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Case Report

Acute Fascioliasis as a Leading Cause of Mortality in Free-Ranging Flocks of Domestic Sheep in Babol Countryside, Mazandaran Province, Iran: A Case Report

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Abstract
In July 2021, the School of Veterinary Medicine at Amol University of Special Modern Technologies was informed of high mortality within a sheep flock. Out of 200 animals, 24 sheep died within a few days. Due to the rapid deaths, poisoning was initially suspected but ruled out after clinical observation, sampling, and necropsy. A full herd assessment was performed, and blood and fecal samples were collected from lambs and adults for laboratory analysis. Blood samples were drawn via jugular venipuncture; fecal samples were examined using the Modified McMaster technique to detect helminth eggs or protozoan oocysts. For hematological and biochemical evaluation, blood was divided into EDTA and plain tubes. Serum analysis revealed a significant elevation in AST levels, indicating hepatic tissue damage associated with the parenchymal migration of juvenile *Fasciola hepatica* during acute fascioliasis. Infected sheep showed a marked reduction in serum SOD activity ($P<0.001$) and a significant increase in MDA levels ($P<0.001$) compared with non-infected animals, reflecting oxidative stress. These findings suggested that environmental and climatic conditions at the time favored the spread of fascioliasis. Considering the zoonotic potential and widespread occurrence of *F. hepatica*, effective control and preventive strategies are essential. Recommended measures include wetland drainage, strategic deworming, and controlling snail and slug populations to limit transmission.

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Introduction

Fascioliasis, primarily caused by *Fasciola hepatica*, is the most important liver fluke is a widespread infection that affects various hosts in the whole of the world and leads to substantial economic losses in all ruminants, especially sheep (1). Also, *Dicrocoelium dendriticum*, another liver parasite commonly known as the small liver fluke, generally exhibits milder clinical symptoms compared to Fascioliasis (1).

In regions with abundant annual rainfall, high humidity, and abundant pastures, chronic fascioliasis is a common cause of this parasitic infection, as seen along the regions on the south coast of the Caspian Sea, Mazandaran, Iran (2, 3). Recent studies conducted in different parts of Iran have indicated that chronic fascioliasis is the most prevalent form of infection, followed by subclinical and acute phases, respectively (1, 3, 4).

The present study is related to the unexpected occurrence of acute fascioliasis in a flock of sheep near the city of Babol, Mazandaran province, Iran, which led to high mortality at different times.

Case Report

In July 2021, a report of unusual mortality and heavy losses in a flock of sheep was reported to the School of Veterinary Medicine at Amol University of Special Modern Technologies from Amir Kala, a small village in the suburbs of Babol City, Mazandaran Province, Iran. Out of a total of 200 sheep in the herd, unexpectedly 24 of them perished within a few days. First, due to the rate of death and the speed of casualties, it was assumed that poisoning had been proposed as a possible reason but by observing and examination of the herd livestock and sampling from blood and feces of the sheep and finally necropsy the dead sheep this hypothesis was rejected. The farm owner revealed that approximately 45 days before the onset of mortality, the flock had been moved to a grass-filled pasture for grazing purposes.

Abnormalities and sudden deaths of several lambs occurred upon the flock's return from the pasture to the farm and after that, mortality in adult sheep also started. As initial symptoms, general weakness, loss of appetite, severe ascites, weight loss, and gradual development of bottle jaw were observed in the herd. Following a thorough examination of the entire flock, after the history and herd examination, stool and blood samples were collected from lambs and sheep and transferred to the Laboratories at the School of Veterinary Medicine for more investigation. Blood samples were obtained via jugular venipuncture, and the Modified McMaster's Fecal Egg Counting Technique was utilized to identify the presence of worm eggs or protozoan parasite oocysts in fecal samples.

In the parasitology laboratory, just a small number of *Moniezia expansa* eggs and *Eimeria ovinoidalis* oocysts were identified in some of the fecal samples. However, the infection rates indicated by the eggs and oocyst counts per gram of feces samples (EPG and OPG) were insufficient to cause the acute phase observed in lambs and adult sheep according to previous studies (5, 6). Simultaneously, meanwhile, the surveys were being carried out, and after a couple of days, again new cases of mortality were reported; Losses of other five lambs and three mature sheep. Abdominal distension in alive sheep suggestive of ascites and severe ascites observed in the abdominal cavity of affected sheep during post-mortem examination (Fig.1).

Fig. 1: Clinical signs of abdominal distension in a live sheep suggestive of ascites (Original)

When an autopsy was done, at first, notable paleness was seen in whole the sheep body and upon surveying the abdominal cavity, remarkable signs of liver infection with fluke larval stages were observed (Fig. 2a, b).

Fig 2a Fig 2b

Fig. 2: (b) Liver tissue section illustrating the migratory pathways of juvenile *Fasciola* spp. in a sheep. (a) Congestion and petechial hemorrhages were observed in the damage during post-mortem examination

After that fasciolosis liver sections were cut into 0.5-centimeter slices using a sharp autopsy knife and immediately fixed in 10% formalin for more study. The sections were subsequently transferred to the clinical pathology Lab for further investigation. Accurate observation of liver tissue revealed a high number of immature *F. hepatica* larvae in different sizes and a small number of *D. dendriticum* ones (Fig. 3 a, b, c).

