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Khalid Ismael Oleiwi
Fallujah University, Vet. Med. College, Iraq

Zaid Salah Hussein
Baghdad University, Vet. Med. College, Iraq

Kefah Oda Salman
Baghdad University, Vet. Med. College, Iraq

Correspondence
Khalid Ismael Oleiwi
Fallujah University, Vet. Med. College, Iraq

Detection of *Fasciola hepatica* in Abu-Ghraib district (Iraq)

Khalid Ismael Oleiwi, Zaid Salah Hussein and Kefah Oda Salman

Abstract
The objective of this investigation was to determine the seropositivity of *Fasciola hepatica* in local sheep selected from Abu Ghraib district/ Baghdad province. To conduct such purpose fecal and blood samples of 220 animals were randomly collected from those sheep reared at the studied area during the period extended between August 2016 and March 2017. Samples were transported directly to the Lab of clinical pathology at college of Veterinary Medicine to perform some important tests that were ELISA, hemogram and leukogram evaluation, fecal examination, serum albumin, total protein and gamma-glutamyltranspeptidase. Out of the 220 samples, sera of 28 (12.73%) animals were found seropositive toward *Fasciola hepatica* post applying ELISA test, but only 6 (2.73%) of these animals exhibits eggs of Fasciola in their feces. Outcome of the hemogram showed significant variations ($P<0.05$) between seropositive and seronegative animals in the values tested such as Hb, MCV and MCHC. On the same line, the leukogram showed significant variations ($P<0.05$) in the tested values of WBC, Neutrophil, Eosinophil and Basophile. In another aspect, results of the biochemical tests showed significant variations ($P<0.05$) in albumin, total protein and GGT values between seropositive and seronegative.

Keywords: *Fasciola hepatica*; sheep; ELISA; fecal examination; ABU Ghraib

Introduction
Fascioliasis also known as distomatosis or liver rot [1] was considered one of the most important worldwide parasitic diseases in the domestic animals [2]. The occurrence and zoonotic nature of fascioliasis elicit the disease as a major global concern affecting all domestic animals especially in regions characterized by intensive cattle production [3]. Fascioliasis is existed in Europe, Africa, Asia, Americas and Oceania. It is a common parasite in ruminants, especially sheep, goats and cattle. Alternative hosts are equine (horses, donkeys and mules) and camelids. Wild herbivorous mammals such as buffalo, deer, wild sheep, wild pigs, various marsupials, rabbits, hares, nutria and monkeys are also considered susceptible hosts [4]. The parasite poses a threat to animal welfare and causes substantial economic losses through mortality, ill-thrift and condemnation of infected livers at the abattoir, predisposition to other diseases, treatment and associated veterinary costs [5]. The parasite can stay inside the definitive host for one to twenty years and in sheep it was recorded up to eleven years [6, 7]. Long-term damage can cause low performance and possibly reduced ability to resist other infections [8]. Records of WHO showed at least 2.4 millions of people in more than 70 countries were infected, whereas, several other millions were at risk and no continent is free from the disease [9]. In fact, the emergence of Fascioliasis in human in recent decades as highly pathogenic zoonotic disease was in part due to the effects of climatic changes, anthropogenic environmental modifications as well as import/export and movements of livestock [10]. These factors may predispose to the increase of occurrence of *Fasciola spp*.
Prevalence of the disease in the world is due to the development of resistance against triclabendazole which is the treatment of choice against this parasitic disease [11, 12, 13, 14, 15, 16]. The ecological changes that occurs in vast regions middle and northwest of Iraq is one outcome of military conflicts that were encountered between the year 2014 and 2015 when several dams and water systems were destroyed accompanied by floods followed by a period of drought [17]. Abu Ghraib district was selected as an example of such ecological changes to identify the impacts of these changes on the prevalence of Fascioliasis in local sheep.

Materials and Methods

A. Studied area and design

During the period of early August 2016 till March 2017 a survey was conducted in twenty-one villages located at Abu Ghraib district/ Baghdad. Randomly two hundred and twenty samples were collected from sheep to determine the seropositivity of the liver flukes in them.

B. Sampling

Blood samples

Using a vacutainer tubes (plain and EDTA coated), two blood samples were collected from the jugular vein of each selected animal. Serum was obtained according to the method previously described [18] and stored at -20 °C in Eppendorf tubes for subsequent serological and biochemical tests. Complete blood picture was conducted using automated hematology analyzer (Genex) while leukogram was performed according to the procedure previously described [18].

