Iranian journal of ruminants health research (2017), 2(1): 1-7.
Doi: 10.22055/ijrhr.2017.14412

Acute-phase proteins and antioxidant status in cows naturally infected by hydatid cysts in the Razavi Khorasan Province

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Abstract

Parasitic infections can be extremely problematic for animals and humans. Host-parasite relationships may be different between species. The purpose of this study was to evaluate Haptoglobin (Hp), Serum amyloid-A (SAA), Alpha -1- acid glycoprotein (AGP), Albumin (ALB), Total protein (TP), Globulin (GLB), Malondialdehyde (MDA), and Total antioxidant capacity (TAC) following natural infection with hydatid cysts in cows. This study was undertaken in a slaughterhouse in Torbate-Heidarie, Razavi Khorasan province, Iran, in January 2016. Four hundred cows were examined. Clinical examination prior to slaughter was done by an expert veterinarian and all clinically healthy cows were used in the study. The age determination was done according to the dental formula. Blood samples were obtained before slaughtering. Eventually, 60 cows with hydatid cysts and 60 apparently healthy cows were included in the study and control groups, respectively. The results indicated that acute phase proteins were not changed in naturally infected cows with hydatid cysts. It could be concluded that host-parasite relationship might be different in species such as cows and sheep. This finding may be due to a difference between species or a different behavior of hydatid cysts in cows and sheep.

Keywords: Abattoir; Acute phase proteins; Antioxidant; Cow; hydatid cysts

Introduction

Health assessment in dairy cows is of great value. In this regard, the optimal care and well-being of dairy cattle, reduction of the losses in productivity caused by diseases, and management errors must be considered. Plasma concentrations

of some proteins will be changed in response to infections and injuries involving inflammation. This class of blood proteins is named Acute-phase proteins (APPs) (Jain et al., 2011). The concentration of APPs such as Haptoglobin (Hp), Serum amyloid-A (SAA), and Alpha -1- acid glycoprotein (AGP) will increase manifold in some inflammation and infectious diseases including clinical and subclinical mastitis (Nazifi et al., 2011), metritis (Huzzey et al., 2009), endometritis (Biswal et al., 2014), traumatic pericarditis, reticuloperitonitis, abomasal displacement (Tóthová et al., 2013), respiratory diseases (Nikunen et al., 2007), diarrhea (Ulutas et al., 2011), and surgically-treated abdominal disorders in dairy cows (Hirvonen and Pyörälä, 1998). The APPs contribute to restoring homeostasis in animals. Probably, the innate immune systems of animals could be areattributed to the mechanism of the APP response (Murata et al., 2004). APPs synthesis of the liver is triggered by different stimuli that lead to an increased serum concentration of positive APPs. Serum concentration of APPS returns to base levels when the triggering factor is no longer present (Petersen et al., 2004). In chronic inflammation and sepsis, stimulation of the liver to increase and decrease protein production may be different (Vary and Kimball, 1992). Parasitic infections are responsible for the condemned organs or carcasses of cows in the world and in Iran (Ahmadi and Meshkehkar, 2011). Iran is an important endemic region for hydatid cyst disease that is caused by infection with the Echinococcus granulosus metacestodes and affects both humans and livestock (Omidi et al., 2016). Cows ingest the ovum while grazing on contaminated ground. These parasites are usually considered after slaughtering and evisceration. Reactions of the cows to infection by Echinococcus granulosus have been poorly studied. In addition, there are interspecies variations in the pattern of host responses to the APPs (Eckersall, 1995).

The present study aimed to assess changes in some blood parameters such as haptoglobin (Hp), serum amyloid-A (SAA), alpha-1-acid glycoprotein (AGP), albumin (ALB), total protein (TP), globulin (GLB), malondialdehyde (MDA), and total antioxidant capacity (TAC) in cows following natural infection with Echinococcus granulosus metacestodes.

