

Analysis of the Crude Antigen of *Hymenolepis nana* from Mice by SDS-PAGE and the Determination of Specific Antigens in Protein Structure by Western Blotting

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Abstract: Protein bands of crude antigens of *Hymenolepis nana* were determined by SDS-PAGE and Western blotting. Thirty Swiss albino mice were allotted into two groups of 15 each as positive (infected with *H. nana*) and negative (non-infected with *H. nana*) groups. The natural infections of *H. nana* and other helminths were determined by centrifugal flotation of faeces. After bleeding, the mice were necropsied and their guts were examined for *H. nana* and other intestinal helminths. Sera from mice were tested by Western blotting and the bands obtained from positive and negative groups were compared. The specific protein band for *H. nana* infection was determined to be 24 kDa.

Key Words: *Hymenolepis nana*, mice, SDS-PAGE, Western blotting

Fare Kökenli *Hymenolepis nana* Somatik Antijenlerinin SDS-PAGE Metoduyla Analizi ve Western blotting Metoduyla Protein Yapısındaki Spesifik Antijenlerin Saptanması

Özet: Bu çalışmada, SDS-PAGE ve Western blotting yöntemleri kullanılarak *Hymenolepis nana* somatik antijeninin protein bantları ortaya çıkarılmıştır. Çalışmada kullanılan 30 Swiss albino fare, pozitif (*H. nana* ile enfekte) ve negatif (*H. nana* ile enfekte olmayan) olarak 15'er hayvanlık iki gruba ayrılmıştır. *H. nana* ve diğer helmint enfeksiyonları ile doğal enfekte farelerin tespiti için fare dışkıları santrifüj flotasyon yöntemi ile incelenmiştir. Farelerden kan alındıktan sonra otopsileri yapılmış, bağırsakları *H. nana* ve diğer helmint enfeksiyonları yönünden kontrol edilmiştir. Pozitif ve negatif fare gruplarından elde edilen serumlar Western blotting yöntemi kullanılarak incelenmiş, elde edilen bantlar karşılaştırılarak *H. nana* enfeksiyonu için spesifik protein bantının 24 kDa olduğu belirlenmiştir.

Anahtar Sözcükler: *Hymenolepis nana*, fare, SDS-PAGE, Western blotting

Introduction

Hymenolepis nana, the dwarf tapeworm, is a common cestode of mice, rats and primates including humans. The life cycle may be either direct or indirect. Nonimmune hosts can be autoinfected; eggs are produced, hatched and complete their life cycle within the intestine of a single host. The indirect cycle utilises arthropods as intermediate hosts (1). *Hymenolepis nana* is a cosmopolitan species and the surveys of human (2-4) and laboratory animals (5-7) in Turkey have shown the prevalence of *H. nana* to range from 0.02 to 14.38% and 13.3 to 100%, respectively.

Currently, the enzyme-linked immunosorbent assay (ELISA) (8-10), immunodiffusion (ID) (10),

immuno-electrophoresis (IEP) (10,11), double diffusion (DD) (11), immunoprecipitation (IP) (8) and indirect immunofluorescent antibody test (IFAT) (12) are used in the diagnosis of *Hymenolepis* spp. infections. Gomez-Priego et al. (9) have used a crude antigenic extract prepared from the scolex and neck regions of adult worms and detected the serum antibodies in human *Hymenolepis nana* infection by ELISA. Researchers have reported that *Hymenolepis nana* infection in humans induces a low but detectable humoral immune response but is not useful for diagnostic purposes. Cheng and Ronald (10) have studied the cross-reactions between crude antigens of larval *Taenia solium* and other helminths of pigs (*Taenia hydatigena*, *Fasciolopsis buski*,

Hymenolepis diminuta and *Dipylidium caninum*) by ID and IEP and detected cross-reactions and false positive results.

In recent years, SDS-PAGE and Western blotting procedures have initiated a new era in immunodiagnosis, greatly reducing cross-reactions (13). Almost all analytical electrophoresis of proteins is carried out in polyacrylamide gels. These techniques were used as a verifying test in the diagnosis of viral and bacterial infections in the beginning, but lately these techniques have been used in the field of parasitology (14,15). Diaz et al. (16) compared the results of an ELISA and an enzyme-linked immunoelectrotransfer blot (Western blotting) assay for the diagnosis of cysticercosis in sera and cerebrospinal fluid (CSF) and their results demonstrate that Western blotting is the best assay available for the diagnosis of cysticercosis in both sera and CSF. In this study, cross-reactivity was evaluated in sera from patients with *Echinococcus granulosus* (hydatid) and *Hymenolepis nana* infections. It was determined that sensitivity in detecting cysticercosis in sera was 94% by Western blotting and 65% by ELISA and the specificity of the Western blotting was 100%, while that of ELISA was 63%.

