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Clinical and parasitological evaluation of pour-on fluazuron and ivermectin for treating canine demodicosis[1]

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ABSTRACT.- Souza C.P., Ramadinha R.H.R. & Scott F.B. 2014**. Clinical and parasitological evaluation of pour-on fluazuron and ivermectin for treating canine demodicosis.** *Pesquisa Veterinária Brasileira 34(11):1094-1100*. Departamento de Parasitologia Animal, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, BR-465 Km 7, Seropédica, RJ 23890-000, Brazil. E-mail: clarissaps@globo.com

The objective of the study was to evaluate the efficacy of pour-on formulations of fluazuron and ivermectin in different therapeutic protocols for treatment of demodicosis by means of quantifying mites with skin scraping, histological and clinical evaluation in dogs. Eighteen dogs with skin scrapings positive for *Demodex canis* were evaluated, divided into three groups. All the animals were treated every 14 days, completing 6 treatments for each animal (days 0, 14, 28, 42, 56 and 70). In group 1, pour-on 2.5% fluazuron was used at the dose of 20mg/kg; in the group 2 pour-on 2.5% fluazuron at a dose of 20 mg/kg in association with pour-on 0.5% ivermectin at the dose of 0.6mg/kg; and in group 3, pour-on 0.5% ivermectin alone was used, at the dose of 0.6mg/kg. The treatment was evaluated and monitored through skin scrapings and clinical follow-up of the lesions every 14 days for 84 days, and through histopathological examination at the end of each treatment protocol. The success rate was defined as the percentage of dogs in each group that had negative skin scrapings after the treatment: this was 16.67% for group 1, and 50% for groups 2 and 3. The reduction in mite counts reached effectiveness of 67.66%, 88.99% and 84.29% for groups 1, 2 and 3 respectively. The Wilcoxon test showed that there was a significant difference between the number of mites before and after treatment in groups 2 and 3. The histopathological examination revealed that only group 1 showed no significant difference in the intensity of infestation between days 0 and 84. Clinically, there was no significant difference between the evaluation before and after treatment in the three groups. Pour-on 2.5% fluazuron and pour-on 0.5% ivermectin were not effective for treating canine demodicosis, either in association or as single therapy, when applied every 14 days for a period of 70 days. Quantification of mites using skin scrapings and histological evaluation proved to be ineffective, either one as sole therapeutic evaluation parameters, for canine demodicosis.

INDEX TERMS: *Demodex canis*, control, fluazuron, ivermectin, pour-on.

RESUMO.- [**Avaliação clínica e parasitológica do fluazuron e da ivermectina *pour-on* no tratamento da demodiciose canina.**] O objetivo do presente estudo foi avaliar a eficácia do fluazuron e da ivermectina *pour-on* em diferentes protocolos terapêuticos no tratamento da demodiciose, através da quantificação de ácaros por raspados cutâneos e exames histológicos, além da avaliação dos cães. Foram avaliados 18 cães com raspados cutâneos positivos para o ácaro *Demodex canis*, divididos em três grupos. Todos os animais foram tratados a cada 14 dias, totalizando seis tratamentos em cada cão (Dias 0, 14, 28, 42, 56 e 70). No grupo 1 foi utilizado fluazuron 2,5% *pour-on* na dosagem de 20mg/kg; no grupo 2 foi empregado fluazuron 2,5% *pour-on* na dosagem de 20mg/kg associado a ivermectina 0,5% *pour-on*, na dosagem de 0,6mg/kg e, no grupo 3, somente ivermectina 0,5% *pour-on* 0,6mg/kg. Raspados cutâneos e acompanhamento clínico das lesões foram realizados a cada 14 dias por 84 dias e realizado exame histopatológico

