

The Indirect Serological Detection of IgG Antibodies against *Fasciola hepatica* in Blood, Milk and Meat Juice of Female Buffaloes in Al-Qadisiya Province

التشخيص المصلي الغير مباشر للجسام المضادة لل *Fasciola hepatica* في الدم والحليب وعصير اللحم في اناث الجاموس في محافظة القادسية

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Abstract

The present study was the first regard based on detection of chronic infections of *F. hepatica* infection in Iraqi female buffaloes. At slaughterhouse, a total of (28) female buffaloes were submitted to the serological indirect ELISA test to detection the specific IgG antibodies against *Fasciola hepatica* by using three different samples involved blood serum, milk and meat juice and the results were (21.43%), (14.29%) and (3.57%), respectively. As well as, the degree of infection between the tested samples was obtained according to the levels of antibody titer. Whilst the mild infection had (10.71%), (7.14%) and (3.57%) in serum, milk and meat juice, respectively; the moderates had (3.57%) for sera and (7.14%) for milk, and the strong infection occurred, only, in blood serum samples (7.14%). The significant differences were detected between the seropositivities samples and, also, between the levels of infection's degree ($P<0.005$).

Keywords: *Fasciola hepatica*, Buffaloes, ELISA, IgG

الخلاصة

تعتبر الدراسة الحالية الأولى من نوعها والتي تعتمد على تشخيص الإصابات المزمنة المصابة بديدان المتورقة الكبدية في اناث الجاموس العراقي . في المجزرة ، خضع (28) رأس من الجاموس للفحص المصلي بتقنية الاليزا الغير مباشر لتحديد الأجسام المناعية IgG المضادة باستخدام ثلاثة أنواع مختلفة من العينات تضمنت مصل الدم والحليب وعصير اللحم وكانت النتائج (21.43%) و (14.29%) و (3.57%) في هذه العينات على التوالي . إضافة إلى ذلك ، فان درجة الإصابة بين هذه العينات المستخدمة في الاختبار تم اعتمادها طبقا مستوى المعايير للأجسام المضادة . فبينما امتلكت الإصابة الطفيفة (10.71%) و (7.14%) و (3.57%) في المصل والحليب وعصير اللحم ، على التوالي ، فقد امتلكت الإصابة المتوسطة (3.57%) في المصل و (7.14%) في الحليب، أما الإصابات القوية فقد تواجدت فقط في عينات مصل الدم وبنسبة (7.14%) . الاختلافات المعنوية كانت متواجدة بين العينات الموجبة مصليا وكذلك بين المستويات المختلفة للإصابة.

Introduction

Fasciola hepatica, or liver fluke, is one of the most commonly a digenetic, flatworm trematode of the genus *Fasciola*, that causing a highly damaging disease, fascioliasis or fasciolosis, in 46 final host's species of domestic and wild vertebrates such as cattle, sheep, goat, buffalo, pigs, horse, dog, cats, macropods, rats, rabbits and many other animals as well as humans (1, 2, 3). Globally, about twenty species of snails, which belong to Lymnaeidae family, are described as potential and alternative intermediate hosts for *Fasciola*. The snail, which lives in marshy and standing water areas, is necessary for parasite to complete its life cycle (4). *Fasciola* is worldwide distribution in tropical and subtropical climate areas causing a considerable economic loss in livestock industry such as anemia, weight loss, growth retardation in young animals, and production's losses in addition to liver condemnation, morbidities, mortalities, and treatments with controls (5, 6, 7). In

