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Studying antigenicity and immunogenicity of *Echinococcus granulosus* metabolites

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Abstract. Antigenicity and immunogenicity of *Echinococcus granulosus* excretory-secretory antigen (ES-Ag) were studied to identify proteins that can be used in the development of test-system for the detection of dogs infested with echinococcosis. Five and/or two dogs were infected orally with viable *E.granulosus* protoscoleces and/or *Cysticercus tenuicolis,* obtained from infested sheep at a slaughterhouse. ES-Ag of *E.granulosus* protoscoleces and its adult form, obtained *in vitro,* possessed pronounced antigenicity towards antisera of infected dogs. Anti-echinococcus sera reacted equally with larvae ES-Ag of two closely related tapeworms. At the same time, antibodies of dogs infected with echinococcosis allowed to differentiate ES-Ag of adult helminths. *Taenia hydatigena* ES-Ag was also characterized by the antigenicity that intensified with the development of the invasion process. It was found that anti-*T. hydatigena* serum cross-reacted with adult echinococcus significantly weaker than vice versa. The protein fractions of the tapeworms that are immunogenic for rabbits were establashed.

Keywords: *Echinococcus granulosus*, excretory-secretory antigen, antigenicity, immunogenicity, ELISA.

Introduction. In recent decades extremely adverse epizootic and epidemic situations on echinococcosis are created in Kazakhstan. According to statistical data of the Republican Veterinary Laboratory of Kazakhstan for the first half of 2013 hydatid disease was diagnosed in 3.1% of cattle and 3.04% of small ruminants of Akmola region. In West- and East-Kazakhstan regions these figures were equal to 3.68% - 2.24% and 3.86% - 2.54%, respectively. High incidence of echinococcosis among sheep have been also registered in Kostanai region (3.34%). The incidence of human echinococcosis in the oblasts such as South Kazakhstan, Almaty, Mangistau, Kyzylorda, Aktobe and West Kazakhstan for 100 thousand population ranges from 4.03 to 10.7.

The source of infection of animals and human with echinococcosis are stray, shepherd and guard dogs which release mature segments filled with eggs into the environment. The main factors that negatively affecting the epidemiological situation on echinococcosis in Kazakhstan are slight funding for dogs deworming, lack of cooperation between sanitary-epidemiological and veterinary services for the prevention of the diseases, and failure to comply with the sanitary and veterinary regulations of keeping animals [1]. In addition, there have been

significant changes in the agricultural sector. Farms are now smaller family units, that is why there are much greater numbers of people and dogs involved in sheep husbandry than previously, and there is a closer interaction[2]. In recent years, infection of urban dogs with echinococcosis has also significantly increased. According to A.M.Abdibekova (2010) infection of urban dogs with *E.granulosus* has reached more than 10% with a fairly high intensity of infestation [3]. In this context, the timely detection of dogs infested with *E.granulosus,* as well as environmental monitoring for the presence of the parasite are an actual problem of veterinary science and practice. As rightly pointed by P. Craig et al. (2015) «advances in diagnostic approaches for definitive hosts and livestock have not progressed equally over the last 20 years» [4]. The sedimentation and counting technique performed with intestinal contents of the sacrificed animals remains the gold standard for detection of *E.granulosus* in definitive hosts. Arecolin purgation is commonly used for *in vivo* diagnosis. However, these methods are not satisfactory due to the fact that the eggs of taeniid species cannot be microscopically differentiated from each other [5]. In the diagnosis of the disease immunological methods are also used such as immunoprecipitation, indirect hemagglutination, immunofluorescent test, and others[6]. However these tests are characterized by low sensitivity. Besides, they have insufficient specificity due to the use of complex helminth antigen. Nowadays, the serological diagnosis of echinococcosis is mainly based on the use of *E.granulosus* hydatid cyst fluid as the antigen. The hydatid cyst fluid contains numerous lipo- and glycoproteins, salts, carbohydrates and lipids, as well as a complex mixture of host proteins, mainly represented by albumin and immunoglobulins, together with proteins released by *E. granulosus* [7-10]. Moreover, serodiagnosis of canine echinococcosis is hampered by antigenic similarities between *E. granulosus* and *Taenia hydatigena* [11], which is parasitizing in the small intestine of dogs and other carnivores. Therefore «available immunodiagnostic tests lack standardization of the target antigen and, in turn, this is reflected on poor sensitivity and specificity of the serological diagnosis» [12]. Thus, the search for adequate antigens of *E.granulosus* is a necessary prerequisite for the development of highly specific and sensitive immunoassays.

