Investigation of Antigenic Specificity against *Cysticercus tenuicollis* Cyst Fluid Antigen in Dogs Experimentally Infected with *Taenia hydatigena*

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Abstract: Antigenic specificity was investigated in dogs experimentally infected with *Taenia hydatigena* using SDS-PAGE and Western blotting. *Cysticercus tenuicollis* cysts from sheep were given to the puppies in the experimental group. Experimental infection was achieved in the animals of the experimental group at 11-13 weeks, and infected animals were necropsied and mature *Taenia hydatigena* worms were collected. Antigen was prepared from the fluids of *C. tenuicollis*. Sera from both experimental and control groups were tested by Western blotting. According to the results of this study performed with fluid antigen from *C. tenuicollis*, the bands of 38 and 42.5 kDa are concluded to be the specific proteins for diagnosis of *T. hydatigena* in dogs.

Key Words: Taenia hydatigena, dog, Western blotting

Taenia hydatigena ile Deneysel Enfekte Köpeklerde Cysticercus tenuicollis Kist Sıvısı Antijenine Karşı Antijenik Spesifitenin Araştırılması

Özet: Deneysel olarak *Taenia hydatigena* ile enfekte köpeklerde antijenik spesifite SDS-PAGE ve Western blot kullanılarak araştırılmıştır. Koyunlardan toplanan *C. tenuicollis* kistleri deneysel gruptaki köpek yavrularına yedirilmiştir. Deneysel gruptaki hayvanlarda 11-13. haftalar arasında deneysel enfeksiyon başarıya ulaşmış ve bu hayvanlara nekropsi yapılarak ergin *T. hydatigena*'lar elde edilmiştir. Antijen, *C. tenuicollis* kist sıvısından hazırlanmıştır. Deneysel ve kontrol gruplarından elde edilen serumlar Western blot yöntemiyle test edilmişlerdir. Sonuçlara göre; *C. tenuicollis* kist sıvısının antijen olarak kullanıldığı bu çalışmada, *T. hydatigena*'nın teşhisinde kullanılabilecek spesifik bantların 38 ve 42,5 kDa ağırlığındaki bantlar olduğu belirlenmiştir.

Anahtar Sözcükler: Taenia hydatigena, köpek, Western blot

Introduction

Taenia hydatigena is typically found in the small intestine of dogs, wolves and other wild carnivores. The intermediate hosts are domestic and wild ruminants particularly sheep. Normally, infection with *T. hydatigena* is not very pathogenic in dogs. However, its larvae, *Cysticercus tenuicollis*, migrate through the liver tissue and cause hemorrhagic and fibrotic tracts known as hepatitis cysticercosa. The prevalence of *T. hydatigena* in Turkey were reported as 15.2% and 32 % from necropsied stray dogs (1,2).

The eggs of *T. hydatigena*, *T. ovis*, *T. serialis*, *T. pisiformis*, and *Echinococcus granulosus* are

indistinguishable, and so fecal examination is of limited value in the determination of which species a dog is infected with. Proglottids of Taenia spp. are passed in the feces and these can be identified in some instances. To improve the diagnostic sensitivity of fecal examinations for *E. granulosus* in control programs, Arecolin purging of dogs with subsequent intensive fecal examination has been widely used. However, the accuracy of this technique is variable, and only about 50% of the infected dogs can be identified (3-5). Dogs artificially infected with *T. hydatigena* excreted highly variable numbers of proglottids per day (0-55) during patency; only 36% were eliminated with feces but 64% without defecation (6). In the same study, for the detection of Taenia eggs,

anal-skin swabs proved to be more reliable than examination of fecal samples using a flotation technique (6). A serological test would be most useful in campaigns to eradicate cestodes because it could specifically determine the type of parasites are present, and the status of infection, that is existing or past. Most previous studies on the immune response of dogs to cestode infections have been concerned with immunization against infection (7). Specific antibodies are detectable in sera of dogs infected experimentally or naturally with Taenia spp. or *E. granulosus* (5,8-11).

There has been no report about determining immunospecific proteins for *Cysticercus tenuicollis* antigens in dogs infected experimentally and monospecifically with *T. hydatigena* tested by Western blotting. Determining specific protein bands for *T. hydatigena* infections in dogs by SDS-PAGE and Western blotting may have importance in the future vaccination and diagnostic kit studies for this cestode. This was the first study of its kind. Thus the aim of the present work was to determine specific protein bands for *T. hydatigena* from the sera of dogs.

