Serological Detection of Fasciola hepatica Antibodies among Cattle and Human in Behera Province, West Delta, Egypt

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Key words: F. Hepatica, Human, Cattle, IHAT, Serology

ABSTRACT:
A total of 184 serum samples were collected including; 92 from slaughtered cattle (23' season) in Abo El Matameer Abattoir, and 92 from random human patients attending a private laboratory in Abo El Matameer District, Behera Province to be examined for presence of antibodies against F. hepatica. Cattle samples were examined by Distomiasis Fumouze kits® (ready to use indirect haemagglutination test kits) and it was found that the total prevalence was 9.8 %. The seasonal prevalence of F. hepatica infection in examined serum samples of cattle revealed that winter and spring seasons showed the highest seasonal prevalence followed by summer season and males (10.8 %) were higher than females (7.4 %). In addition, the highest prevalence was observed in the age group 2 - 4 years (18.18 %) followed by the age group > 4 years (5.88 %). On the other side, the overall detection rate of F. hepatica infection in human was 13.04 % and the highest seasonal prevalence was noticed in winter season (21.74 %) followed by summer and spring season. The sexual distribution of the positive reactors to F. hepatica in human revealed higher prevalence in females (13.3 %) than in males (12.9 %) and the age group 20 - < 40 years (24.4 %) showed the highest prevalence followed by the age group 40 - < 60 years (22.7 %).

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1. INTRODUCTION
One of the neglected food-borne diseases in the international public health arena is fascioliasis. It is a zoonotic disease caused by the trematode Fasciola hepatica. It can infect a wide variety of mammalian hosts, particularly sheep, goats and cattle. Humans become infected after eating aquatic plants on which encysted organisms are present or by drinking contaminated water (Price et al., 1993).

In Egypt, animal and human fascioliasis is an endemic clinical and epidemiological health problem and it occurs throughout the year and affects both genders and all ages are under risk.

Doubtless, understanding the epidemiology of the parasitic diseases and factors affecting their incidence provides the foundation upon which effective prevention and control programs should be established (Soliman, 2008).

The parasitological diagnosis is based on identification of eggs in stool, duodenal contents or bile, also by the recovery of adult worm during surgical exploration, after treatment or at autopsy. However, the eggs may be present in very small number at irregular intervals, hence difficult to be found. Besides, the eggs may be transiently present in stool after ingestion of raw or undercooked liver from infected animals. The direct methods of diagnosing the egg are usually unsatisfactory. The symptoms may be present for several weeks before eggs are recovered in stool. Thus, the serologic tests are the alternative method of confirming early and extrabiliary human fascioliasis (Martinez et al., 1996). However, cross-reactions with other helminthic antigen may confuse the interpretation of the results (Haseeb et al., 2002). Serological tests including IHAT are acting as one of the most accurate, rapid and easy methods for diagnosis of fascioliasis in cattle and man.

So, the aims of the current study are to determine the prevalence of *F. hepatica* in serum samples of slaughtered cattle and human in Abo El Matameer, Behera Province. In addition, study the effect of season, sex and age.

2. MATERIAL AND METHODS

Collection of serum samples:
A. Slaughtered cattle:

About 5 ml of blood were collected aseptically in sterile tubes from 92 cattle (23/season) attending Abo El Matameer Abattoir before slaughtering during the period extended from December 2011 till November 2012. The blood samples were left for 30 minutes at room temperature for clotting then centrifuged at 3000 rpm /15 minutes. Serum was aspirated, labeled and stored at -20°C till examination. The data of each sample including age and sex were registered for epidemiological analysis.

**Distomiasis Fumouze test procedure:**

The test procedures were performed as described by the manual of the manufacture company *Fumouze Diagnostics* that was the reference for the rapid testing and the leader in the world for many manufactured rapid testing products.

**Preparation of 1:40 stock dilution of test serum**

Before use, allow reagents and samples to return to room temperature Deliver in a haemolysis tube and mix: 50 µl of test serum; 1.95 ml of phosphate buffer solution.

**Test execution on microplate:**

- By means of a multi-channel micropipette, deliver 50 µl of phosphate buffer solution in 8 wells of the microplate.
Add 50 µl of serum stock dilution in the 1st well.
Mix with buffer and transfer, preferably by means of a microdilutor (“tulip”), 50 µl from the 1st well into the 2nd well, from the 2nd into the 3rd and so on until the 6th well.
Discard 50 µl from the 6th well.
Add 50 µl of serum stock dilution into the 7th well.
Mix with buffer and discard 50 µl. This 1:80 dilution constitutes the serum control. It serves for the detection of natural anti-sheep agglutinins, which may occur in certain sera.
Carefully shake red blood cells suspensions.
Distribute 1 drop of sensitized red blood cells in the first 6 wells.
Distribute 1 drop of unsensitized red blood cells in the 7th well (serum control).
Distribute 1 drop 01 sensitized red blood cells in the 8th well (reagent control). Its role is to control the validity of buffer and sensitized red blood cells.

