Dose Dependent Effectiveness of Topical Selamectin on Puppies with Ascaridiosis

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SUMMARY

This study evaluated the effects of different doses of Selamectin's topical application on puppies with ascaridiosis. The study involved 12 puppies up to 40-60 days old. Animals were divided into two equal groups; dogs in the group I were treated with topical selamectin at the dose of 6 mg/kg, body weight (bw), the others (Group II) were treated with topical selamectin at the dose of 12 mg/kg, bw. Ascaride eggs were counted before treatment (day 0) and after treatment on the day 7 and 14 using the Mc Master technique. The clinical, hematological, and biochemical findings of day 0, 7, and 14 were also examined. Total red blood cell (RBC) of puppies in group II ($p<0.05$) and hematocrite value in both groups significantly increased on the 14th day compared prior to the treatment ($p<0.01$); leukocyte numbers decreased in the group II on the 7th ($p<0.05$) and 14th ($p<0.01$) days compared prior to the treatment. Differential leukocyte count revealed a significant decrease in the proportion of eosinophil after treatment in both groups on the 14th day ($p<0.01$). Total protein concentrations increased in both groups of treatment regimen on day 7 ($p<0.05$) and 14 ($p<0.01$). But, albumin and globulin concentrations increased only in the group II of treatment regimen on day 14 ($p<0.05$). Egg counts per gram of faeces in both group decreased after treatment but this was more obvious in group II. As a result, it is determined that a considerable improvement was achieved in a shorter time in group treated with 12 mg/kg of selamectin when clinical, haematological, biochemical parameters and egg counts were taken into consideration.

Keywords: *Dog, Ascaridiosis, Selamectin, Effectiveness.*

Askaridiozisli Yavru Köpeklerde Topikal Selamektin Uygulamasının Doza Bağlı Etkinliği

ÖZET

Bu çalışmada, askaridiozisli yavru köpeklerde selamektin'in farklı dozlarda topikal uygulamasının etkinliği araştırıldı. Çalışma 40-60 günlük yaşlarda 12 yavru kopek üzerinde yapıldı. Hayvanlar iki eşit gruba bölündü. Birinci grup köpeklere (grup I) 6 mg/kg/CA dozunda, diğer gruba (grup II) ise 12 mg/kg/CA dozunda topikal selamektin uygulandı. Askarit yumurtaları Mc Master tekniği ile tedavi öncesi (0. gün) ve tedavi sonrası (7. ve 14. gün) sayıldı. Klinik, hematolojik ve biyokimyasal bulgular 0., 7. ve 14 günlerde incelendi. Tedavi öncesiyle karşılaştırıldığında grup II'deki yavru köpeklerde 14. günde eritrosit sayıları ($p<0.05$) ve her iki grupta hematokrit değer ($p<0.01$) önemli derecede arttı. Total lökosit sayıları II. grupta tedavi öncesine göre 7.gün ($p<0.05$) ve 14. günlerde ($p<0.01$) azaldı. Formül lökosit incelendiğinde, her iki grupta tedavi sonrası 14. günde eozinofil oranlarında önemli bir azalma ($p<0.01$) belirlendi. Total protein konsantrasyonları her iki grupta tedavinin 7. ($p<0.05$) ve 14. ($p<0.01$) günlerinde arttı. Fakat, albumin ve globulin konsantrasyonları sadece II. grupta tedavinin 14. gününde arttı ($p<0.05$). Gram dışkıdaki yumurta sayısı tedaviden sonra her iki grupta azaldı. Fakat II.grupta daha çok belirgindi. Sonuç olarak; klinik, hematolojik ve biyokimyasal bulgular ile dışkıdaki yumurta sayısı değerlendirildiğinde, 12 mg/kg selamektinle tedavi edilen grubun (grup II) tedaviye daha kısa sürede cevap verdiği belirlendi.

Anahtar kelimeler: *Köpek, Askaridiozis, Selamektin, Etkinlik*

INTRODUCTION

Ascarids, *Toxocara canis* and *Toxascaris leonine,* are the most common gastro intestinal nematodes in cats and dogs (9, 17). Adult dogs are commonly affected by *T. leonine* and less commonly by *T. canis.* Studies to date report high prevalence of ascaridiosis in both cats and dogs. The figures ranged between 33.6% and 80% and the proportion decreased as animals aged (1, 2, 4). Ascarids are transmitted via oral, intrauterine and lactogen routes among animals. People may contract disease by ingesting contaminated food. Ascarids larvae are either encapsulated in various organs or cause visceral and ocular trauma by migrating (1, 4, 6, 9, 13, 14, 17).