The use of SDS-PAGE and Western blotting against *H. nana* infection in mice, rats and primates has not been reported. Montenegro et al. (17) compared crude antigens of *H. nana* and *Echinococcus granulosus* in humans and they determined that bands 49 and 66 kDa obtained from crude antigens of *H. nana* could be used for the diagnosis of *H. nana* infections in humans.

It has not been determined in mice. The purpose of the present work was to determine specific protein bands from the sera of mice naturally infected with *H. nana*. These research results may provide some basic information required for antigen purification studies.

Materials and Methods

Thirty 3-month-old Swiss albino mice weighing approximately 30 g were used. Their faeces were examined by the technique of ZnCl₂ + NaCl centrifugal flotation of faeces and natural infection of *H. nana* and other helminths were identified. Mice were divided into two groups as positive (infected with *H. nana*) and negative groups (non-infected with *H. nana*) each having 15. Examination of animals for *H. nana* and other

helminth eggs (described above), was carried out three times (days 1, 8 and 15) before necropsy.

Blood samples were taken from positive and negative mice and sera obtained from these mice were stored at -20 °C. After bleeding, the mice were necropsied and examined for *H. nana* and other helminths.

Antigen Preparation

After the necropsy, mature *H. nana* parasites were collected from the small intestine of mice and washed in three changes (30 minutes each) of 0.8% saline. The parasites (0.5 g) were placed in eppendorf tubes to which appropriate amounts of 10% sodium dodecyl sulphate (5 ml) and mercaptoethanol (500 µl) directly aliquoted parasite material were added. Then the antigen solution was shaken for 30 min on a shaker and stored at -20 °C.

Polypeptide analysis

Crude antigens of *H. nana* were separated by SDS-PAGE and proteins were visualised with the silver stain technique and their molecular weights were determined by comparing with molecular weight standards. To determine the most appropriate amount of antigen, a gel (5% stacking + 12% separating) was prepared. To determine the volume of antigen, 5, 10, 20, 30, 40 and 50 µl solutions of antigen were loaded to gel and stained with silver stain. The best bands were obtained by using 20 µl of antigen. In the determination of the molecular weights of protein, one protein standard was used and this was the Sigma wide molecular weight range (M-4038 St. Louis, MO, USA). The preparation of solutions, the procedures of electrophoresis and Western blotting were as described by Sambrook et al. (18).

Antigenic Analysis

Antigenically active components among SDS-PAGE resolved bands were detected by Western blotting. After SDS-PAGE, the proteins were transferred electrophoretically onto nitrocellulose sheets using a transfer blot apparatus. Gels were fixed and stained with Panceau-S to determine molecular weights of the proteins. Nitrocellulose containing transferred samples was incubated overnight at 4 °C in 3% nonfat dried milk, and then rinsed in PBS before 2 hours' incubation with sera containing test antibodies. Following three PBS washes to remove unbound antibody, nitrocellulose sheets were incubated for 60 minutes in horseradish peroxidase conjugated anti-IgG (Sigma Chemical Co., St.

Louis, MO, USA). Unbound conjugate was removed by three PBS washes before the addition of substrate solution containing DAB (3,3'- Diaminobenzidine, Sigma Chemical Co., St. Louis, MO, USA). Bands were visible within 15 minutes and development was stopped by removing the substrate with distilled water and air drying the nitrocellulose.

Results

The intestines of mice were microscopically examined at necropsy. The proportions of mice in the positive group infected with parasites were as follows: 53.3% infected with *H. nana* and *Aspicularis tetraptera*, 20% with *H. nana* and *Syphacia* spp. and 20% with *H. nana*, *Syphacia* spp. and *A. tetraptera*. Only *H. nana* infection was observed in 6.6% of mice. In the negative group, 33.3% of mice were infected with *A. tetraptera*, 26.6% with *Syphacia* spp. and 26.6% with both *Syphacia* spp. and *A. tetraptera*. No helminthic infection was observed in 13.3% of mice (Table).

Ten protein bands were detected between 14 and 66 kDa in polyacrylamide gel cast as separating and stacking gel (Figure 1). One protein band was detected from 15 tested mice sera in nitrocellulose membrane. This band was 24 kDa. No bands were detected in the sera of the negative group (Figure 2).

No bands observed in the nitrocellulose membrane belong to other helminth infections in the positive and negative groups. Therefore, our test results were specific for *H. nana*.

Discussion

In recent years, SDS-PAGE + Western blotting have been widely used in the diagnosis of parasitic diseases.

