THE DETERMINATION OF IMMUNE REACTIVE PROTEINS IN *CYSTICERCUS TENUICOLLIS* CYST FLUIDS BY SDS-PAGE AND WESTERN BLOTTING IN SHEEP

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Summary:

Cysticercus tenuicollis, the larva of *Taenia hydatigena*, is commonly seen in sheep and goats slaughtered in Turkey. In this study, the protein bands were revealed in *C. tenuicollis* cyst fluid antigen by using SDS-PAGE and Western blotting, and immune reactive bands were determined. Ten positive and 10 negative sera for *C. tenuicollis* from sheep and one non-infected sheep serum were tested in this experiment. According to the results, there was only one protein band determined to be immune reactive, which was 36 kDa.

KEY WORDS : *Cysticercus tenuicollis*, sheep, SDS-PAGE, Western blotting.

Résumé : RECHERCHE DES PROTÉINES IMMUNORÉACTIVES DANS LE LIQUIDE DE KYSTE DE *CYSTICERCUS TENUICOLLIS* PAR SDS-PAGE ET WESTERN BLOT CHEZ LE MOUTON

Cysticercus tenuicollis, la larve de *Taenia hydatigena*, est généralement vu chez les moutons et les chèvres abattues en Turquie. Dans cette étude, des bandes de protéines ont été observées dans le liquide antigénique de kystes de *C. tenuicollis* par SDS-PAGE et Western Blot, et des bandes immunoréactives ont été recherchées. Dix sérums de moutons positifs et 10 négatifs vis-à-vis de *C. tenuicollis* et un sérum de mouton non infecté ont été examinés dans cette étude. Selon les résultats, une seule bande de protéines de 36 kDa a été déterminée comme étant immunoréactive.

MOTS CLÉS : *Cysticercus tenuicollis*, moutons, SDS-PAGE, Western Blot.

INTRODUCTION

Cysticercus tenuicollis, the larva of the canine tapeworm *Taenia hydatigena*, migrates through the liver tissue and encysts on the peritoneal membranes of sheep, goat, swine, cattle, and deer. The migratory immature stages of the metacestode are potentially pathogenic for the host and may be overlooked at meat inspection. Massive invasions, such as occur when entire tapeworm segments are ingested, result in acute traumatic hepatitis caused by the migratory cysticerci and may not only lead to morbidity but may also result in the death of the host animal (Soulsby, 1982). The parasite is responsible for economic losses owing to the high levels of liver condemnation in infected animals, and sometimes even small numbers of migrating *T. hydatigena* larvae are capable of precipitating "black disease" in the presence of *Clostridium novyi* (Guralp, 1981).

Cysticercus tenuicollis is quite common in the world. Pathak & Gaur (1982) reported that the prevalence of *C. tenuicollis* in sheep, goat, and swine of India were

37.03, 27.29, 8.3 %, respectively. This parasite had been reported in 16,7 % of sheep in Germany (Hasslinger & Weber-Werrighen, 1988). The prevalence of this parasite in goats was 33.3 % in Nigeria (Nwosu *et al.*, 1996). *Cysticercus tenuicollis*, the larva of *Taenia hydatigena*, is also widespread throughout Turkey. Sarimehmetoglu *et al.* (1993) reported this larva in 31.8 and 28.6 % of slaughtered sheep and goats of Ankara and vicinity, and also it has been reported in 28.0 and 27.9 % of sheep and goats slaughtered in Ankara province (Oge *et al.*, 1999).

Although a few attempts have been made for ante mortem diagnosis of *T. hydatigena* cysticercosis by serological tests these have yielded variable results (Pathak *et al.*, 1984; Deka & Gaur, 1990). Yong & Heath (1984) compared the antigens prepared from cyst fluids or produced *in vitro* culture of *Echinococcus granulosus*, *Taenia ovis*, *T. crassiceps*, and *T. hydatigena* by enzyme-linked immunosorbent assay (ELISA). Using the ELISA, all of antigens were able to detect the presence of larval cestodes, even in sheep with only one or two cysts. None of these antigens was able to detect specific infections only. The role of antibody resistance of sheep to *T. hydatigena* metacestodes was examined using passive transfer of immunoglobulins to newborn lambs, and it was reported that pooled serum from donor lambs which had received one light oral infection did not protect recipients although the donors themselves were immune (Jacobs *et al.*, 1994).