Materials and methods

The study was conducted in January 2016 at a slaughterhouse in Torbat-e Heydariyeh, which is located in the Razavi Khorasan province, 110 km southeast of Mashhad city (35°16′26″N 59°13′10″E). Four hundred cows were examined and their sex and age were recorded. Clinical examination prior to slaughter was done by an expert veterinarian and all clinically healthy cows were used in the study. The age determination was done according to the dental formula. Blood samples were obtained by jugular venepuncture into plain tubes and tubes containing EDTA (1.5 mg/1 ml) as an anticoagulant before slaughtering and serum or plasma was separated following the centrifugation of blood at 750g for 15 min at

room temperature. Samples were stored at -80°C until analysis. Evisceration was done after slaughtering and internal organs were thoroughly examined by visual inspection, palpation, and systematic incisions for the presence of any abnormalities related to Echinococcus granulosus metacestodes.

Biochemical analysis

The samples with hemolysis were discarded. Eventually, Blood samples of sixty cows with hydatid cysts and 60 apparently healthy cows were included in the study and control groups, respectively.

Acute phase proteins (Hp, SAA and Alpha-1-acid glycoprotein) determination

Serum amyloid A (SAA) was measured by a solid phase sandwich-ELISA method with a sensitivity of 0.156 pg/ml (cow-specific kit; Shanghai Crystal Day Biotech Co., LTD, Shanghai, China). Serum levels of Hp were measured using a quantitative sandwich enzyme immunoassay technique by a commercial cow-specific kit (Shanghai Crystal Day Biotech Co., LTD, Shanghai, China). Serum levels of AGP were determined by a commercial cow-specific kit (CUSABIO, China) using ELISA method and the assay sensitivity was reported to be 0.5 ng/ml.

Serum proteins measurement

Serum was analyzed for TP by Biuret method (Commercial kit; Pars Azmoon, Tehran, Iran) and for ALB by the bromocresol green method (Commercial kit; Pars Azmoon, Tehran, Iran). GLB was determined as the difference between serum TP and ALB. Biochemical analyses were done using a standard autoanalyzer (Alpha Classic Model, Sanjesh Diagnostic Company, Iran).

Measurement of malondialdehyde (MDA)

The modified HPLC method was employed to measure MDA. The final product was analyzed by UV spectrophotometer at 532 nm and values were finally expressed as mmol/L.

Measurement of total antioxidant capacity (TAC)

The commercial kit (Labor Diagnostika Nord (LDN) Com, Nordhorn, Germany) was employed to measure TAC. In the end, a color product of the chromogenic substrate (tetramethylbenzidine) appeared. The change in colour was measured calorimetrically at 450 nm and expressed as millimoles per liter (mmol/L).

Statistical analysis

The results were analyzed using SPSS (version 21.0). The Kolmogorov-Smirnov tests were used to verify that normal distribution of the variables. The means of two variables were analyzed using Student's t-tests or Mann-Whitney U-tests in the case of non-normal distributions. Pearson's correlation coefficients were used to analyze the relationship between the different variables. The significance level was set at $P< 0.05$.

Results

Hydatid cysts did not cause any significant changes in the SAA, Hp, AGP, ALB, TP, GLB, MDA, and TAC (Table 1). Table 2 shows the negative and strong interrelationships between MDA and TAC. The ALB and GLB had strong correlations with TP. ALB showed a moderate positive correlation with GLB.

Discussion

Hydatid cyst (cystic echinococcosis) caused by Echinococcus granulosus is an important medical and veterinary problem and a substantial cause of morbidity and mortality in many parts of the world (Craig, et al., 2007). Considerable economic losses in the livestock industries in most areas of Iran are attributed to Hydatid cyst (Ahmadi & Meshkehkar, 2011). The APPs in

Table 1: Evaluated physiological parameters (Independent Samples Test)

Group (n)/ Variable (unit)	Hp (ng/ml)	SAA (µg/ml)	TAC (mmol/l)	MDA (mmol/l)	AGP (pg/ml)	TP (g/dl)	ALB (g/dl)	GLB (g/dl)
Hydatid (60)	0.10±0.04	10.67±4.52	1.32±0.08	5.09±0.13	3.90 ±0.91	7.07±0.47	3.10±0.24	3.97±0.29
Healthy (60)	0.11±0.05	10.21±4.20	1.33±0.06	5.08±0.11	4.07±0.78	7.03±0.42	3.07±0.19	3.96±0.31
P value	NS	NS	NS	NS	NS	NS	NS	NS

