Jentashapir J Health Res. 2017 October; 8(5):e65813. doi: 10.5812/jjhr.65813.

Published online 2017 December 31. **Research Article**

Evaluation of Bilirubin, Phosphorus, Calcium and Cholesterol Levels in Bile of Livers Consistent with Hepatic Fascioliasis

Jasem Saki [1], Iman Khodkar [2,*] and Mohammad Hossien Razi Jalali [3]

[1] Alimentary Tract Research Center; Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[2] Department of Parasitology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, IR Iran
[3] Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, IR Iran

[*] *Corresponding author*: Iman Khodkar, Department of Medical Parasitology, Ahvaz Jundishapur University of Medical Sciences, P. O. Box: 61357-33118, Ahvaz, IR Iran. Tel: +98-9372998140, E-mail: iman.khodkar@yahoo.com

Received 2017 December 02; **Accepted** 2017 December 24.

Abstract

Background: *Fasciola* spp. is endemic in Iran, and even in recent years, some human epidemics of this parasite have been reported in the northern parts of Iran. Biliary fluid is one of the most important substances that is altered by invasion and replacement of the parasite, and it seems that biliary fluid can be used to determine the progression of the disease and the degree of the damage to the liver cells. The aim of this study was to investigate whether there was a relationship between the amount of bilirubin, phosphorus, calcium and cholesterol in the biliary fluid of healthy livers and those in livers infected with *Fasciola* spp.
Methods: A total of 50 samples of biliary fluid from the infected livers and 50 samples of biliary fluid from healthy livestock in terms of presence or absence of contamination were collected.
Results: Direct bilirubin and cholesterol in infected livers is lower than those in healthy livers. Also, the level of calcium in the liver contaminated with the parasite was higher than that of the healthy liver; the difference was statistically significant. The level of phosphorus in the infected livers was higher than that in the healthy liver, however, the difference was not statistically significant.
Conclusions: This study showed that bilirubin, cholesterol, calcium, and phosphorus in the liver consistent with hepatic fascioliasis were altered significantly, indicating valuable effects of the parasites on hepatic cells.

Keywords: Iran, Bilirubin, Cholesterol, Calcium, Phosphorus, *Fasciola hepatica*

1. Background

One of the oldest and most important productive professions in Iran is livestock farming, which has long been the job of many people in this land. Therefore, livestock diseases have always been the concern of livestock farmers and veterinary researchers (1). One of the most common and important diseases of domestic animals in Iran is fascioliasis. It is a common disease between humans and animals and is caused by two species of teratogens called *Fasciola hepatica* and *Fasciola gigantica* (2). Humans are also considered to be a random host in the cycle of these parasites, and in most parts of the world, human cases have been reported. According to the world health organization, nearly 17 million people in the world suffer from the disease, and nearly 180 million are at risk (3). Suitable weather and the presence of intermediate hosts have led to a high prevalence of the disease in parts of the country where livestock are prevalent. *Fasciola hepatica* is endemic in Iran, and even in the recent years, we have seen some human epidemics of this parasite in the northern parts of the country (4). Apart from human cases of fascioliasis, the contamination of livestock with this parasite is also very high and has a huge economic impact on livestock breeders and protein producers (5). *Fasciola hepatica* is located in the final host in two stages, and each of these can cause a host of disturbances:

1. Immigration or parenchymal stage, in which young worms enter the body after passing through the tissues finding their way to the liver. 2. Biliary stage or establishment, in which the worms are mature and begin to ovulation. These steps increase the diameter of the bile ducts, damage the liver tissue, and finally cause impairment in the production of liver enzymes, hemorrhage, anemia, fibrosis, jaundice, and a decrease in the function of the gallbladder in the production of different chemicals and even death (6). In the meantime, biliary fluid is one of the most important substances that is altered by invasion and replacement of the parasite, and it seems that biliary fluid can be used to determine the progression of the disease

and the degree of damage to the liver cells (7), and to examine the changes in the production of bile duct material. The aim of this study was to find a significant relationship between the amount of bilirubin, phosphorus, calcium, and cholesterol in the biliary fluid of healthy livers and those in livers infected with *Fasciola* spp.

2. Methods

This study was carried out in Ahwaz Industrial Slaughterhouse for 3 months. After checking the liver of the livestock by a slaughterhouse expert, 50 samples of biliary fluid from the infected livers and 50 samples of biliary fluid from healthy livestock in terms of presence or absence of contamination were collected. From each gallbladder, between 2 and 5 cc of bile fluid was drawn by sterile syringe, and these specimens were transferred to the laboratory of the faculty of veterinary medicine of Shahid Chamran University at the earliest opportunity.

In this study, diazo photometric method, at a wavelength of 555 nm, was used to measure the amount of bilirubin (8). For the determination of phosphorus content, Molybdenum reduction method (Pars Azmoon kits) was used at 630 nm optical waveguide (9). To measure calcium in the biliary fluid, the o-cresolphthalein (kits of the Zist Shimi Company) was used at a wavelength of 570 nm (10), and cholesterol level was determined by cholesterol oxidase at 546 nm. To measure the quantities of these chemicals, Milton Roy machine Spectronic 501/601 Spectrophotometer was used (11).

2.1. Statistical Analysis

SPSS version 17 was used to compare the results of the two groups.

3. Results

Table 1 lists the values of bilirubin, phosphorus, calcium, and cholesterol in healthy and facioliasis infected animals.

According to the results obtained and their analysis by statistical software, it can be concluded that production of conjugated or direct bilirubin and cholesterol in infected livers is lower than those in healthy livers; there is a significant difference between the two groups. In addition, the level of calcium in the liver contaminated with the parasite was higher than that of the healthy liver; the difference was statistically significant between the two groups. The level of phosphorus in the infected livers was higher than that in the healthy liver, however, the difference was not statistically significant.