Clinical signs are insidious in adult dogs but puppies may exhibit overt clinical signs as abdominal pain, diarrhoea, moaning, ascites, humbacked posture, abnormal coat, dullness, dehydration and retarded growth. If animals are infested by numerous numbers of ascarids larvae can be excreted in faeces and vomit and may also lead to intestinal obstruction (4, 9, 16, 17). Various products such as piperazine, pyrantel, nitroscanate, febendazole, febantel, praziquantel and ivermectin are used for the treatment of ascaridiosis either orally or parenterally (2, 9, 10, 11). Turgut and Ok (16) reported that because most of the puppies were born with *T. canis* infection, the treatment should start before the first transmission of eggs to the faeces when the puppies are two weeks old and that the treatment should be repeated on every two weeks. Effective treatment of ascaridiosis is of paramount importance due to its zoonotic property (9, 13, 14). It is reported that avermectin have a high therapeutic effect and safety margins (11). Selamectin is a new member of avermectin group and reported to be effective against ascarides. However, dose rate this drug is not well studied especially when applied topically. This study was therefore designed to evaluate the effect of topical selamectin on puppies with ascaridiosis.

MATERIAL and METHOD

The study involved 40-60 days old 12 puppies (9 German shepherd and 3 kangal) admitted to the Clinics of the Faculty of Veterinary Medicine, Yuzuncu Yıl University between August 2001 and June 2003. Animals were separated into two equal groups. Grup I (3 German shepherd and 3 kangal puppies) was applied topical selamectin (Stronghold®, Pfizer) at the dose of 6 mg/kg, bw, and group II (6 German shepherd) topically received selamectin at the dose of 12 mg/kg, bw. All animals were clinically examined, and blood and faecal sampled before treatment and on day 7 and 14 after treatment. Faecal samples from all puppies and their mothers (only 4 German shepherd) were examined for the presence of ascarid eggs. Egg counts per gram of faecal samples were determined using Mc Master technique (4). Blood samples were also taken for haematological and biochemical parameters.

Haematological parameters included, haematocrite (by microhematocrite method), haemoglobin (colorimetrically with spectrophotometer), differential leukocyte counts (Giemsa-stained blood smears), total red blood cell (RBC) and total white blood cell (WBC) (classically via thoma slide). Biochemical parameters (glucose, total protein, albumin, globulin, creatinin, total bilirubin, ALP, ALT, AST, GGT) were measured on an autoanalyser (Roche-Cobas Integra 800) using commercial kits. Sodium (Na), potassium (K), and chloride (Cl) levels were measured in ISE (Medica®, USA) apparatus.

Statistical evaluation of the parameters observed before and after treatment in both groups was carried out using Student t-test on SPSS (10).

RESULTS

Clinical examination revealed arched back posture, growth retardation, dullness, constipation, diarrhoea and dehydration in some puppies on arrival (before treatment). Puppies in group II had great improvement on day 7 and showed no clinical signs on day 14 while animals in group I showed clinical improvements only on day 14.

Haematological findings of puppies with ascaridiosis before and after treatment are presented in table 1. Haematological findings revealed a statistically significant increase in haemoglobin concentration, haematocrite, and total RBC after treatment in the group II on day 14 while only haematocrit value significantly increased in group I on day 14. Total WBC count decreased in both groups but it was statistically significant only in group II after treatment. The proportion of eosinophil showed a considerable decrease in both groups after treatment while other haematological parameters were within normal range.

Biochemical parameters are presented in table 2. There were no statistically significant changes in biochemical parameters examined except for total protein, albumin, and globulin concentrations. Total protein levels significantly increased in both groups after treatment while statistically significant rise was noticed in the concentration of albumin and globulin only in group II on day 14.

Egg count per gram of faeces was over one thousand in both groups before the treatment (table 3). Number of eggs significantly decreased in groups II and I after treatment. However, this decrease was considerable in animals of the group II (table 3).

