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Toxocara spp, Ancylostoma caninum and *Trichuris* spp. Mono- and Mixed Infections in Diarrheic Dogs

Adnan Ayan[1]*, Kerem Ural[2], Hasan Erdogan[2], Zeliha Selamoglu[3], Songul Erdogan[2], Pelin Kandemir[1], and Deniz Sude Ates[2]

[1]Department of Genetics, Faculty of Veterinary Medicine, Van Yuzuncu Yil University, Van, 65080, Turkey

[2]Department of Internal Medicine, Faculty of Veterinary Medicine, Adnan Menderes University, Aydın, 09016, Turkey

[3]Department of Biotechnology, Omer Halisdemir University, Nigde, 07058, Turkey

Research Article

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***For Correspondence**

Adnan Ayan, Department of Genetics, Faculty of Veterinary Medicine, Van Yuzuncu Yil University, Van, Turkey.

Tel: +90 4322251128/21549

E-mail: adnanayan@yyu.edu.tr

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ABSTRACT

In this study was firstly aimed to compare the relationship between rutine conventional methods and rapid commercial test (Uranovet test), secondly investigate the shallow prevalence of parasitic etiologies in dogs with diarrhea. For this purpose total of 24 fecal samples taken from dogs by rectal route. Samples were investigated by rutine methods and rapid Uranovet test. Six of twenty four dogs had parasitological etiology with Urano test, whereas 10 of 24 animals had parasites in routine conventional methods. In conclusion, we can promptly say Urano test might be a useful rapid indicator for small animal practitioner for rapidly detecting parasitic agents in diarrheic dogs.

INTRODUCTION

Ancylostoma caninum is an important parasite of all canidae, especially dogs, and is also seen in humans, albeit rarely. Male parasites have a length of 11-13 mm and a width of 0.34-0.39 mm, and females are 14-20.5 mm long and 0.50-0.56 mm wide [1,2]. These parasites are gray or reddish in color depending on the absence or presence of blood in the digestive system and have a very strong structure. One of these parasites is *Toxocara canis* that lives in the small intestine and less often in the large intestine and stomach primarily of dogs, as well as of other flesh-eating animals, such as foxes, wolves, and jackals. In dogs, infection can occur in four different ways; directly, prenatally, galactogenically, and through a paratenic host [3]. Another parasite is *Trichuris vulpis*, a nematode belonging to the family of Trichuridae, which causes the following clinical signs in dogs; diarrhea (usually chronic and contains bloody mucus), abdominal pain, pica, dehydration, weight loss, dry flaky coat, and depression [4].

In addition to causing diseases in dogs, parasitic agents, such as *Toxocara* spp., *Ancylostoma* spp., and *Trichuris* spp. have zoonotic significance for humans. These etiologic agents can lead to the development of cutaneous, visceral and ocular larva migrans and play an important role in the emergence of disease states resulting in eosinophilic enteritis [5,6]. Gaunt and Carr [7]. suggested that considering the zoonotic potentials of these diseases, the primary precaution to be taken in the veterinary field is to determine their prevalence, and the authors underlined the importance of veterinarians' paying special attention to this issue. For this purpose, fecal samples collected from dogs with the complaint of diarrhea and brought to the clinic should be evaluated in terms of clinical parasitology as part of basic laboratory examination [8]. These evaluations can be performed using direct fecal smear microscopy, as well as traditional methods, such as sedimentation and flotation [9-11]. In the latter method, especially in the detection of helminth eggs, it is necessary to use solutions with a higher specific weight than that of parasite eggs; e.g.,

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zinc sulfate, sodium chloride, and sodium nitrate. These solutions can be easily and efficiently prepared and have the advantage of being cost-effective [11]. However, in almost all these methods, the requirement of certain laboratory procedures, such as centrifugation negatively affects the speed of clinical diagnosis.

Innovations that have emerged in rapid diagnostic tests in recent years have led to the modification of procedures on the preparation of solutions in the laboratory to reduce diagnostic time. Especially in the parasitological evaluation of feces, applications that eliminate the requirement of centrifugation are considered to facilitate and accelerate the diagnostic process for veterinarians and have therefore been commonly adopted in this field [12].

One of these applications is the use of the Uranotest Copro method designed to speed up microscopic examination under field conditions. This study aimed to evaluate the reliability of this method in the parasitological examination of fecal samples compared to the results of traditional methods. Therefore, we analyzed parasitic distribution in diarrheic dogs.

