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Role of oxidative stress in concomitant occurrence of *Fasciola gigantica* and leiomyoma in cattle

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ABSTRACT

Fasciola gigantica is a parasitic helminth that predominantly infects the liver and bile ducts of cattle and causes great losses of cattle production in the southwestern regions of Iran. The purpose of the present study was to find out the possible relationship between the extent of liver destruction and oxidative stress by estimating the level of MDA, and SOD and GPX enzymes in the liver of cattle infected with *F. gigantica*. Studies were carried out on 49 infected and 20 healthy livers. Based on the results, the SOD activity of the infected livers was substantially lower than those of the healthy ones. As in the livers with more damage, a significant decrease was observed in the SOD activity. Although the GPX activity of the control livers and the livers with low damage showed no statistically significant variation this value was significantly different between the livers with high injury and the control ones. The MDA level of the infected animals was significantly higher than the non-infected animals. In addition, the level of MDA production showed a parallel correlation with the severity of infection. By histological examination, the lesions in infected livers were varied from tortuous, organized fibrotic areas in or around the hepatic lobules, cirrhosis, and chronic catarrhal cholangitis to fresh migratory tunnels filled with RBC and eosinophils. Multifocal and relatively homogenous populations of densely packed spindle cells with blunt-ended nuclei, arranged in broad interlacing fascicles were noted in samples of 10 infected livers. Based on the histological features by routine and special staining and the results of the immune labeling, the tumors were diagnosed as hepatic leiomyoma. To the best of our knowledge, this is the first report on the concomitant occurrence of *Fasciola gigantica* infection and leiomyoma in cattle. Therefore, there is considerable evidence indicating that the severity of hepatic damage in fasciolosis is causally associated with the extent of intrahepatic oxidative stress. Future research is necessary in order to clarify the complex host–parasite interactions and to better define the oncogenic implications of *F. gigantica* infection.

1. Introduction

Fasciolosis is an important worldwide disease in Veterinary Medicine especially in tropical and sub-tropical countries. Infection with *Fasciola gigantica* is regarded as one of the most common single helminthes infection of ruminants in Asia and Africa (Roberts and Suhardono, 1996). *F. gigantica* is the causative agent of great economic loss in livestock husbandry in Iran and is the main fasciolid species in Khuzestan Province, southwestern Iran. The pathogenic effect of this parasite is extended over a large number of domestic ruminants; cattle, sheep, goats,

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buffaloes and wild herbivores are mostly affected (Hammond and Swell, 1975). The economic importance of this disease is mostly obvious when it causes mortality, but even subclinical infections have been shown to cause high economic losses from reduced feed efficiency, weight loss, reduced milk production, reproductive insufficiency, poor carcass quality and work output in draught animals, condemnation of livers at slaughter and expenditures for anthelmintics using (Vassilev and Jooster, 1991).

Recently, fasciolosis has emerged as an important pathogen of humans worldwide especially in Egypt, Iran and South American countries such as Bolivia, Peru and Ecuador (Mas-Coma et al., 1999). Human cases with liver cirrhoses and ectopic infection in vital organs such as brain, lungs, kidneys and eyes had previously been recorded (Dalimi and Jabarvand, 2005).

Hepatic lesions produced by *Fasciola* in various host species are associated with the number of ingested metacercariae and parasite burden. Migration of the juvenile flukes in liver is associated with mechanical damage to the parenchyma in the form of haemorrhages, necrosis, fibrosis, cirrhosis and in some instances hepatic and post-hepatic jaundice (Kolodziejczyk, 1994). Many investigations indicate that parasitic infections with high tolerance of the host are associated with defense mechanisms which include enhanced generation of reactive oxygen species (ROS) (Boczoń et al., 1996; Sanchez-Campos et al., 1999). Accumulation of these ROS in the cells are capable of damaging biomolecules of which lipids are probably the most susceptible if not controlled by appropriate antioxidant scavenging system. Oxidative destruction of the polyunsaturated fatty acids is particularly damaging because it proceeds as a self-perpetuating chain reaction. One of the toxic end products of lipid peroxidation is malondialdehyde (MDA) (Chessaman, 1993). Cells are particularly sensitive to oxidative stress and are supplied with protective antioxidant mechanism in order to counteract the toxic action of ROS. Two of the main antioxidant enzymes with free-radical scavenging activity are superoxide dismutase (SOD) which catalyzes the dismutation of superoxide anions into molecular oxygen and hydrogen peroxide (H_2O_2), and Glutathione peroxidase (GPX) whose detoxificant action lies in reducing free H_2O_2 to water and O_2 (Guemouri et al., 1991). Free radicals are produced continuously by normal metabolic processes, but their rate of production increases during certain parasitic infections including the infections with *F. hepatica* in sheep (Saleh Mostafa, 2008) and rats (Kolodziejczyk et al., 2005) and *Dicrocoelium dendriticum* in sheep (Simsek et al., 2006) and hamsters (Sanchez-Campos et al., 1999).

By searching the available literature, no previous studies have investigated the oxidative stress status during fascioliasis due to *F. gigantica* in ruminants. Such information will provide an indication of the oxidative response of the infected animals and additional insight into the pathogenesis of the disease. Since, it has been reported that the increased plasma lipid peroxides in hepatic diseases originates essentially from the affected liver (Suematsu and Abe, 1982), this study was therefore carried out to find out the possible relationship between the extent of liver destruction and oxidative stress by estimating the level of MDA, and SOD and GPX enzymes in the liver of cattle infected with *F. gigantica*.

2. Materials and methods

2.1. Study area

This study was done in Khuzestan Province, southwest region of Iran, where fasciolosis is prevalent. Khuzestan Province has a surface area of about 64,236 km^2, between 47 degree and 41 min to 50 degree and 39 min of eastern longitude from prime meridian and 29 degree and 58 min to 33 degree and 4 min of northern latitude from equator. The province has hot and wet summers, mild springs and cold winters. The maximum temperature in summer is around 54 °C while in winters the minimum temperature can fall around −5 °C (mean 25 °C). The average annual rainfall is around 213 mm (Statistical book of Khuzestan Province, 2006).

2.2. Sampling

Forty-nine cattle with liver fasciolosis and 20 healthy cattle were selected from the animals admitted for slaughtering in Ahvaz Slaughterhouse, Khuzestan Province in 2012. Selection of the parasitized animals was solely restricted to those affected with *F. gigantica*. The negative control animals were clinically healthy, free of any parasitic diseases and the carcasses did not show any pathologic changes. All the mature and immature *Fasciola* flukes were collected from the livers and bile ducts according to Anderson et al. (1999) and emerged flukes were morphologically identified. The liver damages were subjectively classified as grades 1 and 2 where grade 1 showed slight or moderate visible scarring in the bile ducts, with occasional presence of parasites in the bile duct and grade 2 had severe scarring with parasites consistently present.

2.3. Preparation of tissues

200 mg of each healthy and infected liver were homogenized in 2 ml potassium phosphate buffer to obtain 10% homogenate. The liver homogenate was then centrifuged at 4000 × *g* for 10 min. The supernatant was used for the experiment.

2.4. Determination of lipid peroxidation levels

Malondialdehyde level in the liver samples were measured using the thiobarbituric acid reaction method of Placer et al. (1966). Quantification of the thiobarbituric acid reactive substances was determined at 532 nm by comparing the absorption to the standard curve of MDA equivalents generated by acid-catalyzed hydrolysis of 1,1,3,3-tetramethoxypropane. The values of MDA were expressed as nmol/g.

2.5. Antioxidant enzyme assay

The determination of the SOD activity was based on the generation of superoxide radicals produced by xanthine