Table 1. Hematological findings of puppies with ascardiosis.

Parameters	Before treatment		After Treatment			
	Day 0		Day 7		Day 14	
	Group I $\overline{X} \pm SX$ (n=6)	Group II $\overline{X} \pm SX$ (n=6)	Group I $\overline{X} \pm SX$ (n=6)	Group II $\overline{X} \pm SX$ (n=6)	Group I $\overline{X} \pm SX$ (n=6)	Group II $\overline{X} \pm SX$ (n=6)
RBC (10^6 mm^3)	5.1±0.2	5.2±0.1	5.6±0.2	6.1±0.2	6.0±0.3	8.6±0.3*
WBC (10^3 mm^3)	14.9±0.5	12.8±0.3	12.6±0.4	10.2±0.6*	10.3±0.5	9.8±0.3**
Haematocrit (%)	26±1.0	28±1.0	28±1.1	32±1.4	32±1.7**	39±1.5**
Haemoglobin mg/dl)	8.6±0.2	8.9±0.1	9.7±0.4	11.4±0.1	10.2±0.1	13.1±0.3
Neutrophil (%)	59±2.0	54±2.3	60±2.0	56±2.6	60±3.0	59±2.6
Lymhocyte (%)	26±1.2	25±1.0	28±2.3	27±1.6	31±2.1	30±1.6
Eosinophil (%)	12±0.9	16±0.7	10±1.6	13±2.1	6±0.6**	7±1.0**
Monocyte (%)	2±0.1	5±0.1	3±0.2	4±0.1	3±0.1	3±0.1
Basophil (%)	1±0.01	0	0	1±0.02	0	1±0.01

*p<0.05, **p<0.01

Table 2. Biochemical findings of puppies with ascaridiosis.

	Before treatment		After treatment			
	Day 0		Day 7		Day 14	
Parameters	Group I $\overline{X} \pm SX$ (n=6)	Group II $\overline{X} \pm SX$ (n=6)	Group I $\overline{X} \pm SX$ (n=6)	Group II $\overline{X} \pm SX$ (n=6)	Group I $\overline{X} \pm SX$ (n=6)	Group II $\overline{X} \pm SX$ (n=6)
Glucose (mg/dl)	108±4.8	112±4.3	98±1.9	110±2.3	97±3.9	93±3.4
Total protein (g/dl)	4.2±0.1	4.4±0.1	4.5±0.1*	4.9±0.1*	4.9±0.1**	5.3±0.1**
Albumin (g/dl)	2.4±0.1	2.6±0.1	2.5±0.1	2.8±0.1	2.7±0.1	3.0 ±0.1*
Globulin (g/dl)	1.8±0.13	1.8±0.10	2.0±0.12	2.1±0.09	2.2±0.12	2.3±0.12*
Creatinine (mg/dl)	0.3±0.01	0.4±0.01	0.4±0.01	0.5±0.01	0.4±0.02	0.5±0.01
T.Bilirubin mg/dl)	0.26±0.01	0.33±0.1	0.30±0.1	0.32±0.02	0.25±0.01	0.29±0.02
ALP (U/L)	241±18.0	289±13.5	297±16.2	337±11.7	222±15.1	302±20.1
AST (U/L)	25±1.5	19±2.2	22±2.0	23±2.0	21±2.5	28±1.2
ALT (U/L)	24±1.6	32±1.7	24±1.3	29±1.2	27±0.8	24±2.1
GGT (U/L)	2.3±0.4	2.7±0.6	2.6±0.7	3.1±0.7	2.8±0.6	2.6±0.7
Na (mmol/L)	142±1.8	141±0.8	143±1.2	151±2.0	149±1.1	148±1.2
K (mmol/L)	5.6±0.2	4.6±0.1	5.3±0.1	5.4±0.1	5.0±0.2	4.7±0.1
Cl (mmol/L)	114±1.1	116±1.0	115±1.1	117±0.9	112±0.8	114±0.4