The aim of research was to study antigenicity and immunogenicity of excretory-secretory antigens (ES-Ag) of *in vitro* reared *E.granulosus* larval and adult forms.

Materials and methods

Experimental animals. Seven outbred female dogs at the age of 3 months were kept under standard conditions in accordance with the "Principles of Good Laboratory Practice (GLP)», and their use and care were approved by the Animal Ethics Committee of the Faculty of Veterinary and Livestock Technology of Seifullin Kazakh Agro-Technical University. Feed and water to laboratory animals were given *ad libitum.* In the premises for keeping dogs a 12-hour cycle of lighting was maintained. The temperature and humidity were controlled daily.

The dogs were vaccinated against distemper, parvovirus enteritis, infectious hepatitis, leptospirosis and adenoviruses (Geksakanivak, "Vetzverotsentr" Russia), treated orally with anthelmintic to eliminate worm parasites. Primary worming was carried out by Vermis-EX (CP-Pharma Pharmaceutical) at the rate of 1 tablet (50 mg praziquantel and 50 mg fenbendazole) per 10 kg bodyweight, and Cestem (Ceva, Spain) at the rate of 0.5 tablet (150 mg febantol, 50mg pyrantel and 50mg praziquantel) for 5 kg bodyweight was used at the last deworming.

Five dogs were infected orally with 8000 viable protoscoleces (*E.granulosus* larvae), isolated from ovine fertile hydatid cysts. The selection of hydatid cysts was performed during the slaughter of sheep at a slaughterhouse "Altyn-taga", located in Tselinograd district, Akmola oblast (Kazakhstan). Two additional dogs were infected orally with *C. tenuicolis* (*T.hydatigena* larvae), obtained from infested sheep.

Blood samples from dogs were taken before experiment (by 35 days after the last deworming), then by 12^{th} , 30^{th} and 35^{th} days post infection (d.p.i.). Dogs infected with *E.granulosus* and *C. tenuicolis* were euthanized for necropsy diagnosis by intramuscular injection of xylazine (2 mg/kg bodyweight; Bioveta, as, Czech Republic) following by an intravenous overdose of anestofol (15 mg/kg bodyweight; LLC "VIC", Russia) on the 35^{th} and 70^{th} d.p.i., respectively. Small intestines were removed post mortem, opened longitudinally and examined directly for presence of tapeworms following the recommendations of the WHO/OIE [13].

Obtaining ES-Ag of E.granulosus protoscoleces and C. tenuicolis. The tapeworms' larvae isolated from infested sheep were cultured in incomplete RPMI-1640 medium (Sigma-Aldrich, USA) supplemented with penicillin 105 IU/L (Simbirskaya veterinary company, Russia), streptomycin 100 mg/L (JSC "Himfarm", Kazakhstan) in CO_2-incubator at 37ºC. The supernatant containing ES-Ag was taken after 2, 5, 9 and 16 days of cultivation and it was concentrated by PEG 6,000 (Sigma-Aldrich, USA) up to protein concentration of 250-1000 mg/mL. Protein content was determined using the standard technique [14].

Obtaining ES-Ag of imaginal E. granulosus and T. hydatigena. The worms were collected from the small pieces of the open gut in sterile Hank's balanced salt solution (Sigma-Aldrich, USA) with gentamicin (PanEco, Russia). The isolated helminths were washed with the same solution, and were transferred to a sterile polystyrene mattresses (Nunc, Denmark) containing 50 ml incomplete RPMI-1640 medium with the above antibiotics. The supernatant was taken after 24 hours of incubation at 37°C, and then it was collected by 5-7 days of cultivation. The medium containing ES-Ag was poured into sterile polypropylene tubes of 50 ml (TPP, Switzerland) and centrifuged at 3000 x g for 10 min. Then, the supernatant was dialyzed against distilled water using dialysis tube at 4°C over night. Resulting antigen was concentrated 10× using ultrafiltration unit (Millipore).

Preparation of E.granulosus somatic antigen (SA). Adult *E.granulosus* were washed with buffered saline (PBS). The worms were homogenized and ultrasonicated (10 cycles for 12 sec. at the frequency of 60 Hz). Sonicated material was centrifuged during 35 min at 3000 x g, and the resulting supernatant was used

as *E.granulosus* SA.

