

EFFECTIVENESS OF SHEEP CRUDE HYDATID CYST FLUID (CHCF) ANTIGEN FOR SERODIAGNOSIS OF HUMAN, SHEEP, MICE AND CATTLE HYDATIDOSIS

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ABSTRACT: The aim of the current study was to assess the usefulness of sheep crude hydatid cyst fluid antigen (CHCF) of *Echinococcus granulosus*, obtained from sheep naturally infected for evaluation of antibody responses of human, mice, sheep and cattle IgG responses. Hydatid cysts obtained from lungs and livers of sheep slaughtered from local abattoirs in Ilam, Iran was used as the source of antigen either for the SDS, Western blotting or ELISA analysis or for experimentally infecting the mice. HCF recovered from cysts was centrifuged at 10000 rpm for 25 minutes. The supernatant was dialysed against PBS, freeze-thawed and used as crude CHCF. There was an increasing in IgG4 subclass level compared to total IgG, IgG2 and IgG3 subclass responses against sheep CHCF. In this analysis all antibody class and subclass responses showed a higher mean OD than that of the cut off indicating that all human sera from patients with hydatidosis recognized reacted with sheep crude hydatid cyst fluid antigen at an acceptable level. The sensitivity and specificity of ELISA using IgG class and IgE, was 100% and 70.8% and also 93.3% and 100% respectively while for the IgG2, IgG3 and IgG4 it was 100%, 93.8%; 95, 100% and 100, 100% respectively. HCFs obtained from sheep were significantly useful in serodiagnosis of hydatidosis similar to that of human and mice and therefore can be used in hydatid cyst diagnosis kit using ELISA and immunoblotting.

INTRODUCTION

The *Echinococcus*' main intermediate host is the sheep and the parasite has as point of localization, the liver and the lungs. *Echinococcus granulosus* which has a worldwide distribution and is responsible for 95% of echinococcosis cases in humans worldwide is estimated to have more than 3 million global cases ([Fotiou et al., 2012](#)). In order to understand how the immune system deals with these parasites it is important to evaluate the immune response of different hosts against the most accessible antigens of such parasites ([Zhang et al., 2003](#); [Khosravi et al., 2012a](#); [Khosravi et al., 2012b](#)). As parasite can habituate in different organs like lung, heart, brain, liver, spleen and spinal cord in asymptomatic form for a period of 20 years, therefore, the diagnosis of disease is so difficult and usually is based on para-clinical methods like serology ([Golassa et al., 2011](#)). As the disease is dangerous and surgery is the only way for the treatment of this disease therefore definitive and immediate diagnosis of hydatidosis is vital for the hosts ([Gulalp et al., 2007](#)). The use of crude hydatid (HCF) cyst fluid antigen for the diagnosis of cystic hydatid (CH) is one method which along with immediate serological investigation can be helpful and effective in rapid treatment of the disease ([Vuitton and Wen, 2007](#)). Rapid and definite diagnosis is really important not only in human but also in animals. To achieve such a goal using the HCF antigens of different hosts including human and animals can be used to evaluate the diagnosis of hydatid cyst as it was reportedly confirmed that the HCF from a given host (human) shows a relatively stronger positive reaction ([Zhang et al., 2003](#)). If serological diagnosis can be based on the use of some highly valid and reliable antigens of different animals, designing the diagnostic kit with such antigens can be a simple, non expensive and available method. Most of the serological tests used in diagnosing the

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hydatid cyst have their own problems like limited availability, different sensitivity and specificity and difficulty in their preparation. Some of these tests need several specific techniques, equipments and experienced personnel. In many countries, the materials, reagents and equipment to perform the IgG-ELISA are readily available, and this technique is probably the best overall choice for use in immunodiagnosis for human CE. Antigens have different fractions e.g. antigen B has 8, 16, 24 and 38 KD with different sensitivities in diagnosis of the disease ([Hernandez-Gonzalez et al., 2012](#); [El-Zayyat et al., 1999](#); [Celik et al., 2009](#)). Also these fractions have different sensitivities in each animal as well, therefore finding an antigen with a fraction which its sensitivity and specificity is high in response to the sera of human or other animals can be a considerable progress in developing a reliable and non-expensive method for diagnosis of HC. In addition there is a good trial to test the reactivity of human, mice and sheep sera to mice HCF antigens in order to analyze such a hypothesis for animals too. The current study was designed to find antibody responses in sera of human, mice, cattle and sheep against the sheep HCF antigens compared to other antigens in order to obtain a better evaluation of this antigen for diagnostic purposes in human and animal hydatidosis.

