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Abstract : Protein bands of hydatid fluids of sheep liver were determined by SDS-PAGE and Western blotting, and this was followed by the determination of specific bands of hydatid cysts for sheep and humans.

For diagnostic purposes, 30 positive and 20 negative sheep sera, 1 commercially obtained non-infected sheep serum, and 10 positive and 10 negative human sera were used in this experiment. According to our results, the specific protein band for hydatid disease in sheep was determined as 116 kDa, while the specific protein bands for hydatid disease in humans were determined as 68 and 8 kDa.

We determined that the diagnosis of hydatid disease in sheep and humans is possible with the use of the purified specific proteins obtained in this study. After putting these research results into practice, there will be no need to import expensive ready-made kits for the diagnosis of hydatid disease in humans.

Key Words : Hydatid cyst, SDS-PAGE, Western blotting

Koyun Kökenli Kist Hidatik Sıvılarının SDS-PAGE Metoduyla Analizi ve Western Blotting Metoduyla Protein Yapısındaki Spesifik Antijenlerin Saptanması

Özet : Bu çalışmada SDS-PAGE ve Western blotting yöntemleri kullanılarak, koyun karaciğer kist hidatik sıvısı protein bantları ortaya çıkartılmış daha sonra da koyun ve insanlar için spesifik kist hidatik protein bantları belirlenmiştir.

Bu amaç için 30 pozitif koyun serumu, 20 negatif koyun serumu, 1 non-enfekte koyun serumu, 10 pozitif insan serumu ve 10 negatif insan serumu kullanılmıştır. Çalışma sonuçlarına göre; kullanılan yöntemler ile koyun kist hidatik hastalığı için spesifik protein bantının 116 kDa, insan kist hidatik hastalığı için spesifik protein bantlarının 68 ve 8 kDa olduğu belirlenmiştir.

Bu çalışmada belirlenen spesifik proteinlerin pürifikasyonu ve bu proteinlerle hazırlanabilecek kitlerle yanlış reaksiyon riski olmaksızın kist hidatik hastalığının koyun ve insanlarda teşhisinin mümkün olabileceği saptanmış, özellikle insanlarda hastalığın teşhisinde yurtdışından getirilen kitlerin kullanılması nedeniyle araştırma sonuçlarının pratiğe aktarılması ile ekonomik boyut kazanabileceğine işaret edilmiştir.

Anahtar Sözcükler : Kist hidatik, SDS-PAGE, Western blotting

Introduction

Echinococcus granulosus is a parasite of the dog, coyote, wolf, and dingo. Its larva is a hydatid cyst in sheep, swine, cattle, man, mice, caribou, kangaroo, etc., and is found in a wide range of anatomical sites such as the lungs, liver, heart, and brain. This disease is widespread in many countries in the world. It is also an important health and economic problem in Turkey (1-3).

The diagnosis of hydatidosis is not possible via routine laboratory procedures and clinical symptoms. In particular, it is very difficult to diagnose newly formed cysts by radiography and ultrasound. Early diagnosis of this disease is very important for successful treatment. Currently, indirect haemagglutination (IHA), indirect fluorescence antibody test (IFAT), immunoelectrophoresis (IEP), counter immunoelectrophoresis (CIEP), double diffusion (DD) and enzyme-linked immuno sorbent assay (ELISA) are used in the early diagnosis of this disease, but they have some disadvantages such as cross-reactions with

other *Taenia* spp. and *Hymenolepis nana,* leading to false positive results. Therefore, it can be said that reliability of these tests is not high (4-8).

Some serologic studies have been carried out in the diagnosis of hydatidosis in humans (9-12). In animals, studies have been carried out in Turkey (13,14). Aykol (13), after using the techniques of CIEP and DD, indicated that the localization of cysts may affect the results in sheep. Şenlik (14) reported that IFAT has a sensitivity of 78.95% and a specificity of 77.3% for hydatidosis in sheep, noting that IFAT is more reliable than IHAT. Nevertheless, he also reported that both tests revealed cross-reaction with *Cysticercus tenuicollis* and *Moniezia* spp., leading to false positive results.

