



Serological Prevalence of Anti-*Fasciola Hepatica* Antibodies in Sheep



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Abstract

THIS STUDY aims to investigate serological prevalence of *F. hepatica* in sheep with estimating the degree of positivity and association to epidemiological risk factors. Totally, 460 sheep were selected and blood sampled to testing the obtained sera by indirect ELISA. The findings revealed that the prevalence of anti-*F. hepatica* IgG antibodies in sheep was 39.78% with existence 1.96% of dubious cases. According to degree of seropositivity, heavy infestation (49.73%) was increased significantly in comparison with mild (13.11%) and moderate (37.16%) infestations. The results of risk factors showed a significant increasing in positivity, Odds ratio and relative risk in sheep of 13-24 months old; and reduction in sheep of ≤6 months old. Subsequently, mild infestation was increased at ≤6 months old; whereas, moderates and heavies were appeared at ≥25 and 13-24 months old, respectively. Seropositivity, Odds ratio and relative risk were higher in Al-Kut than Al-Hay and Al-Numaniyah. Concerning degrees of positivity, mild and moderate infestations were detected at Al-Hay; while, heavy infestation in Al-Numaniyah. No significant variation was found between females and males; however, females appeared at higher risk than males. For degrees of seropositivity, moderates and heavies were elevated significantly in males and females, respectively. In conclusion, this represents the first serological study targets detection of *F. hepatica* in sheep of Wasit province (Iraq). Therefore, additional investigations using the advanced assays (serology and molecular assay) appear necessary to determine the rate of positivity in animals, and areas at higher risk of parasitic contamination and infection.

Keywords: Fasciolosis, Liver fluke, ELISA, Ovine parasitic diseases, Iraq.

Introduction

Fasciola hepatica is a common parasitic liver fluke, which belongs to the Plagiorchiida Order under the Trematoda Class of Platyhelminthes Phylum, which infects various mammals including human causing a disease known as fasciolosis [1]. The parasite is found in all continents except Antarctica, especially in areas of sheep and cattle that infected by eating of water pants contaminated with immature parasite larvae [2]. After reaching of young worms to liver, particularly bile duct, it matured to adult and start to production of eggs [3].

Worldwide, *F. hepatica* has implicated in greatly

economic losses in sheep industry as a result of acute infections, blood loss, sudden deaths and liver damages [4, 5]. Despite adequate flock nutrition, sub-acute fasciolosis may appear as low-fleece quality, rapid loss of body condition, reducing in milk production, and low growth rate; whereas, the chronic cases characterize by bottle-jaw and very poor body condition. Various authors mentioned that the high metabolic demands of advanced pregnancy or early lactation might increase the rate of mortality and decrease the lamb crop in infected ewes [6-8].

For diagnosis, the farm history and epidemiology data might be followed to suggest the fluke risk; however, traditional examination of slaughtered

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animals remains the most definitive indicator. Also, identification of fluke eggs in fecal samples of clinically suspected animals is useful; however, it's time-consuming, laborious and lack of sensitivity, particularly, in animals having low parasitic burdens [9, 10]. In last decade, serological assays such as enzyme-linked immunosorbent assay (ELISA) offers a rapid, valuable and sensitive tool for indication of infection based on positive reaction of anti-fluke antibodies in blood samples [11].

In many countries, laboratory surveillance and slaughterhouse condemnations showed a remarkable elevation being observed further in regions that considered previously at lowered risk or free [12, 13]. Climate changes appeared to play a role in reducing the predictability of geographical and seasonal ranges of flukes. Additionally, the movement of diseased sheep into previously free areas allows the parasite to establish [14]. In Iraq, few serological studies have been conducted in sheep in Duhok [15], Baghdad [16], Al-Qadisiyah [17] and Nineveh [18]. Hence, the current study aims to investigate serological prevalence of *F. hepatica* in sheep, estimate the degree of positivity and identify association to some epidemiological risk factors.

Material and Methods

Ethical approval

The Scientific Committee in College of Medicine (University of Wasit, Wasit, Iraq) was licensed the current study, and the blood samples were collected following the oral agreement of their owners.

Samples and data

Totally, 460 sheep of both sexes and variable ages were selected randomly at areas of Wasit province (Iraq) during March to December (2022). Approximately, 2.5 ml of venous blood was sampled from each study animal using disposable syringes into free-anticoagulant glass tubes that transported in vertical position using of ice-bag box. Post centrifugation, sera were kept frozen until serology [19]. Additional required data concerned to age (<6 months, 6-12 months, 1-2 year and > 2 years), sex (males and females) and region (Al-Kut, Al-Numaniyah, and Al-Hay) were recorded as epidemiological risk factors.

