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Original Article

Efficacy of afoxolaner (NexGard®) on the treatment of myiasis caused by the New World screwworm fly *Cochliomyia hominivorax* (Diptera: Calliphoridae) in naturally infested dogs

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ABSTRACT

New World screwworm (NWS) myiasis is an infestation by *Cochliomyia hominivorax* larvae that consume the living tissue of warm-blooded animals, including humans. Domestic dogs are among the potential hosts of these flies that lay their eggs on the edges of wounds. NWS myiasis cases can be fatal if untreated. Treatment with parasiticides must be fast-acting, long-lasting and show 100% efficacy, since open wounds can be reinfested. Afoxolaner is a molecule from the isoxazoline family with proven ectoparasiticide action against fleas, ticks and mites in dogs. Fourteen healthy client-owned dogs, naturally infested by *C. hominivorax* larvae, were treated with afoxolaner (NexGard®) as per label recommendations, providing at least the minimum dosage of 2.5 mg/kg. Maggot infestations were classified as light (fewer than 10 larvae), mild (from 10 to 20 larvae) or severe (more than 20 larvae), according to the number of larvae found in the wound and/or collected from the ground after treatment. Twenty-four hours post-treatment, infested lesions were carefully inspected and collected larvae were counted and classified as live or dead. All maggots were identified as second and third instar larvae of *C. hominivorax* and were found dead within 24 h after treatment, demonstrating 100% larvicidal efficacy against *C. hominivorax*.

1. Introduction

The New World screwworm (NWS) fly, *Cochliomyia hominivorax* (Coquerel, 1858) (Diptera: Calliphoridae) causes an obligatory myiasis in warm-blooded animals, including humans. Myiasis is defined as the infestation of live vertebrate animals by dipterous larvae, which, at least for a certain period, feed on the host's dead or living tissue, liquid body-substances, or ingested food (Zumpt, 1965). *C. hominivorax* was originally distributed from the southern United States to Argentina, including the Caribbean (Hall and Wall, 1995). The sterile-insect technique eradicated the NWS in the U.S.A., Mexico and Central America (Lindquist et al., 1992; Knipling, 1995; Wyss, 2006). Currently, *C. hominivorax* is still endemic in the Caribbean and South America, except Chile (Welch, 2019).

Fertile female NWS flies lay their eggs on the margin of live tissues that have been naturally or accidentally damaged (bleeding wounds, tick bites, chronic mucosal or skin ulcers for example) and near orifices with purulent discharge (Guimarães and Papavero, 1999; Mendes-de-Almeida et al., 2007; Rodríguez-Hidalgo et al., 2019). *C. hominivorax* is a major cattle pest and causes significant impairment to the welfare of infested animals and important economic losses to farmers that are estimated to be 340 million USD/year in Brazil (Grisi et al., 2014). Other livestock (swine, sheep, goats), dogs, cats, wildlife and even humans are also susceptible hosts (Coronado and Kowalski, 2009; Pezzi et al., 2019; Rodríguez-Hidalgo et al., 2019). In dogs, predisposing factors are open wounds (e.g., trauma, bites, fight, etc.), dermatological disorders (e.g.,

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pododermatitis, otitis, dermatitis, etc.) and conditions of neglect (such as neglected post-surgical intervention, ulcerated tumor, decubitus ulcers, etc.) (Cramer-Ribeiro et al., 2003; Leal et al., 2019). The diagnosis of myiasis is based on clinical evidence, through the visualization of fly larvae in the tissue associated with a characteristic necrotic smell. Screwworm myiasis can worsen existing open wounds and secondary bacterial infections can occur (Marcondes and Thyssen, 2017; CAPC, 2017). Animals display discomfort, are depressed and prostrated. In addition, severe infestations can lead to anorexia, weight loss and to the death of the host.

Various topical and systemic drugs are used for the treatment of myiasis in small animal practices (macrocyclic lactones, carbamates, organophosphates and nitenpyram) (Han et al., 2018; Mendes-de-Almeida et al., 2007; Oliveira et al., 2019, 2018). More recently, other classes of parasiticides, such as spinosyns and isoxazolines, have been shown to be effective in the treatment of screwworm myiasis (Han et al., 2018; Oliveira et al., 2019, 2018).