Sodium dodecilsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed by the standard procedure using a vertical electrophoresis apparatus (Bio-Rad, USA) by the standard procedure[15]. The molecular weight of protein was determined using the software Photo-Capt Version 12.4 of "Vilber Lourmat".

Testing antigenicity of tapeworms' antigens was carried out by standard protocol of indirect ELISA using polystyrene 96-well flat-bottomed plates (Alto, Italy). The plate wells were sensitized by the antigens in the concentration of 0.005 mg/ml at 4ºC overnight. Samples of infected dogs sera at 0.1 ml diluted in the wells starting from 1:100 and incubated at 37°C for 60 min. Then, the wells were washed 3 times with phosphate buffered saline-tween-20 to remove unbound components of the reaction. Immune complex was detected using antispecies conjugate (Jackson ImmunoResearch, USA) and its substrate - orthophenylenediamine (Sigma, USA). ELISA results were recorded using a spectrophotometer with vertical flow light (96 ASYS Expert, Austria) at a wavelength of 492 nm. ELISA result was considered positive if the optical density (OD) of the well with antiserum was higher at least 2 times as compare to the OD for the control well with dog's serum obtained before infection.

In order to study immunogenicity of *E.granulosus* ptotoscoleces and/or *E.granulosus* imago ES-Ag for rabbits two methods of immunization have been used. Briefly, ES-Ag in an amount of 100.0 ug was mixed with Freund's complete adjuvant until a thick emulsion. Then, immunogen was injected subcutaneously at several points of the back. The immunization was repeated on the 14^{th} and 28^{th} days by intramuscularly administration of ES-Ag in the same dose with Freund's incomplete adjuvant. On the 35^{th} day subcutaneous booster injection was carried out by injecting 50 ug of immunogen without adjuvant, and after 7 days blood collection was performed. Blood was placed in an incubator for coagulation. The resulting serum was drained and clarified by centrifugation. The second method was distinguished by the fact that on the 35^{th} day of immunization booster injection was given intravenously in a volume of 10 ug of ES-Ag without adjuvant, and 3 days later blood sampling was carried out.

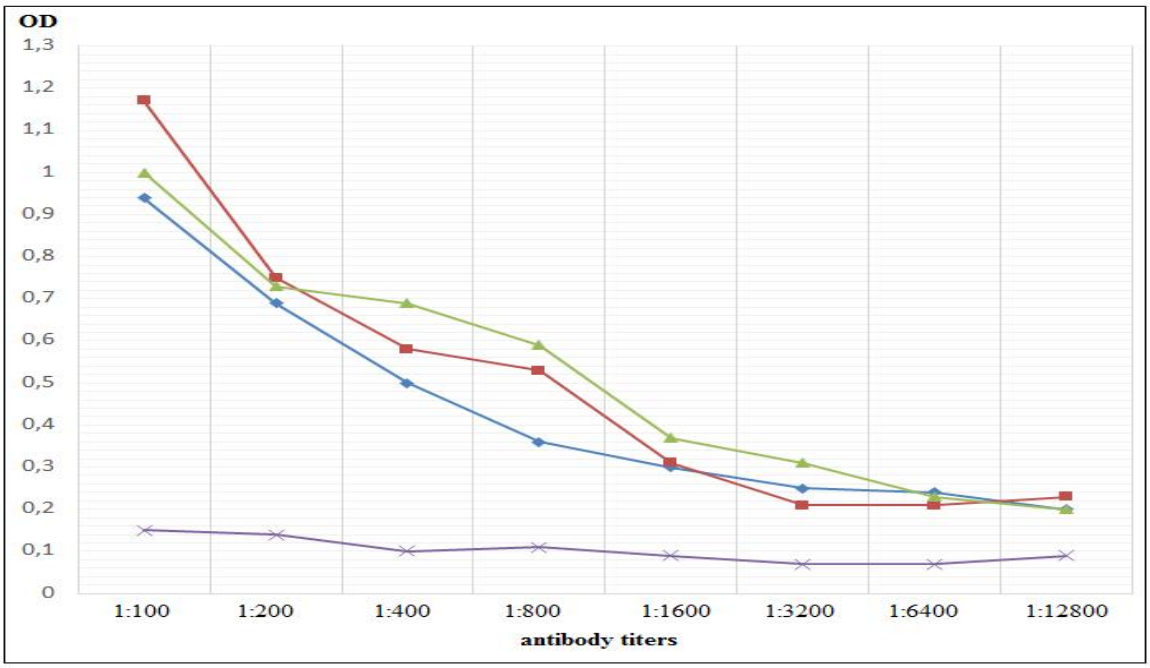
Western blot analyses. SDS-PAGE was performed as described above. The resolved proteins were transferred onto nitrocellulose membranes (Watman Nytran Supercharge Aldrich, USA) and immunoblotting was carried out as described previously [16]. Detection of specific protein bands of parasite's ES-Ag was performed with serum of infested dog and/or hyperimmunized rabbit.

