



Detection of *Echinococcus* spp. Coproantigens in Dogs in Van Province Using the ELISA Method

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ABSTRACT

This study was carried out to determine the prevalence of *Echinococcus* spp. in stray dogs in Van province by copro-antigen ELISA. Fecal samples were obtained from 150 dogs. Firstly, it was examined for *Taenia* spp. eggs by zinc sulphate flotation technique. After that presence of *Echinococcus* spp. coproantigens was examined by coproantigen ELISA test using a commercial coproantigen ELISA kit (Combined kit) (COMBINED BIOTECH CO., LTD, Shenzhen, P. R. China). 15.3% of the dogs were infected with *Taenia* spp. The prevalence of *Echinococcus* spp. coproantigen in dogs was 29.3%. In the fecal examination, dogs were also found to be infected with *Toxascaris leonina* (13.3%), *Toxocara canis* (11.3%), *Ancylostoma caninum* (2.6%) and *Trichuris vulpis* (0.6%). This study is the first to investigate of *Echinococcus* spp. in dogs in Van using the coproantigen ELISA technique.

Keywords: Coproantigen ELISA, Dogs, *Echinococcus* spp., Prevalence, Van.

ÖZ

Van İlinde Köpeklerde *Echinococcus* spp. Koproantijenlerinin ELISA Yöntemi ile Belirlenmesi

Bu çalışma, Van ilinde sokak köpeklerinde *Echinococcus* spp.'nin yayılışını koproantijen ELISA yöntemi ile tesbit etmek amacıyla yapılmıştır. Toplam 150 köpek dışkısı önce *Taenia* spp. yumurtaları yönünden çinko sülfat flotasyon metoduyla muayene edilmiştir. Dışkı örneklerinde *Echinococcus* spp.'nin koproantijenlerinin varlığı ticari bir koproantijen ELISA kiti kullanılarak (Combined kit) (COMBINED BIOTECH CO., LTD, Shenzhen, P. R. China) incelenmiştir. Köpeklerin % 15.3'ünün *Taenia* spp. ile enfekte olduğu tespit edildi. Köpeklerde *Echinococcus* spp.'nin koproantijen prevalansı % 29.3 oranında bulundu. Dışkı muayenesinde köpeklerin *Taenia* spp. haricinde *Toxascaris leonina* (% 13.3), *Toxocara canis* (% 11.3), *Ancylostoma caninum* (% 2.6) ve *Trichuris vulpis* (% 0.6) ile de enfekte olduğu görülmüştür. Bu çalışma Van'da köpeklerde *Echinococcus* spp.'nin koproantijen ELISA tekniği ile araştırıldığı ilk çalışmadır.

Anahtar Kelimeler: Koproantijen ELISA, Köpek, *Echinococcus* spp., Prevalans, Van.

INTRODUCTION

Zoonotic parasitic diseases cause a important public health problem in underdeveloped and developing countries. Parasitic diseases such as hydatidosis have been described in Türkiye since 1861 and are among the most important zoonotic diseases. Considering the impact of *Echinococcus* species causing echinococcosis on intermediate hosts, the development of reliable diagnostic methods for detecting the parasite in definitive hosts remains crucial. Besides necropsy, which is the most reliable diagnostic method but ethically problematic in terms of animal rights, various diagnostic techniques are available (Şenlik 2004).

Arecoline purgation, a method used for diagnosing *Echinococcus* species in definitive hosts, has advantages such as the absence of cross-reactions and the ability to

directly observe adult parasites. However, it also has disadvantages, including the risk of environmental contamination with eggs, potential side effects in treated dogs such as tremors, vomiting, and loss of consciousness, and its contraindication in pregnant definitive hosts (Acıöz 2008). In classical flotation or Telemann methods, *Echinococcus* eggs cannot be morphologically distinguished from *Taenia* species under a microscope, making species-specific diagnosis impossible. The presence of specific *Echinococcus* spp. antigens in feces is detected using the coproantigen Enzyme-Linked Immunosorbent Assay (ELISA) method. The purpose of fecal antigen detection is to identify coproantigen presence during both prepatent and patent periods. The main significance of this method lies in enabling early diagnosis before the parasite reaches the stage of egg shedding and

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environmental contamination, thereby allowing timely treatment (Tinar 2006). For this reason, two alternative diagnostic methods are employed: specific coproantigen ELISA and coproDNA detection. The aim of the coproantigen ELISA method is to detect somatic, secretory, or excretory antigens of the parasite. The specificity and sensitivity of this test are considerably high (Şenlik 2004).