Table 2: Intercorrelations (Pearson's) between evaluated physiological parameters

		1	2	3	4	5	6	7
1	Hp	1						
2	SAA	-0.032	1					
3	TAC	-0.033	-0.042	1				
4	MDA	0.041	0.070	**-0.981****	1			
5	AGP	0	0.036	0.123	-0.078	1		
6	TP	0.022	-0.071	-0.098	0.077	-0.002	1	
7	ALB	0.041	-0.033	-0.082	0.069	0.020	**0.805****	1
8	GLB	0.002	-0.081	-0.086	0.064	-0.018	**0.900****	**0.467****

ruminants are poorly described (Ceciliani et al., 2012). It should be noted that reactions of the cows to infection by Echinococcus granulosus have been poorly studied.

Production of some APPs such as SAA, Hp and AGP by the liver occurs following the response of the host's body to inflammation (Gruys et al., 2005).

In the present study, no significant changes were found in the SAA, Hp, AGP, ALB, TP, GLB, MDA, and TAC. In a study by Sevimli et al. (2015) on a group of twenty-seven cows with hydatid cysts and eight apparently healthy cows, an increase in SAA was seen (SAA level of 7.51 ± 0.41 μg/ml, V.S. 4.84 ± 0.51 μg/ml in the infected and control group, respectively), but the serum Hp level of cows with hydatid cysts were found to have decreased (Hp level of 2.08 ± 0.65 ng/ml, V.S. 3.87 ± 0.91 ng/ml in the infected and control group, respectively). In the present study, sixty cows with hydatid cysts and sixty apparently healthy cows were evaluated. In the infected and healthy group of cows, the serum level of SAA was found to be 10.67 ± 4.52 μg/ml, V.S. 10.21±4.20 μg/ml. respectively). The serum level of Hp in cows with hydatid cysts was 0.10±0.04 ng/ml while in the healthy cows, it was 0.11±0.05 ng/ml. In a study on twelve goats with mixed helminth infections, elevated levels of SAA and Hp were found in the infected group (Ulutaş et al., 2007). Increases in the SAA, Hp, AGP TP, GLB, MDA and decreases in the ALB and TAC were found in a study conducted on 70 sheep with hydatid cysts against 65 healthy sheep (Omidi et al., 2016). In the present study, probably, the longtime exposure to parasites may be the reason for some adaptation to the existence of hydatid cysts. Another hypothesis refers to the difference between biochemical parameters within hydatid cyst fluids of cows and other animals. Cystic fluids of different host origins may be different in the concentration of some parameters (Shaafie et al., 1999). Immunology, metabolism, and physiology of Echinococcus granulosus may alter with biochemical substances within hydatid cysts (Radfar & Iranyar, 2004). Radfar et al. (2012) stated that biochemical composition of hydatid cyst fluids was not related to the cyst location. Alterations in the metabolism of Echinococcus granulosus in different species of intermediate hosts may lead to parasite survival in different conditions (Radfar & Iranyar, 2004). The APPs response is non-specific and it develops after an injury, regardless of the pathogenic factor. It must be noted that the function of most APPs has not been totally elucidated (Gruys, et al., 2005).

It is concluded that host-parasite relationship might be different in species such as cows and sheep. These findings are strongly different with the changes of acute phase proteins in naturally infected sheep with hydatid cysts in a previous study (Omidi et al., 2016). This finding may be due to the difference between species or different behaviors of hydatid cysts in cows and sheep. More actions are recommended as how to control the disease in Iran. These include the treatment of stray dogs, doing epidemiological

studies, along with finding new diagnostic methods.

Acknowledgments

The authors gratefully acknowledge the financial support for this work provided by the Vice Chancellor for Research at Shiraz University.

Conflict of interest

The authors declare that they have no conflict of interest.

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