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ao final de cada protocolo terapêutico. A taxa de sucesso foi definida pela porcentagem de cães em cada grupo com raspados negativos ao final do tratamento, que foi 16,67% para o grupo 1 e 50% para os grupos 2 e 3. A redução na contagem no número de ácaros alcançou eficácia de até 67,66%; 88,99% e 84,29%, nos grupos 1, 2 e 3, respectivamente. O teste de Wilcoxon mostrou que houve diferença significativa entre a quantidade de ácaros antes e após o tratamento nos grupos 2 e 3. No exame histopatológico apenas o grupo 1 não apresentou diferença significativa na intensidade da infestação entre os dias 0 e 84. Clinicamente não houve diferença significativa entre as avaliações antes e após o tratamento dos três grupos. O fluazuron 2,5% *pour-on* e a ivermectina 0,5% *pour-on* associados ou como terapia única, não foram eficazes no tratamento da demodiciose canina, quando aplicados a cada 14 dias em um período de 70 dias. A quantificação de ácaros através do exame parasitológico em raspado cutâneo e em exame histopatológico demonstrou-se ineficaz como parâmetro isolado de avaliação pós-terapêutica para demodiciose canina.

TERMOS DE INDEXAÇÃO: Demodiciose canina, *Demodex canis*, controle, fluazuron, ivermectina, *pour-on*.

INTRODUCTION

Demodectic mange or canine demodicosis is an inflammatory parasitic skin disease caused by excessive proliferation of the mite *Demodex canis*, which lives in hair follicles and sebaceous glands. These parasites are transmitted from mother to offspring, which also inherit a specific cell-mediated condition of immunodeficiency (Heine et al. 2005, Delayte et al. 2006).

It has now been shown through molecular techniques that all dogs, even without lesions, house a small population of mites on their skin. Their immune systems tolerate the presence of these parasites and keep them under control. But at some point, the number of mites can increase, thereby developing a pathogenic role in relation to the host (Ravera et al. 2013).

Routinely, infestation is diagnosed through viewing the mite *D. canis* under an optical microscope in parasitological examinations of hair plucking or skin scrapings, or histopathological examination (Larsson 1989).

Several drugs have been used for treating canine demodicosis. Amitraz and macrocyclic lactones are the most common drugs and they are used in different therapeutic schemes. In general, they show varied levels of effectiveness and they may generate side effects that make it impossible to complete the therapy. Another negative point is that some protocols are not very practicable, which decreases the pet owners' compliance. For this reason, it is necessary to search for alternative therapies, due to the possibility of failure or impracticality of the currently recommended treatments (Mueller et al. 2012).

Ivermectin was the first macrocyclic lactone to be marketed and has been regularly used for treating various infestations in small animals (Lynn 2003). In the 1990s a pour-on 0.5% formulation became available for the treatment of ecto and endoparasites in cattle, which highlighted the practicality of topical therapy (Paradis & Pagé 1998).

A recent advance in the effort to control ectoparasites on animals came through the development of insect growth inhibitors. Fluazuron is in this class and it is considered to be a good drug for controlling ticks on ruminants, due to its high specificity, low mammalian toxicity, effectiveness at low concentrations and long-lasting residual effect against reinfestation (Kryger et al. 2005). The action and safety of this antiparasitic drug has now also been tested against fleas and ticks on dogs, with promising results (Vieira 2009, 2012, Oliveira et al. 2012).

The objective of the present study was to evaluate the efficacy of pour-on formulations of fluazuron and ivermectin in different therapeutic protocols for treating demodicosis by means of quantifying mites using skin scraping, histological and clinical evaluations on dogs.

MATERIALS AND METHODS

The study was approved by the Ethics Committee on Animal Experimentation of the Universidade Federal Rural do Rio de Janeiro (CEUA-FAPUR/UFRRJ). Eighteen dogs of both sexes and various ages and breeds that presented clinical and laboratory diagnoses of localized and generalized demodicosis were evaluated. These dogs were treated at the Dermatology service of the Federal Rural University of Rio de Janeiro Veterinary Hospital, over a period of 20 months.

The initial diagnosis was made through skin scrapings of two areas with lesions, each of 1cm^2, and these were delineated on the dog's skin using a marking pen. As close as possible to this site, still within the lesional area, a biopsy was performed under local anesthesia using a punch of 8 mm of diameter on order to collect skin material on day 0. The fragment collected was fixed in a solution of 10% formalin and was sent for routine histology processing and hematoxylin-eosin staining.

Dogs that presented one to four small, circumscribed areas of alopecia were considered cases of localized demodicosis. All the animals that presented at least five small damaged areas (<100 cm^2 of the body) or were affected over an entire region of the body (>100 cm^2) or had an entire limb compromised (Mueller et al. 2009) were deemed to have the generalized form of infestation.

