Prevalence of fasciolosis in ruminant in Karbala city

Khetam L Hussain and Zainab R Zghair

Abstract
The total number of the examined cow was (500) divided into 296 and 204 male and female, respectively and number of infected was 8, 2 male and 6 female. Infection in cow was 3.61% (0.67 to 2.94%) for male and female, respectively. In sheep the number of examined was (448), divided into 220 male and 228 female, but the total infected ones was (13), divided into 5 male and female 8. Infection in sheep was 5.77% (2.27 to 3.5%) for male and female. The pathological examination of liver of cattle infection with *Fasciola* shows presence of fibrosis around bile ducts with cellular infiltrations, in sheep the portal area in some sections there are flukes with different stages surrounded by inflammatory cells with fibrous tissue, and different stages of immature flukes with mechanical destruction of liver tissue.

Keywords: Fasciolosis, ruminant, pathological

Introduction
Fasciolosis or liver fluke it is zoonotic disease caused by *fasciola hepatica* and *fasciola gigantica* which remain economically important parasitic disease of livestock [1]. In old year the fasciolosis in human was limited to the people within well-defined watershed boundaries, but recently it has been recognized in many countries [2], approximately seventeen million people have been infected with *fasciola* and about 180 million people at hazard over the world [3].

There are many studies of fasciollosis in Iraq some of these study reported that the rate of infection in Basra city was 7.2% in sheep and 8.3% in cattle [4]. In Iran the rates of liver condemnation was 100% in cattle [5]. In Ethiopia the prevalence of fasciolosis was 62.7% in sheep [6], and 17.2% in goats [7]. Also these disease reported in human in many countries like Iraq [8], Iran [9], Syria [10].

Fasciolosis in animal cause severe and enormous economic losses throughout the world due to morbidity, mortality, susceptibility to secondary infections, cost of treatment and control, poor carcass quality, reduced weight gain, and also a condemnation of infected liver, [11]. While in human cause inflammation and swelling of bile duct and liver and cause increase seriousness of the health because it cause cancer disease [12], and the aims of the study are:

1. Determine prevalence of Fasciolosis in human, sheep and cattle in Karbala Province.
2. Identification the morphology of *fasciola*.
3. Investigate the gross and histopathological change in liver of sheep and cattle.

Materials and methods

Samples Collection
A weekly visit was made to the slaughter house, local Butcher and rural place in a different area at Karbala province and varied between [1-3] times per week, in the period between November 2016 to April 2017.

Collection and storage of samples
Liver sample
For each sheep and cattle slaughtered, the abattoir workers and local shopper assisted in bringing the infected liver. The livers were thoroughly examined alongside, it was located on a clean plank and firstly the liver and bile duct were inspected to detect presence of fasciola species by applying the routine inspection procedures to internal organ, if there is indication of fascioliasis is found, they were classified as immature or mature and gross lesions were recognized [13]. The primary examination included visualization and palpation of the organs,
while secondary examination included more incision of liver and bile duct opening. For generalized fasciola infection (liver fluke) made incision in different parts of the liver to confirm the presence of fluke in liver parenchyma, the cut liver was pressed in order to flukes squeeze out from the tissue and smaller bile ducts. Each collected flukes from animal were examined and classified on the basis of shape and size (Soulsby, 1982). Then parts of the affected organ and worm were sampled into 10% formalin for histological lesion characterization.

| Formaldehyde 40% 10 ml |
| Tape water 90 ml |

Results

Epidemiological study

All liver samples that collected in this study were under different condition, like; type of animals, month and sex. The total number of the examined cow was (500) divided into 296 and 204 male and female, respectively, while the total number of infected was 8, divided into 2 and 6 male and female, respectively. The total percentage infection in cow was 3.61% (0.67 to 2.94%) for male and female, respectively. The statistical analysis showed that there were no significant differences under (P<0.05) between percentage of infection in male and female cow (0.42). Table (1).

In sheep the total number of the examined was (448), divided into 220, 228 male and female, respectively, but the total infected ones was [13], divided into 5, 8 for male and female, respectively. The total percentage infection in sheep was 5.77% (2.27 to 3.5%) for male and female, respectively. The statistical analysis showed that there were no significant differences under (P<0.05) between percentage of infection in male and female cow (0.28). Table (1). In cow the highly percentage of infection was in April (4.12%), while the low percentage was in November (0.00%), table (2). In sheep the highly percentage of infection was in March (6.84) while the low percentage was also in November (0.00%), table (3).

<p>| Table 1: Percentage of infection in Cow According to the sex |</p>
<table>
<thead>
<tr>
<th>Sex</th>
<th>No. of Exam.</th>
<th>No. of Inf.</th>
<th>Percentage of infection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>296</td>
<td>2</td>
<td>0.67</td>
</tr>
<tr>
<td>Female</td>
<td>204</td>
<td>6</td>
<td>2.94</td>
</tr>
<tr>
<td>Total</td>
<td>500</td>
<td>8</td>
<td>3.61%</td>
</tr>
<tr>
<td>Chi-Square p-value</td>
<td>---</td>
<td>---</td>
<td>0.895 NS</td>
</tr>
</tbody>
</table>

NS: Non-significant.