Research results

The antigenicity of adult *E.granulosus* ES-Ag was identified by indirect ELISA using sera samples of experimental dogs (Fig.1).

Fig. 1 shows that imaginal echinococcus *ES-Ag* possesses expressed antigenicity to antibodies of infected dogs. Thus, antibody titer of the experimental animals specific for the antigen achieved 1: 1600-1: 3200 by the 12^{th} d.p.i. and it was maintained at this level until 35^{th} d.p.i.

It should be noted that the medium antibody titer of infected dogs' sera was somewhat higher against *E.granulosus* protoscoleces ES-Ag than that of eponymous antigen of adult tapeworm. For example, on the 35th d.p.i. 3 dogs showed antibody titer against metabolites of protoscoleces in the range of 1: 3200 - 1: 6400, while specific antibodies in the sera of the other animals were detected up to dilution of 1:12 800 -1:102 400.



- 12th d.p.i.; - 30th d.p.i.; - 35th d.p.i.; - before infection

Fig.1- The antigenicity of adult *E.granulosus* ES-Ag by ELISA

In the group of dogs, infected with hydatigenic teniasis, antibody titers against the homologous tapeworm ES-Ag in the course of serological assay (12th, 30th and 35th d.p.i.) by ELISA had a clearly expressed tendency to growth- 1: 6400 1: 25,600 and 1: 51,200, respectively. It was found that antibodies against *T. hydatigena* weakly cross-reacted with *E.granulosus* protoscoleces ES-Ag and eponymous antigen of adult worms up to titer of 1: 200 and 1: 400, respectively.

The specificity of anti-echinococcus sera obtained from experimental dogs was studied by using ES-Ag of larval and adult forms of tapeworms (Table 1).

Table 1 - The specificity of antisera of dogs infected with echinococcosis

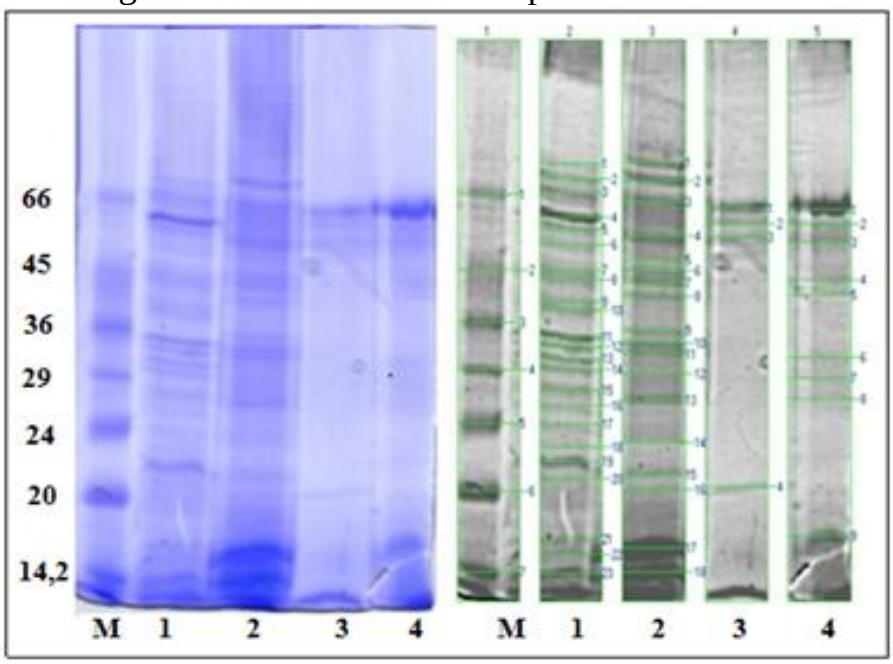
ES-Ag of worms	The titer of anti-echinococcus sera				
	№1	№2	№3	№4	№5
Adult *E. granulosus*	1:3200	1:6 400	1:3200	1:3200	1:12 800
E. granulosus protoscoleces	1:102 400	1:102 400	1:25 600	1:102 400	1:102 400

T. hydatigena	1:800	1:800	1:400	1:400	1:800
C. tenuicolis	1:102 400	1:102 400	1:25 600	1:12 800	1:102 400
Note - Each serum was tested fivefold. The table shows the predominant titers.					

