

Prevalence of *Fasciola hepatica* in Goats and Sheep by Using ELISA in Sera and Milk in Duhok, Iraq

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ABSTRACT

An indirect ELISA technique was used to determine the prevalence of infection in sera and milk samples among goats and sheep in Duhok, Kurdistan Region, Iraq. Results of the present study shows that the prevalence of infection was higher in sera compared to milk samples among goats and sheep as follow: (6.3, 4.2%) and (8.9, 5.6%) respectively and there was no statistical significance difference in goats at P value =0.42, also there was no statistically differences at P value= 0.22. ELISA results also shows that the prevalence according to the age among goats and sheep were higher in animal aged > 2 years in both sera and milk samples were (7.5, 5.3%) and (11.3, 7%) respectively and there was no significant difference between age groups of goats and sheep by ELISA. The present study is the first study done to determine the prevalence of *F. hepatica* in both sera and milk samples among local goats and sheep. Continuously screening of sheep and goats for Fascioliasis and using of anthelmintic to control the infection in small ruminants is necessary.

Keywords: *Fasciola hepatica*, ELISA, Sera and Milk of small Ruminants and Duhok

Introduction

Fasciola hepatica is a flat worm trematode of the liver of domestic animals and commonly ruminants as well as humans. *Fasciola hepatica* is under the class Trematoda, and that causing a disease is called fascioliasis or fasciolosis (1). *Fasciola hepatica* has an indirect life cycle, the final host for *Fasciola hepatica* small and large ruminants and occasionally man and its intermediate host is the snail. There are about twenty species of snails through the world (2). Basically, the prevalence of the disease is linked to a mollusc of the genus *Lymnaea* species which serves as the intermediate of the agent server to host. Infective stage for fascioliasis is Metacercariae and the disease is transmitted via ingestion of contaminated grasses containing Metacercaria or drinking of water containing

Metacercaria. There are two forms of diseases including acute form or parenchymal which involves the subsequent migration of small juvenile in liver and causing liver damage, hepatomegaly, hemorrhage and necrosis and the second form is chronic form biliary form and this form is occur due to mature flukes, which cause the obstruction of bile duct, cholangitis, jaundice, anemia and fibrosis and this result in the hardened liver (3). There some factors are associated with development of infection such species of snail, environmental factors including rainfall, temperature and humidity (4)

Fasciola hepatica is distributed through the world mainly in tropical and subtropical climate regions and causing economic loss in livestock such as weight loss, anemia, decrease in body weight mainly in young animals, loss of milk production, also cause destruction of hepatic tissue and the bile duct (5, 6, 7). The traditional diagnosis of fasciolosis is by detection of eggs in feces (8), but this method of diagnosis does not detect the early stage prepatent infection. There are several types of ELISA are available to improve the diagnosis of both early and chronic fasciolosis (9). ELISA has the ability to detect the early diagnosis of fascioliasis by detection of *F. hepatica* circulating antibodies against an excretory-secretory antigen

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in early stage of infection (10, 11). The use of corticosteroid with flukicide give quickest and the best response to treatment of fasciolosis, This could be explained that the use of corticosteroid assisted the flukicide in preventing immune damage and fibroplasias inflicted by the liver flukes beside to improvement metabolic actions of the animal (12). In Iraq, the prevalence of *F. hepatica* in sheep and goats in Sulaymaniyah Province was 15% by using PCR (13), While in Abu-Ghraib district the prevalence of *F. hepatica* in local sheep was 12.73% by using ELISA (14), in other hand, in Kirkuk province, the prevalence of *F. hepatica* in sheep and goat was 72% and 11% by sedimentation technique respectively (15). In Turkey, the prevalence of *F. hepatica* in sheep was 7.50% by using ELISA (16). In Iran, the rate of infection was 32% by using sedimentation method (17). The aim of the present study was for detection of antibodies against *F. hepatica* in milk and sera of sheep and goat in Duhok City, Kurdistan Region, Iraq by using indirect ELISA.

Materials and Methods

This present study was carried out at the Duhok Province, Kurdistan region, Iraq. Duhok is located at a strategic location between two mountain ranges Bekhair to the northeast and Zaiwa to the southeast. Duhok city has dry summers and mild to cool and wet winters.

An overall of 142 adult female goats and 180 adult female sheep were selected randomly from different areas in Duhok Province, during a period of March to Jun of 2015. Five milliliters of blood were collected from the jugular vein by a sterile syringe from each animal including the positive control, the blood samples were collected into a clean test tube without anticoagulant and centrifuged at 300 rpm for 5-10 minutes. The sera were separated and transferred to label multiple clean Eppendorf tubes with animal information then stored at -20°C until analyzed by ELISA.

