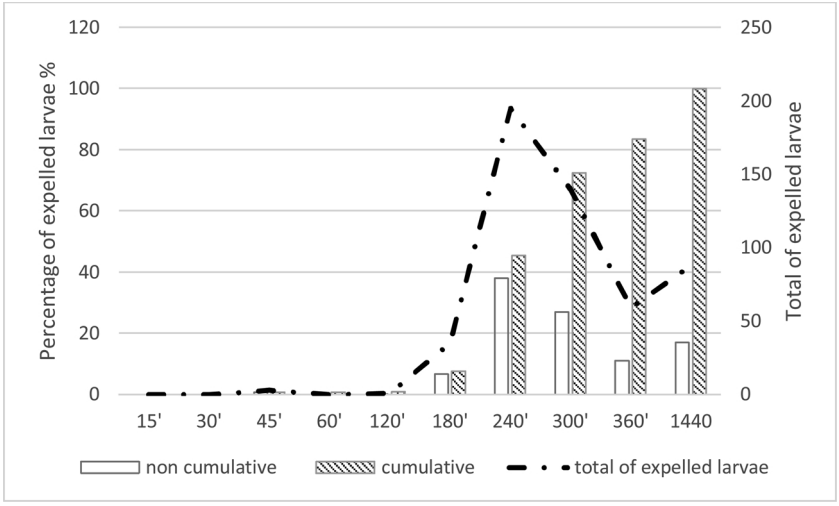

Fig. 1. Nnumber of expelled *Cochliomyia hominivorax* larvae per observational period after treatment with sarolaner (Simparica®) in dogs.

Compliance with ethical standards

All investigations comply with the current laws of the country in which they were performed.

Declaration of Competing interests

The authors declare that they have no competing interests.

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