# Sero-Diagnosis of Anti-Fasciola hepatica IgG-Antibodies in Blood and Milk of Cattle, Goats and Sheep by Using an Indirect-ELISA

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## Abstract

The present study was aimed to evaluate the prevalence of anti- F. hepatica IgG-antibodies in blood and milk of cattle, goats and sheep by using an indirect-ELISA. The study was performed in different areas in Al-Qadisiyah province, during a period of April to September / 2016, and involved totally 388 milking animals; 113 cattle, 130 goats and 145 sheep. Overall results reported that 34.54% and 23.71% of blood and milk samples, respectively, were seropositives for anti- F. hepatica antibodies. Among study's animals, prevalence of seropositive in blood and milk, respectively, was 46.02% and 32.74% in cattle, 26.15% and 14.62% in goats, and 33.1% and 24.83% in sheep. Significant differences were detected between positive results of blood and milk as well as between study's animals (P>0.05). In regarding to intensity of infection, the mild infections (52.99% and 60.87%) were more appearance than moderates (33.58% and 29.35%) and severe infections (13.43% and 9.78%) in blood and milk, respectively. In cattle, it's noted that the moderate infections were appeared, relatively, more obvious; while in sheep and goat, mild infections reported a high incidence than other levels of infections.

**Keywords:** Fasciola hepatica, ELISA, Blood, Milk, Cattle, Goat, Sheep

# التشخيص المصلي للاجسام المضادة – IgG لديدان المتورقة الكبدية في الدم والحليب للابقار والماعز والاغنام بأستعمال الاليزا غير المباشر

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**الخلاصة**

هدفت الدراسة الحالية الى تقييم انتشار الاجسام المضادة – IgG لديدان المتورقة الكبدية Fasciola hepatica في الدم والحليب للابقار والماعز والاغنام باستعمال الاليزا غير المباشر . اجريت الدراسة في مناطق مختلفة من محافظة القادسية خلال الفترة من نيسان الى ايلول وشملت اجماليا ٣٨٨ حيوان مدر للحليب ؛ ١١٣ ابقار و ١٣٠ ماعز و ١٤٥ اغنام . سجلت النتائج الكلية ان ٣٤.٥٤% و ٢٣.٧١% من عينات الدم والحليب ، على التوالي ، كانت موجبة مصليا للاجسام المضادة لديدان المتورقة الكبدية . خلال حيوانات الدراسة ، بلغ معدل انتشار الاصابات الموجبة مصليا في الدم والحليب ، على التوالي ، ٤٦.٠٢% و ٣٢.٧٤% في الابقار ، ٢٦.١٥% و ١٤.٦٢% في الماعز ، و ٣٣.١% و ٢٤.٨٣% في الاغنام .

مايتعلق بشدة الاصابة ، ظهرت الاصابات الخفيفة (٥٢.٩٩% و ٦٠.٨٧%) اكثر من الاصابات المتوسطة (٣٣.٥٨% و ٢٩.٣٥%) والاصابات الشديدة (١٣.٤٣% و ٩.٧٨%) . في الابقار ،لوحظ ان الاصابات المتوسطة ظهرت ، نسبيا ، بوضوح

اكثر ؛ اما في الماعز والاغنام ، سجلت الاصابات الخفيفة معدل حدوث اعلى من بقية مستويات الاصابة .

الكلمات المفتاحية : ديدان متورقة كبدية ، اليزا ، الدم ، الحليب ، الابقار ، الماعز ، الاغنام