Five milliliters of milk samples were collected from the lactating goats and ewes. After centrifugation at 3000 rpm for 5-10 minutes,

defatted skim milk samples were separated, labeled and stored at -20°C until analyzed by ELISA. The Indirect ELISA was used for the serodiagnosis of *Fasciola hepatic* (special kit for *Fasciola hepatica*) in goats in both sera and milk samples by detection of IgG (By Bio-X Diagnostic – German).

Statistical Analysis

The present study results were analyzed by using of descriptive statistics including means and percentages. Chi-square (X^2) was used to determine the prevalence of infection and assess of the difference in prevalence with sera and milk in both goats and sheep (18).

Results and Discussion

Fasciolosis can cause significant economic losses in small and large ruminants, because causing damage of liver and bile duct and resulting in hepatomegaly and anemia and decrease in milk production (4).

The indirect ELISA technique was used for the detection of antibodies against *Fasciola hepatica* in both serum and milk samples. A total of 142 serum samples of goats only 9 (6.3%) sample was seropositive by ELISA and out of 142 milk samples; the seropositivity was 4.2% by ELISA as shown in Table 1.

There are no significant differences of seropositivity between types of samples at P-value=0.42. The study shows a high prevalence of fascioliasis in serum samples compared to milk samples in both goats and sheep and this is due to high amount of antibodies (IgG) in serum against *F. hepatica* was higher in serum compared in milk, as well as IgG appear firstly in serum than in milk.

There are many factors effects on the titer of IgG in milk such as lactation stage, phase of infection (acute or chronic phase), severity of infection and production of milk (19).

The results of the current study in goats were lower than the results in Abu-Ghraib district, where the prevalence was 11% by sedimentation technique (15).

Table 1. The seropositivity of anti *F. hepatica* IgG antibodies in Sera and Milk of goats by ELISA (No =142).

Type of sample	Seropositive		Sero-negative		P Value
	No.	%	No.	%	
Serum	9	6.3	133	93.7	0.42
Milk	6	4.2	136	95.8	

Serological results also show that prevalence in serum in goat (6.3%) was lower than the prevalence in sheep (8.9%). There is another study agreed to the present study results were done in Nigeria, and they found that the prevalence of infection in sheep and goats were 0.32% and 0.23% respectively (20).

The results of the ELISA for the estimation of the antibodies according to the age of the goats are shown in Table 2. The seropositivity was 2 (4.2%) and 7 (7.5) in animals aged ≤ 2 years and > 2 years respectively, there is no significant difference between age groups (P =0.44).

Table 2. The Seropositivity of Anti *F. hepatica* IgG antibodies in Sera of Goats by ELISA according to the age

Age (Year)	Total Number (142)	Seropositive		Seronegative		P Value
		No.	%	No.	%	
≤ 2	48	2	4.2	46	95.8	0.44
> 2	94	7	7.5	87	92.5	

The seropositivity of antibodies to *F. hepatica* in milk which was no statistically difference (P= 0.36) between different age groups as detailed in

table3. The rate was higher in the age group > 2 years 5(5.3%) as compared in age with ≤ 2 years 1(2.1%).

Table 3. The Seropositivity of Anti *F. hepatica* IgG Antibodies in Milk of Goats by ELISA according to the age

Age (Year)	Total Number (142)	Seropositive		Seronegative		P value
		No.	%	No.	%	
≤ 2	48	1	2.1	47	97.9	0.36
> 2	94	5	5.3	89	94.7	

Table 4 Shows the seropositivity of antibodies to *F. hepatica* in both milk and sera of sheep which was 16 (8.9%) and 10 (5.6%) respectively. There are no significant differences of seropositivity between types of samples (P=0.22).

The higher prevalence of infection in sheep may

be due to the type of grazing; goats depend on leaves of woody plant ant trees, while sheep depend on their graze on the ground plants and infective stage of *F. hepatica* is metacercaria where are commonly found on it. Therefore, in sheep, the risk of infection with metacercaria is

greater than in goats (21). A study was done in Mazandaran, northern Iran, who they found that *F. hepatica* is more dominant in sheep than goats (22). The prevalence of *F. hepatica* in sheep in the present study was lower than those studies done in Sulaymaniyah Province, Abu-Ghraib district and Kirkuk province, which were 15%, 12.73% and

72% by using PCR, ELISA and sedimentation technique respectively (13, 14, 15). Although, the result of the present study was lower than those results proved in Iran, which were 32% by sedimentation technique (17). On the other hand, the prevalence was lower than those recorded in Turkey 7.5% by ELISA (16).