Fecal Samples

Fecal samples of 5-10g were collected directly from the anus of each selected sheep and transferred using plastic containers with optimum condition to the lab of internal and preventive veterinary medicine department.

Direct fecal smear was performed following procedure previously described [19] and the sedimentation fecal examination was conducted [20].

C. Antibodies detection of *Fasciola hepatica*

Detection test was performed by applying ELISA. The procedure was performed following the instructions of kit Manufacture Company (Mono screen Ab ELISA *Fasciola hepatica* Bio-X Diagnostics).

D. Biochemical tests

Total protein was estimated using commercial kit (total protein biuret method) provided by BIOLABO SAS (France). Serum albumin was calculated according to the instructions of the commercial kit (ALBUMINBROMOCRESOL GREEN) provided by Biosystems reagents & instruments (Spain)

The glutamyl transpeptidase (GGT) was calculated according to the instructions of the commercial kit provided by Cyber Diagnostic (Belgium).

E. Statistical analysis

The statistical analysis system- (SAS) (2012) program was used to analyze the differences in studied parameters. Chi-square test was used to compare between obtained percentages in the current study.

Results

Sera samples that were obtained from 220 sheep at Abu – Ghraib district/Baghdad province showed only 28 (12.73%) sera positive to the applied tests. For fecal examination, only 6 (2.73%) fecal samples were containing the *Fasciola* eggs, while 214 (97.27%) fecal samples of the rest were negative. Results of hemogram showed significant variations ($P<0.05$) between seropositive and seronegative animals in tested values such as Hb, MCV, MCH and MCHC (Table 1).

Hematological findings of differential leukocyte count for seropositive and seronegative animals showed significant variation ($P<0.05$) between them in the values of WBC (neutrophils, eosinophils and basophils) (Table 2).

Findings of the biochemical tests showed significant variations ($P<0.05$) in values of albumin, total protein and GGT between seropositive and seronegative animals (Table 3)

Table 1: Hemogram analysis of seropositive and seronegative samples

Groups	Mean ± SE				
	RBC 10*12/L	Hb (g/dl)	PCV (%)	MCV fl	MCH pg
Seropositive	7.23 ± 0.17	8.03 ± 0.23	26.78 ± 0.91	37.33 ± 0.64	11.97 ± 0.97
Seronegative	7.11 ± 0.08	8.86 ± 0.10	24.61 ± 0.58	34.84 ± 0.35	14.54 ± 0.46
T-Test	0.446	0.549 **	3.101	1.864 **	2.473 *
P-value	0.605	0.0034	0.169	0.009	0.0418

* Means there is significant difference ($P<0.05$) ** means there is significant difference ($P<0.01$)

Table 2: Leukogram analysis of seropositive and seronegative samples

	Mean ±SE		Significance
	Seropositive	Seropositive	
WBC	9020.00 ± 387.29	9020.00 ± 387.29	*
Neutrophil	3633.64±163.34	3633.64±163.34	*
Lymphocyte	4003.21±213.87	4003.21±213.87	
Monocyte	196.07±16.08	196.07±16.08	
Eosinophil	1226.10±40.57	1226.10±40.57	*
Basophile	76.46±9.37	76.46±9.37	*

* Means there is significant difference ($P<0.05$)

Table 3: Biochemical analysis of seropositive and seronegative samples

	Mean ±SE		Significance
	Seropositive	Seropositive	
Albumin	15.94 ± 0.73	15.94 ± 0.73	*
Total Protein	49.13±1.28	49.13±1.28	*
GGT	138.17±8.43	138.17±8.43	*

* Means there is significant difference ($P<0.05$)

Discussion

ELISE results

Current findings indicated that ELISA technique was the ideal test for the present investigation because of its higher sensitivity as well as its simplicity and time saving when applying this technique and such findings was in a good agreement with previous records [21].

The hypothesis is that detectable circulating antibody against *F. hepatica* can be measuredin sera of infected animals as early as two-week post infection [22], furthermore these antibodies may be persisted for a six month after anthelmintics treatment [23] so that serological tests could be the most significant method for fascioliasis surveillance for the live animals.