## Introduction

Fasciolosis is a parasitic liver infection of wild and domesticated ruminants as well as humans, occasionally, which caused by digenean trematodes of the genus Fasciola, included mainly F. hepatica and F. gigentica species (1). However, F. hepatica is a great global health problem, particularly, in countries with temperate climates including Iraq causing high production losses in livestock as a result of condemned liver, reductions in weight gain, milk yield and fertility (2). Clinically, the disease is occurred as acute or sub-acute outbreaks in cattle, sheep and goats, and reported frequently as a chronic debilitating disease (3) Although, the microscopic examination of feces by concentration methods is common practically to detect parasite eggs, it's still not effective until at least 10-12 weeks post-infection (4). In addition, these traditional diagnostic techniques have several drawbacks as they less sensitive, hard to perform, requires an appropriate amount of feces, unable to diagnosis infection in early stage, and in chronic infections, the sporadic releasing of eggs in feces leading to misdiagnosis of infection (5). Since 1950's, many immunological diagnostic techniques were recommended and licensed for the early diagnosis of disease during a migratory phase of parasite or even in chronically stage of infection (6). Nonetheless, most of these techniques have limited values because of their low sensitivity and specificity, time consuming, expensive, difficulty to read and provide variable results (7). After introduction of enzyme-linked immunosorbent assay (ELISA), numerous types of this assay were developed and modified for detection of fasciolosis by using of different antigens such as the whole excretory-secretory products of F. hepatica (ES) (8), many purified recombinants as cathepsins (PRC) (9), and coproantigen-ELISA (10). Recently, the indirect ELISA (ES) was applied successfully due to an absence the relevant cross reactions with the gastrointestinal nematodes, high degree of repeatability, simplicity, easy automation, superiority, practicability with very high sensitivity and specificity that could be reached to 100% in detection of natural and experimental infections (11).

Hence, this study was conducted toward detection the prevalence of specific antibodies against fasciolosis caused by Fasciola hepatica in milk and sera samples of cattle, sheep and goat in Al-Qadisiyah province, by using an indirect-ELISA.

## Materials and methods

From different areas in Al-Qadisiyah province, and during a period of April to September / 2016, an overall of 388 adult females, comprised of 113 cattle, 130 goats and 145 sheep were included for this study. From each animal, 50 ml of milk samples were drained into specific bottles that containing a broad spectrum antibiotic with preservative microtabs, and 5 ml of blood samples were collected from jugular vein free-anticoagulant vacutainer tubes, and both samples were numbered and transported to the lab by using a cooled keeper. At lab, the blood and milk samples were centrifuged (4000 rpm/15 min.), then, the obtained sera and skimmed milk were saved into numbered 1ml eppendorf-microtubes and kept at -20°C until tested (12, 13).

For detection of specific IgG anti-Fasciola hepatica antibodies in sera and milk samples, the indirect-ELISA kit of (BIO-X Diagnostics, Belgium) were used. According to manufacturer instructions, the samples were prepared, diluted, incubated and, finally, read at 450nm of an optical density (OD) by using a computerized ELISA-reader (BioTek-USA). Subsequently, the test was considered as validating when the positive control antigen yielded a difference in OD greater than 0.800, whereas, the OD of milk and sera samples was submitted to this formula: Percent positivity (PP) = Mean OD $_{\text{Samples or Negative control}}$ × 100 / Mean OD $_{\text{Positive Control}}$ , and the results of PP were interpreted as follow: [PP < 0.0150 = Negatives, PP ≥ 0.150 = Mild positives, PP ≥ 0.450 = Middle positives, PP ≥ 0.750 = Severe positives. All obtained data were classified and analyzed by two of computerizing programs, Microsoft Office Excel (2007) and IBM/SPSS (v.23). Descriptive statistics and Chi-square ($x^2$) were used to detect the significant differences between the positive results of milk and sera in cattle, sheep and goats at a level of P≤0.05 (14).

## Results

Of 388 tested animals, the total results for examination of blood and milk samples by an indirect-ELISA were revealed on, respectively, 134/388 (34.54%) and 92/388 (23.71%) seropositive animals with anti-F. hepatica IgG-antibodies (Table 1).