The dogs were divided into localized and generalized demodicosis cases and therefore randomly placed into one of the three groups, with six animals each group.

The group 1 received a pour-on application of 20mg/kg of 2.5% fluazuron (Acatak®; Novartis Animal Health) arthropod growth inhibitor formulation, as used for cattle. The drug was administered with the aid of a disposable hypodermic syringe at a dose of 20mg/kg, along the back of the dogs.

The animals of the group 2 were treated with fluazuron at 20mg/kg and also with pour-on 0.5% ivermectin (Ivomec®; Merial Animal Health) at a dose of 0.6mg/kg. Both drugs were administered at the same time in parallel on opposite sides along the back of each dog, using a disposable hypodermic syringe for each product.

The treatment for the group 3 consisted of pour-on 0.5% ivermectin at 0.6mg/kg.

All the dogs were treated every 14 days for 70 days, thus completing a total of six treatments for each animal (days 0, 14, 28, 42, 56 and 70). The total treatment time was established in accordance with reports in the literature that indicate that after two to three months of therapy, there is already a response regarding the effectiveness of a particular drug (Schwassmann et al. 1997).

Possible side effects relating to the treatment were evaluated. The animals were weighed to calculate the volume that should be administered, and at every new administration, the weight was measured again and the volume adjusted.

For evaluation and follow-up treatment, two skin scrapings of 1cm^2 each were repeated on all the dogs every 14 days, for 84 days, always in the same areas of the skin. The different stages of *D. canis* were identified, counted and compared on each evaluation day, as recommended by Mueller et al. (2012).

On day 84 another sample of skin was collected from each animal at the same area where biopsy was performed at the beginning of the protocol. The intensity of infestation seen in the histopathological examination, both before and after treatment, was determined using the score recommended by Caswell et al. (1995), where (0) indicates no mites seen in the examination, (1) rare fragments of mites (slight infestation), (2) 2 to 4 mites per slide (mild infestation), (3) several mites, but three or less per follicle (moderate infestation) and (4) more than three mites per hair follicle (severe infestation).

At the initial and final consultations (days 0 and 84), the main skin lesions of the dogs were examined and there were evaluated: alopecia, dyschromia (erythema and/or hyperpigmentation), crusts, seborrhea, papules and pustules, and swelling. The intensity of these alterations was classified as: (0) absent, (1) slight, (2) intermediate or (3) severe (Heine et al. 2005, Delayte et al. 2006). These parameters were used for clinical evaluation of the effectiveness of the proposed therapeutic protocols.

Dogs with secondary bacterial infection diagnosed through cytological examination using skin imprints, which were stained by means of fast panoptic (Instant Prov), were treated with systemic antibiotic: cephalexin at a dose of 30mg/kg every 12 hours. Antibiotics were used until bacteria were no longer found in cytology, or any lesions suggestive of pyoderma.

The effectiveness of therapeutic protocols based on reduction of the number of mites in relation to the number prior to treatment was determined by means of the geometric mean, calculated using the following formula:

$$\frac{\text{(Geometric mean before treatment - geometric mean after treatment) x100}}{\text{Geometric mean before treatment}}$$

The success rate was defined as the percentage of dogs in each group that presented negative skin scraping tests (Fourie et al. 2007).

The results obtained from the skin scraping tests, clinical evaluation scores and histological examinations were statistically compared before and after treatment using the Wilcoxon test for differences between ordered pairs (Sampaio 2002).

All the animals were monthly monitored for twelve months following the withdrawal of the treatment protocols, which is the time period suggested in the literature for monitoring recurrence of clinical disease in dogs (Gortel 2006, Mueller et al. 2012).

RESULTS

The Table 1 shows the ages of the dogs studied at the time of appearance of the lesions and at the beginning of the treatment; data about sex, breed and length of fur of the dogs were also included.

Results from therapy using pour-on 2.5% fluazuron

The dogs included in this treatment protocol were identified by the numbers 1 to 6 (Group1). The evaluations performed at the beginning and during the treatment, through the numbers of mites in the skin scraping tests, are described in Table 2. The reduction in the count of the number of mites reached levels that ranged from 38.90 to 67.66% in the six evaluations after the therapy began. The success rate at the end of treatment was 16.67% (1/6) (Table 2). There was no significant difference between the numbers of mites before and after the therapy.