In recent years, SDS-PAGE and Western blotting have created a new era in immunodiagnosis, which greatly reduces cross-reactions (15). Almost all analytical electrophoresis of proteins is carried out in polyacrylamide gels. The strong anionic detergent SDS is used in combination with a reducing agent and heat to dissociate the proteins before they are loaded on the gel. The denatured polypeptides bind SDS and become negatively charged. SDS-polypeptide complexes migrate through polyacrylamide gels in accordance with the size of the polypeptide. At saturation, approximately 1.4 g of detergent is bound per gram of polypeptide. By using markers of known molecular weight, it is therefore possible to estimate the molecular weight of the polypeptide chain (4).

SDS-PAGE and Western blotting were used as verification tests in the diagnosis of viral and bacterial infections in the beginning, but lately these techniques have been used in the field of parasitology. However, they still need to be standardized like other applications (4,16). It has been reported that different results are obtained in the fractionating of proteins even when the same antigens are used. These differences may be due to electronic equipment, chemical reagents or application procedures (10).

This technique has been reported to give very sensitive and specific results in human studies (16,17). In humans infected with hydatidosis, this technique greatly reduces the risk of cross-reaction of immunoblotting, which is a very important step in the early diagnosis of the disease (18-20).

Kanwar et al. (20) reported that 15 protein fractions with molecular weights of 8-116 kDa were detected in hydatid cyst fluid taken from sheep, goats, pigs and humans, and antibody responses were developed against 12 protein fractions of sheep hydatid fluid in humans infected with hydatidosis. Researchers have reported that antibody responses develop in the sera of hydatidosis patients against bands 16, 24, 38, 45 and 58 kDa, but these bands give cross-reactions with the sera of humans infected with other parasites. However, specific antibody responses in humans with hydatidosis have been detected against 2 antigen fractions, 8 and 116 kDa (20). Maddison et al. (21) reported that band 8 kDa is more specific than the others. In addition, some researchers have pointed out that antibody responses against bands 12, 16, 20, 37, 38 and 48 kDa in the sera of patients infected with hydatidosis are also specific (18,19,22-25).

Heath and Lawrence (5) obtained bands 23 and 25 kDa from egg onchospheres of *Echinococcus granulosus* by SDS-PAGE, and then tested these proteins on sheep. Specific antibodies which cause lysing of oncospheres were developed in the sera of these sheep.

Twenty bands in the range 8-120 kDa have been detected in hydatid fluids of human, sheep, and cattle by SDS-PAGE (10). The same researchers also reported that antibody responses developed against 5 of these bands, 116, 98, 68, 57 and 45 kDa, in the sera of both humans and cattle infected with hydatidosis.

Antigenic profiles of protoscolices, fluid, and germinal epithelia of cyst hydatids were studied by Western blotting in another study in Turkey (26). In this study, sera taken from patients who had previously had surgery were analyzed. Antigenic determinants (8, 20, 45, 57, 68 kDa) were recognized by antibodies. Similar results have been obtained in studies on hydatid fluids of cattle and sheep (26).

Immunoblotting is a good confirmation test for hydatidosis, but further studies must be carried out to detect more specific bands (18-20,23).

Materials and Methods

Serum samples were obtained from sheep with or without hydatidosis brought to municipal slaughterhouses of Kazan and Kızılcahamam. At the same time, faecal samples and organs from these sheep were examined for fasciolosis, dicrocoeliosis, paramphistomosis, trichostrongylosis, trichuriosis and cysticercosis.

Besides these serum samples, non-infected sheep serum (Sigma S-3772 St. Louis, MO, USA) was obtained to verify the negativity of the sera of control animals. Positive and negative human sera were supplied by the Department of Microbiology, Gülhane Military School of Medicine, Ankara. The positivity of the positive sera was also confirmed by radiological and ultrasonographic procedures.