MATERIAL AND METHODS

This study was carried out on 24 diarrheic dogs brought to Adnan Menderes University, Faculty of Veterinary Medicine, Department of Internal Medicine located in Aydın, Turkey. The fecal samples were directly collected from the rectum, placed in containers, and numbered. The age, breed and sex of each dog were recorded in the protocol book. Routine examination was undertaken using the Fülleborn flotation and modified Benedek sedimentation methods. The samples were first macroscopically examined for cestode rings. For Fülleborn's flotation, 3-5 g of fecal samples were placed in a container, to which a small amount of saturated saline was added, and the mixture was stirred and crushed with a stick. The resulting suspension was filtered into another container, which was filled with saline again. Two coverslips were placed on the liquid, parallel to the surface. After 15-20 minutes, the coverslips were lifted using forceps without immersing them in the liquid and placed on a clean slide. This procedure was repeated for all samples. The samples were then examined under a microscope. For the modified Benedek sedimentation method, approximately 3 g of fecal sample was placed in a container, followed by the addition of a small amount of tap water. After the feces had been thoroughly crushed by a stick, more water was added to make it homogeneous. The resulting suspension was filtered into a petri dish using a tea strainer and tap water was added. After leaving it to settle for 15-20 minutes, the top liquid layer was removed without disturbing the sediment on the bottom of the dish. Water was added and the same procedure was repeated until the top liquid layer was completely clear. Then, the sediment collected at the bottom was examined under a microscope for all samples [13-16]. **Figure 1A-1E** presents images related to this procedure.

For the application of Uranotest Copro, the test protocol specified by the manufacturer was followed: After opening the bottle, two measures of fecal sample were placed into the bottle using a plastic scoop; then, the bottle was tightly closed. Before shaking, the sealing cap was removed, and the gas was released by applying a light pressure. Then, the bottle was closed again and shaken until a homogeneous mixture was obtained. The bottle was opened and placed in the hole designed for this purpose with the open end facing downward. After settling for 15 minutes, the bottle was removed and the first drop of the sample was discarded by gently pressing the bottle. Then, 1-2 drops of the sample were taken and placed on a slide covered with a coverslip to be examined under a microscope. **Figures 2 and 3** present the researchers and application procedure for Uranotest Copro.

Figure 1: (A and B) During flotation applications, (C-E) During Sedimentation applications

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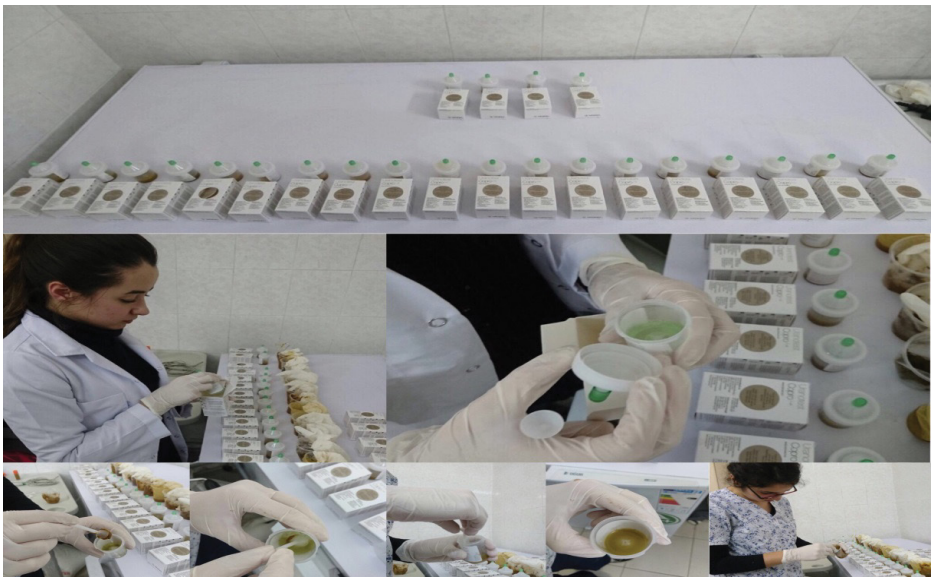

Figure 2: Researchers during the application of uronotest and application procedure.

Figure 3: Researchers during the application of uronotest and application procedure.

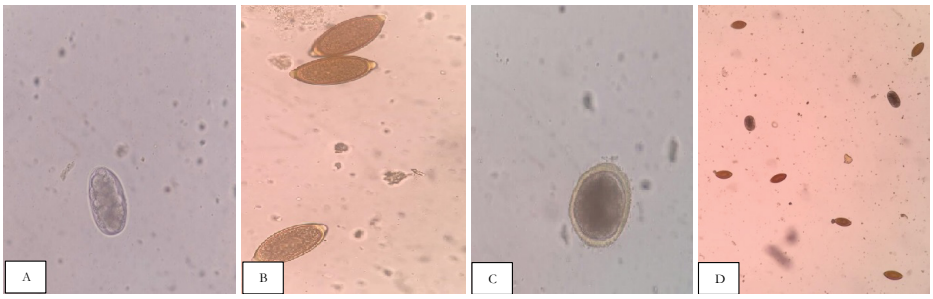

Figure 4: A: *Ancylostoma caninum*, B: *Trichuris* spp, C: *Toxocara* spp, D: *Ancylostoma caninum*, *Trichuris* spp

Table 1. Demographic information according to the cases (positive ones) and distribution according to the parasitic factors in which they are infested.

