Liverfluke Coprological Cross-Sectional Survey in Cattle, Sheep and Goats in Sharazur District Kurdistan- Iraq

Kwestan Najm Ali¹, Hardi Fattah Marif ¹, Nawroz Akram Kakarash² and Hawsar Othman Mohammed³

¹ Department of Internal Medicine and Clinic, College of Veterinary Medicine, University of Sulaimani, Sulaimani City, Kurdistan Region, Northern Iraq.
² Department of Anatomy and Pathology, College of Veterinary Medicine, University of Sulaimani, Sulaimani City, Kurdistan Region, Northern Iraq.
³ Department of Basic Sciences, College of Veterinary Medicine, University of Sulaimani, Sulaimani City, Kurdistan Region, Northern Iraq.

*Corresponding author: E-mail address: kwestan.ali@univsul.edu.iq, Phone number: +964-07701598448

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Cross-sectional coprological survey was conducted to know the prevalence of liver flukes in cattle, sheep and goats in Sharazur district Kurdistan- Iraq from June 2018 to March 2020. Parasitological examination of fecal samples collected from 685 animals from several field (280 sheep, 245 goats and 160 cattle) was done by using sedimentation method (Fecal Egg Count Reduction Test- FECRT). We revealed that an overall Fasciola species prevalence were (49.48%). Liver fascioliasis was documented highly in sheep (55.71%), followed by cattle (47.5 %) and goats (43.67%). Risk factors such as age and sex showed a significant effects on the prevalence of liver flukes (P<0.05). A higher prevalence rate was noticed and identified in animals older than 3 years old (57.66%) and it was higher than those found in middle age (47.71%) and in young animals (31%). The prevalence of female Fascioliasis was (52.74%) and higher than male Fasciolasis which was (40%).

Keywords: Fasciola gigantica, Fasciola hepatica, Foodborne diseases, Snails, Sharazur district.
Introduction

Fascioliasis is one of the crucial food-borne parasitic diseases in the class trematoda. It is one of the group of neglected atropical diseases (NTDs). According to the WHO roadmap for minimizing the public health problems of NTDs, Food-borne trematodiases are one of the 17 NTDs included (1). It is also known as the most widely distributed disease among zoonotic diseases, and it has a great impact on economic loses in animal industry in the world especially in cattle and sheep. This parasitic disease is very common in ruminants, particularly in cattle, sheep, buffaloes, swine and goats and also in human beings (2). The causative agent is the endoparasitic trematodes of the genus Fasciola (3). lives in the bile ducts (4). The two most common species of liver flukes are Fasciola hepatica and F. gigantica. They can cause hepatobiliary system infection in cattle and sheep, and also have an impact on public health. It has been documented that F. gigantica is causing liver fluke infection in tropical areas whereas, F. hepatica is mostly reported in temperate climates (3). F. gigantica is found in Asian and African countries. While, F. hepatica found worldwide. Moreover, F. hepatica is the main cause of human fascioliasis and it has recently been reported as an emerging and re-emerging zoonotic disease in many countries (5).

This disease has been stated as a significant disease in livestock-rearing areas, because it has major effects on the animal husbandry industries (6). Recently climate change increases Fascioliasis in livestock animals globally, because it can messuer as s contributing factor for the disease burden. This can be expalied by wetter summers and warmer winters as well as supporting the larger population of intermediate host (mud snail). Climate change can also has an impact on disease management (sheep treatment only, cattle will not be treated and veterinary interaction limitation). Climate change may cause treatment and chemical control resistance and moving livestock animals in the absence of commercial vaccines (7). Abdominal pain is one of the most common clinical signs of the parasite infections. This occurs bacuase of the movement of the young parasite within the liver and the bile ducts. Extensive damage of the liver is occurring because of this migration and may cause portal cirrhosis, fever, nausea, vomiting, hepatomegaly, hepatic tenderness, and eosinophilia are also common. (8, 9).

Lowered weight gain, anemia, reduced animal productivity, reduced milk yiled and meat production and lowered feed conversion efficiency are seen in the infected animlas (10). Diagnosis can be difficult without clear clinical manifestations. Fasciola is diagnosed by Fecal Egg Count Reduction Test (FECRT), detecting antibody in serum and milk and feces. (11, 12, 13). Imaging techniques, such as computed tomography (CT) and ultrasonography (US), endoscopic retrograde
cholangiopancreatography (ERCP) and sphincterotomy have been used for diagnosis (14, 15, 16). The most effective and widely used antihelmintic for the treatment of fascioliasis in animals is triclabendazole (TCBZ). This drug is highly effective for mature and immature stages of the parasite (17). It has been recommended by CDC that TCBZ is the first line agent that used for the treatment of fascioliasis in humans, and this may have some side-effects such as dizziness, fever and abdominal pain one week after the using of the treatment (18). Other drugs, such as pain killers, can also be used to treat some symptoms such as pain and diarrhea. Surgical operation may be necessary in very few cases where cholangitis, and bile duct infection in the liver has developed. The amis of the present study are the establishment the prevalence rate of fascioliasis in cattle, sheep and goats in Sharazur district Kurdistan Region-Iraq. Moreover, to investigate the possible effects of sex and species on the prevalence of fascioliasis among sampled ruminants.

