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Efficacy of Ivermectin Pour-On Administration Against Natural *Dirofilaria immitis* Infestation in Native Dogs of East-Azerbaijan Province, Iran

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Abstract: One of the real problems that cause the public health risks in small animal yearly is parasitic diseases and some of these parasites are zoonoses. To overcome these problems, the use of antiparasitic drugs is necessary. Ivermectin is a broad spectrum antiparasitic agent and different routes of its administration such as injection, oral and pour-on were used. The aims of the current study were evaluation of the efficacy of ivermectin pour-on administration against natural *Dirofilaria immitis* infection in native dog and also determination the prevalence rate of this parasite in East-Azerbaijan province, Iran. In the present study, 60 native dogs were investigated by detection and enumeration of circulating microfilariae of *Dirofilaria immitis* were carried out at each visit by the modified Knott test. After confirming the infection with worms, ivermectin (0.5 mg kg^{-1}) pour-on was administrated to infected dogs. Blood examination was repeated in 1, 7, 21 and 28 days post treatment. Results showed that total prevalence of *Dirofilaria immitis* infection was 71.66% in native dog of East-Azerbaijan province, Iran. Efficacy rate of ivermectin pour-on was 31.42, 63.49, 88.66 and 98.54% in 1, 7, 21 and 28 days, respectively. In conclusion, the effect of this drug against *Dirofilaria immitis* resulted in reduction in Knott test exceeded 98% so, this drug can be used in antiparasitic program in dog. Further investigations are necessary to evaluate the drug effect on other nematodes and parasitic infections.

Key words: *Dirofilaria immitis*, knott test, ivermectin, topical formulations, dog, Iran

INTRODUCTION

Infections with gastrointestinal nematodes and heart worm are very common on native dog in Iran and all over the world. Parasitic infections of dogs are major factors responsible for public health losses through increased mortality and public health risk and cause zoonotic diseases in human and animals (Kozen *et al*., 2008; Ahmad *et al*., 2011; Snyder *et al*., 2011; Ashraf *et al*., 2008; Obiukwu and Onyali, 2006). Parasites cause the dogs to be unthrifty which may include the loss of weight. Due to parasitism, the animals become susceptible to other health problems which can lead to death.

Many researches for prevalence rate of gastrointestinal parasites and heart worm all over the world have been reported but researches for effect of anti parasitic drugs by different administration routes is low and in Iran the study on present subject has not been done (Georgi *et al*., 1990; Kassai, 1999; Mandal, 2006; Soulsby, 1986). Medicine combinations such as pyrantel pamoate, nitroscanate, milbemycine, ivermectin, selamectin, moxidectin, praziquantel, pyrantel embonate and febantel, ivermectin and pyrantel pamoate have been used in dogs to treat gastrointestinal cestodes and nematodes in the recent years (Kozen *et al*., 2008). Macrocyclic lactones such as avermectins and milbemycins, ascarids included as well show a perfect anti-parasitic activity against nematodes. Various formulations of these compounds are used all over the world for many animal groups such as dogs, cattle, sheep, pig and horse (Kozen *et al*., 2008; Snyder *et al*., 2011). Ivermectin is a member of the macrocyclic lactone class of endectocides.

It is labeled for the treatment of internal and external parasites in dogs, cats, horses, pigs, sheep, cattle and birds. Subcutaneous and topical formulations are available for use in animals at a dose of 0.2 and 0.5 mg kg^{-1} body weight, respectively. Ivermectin is a highly potent broad-spectrum anthelmintic that is widely used in different animals. It is available in injectable, oral and topical formulations for use in animals (Kozen *et al*., 2008; Ahmad *et al*., 2011; Snyder *et al*., 2011; Vermunt *et al*., 1995; Williams *et al*., 1997; Khayatnouri *et al*., 2011; Ashraf *et al*., 2008; Obiukwu and Onyali, 2006).