Very carefully homogenize wells content:
Either manually, by lateral thrumming on the edges of the microplate, place flatwise or with a vibrator-shaker for microtitration plates (for example 1300 rpm for 10 seconds). Then allow the plate to remain motionless, protected from vibrations.

Read the reaction 2 hours later.
Adsortion of natural anti-sheep agglutinins in case of agglutination of serum control
Introduce into a tube and mix: -0.1 ml of serum and 0.3 ml of adsorbent.
Incubate for 60 min at room temperature.
Centrifuge at 2000 rpm for 15 min.
Collect the supernatant fluid; the serum is then 1:4 diluted.
Dilute the supernatant fluid 1: 1 0 with the phosphate buffer solution to obtain a 1:40 adsorbed stock dilution.
Follow the protocol of test execution on microplate by replacing the stock dilution by the adsorbed stock dilution.

How to read the test results:
Negative Reaction: No Haemagglutination was indicated by presence of a more or less wide ring in well bottom.
Positive Reaction: Haemagglutination was indicated by the presence of a reddish-brown film in well bottom; sometimes, presence of a thin peripheral ring.

How to interpret the test results:
Titre < 1:160 indicate non significant reaction that may be due to probable absence of distomiasis so repeat the test 2 or 3 weeks later and associate a counter immunoelectrophoresis or an immunoelectrophoresis.

Titre = 1:160 indicate equivocal reaction so repeat the test 2 or 3 weeks later and associate a counter immunoelectrophoresis or an immunoelectrophoresis.

Titre ≥ 1:320 indicate significant reaction in favour of acute distomiasis.

3. RESULTS
1. Detection of antibodies against F. hepatica in examined serum samples of slaughtered cattle in relation to season of the year.
The recorded results in Table (1) clarified that the overall prevalence of antibodies against F. hepatica in examined serum samples of slaughtered cattle by IHAT was 9.78 % (9 out of 92). In addition, they revealed that the highest seasonal prevalence was observed in winter and spring seasons (13.04 %) followed by summer season (8.69 %) then autumn season (4.34 %).
Table (1): Prevalence of *F. hepatica* in the examined serum samples of slaughtered cattle in relation to season of the year

<table>
<thead>
<tr>
<th>Season of the year</th>
<th>No. of examined samples</th>
<th>+ve</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td>23</td>
<td>3</td>
<td>13.04</td>
</tr>
<tr>
<td>Spring</td>
<td>23</td>
<td>3</td>
<td>13.04</td>
</tr>
<tr>
<td>Summer</td>
<td>23</td>
<td>2</td>
<td>8.69</td>
</tr>
<tr>
<td>Autumn</td>
<td>23</td>
<td>1</td>
<td>4.34</td>
</tr>
<tr>
<td>Total</td>
<td>92</td>
<td>9</td>
<td>9.78</td>
</tr>
</tbody>
</table>

2. Detection of antibodies against *F. hepatica* in examined serum samples of slaughtered cattle in relation to sex.

Tabulated data in Table (2) showed that higher prevalence of *F. hepatica* antibodies was observed in males (10.8 %) than in females (7.4 %).

Table (2): Prevalence of *F. hepatica* in the examined serum samples of slaughtered cattle in relation to sex

<table>
<thead>
<tr>
<th>Sex</th>
<th>No of examined samples</th>
<th>+ve</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>65</td>
<td>7</td>
<td>10.8</td>
</tr>
<tr>
<td>Females</td>
<td>27</td>
<td>2</td>
<td>7.40</td>
</tr>
<tr>
<td>Total</td>
<td>92</td>
<td>9</td>
<td>9.78</td>
</tr>
</tbody>
</table>

3. Detection of antibodies against *F. hepatica* in examined serum samples of slaughtered cattle in relation to age groups.

The age wise positivity rate of *F. hepatica* infection in examined serum samples of slaughtered cattle was tabulated in Table (3) and revealed that the highest prevalence was observed in the age group (2 - 4 years) (18.18 %) followed by the age group (> 4 years) (5.88 %) and lastly the age group (< 2 years) (4.76 %).