buffaloes, it has been asymptomatic, subclinical or chronic and, adversely, affecting on food conversion efficiency, weight gain, reproductive cycle and productivity. Previously, the gold standard method in the diagnosis of infection was based on the suspected clinical signs and confirmed by the coprological tests (sedimentation and floatation fecal) that depends on eggs detection (8). Although, this method is simple and confirmatory but it's useless at the low levels of adult fluke burden and cannot detect the infection in a pre-patent period, because the parasite's eggs are, only, persist in feces if the flukes were already matured after (10-14) weeks post infection (9). Therefore, the necessity for an alternative technique other than a classical coprology has appeared from decades (8). Recently, a number of serological tests are developed, such as complement fixation test (CFT), immune-fluorescence technique (IFAT), counter immune electrophoresis (CIE) and enzyme-linked immunosorbent assay (ELISA), which have been utilized for the diagnostic and prevalence studies in lived animals. These serological techniques can be test a large number of animal's sera at a few time as well as, have, the ability to early diagnosis of infection by detection of circulating antibodies against an excretory-secretory antigen of *F. hepatica* that produced during the early stage of infection (10, 11, 12). The indirect ELISA test is consider as one of the best serological tests due to the simplicity, sensitivity, specificity, automated reading, and appropriateness for use in epidemiological surveys especially in endemic areas (13, 14). In Iraq, the number of studies about buffaloes related to enumeration, distribution, diseases, amount of milk and meat productions is very a few, limited and without update. The present study was dealt with the *F. hepatica* infection by using an advanced serological technique (indirect ELISA) that licensed by Swedish veterinary diagnostic company (SVANOVA). This technique is based on the reaction between the antibodies of infected animals with a crude excretory / secretory (ES) antigen or with purified / recombinant antigen. The results of SVANOVA ELISA kit has 98 and 96 % sensitivity and specificity, respectively, and can support the implementation of control measures in the farm. "Monitoring and controlling herd infection to an acceptable level with no loss of production is a new way to manage fasciolosis. The SVANOVIR® *F. hepatica*-Ab is a novel tool that provides hard facts for optimizing pasture management and treatment strategies which are the two most commonly used control measures" (8, 15, 16, 17). The main goals of this study were to:

1. Detection the more practical morbidity rate for *F. hepatica* infection in buffaloes in Wasit province / Iraq, by using an advance serological test.
2. Evaluation the efficacy of this test in detection of specific antibodies against *F. hepatica* in blood, milk and meat juice of buffaloes for first time in Iraq.
3. Provide an additional data about buffalo's *F. hepatica* infections for treatment, control and future epidemiological studies.

Material and Methods

The study was conducted in slaughterhouses of Al-Qadisia province / Iraq, from May-2015 to February-2016, to detect the IgG antibodies against *Fasciola hepatica* infection in buffaloes at these areas. Randomly, a total of 28 females were submitted to collection of samples that included blood, milk (collected before slaughter) and meat (collected after slaughter).

Collected serum samples from theses slaughtered females were stored in 1ml micro-tubes (eppendorf) and kept in deep freeze at (-20°C) until use. From each one, 5 µL of serum was required to each well examine by an indirect ELISA (18, 19).

Fifty ml of milk was collected from each female by using a special sampling bottle that containing a broad spectrum with and preservative micro-tabs. The samples were centrifuged for 15 minutes at (2000 × g) to remove the lipid layer, imbibed under the fat layer and transferred to 1.5 ml eppendorf tubes and frozen at (-80°C) until tested. 100µL of skim milk is required for each sample well to examine with an indirect ELISA (20).

From each examined animal, about (5-10) grams of skirt muscle sample was collected, kept in container and stored at (-20°C) overnight, then allowed to thawed, and the meat juice formed by gently squeezes the meat sample. Meat juice was centrifuged for 15 minutes at (2000 × g) and

transferred to 1ml eppendorf tubes and frozen at (-80°C) until tested. 10 µL of meat juice is required for each sample well to examine with an indirect ELISA (15).

All data was analyzed the differences between the variables, which regarded, as statistically, significant by using the IBM SPSS v.23. Chi-square (χ^2) test was used to determine the prevalence of infection and assess of the difference in prevalence with blood, milk and meat juice. The statistical significance was set at $P < 0.05$ (21).

Results

Out of 28 examined buffaloes, six (21.43%), four (14.29%) and one (3.57%) females were positive for antibodies against *F. hepatica* in blood, milk and meat, respectively, (Table 1).

In (Table2), that deal with the degree of infection in seropositive buffaloes, 3/6 (10.71%), 2/4 (3.57%) and 1/1(7.14%) females that had the mildly infection in blood, milk and meat, respectively; while 1/6 (7.14%) and 2/4 (7.14%) buffaloes were with the moderately infection in blood and milk, respectively, only. No samples were found to be strongly positive in milk and meat except in blood serum that had 2/6 (3.57%) animals showed a high rate of antibodies titration against *F. hepatica* infection.