**Materials and Methods:**

2.1. Study area

The present study was carried out in (656 KM²), located in the Eastern of Sulaimani city. The area is mostly flatty and some parts has either hilly or mountainous and extends from (450-600 M) above the sea level. There is only one lake and many rivers in the area. The area is also characterized with high humidity and high water fall with about (500-700 mm) yearly. The rain usually starts from the early autumn to the late spring. Weather is very dry in summers and rainy in winters which is a Mediterranean climate.

2.2. Sample sizes and sample distribution

The samples were taken from 10 ovine farms (fecal samples from 280 animals), 9 caprine farms (fecal samples from 245 animals) and 7 bovine farms (fecal samples from 160 animals) in all parts of the study area from June 2018 to March 2020. Samples were taken randomly from 510 female and 175 male animals from three different groups of age (100 samples < 1 year, 285 samples 1-3 years and 300 > 3 year). All farms were selected to be equally distributed in the study area. For this reason we used Google map to divide the study area. It is important to say that there were many bovine, caprine and ovine pasturing farms. Samples were directly sent to the Laboratory of the Veterinary Teaching Hospital for testing.

2.3. Laboratory procedure

Fecal Egg Count Reduction Test (FECRT), a sedimentation method was used to determine the numbers of fluke eggs/ gram of feces. The samples were mixed well and 10g of feces was weighed out and mixed with a little water in a 500ml beaker. The beaker was then top up with water. Three sieves (38µm, 150µm and 500µm)
were stacked with the smallest aperture at the bottom and largest at the top. The fecal water was slowly passed through the sieves followed by thorough washing with water until the water was run clear from the bottom sieve. The 500µm sieve was removed and washed through the remaining two sieves were repeated. The 150µm sieve was removed and the retentive on the surface of the 38µm sieve washed and the remaining contents backwash into a 500ml beaker. The beaker was topped up with water and left to stand for 4 minutes. The supernatant was poured off leaving approximately 100ml of sediment and then the beaker was refilled with water and left to stand for a further 4 minutes. This process was repeated until the supernatant was cleared. When clear the supernatant poured off to 100ml or less if possible without losing any sediment and the remaining contents transferred into a large square Petri dish. Two drops of Methylene blue was added and the number of F. hepatica eggs counted using a dissecting microscope. Results were given in the number of eggs/gram of feces calculated was calculated by dividing the total number of eggs by 10. (19)

2.4. Statistical analysis
Variables were introduced into the statistical analysis were age and sex in three different animal species (sheep, goats and cattle) using SPSS v24, M.S. Excel (for graphs). The differences were considered statistically significant when P < 0.05 with 95% confidence intervals (CI). The differences between age and sex of the different animal species were compared statistically using two-way analysis of variance, followed by post hoc (LSD).

Results and Discussion
Among the 685 fecal samples in all animal species with different ages and sex tested for liver fluke eggs 339 showed positivity, leading to an overall of 49.48% (95% CI). The percent positivity of liver fluke varied significantly (p < 0.05) among all species: 55.71%, 43.67% and 47.5 % in sheep, goats and cattle respectively (Table1). In addition, the statistical analysis showed that age and sex affected the percent positivity of liver fluke infection in all animal species. Positivity in percentage was higher in females (52.74%) (95% CI) than in males (40%) (95% CI) (p < 0.05) (Figure1). Moreover, percent positivity in animals older than 3 years old (57.66) was significantly (p < 0.05) higher than those in middle age (47.71%) and in young animals (31%) as shown in (Table1).
Table 1: Prevalence the infection rate of Fasciola spp among sheep, goats and cattle according to sex and ages by Faecal Egg Count Reduction Test (FECRT).

<table>
<thead>
<tr>
<th>Factor</th>
<th>Animals</th>
<th>Sample size</th>
<th>Number of positive samples</th>
<th>Percent positivity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Sheep</td>
<td>280</td>
<td>156</td>
<td>55.71</td>
</tr>
<tr>
<td></td>
<td>Goats</td>
<td>245</td>
<td>107</td>
<td>43.67</td>
</tr>
<tr>
<td></td>
<td>Cattle</td>
<td>160</td>
<td>76</td>
<td>47.5</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>175</td>
<td>70</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>510</td>
<td>269</td>
<td>52.74</td>
</tr>
<tr>
<td>Age (year)</td>
<td>&lt; 1 year</td>
<td>100</td>
<td>31</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>1-3 year</td>
<td>285</td>
<td>136</td>
<td>47.71</td>
</tr>
<tr>
<td></td>
<td>&gt; 3 year</td>
<td>300</td>
<td>172</td>
<td>57.33</td>
</tr>
</tbody>
</table>

Figure 1: Total number of samples tested by fecal egg count reduction test (FECRT) in three different species of animal with sex and ages (year). Error bars indicate the 95% confident intervals.