Through histological examination, it was found that there was no significant decrease in the intensity of infestation from the beginning (day 0) to after the treatment (day 84) (Table 3).

Clinical evaluation of the lesions showed that the treatment failed to resolve the skin alterations caused by the mite *Demodex canis*. There was no significant difference between the clinical observations made before treatment and at the end of the treatment protocol (Table 4).

Results from therapy using pour-on 2.5% fluazuron and pour-on 0.5% ivermectin

The dogs included in this treatment protocol were identified by the numbers 7 to 12 (Group 2).. The reduction in the number of mites reached levels that ranged from 67 to 88.99% in the six evaluations conducted after the therapy protocol began. The success rate at the end of the treatment time was 50%. There was a significant difference in the numbers of mites from before to after the therapy (Table 2).

Through histological examination, it was found that there was no significant decrease in the intensity of infestation from before (day 0) to after the treatment (day +84) (Table 3).

Clinical evaluation of the lesions showed that the treatment was partially effective for controlling the skin alterations caused by *D. canis*. Dogs 7, 9 and 10 (3/6) presented clinical failure, dogs 11 and 12 (2/6) showed clinical improvement and dog 8 (1/6) presented clinical cure. There was no significant difference between the clinical observa-

Table 1. Characteristics and history of 18 dogs with demodicosis used in evaluating different treatment protocols for infestation control

Dogs	Gender	Breed	Length of hair	Age at the appearance of lesions (months)	Age at beginning of treatment (months)
1	Male	Mongrel	Short	4	6
2	Female	Mongrel	Short	4	6
3	Male	Yorkshire	Long	24	36
4	Female	Mongrel	Medium	7	8
5	Male	Poodle	Long	6	30
6	Female	Pug	Short	7	9
7	Female	Mongrel	Medium	32	84
8	Male	Mongrel	Long	3	5
9	Male	Mongrel	Medium	24	48
10	Female	Mongrel	Medium	2	7
11	Female	Pinscher	Short	35	38
12	Male	Mongrel	Medium	14	24
13	Male	Pinscher	Short	3	6
14	Female	Mongrel	Short	4	6
15	Female	Mongrel	Medium	10	36
16	Male	Bulldog	Medium	4	6
17	Male	Pinscher	Short	7	8
18	Male	Bull Terrier	Medium	48	84

Table 2. Quantity of *Demodex canis* mites found in skin scraping tests on the dogs evaluated by means of three therapeutic protocols over the 70 days of treatment

Groups	Dogs	Measurement method	Quantity of *Demodex canis* (different stages)						
			Day 0	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84
Group 1[a]	1		429	193	184	214	124	96	78
	2		55	16	22	16	14	13	13
	3		25	10	8	31	61	11	13
	4		2	1	0	1	4	2	0
	5		20	16	15	15	17	11	10
	6		88	79	39	29	15	22	18
		Geometric mean[d]	35.71	18.42	16.33	18.94	21.82	13.71	11.55
		% Reduction	-	48.42	52.56	46.96	38.90	61.61	67.66
		% Success rate	0	0	16.67	0	0	0	16.67
Group 2[b]	7		8	6	10	6	2	1	1
	8		3	0	0	0	0	0	0
	9		2	3	4	4	3	0	0
	10		54	8	12	7	6	5	4
	11		72	10	2	1	0	0	0
	12		12	1	3	4	2	1	1
		Geometric mean	11.44	3.36	3.77	2.96	2.04	1.31	1.26
		% Reduction	-	70.63	67	74.13	79	88.55	88.99
		% Success rate	0	16.67	16.67	16.67	33.33	50	50
Group 3[c]	13		2	1	0	0	0	0	0
	14		8	6	6	7	11	12	2
	15		14	13	3	2	0	0	0
	16		3	2	6	5	5	2	1
	17		12	25	3	1	1	0	0
	18		365	103	68	45	38	32	22
		Geometric mean	11.97	8.59	5.29	3.83	3.58	3.03	1.88
		% Reduction	-	28.24	55.81	68	70.10	74.69	84.29
		% Success rate	0	0	16.67	16.67	33.33	50	50

[a] dogs treated with pour-on 2.5% fluazuron; [b] dogs treated with pour-on 2.5% fluazuron and pour-on 0.5% ivermectin; [c] dogs treated with pour-on 0.5% ivermectin; [d] $p>0.05$.

tions made before treatment and at the end of the treatment protocol (Table 4).