Hydatid fluid obtained from sheep liver fluid was centrifuged at 10 000xg for 30 minutes (+4°C) to remove protoscolices. The supernatant was filtered through a 0.45 mm membrane filter. Filtered solution was dialyzed against distilled water at +4°C for 36 hours. This fluid was then re-dialyzed with polyethylenglycol 400 to concentrate. To determine the volume of antigen, 5, 10, 20, 30, 40, 50 ml solutions of antigen were loaded on gels and stained. The best bands were obtained by using 40 ml of antigen. In the determination of molecular weights of protein, 2 different protein standards were used. One was Owl Unstained Standards High Range Kit (ER-111-H Woburn, MA, USA), and the other was Owl Unstained Standards, Low Range Kit (ER-111-L Woburn, MA, USA). The preparation of solutions, and the procedures of electrophoresis and Western blotting were applied according to Sambrook et al. (27).

To determine specific protein bands and cyst fluid antigens, separating and stacking gel was subjected to SDS-PAGE. Proteins were separated according to their molecular weights. Later on, the antigens were transferred from gel to nitrocellulose membranes. The protein standard was loaded to the first combination, and stained with Ponceau -S. Membranes with blotted antigens were cut into strips. The strips were washed and incubated with test sera (human and sheep). After repeated washings, the strips were further incubated with peroxidase labelled antibodies against human IgG (Sigma A-8775 St. Louis, MO, USA) and sheep IgG (Sigma A-3415 St. Louis, MO, USA), and were developed with substrate (DAB peroxidase substrate Sigma D-4293 St. Louis, MO, USA). And also, after separating antigenic proteins by SDS-PAGE, the obtained gel was silver stained.

Results

In this study 9 specific protein bands detected at molecular weights of 200,116,98,68,58,38,24,16 and 8 kDa (Figure 1). In nitrocellulose membrane, 2 to 5 bands were seen in all 30 positive sheep sera. The band of 116 kDa was seen in 29 out of 30 sera. The band 98 kDa was seen in 25 sera, 68 kDa in 17 sera, 58 kDa in 11 sera and 38 kDa in 12 sera (Table 1).

Two to 4 bands were seen in 20 negative sheep sera while 98 kDa was detected in all serum samples, 68 kDa in 17 sera, 58 kDa in 11 sera and 38 kDa in 14 sera. The band 116 kDa was detected in only 1 negative serum (Table 2).

Non-infected sheep sera were tested via Western blotting 2 bands in the molecular weights of 98 and 68 kDa were revealed. Two bands in the molecular weights 68 and 8 kDa were detected in the nitrocellulose membrane in 10 positive human sera. These bands were not seen in all 10 samples of negative sera. This shows that there are 2 specific bands in humans tested by Western blotting by using the antigen prepared from sheep hydatid cyst fluid (Table 3).

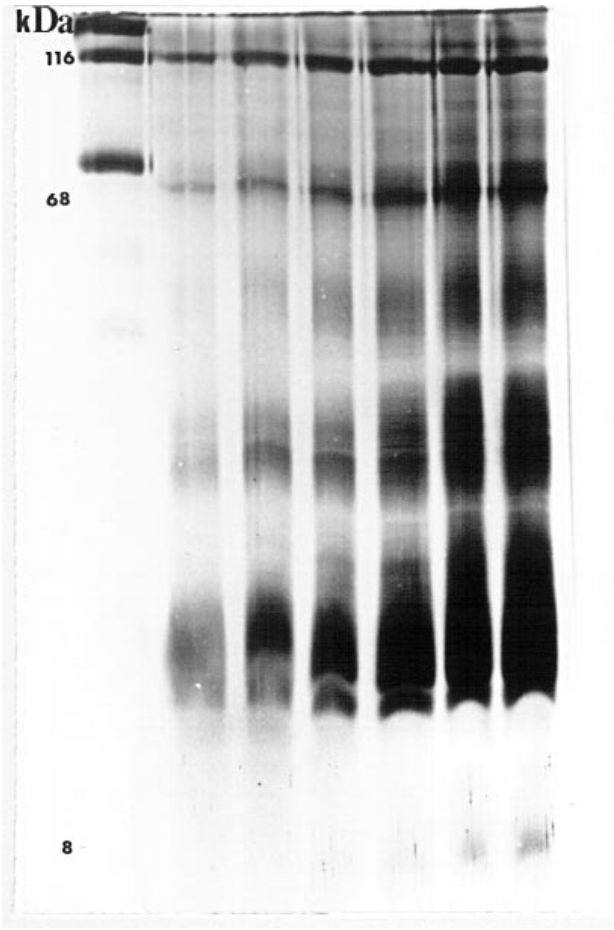

Figure 1. Protein bands separated by SDS-PAGE in cyst hydatid fluids of sheep.