In Van province, no data was found on the prevalence of *E. granulosus* in dogs until 2018, and only Orhun and Ayaz (2006) reported that they encountered *Taenia* spp. at a rate of 14.8% in dogs until this date. In 2018, Oğuz et al. (2018) reported an *E. granulosus* prevalence of 4% in Van using the copro-PCR method. No other studies have investigated the prevalence of *Echinococcus* spp. in dogs. This study aims to investigate the prevalence of *Echinococcus* spp. in stray dogs in Van Province using the coproantigen ELISA method.

MATERIAL AND METHODS

This study was conducted within the framework of a research project approved by the Van Yüzüncü Yıl University Animal Experiments Local Ethics Committee under the decision dated 05.11.2015 and numbered 2015/12.

Fecal samples used in the study were gathered from 150 stray dogs in Van Province, comprising 91 males and 59 females. The dogs were categorized into two groups based on age: those younger than one year (<1) and those aged one year or older (≥1). The collected fecal samples were placed in sealed plastic bags, labeled, and transported to the Parasitology Department Laboratory of the Faculty of Veterinary Medicine at Van Yüzüncü Yıl University. To ensure the inactivation of eggs, the samples were stored at -80 °C until further examination.

Fecal samples were first viewed macroscopically for the existence of cestode proglottids. Subsequently, 4-5 grams of each sample were analyzed using the zinc sulfate (ZnSO₄) flotation method (density = 1.50) to detect *Taenia* spp. and other helminth eggs. The remaining samples were then examined to existence of *Echinococcus* spp. using the copro-antigen ELISA method.

To determine *Echinococcus* spp.-specific coproantigens in the fecal samples, a commercial ELISA kit (Combined Kit) (Shenzhen COMBINED BIOTECH CO., LTD, P.R. China) was used. The test procedure was carried out properly the manufacturer's directive.

Statistical Analysis

For statistical analysis, the chi-square test was applied using the SPSS software package to determine the significance of differences between groups. A p-value of <0.05 was considered statistically significant.

RESULTS

Taenia spp. eggs were found in 23 of 150 stools examined (15.3%) (Figure 1). Using the coproantigen ELISA method, 44 dogs (29.3%) were found to be infected with *Echinococcus* spp. (Figure 2).

As shown in Table 1, *Echinococcus* spp. coproantigens were not detected in the fecal samples of 10 dogs in which *Taenia* spp. eggs were identified. Additionally,



Figure 1: *Taenia* spp. egg (Original).

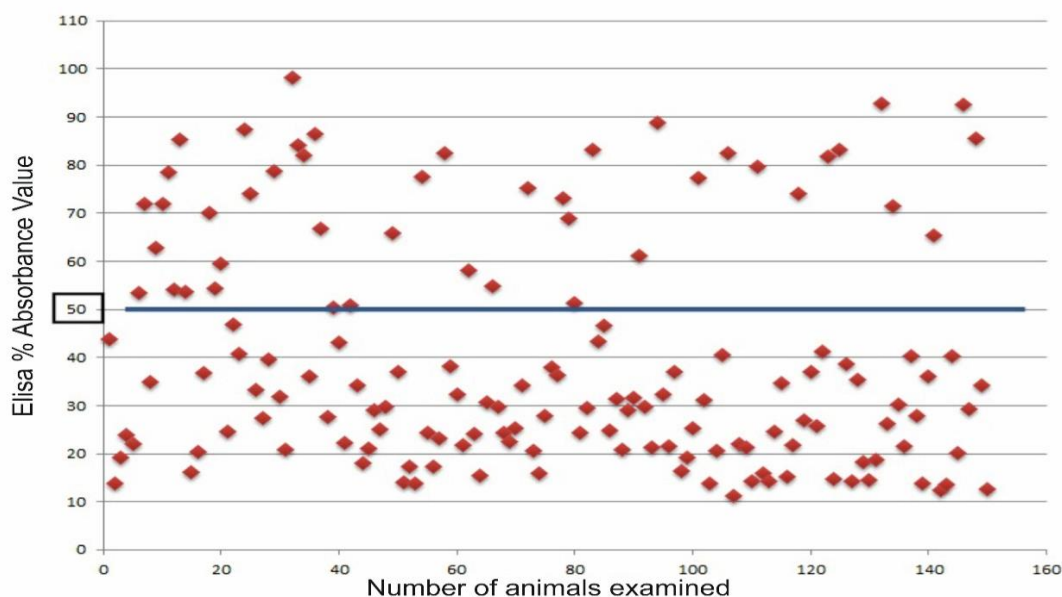


Figure 2: The absorbance values of positive and negative samples in Coproantigen ELISA.