Fig 3a Fig 3b Fig 3c

Fig. 3: (a) Juvenile *Fasciola* spp. Isolated from the liver of a naturally infected sheep. (b) *Dicrocoelium dendriticum* isolated from the bile ducts of the liver in a naturally infected sheep. (c) Hyperplasia and dilation of the bile duct caused by the presence of *D. dendriticum* in the liver of a sheep

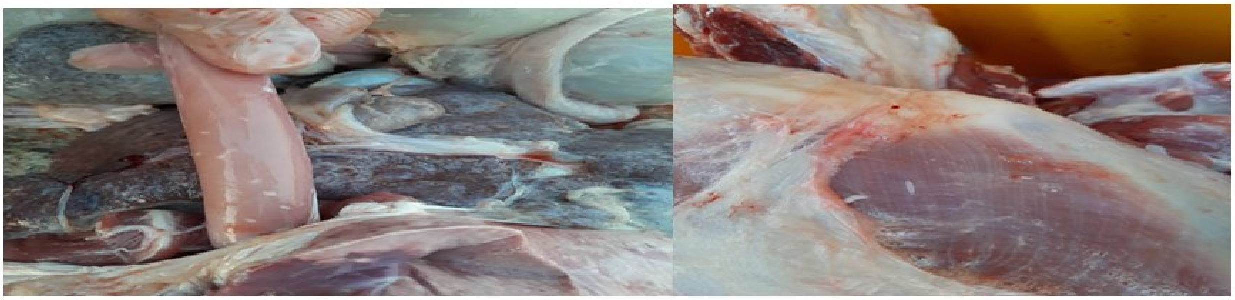

Fig 4a Fig 4b

Fig. 4: (a). Macroscopic sarcocysts observed in the esophageal tissue and (b) the shoulder muscle of an infected sheep

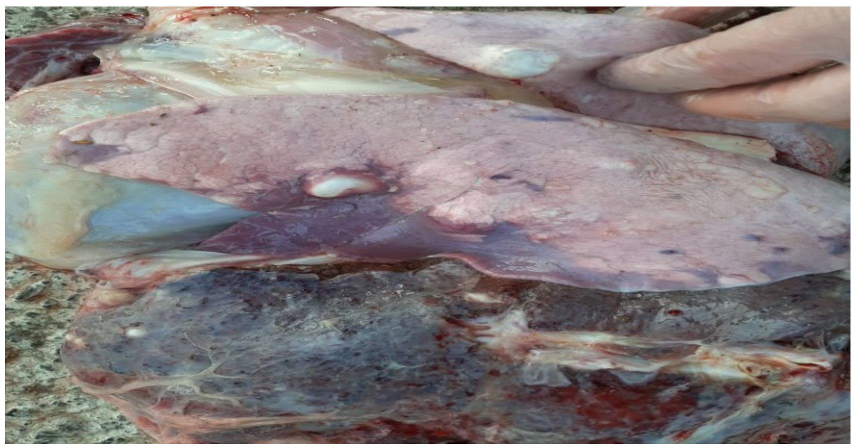

Fig. 5: Hydatid cyst in the lung tissue of an infected sheep, observed during post-mortem examination

For hematological analysis blood samples collected from the jugular vein of infected and healthy sheep were divided, one into a tube containing ethylene diamine tetra acetic acid dipotassium salt (EDTA-K2) for hematological examination and the second in the tube without the anticoagulant for serum biochemical parameters. Serum was collected at 1500g for 10 min and samples were stored at −20°C until further analysis. Serum activities of aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), concentrations of total protein (TP), albumin (ALB), and globulin (GLB) concentration were measured by using an automatic analyzer with commercial kits. The formation of thiobarbituric acid in serum was assessed for Measurements of Malondialdehyde (MDA) using an original method. Briefly, serum samples were mixed with 20% trichloroacetic acid and the mixture was centrifuged. Then, the supernatant was heated with thiobarbituric acid at 90°C. Its absorbance was measured at 532 nm. The values were expressed as nmol/ml using a molar extinction coefficient of 1.56 × 10infet5 M-1. Commercially available Nasdox kits (Navand Salamat Co., Iran) were used to estimate SOD in the serum following the manufacturer's guidelines (Figs. 4,5).

The results of the hematological analysis of infected sheep are summarized in (Table 1).

Table 1: Hematological parameters of *F. hepatica* infected and non-infected Sheep (mean ± SD)

Test	Infected sheep	Non infected
W.B.C (×10³/µL)	33±16.34[a]	15± 3.4[b]
R.B.C (×10⁶/µL)	6.4±1.12[a]	13.89±2.6[b]
Hemoglobin (g/dl)	6.25± 1.01[a]	12.72±3.1[b]
Hematocrit (%)	27.1± 3.21	29.34±4.5
M.C.V (fL)	42.45±1.78	43.92±6.1
M.C.H (pg)	9.75±2.11	10.56±3.2
M.C.H.C	34.9± 3.22	34.01±5.2

Values in rows with different superscripts are significantly different (*P*<0.01)

RBC numbers and hemoglobin concentration decreased significantly in infected sheep, but MCHC, PCV, and MCV remained unchanged whereas total WBC count was increased significantly in infected sheep. Urea, Creatinine, Uric Acid, Globulins, and AST increased significantly but Albumin decreased (Table 2).