Afoxolaner belongs to the latest class of ectoparasiticides, the isoxazolines, and is administered at the minimum effective dosage of 2.5 mg/kg to treat and/or prevent fleas (Hunter et al., 2014; Dryden et al., 2015), ticks (Dumont et al., 2014; Mitchell et al., 2014; Kunkle et al., 2014); and mites (Beugnet et al., 2016a; Beugnet et al., 2016b; Carithers et al., 2016) infestations in dogs. Afoxolaner acts systemically and binds to chloride channels, gated by the neurotransmitter GABA, thereby blocking the flow of chloride ions across nerve cell membranes and inhibiting the firing of new action potentials (Shoop et al., 2014). Binding to glutamate-gated chloride channels has also been demonstrated (Garcia-Reynaga et al., 2013). Prolonged afoxolaner-induced hyperexcitation results in uncontrolled activity of the central nervous system and death of insects and acarines (Shoop et al., 2014). As *C. hominivorax* larvae feed on living tissues and the wound fluids, we hypothesized that afoxolaner could kill larval stages that feed on afoxolaner treated dogs, as observed for other isoxazolines (Han et al., 2018; Oliveira et al., 2019).

The objective of the study was to determine the efficacy of afoxolaner after a single oral treatment in dogs naturally infested by *C. hominivorax* larvae.

2. Materials and methods

The study was conducted in the urban and peri-urban metropolitan region of Campinas, São Paulo State, in Brazil, between March 2017 and April 2018. All dogs were managed similarly and with due regard for their well-being. Dogs were handled in compliance with Boehringer Ingelheim Institutional Animal Care and Use Committee (IACUC) approvals and the study met the CONCEA (Brazil's National Council for the Control of Animal Experimentation) animal welfare requirements.

Fourteen healthy client-owned dogs (7 males and 7 females) between 2 and 15 years old and weighing between 10.2 and 43 kg were included in the study. Dogs were fed, housed and managed by their owners.

All dogs were naturally infested by *C. hominivorax* larvae. Diagnosis was based on observation of live larvae. The number of larvae present in the wound was visually estimated before treatment and the location of the lesions was recorded. All dogs were weighed prior to treatment administration and treated orally with a single treatment of afoxolaner (NexGard®, Boehringer Ingelheim Animal Health), as per label recommendations, providing at least the minimum dosage of 2.5 mg/kg. All dogs were kept under supervision in an enclosed area for 24 h after treatment (e.g., kennel, room inside the dog owner's house or veterinary clinic). Then, lesions were carefully inspected: all larvae were mechanically removed from the wound and/or collected from the ground, below or around the dog, if they were expelled. The larvae were then counted and classified as live or dead. Larvae were classified as live if they were motile or dead if they were not motile, dehydrated or dry. If multiple dogs from the same household were included in the study, the veterinarian examined the lesions of the dogs previously enrolled when including a new one. Infestations were classified as light, mild or severe if fewer than 10, 10 to 20, and more than 20 larvae were recovered after treatment, respectively. The collected larvae were stored in individual vials containing a 10% formalin solution and sent to the Laboratory of Integrative Entomology, Department of Animal Biology, UNICAMP for larval instar determination and species confirmation, following the morphological characteristics provided by Marcondes and Thyssen (2017).

Larvicidal efficacy was calculated for each dog as: [(the number of dead larvae at 24 h – number of live larvae at 24 h)/number of dead larvae at 24 h] x100. The mean % larvicidal efficacy was calculated for the group as: [(the total number of dead larvae at 24 h – total number of live larvae at 24 h)/total number of dead larvae at 24 h post-treatment] x 100.

3. Results and discussion

No adverse events were recorded during the study. The lesions were distributed in different body areas: ear (1), face (1), oral cavity (1), neck (2), legs (3), thorax (1), abdomen (1), tail (1), perianal (1) and base of nails (2). Three, four and seven dogs presented light (2.0 ± 1.7 larvae), moderate (14.0 ± 3.7 larvae) and severe (117.7 ± 122.6 larvae) infestations, respectively. Three dogs were infested with more than 150 larvae (159, 195 and 346 larvae). One of them (Dog n°1) was administered a non-steroidal anti-inflammatory (meloxicam) and antibiotic (penicillin) due to the large inflammation of the lesion within 24 h post-treatment. Three dogs (Dogs n° 1, 4 and 7) displaying aggressive behavior had to be anesthetized with a combination of ketamine and xylazine or a combination of tiletamine and zolazepam for the post-treatment clinical examination. The dog with the highest larval count presented multiple lesions, including an extensive lower limb necrotic lesion with bone exposure.

All collected maggots were found dead 24 h after treatment. They were all expelled (Fig. 1) and/or mechanically removed and identified as second and third instar larvae of *C. hominivorax* (Table 1). Afoxolaner demonstrated 100% larvicidal efficacy against *C. hominivorax*.