Table 1: Results of fecal examination (*Taenia* spp.) and coproantigen ELISA (*Echinococcus* spp.) tests.

Number of Examined Dogs	150	
	Number	%
Fecal Examination + Coproantigen ELISA +	13	8.6
Fecal Examination + Coproantigen ELISA -	10	6.6
Fecal Examination - Coproantigen ELISA +	31	20.6

Echinococcus spp. coproantigens were detected in the fecal samples of 31 dogs in which *Taenia* spp. eggs were not observed. The distribution of *Echinococcus* spp. according to age and sex, based on the coproantigen ELISA results, is presented in Table 2. A statistically significant difference was found among age groups in *Echinococcus* spp. positive dogs, whereas no significant difference was observed in terms of sex ($p>0.05$). The infection rate was higher in dogs aged one year or older compared to those younger than one year, and this difference was statistically significant ($p<0.05$, $p=0.043$).

Table 2: Distribution of *Echinococcus* spp. infection by age and sex.

Parameters	Number of Dogs Examined	Coproantigen Positive	
		Number	%
* Age Group			
<1	41	7	17.1
≥1	109	37	33.9
**Sex			
Male	91	29	31.9
Female	59	15	25.4

* $p<0.05$, ** $p>0.05$.

In fecal examination, eggs of other helminth species were also found in addition to *Taenia* eggs (Table 3). The distribution of helminth species detected in dog feces according to age and sex, based on fecal examination, is presented in Table 3. *Toxascaris leonina* and *Toxocara canis* infections were more frequently observed in dogs younger than one year compared to those aged one year or older, and this difference was found to be statistically significant ($p<0.05$). Among helminth infections, a statistically significant difference was observed only in *T. canis* infection between male and female dogs ($p<0.05$, $p=0.023$).

Table 3: Distribution of helminth infections by age and sex.

Age	Number of Dogs Examined	Helminth Species				
		<i>Taenia</i> spp.	<i>T. leonina</i>	<i>T. canis</i>	<i>A. caninum</i>	<i>T. vulpis</i>
<1	41	3 (7.3)	^a 10 (24.4)	^b 9 (22.0)	2 (4.8)	-
≥1	109	20 (18.3)	10 (9.2)	8 (7.3)	2 (1.8)	1 (0.9)
Sex						
Male	91	15 (16.5)	13 (14.3)	6 (6.6)	1 (1.1)	1 (1.1)
Female	59	8 (13.6)	7 (11.9)	^c 11 (18.6)	3 (5.1)	-
Total	150	23 (15.3)	20 (13.3)	17 (11.3)	4 (2.7)	1 (0.7)

^a: $p<0.05$ ($p=0.015$), ^b: $p<0.05$ ($p=0.012$), ^c: $p<0.05$ ($p=0.023$).

DISCUSSION AND CONCLUSION

Echinococcus granulosus, which causes larval echinococcosis in many mammalian species, particularly ruminants, is a significant public health concern in numerous countries worldwide (Saygı 1998; Umur 2003).

Different research have been made on the epidemiology of *Taenia* spp. at dogs in various countries, including Turkey, obtaining diverse results. The studies on the prevalence of *Taenia* species in dogs have reported rates ranging from 2.4% to 18% (Fok et al. 2001; Beiromvand et al. 2013). In Türkiye, the prevalence of *Taenia* spp. eggs detected through traditional microscopic examination has been reported as follows: 7.5% in Aydın (Ünlü and Eren 2007), 14.8% in Van (Orhun and Ayaz 2006), 14.2% and 27% in Ankara (Ayçiçek et al. 1998; Öge et al. 2017), 28% in Muş (Acıöz 2008), 0.4% in Samsun (Gürler et al. 2015), 2.9% in Afyon, 23.9% in Eskişehir (Kozan et al. 2007), and 3.8% in

Diyarbakır (İpek and Koçhan 2017). In this study, *Taenia* spp. eggs were detected in 15.3% of dog feces using the flotation technique. This prevalence is higher than that reported in Samsun (Gürler et al. 2015), Afyon (Kozan et al. 2007), Diyarbakır (İpek and Koçhan 2017) and Aydın (Ünlü and Eren 2007) but aligns with previous findings from Ankara (Ayçiçek et al. 1998; Öge et al. 2017), Muş (Acıöz 2008), Eskişehir (Kozan et al. 2007) and Van (Orhun and Ayaz 2006).