Table (1): Seroprevalence of *F. hepatica* in Blood, Milk and Meat Juice in Female Buffaloes

Type of Samples	Seropositives in 28 Buffaloes		Seronegatives in 28 Buffaloes	
	No.	%	No.	%
Blood Serum	6	21.43 ^a	22	78.57
Milk Bulk Tank	4	14.29 ^b	24	85.71
Meat Juice	1	3.57 ^c	27	96.43

The difference in small letters, vertically, refers to significant differences at level $P < 0.05$

Table (2): Degree of Infection in Seropositive Buffaloes According to Type of Sample

Type of Samples	Seropositives in 28 female buffaloes						Total No.
	Mild		Moderate		Strong		
	No.	%	No.	%	No.	%	
Blood Serum	3	10.71 ^a	1	3.57 ^b	2	7.14 ^a	6
Milk Bulk Tank	2	7.14 ^b	2	7.14 ^a	0	0 ^b	4
Meat Juice	1	3.57 ^c	0	0 ^c	0	0 ^b	1

The difference in small letters, vertically, refers to significant differences at level $P < 0.05$

Discussion

The serological ELISA detection of antibodies against *Fasciola hepatica* was conducted in 3 different samples involved blood serums, milk bulk tank and the meat juice. In Iraq, although several studies were concerned with the diagnosis of *F. hepatica* in cattle, sheep, buffaloes and goat; but most of them have been done by floatation and sedimentation, and only a few studies dealt with the advanced techniques, and the results ranged from 0.174 to 34 % (22, 23, 24, 25, 26, 27, 28, 29). In buffalo, although the subclinical or chronic form of fascioliasis, ELISA showed that it's a reliable and dependable method in screening studies (30, 31, 32).

In this study, the overall prevalence revealed a high rate of infection by using the sera when comparing the results of ELISA test between them (table 1), and this mean that the quantity and / or quality of serum's IgG antibodies that available for reaction with *Fasciola* antigen were exceeded on those in milk and meat juice. As well as, the degree of infection, which has been established in depending on the levels of antibody's titration in seropositive buffaloes, was reported some variations in severity of infection in the examined buffaloes (table 2). (33) reported that the antibodies appear earlier in serum than in milk, and the concentration of antibodies in serum is about 30 times greater than in milk. The factors that can affect on titration of the parasite-specific

antibodies in milk are multifactorial such as the numbers with relative seropositivity of contributors, stage of lactation, stage of infection, severity of illness and milk production (34). Also, it's important to note that the negative results from milk don't mean that the animal is definitively free from the parasitic infection, because that all ELISA types have a threshold for antibody's concentration that must be achieved before assaying the results. However, the correlating of the percentages in infected animals with bulk milk score can be challenging (16). In addition, the using of milk, as solely sample, may cause further faults because it clearly doesn't included the contributions from non-lactating females that canceled from the milking collect due to a disease or treated with drugs that require milk discarding (35). In meat juice, the decreasing in antibody levels may be explained by the fact that some types of immunoglobulin don't have the ability to cross through the membrane of bodily cells (36).

Finally, the bulk milk and meat juice ELISA were subject to the same shortcomings as in serum ELISA because there can be a significant delay between the onset of infection and detection of the antibody and /or a presence of lag between the elimination of parasite and the identical reduction in antibody's titer, and these in turn may be influenced by the treatment, re-infection, host immune response and a clearance of parasite (37, 38, 39). However, the early diagnosis of infection can create an opportunity to intervention with the specific anthelmintics that have a high degree of efficiency and decreasing the economic losses (40, 41).

In conclusion, the study facing more one problem, especially, these related to the current data and related references. However, the result of this study reported a high rate of infection with *F. hepatica* in Iraqi buffaloes, especially with serum ELISA test, as well as the receptivity of milk to revelation the specific antibodies with possibilities using it as alternative sample for blood serums in detection of infection because it could provide an information, timely, about the exposure status to parasite. In contrary, meat juice showed that it difficult to applied, practically, in lived animals and can't be exhibited the actual infection rate and, therefore, its suitable to used in academic or epidemiological studies.

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