Veterinary Parasitology 195 (2013) 131–135

Contents lists available at SciVerse ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Oxidant/antioxidant status in cattle with liver cystic echinococcosis

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ARTICLE INFO

Article history:
Received 26 November 2012
Received in revised form 20 January 2013
Accepted 22 January 2013

Keywords:
Antioxidants
Oxidative stress
Cattle
Cystic echinococcosis

ABSTRACT

The objective of the present study was to evaluate the changes of antioxidants and oxidative stress markers in cattle with cystic echinococcosis (CE). Thirty cattle with liver CE along with 30 healthy cattle were used for the study. Parasitized cattle presented a significantly higher lipid peroxidation assessed by the malondialdehyde (MDA) compared with healthy animals ($P<0.05$). A significantly lower erythrocyte superoxide dismutase (SOD) and glucose 6-phosphate dehydrogenase (G6PD), and a significantly higher erythrocyte glutathione peroxidase (GPx) in the parasitized group, were observed when compared with healthy group ($P<0.05$). No significant differences were observed for serum total antioxidant status (TAS), zinc, copper and iron between parasitized and healthy groups. The results obtained in this study suggest that CE in cattle induces changes in the activity of antioxidant enzymes. These changes render host cells susceptible to oxidants and exaggerate the generation of free radicals with a consequent lipid peroxidation enhancement.

1. Introduction

Echinococcosis is a zoonotic infection caused by cestode species of the genus Echinococcus. Cystic echinococcosis (CE), caused by *Echinococcus granulosus*, has shown that occur on all continents and in at least 100 countries (Eckert and Deplazes, 2004; Borji et al., 2012a). CE in farm animals causes considerable economic problems due to productions decrease and loss of the edible liver (Eckert and Deplazes, 2004; Borji and Parandeh, 2010; Borji et al., 2012b). Although there are two species of echinococcus that are found in cattle, only *E. granulosus* (G1) has been found in Iranian cattle (Harandi et al., 2002; Sharbatkhori et al., 2010).

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are formed as products of normal cellular metabolism. At low to moderate concentrations, nitric oxide (NO) and superoxide (O_2^-) free radicals are involved in signal transductions between cells. When generated at higher concentrations in disease states, these free radicals (and even more potent oxidative metabolites that they produce) can overwhelm protective systems within the body, producing cellular injury and/or destruction (Harvey, 2010). Oxidative stress resulting from increased production of ROS and RNS, and/or a decrease in antioxidant defense, leads to damage of biological macromolecules and disruption of normal metabolism and physiology (Trevisan et al., 2001). Oxidative stress plays a crucial role in the pathogenesis of various diseases in cattle (Miller et al., 1993a,b). In the parasitic infections, host reacts to the presence of the parasites using potentially destructive factors (Woodbury et al., 1984), such as free radicals, which damage the parasite and generate oxidative stress. Several studies have reported on the presence of oxidative stress animals infected with parasites. (Sanchez-Campos et al., 1999; Derda et al., 2004; Simsek et al., 2006; Saleh, 2008; Saleh et al., 2009). Free radicals, especially NO, play a key role in host defense against echinococcosis (Touil-Boukoffa et al., 1998; Amri et al., 2007), but when generated at high levels they can result in oxidative damage

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http://dx.doi.org/10.1016/j.vetpar.2013.01.018

(Zeghir-Bouteldja et al., 2009). Oxidative stress has been reported in human (Koltas et al., 2006; Ersayit et al., 2009), camel (Heidarpour et al., 2012a) and sheep (Heidarpour et al., 2012b) with CE.

When free radicals are produced faster than they can be safely neutralized by antioxidant mechanisms, oxidative stress results (Sies, 1991). The cells contain a variety of antioxidants mechanisms that play a central role in the protection against reactive oxygen species (Halliwell, 1991). Antioxidants can be broadly defined as any substance that delays, prevents or removes oxidative damage to target molecules (Halliwell and Gutteridege, 2007). The antioxidant system consists of antioxidant enzymes [superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glucose 6-phosphate dehydrogenase (G6PD)], and non-enzymatic substances like metal-binding proteins (transferrin, ceruloplasmin, and albumin), vitamins (alpha-tocopherol, ascorbate, and beta-carotene), and trace elements (iron, copper and zinc) (Halliwell, 1994).