Researches about the *E. granulosus* prevalence in dogs across various countries have reported the following infection rates: 56% in Azerbaijan (Chobanov et al. 1991), 38% in Iraq, 14% in Jordan, 15% in Saudi Arabia (Dar and Alkarmi 1997), 36.19% in Iran (Mehrabani et al. 1999) and 0.012% in Cyprus (Dakkak 2010). Regarding *Echinococcus multilocularis* in definitive hosts, studies have reported prevalence rates of 47-56% in foxes in Switzerland (Gottstein et al. 2001), 22.9% in Iran (Zariffard and

Massoud 1998), 37.5% in Poland (Machnicka et al. 2003) and 21% in Canada (Kotwa et al. 2019). In dogs, infection rates have been reported as 0.24% to 1.3% in Germany (Dyachenkoa et al. 2008; Mueller and Partridge 1974), 0.3% in Switzerland (Deplazes et al. 1999), 1.07% in Japan (Morishima et al. 2006), 1.5% in Poland (Karamon et al. 2019), 0.8% in Lithuania (Bruzinskaite et al. 2009), 0.8% in France (Umhang et al. 2014) and 2.8% in Slovakia (Antolová et al. 2009).

In Türkiye, studies about *E. granulosus* prevalence in dogs have utilized necropsy between 1963 and 1998, while after 2007, serological or molecular methods have been employed to detect the parasite's antigen or DNA in dog feces. The 29.3% prevalence of *Echinococcus* spp. detected in stray dogs in this study is lower than that reported in Ankara (44% by Doğanay 1983; 54.5% by Zeybek et al. 1992) and Kars (40.5% by Umur and Arslan 1998). However, it is higher than findings from İzmir (5.5% by Üner 1989), Elazığ (3.33% by Taşan 1984), Muş (9% by Acıöz 2008), Antakya (8.86% by Güzel et al. 2008), İstanbul (0.8% by Öter et al. 2011), Van (4% by Oğuz et al. 2018) and Ankara (0.94% by Ayçiçek et al. 1998). The results are comparable to those reported in Bursa (36% by Tınar et al. 1989), Kayseri (24% by Şahin et al. 1993), Adana (24.72% by Demirkazık et al. 2007), Konya (28.33% by Aydenizöz 1997), Sivas (28% by Ataş et al. 1997) and İstanbul (22.72% by Merdivenci 1963).

Prevalence variations may be attributed to factors such as whether the dogs are stray or owned, proximity to slaughterhouses, likelihood of contact with infected organs, density of intermediate hosts, and socio-economic and cultural characteristics of the region. Additionally, differences in sample size and detection methods used in studies may contribute to these variations. The high *Echinococcus* spp. prevalence detected in this study is likely related to the fact that all sampled dogs were stray and many were found near slaughterhouses.

In this study, when the prevalence of *Echinococcus* spp. was evaluated between male and female dogs, no significant difference was observed, similar to the findings of Lopera et al (2003), Güzel et al (2008), Seres and Cosma (2008), Seres et al (2010) and Karamon et al (2019). Moro et al (2005) reported that female dogs were more infected than male dogs. When we evaluated in terms of age groups, *Echinococcus* spp. infection was found more frequently in dogs one year old and over one year old in this study and this was found to be statistically significant ($p < 0.05$). In parallel with our findings, Seres and Cosma (2008), Seres et al. (2010) and Karamon et al. (2019) also reported that they encountered more infections in older animals than in younger ones. Lopera et al. (2003) and Öge et al. (2017) detected that there was no important variation between age groups and infection prevalence.

Among the 31 fecal examples in that no *Taeniid*-type eggs were determined, *Echinococcus* spp. coproantigens were identified. This may be explained by factors such as the prepatent period of infection, irregular egg shedding, or the small size of eggs and segments, which may lead to underestimation due to the low sensitivity of fecal examination methods (Deplazes 2006; Şenlik 2013). Boğa (2012), detected *E. granulosus* DNA in a fecal sample that tested negative for *Taeniid*-type eggs through multiple microscopic examinations, demonstrating that the risk of *E. granulosus* infection persists even in the absence of visible eggs or segments. Similarly, Güzel et al. (2008) reported that they found *Taenia* spp. eggs in 7 of 79 stool samples, but only one of them was positive with

coproantigen ELISA and that they did not find *Taenia* spp. eggs in any of the six coproantigen-positive stools. Zare-Bidaki et al. (2009) reported that only three out of 27 coproantigen-positive canine samples contained *Taenia* spp. eggs, emphasizing that coproantigen presence reflects active intestinal infection with adult parasites, making coproantigen ELISA one of the most effective immunological methods for diagnosing *Echinococcus* infections.