Table 2: Biochemical parameters of *F. hepatica* infected and non-infected Sheep (mean ± SD)

Parameter	Infected	Non infected
Urea (Mg/dl)	40.25 ± 9.75[a]	19.11± 1.12[b]
Creatinine (Mg/dl)	3.23 ±0.84 [a]	1.90 ± 0.6[b]
AST (IU/l)	435.25 ±25.75[a]	280 ±17.92[b]
Total Protein (G/dl)	7.37 ±0.82	7.7±0.84
Albumin (G/dl)	1.95 ±0.77 [a]	2.4 ±0.97[b]
Globulins (G/dl)	5.42 ±1.23 [a]	4.1 ±1.01[b]
GGT (IU/l)	47.75 ± 12.16	45 ± 11.32

Values in rows with different superscripts are significantly different (*P*<0.01)

Infected sheep had a significant reduction in SOD activity of the serum compared to the non-infected sheep (*P*<0.001) while MDA levels increased in Infected sheep compared to non-infected sheep (P<0.001) (Table 3).

Table 3: Oxidative stress markers in blood serum of *F. hepatica* infected and non-infected Sheep (mean ± SD)

Variable	infected (Mean ± S.E)	Non infected (Mean ± S.E)
MDA serum(nmol/ml)	70.37 ± 0.76	40.74 ± 0.82
SOD serum u/ml	250.29 ± 1.0	201.87 ± 077

Values in rows with different superscripts are significantly different (*P*<0.01)

The results of serum biochemistry clarified a significant increase in AST levels. AST levels have increased in acute fascioliasis throughout the parenchymal migration of juvenile forms of *F. hepatica*. As stated in previous studies there is normochromic anemia and hypoalbuminemia in acute fasciolosis; our results are consistent with that report (7, 8). During young flukes migrating the liver parenchyma feeds on blood and with the aid of secreted proteases, causes excessive hemorrhaging. Furthermore, reports have shown that fascioliasis in buffaloes affects not only the liver but also other distant tissues, particularly the kidneys (9). In our study, elevated serum urea and creatinine in the infected sheep with *F. hepatica* showed kidney injuries in our study (P<0.05).

SOD is one of the main antioxidant enzymes that prevent oxidative stress. The increase in oxidative stress during *Fasciola* infection in the liver is because many immune cells have arrived to reduce necrosis, repair damaged cells, and induce fibrogenesis. It is known that the elevated serum MDA could cause injuries in the kidneys and lead to the generation of reactive oxygen release (ROS) (10). In the present study, MDA levels increased in infected sheep compared to non-infected sheep (P<0.001) (Table 3). The histopathological survey, combined with the observation of clinical symptoms and histopathological findings indicates that acute fascioliasis was the most probable cause of heavy mortality and economic losses in the sheep herd.

The study revealed that the herd had grazed in a pasture near a ranch for approximately a month. This enclosed pasture had been regularly utilized by various herds over the years. It was discovered that another herd from a different sheep husbandry had grazed there ten days before the flock's arrival. On the south coast of the Caspian Sea, in Iran, the first infection by these flukes typically occurs in mid-spring. In this study, it is hypothesized that upon the return of the livestock from the mentioned rangeland, the presence of potential metacercaria remaining from the previous herd in the forage around wet and irrigated areas, along with favorable climatic conditions for snail growth, facilitated the infection of animals with fascioliasis.

The presence of at least 12 rainy days per month and an average daily rainfall exceeding one millimeter is necessary for the occurrence of fascioliasis. According to the four regions defined by Sherman the southern coast of the Caspian Sea, with its average temperatures ranging from 8 to 26 °C and annual rainfall between 60 and 150 cm, offered the most favorable environment for the spread of fascioliasis. From June to September mostly, the highest amount of fascioliasis was reported in Iran (11). Based on this information, both the location and time of the infection were so suitable for the prevalence of fascioliasis. Considering the high prevalence of fascioliasis and reporting human infection cases, control and prevention of that is very important. Prevention methods should be based on wetland drainage, strategic treatments, and snail and slug control (12).

Targeted and strategically timed treatments are considered the best approach to control *Fasciola* infections. Since this infection is prevalent in Iran, especially along the Caspian coast, three treatments are recommended in late spring, early summer, and late autumn. Migratory sheep should receive two treatments: one in early spring before migrating to summer highland pasture and the other in mid-autumn after returning from summer pastures. If the temperature and rainfall conditions in autumn are suitable, a third treatment should be administered in late autumn or early winter (4, 5).

According to previous studies and the present report the high prevalence of fascioliasis in the northern regions of Iran, it is essential to implement stricter measures to prevent and control this parasitic infection, involving both health and government organizations and more comprehensive research.

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Conflict of Interest

The authors declare that there is no conflict of interests.

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