Table 1. Protein bands detected by Western blotting in the sera of sheep with hydatidosis.

Animals	Localization of cyst	Other helminthic infections*	Bands				
			116 kDa	98 kDa	68 kDa	58 kDa	38 kDa
1	Liver, Lungs	Trc.	+	+	+	+	-
2	Liver, Lungs	Trc.	+	+	+	+	-
3	Liver, Lungs	Trc.	+	-	+	+	+
4	Liver, Lungs	-	+	-	-	+	-
5	Liver, Lungs	Trc., Paramph.	+	+	-	-	-
6	Liver, Lungs	Trc., Cyst.	+	+	-	-	-
7	Liver, Lungs	Trc.	+	+	-	-	-
8	Liver, Lungs	Trc., Dic	+	+	-	-	+
9	Liver, Lungs	Trc.	+	+	+	-	-
10	Liver, Lungs	Trc.,Trich.	+	+	+	+	-
11	Liver, Lungs	Trc.,Trich.	+	-	-	-	+
12	Liver, Lungs	Trc., Cyst	+	-	+	+	-
13	Liver, Lungs	Trc., Fas.	+	+	+	-	+
14	Liver	Trc.	+	+	+	-	-
15	Liver	Trc.	+	+	-	-	+
16	Liver	Trc.	+	-	+	+	-
17	Liver	Trc.	+	+	+	-	+
18	Liver	Trc.	+	+	+	-	+
19	Liver	Trc., Dic., Paramph	-	+	-	+	-
20	Liver	Trc.	+	+	-	-	+
21	Liver	Trc.	+	+	+	+	+
22	Liver	Trc., Cyst.	+	+	-	+	+
23	Liver	-	+	+	-	-	-
24	Liver	Trc., Trich.	+	+	+	-	+
25	Liver	-	+	+	+	-	-
26	Liver	Trc., Fas., Dic.	+	+	+	-	-
27	Liver	-	+	+	-	-	-
28	Liver	Trc.	+	+	+	+	-
29	Liver	Cyst.	+	+	+	-	+
30	Liver	Trc., Paramph.	+	+	-	-	-
Number of positive bands			29	25	17	11	12
Percentage of positive bands			96.7	83.3	56.7	36.7	40

* Other helminthic infections: Trc.: *Trichostrongylidae*, Paramph.: *Paramphistomum* sp., Trich.: *Trichuris* sp., Fas.: Fasciola sp., Dic.: Dicrocoelium dendriticum, Cyst.: Cysticercus tenuicollis

Table 2. Bands detected by Western blotting in the sera of sheep from the control group.

Animals	Other helminthic infections*	Bands				
		116 kDa	98 kDa	68 kDa	58 kDa	38 kDa
1	Trc.	-	+	+	+	+
2	-	-	+	-	+	+
3	Trc., Trich., Dic.	-	+	+	-	-
4	Trc.	-	+	+	+	+
5	Trc.	-	+	+	-	+
6	-	-	+	+	-	-
7	Trc., Paramph.	-	+	+	-	-
8	Trc.	-	+	+	+	-
9	Trc.	-	+	+	+	+
10	Trc., Fas	-	+	-	-	+
11	-	-	+	+	+	+
12	Trc.	-	+	+	+	+
13	Trc.	-	+	+	-	+
14	Trc.	-	+	+	-	+
15	Cyst.	-	+	-	+	-
16	Trc.	-	+	+	-	+
17	-	-	+	+	+	+
18	Trc., Paramph.	+	+	+	+	-
19	Trc.	-	+	+	+	+
20	Cyst.	-	+	+	-	+
Non-infective sheep serum		-	+	+	-	-
Number of positive bands		1	20	17	11	14
Percentage of positive bands		5	100	85	55	70

* Other Helminthic Infections: Trc.: *Trichostrongylidae*, Paramph.: *Paramphistomum* sp., Trich.: *Trichuris* sp., Fas: Fasciola sp., Dic.: Dicrocoelium dendriticum, Cyst.: Cysticercus tenuicollis

Table 3. Specific bands detected by Western blotting in the sera of hydatid patients and the control group.