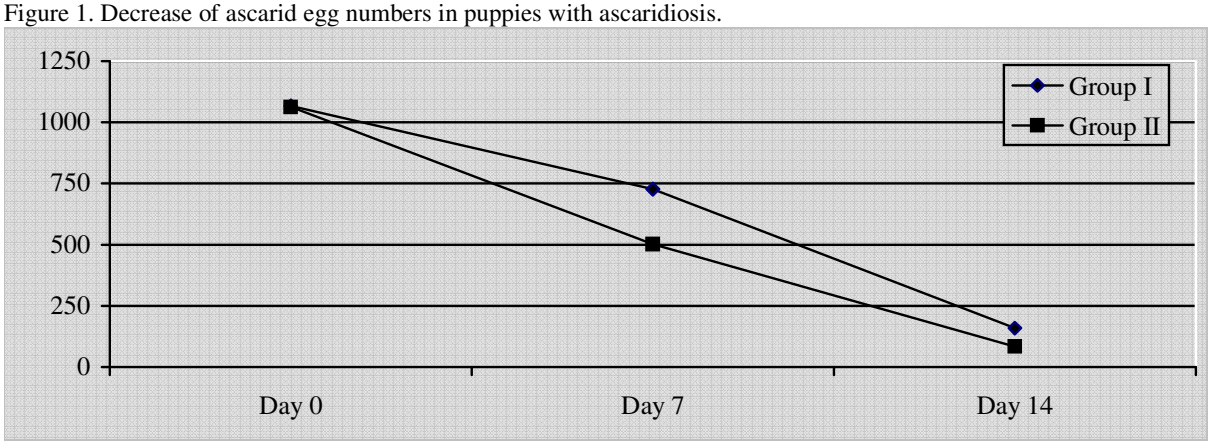
*p<0.05, **p<0.01

Table 3. Ascarid egg numbers of puppies with ascardiosis.

Before treatment		After treatment			
Day 0		Day 7		Day 14	
Group I $\overline{X} \pm SX$ (Range) (n=6)	Group II $\overline{X} \pm SX$ (Range) (n=6)	Group I $\overline{X} \pm SX$ (Range) (n=6)	Group II $\overline{X} \pm SX$ (Range) (n=6)	Group I $\overline{X} \pm SX$ (Range) (n=6)	Group II $\overline{X} \pm SX$ (Range) (n=6)
1067.5±54.18 (850-1170)	1063.3±67.31 (780-1240)	726.6±45.94* (600-850)	501.6±38.59** (420-630)	158.3±15.79** (100-210)	83.3±11.45** (50-120)

*p<0.01, **p<0.001

Figure 1. Decrease of ascarid egg numbers in puppies with ascaridiosis.



DISCUSSION and CONCLUSION

Epidemiological studies indicate high frequency of ascarides in domestic carnivores and that the parasite is an important zoonotic agent (4, 6, 9, 13). Therefore effective and appropriate treatment is important not only for health and welfare of animals but also for the public health.

In this study selamectin was used to treat puppies with ascaridiosis. Clinical findings observed in puppies were in accordance with that reported for toxocariasis (16, 17). The group treated with 12 mg/kg of selamectin had greater improvement in clinical signs as well as in the decrease of egg counts per gram. This was supported by the improvements in haematological as the proportion of eozinophil decreased and total RBC and haematocrit increased to the normal values (Table 1) (8, 15). It is thought that the changes in haematological parameters might be related with appetite and nutrition improvement due to improvement of clinical symptoms in dogs with ascaridiosis after treatment. In this study decrease in total leucocyte numbers (especially group II) after treatment might be related to defence mechanisms against ascarids by the effectiveness of the treatment and/or with the decrease of the eozinophil numbers partially increased

before the treatment. The observed increase in eozinophil levels prior to the treatment is a characteristic of all helminth infections. It is reported that during parasite infections, eozinophils play an important role in immune response (5).

Biochemical parameters examined did not change apart from total protein, albumin and globulin values in this study. This finding is in harmony with the reporting of Sommerfelt et al. (15). But even these changes were within reference values reported for dogs. Decrease in total protein, albumin and globulin before treatment might be attributed to any liver damage as reported by others or alternatively due to malnutrition resulting from heavy infection (Table 2) (3, 6)

Animals in this study were heavily infected, as the number of eggs per gram was greater than 1000 epg (Table 3) (17). However there was a significant decrease as the treatment prolonged and dose increased. This finding adds further credence to the reliability of selamectin as reported previously (Figure 1) (11).