Fascioliasis is considered to be a major issue in public health specially for farmers and veterinarians globally. This because of the great impacts on the animal products. Furthermore, it has been reported that anthelmintic treatment is not always effective because of the development of the drug resistance (17, 20). Risk factor identification of the fluke infection may cause the progression and development of
a good control strategies and treatment protocol measures for minimizing of the fluke infection this will cause the effectiveness of the animal production. (21).

In Iraq, many factors affect the distribution of the fascioliasis in various parts of the country, such as old-style and not standard animal agriculture and the great populations of the snail. Fascioliasis deemed to be one of the crucial health problem in the study area. It has economical and social influences of the people in Iraq (22).

The rate of the fluke infection in the current study in Sharazur district Kurdistan region-Iraq were high (49.48%), this may be due to that the area is very wet and has many rivers. The management system of the livestock animals and using water resources directly from rivers can also be considered as the potential factor of the flukes life cycle (23, 24). Moreover, the disease is chronic and still there is no control program by local authorities in Kurdistan Region for fascioliasis. The result is higher than that previously reported by (25) in Sindh Province of Pakistan (42.06%), (26), (25.46%) in Punjab-Pakistan, (27), (37%) in Zimbabwe, (28), (26%) in Kenya, (29), (46%) in Zambia, (30), (14.7%) in Egypt, (31), (10.92%) in Malang District – East Java, (32), (23.18 %) in Ireland, (33), (5.8%) in Batu City- Indonesia, (34), (0.87%) in Al- Najaf-Iraq, (35), (0.50%) in Kirkuk-Iraq, (36), (1.7%) in Kermanshah-Iran, (37), (3.28%) in Kashan-Iran, and (38), (0.56%) in Arak- Iran. Moreover the result is lower than that previously documented by (39) (65%) in Ireland.

According to the species of animals the result showed that sheep had a highest prevalence rate which was (55.71%), to the disease than cattle (47.5%) and goat (43.67%), this was in agreement with the data obtained by (40) in sheep (7.1%) and goats were (3.9%) respectively, (41) in sheep (19%), cattle (17.8%), goat (11.5%), respectively, and (42) in sheep (5.7%) and goat (1.6%). The prevalence in sheep is high. This may be because of the animal grazing habits. Goats are mostly grazing on trees which is free from flukes however, sheep eating plants on the ground, where full of fluke infective stage (43, 44, 45 and 46). This is what researchers investigate in Morocco (47) and in Argentina (48). Availability of suitable habitat for snails as intermediate hosts, temperature and humidity are the main factors to consider in the epidemiology of fasciolosis (49).

The age has great effect (p < 0.05) on the rate of propagation of the disease. The prevalence of the disease is elevated with the age of the animals, the infection rate of Fasciola was the highest above 3 years (57.66%) while it was (47.71%) in 1-3 years age, and lowest in under 1 year age animals which was (31%). The high rate in the prevalence of this parasite with the age has been documented by (50). This may be due to the reason that animals do not develop
resistance against fluke infection with the increment of age (51). Research carried out by (52) stated that clinical signs and lesions in the liver can mostly be seen in the animals with old ages because they have more exposure to the parasite infective stage (metacercaria) (52). This can be explained by young animals are mostly stayed around the farms and feed in door, so that they have less chances to be exposed with the fluke metecercaria compared to adults (53).

In the present study we confirmed that the animal sex has significant effects (p < 0.05) on the occurrence of fascioliasis in the study area which is female has greater chance of affected by fascioliasis than male 269 (52.74% and 70 (40%) respectively. This study finding is in line with reports of (54) was recorded (39.7%) and (16.9%) in female and male animals, respectively, (55), (51.09%) and (50.39%) was recorded in female and male animals respectively, (56), (70.6%) and (36.06%) was recorded in female and male animals respectively. (57) who documented a higher prevalence in females (41.3%) than males (13.8%) and by (58) in females (46.99%) and males (19.96%). This may be due to the fact that females have pregnancy and lactation stress as well as spending more time in the pasture for grazing. (59). Many intrinsic risk factors such as genetics, physiology, and immunology and extrinsic risk factors such as environment and management practices present between male and female cattle. Hormonal and stress influences are considered to have effects on the higher percentage of the fluke infection in the females as stress will lead to immune-suppression (57).

**Conclusion**

The current findings showed that fascioliasis was more common and has major impacts on economical losses in animal industry. This parasitic disease is mainly affecting ruminants in the study area. Hence, treatment and control strategies should be designed to reduce the prevalence liver flukes. This study also gives data for the further monitoring protocol in the treatment and prevention of the fluke infection in the region. Some factors associated with the prevalence of fascioliasis were noticed, including age, sex, and species of the infected animals.

**Conflict of Interest**

The authors disclose no conflicts related to the present study.

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