Serology

As described in the manufacturer instructions of the Monoscreen AbELISA *Fasciola hepatica* Kit (Bio-X Diagnostics, Rochefort-Belgique), sera were thawed, diluted in the Dilution Buffer (1:100), and processed by adding of positive and negative control solution to the first 1-4 Wells of the plate, and then

adding of sera to followed Wells. After incubation, the plate was washed then the Conjugate and a Peroxidase-labeled anti-IgG1 monoclonal antibody was added. After incubation, the plate was washed, TMB was added, plate was re-incubated, and then the Stop Solution was added. Optical density (OD) was read in the ELISA Microplate Reader (Bio Tek, USA) at 450 nm. The degree of positivity was interpreted as (0) for negative *F. hepatica* infestation, (+/-) for dubious findings, (+1) for low-grade infestation, (+2) for Moderate infestation and (+3) for heavy infestation.

Statistical analysis

The obtained data were analysed using the GraphPad Prism (version 6.0.1) Software. *One- and Two-Way ANOVA* were served to detect significant variation between the degrees of positivity; whereas, Odds-Ratio and Relative Risk were measured to evaluate the levels of risk among study animals. Values were represented as number (percentage) [No. (%)], and differences were considered significant at $P < 0.05$ [20]. In tables, variation in large horizontal and/or small vertical letters referred to a significance ($P < 0.05$); while in figures, the presence of (*) was referred to a significant variation ($P < 0.05$).

Results and Discussion

Total results and degree of positivity

In this study, the seroprevalence rate of IgG antibodies against *F. hepatica* in sheep sera tested by ELISA was 39.78% (183 / 460) with existence 1.96% (9/460) of dubious cases and 58.26% (268%) as negatives ($P < 0.0091$), (Figure 1). The findings of degree seropositivity revealed that the prevalence rate of heavy infestation was increased significantly [49.73% (91/183)] when compared to mild [13.11% (24/183)] and moderate [37.16% (68/183)] infestations of *F. hepatica* among positive study sheep ($P < 0.0161$), (Figure 2).

Fasciola hepatica is potentially fatal parasite of sheep throughout the world, as it directly linked to the habitat. The findings of this study revealed that 39.72% of sheep was positives to anti-*F. hepatica* IgG antibodies with high prevalence of heavy infestation in comparing to mild and moderate infestations. This indicates the frequent previous and recent exposure of study sheep to the parasite. In comparison with the national studies, there were 33.1% in Al-Qadisiyah [17], 12.73% in Baghdad [16], 8.9% in Duhok [15], and 8.88% in Nineveh [18]; whereas globally, prevalence rate of fasciolosis was 60.2% in Australia [21], 78.4% in Greece [22], 39.2% in Pakistan [23], 82.13% in Sweden [24],

32.4% in Turkey [25], and 41.5% in Columbia [26]. This variation in nationally and globally prevalence of *F. hepatica* might be attributed to differences in geographical and environmental condition, management and treatment/control protocols, number of studied animals, time and method of sampling, type of serological assay and targeted antigens. Rinaldi et al. [27] highlighted the importance of seasonality, rainfall and temperature in extension the recurrence of infections.

Several previous and recent serological studies; carried out in cattle, goats and sheep, have demonstrated the high efficacy of ELISA in earlier diagnosis of *F. hepatica* than coprological test (fecal eggs count) that cannot identify the existence of *Fasciola* eggs up to 2-3 months after infection [28-31]. Gröning et al. [32] mentioned that antibodies could detect with 2-4 weeks post infection, and still higher for at least 1.5 year. In one study, the findings showed that the specificity of ELISA is 100% in comparison with traditional coprological and molecular assays [24]. However, detection of antibodies may be related to presence of active infections or previous exposure [23].

Epidemiological risk factors

The findings showed that the positive rate of *F. hepatica* as well the Odds ratio and relative risk values were increased significantly ($P < 0.05$) in sheep aged 13-24 months; while significant decreases ($P < 0.05$) were seen in animals aged ≤ 6 months when compared to those of ≥ 25 months and 7-12 months age old (Table 1). Regarding the degree of seropositivity, mild infection was increased in sheep of ≤ 6 months old; whereas, moderate and heavy infestations were elevated significantly in sheep of ≥ 25 months and 13-24 months, respectively (Table 2).