The Food and Drug Administration-Center for Veterinary Medicine (FDA-CVM) approvals in the united states in the late 1980s and early, 1990s of the macrocyclic lactones, ivermectin and milbemycin oxime and intended to be administrated orally at sequential monthly intervals during the heart worm transmission season,

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led to substantial improvements in chemoprophylaxis to prevent infection with *Dirofilaria immitis* (Snyder *et al*., 2011). Macrocyclic lactones are safe, effective and convenient drugs for prevention of heart worm diseases in virtually all dogs when used as instructed. While 100% prevention was not obtained for either macrocyclic lactones with this recent heart worm field isolate, it should be emphasized that these products are not intended to be used for just one month during the heart worm transmission season (Snyder *et al*., 2011).

The most important GI nematode and heart worm responsible for public health losses through increased mortality and public health risk and cause zoonotic diseases in human and animals (Kozen *et al*., 2008; Ahmad *et al*., 2011; Snyder *et al*., 2011; Ashraf *et al*., 2008; Obiukwu and Onyali, 2006). The objective of the present study is the evaluation of the effect of ivermectin pour-on administration against natural *Dirofilaria immitis* nematode infections and determination of its prevalence rate in native dogs. This study is the first report in Iran.

MATERIALS AND METHODS

In present study, a total number of 60 native dogs to *Dirofilaria immitis* infestation, from different region of East-Azerbaijan province, Iran was subjected for blood examination and Knott test. Ivermectin was administrated to treat infected animals at a dose of 0.5 mg kg^{-1}. Also, pour-on form of 0.5% ivermectin powder in isopropyl alcohol was made. Before and after treatment of dogs, 3 blood samples of each animal were taken for blood examination and microfilariae count was recorded. Blood examination in days 1, 7, 21 and 28 after treatment were repeated. In the present study, Knott test were used for microfilariae count.

Detection and enumeration of circulating microfilariae of *Dirofilaria immitis* were carried out at each visit by the modified Knott test, according to Bazzocchi *et al*. (2008). Briefly, 1 mL venous blood was mixed with 10 mL of 2% buffered formalin and centrifuged for 5 min at 2000 rpm. A 100 μL of sediment was mixed with equal parts of a 1:1000 methylene blue stain. An aliquot of 20 mL of stained sediment was placed on a slide, covered with a coverslip and examined under a microscope. The number of microfilariae was multiplied by 10x and expressed as microfilariae/mL (Bazzocchi *et al*., 2008). Ivermectin efficacy was calculated according to the following equation:

$$\text{Drug efficacy}(\%) = \frac{P - R}{P} \times 100$$

Where:

R = The average number of microfilariae in mL of blood sample after treatment

P = The average number of microfilariae in mL of blood sample before treatment

Data were analyzed by non-parametric Crosscal-walis and $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

The results of present study indicated that 43 dogs from a total of 60 were infected with *Dirofilaria immitis* with a prevalence rate of 71.66%. Average number of enumerated microfilariae in infected non treated animals was 4613. The average number of enumerated microfilariae in blood samples after treatment with pour on ivermectin has been shown in Table 1. Reduction percentages in microfilariae count after 1, 7, 21 and 28 days of treatment with ivermectin were 31.42, 63.49, 88.66 and 98.54%, respectively (Table 1).

According to results of Crosscal-walis test, it is possible to determine which pour on administration of ivermectin decreases the natural infestation of dogs with *Dirofilaria immitis*. The efficacy rate of ivermectin on this parasite is >8%. Recently, ivermectin has different drug shapes. Half time of intra venal administration of ivermectin with dose of 300 μg kg^{-1} in cattle is 2.8 day but in subcutaneous administration with dose of 200 μg kg^{-1} is 8 days and also has been shown that the effect of sustained-release administration of this drug in cattle is more than to oral and subcutaneous administration (Reinemeyer and Courtney, 2001) but in dog any research was not done. The important base in use of antiparasitic drug is the increase of contact time of drugs with parasites rather than increase the dose of these drugs (Georgi *et al*., 1990; Kassai, 1999; Reinemeyer and Courtney, 2001; Soulsby, 1986; Urquhart *et al*., 2003). This subject has been demonstrated that ivermectin with dose of 1 mg kg^{-1} (Oral or injection) have effective antiparasitic role in veterinary. The dose of this drug in cattle for oral and subcutaneous administration is 0.2 mg kg^{-1} and for pour on administration is 0.5 mg kg^{-1}; these doses of