Table (3): Prevalence *F. hepatica* in the examined serum samples of slaughtered cattle in relation to age groups

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>No of examined samples</th>
<th>+ve</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 2</td>
<td>42</td>
<td>2</td>
<td>4.76</td>
</tr>
<tr>
<td>2 - 4</td>
<td>33</td>
<td>6</td>
<td>18.18</td>
</tr>
<tr>
<td>&gt; 4</td>
<td>17</td>
<td>1</td>
<td>5.88</td>
</tr>
<tr>
<td>Total</td>
<td>92</td>
<td>9</td>
<td>9.78</td>
</tr>
</tbody>
</table>

4. Detection of antibodies against *F. hepatica* in examined serum samples of human in relation to season of the year.

The data presented in Table (4) firstly clarified that the overall prevalence of *F. hepatica* infection in 92 examined human serum samples was 13.04 % and secondly, it was noticed that the highest detection rate was recorded in winter season (21.74 %) followed by summer and spring season (13.04 %) and lastly autumn season (4.35 %).
Table (4): Prevalence of *F. hepatica* in the examined serum samples of human in relation to season of the year

<table>
<thead>
<tr>
<th>Season of the year</th>
<th>No. of examined samples</th>
<th>+ve</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td>23</td>
<td>5</td>
<td>21.74</td>
</tr>
<tr>
<td>Spring</td>
<td>23</td>
<td>3</td>
<td>13.04</td>
</tr>
<tr>
<td>Summer</td>
<td>23</td>
<td>3</td>
<td>13.04</td>
</tr>
<tr>
<td>Autumn</td>
<td>23</td>
<td>1</td>
<td>4.35</td>
</tr>
<tr>
<td>Total</td>
<td>92</td>
<td>12</td>
<td>13.04</td>
</tr>
</tbody>
</table>

5. Detection of antibodies against *F. hepatica* in examined serum samples of human in relation to gender.

The sexual distribution of the positive reactors to *F. hepatica* in examined serum samples of human was recorded in Table (5) and revealed higher prevalence in females (13.3 %) than in males (12.9 %).

Table (5): Prevalence of *F. hepatica* in the examined serum samples of human in relation to gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>No of examined samples</th>
<th>+ve</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>62</td>
<td>8</td>
<td>12.90</td>
</tr>
<tr>
<td>Females</td>
<td>30</td>
<td>4</td>
<td>13.33</td>
</tr>
<tr>
<td>Total</td>
<td>92</td>
<td>12</td>
<td>13.04</td>
</tr>
</tbody>
</table>

6. Detection of antibodies against *F. hepatica* in examined serum samples of human in relation to age groups.

The effect of age on the prevalence of *F. hepatica* infection in examined serum samples of human was illustrated in Table (6) revealed that the highest prevalence was noticed in the age group (20 - < 40 years) (24.4 %) followed by the age group (40 - < 60 years) (22.7 %) and finally the age group (< 20 years) (12.1 %).

Table (6): Prevalence of *F. hepatica* in the examined serum samples of human in relation to age group

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>No of examined samples</th>
<th>+ve</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>30</td>
<td>2</td>
<td>12.1</td>
</tr>
<tr>
<td>20 - 40</td>
<td>42</td>
<td>8</td>
<td>24.4</td>
</tr>
<tr>
<td>&gt; 40 - 60</td>
<td>20</td>
<td>2</td>
<td>22.7</td>
</tr>
<tr>
<td>Total</td>
<td>92</td>
<td>12</td>
<td>13.04</td>
</tr>
</tbody>
</table>

4. DISCUSSION

Although, *F. gigantica* is one of the commonest parasitic infections in livestock in Egypt but WHO, (2012) reported that human cases occurred occasionally but are now increasingly reported from Europe, the Americas and Oceania (where only *F. hepatica* is transmitted) and from Africa and Asia (where the two species *F. gigantica* and *F. hepatica* overlap) so, it is preferable to study the epidemiology of *F. hepatica* too in Egyptian livestock and human. Based on the previous fact, the current study was carried out.

The obtained results in Table (1) clarified that the overall rate prevalence of antibodies against *F. hepatica* in examined serum samples of slaughtered cattle by IHAT was 9.78 % (9 out of 92). This result was extremely lower than that obtained by Samaha, (1989) (68.75 %), Levieux et al., (1992) (48 %) and Morrondo et al. (1997) (64.7%). In addition, it was lower than Abdel-Rahman, (2002) (14 %), El-Shazly et al.,
(2002) (12.31%), Ansari-Lari and Moazzeni (2006) (21 %), Munguía-Xochihua et al., (2007) (24.4 %) and Kozłowska-Łój (2011) (21.24%). The result may be attributed to the fact that F. hepatica was less common in Egypt compared to F. gigantica that may be investigated in the previous studies or may be due to the regular administration of anthelmintics that was done by cattle owners in Abo El Matameer Districts and regular veterinary medical campaigns aimed to cure cattle. On contrary, the obtained result in the current study was higher than that obtained by Haridy et al., (1999) (3.54 %). The seasonal prevalence of F. hepatica infection in examined serum samples of slaughtered cattle revealed that the highest seasonal prevalence was observed in winter and spring seasons (13.04 %) followed by summer season (8.69 %) then autumn season (4.34 %). This result was in harmony with that obtained by Phiri et al., (2005) who noticed that all cattle, regardless of age, had higher fluke abundances in the post-rainy season (39.1% young and 42.1% adult) while the lowest rates were in the hot dry season (13.3% young and 14.3% adult).