**Table (1): Total seropositive results of blood and milk by indirect-ELISA**

| Samples | | Total No. | Seropositivity | Seronegativity |
|---|---|---|---|---|
| 1 | Blood | 388 | 134 (34.54%) $^{A}$ | 254 (65.46%) |
| 2 | Milk | 388 | 92 (23.71%) $^{B}$ | 296 (76.29%) |

**Variation in large vertical letters refers to significant difference**

Among study's animals, the results of testing blood and milk samples, respectively, in 113 cattle, 130 goats and 145 sheep; showed that 46.02% and 32.74% of cattle, 26.15%14.62% of goats, and 33.1% and 24.83% of sheep were seropositives (Table 2)

**Table (2): Among study's animals, positive results of blood and milk**

| Animals | | Total No. | Blood | Milk |
|---|---|---|---|---|
| 1 | Cattle | 113 | 52 (46.02%) $^{Aa}$ | 37 (32.74%) $^{Ba}$ |
| 2 | Goat | 130 | 34 (26.15%) $^{Ac}$ | 19 (14.62%) $^{Bc}$ |
| 3 | Sheep | 145 | 48 (33.1%) $^{Ab}$ | 36 (24.83%) $^{Bb}$ |
| | Total | 388 | 134 (34.54%) | 92 (23.71%) |

**Variation in small horizontal and large vertical letters refer to significant differences**

Overall seropositive results of study's animals were detected significant increasing in specific IgG-antibodies of blood where compared to milk (P>0.05), (Figure 1).

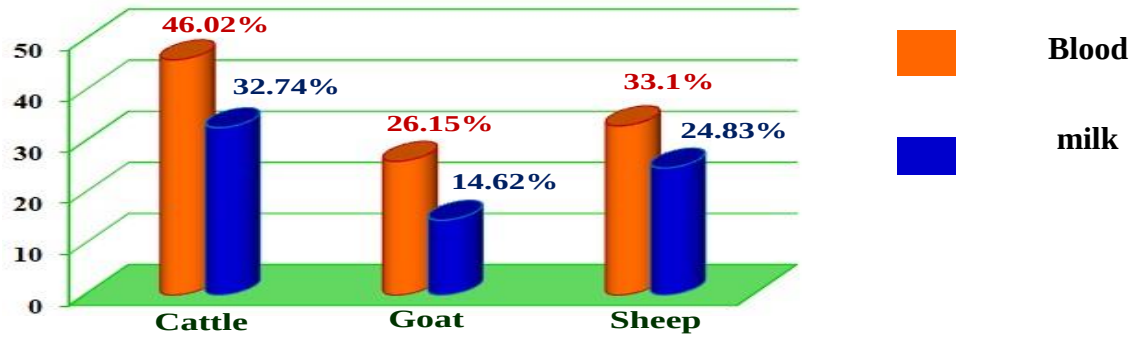


**Figure (1): Total positive results of blood and milk among study's**

**Accord**ing to manufacturer instructions, seropositive results were distributed to 3 levels of infection's intensity; mild, moderate, and sever; which reported 52.99%, 33.58% and 13.43% in blood; and 60.87%, 29.35%, and 9.78% in milk samples, (Table 3). Among both samples, mild infections were more prevalent than moderate and severe infections (Figure 2).

**Table (3): Total blood and milk seropositives among levels of infection's intensity**

| | Samples | Total Positives | Mild | Moderate | Severe |
|---|---|---|---|---|---|
| 1 | Blood | 134 | 71 (52.99%) $^{Ab}$ | 45 (33.58%) $^{Ba}$ | 18 (13.43%) $^{Ca}$ |
| 2 | Milk | 92 | 56 (60.87%) $^{Aa}$ | 27 (29.35%) $^{Bb}$ | 9 (9.78%) $^{Cb}$ |