The data of Table 1 demonstrate that anti-echinococcus sera reacted equally with larval ES-Ag of two tapeworms. However, antisera of dogs infected with echinococcosis allowed to differentiate imago metabolite products of these closely related taeniid species. For instance, antibody titers of infected dogs against homogeneous adult parasite's ES-Ag were within 1: 3200 - 1: 12800, while positive ELISA results to the same antigen of *T. hydatigena* were registered in a considerably lower titers (1:400-1:800).

The protein composition of the antigenic preparations is represented in Figure 2.

Twenty three protein fractions with the molecular weights (mol.w.) from 14 kD to 75 kD were detected in the electrophoregram of protoscoleces ES-Ag, while the same antigen of adult parasite had a total of 18 bands with mol.w. from 14.0 kD to 75 kD. Three proteins with the mol.w.: 75kD; 70kD and 33 kDa were common to both stages of echinococcus development.

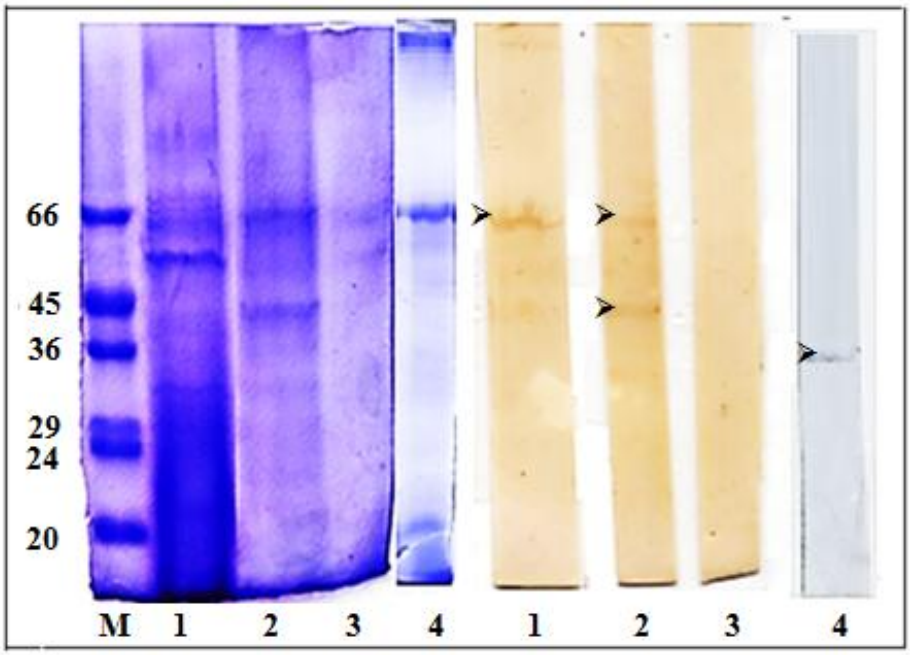


M- Marker proteins; 1-*E.granulosus* protoscoleces ES-Ag; 2- *E.granulosus* imago ES-Ag; 3- *E.granulosus* SA; 4-*T.hydatigena* ES-Ag.

Fig.2 – Electrophoregram of tapeworms' ES-Ag

In the protein profile of adult *E.granulosus* SA only 4 bands are found. Moreover, two protein fractions having mol.w. 57.0 kD and 20 kD are also identified in the composition of protoscoleces ES-Ag. The protein spectrum of ES-Ag *T.hydatigena* consisted of 9 bands (17 kD-61 kD). Among them 5 fractions have been also found in the electrophoregram of homologous *E.granulosus* antigen.

Western blot analysis of the protoscoleces and/or adult *E. granulosus* antigens by blood serum of infected dog is shown in Figure 3.