The determination of products of peroxidative damage to macromolecules and antioxidants are useful markers for the oxidative stress and antioxidant status, respectively. There is, to our knowledge, no study that evaluates the antioxidants status in cattle parasitized with CE. Such information will provide an indication of the antioxidant changes in parasitized animals. The present study aimed to assess the level of antioxidants including serum total antioxidant status (TAS), zinc, iron and copper and erythrocyte GPx, SOD and G6PD in cattle with liver cystic echinococcosis caused by *E. granulosus*. The concentration of serum malondialdehyde (MDA), as a biomarker of lipid peroxidation, was also determined in the parasitized animals.

2. Materials and methods

2.1. Animals

Thirty cattle (3–8 years) with liver CE along with 30 healthy cattle were selected from animals admitted for slaughtering at slaughterhouse located in Mashhad, Iran. The selection of parasitized animals was restricted to those affected with liver CE only. Also, the negative control animals did not show any pathology and parasite in the carcass and blood samples.

2.2. Sampling

Blood was taken from the jugular vein. Five milliliters of blood anticoagulated with disodium-EDTA was used for parasitological examination and preparation of erythrocyte haemolysate and 5 ml transferred to plain tube for serum separation. All tubes were immediately placed on ice and were transferred to the laboratory. Plain tubes were centrifuged at $1800 \times g$ for 10 min followed by removal of serum. Serum was stored at $-20\,^{\circ}C$ until analysis. For preparation of erythrocyte haemolysate, blood samples were centrifuged at $800 \times g$ for 15 min at $4\,^{\circ}C$. The plasma and buffy coats were removed by aspiration. The packed erythrocytes were resuspended in phosphate-buffered saline (PBS, pH 7.4) and were centrifuged again and the supernatants were discarded. This process was repeated two times. Finally, a 1:10 dilution of erythrocyte hemolysate was prepared in ice-cold distilled water

2.3. Parasitological examination

The blood samples with anticoagulant were used to prepare thin blood smears for parasitological examination. Blood smears were fixed with methanol for 5 min, stained with Giemsa at a dilution of 5% in buffer solution for 30 min, and then examined for the presence of blood parasites under light microscopy. The blood smears were recorded as negative if no parasites were observed in 200 oil-immersion fields ($1000\times$). In post-mortem inspection, the carcass was carefully examined for any pathology and parasite. The animals with liver CE and no other pathology and parasite in the carcass and blood samples were selected as parasitized group. The negative control animals did not show any pathology and parasite including *E. granulosus* in the carcass and also any parasite in the blood samples.

2.4. Biochemical analysis

The amounts of serum iron, copper and zinc were measured by commercial kits (Pars Azmoon, Iran for iron; Giesse Diagnostics, Italy for zinc; EliTech diagnostics, France for copper) using an autoanalyzer (Biotecnica, Targa 3000, Rome, Italy). Control serum (Randox control sera, Antrim, UK) was used for controlling measurement accuracy.

Total antioxidant status (TAS) of the blood serum was determined based on the suppressing activity of antioxidants on producing a colored radical cation (Miller et al., 1993a,b). For serum TAS measurement, commercial test kit (TAS test kit, Randox Laboratories Ltd. G.B) was used. The activity of GPx was measured according to Pagalia and Valentine (1967) using a commercially available kit (Ransel test kit, Randox Laboratories Ltd. G.B.). SOD activity was measured by a modified method of iodophenyl nitrophenol phenyl tetrazolium chloride (INT) (Ransod test kit, Randox Laboratories Ltd. G.B.). G6PD activity was measured by commercially available kits (G6PD test kit, Randox Laboratories Ltd. G.B.). This method is based on that of Komberg et al. (1955). Lipid peroxidation (as MDA) levels in serum samples were measured using the thiobarbituric acid reaction method of Placer et al. (1966). The values of lipid peroxidation were expressed as nano moles (nm) of MDA per ml of serum using a molar extinction coefficient of pure MDA as 1.56×10^5.

2.5. Statistical analysis

Statistical analysis was conducted using SPSS for windows (release 16, SPSS Inc., Chicago, IL) with a P value of <0.05 as statistically significant. Data were expressed as mean $\pm$ standard deviation (SD). Independent-samples