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A comparison had been made of the interactions between passively transferred and actively acquired immunity in regulating populations of *T. hydatigena* and *T. ovis* (Gemmell *et al.*, 1990). A qualitative difference between the species was the destruction of larval *T. ovis* prior to their establishment (pre-encystment immunity) and that of *T. hydatigena* after they had become established in the challenged lambs. Passive, like active immunity, is a density-dependent constraint. It plays an important role in the population regulation of *T. ovis* but not of *T. hydatigena* (Gemmell *et al.*, 1990). Generally Western blotting studies have been conducted on zoonotic cestods such as *Echinococcus granulosus*, *T. solium*, *T. saginata* (Ralston & Heath, 1995; Ko & Ng, 1998; Burgu *et al.*, 2000; Doganay *et al.*, 2000). In those studies, different specific protein bands were reported, and also *T. hydatigena* or *C. tenuicollis* antigens were tested for cross-reactions in those studies (Ralston & Heath, 1995; Ko & Ng, 1998). Determining specific protein band for *T. hydatigena* infections in sheep by Western blotting may have importance in the future vaccination and diagnostic kit studies for this cestode or its larva and other sheep and dog cestods.

MATERIALS AND METHODS

The cysticerci of *T. hydatigena* were collected from infected sheep brought to municipal slaughterhouse of Kazan, Ankara. The cysticerci were

thoroughly washed in distilled water and their fluid, scolices, and membranes were aseptically collected by puncturing the cysts. The fluid was centrifuged at 9.000 g for 30 minutes at 4° C. The supernatant was filtered through 0.45 µm membrane filter. Filtered solution was dialyzed against distilled water at 4° C for 24 hours, and this solution was stored as antigen at - 70° C until it was used. Blood samples were collected from 10 sheep infected with *C. tenuicollis* and 10 non-infected sheep. Sera were separated after centrifugation at 8.000 g for 15 minutes and finally stored - 20° C. To determine the appropriate volume of antigen for Western blotting, 5, 10, and 15 µl of antigen were run on 15 % SDS-PAGE. Because of capability of seeing proteins in the gel, there was no need to perform a protein concentration technique. Following electrophoresis, the gel stained with silver stain (Owl Scientific, Inc., USA). The best bands were observed by using 15 µl of antigen in the gel. In the determination of molecular weights of proteins, two different protein standards were used. These were Owl Unstained Standards High and Low Range Kits (ER-111-H and ER-111-L, Woburn, MA, USA). Molecular weights of protein bands observed on the gel were calculated.

Antigen ran by SDS-PAGE transferred onto nitrocellulose membrane (Sigma Chemical Company, USA). After blocking in PBS containing 3 % non-fat milk powder and 0,2 % Tween-20 overnight, the membrane strips were probed at room temperature with *C. tenuicollis* infected and negative sera, and also commercially obtained non-infected sheep sera (S-3772, SIGMA Che-

No. of infected animals	Localization of <i>C. tenuicollis</i>	Localization of cyst hydatid	Protein bands (kda)		
			66	36	27.5
1	Mesenterium	-	+	+	+
2	Mesenterium	-	+	+	+
3	Rumen	Liver	+	+	+
4	Mesenterium	-	+	+	+
5	Mesenterium, rumen	Liver	+	+	+
6	Mesenterium	-	+	+	+
7	Mesenterium	-	+	+	+
8	Mesenterium, rumen	Liver, lungs	+	+	+
9	Mesenterium	-	+	+	+
10	Mesenterium	-	+	+	+
No. of control animals (negative)					
1	-	-	+	-	+
2	-	Liver	+	-	+
3	-	-	+	+	+
4	-	Liver	+	-	+
5	-	-	+	-	+
6	-	-	+	-	+
7	-	-	+	-	+
8	-	-	+	-	+
9	-	-	+	-	+
10	-	-	+	-	+

Table I. - Information about carcass examinations of sheep and detected protein bands.

mical Company, USA) diluted 1:25 in blocking solution for two hours. The membranes were washed with PBS, incubated with a 1:3,000 dilution of horseradish peroxidase conjugated rabbit anti-sheep anti-serum (A-3415, Sigma Chemical Company, USA) in PBS solution for one hour at room temperature washed again and developed with DAB peroxidase substrate (D-4293, Sigma Chemical Company, USA). The preparation of solutions, the procedures of electrophoresis and Western blotting were applied according to Sambrook *et al.* (1989).

RESULTS

During carcass examination, hydatid cysts were seen in three of 10 *C. tenuicollis* infected sheep. Hydatid cysts were also seen in two of 10 sheep negative for *C. tenuicollis* (Table I). In this study 16 protein bands in antigen were revealed in the gel stained by silver stain technique (Fig. 1). While the bands of

6.1, 14, 16, 23, 27.5, 36, 45, 55, 66, 116 kDa had strong appearance in the gel, the remaining six bands had weak appearance.