Materials and Methods

Animals

The animals used in this study were kept in the experimental animal unit of Faculty of Veterinary Medicine at Ankara University from April to October, 2000, and treated humanely. Nine 2- to 3-month-old puppies were examined for intestinal parasites by routine coprological procedures. All puppies were treated with ivermectin (0.2 mg/kg). The following day, a commercial anthelmintic containing preziquantel (50 mg), pyrantel pamoate (140 mg), and oxantel pamoate (545 mg) was administered to all puppies, and they were vaccinated (Biocor Animal Health Inc.) against viral infections. Three weeks after the treatment, 6 of the 9 puppies were infected with metacestodes of *T. hydatigena* as previously described (6). Each dog except one received 5 metacestodes freshly isolated from slaughtered sheep. The remaining 3 puppies were kept as controls. For the infected puppies the prepatent period was determined by daily examination for proglottid and egg excretion. Blood samples were collected during the study, and after separation from the clot by centrifugation the sera were stored at -20 °C until required for testing.

Antigen

Cysticercus tenuicollis cysts were washed thoroughly in distilled water. The fluid, scolices, and membranes were aseptically collected by puncturing the cysts. The fluid was centrifuged at 13.000 rpm for 30 minutes at 4 °C. The supernatant was filtered through a 0.45 μ m membrane filter. Filtered solution was dialyzed against distilled water at 4 °C for 24 hours, and stored as antigen at -70 °C until it was used.

Western blotting

The antigen was separated by SDS-PAGE. Proteins were visualized with silver stain technique and their molecular weights were determined by comparing with molecular weight standards (Sigma wide molecular weight range- M-4038, Sigma Chemical Co., USA). To determine the most appropriate amount of antigen, a gel (5% stacking + 15% separating) was prepared. The gel was loaded with 10, 15, 20, 25, 30, and 35 μ l of antigen and 30 μ l of antigen was found to be the best amount for this study. SDS-PAGE, Western blotting and preparation of solutions were performed as described by Sambrook et al. (12).

Antigenically active components among SDS-PAGE resolved bands were detected by Western blotting. After proteins were transferred SDS-PAGE. the electrophoretically onto a nitrocellulose sheet using transfer blot apparatus. Nitrocellulose membranes containing transferred samples was incubated overnight at 4 °C in 3% nonfat dried milk, and then rinsed in PBS before 2 hours of incubation with sera containing test antibodies. Following 3 PBS washes to remove unbound antibody, nitrocellulose sheets were incubated for 1 hour in horseradish peroxidase conjugated anti-IgG (A-8917, Sigma Chemical Co., USA). Unbound conjugate was removed by 3 PBS washes before addition of substrate solution containing DAB (3,3'- Diaminobenzidine, D-4293, Sigma Chemical Co., USA). Bands were visible within 15 minutes and development was stopped by removing substrate with distilled water and air drying the nitrocellulose.

Results

Infections

According to fecal examination results before treatment with anthelmintics and experimental infection,

Toxocara canis eggs were seen in all puppies except number 3 (Table 1). After treating animals with anthelmintics, no parasite eggs were present. Data showing parasites recovered at necropsy from the experimentally infected dogs after infection are shown in Table 2. Parasites were recovered from all puppies in the experimental group. Details of numbers, lengths and wideness of *T. hydatigena* parasites are also shown in Table 2.

Western blotting analysis of the antigen

A large number of protein components were apparent in *Cysticercus tenuicollis cyst fluid* antigen when analyzed by SDS-PAGE. This antigen was immunoblotted by using sera from both experimental and control groups. While the bands of 116, 66, 42.5, 38, and 26 kDa were observed in the nitrocellulose membranes in the sera of infected group (Figure 1), only the bands of 116, 66, and 26 kDa were observed in the sera of the control group (Figure 2). A component of 42.5 kDa was observed to immunoreact with all of the sera from *T. hydatigena* infected dogs and none of the sera from cestode-free dogs. The band of 38 kDa was only seen in the sera of 4 of the 6 infected animals and in none of the control animals. The 38 and 42.5 kDa bands were determined to be immunoreactive bands.