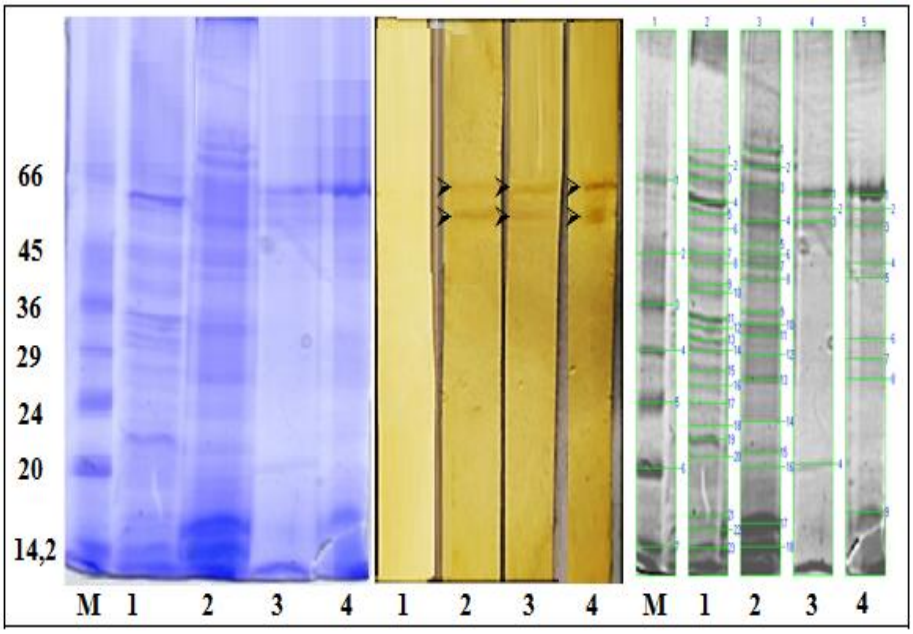


M- Marker proteins; 1-Protoscoleces ES-Ag; 2-Imago ES-Ag; 3-Imago SA; 4-*T.hydatigena* ES-Ag

Fig.3 - Western blot analysis of *E.granulosus* antigens

Western blot results showed that among the proteins of protoscoleces ES-Ag only one fraction with a mol.w.of 64 kDa possesses antigenicity, whereas the protein bands of eponymous antigen of adult worm with the mol.w. of 64 kDa and 43 kDa had the ability to bind specific antibodies. Antibodies of echinococcosis infested dog cross-reacted with the protein of *T.hydatigena* ES-Ag having a mol.w.of 36 kDa. It is important to note that the given protein fraction was not detected in electrophoregram.

Immunoblotting on the nitrocellulose membrane using replicas of tapeworms' antigens and serum of dog, infected with hydatigenic teniasis, showed the antigenicity of the protein fractions of *T.hydatigena* ES-Ag with the mol.w. of 57 kDa and 52 kDa (Figure 4).



M-Marker proteins; 1-*E. granulosus* protoscoleces ES-Ag; 2 -*E.granulosus* imago ES-Ag;3 - *E.granulosus* SA; 4-*T.hydatigena* ES-Ag

Fig.4- Western blot analysis of tapeworms' antigens

As can be seen from Fig.4 anti-taenia antibodies had the specificity to the similar protein bands of *E.granulosus* SA and/or ES-Ag.

The results of studying immunogenicity of *E. granulosus* protoscoleces ES-Ag for rabbit and specificity of obtained antiserum are shown in Table 2.

Table 2 shows that protoscoleces ES-Ag caused strong immune response in the form of antibody production. For example, antibodies specific to homologous antigen were detected up to a titer of 1: 819 200. The activity of antiserum against antigen of adult worm was considerably lower (1:51 200). It is necessary to note that antiserum obtained by the used scheme allows to differentiate *E.granulosus* protossoleses ES-Ag from analogous antigens of *C. tenuicolis* (1: 6400) and *T. hydatigena* (1:25 600).