Previous studies showed that isoxazolines - oral afoxolaner and sarolaner - reached 100% efficacy against *Chrysomya bezziana* (Villeneuve) (syn., Old World screwworm) and *C. hominivorax* larvae 24 h post-treatment, respectively (Han et al., 2018; Oliveira et al., 2019). Spinosad also reached 100% efficacy after a 24 h-period against *C. bezziana* (Han et al., 2018). Other insecticidal treatments, such as nitenpyram and spinosad + milbemycin oxime, achieved complete larval killing against *C. bezziana* within 6 to 7 h, respectively (Han et al., 2018). Two oral administrations of nitenpyram at 6 h-interval demonstrated 100% larvicidal efficacy against *C. hominivorax* (Correia et al., 2010). Nitenpyram is indicated for the treatment of flea infestation and its efficacy persists for only 48 h (Dobson et al., 2000; Rust et al., 2003),

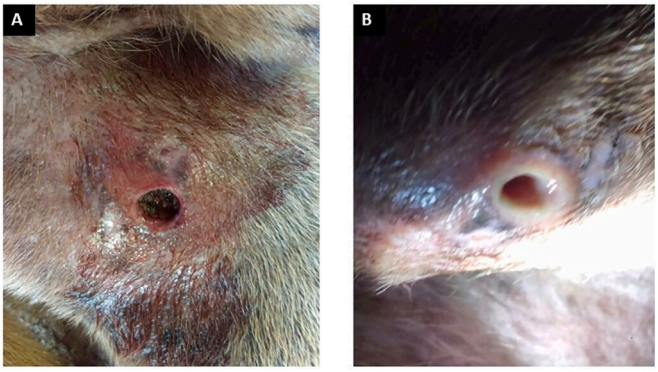

Fig. 1. Moderate myiasis lesion in the inguinal fold (Dog n°4). A) Pre-treatment; B) 24 h post-treatment: all larvae were dead and expelled

Table 1
Lesion location and severity, pre- and post-treatment larvae count and larvicidal efficacy of afoxolaner (NexGard) against *C. hominivorax* larvae in naturally infested dogs.

Animal ID	Lesion location	Lesion Severity[a]	Pre-treatment larvae count estimate	Post-treatment live larvae count	Post-treatment dead larvae count			Larvicidal efficacy (%)
					(Number per instar)		Total	
					L2	L3		
1	Ear	Severe	>200	0	51	144	195	100
2	Thorax	Severe	>20	0	11	33	44	100
3	Face	Light	<10	0	–	–	0[b]	100
4	Leg	Moderate	>10	0	–	13	13	100
5	Perianal	Light	<10	0	–	3	3	100
6	Leg	Moderate	>10	0	–	14	14	100
7	Abdomen	Severe	>10	0	–	21	21	100
8	Neck	Severe	>50	0	1	32	33	100
9	Tail	Light	<10	0	–	3	3	100
10	Nail base	Severe	>10	0	–	26	26	100
11	Neck	Severe	>50	0	–	159	159	100
12	Nail base	Moderate	>5	0	10	–	10	100
13	Oral	Moderate	>20	0	–	19	19	100
14	Leg	Severe	>500	0	11	335	346	100
Total				0	84	802	886	100

[a] The number of larvae in each wound was visually estimated before treatment. According to the number of larvae recovered in the wound and/or collected from the ground after treatment, the severity of the lesion was classified as light (fewer than 10 larvae), moderate (from 10 to 20 larvae) or severe (more than 20 larvae).
[b] No larvae were recovered after treatment: they were all expelled from the wound before the post-treatment larvae count and were not recovered from the ground because of the low number of larvae pre-treatment.

which may not provide a long-lasting protection in case of continuous re-infestations. Myiasis can cause serious tissue damage and can be fatal if untreated, thus, its treatment with parasiticides must kill quickly the larvae, i.e. in less than 24 h for pathogenic species like *C. hominivorax*. Since reinfestations of the same lesion are possible, long-lasting formulations may provide protection on top of their curative action. Given that afoxolaner provides a preventive efficacy against fleas and ticks for a month (Hunter et al., 2014; Dryden et al., 2015; Dumont et al., 2014; Mitchell et al., 2014; Kunkle et al., 2014), its use against *C. hominivorax* infestation could not only cure infested dogs, but could also provide protection against maggot reinfestations.