Group (numbers)	Bands (%)	
	68 kDa	8 kDa
Patient group (10)	10 (100%)	10 (100%)
Control group (10)	-	-

In the macroscopical examination of 30 sheep for positive controls, hydatid cysts were seen in both the lungs and livers of 13 animals. Hydatid cysts were seen in the livers of 17 animals. *Cysticercus tenuicollis* was seen in 4 out of these 30 animals and 2 out of 20 negative control animals.

Faecal examinations of positive and negative control group animals were carried out by sedimentation and flotation procedures. According to these examinations, the eggs of *Trichostrongylidae* spp. were found in 25 sheep, *Dicrocoelium dendriticum*, *Paramphistomum* spp. and *Trichuris* spp. in 3 positive control animals, and *Fasciola* spp. in 2 positive control animals (Table 4).

During the examinations of 20 negative controls, the eggs of *Trichostrongylidae* spp., *Paramphistomum* spp., *D. dendriticum* and *Trichuris* spp. were seen in 14, 2 ,1 and 1 sheep respectively.

The eggs of *Trichostrongylidae* spp. and *Paramphistomum* spp. were detected in a sheep from which negative sera was taken. The band with a molecular weight of

Table 4. Bands detected by Western blotting in the sera of sheep infected with different helminths.

Diagnosed diseases (case number)	Number of detected bands (%)				
	116 kDa	98 kDa	68 kDa	58 kDa	38 kDa
Hydatidosis (30)	29 (96.7%)	25 (83.3%)	17 (56.7%)	11 (36.7%)	12 (40%)
Cysticercosis (6)	4 (75%)	5 (83.3%)	3 (50%)	3 (50%)	3 (50 %)
Trichostrongylosis (39)	25 (64.1%)	36 (92.3%)	28 (71.8%)	18 (46.2%)	22 (56.4%)
Trichuriosis (4)	3 (75%)	3 (75%)	3 (75%)	1 (25%)	2 (50%)
Fasciolosis (3)	2 (66.7%)	3 (100%)	2 (66.7%)	-	2 (66.7%)
Dicrocaeliosis (4)	2 (50%)	4 (100%)	3 (75%)	1 (25%)	1 (25%)
Paramphistomosis (5)	3 (60%)	5 (100%)	2 (40%)	2 (40%)	-

116 kDa was obtained from this sera. There was no available information about the human patients from which positive and negative sera were obtained.

According to the results, the specific band was 116 kDa determined by Western blotting by using the antigen prepared from the cyst fluid of sheep liver (Figure 2). This band was revealed in 29 out of the 30 positive sheep sera. This band was not revealed in the commercially obtained non-infected sheep sera. This band was detected in 1 of 20 negative sheep sera, which might be due to some hidden cysts in this animal identified as negative. The fact that the band 116 kDa was not observed in 1 positive serum might be due to some unfortunate mistakes in application. This sample was not re-tested because of a lack of serum. The other bands (98, 68, 58 and 38 kDa) which were seen in both positive and negative control sera have no antigenic specificity for hydatid disease.

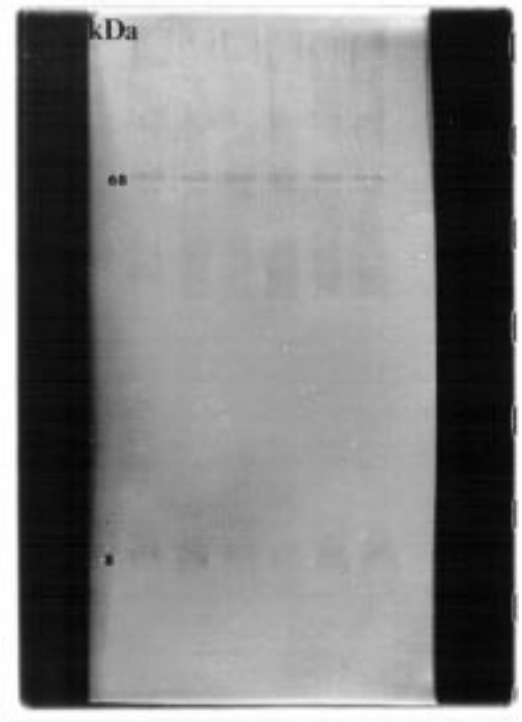

Figure 2. Bands detected by Western blotting in positive (A) and negative (B) sheep sera.