Table 1. Values of Bilirubin, Phosphorus, Calcium and Cholesterol in Healthy and Hepatic Facioliasis Infected Animals

Sample	Measured Values in Healthy Livestock	Measured Values in Infected Livestock	P Value
Bilirubin conjugate (direct), mg/dL	7.9	6.2	< 0.05
Cholesterol, mg/dL	60.7	53.6	< 0.05
Calcium, mg/dL	97/15	18/02	< 0.05
Phosphorus, mg/dL	12.8	14	> 0.05

4. Discussion

One of the main sources for providing animal protein in Iran is sheep, which are a very good hosts for the *Fasciola hepatica* parasite and considered as the main host in the life cycle of this parasite. The breeding of sheep in Iran plays a very important role in the economy of rural communities and hence the recognition and research of diseases involving domestic livestock, especially sheep, should receive scholarly attention (2, 12). In Iran, livestock breeders rely on traditional methods of breeding sheep, and the risk of infection with fascioliasis seems to be high in such communities due to the suitable environments for parasite growth and the close contact the livestock have with their surroundings. The world health organization has recently announced that Iran is one of the six countries with a high incidence of human parasite infection, which has become a health problem (13). One of the provinces with a high prevalence of fascioliasis in sheep is Khuzestan, and studies by researchers suggest that the prevalence of *Fasciola hepatica* parasites in slaughtered sheep in Khuzestan province is about 35% (14).

As we know, during different stages of the disease including migration (acute form of the disease), replacement (chronic form of the disease), and feeding, the parasite causes a wide range of harms to the host, and one of these is the changes in the chemicals in the bile. In the chronic form of the disease due to the mechanical, toxic and clotting activity of the parasite in the bile ducts, in addition to damage to the biliary duct epithelial cells, the quantities of secreted material in the biliary fluid also undergo changes (15). However, studies have shown that in this phase, the amount of secreted chemicals in the bile returns to the normal state due to the restoration of the liver, tissues, and the process of producing liver enzymes. It seems that the study of the volume of secretion in biliary fluid is not a good indicator for determining whether the host is infected with the parasite, especially since in most cases of parasite infections in sheep, the chronic form of

the disease develops. However, in the stages of the disease, even in its chronic form, using these changes and their careful examination can make us aware of the progress and stage of the disease at which the host is and the effect of these changes on the livestock living process (16).

Most of the studies done on changes in the level of macro- and micro-minerals in sheep have examined blood samples, and few studies have looked at the extent of changes in these elements in biliary fluid, and the present study can open up new horizons for understanding the biochemical changes of biliary fluid in sheep infected with fascioliasis.

Biliary fluid contains 97% water, 0.7% bile salts, 0.2% bilirubin, 0.51% fat, and minor amounts of non-organic salts. Biochemical changes in this fluid can reduce the absorption of lipids, and usually liver damage is one of the major causes of these changes (15, 17, 18). Most of the studies carried out on the elements secreted in biliary fluid have focused on blood samples, and in these studies changes in biliary fluid elements have been shown. The present study also showed that these elements also decreased, and the results are dependable (15, 19, 20). Studies have shown that direct bilirubin levels in the sheep sera from liver-damaging diseases increase due to the direct release of bilirubin in the plasma, however, in spite of this, direct bilirubin levels reduce due to damage of the parasite to liver cells, which is confirmed by the results of the present study (21). As expected, in the study of bile ducts, we observed an increase in calcium levels, which was not unexpected. Due to the damage that the parasite causes to the bile ducts and the liver, and considering calcium secretion for restricting and calcification of the sites invaded by parasites, the higher amounts of calcium levels are likely to be observed in livestock infected with *Fasciola hepatica* (22).

Bile acids are mainly made by cholesterol, and the amount of plasma or serum cholesterol can be a good indicator of the damage in the liver. That is, plasma cholesterol increases in the early stages of liver diseases and diseases related to biliary obstruction, and then in the chronic phase of the disease, a decrease in cholesterol can be observed. According to the results of this study on infected animals, it can be said that these livestock have been affected by the chronic phase of the disease, and the hypothesis that the disease of the sheep exposed to Fasciola hepatitis progresses towards a chronic level is confirmed (23, 24). The presence of macromineral phosphorus is essential for the growth, maintenance and regeneration of cells, and its role in the production of phospholipids constituting the cell wall has also been proven. After calcium, phosphorus is the most important element in the body and is involved in many physiological actions there. It seems that decreased level of phosphorus in the bile duct can also be related to the low level of cholesterol in the biliary fluid (25). Therefore, it can be concluded that the reduction of some elements in the biliary fluid has an effect on the production of other bile ducts as well. Although phosphorus did not reduce significantly in the livestock of our study, it is an important role in the production of phospholipids cannot be easily ignored (26).

4.1. Conclusions

Studies dealing with the changes in the biliary fluid are scarce since such studies are costly and difficult to conduct. The present study showed that biliary fluid elements changed significantly in fasciolasis. However, due to the complexity of the evolution of this parasite, and the impact of environmental, genetic, and nutritional conditions on the pathogenicity of the parasite in the host body, further studies are needed to confirm these results.

Acknowledgments

We are grateful to the sincere cooperation of the slaughterhouse personnel, Mrs. Farnoosh Shadenush, a PhD student, Mr. Nasouti, a medical student, and Mr. Gholamreza Bahmam for collecting samples.

Footnote

Funding/Support: The financial support of this study was provided by the office of vice-chancellor for research and technology development of Ahvaz Jundishapur University of Medical Sciences, by the grant code RDC-9401.

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