Present obtained data showed only 28(12.73%) samples which were out of 220 sheep seropositive to the applied test and such records were lesser than those recoded previously in Iraq [24]. The latter reference conducted serosurveillance in sheep and cattle in southeast of Iraq and found a percentage up to 67.7% of 43 examined sheep that were seropositive to Fasciola. also higher prevalence was recorded up to 35% in 20 examined sheep in Babylon province at the middle of Iraq [25] but differently using the same test on jaundiced slaughtered sheep in Duhok abattoir at the northern of Iraq found only 16.8% of them were seropositive for F. hepatica [26]. Regardless the influences of sampling origin and size in the previous studies in Iraq the significant variation in results with present investigation was considerably due to the drought that take place in the studied area as an outcome of blocking and destroying of several dams during the military conflicts with ISIS. Hence they changed the ecological factors that play a role in the development of both *Fasciola spp*. and their intermediate hosts.

Studies concerned in the neighbor countries such as Turkey recorded higher seropositive rate than the present investigation [27, 28]. The latter references recorded 93% and 31.4%, respectively when they studied the seropositivity in sheep and cattle. On the same line, the seropositive to *F. hepatica* was 28.7% in Southwestern of Iran [29]. These variations affirmed that the incidence of fascioliasis is much higher in the neighboring countries compared to Iraq in spite of the lack of reliable anthelmintic controlling regimes in Iraq during the last decade.

Fecal examination results

Although serological investigations were generally more practical for surveillance of most animal diseases and possess a reliable outcome but the shortcoming of their results reveals the exposure of animal (s) to a particular antigen regardless its non-pathogenic or vaccine nor determine the exact time of the this exposure. This may be the reason beyond the variation between the seropositivity compared to fecal examination of the same animals when sedimentation fecal examination exhibit that only 2.73% (six samples) were containing the eggs of Fasciola in their feces. Beside that many previous studies proved doubt results of fecal examination due to the intermittent and highly variable egg shedding patterns by individual worms [30, 31]. However, in Fascioliasis the endemicity and the chronic nature of the disease in Iraq obligate the involvement of confirmatory tests since the specific antibodies may persist for six months after treatment [23].

In the present work, sedimentation fecal examination was applied to determine theincidence of the disease since it is more sensitive than the direct smear yet not reveal the accurate incidence of disease when compared to the postmortem examination of livers and this could be the reason of the lesser results compared to previous records [32] when he recorded 26.3% of slaughtered sheep (363 animals) in kirkuk abattoir suffer liver parasitism. Similarly, records in south of Baghdad [38] found 27.4% of 84 slaughtered sheep were contain liver flukes. On the same line, many studies showed lower percentages compared to the present findings [33, 34]. Regardless the influence of diagnostic method or the sampling size, the source of these samples is considered a substantial factor in any outcome since both of parasite and its intermediate host affected by surrounding ecosystem and this may be the main reason of variation in the incidence of fascioliasis taking in consideration the vast differences in climate across Iraqi different areas. This reason also interpret the higher incidence of fascioliasis that was recorded in slaughtered sheep in southwestern (28.7%) [29] And records of slaughtered sheep in northeast of Iran (8.57%) [35].

Hemogram and leukogram results

Statistical analysis of hemogram results showed significant variations ($P<0.05$) in values of Hb, MCHC, MCV and MCH and such decreased values indicated hypochromic macrocytic anemia due to the chronic fascioliasis.

Many previous studies [36-40] illustrated the reason of such findings intra-hepatic hemorrhage and parenchymal damage caused by the migration of immature flukes and their feeding which estimated by 03-0.5 ml per fluke. These findings were parallel to previous studies [41, 42] that were naturally and experimentally demonstrated the eosinophilia and basophilia in sheep fascioliasis. The present study support previous findings when Statistical analysis reveals significant differences between seropositive and seronegative animals in values of WBC (neutrophils, eosinophils and basophils). Other researchers [43-45] attributed such elevations to the reaction and sensitivity of the host against secretory products of the parasite.

Biochemical Tests

Results of the present investigation affirmed many previous studies [46-51] concerning effects of fascioliasis on albumin and total protein levels. The significant decrease ($P<0.05$) in both parameters in seropositive sheep was due to the destructive effects of the parasite on hepatic parenchyma which lead to insufficiency in utilization and retention of nitrogen and albumin synthesis.

To exclude malnutrition and other internal parasitism influence that cause hypoproteinemia other than hepatic origin, GGT was measured in both seropositive and seronegative samples. The elevation of this enzyme in seropositive compared to seronegative animals refers to elevation of this enzyme in chronic fascioliasis and such observations were in accordance with those mentioned previously on fascioliasis types [5].

Conclusions

The seropositive and fecal examination affirmed the indemnity of the disease in Iraq however the disease is chronic in nature in Abu Ghraib district with low incidence and this could be one of the outcome of ecological changes takes place in this area.

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