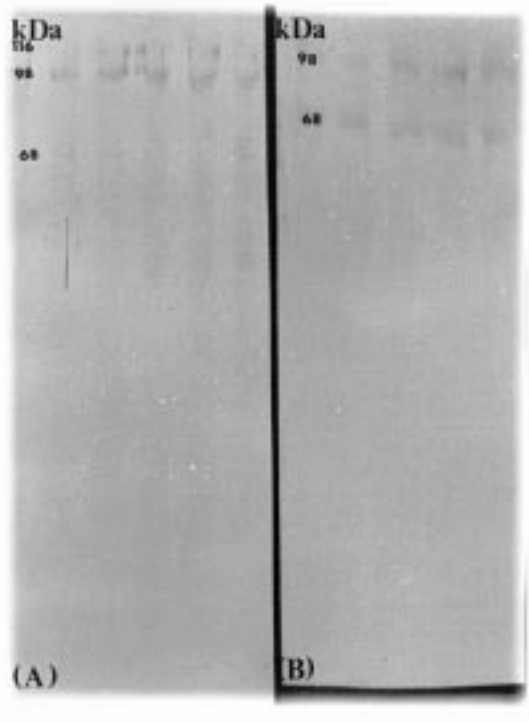

Figure 3. Specific bands detected by Western blotting in positive human sera.

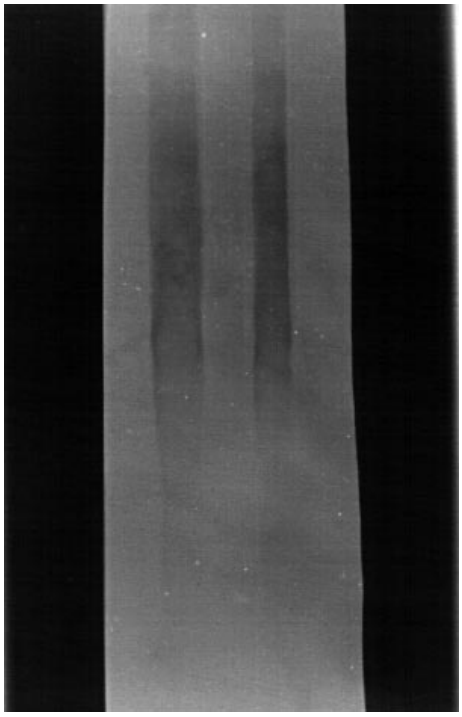

Figure 4. Strips detected by Western blotting in negative human sera.

In humans, 2 bands (68 and 8 kDa) are specific for hydatid disease. We reached this conclusion because the data (Figure 3) from all 10 samples of human sera revealed these 2 bands. No bands were revealed in the nitrocellulose membrane in 10 negative sera (Figure 4). The reason of not seeing many bands in human sera is that humans are not frequently infected with bacterial and viral infections as much as animals.

Discussion

In recent years, SDS-PAGE and Western blotting have been widely used in the diagnosis of parasitic diseases (4). Kanwar et al. (20) reported that sera from surgically confirmed cases of hydatidosis reacted with 12 polypeptides with molecular weights of 8-116 kDa by Western blotting with hydatid antigens. Polypeptides of 8 and 116 kDa were recognized by all hydatidosis sera but not by any sera from patients with cysticercosis, other parasitic infections or viral hepatitis, or from healthy controls. Thus, the recognition of 8 and 116 kDa hydatid antigens by a patient's serum appears to be a specific that confirms a clinical diagnosis. Maddison et al. (21) reported that specific 8 kDa is the most specific band in the diagnosis of hydatidosis in humans.