Results from therapy using pour-on 0.5% ivermectin

The dogs included in this treatment protocol were identified by the numbers 13 to 18 (Group 3). The reduction in the number of mites reached levels that ranged from 28.24 to 84.29% in the six evaluations conducted after the treatment began. The success rate at the end of treatment was 50%. There was a significant difference in the numbers of mites from before to after the therapy (Table 2). Through histological examination, it was found that there was no significant decrease in the intensity of infestation from before (day 0) to after the treatment (day +84) (Table 3).

Clinical evaluation of the lesions showed that the treatment was partially effective for controlling the skin alterations caused by *D. canis*. Dogs 14 and 16 (2/6) presented clinical failure, dogs 15 and 18 (2/6) showed clinical improvement and dogs 13 and 17 (2/6) presented clinical cure. There was no significant difference between the clinical observations made before treatment and at the end of the treatment protocol (Table 4).

Dogs 1, 2, 3, 5 (group 1), 10, 11 (group 2), 14, 15, 16 and 18 (group 3) (10/18, 55,6%) received systemic antibiotic therapy until remission of pyoderma and, for some animals, this treatment continued throughout the trial period.

In dogs 1, 2, 3, 5, 6 (group 1), 7, 10, 12 (group 2), 14, 16 and 18 (group 3) (11/18, 61,1%) mites could be seen in the skin scraping, and dogs 4, 9 and 15 (3/18, 16,67%) still presented clinical alterations on the skin on the last day of evaluation. These dogs were then started on treatment with oral ivermectin at a daily dose of 0.6 mg/kg until complete remission of lesions and until they presented negative skin scrapings, which took up to four months of treatment for some animals.

The dogs were monitored for 12 months after the treatment protocol ended, through periodic reviews or telephone contacts, or even through photos sent by the owners.

Dogs 8, 11 (group 2), 13 and 17 (group 3) (4/18, 22,2%), which did not receive any drugs other than the initially instituted protocol, did not show any clinical signs of recurrence after 12 months.

None of the dogs presented any side effects relating to the treatment during the evaluation period of this study.

DISCUSSION

Fluazuron is an insect growth inhibitor that belongs to the group of chitin synthesis inhibitors, also called benzoylphenylureas. It does not have any direct action that causes the death of insects and mites but, rather, it interferes with the molting and hatching processes of the parasites, thereby interrupting their life cycle. Fluazuron has been used especially for controlling *Rhipicephalus (Boophilus) microplus* (Graf 1993). Vieira (2009) and Oliveira et al. (2012) also

Table 3. Clinical presentation of demodicosis and quantity of Demodex canis in histopathological examinations on the dogs used in the three therapeutic protocols, before treatment and after 84 days of evaluation

Dogs		Clinical presentation of demodectic mange	Intensity of infestation in histopathological examination	
			Before treatment	After treatment
Group 1[a]	1	Generalized	4[i]	4
	2	Generalized	3[h]	3
	3	Generalized	4	4
	4	Localized	0[e]	0
	5	Generalized	4	4
	6	Localized	3	3
	Arithmetic mean[d]		3.5	3.5
Group 2[b]	7	Generalized	4	4
	8	Localized	1[f]	0
	9	Generalized	3	0
	10	Generalized	3	2 g
	11	Generalized	4	1
	12	Localized	4	2
	Arithmetic mean		3.17	1.5
Group 3[c]	13	Localized	3	2
	14	Generalized	3	2
	15	Generalized	4	3
	16	Generalized	3	2
	17	Localized	3	0
	18	Generalized	3	2
	Arithmetic mean		3.17	1.83

[a] dogs treated with pour-on 2.5% fluazuron; [b] dogs treated with pour-on 2.5% fluazuron and pour-on 0.5% ivermectin; [c] dogs treated with pour--on 0.5% ivermectin, [d] p>0.05; [e] 0 = no mites; [f] 1 = slight infestation; [g] 2 = mild infestation; [h] = moderate infestation; [i] 4 = severe infestation.

demonstrated the potential of pour-on fluazuron against different stages of *R. sanguineus*, at diverse concentrations.