Table 2- The immunogenicity of *E. granulosus* protoscoleces ES-Ag and specificity of antiserum by indirect ELISA

Dilutions of antiserum	Antigens used for coating solid phase							
	E. granulosus protoscoleces ES-Ag		Adult *E. granulosus* ES-Ag		*C. tenuicolis* ES-Ag		*T. hydatigena* ES-Ag	
	antiserum	negative serum	antiserum	negative serum	antiserum	negative serum	antiserum	antiserum
	The mean OD of the wells, 492 nm*							
1:100	1.417	0.011	0.735	0.011	0.115	0.006	0.533	0.015
1:200	1.309	0.010	0.486	0.012	0.193	0.014	0.587	0.028

1:400	1.278	0.005	0.553	0.013	0.193	0.014	0.499	0.043
1:800	1.237	0.007	0.598	0.014	0.153	0.025	0.367	0.025
1:1600	1.191	0.011	0.508	0.014	0.118	0.017	0.248	0.017
1:3200	1.069	0.002	0.342	0.017	0.076	0.018	0.167	0.010
1:6400	0.856	0.007	0.303	0.019	0.045**	0.034	0.111	0.029
1:12800	0.774	0.010	0.209	0.021	0.031	0.023	0.078	0.057
1:25600	0.265		0.153		0.016		0.065**	
1:51200	0.218		0.051**		0.024		0.043	
1:102400	0.162		0.061		0.020		0.031	
1:204800	0.133		0.041		0.023		0.019	
1:409600	0.065		0.030		0.019		0.015	
1:819200	0.039**		0.073		0.026		0.013	
Notes: *-The table shows the average OD of the three assays; ****** - OD indicating antibody titer								

The immunogenicity of *E. granulosus* ES-Ag and specificity of rabbit serum antibodies are shown in Table 3.

Table 3- The immunogenicity of adult echinicoccus ES-Ag and antiserum specificity by indirect ELISA

Dilutions of antiserum	Antigens used for coating the solid phase							
	E.granulosus protoscoleces ES-Ag		Adult *E.granulosus* ES-Ag		*C. tenuicolis* ES-Ag		*T.hydatigena* ES-Ag	
	antiserum	negative serum	antiserum	negative serum	antiserum	negative serum	antiserum	antiserum
	The mean OD of the wells, 492 nm*							
1	2	3	4	5	6	7	8	9
1:100	0.658	0.014	0.879	0.015	0.282	0.030	0.370	0.008
1:200	0.591	0.015	0.871	0.014	0.217	0.011	0.391	0.010
1:400	0.487	0.007	0.823	0.011	0.171	0.012	0.313	0.007

Continuation of Table 3

1	2	3	4	5	6	7	8	9
1:800	0.406	0.005	0.591	0.012	0.140	0.013	0.263	0.006
1:1600	0.213	0.011	0.568	0.012	0.120	0.013	0.200	0.007
1:3200	0.117	0.009	0.396	0.009	0.075	0.024	0.142	0.004
1:6400	0.129	0.003	0.246	0.016	0.077	0.018	0.110	0.013
1:12800	0.102	0.017	0.227	0.015	0.060**	0.029	0.071	0.016
1:25600	0.050		0.081		0.022		0.052	
1:51200	0.041**		0.062		0.022		0.029**	
1:102400	0.035		0.042		0.023		0.020	
1:204800	0.024		0.031		0.015		0.017	

1:409600	0.021		0.084		0.015		0.018	
1:819200	0.015		0.040**		0.015		0.010	
Notes: * - The table shows the average OD of the three assays; ** - OD indicating antibody titer								

As can be seen from Table 3, *E.granulosus* ES-Ag also indicated its high immunogenicity for rabbit immune system, and at the same time obtained antiserum allowed to distinguish of echinococcus from closely related taeniid species - *T.hydatigena.*

In order to identify immunogenic protein fractions the electrophoretogram of tapeworms' antigens was transferred to a nitrocellulose membrane and stained immunochemically using antiserum of rabbit immunized with adult echinococcus ES-Ag. The results of Western blot analyses showed that in the structure of parasite's antigens (protoscoleces and/or *E.granulosus* ES-Ag, *E.granulosus* SA and *T.hydatigena* ES-Ag) there are two common fractions with the mol.w. of 40 kD and 29 kD possessing immunogenicity for rabbits. It should be noted, the latter protein band in the electrophoregram of *E. granulosus* SA did not stained but it was detected by immunoblotting. Western blot allowed to reveal immunogenic protein in the composition of adult *E.granulosus* ES-Ag with a mol.w. of 64 kD. The protein with a mol.w. of 22 kD , that is common for all used echinococcus antigens, has also possessed immunogenicity.

Discussion and conclusions

The research results indicate a high diagnostic value of protoscoleces and/or adult *E. granulosus* ES-Ag that are synthesized *in vitro*. Antigenicity of these preparations in relation to antibodies of experimentally infected dogs had been gradually strengthened with the development of invasive process, and this feature was better expressed by protoscoleces ES-Ag.