MATERIALS AND METHODS

This is an analytical case-control study using human crude HCF as the source of antigen for performing ELISA and Western blotting. Sample sera used in present work were collected from patients who recently had hydatid surgery in hospitals of Tehran, Hamadan and Ilam cities as human case group together with some human or animal sera with no history of hydatidosis with negative HCF using ELISA and IFAT as control group. Briefly, the required antigen was extracted and prepared from human hydatid fluid cysts. HCF recovered from cysts was centrifuged at 10000 rpm for 25 minutes. The supernatant was dialysed against PBS, freeze-thawed and used as crude CHCF. 30 positive samples sera from human sources together with 30 mice, sheep and cattle were used as the case together with 30 healthy sera as control group. Hydatid cyst fluid antigen preparation was carried out according to the procedure described by Mamuti, et al, with slight modifications. ELISA method was carried out as described by Verastegui.

RESULTS

3.1. ELISA analysis results

3.1.1. The human antibody responses to sheep crude hydatid cyst fluid antigen (CHCF)

As the mean OD of human IgG class and subclass (IgG2, IgG3, IgG4) together with human IgE against sheep CHCF was evaluated there was an increasing in IgG4 subclass level compared to total IgG, IgG2 and IgG3 subclass responses. In this analysis all antibody class and subclass responses showed a higher mean OD than that of the cut off indicating that all human sera from patients with hydatidosis recognized reacted with sheep crude hydatid cyst fluid antigen at an acceptable level (Table 1). ANOVA analysis showed that the differences between human antibody responses against the sheep antigen was statistically significant ($P < 0.0001$). The sensitivity and specificity of the test using IgG class and IgE, was 100% and 70.8% and also 93.3% and 100% respectively while for the IgG2, IgG3 and IgG4 it was 100%, 93.8%; 95, 100% and 100, 100% respectively.

Table 1: Mean OD levels of human IgG class and subclass and IgE against CHCF of sheep

OD	Mean	SD	Minimum	Maximum	Number	Range	Cut off
IgG	0.26	0.06	0.08	0.33	30	0.26	0.12
IgG2	0.21	0.1	0.08	0.42	30	0.33	0.08
IgG3	0.25	0.16	0.03	0.71	30	0.68	0.11
IgG4	0.7	0.12	0.51	0.87	30	0.36	0.14
IgE	0.31	0.08	0.22	0.48	30	0.26	0.12

3.1.2. The sheep antibody responses to sheep crude hydatid cyst fluid antigen (CHCF)

All 30 sheep sera had IgG titer higher than the cut off indicating the positivity of sera against CHCF of sheep (Table 2) as was seen for the sheep sera against the other CHCF of the other origins. The highest mean OD value of sheep IgG was seen against the mice and sheep CHCF respectively (Figure 1). The sensitivity and specificity of the ELISA for these antibodies was 100 and 100% respectively.

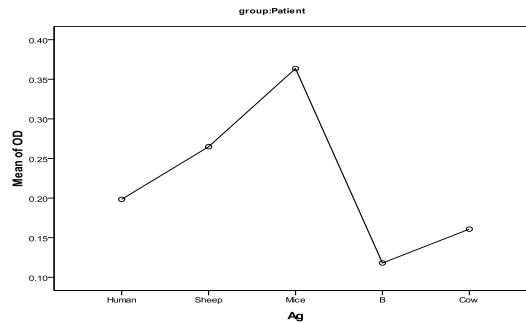


Figure 1: Sheep IgG responses to CHCF of human, sheep, mice, B and cow.

Table 2: Mean OD levels of sheep IgG class against CHCF of Balb/ mice

OD	Mean	SD	Minimum	Maximum	Number	Range	Cut off
IgG	0.26	0.02	0.23	0.3	30	0.07	0.06

3.1.3. The mice antibody responses to sheep crude hydatid cyst fluid antigen (CHCF)

The highest response belonged to mice IgG2b against the sheep CHCF though the IgG, and IgE also strongly showed higher titer than that of the cut off (Table 3). ANOVA showed significant differences for the sheep antibody responses against the mice antigens. The sensitivity and specificity of ELISA for all antibodies except for the IgGAM that was 50 and 60% respectively, was 100%.