Köksal et al. (10) tested the cyst fluid of humans, sheep and cows by SDS-PAGE (10). They found 20 bands of different molecular weights ranging from 8 to 120 kDa. Antibody responses developed in preop serum samples of patients against 5 specific bands (45, 57,68, 98 and 116 kDa) in both human and cow cyst fluids. They did not reveal 8 kDa in nitrocellulose membrane electric current. This study was similar to our study in finding 68 kDa in all 10 human serum samples. Şaşmaz et al. (26) compared the stage-specific antigens of the parasite (human-originated protoscolex, hydatid cyst fluid and germinal membrane) using the serum from patients with surgically confirmed hydatid disease. They described 5 antigenic bands (8, 20, 45, 57 and 68 kDa) that were recognized by specific human antibodies in western blotting analysis. The same antigenic profiles observed in the cyst fluid bovine and sheep samples. However, some researchers (18, 19, 22-25) reported that immune responses to antigenic bands 14, 16, 20, 37, 38 and 48 kDa were specific in hydatid disease patients. Even studying with the same antigens, it is possible to get different results in SDS-PAGE. These differences might be due to preparing antigenic solutions or using chemical reagents of different quality and quantity (10).

We conclude that the determination of specific antigenic bands for human and sheep hydatid disease was successfully achieved in this study.

References

1. Barış, İ., Şahin, A., Bilir, N., Kalyoncu, A.F., Emri, A.S., Akhan, O., Barış, B., Çopur, A.S. ve Selçuk, Z.T.: Hidatik Kist Hastalığı ve Türkiye' deki Konumu, Türkiye Akçiğer Hastalıkları Vakfı Yayını No:1, Ankara 1989.
2. Güralp, N.: Helmintoloji, Ank. Üniv. Vet. Fak. Yayınları, 368. İkinci baskı, 1981.
3. Morris,D.L. and Richards,K.S.: Hydatid Disease. Butterworth-Heinemann Ltd. Linarcre House, Jordan Hill, Oxford OX2 8DP, 1992.
4. Altıntaş, N.: SDS-Polyacrylamide gel elektroforezi ile proteinlerin seperasyonu, T. Parazitol. Derg., 1991, 2,119-129.

5. Heath, D.D. and Lawrence, S.B.: Antigenic polypeptides of *Echinococcus granulosus* oncospheres and definition of proctetive molecules, Parasite Immunol., 1996, 18, 347-357.

6. March, F., Enrich, C., Mercader, M., Sanchez, F., Munoz, C., Coll, P. and Prats, G.: *Echinococcus granulosus*: Antigen characterization by chemical treatment and enzymatic deglycosylation, Exp. Parasitol., 1991, 73, 433-439.

7. Njeruh, F.M., Okela, G.B.A. and Gathuma, J.M.: Usefulness of indirect haemoglutination (IHA) and enzyme-linked immunosorbent assay (ELISA) in the diagnosis of human hydatidosis, E. Afr. Med. J., 1989, 66,310-314.

8. Hira, P.R., Hahr, G.M., Shweiki, H.M. and Behbehani, K.: An enzyme-linked immunosorbent assay using an arc 5 antigen for the diagnosis of cystic hydatid disease, Ann. Trop. Med. and Parasitol., 1990, 2, 157-162.

9. Genç, S.: İnsan hidatidosisinin tanısında pasif hemaglutinasyon ve Weinberg reaksiyonlarının değeri, Mikrobiyoloji Bülteni., 1976, 10,215-231.

10. Köksal, F., Serin, M.S., Kekeç, Y. ve Sadr, Y.E.: İnsan ve hayvan kökenli kist hidatik sıvılarının SDS-PAGE metoduyla analizi ve Westernblot metodunun klinik önemi, T. Parazitol. Derg., 1995, 19,221-229.

11. Yalçıntaş, İ. ve Çolakoğlu, S.: Kist hidatik teşhisinde indirekt hemaglutinasyon testinin değeri, Çukurova Üniv. Tıp Fak. Derg., 1973, 3,152-155.