	Positive		
Method	**Flotation**	**Sedimentation**	**Urano Test**
Preparate 1	*A. caninum Trichuris* spp.	-	*A. caninum Toxocara* spp. *Trichuris* spp.
Preparate 3	*A. caninum*	-	-
Preparate 6	*Toxocara* spp.	-	*Toxocara* spp.
Preparate 7	*Toxocara* spp.	-	
Preparate 8	*Toxocara* spp.	-	*Toxocara* spp.
Preparate 12	*Toxocara* spp.	-	*Toxocara* spp.
Preparate 13	*Toxocara* spp.	-	*Toxocara* spp.

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Preparate 16	*A. caninum*	-	-
Preparate 19	*Trichuris* spp.	-	-
Preparate 23	*A. caninum*	-	*A. caninum*

RESULTS

The microscopic fecal examination using flotation method revealed one or more parasitological agents in 10 of the 24 diarrheic dogs while Uranotest identified the presence of these agents in the six of these 10 dogs which diagnosed by using flotation method. The striking finding was that case 1 had a co-infection caused by two different parasitological agents according to the flotation method whereas three different parasitological agents were identified by Uranotest for the same case (*A. caninum, Toxocara* spp*, Trichuris* spp.) **(Table 1)**. **Figure 4A-4D** shows all the parasite eggs that were identified. A. caninum eggs were detected in four cases, *Toxocara* spp eggs in six cases, and *Trichuris* eggs in two cases. **Table 1** shows the distribution of parasitological agents by case.

DISCUSSION

The prevalence of T. canis was found to be 36.5% in China, 4.4% in Japan, 13.3% in Mexico, 41.1% in Nigeria, and 11.4% in Venezuela [17-21]. In studies conducted in Turkey, the prevalence of this parasite was reported to be 13.2% in Ankara, 35.7% in Kars, and 13.9% in Van [22-24]. A study on dogs undertaken Japan, Hungary, Greece, Dutch, Brazil, Venezuela, Nigeria showed that the rate of *Trichuris* ranged from 0.39 to 23.3% [17,20,25-29]. In Turkey, *Trichuris* spp. were found at a rate of 6.61% in Elmadağ district of Ankara and 18% in Bursa [30,31]. *Ancylostoma caninum* was detected at 65.6% in Nigeria [18]. In Venezuela, *Ancylostoma* spp. were identified in 24.5% of the investigated cases [20]. In Turkey, the rates for *A. caninum* were reported to be 8.7% for Van and 0.8% for Konya [22,32]. Fok et al. [25] conducted a study in animal shelters in the east and north of Budapest, but instead of the saturated saline we used, the authors utilized saturated MgS04 + saturated Na2S203 solutions for the flotation method. They identified parasitic eggs belonging to *Toxocara* canis (24.3-30.1%), *Trichuris vulpis* (20.4-23.3%), and Ancylostomatidae (8.1-13.1%) [23,24,26-29].

This study aimed to (a) identify the distribution of nematode infections in diarrheic dogs in the Aydın region, Turkey, (b) underline the importance of considering nematode infections in the etiology of cases encountered by independent clinicians, and (c) demonstrate the benefits of combining the native examination, sedimentation, floatation and Uranotest Copro methods for diagnostic evaluation. According to the comparative results, the flotation method showed the presence of one or more parasitological agents in 10 of the 24 diarrheic dogs. When the examination was enriched with Uranotest, six of these 10 dogs were found to have parasitological agents. A noteworthy finding was that although the flotation method revealed co-infection caused by two different parasitological agents, Uranotest was able to identify three agents for the same case (*A. caninum, Toxocara* spp*, Trichuris* spp.). *A. caninum* eggs were detected in four cases, *Toxocara* spp. eggs in six cases, and *Trichuris* eggs in two cases.

Hookworm dermatitis is a relatively uncommon cutaneous disease due to the larval migration of *Ancylostoma caninum*, *Ancylostoma braziliense*, and *Uncinaria stenocephala* [8,33,34]. This disease is assumed to be associated with poor hygiene conditions, which increases the number of infected larvae, and it occurs as a hypersensitivity reaction related to dermatitis larvae migration [35]. Third-stage larvae penetrate into the skin upon contact [8,33,35]. Clinical indications associated with hookworm dermatitis include erythematous papules, erythema, swelling, alopecia, and lichenification. Typical clinical changes occur on the soles of the feet after natural contact with the ground due to the texture of the margin of the sole being soft and separated from the epidermal layer. Distal extremities and other related anatomical structures are often in contact with soil [8,33,35]. The detection of *A. caninum* eggs in four diarrheic dogs in the current study indicates the need to consider this parasitic agent in the diagnosis and treatment of dogs with this symptom.

CONCLUSION

In conclusion, the different parasitic agents identified by Uranotest and traditional methods, such as flotation indicate that modifying the protocols in the diagnosis and treatment of diarrheic dogs may provide benefits. It is possible to achieve a diagnosis based on the results of the flotation method or by enriching this method with Uranotest.

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