The results of this study suggests that the dose rate of 12mg/kg bw is more reliable and effective on ascarides as faster and significant clinical improvements and significant decrease in egg count in animals treated with the mentioned dose arte was evident.

REFERENCES

1. Ayaz, E., Değer, S., Gül, A., Yüksek, N. (2001): Van kedilerinde helmintlerin yayılışı ve halk sağlığı yönünden önemi. Türkiye Parazitoloji Dergisi, 25 (2): 166-169.

2. Barbabosa, I.M., Tsuji, O.V., Cabello, R.R., Cardenas, E.M.G., Chasin, O.A. (2003): The prevalance of *Toxocara cati* in domestic cats in Mexico City. Veterinary Parasitology, , 114: 43-49.

3. Done, J.T., Richardson, M.D., Gibson, T.E. (1960): Experimental visceral larva migrans in the pig. Res. Vet. Sci., 1, 133-151.

4. Habluetzel, A., Traldi, G., Ruggieri, S., Attili, A.R., Scuppa, P., Marchetti, R., Menghini, G., Esposito, F. (2003): An estimation of *Toxocara canis* prevalence in dogs, environmental egg contamination and risk of human infection in the Marche region of Italy. Veterinary Parasitology, 113**,** 243-252.

5. Halliwell, R.E.W., Gorman, N.T. (1989): Veterinary Clinical Immunology. First Edition, W.B. Saunders Company, Philadelphia.

6. Helwigh, A.B., Lind, P., Nansen, J. (1999): Visceral larva migrans: migratory pattern of *Toxocara canis* in pigs. International Journal for Parasitology, 29, 559-565.

7. Hopkins, T.J., Gyr, P., Hedemann, P.M. (1988): Nematocidal and cestocidal efficacy of a tablet formulation containing febantel, pyrantel embonate and praziquantel in dogs. Vet. Med. Rev., 59, 71-75.

8. Jain, N.C. (1993): Essentials of Veterinary Hematology. Lea & Febiger, Philadelphia., 19-20.

9. McTier, T.L., Siedek, E.M., Clemence, R.B., Wren, J.A., Bowman, D.D., Hellmann,K., Holbert, M.S., Murphy, M.G., Young, D.R., Cruthers, L.R., Smith, D.G., Shanks, D.J., Rowan, T.G., Jernigan, A.D. (2000): Efficacy of selamectin against experimentally induced and naturally acquired ascarid (*Toxocara canis* and *Toxascaris leonina*) infections in dogs. Veterinary Parasitology, 91 (3-4), 333-345.

10. Norusis, J.M. (1986): SPSS/PC. SPSS inc. Chicago,

11. Novotny, M.J., Krautmann, M.J., Ehrhart, J.C., Godin, C.S., Evans, E.I., McCall, J.W., Sun, F., Rowan, T.G. (2000): Safety of selamectin in dogs. Veterinary Parasitology, 91 (3-4), 377-391.

12. Payne, P.A., Ridley, R.K. (1999): Strategic use of ivermectin during pregnancy to control *Toxocara canis* in greyhound puppies. Veterinary Parasitology, 85, 305-312.

13. Robertson, I.D., Thompson, R.C. (2002): Enteric parasitic zoonoses of domesticated dogs and cats. Microbes and Infection, 4, 867-873.

14. Six, R.H., Sture, G.H., Thomas, C.A Clemence, R.G., Benchaoui,H.A., Boy, M.G., Watson, P., Smith, D.G., Jernigen, A.D., Rowan, T.G. (2000): Efficacy and safety of selamectin against gastrointestinal nematodes in cats presented as veterinary patients. Veterinary Parasitology, 91 (3-4): 321-331.

15. Sommerfelt, I.E., Santillan, G., Lopez, C., Ribicich, M., Franco, A.J. (2001): Immunological and hematological response in experimental *Toxocara canis*-infected pigs. Veterinary Parasitology, 96, 127-134.

16. Turgut, K., Ok, M. (2001): Kedi ve Köpek Gastroenterolojisi. Bahçıvanlar Basım Sanayi A.Ş., Konya.; 296-300.

17. Urquart, G.M., Armour, J., Duncan, J.L., Dunn, A.M., Jennings, F.W. (1996): Veterinary Parasitology. 2 nd ed., Blackwell Scientific Publications, Oxford. 53-73