Table 4. The Seropositivity of Anti *F. hepatica* IgG antibodies in Sera and Milk by ELISA in Sheep (No=180)

Type of sample	Seropositive		Seronegative		P Value
	No.	%	No.	%	
Serum	16	8.9	164	91.1	0.22
Milk	10	5.6	170	94.4	

The seropositivity of antibodies to *F. hepatica* in sera which was no statistically differences (P = 0.52) between different ages groups of ewes by

ELISA. The rate was higher in the age group > 2 years 13 (11.3%) as compared in age with ≤ 2 years 3(4.6%) as cleared in Table 5.

Table 5. The Seropositivity of Anti *F. hepatica* IgG antibodies in Sera of Sheep by ELISA according to the age

Age (Year)	Total Number (180)	Seropositive		Seronegative		P Value
		No.	%	No.	%	
≤2	65	3	4.6	62	95.4	0.52
> 2	115	13	11.3	102	88.7	

The seropositivity of antibodies to *F. hepatica* in milk which was highly statistically differences (P= 0.27) between different ages groups of ewes by

ELISA. The rate was higher in the age group > 2 years 8 (7%) as compared in age with ≤ 2 years 2(3.1%) as revealed in Table 6.

Table 6. The Seropositivity of Anti *F. hepatica* IgG antibodies in Milk of Sheep by ELISA according to the age.

Age (Year)	Total Number (180)	Seropositive		Seronegative		P Value
		No.	%	No.	%	
≤2	65	2	3.1	63	96.9	0.27
> 2	115	8	7	107	93	

There is another result in the present study shows that the higher prevalence in sheep and goats in the age group > 2 years as compared in age with ≤ 2 years. This agree with result of a study was done in Nigeria (20).

This may be due to the nature and physiology of lambs and kids compared to the adult; they graze less and or may be due to the lactating may decrease the immunity (21).

Conclusion

Fasciola hepatica is commonly parasitized in sheep and goats in Duhok province, Kurdistan region, Iraq and is an important source of liver infection economic loss in small ruminants in Duhok city. Destruction of snail it is an important way for limitation the parasite infection is intermediate host for *F. hepatica*, therefore should be taken as a control measure. Periodic diagnosis of sheep and goats and using of anthelmintic to get the maximum effect on controlling of infection in small ruminants.

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Conflict of interest

The author declares that the work was carried out without any commercial or financial relationships, which could be established as a possible conflict of interest.

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انتشار المتورقة الكبدية *Fasciola hepatica* في الماعز والأغنام باستخدام اختبار الـ ELISA في الأمصال والحليب في مدينة دهوك، العراق

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الخلاصة

تم استخدام تقنية الـ ELISA غير المباشرة لتحديد معدل انتشار العدوى في عينات المصل والحليب بين الماعز والأغنام في مدينة دهوك، إقليم كردستان، العراق. أظهرت نتائج الدراسة الحالية أن معدل انتشار الإصابة كان أعلى في عينات المصل مقارنة بعينات الحليب بين الماعز والأغنام وكما يلي: (6.3، 4.2%) و(8.9، 5.6%) على التوالي، ولم تكن هناك فروق ذات دلالة إحصائية في الماعز عند قيمة $P = 0.42$ وعند قيمة $P = 0.22$. أظهرت نتائج اختبار ELISA أيضاً أن معدل الإصابة وفقاً للعمر بين الماعز والأغنام كان أعلى في الحيوانات التي تقل أعمارها عن سنتين في عينات المصل والحليب حيث كانت (5.3، 7.5%) و(7، 11.3%) على التوالي، من ناحية أخرى لم تكن هناك فروقات معنوية بين الفئات العمرية المختلفة للنجاح باستخدام اختبار ELISA. في الختام، تعد الدراسة الحالية هي الأولى التي أُجريت لتحديد انتشار *F. hepatica* في كل من عينات المصل والحليب بين الماعز والأغنام. الفحص المستمر للأغنام والماعز للكشف عن داء المتورقات واستخدام طارد للديدان للسيطرة على العدوى في المجترات الصغيرة كانت ضرورية.

الكلمات المفتاحية: المتورقة الكبدية، مصل الدم وحليب المجترات الصغيرة ودهوك.