<p>| Table 2: Percentage of infection in sheep According to the sex |</p>
<table>
<thead>
<tr>
<th>Sex</th>
<th>No. of Exam.</th>
<th>No. of Inf.</th>
<th>Percentage of infection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>220</td>
<td>5</td>
<td>2.27</td>
</tr>
<tr>
<td>Female</td>
<td>228</td>
<td>8</td>
<td>3.50</td>
</tr>
<tr>
<td>Total</td>
<td>448</td>
<td>13</td>
<td>5.77%</td>
</tr>
<tr>
<td>Chi-Square p-value</td>
<td>---</td>
<td>---</td>
<td>0.936 NS</td>
</tr>
</tbody>
</table>

NS: Non-significant.

<p>| Table 3: shows monthly distribution of fasciola in cow in Karbala province. |</p>
<table>
<thead>
<tr>
<th>Month</th>
<th>No. of Exam.</th>
<th>No. of Inf.</th>
<th>Percentage of infection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>November 2016</td>
<td>117</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>December</td>
<td>137</td>
<td>1</td>
<td>0.73</td>
</tr>
<tr>
<td>January</td>
<td>78</td>
<td>1</td>
<td>1.28</td>
</tr>
<tr>
<td>February</td>
<td>46</td>
<td>1</td>
<td>2.17</td>
</tr>
<tr>
<td>March</td>
<td>49</td>
<td>2</td>
<td>4.08</td>
</tr>
<tr>
<td>April 2017</td>
<td>73</td>
<td>3</td>
<td>4.11</td>
</tr>
<tr>
<td>Total</td>
<td>500</td>
<td>8</td>
<td>12.37%</td>
</tr>
<tr>
<td>Chi-Square p-value</td>
<td>---</td>
<td>---</td>
<td>1.803 NS</td>
</tr>
</tbody>
</table>

NS: Non-significant.

Gross pathological lesion of infected liver

The gross pathological changes observed in this study in sheep and cattle livers were almost similar, except in cattle there are severe calcification in chronically infected livers (figure 1). The commonly observed gross lesions include: Pin-point hemorrhages on the parietal surface of the liver (figure 2). The liver of sheep become pale, firm, swollen and irregularly outlined with tough consistency (figure 3). Liver appeared tense with fibrous exudates (figure 4). The mature flukes were also observed in the migratory tunnels of the liver parenchyma. In cattle hard, brown and dark color liver with multiple abscesses that surrounded by hyperemic zone (figure 1).

Fig 1: Dark liver of cow show sever calcification due to fasciola infection

Fig 2: pin-point hemorrhage on the parietal surface of cow liver and present of mature Fasciola gigantica.
Fig 3: show the firm swollen sheep liver have sever necrotic area due to fasciola infection

Fig 4: sheep liver become tense with fibrous connective tissue due to Fasciola hepatica infection

Fasciola morphology
The adult liver flukes are leaf like, grayish brown in color and flattened in its morphology. There are highlighted differences between two species, the *F. gigantica* is more transparent than *F. hepatica* also the *fasciola gigantica* is more elongated and narrow than the *fasciola hepatica*, And the measurement is 5x2 cm, 3x2.5cm for *F. gigantica* and *F. hepatica* respectively like in picture. In the anterior region these adult liver flukes have small oral sucker and near the shoulder large ventral sucker are localized and between these two sucker genital pores are present, like in picture and .

Fig 5: Measurement of Fasciola gigantica (A) and fasciola hepatica (B)

Fig 6: Wild microscope 40x of the anterior ventral surface of an adult liver fluke.

Fig 7: Show the external structure of adult fluke, OS (Oral sucker), VS (Ventral sucker).

Histopathological study
The pathological examination of liver of cattle infection with *Fasciola* shows presence of fibrosis around bile ducts with cellular infiltrations especially kupffer cells and fibroblasts in addition to congestion of blood vessels (BV) with eosinophiles and mast cells (fig.8). There is necrosis in portal area and fibrosis with some encapsulated around degenerating flukes with infiltration of inflammatory cells in some sections (fig.9), in sheep the portal area in some sections there are flukes with different stages surrounded by inflammatory cells with fibrous tissue, congestion of BV as in figure, and mature flukes in the portal area, and different stages of immature flukes with mechanical destruction of liver tissue revealed in figure.
Fig 8: Histopathological section in liver of cattle infection with *Fasciola* shows fibrosis around bile ducts with cellular infiltrations especially kupffer cells and fibroblasts in addition to congestion of blood vessels (BV) with eosinophiles and mast cells (H&EX400).