In the study, 106 dog fecal examples were detected to be negative for *Echinococcus* spp. coproantigen. However, it was determined that the feces of 10 of these coproantigen-negative dogs were infected with *Taenia* spp. eggs. Since no confirmation in this study was conducted using necropsy, purgation, or PCR, and the parasite burden was not determined, making comparisons is neither appropriate nor simple. Deplazes et al. (1999) reported that the sensitivity of the coproantigen ELISA test increases in direct proportion to the parasite burden, whereas Sivashi and Motamedi (2006) stated in their study that there was no important variation between high and low infection intensity category.

In recent years, numerous studies have investigated *E. granulosus* prevalence in various countries using coproantigen ELISA. In Peru, *E. granulosus* was detected at a rate of 82% using the coproantigen ELISA method and 34% using the arecoline purgation method (Lopera et al. 2003). In Tunisia, the prevalence was reported as 89.2% by necropsy, 43–76.9% by arecoline purgation, and 82.8% by coproantigen ELISA (Lahmar et al. 2007). In Libya, necropsy studies reported a prevalence of 25.8%, whereas coproantigen ELISA detected a prevalence of 21.6% (Buishi et al. 2005). *Echinococcus granulosus* was detected at a rate of 19.2% in Romania (Seres et al. 2010), 21.6% (Zare-Bidaki et al. 2009) in Iran and 1.1–4.9% (Christofi et al. 2002) in Cyprus using the coproantigen ELISA method. In Türkiye, the presence of *E. granulosus* antigen in dog feces was reported at rates of 8.86% in Antakya (Güzel et al. 2008), 15% in İzmir (Yolaşmaz et al. 2001), and 24.72% in Adana (Demirkazık et al. 2007). In the present study, *Echinococcus* spp. infection was detected in 29.3% of stray dogs in Van using coproantigen ELISA.

In numerous studies, *Echinococcus* spp. coproantigens in dogs have been investigated using either commercial ELISA kits or non-commercial "in-house ELISA" tests, with varying specificity and sensitivity values reported for these assays. Some of the commercial kits used in these studies, such as Chekit Bommeli (Switzerland) and Genzyme Virotech GmbH (Germany), are no longer in production and therefore unavailable. Currently available kits, including the "Combined kit" (Shenzhen Combined Biotech Co. Ltd, Guangdong), the "Haitai kit" (Zhuhai Special Economic Zone Haitai Biological Pharmaceuticals Co. Ltd, Guangdong), and the "Tiankang kit" (Xinjiang Tiankang Animal Husbandry Biotech Co. Ltd, Xinjiang), are of Chinese origin and exhibit variable sensitivity and specificity. Different studies have reported specificity rates of 80–98% and sensitivity rates of 62–83% for the Chekit Bommeli coproantigen ELISA kit (Deplazes et al. 1994; Deplazes et al. 1995; Zariffard et al. 1999; El-Shehabi et al. 2000). Huang et al. (2014) compared the performance of three different coproantigen ELISA kits with necropsy results in both naturally and experimentally infected dogs with *E. granulosus*. Their study reported that the Tiankang kit detected 84% of experimental infections and 60% of natural infections, with a false-positive rate of 7%. The Haitai kit detected 10% of experimental infections and 60% of natural infections, with a false-positive rate of

20%. The Combined kit detected 53% of experimental infections and 100% of natural infections, with a false-positive rate of 33%. It has been reported that the type of antibody used in the tests (Huang et al. 2014) and the composition of the fecal extract solution (Benito et al. 2006) may influence test results. Therefore, further studies are needed to evaluate the specificity, sensitivity, and reproducibility of ELISA tests.

In conclusion, in Van, 15.3% of stray dogs were found to be infected with *Taenia* spp. based on zinc sulfate flotation, while 29.3% were positive for *Echinococcus* spp. based on coproantigen ELISA. In 70.45% of coproantigen-positive samples, *Taenia* spp. eggs were not detected, reinforcing the potential public health risk posed by dog feces. Therefore, periodic anthelmintic treatments should be administered, considering the dietary habits of dogs. Additionally, infected intermediate host organs should be properly disposed of, and public awareness regarding *Echinococcus* infections in both intermediate and definitive hosts should be increased. While the copro-antigen ELISA technique utilize in this research has advantages such as ease of use, applicability to large sample numbers, and early detection of prepatent infections, its specificity and sensitivity should be further optimized to reduce cross-reactions.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

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AUTHOR CONTRIBUTIONS

Idea / Concept: PKV, NÖ
Supervision / Consultancy: NÖ
Data Collection and / or Processing: PKV
Analysis and / or Interpretation: PKV, NÖ, BO
Writing the Article: PKV, NÖ
Critical Review: NÖ, BO

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