**Variation in large horizontal and small vertical letters refer to significant differences**

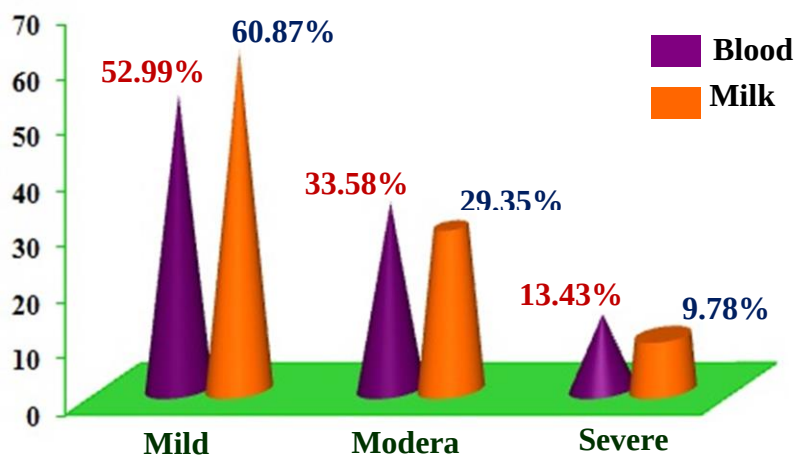


**Figure (2): Levels of infection's intensity among blood and milk**

In regarding to study's animals, seropositive results of cattle were appeared to have a significant increasing (P>0.05) in moderates (48.08% and 48.65%) than mild (30.77% and 35.14%) and severe infections (21.15% and 16.22%) in blood and milk results, respectively (Tables 4 and 5).

In goats, mild (67.65% and 84.21%) was more prevalent than moderate (26.47% and 15.79%) and severe infections (5.88% and 0%), among blood and milk (P>0.05).

Also, sheep reported significant increasing in mild (66.67% and 75%) than moderate (22.2% and 16.67%) and severe infections (10.42% and 8.34%) in both samples (P>0.05).

**Table (4): Intensity of infection among blood of seropositive animals**

| | Positives | Cattle | Goat | Sheep | Total |
|---|---|---|---|---|---|
| 1 | Mild | 16 (30.77%) Bb | 23 (67.65%) Aa | 32 (66.67%) Aa | 71 (52.99%) a |
| 2 | Moderate | 25 (48.08%) Aa | 9 (26.47%) Bb | 11 (22.2%) Cb | 45 (33.58%) b |
| 3 | Severe | 11 (21.15%) Ac | 2 (5.88%) Cc | 5 (10.42%) Bc | 18 (13.43%) c |
| | Total | 52 | 34 | 48 | 134 |

**Variation in large horizontal and small vertical letters refer to significant differences**

**Table (5): Intensity of infection among milk of seropositive animals**

| | Positives | Cattle | Goat | Sheep | Total |
|---|---|---|---|---|---|
| 1 | Mild | 13 (35.14%) Cb | 16 (84.21%) Aa | 27 (75%) Ba | 56 (60.87%) a |
| 2 | Moderate | 18 (48.65%) Aa | 3 (15.79%) Bb | 6 (16.67%) Bb | 27 (29.35%) b |
| 3 | Severe | 6 (16.22%) Ac | 0 (0%) Cc | 3 (8.34%) Bc | 9 (9.78%) c |
| | Total | 37 | 19 | 36 | 92 |

**Variation in large horizontal and small vertical letters refer to significant differences**

## Discussion

In Iraq, several studies have been performed on detection of Fasciola hepatica in cattle, goats and sheep using classical diagnostic methods such as post-mortem inspection, and egg concentration (floatation and/or sedimentation) (15, 16, 17). However, this study was the first one directed with detection a seroprevalence of Fasciola infections, serologically, in living cattle, sheep and goats using of an indirect-ELISA. The total results showed that the prevalence of F. hepatica in both blood and milk samples were (34.54%) and (23.71%), respectively (Table 1).

Overall positive results of blood samples in all tested animals (cattle, goats and sheep) were, significantly, higher than those reported in milk samples (Table 2). This means the quantity and quality of serum antibodies which available for reaction with F. hepatica antigen were exceeded on those available in milk samples (18). Nonetheless, milk samples can be used for detecting of F. hepatica infection in herds, inexpensively, and provide useful information about the status of some diseases as fasciolosis (19).