Table 1: Average no. of microfilariae in blood samples before and after treatment with pour on ivermectin and percentages of microfilariae count reduction

Parameters	Values
Before treatment	4613
After treatment (days)	
1	3142 (31.88%)
7	1684 (63.49%)
21	523 (88.66%)
28	67 (98.54%)

ivermectin have potent anthelmintic effect between 97-100% on adult form and 4th stage larvae of haemonchus, ostertagia, cooperia, trichostrongylus, strongyloides, bonostomum, nematodirus, trichuris, oesophagostomum, dictyocaulus and chabertia ovina and some arthropods (Georgi *et al*., 1990; Kassai, 1999; Reinemeyer and Courtney, 2001; Soulsby, 1986; Urquhart *et al*., 2003) therefore, we administrated ivermectin pour on with 0.5 mg kg^{-1} dosage in dogs. According to findings of previous researches, tablet form of ivermectin with dose of 0.4 mg kg^{-1} causes reduce in eggs in feces during 10 weeks after treatment but has not protective role for reinfection of cattle (Egerton *et al*., 1981; Garg *et al*., 2007; Reinemeyer and Courtney, 2001). Subcutaneous administration of ivermectin with dose of 0.2 mg kg^{-1} and pour-on of that with 0.5 mg kg^{-1} dose have high effective role for control of parasites also have important protective role for reinfection in cattle. Also according to findings of researchers, administration of ivermectin with dose of 0.5 mg kg^{-1} has high effect between 95-100% on haemonchus, oesophagostomum and bunostomum (Egerton *et al*., 1981; Garg *et al*., 2007; Reinemeyer and Courtney, 2001) and also on boophilus, damalina and others arthropods (Barth and Preston, 1988; Borges *et al*., 2008; Colwell and Jacobsen, 2002; Lonneux *et al*., 1997; Marley *et al*., 1993; Reinemeyer and Courtney, 2001).

According to findings of Sharma *et al*. (1990), the efficacy of ivermectin against *Ascaridia galli* infection was evaluated in chickens under controlled laboratory conditions. The chicks in the treated group were subcutaneously injected with ivermectin at a dose of 0.3 mg kg^{-1} body weight. The fall in post-treatment faecal egg counts was 81 and 92% in birds treated on days 10 and 35th, respectively. The drug was found to be 90 and 95% effective against immature and adult worms, respectively (Sharma *et al*., 1990).

In present study, the drug effect was observed 28 days after treatment by pour on ivermectin administration on *Dirofilaria immitis* 98.54% determined. There are studies where macrocyclic lactones such as ivermectin, moxidectin and selamectin were used for the treatment of nematodes in dogs. A dose of 0.2 mg kg^{-1} ivermectin administered subcutaneously and moxidectin were reported to be 100% effectual against *Toxacara canis*. Also shown that selamectin administered topically at a dose of 6 mg kg^{-1} to dogs infected with *Toxacara canis* decreased the faeces egg number by (EPG) 99.7% (Kozen *et al*., 2008). But in dog, any research was not done about pour-on administration of ivermectin. Studies showed that conduction of eight trials in dogs to determine the efficacy of ivermectin and pyrantel pamoat against *Dirofilaria immitis*, *Ancylostoma caninum*, *Uncinaria stenocephala*, *Toxocara canis* and *Toxascaris leonina*. Three studies involved induced infection with *Dirofilaria immitis* and five studies involved induced or natural infection with hookworms and ascarids.

Efficacy of the combined product against *Ancylostoma caninum* was 98.5%. In the intestinal parasite trials each individual component was found not to interfere with the anthelmintic action of the others. Also, declare that pyrantel pamoate was 99.6 and 93.87% effective, respectively against *Ancylostoma caninum*. No side effects were observed (Ashraf *et al*., 2008).