Three dogs included in the present study were housed together in the same household but were not treated on the same day. The dog which was first included and treated (Dog n° 4), was not reinfested during the study, while the two other dogs were found with myiasis infestation later on, demonstrating that adult flies were active and could potentially infest new hosts. Dog n° 7 was re-examined 10 and 18 days after treatment. On Day 10, the dog n° 10 was infested with myiasis, demonstrating that adult flies were active (Fig. 2). However, no new infestation was reported on Dogs n° 4 and n° 7, and on Day 18, on the former lesions. The chronological order of these events is described in the Fig. 3. Similarly, Dog n° 8 presented an extensive lesion on the back of his neck, that was reported to be repeatedly infested by *C. hominivorax* larvae. On Day 29, the lesion was almost completely healed and no new infestation was reported within this time (Fig. 4).

The known persistency of afoxalaner, its plasma protein binding and its half-life of 9–14 days (Letendre et al., 2014), explains the protection against new flea and tick infestations for 30 days. The same long-lasting effect is probable against other ectoparasitosis including myiasis. However, further studies are needed to confirm these findings.

Effective treatment of myiasis includes removal of larvae, lesion cleaning with an antiseptic solution, and surgical debridement to remove necrotic tissue and enhance wound healing. Anti-inflammatory may be used to reduce inflammatory reaction and antibiotic therapy (based on culture and sensitivity) may be used to treat eventual secondary bacterial infections (Saari et al., 2019). Even though, *C. hominivorax* is currently endemic in South America, its introduction and spread into non-endemic areas remains possible, and is facilitated by climate change and global transport (Hall et al., 2016). In 1988, the New World screwworm fly was reported in Libya, probably due to the importation of livestock from South America. More than 200 people were infested, as well as livestock, causing economic losses for the farmers. In 1992, Libya was officially declared screwworm-free after a large international campaign of sterile fly release (Lindquist et al., 1992). More recently, in 2016, an outbreak of *C. hominivorax* occurred in the Florida Keys in Key deer (*Odocoileus virginianus clavium*) and caused

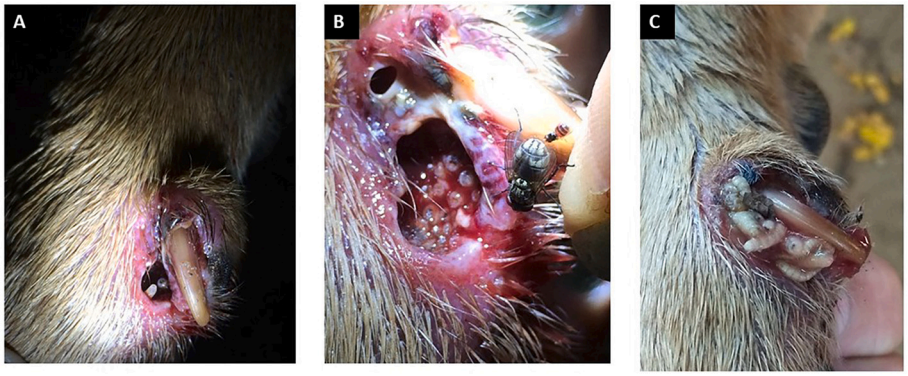

Fig. 2. Severe myiasis lesion of the nail apparatus (Dog n°10). A and B) Pre-treatment; C) 24 h post-treatment: all larvae were dead and expelled