Fig 9: Histopathological section in liver of cattle infection with *Fasciola* shows necrosis in portal area and fibrosis with some encapsulated around degenerating flukes with infiltration of inflammatory cells (H&EX100).

Fig 10: Histopathological section in liver of sheep infection with *Fasciola* shows portal area that contain flukes with different stages surrounded by inflammatory cells with fibrous tissue, congestion of BV (H&EX100).

Fig 11: Histopathological section in liver of sheep infection with *Fasciola* shows mature flukes in the portal area, and different stages of immature flukes with mechanical destruction of liver tissue (H&EX100).

**Discussion**

The incidence of parasitic infection among female cows and sheep was higher than that of males. This result coincided with all previous studies that conducted field surveys in the massacres to detect parasitic diseases that need a long time to appear and why. This is due to the fact that females are not slaughtered except at large ages and thus the chance of being infected with parasitic infection is longer than that of males who are often slaughtered with small ages, and this percentage is high when compare with at Baghdad which find the percentage in cow, sheep were 3.3, 0.42, and also higher tha in 1987 Mahdi and Al-Baldawy found that the percentage of infection with *F. gigantica* in sheep and cow (0.72 and 3.3%) respectively.

In 2008 a study at Karbala province at the slaughter house recorded that the percentage of infections with liver fluke in sheep, cow and was 1.62, 36.73 respectively.

In 2016 in Basra city the study show that the percentage of infection found to (0.82, 3.33%) in cows for males and females respectively, but this result is lower than in Study done at Baghdad province at Sheik Omar slaughter house found the percentage of infection with fascioliasis in cow and sheep was 27, and 7.1% respectively.

Infection in cow and sheep liver examined in the winter months is due to the predators to infection in the summer months and the beginning of the fall season, which are small due to drought conditions and high temperatures, which have negative effects on the activity of water snails.

The gross pathological changes observed in the chronically infected livers was pin-point hemorrhages on the parietal surface. These findings are partially in agreement with the report of, as they reported hemorrhages on the surface of the liver in chronic fasciolosis from dairy farm. The pinpoint hemorrhagic foci on the surface represented the points of entrance of the immature parasites into the liver structure. In agreement with report of. Chronic cases of the current study also revealed that the livers were firm, tough in consistency and the cut section showed evidence of calcification while the affected ducts were enlarged/distended, thickened and cholangitis with adult fluke in the ducts. These might be because of the immunological reaction of macrophages and lymphocytes infiltration that merges with...
fibrotic healing of the necrotic areas during the later stage of fasciolosis. Progressive irritation by the adult flukes wandering in the biliary tract cause biliary inflammation like hyperplastic cholangitis, ductular wall thickening, and intra- and extrabiliary biliary dilation [26]. The differences in calcification might be due to species variation showing the possibility of a direct relationship to host resistance. It is also probable that the calcification seen in cattle interferes with feeding habits of fluke and these effects are noted in its size. According to [27], the liver is enlarged in response to acute inflammation as the wandering juvenile fluke mechanically damage the parenchyma. [24], also stated at the cut surface, the liver is irregular, firm and edematous with hemorrhagic channels and adhered fibrosis in the hepatic parenchyma and also fibrinous tags on the capsule and exudates between organs. These authors justified fibrinous tags on the capsule are due to capsular reaction to the flukes penetrating it. Supporting the current study [27].

In this study The adult liver flukes are leaf like and grayish brown in color flattened in its morphology. There are highlighted differences between two species. The *fasciola gigantica* is more elongated and narrow than the fasciola hepatica, this agree with [20] and [29] and the measurement is 5x2 cm, 3x2.5cm for *F. gigantica* and *F. hepatica* respectively.. The result agree with [30] who reported *F. gigantica* from Australia as leaf shaped, 24 to 76 mm by 5 to 13 mm. It was when fresh. While, F. hepatica has a morphology similar to *F. gigantica* measuring 18 to 32 mm long and 7 to 14 mm wide. In the anterior region these adult liver flukes have oral sucker and near the shoulder ventral sucker are localized and between these two sucker genital pore are present and this agree with [13], histopathological examination of positive case with chronic infection revealed pathological changes in liver by aggregation of inflammatory cells around the blood vessels forming nests of inflammatory cells with congestion of blood vessels [32].

Conclusion
The statistical analysis showed that there were a no significant differences under (*P*<0.05) between the percentage of infection in male and female cow (0.42). In cow the highly percentage of infection was in April (4.12%), while the lowest percentage was in November (0.00%). The statistical analysis showed that there were no significant differences under (*P*<0.05) between percentage of infection in male and female cow (0.28). In sheep the highly percentage of infection was in March (6.84) while the low percentage was also in November (0.00%). The results showed that the infection of sheep with fascioliosis in sheep more than in cattle, and infection in female more than male, also infection occur in spring season.

References
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