Because the mite *D. canis* belongs to the same taxonomic class as ticks (Arachnida), it was considered possible that fluazuron might have some efficacy against these parasites.

The results obtained through pour-on application of fluazuron confirm what was previously found through testing lufenuron, which is another compound in the same group of chitin synthesis inhibitors. Lufenuron was also used for treating canine demodicosis and was considered to be ineffective (Schwassmann et al. 1997). This drug was developed to control fleas on cats and dogs, and it is administered as pills. Schwassmann et al. (1997) suspected that the failure of oral lufenuron against mites was associated with small penetration of the drug into the skin. There is also the possibility that the arthropods may have different mechanisms of chitin synthesis, since it has been proven that lufenuron provides excellent results in environmental control of different stages of fleas' life cycle, and fluazuron used orally also has shown good efficacy in controlling larvae and nymphs of *R. sanguineus* (Vieira 2012). Thus, it is believed that even if administered orally, fluazuron would not show good efficacy against *Demodex canis*.

In recent decades, ivermectin has been extensively evaluated for treating canine demodicosis in various protocols, especially orally, and it is one of the most widely used drugs in the world (Mueller et al. 2012). Efficacy of up to 89.7% has been reported when administered orally every day, but with treatment protocols of duration of up to 150 days (Ristic et al. 1995, Delayte et al. 2006). The present study evaluated the therapeutic potential of ivermectin applied using a pour-on formulation separately and in combination

Table 4. Clinical signs observed in the dog's skin in the three treatment protocols evaluated, before (day 0) and at the end (day 84) of treatment

	Animals	Clinical signs											
		Alopecia		Dyschromia		Crusts		Seborrhea		Papule / Pustule		Edema	
		Day 0	Day 84	Day 0	Day 84	Day 0	Day 84	Day 0	Day 84	Day 0	Day 84	Day 0	Day 84
Group 1[a]	1	2[f]	2	3[g]	3	2	3	1[e]	1	0[d]	0	0	0
	2	3	3	2	2	1	0	1	1	0	0	0	0
	3	1	2	3	3	3	3	0	0	0	0	0	0
	4	1	1	0	0	1	1	0	0	0	0	0	0
	5	2	3	3	3	2	3	1	1	0	0	3	3
	6	1	1	1	1	0	0	0	0	1	1	0	0
p value		0.1797		0		0.5930		0		0		0	
Group 2[b]	7	2	2	3	3	1	0	1	2	0	0	0	0
	8	1	0	0	0	0	0	0	0	0	0	0	0
	9	1	1	1	0	0	0	1	1	0	0	0	0
	10	1	1	1	1	0	0	3	2	0	0	0	0
	11	2	0	3	0	1	0	1	0	0	0	0	0
	12	2	1	2	1	2	0	0	0	0	0	0	0
p value		0.1088		0.1088		0.1088		0.5930		0		0	
Group 3[c]	13	2	0	0	0	0	0	1	0	0	0	0	0
	14	2	3	2	2	0	2	0	0	1	0	0	0
	15	3	1	2	0	2	0	2	0	0	0	0	0
	16	2	2	2	2	0	0	1	0	3	3	0	0
	17	1	0	1	0	1	0	0	0	0	0	0	0
	18	3	2	2	1	3	1	0	0	0	0	3	0
p value		0.1380		0.1088		0.4652		0.1088		0.3173		0.3173	

[a] dogs treated with pour-on 2.5% fluazuron; [b] dogs treated with pour-on 2.5% fluazuron and pour-on 0.5% ivermectin; [c] dogs treated with pour-on 0.5% ivermectin; [d] 0 absent; e 1 slight; [f] 2 intermediate; [g] 3 severe.

with fluazuron, also in a pour-on formulation, applied at the dose of 0.6mg/kg that is usually used in applications via other routes, but at intervals of 14 days for a period of 84 days.