Discussion

Infection with *T. hydatigena* is not very harmful to dogs. However, its larvae, *C. tenuicollis*, migrate through the liver tissue and cause hemorrhagic and fibrotic tracts known as Hepatitis cysticercosa. Various prepatent periods for *T. hydatigena* were given by different authors. The prepatent period for this parasite was reported as 51 days (4) or 56 days (13,14). Neveu-Lemarie (15) and Güralp (16) reported this period as 8-12 and 10-12 weeks, respectively. In addition, in our study, the prepatent period was 11 to 13 weeks. *Taenia hydatigena* proglottids 7-8 cm in lengths were seen in the feces of the dogs. The prepatent period of *T. hydatigena* seem to be longer when it was compared to other studies. It was thought that delays in the development of

Table 1. Fecal examination results from the groups of puppies

Groups	Dog no. and sex	Parasite Species			
		Sedimentation	Salty water flotation	Teleman	
	1 (female)	-	_	-	
Experimental	2 (female)	-	Toxocara canis	T. canis	
	3 (female)	-	Isospora canis	-	
	4 (female)	-	-	-	
	5 (female)	-	T. canis	T. canis	
	6 (male)	-	T. canis	T. canis	
Control	7 (male)	-	T. canis	T. canis	
	8 (male)	-	T. canis	T. canis	
	9 (male)	-	T. canis	T. canis	

Table 2. The necropsy results of dogs in the experimental group

Dog no.	Prepatent period (weeks)	The number of given <i>C. tenuicollis</i>	The number of obtained <i>T. hydatigena</i>	Average lengths of <i>T. hydatigena</i> (cm)	Average widths of <i>T. hydatigena</i> (mm)
1	12	5	4	161.25	6.75
2	13	5	4	163.75	6.25
З	12	5	4	141.25	5.75
4	13	5	4	132.50	5.25
5	11	7	4	108.25	4.75
6	12	5	3	191.66	7.33



Figure 1. Western blotting of *C. tenuicollis* cyst fluid antigen using sera from the experimental group.

parasites could be the due to the age of the dogs, and possibility to the existence of different strains of *T. hydatigena* in the Ankara region or throughout Turkey. Although 5 *C. tenuicollis* cysts were given to each dog except number 5, which was given 7 *C. tenuicollis* cysts, only 4 adult *T. hydatigena* developed in the intestines of the dogs except number 6, in which only 3 adult cestodes developed. This could be explained by the youthfulness of the dogs, whose intestines had not much capacity for growth of cestodes in large numbers.

Very few serological studies have examined T. *hydatigena* and its larvae *C. tenuicollis* in dogs. Those studies were mostly ELISA studies and were carried out by Heath et al. (3), Jenkins and Rickard (10), and Deplazes et al. (17). On the other hand, some studies



Figure 2. Western blotting of *C. tenuicollis* cyst fluid antigen using sera from the control group.

were performed in sheep (18-21), pigs (22), cattle (23,24), and humans (25). The aim of these studies was mostly to understand the immunologic relationship and measure cross-reactions between *C. tenuicollis* and other taenid cestodes. There are also some studies to diagnose some other cestodes using C. tenuicollis antigens. Hydrophobic antigens from *C. tenuicollis* cyst fluids were used to diagnose T. saginata in cattle (23,25,26). In those studies, researchers partially achieved their goals. Berezhko and Romanenko (27) studied antigens of four species of the genus Taenia by means of immunodiffusion reaction in agar gel with the use of hyperimmune sera. They had established that extracts of the studied cestodes contain a great number of antigens, which during parental administration cause a synthesis of antibodies in rabbits.

In our study, only five protein bands appeared when the antigen was immunoblotted. Those bands were 26.8, 38, 42.5, 66, and 116 kDa. Two of these bands (38 and 42.5 kDa) were immunospecific for *C. tenuicollis* antigens in dogs monospecifically infected with *T. hydatigena*. Purifying the proteins of 38 and 42.5 kDa in the future studies using Prep-Cell, Rotofor-Cell or Gel Eluter could be more useful in the diagnosis of *T. hydatigena*.

We conclude that experimental infection of dogs with *C. tenuicollis* cysts to obtain mature *T. hydatigena* worms

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was successfully achieved and also the immunoreactive proteins were determined in *C. tenuicollis* cyst fluid antigens in experimentally and monospecifically infected dogs.

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