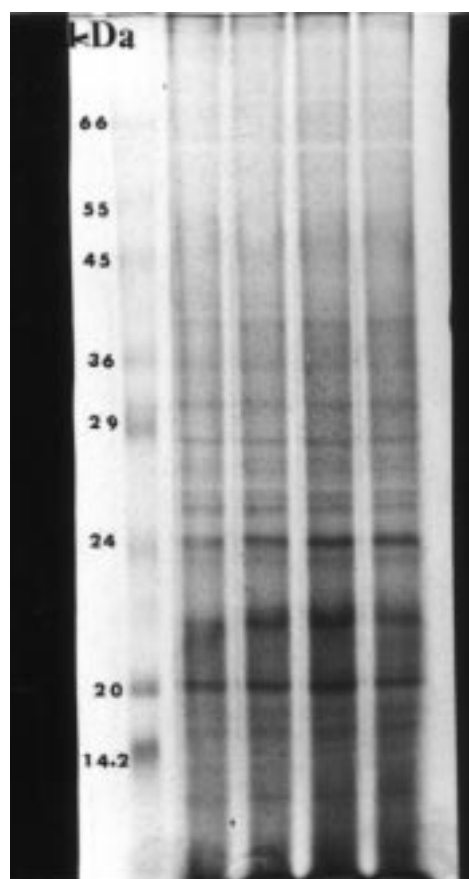


Figure 1. Detected protein bands in crude antigens of *H. nana* by SDS-PAGE

Western blotting greatly decreased the risk of cross-reactions in studies carried out in humans and animals with parasites (14).

Surveys of different helminth infections by SDS-PAGE and Western blotting (19,20) have shown that some specific protein bands obtained from both human and animal sera have closer molecular weights. Montenegro et al. (17) investigated the diagnostic importance of

Groups	Helminth Species	The number of infected mice (%)	Bands 24 kDa
Positive	<i>H. nana</i> , <i>A. tetraptera</i> , <i>Syphacia</i> spp.	3 (20)	+
	<i>H. nana</i> , <i>A. tetraptera</i>	8 (53.3)	+
	<i>H. nana</i> , <i>Syphacia</i> spp.	3 (20)	+
	<i>H. nana</i>	1 (6.6)	+
	<i>A. tetraptera</i> , <i>Syphacia</i> spp.	4 (26.6)	-
Negative	<i>A. tetraptera</i>	5 (33.3)	-
	<i>Syphacia</i> spp.	4 (26.6)	-
	-	2 (13.3)	-

Table. Worm burden and detecting specific protein bands in positive and negative groups of mice

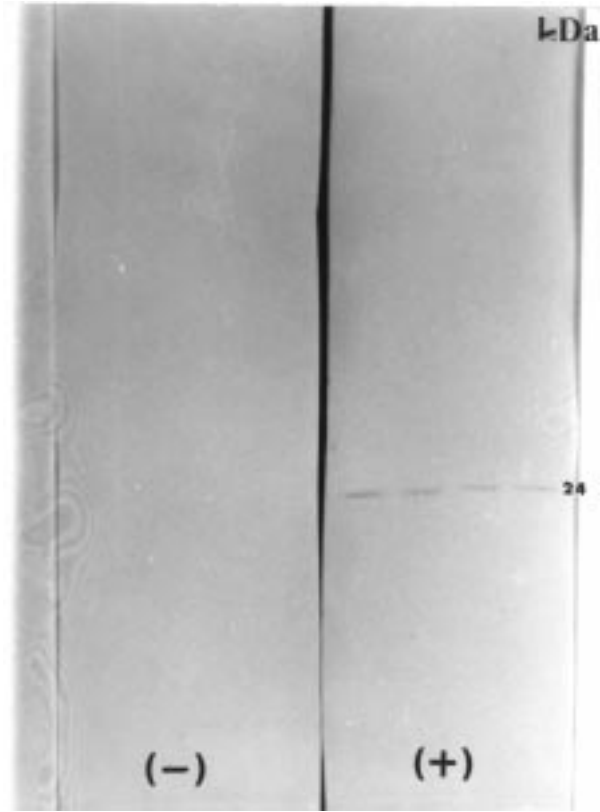


Figure 2. Detected bands in the sera of mice positive and negative for *H. nana* by Western blotting

species specific and cross-reactive components of *Taenia solium*, *Echinococcus granulosus*, and *Hymenolepis nana*. They determined that the bands of 49 and 66 kDa obtained from crude antigens of *H. nana* could be used for the diagnosis of *H. nana* infections in humans. One specific band was detected in this study. The molecular weight of this band was 24 kDa. When the results of Montenegro et al. (17) and ours are compared, no similarities are observed between the protein bands of human and mice.

Ito and Onitake (12) analysed changes in the surface antigens (oncosphere, cysticeroid, adult scolex and adult strobila) of *Hymenolepis nana* during differentiation and maturation in mice and detected that the antibody responses were always delayed compared with the differentiation and maturation of the parasite. We conclude that specific protein bands were determined in crude antigens obtained from *H. nana* as 24 kDa. According to our study, crude antigen in serologic tests will give reliable results. However, specific proteins should be purified by modern equipment, such as Prep-cell, Rotofor-Cell, or Gel Eluter, to obtain the most specific diagnosis in *H. nana*.

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