The topical formulation of ivermectin has already been compared with the oral product among goats, and this demonstrated that percutaneous administration promotes prolonged persistence of the drug in plasma. However, the bioavailability is significantly lower than is seen with oral administration (Scott, Kinabo & McKellar 1990). In the present evaluation, no satisfactory results supporting the hypothesis that it might be possible to administer pour-on ivermectin over a longer treatment interval than could be done through oral administration were obtained, over the treatment period that had been established.

The pour-on formulation of ivermectin was previously tested at a dose of 1.5mg/kg applied three times a week, but only among dogs with chronic and generalized demodicosis and with a history of unsuccessful treatment with amitraz. A reduction in the severity of clinical signs and up to 75% in the number of mites in skin scrapings was observed, but the effectiveness according to the recurrence rate after 12 months of monitoring was 8% (Paradis & Pagé 1998). The disease is considered to be chronic and generalized when it persists for at least six months with involvement of no less than 50% of the dog's body or involvement of the four limbs. This form has been reported as being difficult to treat and the outcome is often frustrating (Paradis & Pagé 1998, Fourie et al. 2007). Dogs 3, 5, 7, 9, 12, 15 and 18 (7/18) had been developing clinical signs of infestation for at least six months prior to being enrolled into the study and they presented the generalized form of demodicosis. With the exception of number 12, in which the disease was presented in the localized form, these animals were considered to be chronic cases. None of these dogs showed complete clinical remission and/or absence of parasites in the diagnostic examinations at 84 days of evaluation.

There is a need for further studies on the pharmacokinetics in dogs that are administered different formulations of ivermectin. The pour-on formulation used at the conventional dose every 15 days for treating canine scabies (Paradis et al. 1997) has demonstrated excellent results. This report makes us believe that there is a reasonable level of systemic absorption and dispersion in dogs' skin.

In each of the three groups, there were two animals with the localized form and four animals with the generalized form of canine demodicosis. Several literature reports have commented on the difficulty of treating generalized mange, in comparison with localized mange. It has even been cited that 10% of the localized cases may have spontaneous remission of clinical signs. Nonetheless, this does not occur routinely in clinical practice and there are no scientific data to support this finding (Mueller et al. 2009). In the present study, different therapeutic responses were observed, regardless of the clinical presentation of the demodicosis. Even among the six dogs with localized mange (numbers 4, 6, 8, 12, 13 and 17), two of them showed clinical failure at the end of the treatment (Dogs 4 and 6).

The duration of treatment required for evaluating the therapeutic protocols in the present study needed to be determined. This was established in accordance with the recommendation from Schwassmann et al. (1997), who stated that after two to three months of therapy, it was possible to observe whether the medication had been effective or not. On the other hand, there are comments in the literature indicating that, for clinical remission of the lesions and absence of parasites in the skin scrapings, the treatment may be very lengthy. Moreover, premature termination may be the major cause of treatment failure (Gortel 2006). Differences in the duration of treatment protocols are reported by some authors, such as Delayte et al. (2006), who obtained the first negative skin scraping result after 90 days of treatment and parasitological clearance after 130 days. Ristic et al. (1995) obtained these same results at 45 and 70 days, respectively. It is possible that, with continuity of the treatment, or if the drugs were applied more often, better clinical and parasitological results might have been observed among the animals studied.

The results found in this study allowed us to observe that although groups 2 and 3 showed significant reductions in the numbers of mites between day 0 and day 84, this was not translated into effective reduction of the general clinical signs among these animals. In other cases, the dogs may present clinically normal results after treatment, but still demonstrate mites in skin scraping tests (Mueller et al. 2012). Thus, studies evaluating therapeutic protocols against demodicosis should not use observation of the presence or absence of *Demodex* mites solely, or clinical appearance alone. Both parameters (clinical and parasitological) must be considered in interpreting the results and in determining whether therapeutic success was achieved.

CONCLUSIONS

Pour-on 2.5% fluazuron and pour-on 0.5% ivermectin, either in association or as single therapies, were not effective for treating canine demodicosis when applied every 14 days over a period of 70 days.

Quantification of mites by means of histological examination and skin scraping proved to be ineffective when used as a sole parameter for therapeutic evaluation of canine demodicosis.

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