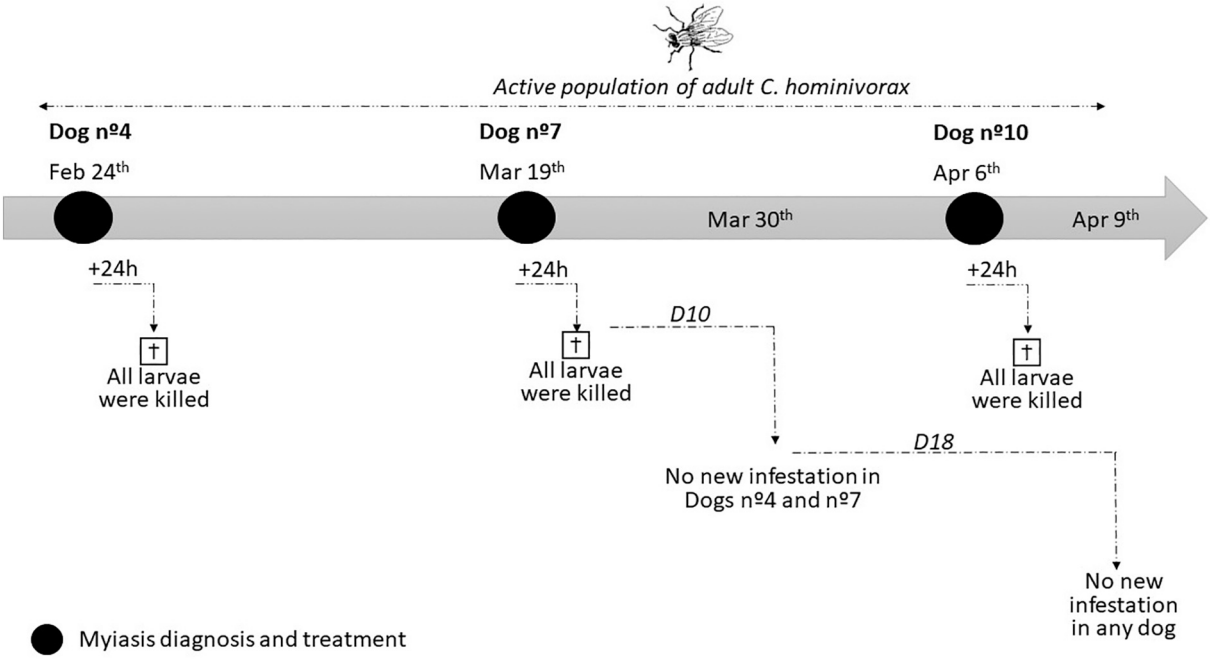

Fig. 3. Chronological order of *Cochliomyia hominivorax* infestations, diagnosis and treatment, in three dogs of the same household.

Fig. 4. Severe myiasis lesion on the dorsal neck (Dog n°8). A) Pre-treatment; B) 24 h post-treatment: all larvae were dead and expelled; C) 48 h post-treatment; D) 29 days post-treatment: the wound was almost fully healed

the loss of 15% of the total Key deer population, a federally listed endangered species. Cases were also reported in companion animals (3 dogs, 2 cats and 1 pet pig) and wildlife (1 raccoon). One year later, the outbreak was ended after sterile fly releases (Hennessey et al., 2019).

The control of *C. hominivorax* is essential due to its medical importance, its distribution in South America and the risk of reintroduction into non-endemic areas. In endemic regions, *C. hominivorax* is commonly found in dogs: in Brazil, 184 out of 190 veterinary practices declared having screwworm infestations cases in their activity in Rio de Janeiro in 2000 (Cramer-Ribeiro et al., 2003). In 2009, 40 cases of myiasis were identified across 90 small animal practices from the Federal District: *C. hominivorax* was the most abundant parasite with 37 cases observed in dogs (Cansi and Demo, 2011). A recent survey assessed the occurrence of *C. hominivorax* in Brazil from 1975 and 2017, and dogs were the second most infested domestic species after cattle, although the occurrence of myiasis in pets is certainly underestimated in all states (Costa-Júnior et al., 2019). In Venezuela and Panama, dogs were also the second most infested domestic species after cattle, with ~ 200 cases between 1999 and 2006 (Coronado and Kowalski, 2009) and 383 cases between 2002 and 2005 (Bermúdez et al., 2007), respectively. Recently, the first case of *C. hominivorax* in dogs has been reported in Colombia (Muñoz et al., 2020). In addition, domestic and wild animals represent a reservoir for human myiasis (Barros and Bricarello, 2020), therefore, strategies to control the New World screwworm population are essential. In companion animals, insecticidal treatments such as afoxolaner, which present speed of kill and 100% efficacy against *C. hominivorax* larvae, are beneficial to owners and veterinarians.

4. Conclusions

A single treatment of afoxolaner (NexGard®) demonstrated 100% efficacy for the treatment of light to severe myiasis caused by second and third instar larvae of *C. hominivorax* in dogs.

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Animal welfare declaration

We, the authors, declare that during the study entitled "Efficacy of afoxolaner (NexGard®) in the treatment of the New World screwworm flies *Cochliomyia hominivorax* (Diptera: Calliphoridae) in naturally infested dogs" the animals were handled in compliance with Boehringer Ingelheim Institutional Animal Care and Use Committee (IACUC) approvals and the study met the CONCEA (Brazil's National Council for the Control of Animal Experimentation) animal welfare requirements.

Declaration of Competing Interest

The authors are either employees or contractors of Boehringer Ingelheim Animal Health. NexGard® is a trademark of Boehringer Ingelheim Animal Health.

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