Significantly, higher rates of positivity, Odds ratio and relative risk of *F. hepatica* were seen in sheep of Al-Kut than Al-Hay and Al-Numaniyah (Table 3). Concerning the degree of seropositivity, the mild and moderate infestations were detected significantly in Al-Hay; while, heavy infestation was observed significantly in sheep of Al-Numaniyah (Table 4).

Although, the positive rates of *F. hepatica* infestation in both females and males were differed insignificantly ($P > 0.05$), females were appeared significantly at higher risk of infection than males (Table 5). Relation to degrees of seropositivity, values of mild infestation differed insignificantly ($P > 0.05$) among males and females; however, values of moderate infestation was elevated significantly ($P < 0.05$) in males, while heavy infestation was showed significantly in females (Table 6).

The prevalence of fasciolosis in ruminants is correlated largely with age. Our findings showed that sheep of ≤ 6 months were at lowered levels of positivity, which might belong to either the role of maternal immunity that could last up to 12 weeks [8]; while low value of seropositivity in lambs of 7-12 months might reflect the fact that in Iraq, most owners kept their lambs indoors with receiving a particular nutritional and therapeutic attentions for their high economic benefits. Significant elevation in anti-*F. hepatica* antibodies at 13-24 months might be caused by the exposing of pastured sheep to a high concentration of parasite and development of immunity against it; while, reducing of seropositivity in older animals may occur by lowering of resistance due to environmental stressors and attenuation of immune system [23]. High infection pressure is more prevalent in old animals due to longer periods pasture exposure [27, 33, 34].

Significantly differences in positive rates of *F. hepatica* infection and distribution of degrees of positivity were showed in study regions (Al-Kut, Al-Hay and Al-Numaniyah). This variation might follow differences in potential resistance to anthelmintics, control strategies, and management or combination of them. Habitat difference between sheep farms could affect the prevalence of *F. hepatica* because good environment provide less suitable condition to survival metacercaria or snails [35]. Valderrama Pomé [36] mentioned that breeding in pasture might favorable for existence of parasite, because animals having direct contact with the infective form.

In the current study, positive rate of infection showed that no significant variation was found between females and males [18, 37, 38]. These findings means that either *F. hepatica* does not associated with sex of animals [23], or both sexes exposed to the same risk factor of infection such as contaminated grass [39]. However, statistical analysis recorded that the study sheep females were at higher risk of heavy infestation than males. In female sheep, attenuated immune system due to stress caused by gestation and lactation, as well as, advanced age may contribute to increasing of seropositivity.

Conclusions

The results indicate that the parasite has become endemic and frequent exposure of sheep populations in studied areas to the parasite. This study recommends to using of ELISA due to the great volubility in detection of infection throughout short-time disregarding the size of samples. Also, the served ELISA kit was provided additional and valuable data through detection the degrees of seropositivity, and then, it can presumed the risk at which the study animals are exposed. However, the

combination of traditional method (fecal eggs count) and serological assay or molecular assay might increase the sensitivity and specificity of the results. Effective strategic parasite control programs for sheep and other domestic animals need to be based on epidemiological data.

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Conflicts of interest

There are no conflicts to declare.

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No external funds were received (private funding).

Data availability

All data included within the manuscript.

Authors' contribution

HAJG: Serological examination of sera. AHKS: Collection of blood samples. IME: Statistical analysis of obtained results. The authors were approved the final copy of manuscript.

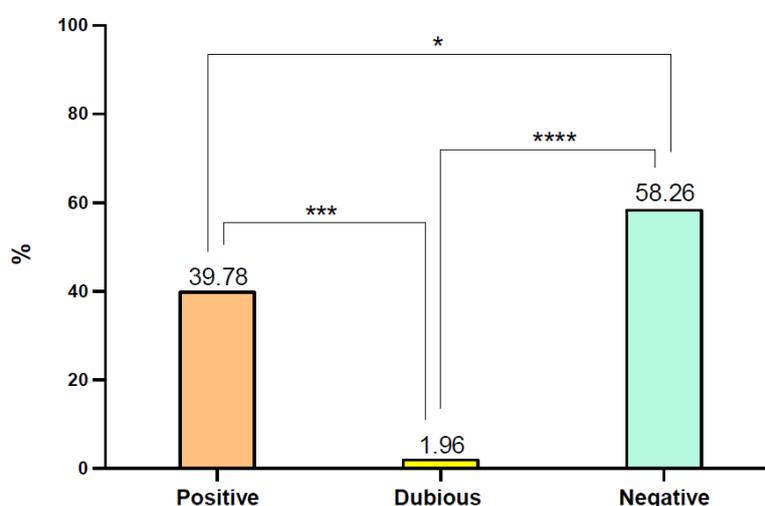


Fig. 1. Total results of serology for testing 460 sheep by iELISA; Positive (No: 183), Dubious (No: 9), Negative (No: 268)