Their molecular weights were between 6.1 and 116 kDa. When the proteins were transferred to nitrocellulose membrane, three of the protein bands which were at the molecular weights of 27.5, 36, and 66 kDa were observed in all of *C. tenuicollis* infected animals tested (Table I, Fig. 2). The bands of 27.5 and 66 kDa were observed in all of the negative sera tested (Table I, Fig. 3), and also these two bands were also observed in commercially obtained non-infected sheep serum. In one of the negative sera from a control sheep, the band of 36 kDa, which were also observed in positive sera was also seen. This mistake might be due to a hidden *C. tenuicollis* cyst in sheep that the serum obtained. According to the results, 36 kDa component had a specific reactivity with antibodies in the sera of *C. tenuicollis* infected sheep, and it can be said that 36 kDa is a specific immune reactive band for larval *T. hydatigena* infections.

Fig. 1. – Detected protein bands in *C. tenuicollis* antigens by using SDS-PAGE.

Fig. 2. – Detected protein bands in the sera of sheep infected with *C. tenuicollis*.

Fig. 3. – Detected protein bands in the sera of sheep negative for *C. tenuicollis*.

DISCUSSION

In recent years, SDS-PAGE and Western blotting have been widely used in the serologic studies of cestods. Generally, these studies mostly concentrated on cestods which are harmful to humans such as *E. granulosus*, *T. solium*, and *T. saginata*. Although some ELISA works had been done for *T. hydatigena* metacestodes in sheep (Yong & Heath, 1984; Jacobs *et al.*, 1994), no report was encountered regarding Western blotting studies for this parasite in sheep. Therefore, we didn't have the opportunity of comparing our results to that of other studies.

In this study, the band of 36 kDa was determined as an immune reactive for larval *T. hydatigena* in sheep. One of the sera grouped as negative control group gave reactivity with 36 kDa. This mistake might be due to a hidden *C. tenuicollis* cyst which we couldn't see in the carcass examination of the sheep in the slaughterhouse. Several studies reported that *C. tenuicollis* cyst fluid antigen or adult *T. hydatigena* somatic antigen cross-reacted with other adult or larval cestode antigens

(Hayunga *et al.*, 1991; Rhoads *et al.*, 1991; Bogh *et al.*, 1995). Because of these cross-reactions, *C. tenuicollis* cyst fluid was used as an immunodiagnostic reagent for bovine and human cysticercosis (Hayunga *et al.*, 1991; Hayunga *et al.*, 1992; Rhoads *et al.*, 1991; Bogh *et al.*, 1995). Larval *T. hydatigena* cyst fluid was also thought as an immunodiagnostic reagent for human hydatidosis (Monzon *et al.*, 1985). Although researchers do not have success until now, they still go on working on adult or larval *T. hydatigena* cyst fluid antigens, which contain similar epitopes with some other cestods. They propose that they can use these antigens in case they may have difficulties in finding other cestode or metacestodes parasites to prepare antigens in the diagnosis of those parasites.

Cross-reactions between crude antigens of larval *Taenia solium* and other cestods of pigs were studied by using the technique of immunoelectrophoresis (Cheng & Ko, 1991). The crude antigens extract contained 48 and 66 kDa. The crude extract also contained 95 kDa which was also found in larval *T. hydatigena* antigen. The sensitivities of the antigenic fractions to rabbit and pig antiserum against *T. solium* were tested by immunoblotting by Cheng & Ko (1992). They found that *T. solium* extracts contain the bands of 95 and 105 kDa which were cross-reacting with *T. hydatigena*. Ko & Ng (1998) tested excretory/secretory products of larval *T. solium* as diagnostic antigens for porcine and human cysticercosis by ELISA, SDS-PAGE, and EITB. Excretory/secretory antigens cross-reacted with the antiserum against larval *T. hydatigena* of pigs. Three host-like molecules with molecular masses 43, 58, and 66 kDa were present in the E/S products (Ko & Ng, 1998). In our study 66 kDa protein band was also found, but it was not specific and it reacted with both positive and negative sera. Our Western blotting analysis indicated that the specific reactivity with antibodies in sera of *C. tenuicollis* infected sheep was associated with 36 kDa component.

Determining the immune reactive protein band for *T. hydatigena* infection in sheep by Western blotting may have importance in the studies concerning eradication of sheep and dog cestods via serologic studies in the future. This immune reactive band, 36 kDa, should be purified to use in the diagnosis of this parasite.

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