Data illustrated in Table (2) showed that the frequency of detection of antibodies against F. hepatica in examined serum samples of slaughtered cattle was higher in males (10.8 %) than in females (7.4 %).

The age wise positivity rate of F. hepatica infection in examined serum samples of slaughtered cattle was tabulated in Table (3) and revealed that the highest prevalence was observed in the age group 2-4 years (18.18 %) followed by the age group season (13.04 %) and lastly autumn season (4.35 %). The obtained result disagreed with that recorded by Marie (1992) who recorded higher prevalence in summer season (8.6 %) followed by spring, autumn and lastly winter season. On contrary, it agreed with that obtained by Abdel-Rahman, (2002) who recorded that fascioliasis prevalence increased in winter season.

The sexual distribution of the positive reactors to F. hepatica in examined serum samples collected from human patients was found in Table (5) and revealed higher > 4 years (5.88 %) and lastly the age group < 2 years (4.76 %). This result was in harmony with that obtained by Geurden et al., (2008) who examined a total of 334 stool and 239 blood samples collected from calves younger than 3 months, animals between 3 and 24 months and adult cows and found that in animals between 3 and 24 months (n=176) and in adult cows (n=90), the prevalence of Fasciola was 28% and 39%, respectively.

The data presented in Table (4) firstly clarified that the overall prevalence of F. hepatica infection in 92 examined human serum samples was 13.04 %. This result agreed with Bjorland et al. (1995) who mentioned that F. hepatica was a common and important parasitic of cattle which can transmitted to human which can recognized by fever and abdominal pain and serum IgG antibodies of F. hepatica. The obtained result was higher than that obtained by Abdel-Rahman, (2002) (4 %) and Freites et al., (2009) (3.9 %). On contrary, it was lower than that recorded by González et al., (2011) (24.4 %). Obtained results in the current study confirmed the role of cattle in transmission of F. hepatica infection in human in Abo El Matameer district, Behera province so control measures should be accounted in order to combat such infection in cattle and subsequently decrease human infection.

The tabulated data in Table (4) also showed the effect of season on the rate of detection of F. hepatica infection in examined serum samples of human. It was noticed that the highest detection rate was recorded in winter season (21.74 %) followed by summer and spring prevalence in females (13.3 %) than in males (12.9 %). This result was in harmony with that obtained by Samaha (1989) who found that prevalence of fascioliasis was higher in females (7.96 %) than in males (5.85%), Apt et al. (1992) who found that women were more frequently infected than men, Marie (1992) found that the incidence of infection in males and females was 6.4 and 8 %, respectively and González et al., (2011) who recorded higher prevalences in females.

The effect of age on the prevalence of F. hepatica infection in examined serum
samples of human was illustrated in Table (6) revealed that the highest prevalence was noticed in the age group 20 - < 40 years (24.4 %) followed by the age group 40 - < 60 years (22.7 %) and finally the age group < 20 years (12.1 %). The obtained result may be explained due to young children to help their parents in agricultural activities and in the tending of animals that is common practice in the country. Thus adults and children, whilst performing these activities stay away from home for many hours. This leads them to drink fresh water and to eat vegetables such as watercress which are soaked with waters that may be contaminated with metacercaria of F. hepatica. This result was not in harmony with that obtained by Samaha, (1989) who recorded that all ages were infected with Fasciola and the higher infestation was restricted to the age ranged from 10 to 20 years and Apt et al., (1992) who found that 51.2 % of the positive samples less than 15 years of age.

Based on the results obtained in the current study, it is concluded that fascioliasis antibodies are found in the examined sera of cattle slaughtered in Abo El Matameer abattoir confirming the role of cattle in transmitting this parasite to man. So, suggestive control measures should be undertaken to avoid spreading of disease from cattle to man including regular examination of cattle serum samples for detection of the presence of fascioliasis and treatment of positive reactors, hygienic disposal of all animal wastes should be done and proper cleaning and disinfection of cattle houses should be applied. Moreover, application of control measures to eradicate transmitting vectors, snails

5. REFERENCE