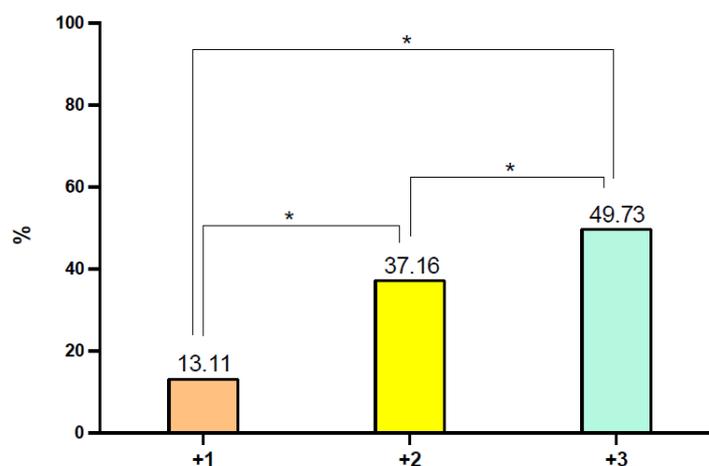


Fig. 2. Degrees of seropositivity among 183 infected sheep; +1 (No: 24), +2 (No: 68), +3 (No: 91)

TABLE 1. Association of seropositivity to age of study sheep (Total No: 460)

Group	Total No.	Positive	Odds-Ratio	Risk
≤ 6 months	25	2 (8%) ^d	0.122 ^d	0.192 ^d
7-12 months	77	20 (25.97%) ^c	0.474 ^c	0.61 ^c
13-24 months	108	54 (50%) ^a	1.73 ^a	1.36 ^a
≥ 25 months	250	107 (42.8%) ^b	1.319 ^b	1.14 ^b

Variation in small vertical letters referred to significant differences (P<0.05).

TABLE 2. Association of degree of seropositivity to age of study sheep (Total No: 183)

Group	Total No.	Degree		
		+1	+2	+3
≤ 6 months	2	2 (100%) ^{Aa}	0 (0%) ^{Bc}	0 (0%) ^{Bc}
7-12 months	20	3 (15%) ^{Cb}	6 (30%) ^{Bb}	11 (55%) ^{Aa}
13-24 months	54	7 (12.96%) ^{Cb}	14 (25.93%) ^{Bb}	33 (61.11%) ^{Aa}
≥ 25 months	107	12 (11.21%) ^{Bb}	48 (44.86%) ^{Aa}	47 (43.93%) ^{Ab}
Total	183(39.78%)	24 (13.11%)	68 (37.16%)	91 (49.73%)

Variation in smallvertical and large horizontal letters refer to significant differences (P<0.05).

TABLE 3. Association of seropositivity to region of study sheep (Total No: 460)

Group	Total No.	Positive	Odds-Ratio	Risk
Al-Kut	160	87 (54.38 %) ^a	2.531 ^a	1.7 ^a
Al-Hay	150	61 (40.67%) ^b	1.055 ^b	1.033 ^b
Al-Numaniyah	150	35 (23.33%) ^c	0.333 ^c	0.482 ^c

Variation in small vertical letters referred to significant differences (P<0.05).

TABLE 4. Association of degree of seropositivity to region of study sheep (Total No: 183)

Group	Total No.	Degree		
		+1	+2	+3
Al-Kut	87	2 (2.3%) ^{Cc}	25 (28.74%) ^{Bc}	60 (68.97%) ^{Ab}
Al-Hay	61	17 (27.87%) ^{Ba}	41 (67.21%) ^{Aa}	3 (4.92%) ^{Cd}
Al-Numaniyah	35	5 (14.29%) ^{Bb}	2 (5.71%) ^{Cd}	28 (80%) ^{Aa}
Total	183	24 (13.11%) ^{Cb}	68 (37.16%) ^{Bb}	91 (49.73%) ^{Ac}

Variation in smallvertical and large horizontal letters refer to significant differences (P<0.05).