Table 3: Mean OD levels of mice IgG class against CHCF of sheep

OD	Mean	SD	Minimum	Maximum	Number	Range	Cut off
IgG	0.3	0.04	0.25	0.37	30	0.12	0.06
IgG2b	0.61	0.24	0.25	0.89	30	0.64	0.11
IgE	0.26	0.06	0.16	0.36	30	0.19	0.04
IgGAM	0.3	0.12	0.21	0.61	30	0.4	0.27

3.1.4. The cattle antibody responses to sheep crude hydatid cyst fluid antigen (CHCF)

The mean OD level of IgG antibody in cattle sera samples against the sheep CHCF was not higher than that for the cut off.

3.2. SDS analysis results

The SDS gel electrophoresis showed that the mice CHCF has fractions of 6, 20, 24, 26, 29, 45, 55, 66 and 116 kD which is mostly similar to fractions of the human CHCF antigen (Figure 2).

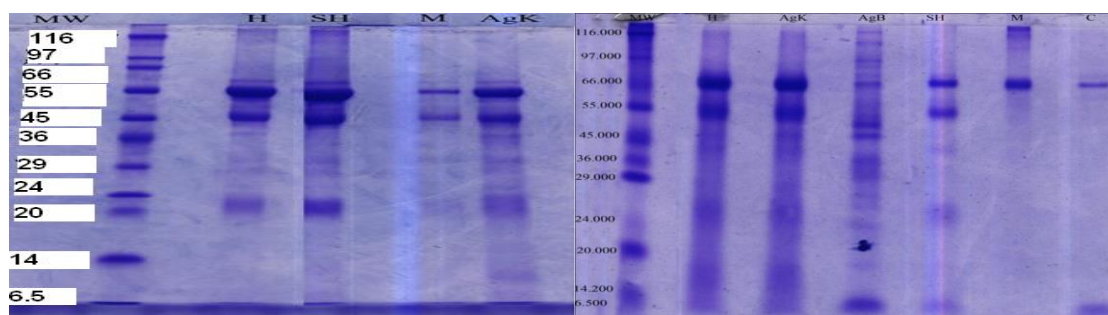


Figure 2: Gel electrophoresis SDS results of different antigens.

MW (molecular weight), H (human), AgK (antigen of diagnosing kit of hydatidosis), SH (sheep), M (mice), c (cow).

3.3. Western blotting analysis results

Sera of sheep showed a significant response against human, sheep and mice CHCF in western blotting analysis (Figure 3). These sera mostly detected a 66 kD fraction in all three antigens.



Figure 3: IgG antibody responses of sheep against human, sheep, antigen B, mice and cow CHCF. H(human), SH(sheep), Ag(Antigen B), M(mice), AgK(antigen of hydatid cyst diagnosing kit), C(cow).

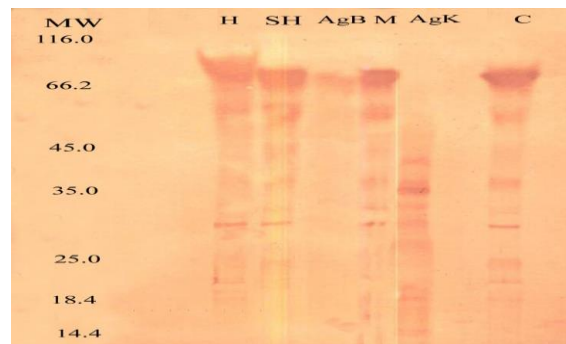


Figure 4: IgG antibody responses of human against human, sheep, antigen B, mice and cow CHCF. H (human), SH (sheep), Ag (Antigen B), M (mice), AgK (antigen of hydatid cyst diagnosing kit), C (cow).

For the human IgG antibody against sheep CHCF antigen, the results of the western blotting analysis showed a strong reaction against this antigen at 66 kD as was seen for the human, antigen B, and cow CHCF though some reactions was also seen at 35 and 25 kD (Figure 4).