Also, studies showed that Eprinomectin (100 μg kg^{-1}) was given to treatment group dogs orally and eggs per gram were determined in the faeces on the day of pre-treatment and the 2, 4, 6, 8 and 10th days of posttreatment. No side effects associated with nervous, respiratory, gastrointestinal systems and some haemotological parameters were observed. Also, eprinomectin was determined to be 100% effectual against *Toxacara canis* (Kozen *et al*., 2008). In other study, contaminated dogs with *Toxocara canis* that were treated with ivermectin and levamisole hydrochloride, respectively.

The efficacy of the drugs was calculated on the basis of reduction in the number of ova discharged in faeces. Those results showed that ivermectin and levamisole hydrochloride were 97.3 and 97.4% effective, respectively. Levamisole hydrochloride is much cheaper than ivermectin and it was slightly more effective. Also, ivermectin at the rate of 0.2 mg kg^{-1} body weight was 97.3% effective on 18th day (Ahmad *et al*., 2011).

In study of Snyder *et al*. (2011) in evaluation of effect of ivermectin and milbemycin oxime in experimental adult heart worm (*Dirofilaria immitis*) infection of dogs showed that two drugs <100% effective against a recent heart worm field isolate, supporting the hypothesis that the effectiveness of a single dose of those preventives can vary (Snyder *et al*., 2011). Cunningham *et al*. (2006) showed that the covered-rod silicone implant containing ivermectin containing 7.3 mg of ivermectin was 100% effective in preventing experimental infection with *Dirofilaria immitis* larvae and resulted in negative results for heartworm antigen in a field trial. This product has the potential to alleviate poor owner compliance with monthly prevention regimens (Cunningham *et al*., 2006). Obiukwu and Onyali (2006) in the study of comparative efficacy of ancylol, ivermectin, mebendazole and piperazine against *Ancylostoma caninum* in experimentally infected pups showed that ancylol at both normal (1 mg/kg/bw) and elevated dose level

(1.5 mL/kg/bw) showed 93.15 and 93.87% (based on worm count) and 93.13 and 93.75% (based on epg count), respectively. Where as ivermectin at normal dose level (1 mL/50 kg) and elevated dose level (1.5 mL/50 kg) was found to be effective. The resuls were 79.48 and 86.81% based on worm count and 89.44 and 92.50% based on epg count. Mebendazoe and piperazine even at elevated dose level was observed ineffective.

Pups treated at normal and elevated dose level revealed acute toxicosis whereas those treated with Mebendazole showed cough and vomiting tendencies which later subsided and also, there was no risk involved in the adminisration of the drugs (Obiukwu and Onyali, 2006). In other study by Williams *et al*. (1999), on comparison the effect of pour-on administration of ivermectin, doramectin, eprinomectin and moxidectin in cattles, they observed that maximum and minimum effect was with eprinomectin and ivermectin, respectively.

Whang *et al*. (1994) reported which pour-on and injection administration of Moxidectin has positive effect >90% on gastrointestinal nematodes and significant different between these two types of administration were not reported. Skogerboe *et al*. (1999) and Rehbein *et al*. (1999) reported that pour-on administration of ivermectin during rain has antiparasitic effect >90% and rain has not specific effect on reduction the role of ivermectin (Rehbein *et al*., 1999; Rolfe *et al*., 1997; Skogerboe *et al*., 1999).

In fact pour-on administration of ivermectin is very easy for farmers and so far, any specific side effects of ivermectin administration have not been reported (Hooke *et al*., 1997; Reinemeyer and Courtney, 2001). Collectively, ivermectin is very effective drug for control of gastrointestinal parasites and heart worm in animals and its use is very easy and has not need specific tools. Effect of pour-on administration of ivermectin on other helminths and arthropods needs more studies.

CONCLUSION

The effect of ivermectin pour-on against *Dirofilaria immitis* resulted in reduction in microfilariae count exceeded 98% so, this drug can be used in antiparasitic program in dogs. Further investigations are necessary to evaluate the drug effect on other nematodes and parasitic infections.

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