TABLE 5. Association of seropositivity to sex of study sheep (Total No: 460)

Group	Total	Positive	Odds-Ratio	Risk
Female	329	134 (40.73%) ^b	1.149 ^a	1.088 ^a
Male	131	49 (37.4%) ^b	0.871 ^b	0.919 ^b

Variation in small vertical letters referred to significant differences (P<0.05).

TABLE 6. Association of degree of seropositivity to sex of study sheep (Total No: 183)

Group	Total No.	Degree		
		+1	+2	+3
Female	134	19 (14.18%) ^{Cb}	31 (23.13%) ^{Bc}	84 (62.69%) ^{Aa}
Male	49	5 (10.2%) ^{Bb}	37 (75.51%) ^{Aa}	7 (14.29%) ^{Bc}
Total	183	24 (13.11%) ^{Cb}	68 (37.16%) ^{Bb}	91 (49.73%) ^{Ab}

Variation in small vertical and large horizontal letters refer to significant differences ($P < 0.05$).

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الانتشار المصلي لأضداد المتورقة الكبدية في الأغنام

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 ٢ فرع الاحياء المجهرية - كلية الطب البيطري - جامعة واسط - واسط - العراق .
 ٣ فرع الطفيليات - كلية الطب البيطري - جامعة البصرة - البصرة - العراق .

تهدف الدراسة الحالية إلى التحقق من الانتشار المصلي لمرض المتورقة الكبدية (*F. hepatica*) في الأغنام وتحديد درجة الإصابة الموجبة وارتباطها ببعض عوامل الخطر الوبائية . تم إجمالاً اختيار ٤٦٠ من الأغنام والتي خضعت لسحب عينات الدم وجمع الأمصال لغرض فحصها بواسطة فحص الادمصاص المناعي المرتبط بانزيم (ELISA) غير المباشر . أظهرت النتائج أن معدل انتشار الأجسام المضادة للمتورقة الكبدية في الأغنام بلغت ٣٩,٧٨٪ مع وجود ١,٩٦٪ من الحالات المشكوك فيها . وفقاً لدرجة الإيجابية المصلية ، ظهرت الإصابة الشديدة (٤٩,٧٣٪) بشكل ملحوظ مقارنة مع الإصابة الخفيفة (١٣,١١٪) والمتوسطة (٣٧,١٦٪) . أظهرت نتائج عوامل الخطر ارتفاعاً معنوياً في الاصابات الموجبة ونسبة الأرجحية والخطر النسبي في الأغنام بعمر ١٣ - ٢٤ شهراً؛ وانخفاضاً في عدد الأغنام التي يبلغ عمرها ٦ أشهر . كذلك ، لوحظ زيادة معدل الإصابة الخفيفة عند عمر أقل من ٦ أشهر؛ بينما ظهرت الاصابات المتوسطة والشديدة عند عمر ≥ 25 و ١٣-٢٤ شهراً على التوالي . بشكل ملحوظ، بلغت الاصابات الموجبة المصلية ونسبة الأرجحية والخطر النسبي أعلى في الكوت منها في الحي والنعمانية . وفيما يتعلق بدرجات الاصابات الموجبة ، فقد تم رصد إصابات خفيفة ومتوسطة في الحي ؛ بينما شديدة الإصابة في النعمانية . لم يتم العثور على اختلاف كبير بين الإناث والذكور الموجبة الإصابة ؛ مع ذلك، يبدو أن الإناث أكثر عرضة للخطر من الذكور . بالنسبة لدرجات الاصابات الموجبة المصلية ، شوهدت الإصابة الخفيفة بشكل غير ملحوظ في الإناث والذكور ، ولكن المتوسطة والشديدة كانت مرتفعة بشكل ملحوظ في الذكور والإناث ، على التوالي . تستنتج هذه الدراسة المصلية الأولى أهداف الكشف عن المتورقة الكبدية في أغنام محافظة واسط (العراق) . ومن هنا ، يبدو من الضروري إجراء دراسات إضافية باستخدام التقنيات المتقدمة (المصلية و الجزيئية) لتحديد معدل الإصابة الموجبة في الحيوانات والمناطق الأكثر عرضة لخطر التلوث الطفيلي والعدوى .

الكلمات الدالة : داء المتورقة ، ديدان الكبد ، فحص المقايسة المناعية الانزيمية ، امراض الاغنام الطفيلية ، العراق.