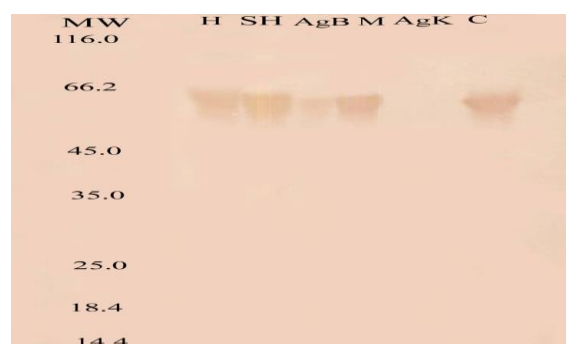


Figure 5. IgG antibody responses of mice against human, sheep, antigen B, mice and cow CHCF. H (human), SH (sheep), Ag (Antigen B), M (mice), AgK (antigen of hydatid cyst diagnosing kit), C (cow).

Western blotting analysis with mice serum also recognized human, sheep, antigen B and cow CHCF (Figure 5) mostly at 66 kD. Human IgE responses also recognized human, sheep, antigen B and mice CHCF at the same molecular weight indicating a similarity of human and sheep reactions to different antigens (Figure 6).



Figure 6. IgE antibody responses of human against human, sheep, antigen B, mice and cow CHCF. H (human), SH (sheep), Ag (Antigen B), M (mice), AgK (antigen of hydatid cyst diagnosing kit), C (cow).

DISCUSSION

As there are some strong cross-reaction between the human, sheep, mice and cattle antibodies mostly at 66 kD fraction of CHCF antigen there is a considerable usefulness of the sheep CHCF for evaluation the sera of different hosts against hydatid cyst. Where mice IgG2b was used it also recognized antigen of sheep CHCF at either 66 or 116 kD which was similar to the reaction of human total IgG, and IgG class . In other words the highest titer of most antibodies studied here was seen against the 66 kD fraction of most antigens such as sheep CHCF antigen. Although the reaction of most of these antibodies was strongly directed against the 66 kD fraction of sheep but some subclass antibodies such as IgG4 showed a stronger response against this fraction than the others. This result was similar to that found by other authors ([Celik et al., 2009](#); [Siracusano et al., 2004](#); [Khabiri et al., 2006](#); [Wen and Craig, 1994](#); [Dreweck et al., 1997](#); [Grimm et al., 1998](#)). Having this interesting result it can be concluded that human IgG4 antibody can be used as a suitable candidate for the diagnosing and research purposes where the CHCF of sheep is being used. In order to be able to discuss the intensity of the reaction, we calculated a ratio for each antibody by dividing the mean OD value of it against the sheep CHCF into the mean OD value of its cut off. This ratio for sheep IgG against human CHCF was about 3 and for the sheep IgG against sheep CHCF about 4.3 indicating the preference of sheep antigen over the human for analyzing of sheep hydatidosis. Such ration pattern was also seen for the human IgG against human CHCF with 10.5 and human IgG against the sheep CHCF which was 5. We observed also the highest sensitivity and specificity of either human or sheep antibody against the sheep CHCF using ELISA and western blotting and this can confirm the suitability of sheep antigen for diagnosing and research purposes though the others like human and mice are appropriate too. Our results for the human IgG and IgE responses against the CHCF are in accordance with the results of [Sbihi et al., \(1997\)](#) and [Grimm et al., \(1998\)](#). ANOVA analysis also found significant differences for the IgG and IgG4 responses to sheep CFCH compared to the other antibodies again confirming these antibodies as good candidate antibodies for evaluating the hydatidosis.

CONCLUSION

We concluded that the cross reaction of antibodies of human and animals against the sheep CHCF is obviously existed and sheep CHCF antigen is a good candidate for the hydatid cyst diagnosing in human, sheep, cow and even mice. The 66 kD fraction of this antigen is the best re-actable part of it. IgG total and IgG4 in human and IgG total in sheep and cow and IgG2b in mice are the best antibodies for evaluation or diagnosing purposes. ELISA and western blotting are reliable enough when using sheep CHCF antigen as the source of antigen in either research or diagnostic evaluation programs.

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