As expected, anti-echinococcus sera cross-reacted with the antigens of closely related taeniid species - *T. hydatigena*. It should be emphasized, that anti-echinococcus sera reacted equally with larval ES-Ag of two tapeworms. At the same time, antibodies of dogs infected with echinococcosis allowed to differentiate ES-Ag of adult parasites. Perhaps, in the course of ontogenetic development of worms substantial changes occur in the antigen composition of the metabolite products. Apparently, the adult helminths begins to produce species specific antigens at reaching their imaginal stage.

T.hydatigena ES-Ag was also characterized by the antigenicity property that intensified with the development of the disease. It is noteworthy that the cross-reaction of anti-taenia serum with echinococcus antigens was expressed much weaker than vice versa. Apparently, *T.hydatigena*, as the largest tapeworm of dogs, has a more complex metabolite composition which leads to weakening of the immune response to common antigens. Thus, adult *E. granulosus* ES-Ag, derivated from the culture medium, can be used in serological diagnosis of canine echinococcosis.

ES-Ag of larval and adult echinococcus have the protein fraction 64 kD that possess antigenicity towards canine antiserum. This protein was also immunogenic for a rabbit. Only 43 kD protein of adult parasite expressed antigenicity for canine antibodies. Both species of adult tapeworms showed the presence of cross-reactive proteins 52 kD and 57 kD in their ES-Ag. It is interesting to note that the protein 22 kD which common for larval and adult forms of echinococcus, as well as for its SA, proved to immunogenic for the rabbit, although it was not detected by anti-serum of infected dog. Apparently, this phenomenon can be explained by species related traits of the immune response to echinococcus antigens. The obtained results demonstrate the possibility of isolation of proteins specific for *E. granulosus* and using them for improving serological diagnosis of the disease.

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Түйін

Бұл жұмыста эхинококкозбен инвазияланған иттерді анықтауға арналған тест-жүйесін әзірлеуде қолдануға болатын ақуыздарды анықтау мақсатында, *Echinococcus granulosus* экскреторлы-секреторлық өнімінің иммуногенділігі мен антигенділігі зерттеу нәтижелері келтірілген. Иттер екі топқа бөлініп, ет комбинатында қойларды сойғанда алынған *E.granulosus* және *Cysticercus tenuicolis* тірі протосколекстерімен – *Taenia hydatigena* балаңқұрттарымен пероральді жолмен жұқтырылды. *In vivo* жағдайында жиналған протосколекстік және ересек формалы *E.granulosus* экскреторлы-секреторлық антигендері (ЭС-Аг), эхинококкозбен жұқтырылған иттердің қан сарысуларына антигенділік танытқаны анықталды. Екі цестодалардың балаңқұрттарының ЭС-Аг айқын телімсіз байланыс көрсетті, имаголық антигендермен салыстырғанда *T.hydatigena* ЭС-Аг де сипатталған антигенділік, инвазиялық үрдіс дамыған сайын күшейе түсті. Анти-тенииоздік қан сарысуы эхинококк антигендерімен телімсіз байланысы әлсіз болды, анти-*E.granulosus* сарысуының *T.hydatigena* мен телімсіз байланысына қарағанда. Екі гельминттің экскреторлы-секреторлық антигендерінің қояндарға иммуногенділігі бар ақуыз фракциялары анықталды.

Summary

Antigenicity and immunogenicity of *Echinococcus granulosus* excretory-secretory antigen (ES-Ag) were studied to identify proteins that can be used in the development of test-system for the detection of dogs infested with echinococcosis. Five and/or two dogs were infected orally with viable *E.granulosus* protoscoleces and/or *Cysticercus tenuicolis*, obtained from infested sheep at a slaughterhouse. ES-Ag of *E.granulosus* protoscoleces and its adult form, obtained *in vitro*, possessed pronounced antigenicity towards antisera of infected dogs. Anti-echinococcus sera reacted equally with larvae ES-Ag of two closely related tapeworms. At the same time, antibodies of dogs infected with echinococcosis allowed to differentiate ES-Ag of adult helminths. *Taenia hydatigena* ES-Ag was also characterized by the antigenicity that intensified with the development of the invasion process. It was found that anti-*T. hydatigena* serum cross-reacted with adult echinococcus significantly weaker than vice versa. The protein fractions of the tapeworms that are immunogenic for rabbits were establashed.

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