12. Yazıcıoğlu, A. ve Dinçer, H.: Kist hidatik ve Weinberg reaksiyonu, Mikrobiyoloji Bülteni, 1971, 5,273-281.

13. Aykol, F.: Tabii Enfekte Koyunlarda Kist Hidatiğin Counterimmunoelektroforesis (CIEP) ve Çift Diffuzyon (DD) Testlerle Karşılaştırmalı Teşhisi, (Doktora Tezi), Ankara Üniv. Sağlık Bilim. Enst., 1986.

14. Şenlik, B : Bursa Yöresi Koyunlarında Indirekt Floresan Antikor (IFA) ve Indirekt Hemaglutinasyon (IHA) Testleriyle Hidatidoz'un Sero-Prevalansı Üzerine Araştırmalar, Uludağ Üniv. Sağlık Bilim. Enst., 1998.

15. Sharma, S.D., Mullenax, J. and Araujo, F.G.: Western blot analysis of the antigens of T.gondii recognized by human IgM antibodies, J. Immunol., 1987, 131, 977-978.

16. Towbin, H., Staehelin, T. and Gordon, J.: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications, Proc. Natl. Acad. Sci. USA., 1979, 76, 4350.

17. Su, X. and Prestwood, A.K.: Dot Elisa mimicry western blot test for the detection of swine trichinellosis, J. Parasitol., 1991, 77, 76-82.

18. Facon, B., Chamekh, M., Dissous, C. and Capron, A.: Molecular cloning of an *Echinococcus granulosus* protein expressing an immunogenic epitope of antigen 5, Mol. Biochem. Parasitol., 1991, 45,233-240.

19. Fadwa, M.A. and Knobloch, J.: Isolation and partial characterization of species-specific and cross-reactive antigens of *Echinococcus granulosus* cyst fluid, Mol. Biochem. Parasitol., 1989, 37,101-108.

20. Kanwar, J.R., Kaushık, S.P., Sawhney, I.M.S., Kamboj, M.S., Mehta, S.K. and Vınayak, V.K.: Specific antibodies in serum of patients with hydatidosis recognised by immunoblotting, J. Med. Microbiol., 1992, 36,46-51.

21. Maddison, S.E., Slemenda, S.B., Schantz, P.M., Fried, J.A., Wilson, M. and Tsang, V.: A specific diagnostic antigen of *Echinococcus granulosus* with an apparent molecular weight of 8 kDa, Am. J. Med. Hyg., 1989, 40,377-383.

22. Chamekh, M., Facon, B., Dissous, C., Haque, A. and Capron, A.: Use of a monoclonal antibody specific for a protein epitope of *Echinococcus granulosus* antigen 5 in a competitive antibody radioimmunoassay for diagnosis of hydatid disease, J. Immunol. Methods., 1990, 134,129-137.

23. Leggatt, G.R. and McManus, D.P.: Identification and diagnostic value of a major antibody epitope on the 12 kDa antigen from *Echinococcus granulosus* (hydatid disease) cyst fluid, Parasite Immunol., 1994, 16,87-96.

24. Profumo, E., Ortana, E., Rigano, R., Gioia, I., Notargiacomo, S., Loppolo, S. and Siracusano,A.: Cellular and humoral responses to antigenic subunits of *Echinococcus granulosus* cyst fluid in hydatid patients, Parasite Immunol., 1994, 16, 393-398.

25. Sanchez, F., March, F., Mercader, M., Coll, P., Munoz, C. and Prats,G.: Immunochenical localization of major hydatid fluid antigens in protoscolices and cyst of *Echinococcus granulosus* from human origin, Parasite Immunol., 1991, 13, 583-592.

26. Şaşmaz, E.: Hashempoor, G.R., Bahiar, İ.H. ve Yuluğ, N., *Echinococcus granulosus*' un karşılaştırmalı antijenik analizi. T.Parazitol. Derg., 1995, 19, 83-87.

27. Sambrook, J., Fritsch, E.F. and Manniatis, T.: Molecular Cloning: A Laboratory Manual, 15 section, p. 18.47-18.76. Cold. Spring Harbor, New York, 1989.