In general, the results of this study were much more than those obtained from previous studies carried out in Iraq by (16) 0.5%, (20) 3.9%, and (21) 9.93-16.8%. In last 20 years, F. hepatica infections were evidenced a significant increasing in its prevalence, and detected in areas where it was previously considered unlikely (22). In cattle, the disease reported (25.9%) in Iran (23), (8.6%) in Saudi Arabia (24), (0.5-25%) Turkey (25), (14.3%) Tunis (26), (24.3-90.7%) Ethiopia (27), (24.4%) Mexico (28) and (100%) in Bolivia (29); in sheep, (6.4-23%) in Algeria (30), (5.3-31.2%) in Iran (23), (3.2%) in Jordan (31), (30.6) in Mexico (29), (35) in Tunis (26); whereas in goats, it's reported (4.9-64.3%) in Iran (23), (43%) in Mexico (28) and (68.4%) in Tunis (26). Hence, the evident variation in prevalence of F. hepatica infections in most countries could because using of different diagnostic tests, variation in numbers of studied animals, areas involved in a study which might abundance suitable environmental conditions for development of pathogen life cycle, lack of attention, retrogression of veterinary services and absence of active or incorrect using of therapies (24, 32).

Regarding to type of animals, study's cattle appeared to be more exposed for infection with F. hepatica than sheep and goats (Table 2, Figure1). Age was appeared to be as one of the main factors that might interpret these results, because of the cattle have a lifetime rate more than goats and sheep, subsequently, increasing the chances of exposure to pathogens or diseases (33, 34). Other factors that including the size of samples, origin of animals,

grazing habits or source of drinking water may facilitate the transmission of infection, and regions where the irrigation areas were permit in presence of fasciolosis more than non-irrigated areas (35, 36). Also, (28, 37) who mentioned to that goats and sheep were tend to develop a weak or unsuitable immune response against F. hepatica, and this can be resulting in high mortalities during acute phase of fasciolosis, or sharing low level of antibodies that couldn't detectable by serological tests. Whereas, (38) preferential hosts for F. showed that cattle and sheep were appeared as the hepatica infection and the goats were fewer receivers and less parasitized.

In this study, the seropositive results of blood and milk samples analyzed by an indirect-ELISA were divided into 3 levels of infection's intensity. Totally, the results revealed that the mild levels were more prevail than other levels of infection (moderate and severe), (Table 3, Figure 2). The frequent contact of liver flukes with an animal might enhance the development of certain immunity and providing partial protection by reducing the number of adult Fasciola (39). Also, the low parasitic burden or flukes during a pre-patent period don't allow confirmation of infection because in that period no Fasciola eggs can be detected (40). Hence, indirect-ELISA was considered as a liable and dependable diagnostic method for detecting of F. hepatica antibodies from the second weeks post infection with high sensitivity, and providing an early diagnosis if compared with other diagnostic techniques particularly the coprological methods (41). Nonetheless, the presence of low levels of antibodies in some cases might not be always indicated an active fluke infection inside the host, and the kinetics of antibodies might be induced more variability than the infection intensity (42). Though, the kinetics and intensity of cellular response were similar during infection of animal with 5 or with 150-250 metacercariae (43).

Among study's animals, the levels of infection intensity revealed with both samples that cattle were, dominantly, afflicted with the moderate type of infection, whereas in goats and sheep, the mild infection was the more prevalent type (Table 4, 5). This meaning that either cattle more sensitive for gaining infections, exposed frequently for sources of parasite burdens, or related to age as cattle have a prolong life-span (44, 45).

In conclusion, the present results was indicated an efficacy of indirect-ELISA in detecting of specific anti-Fasciola hepatica antibodies in both blood and milk samples. In addition, the obtained results detected the high prevalence of fasciolosis among study's animals (particularly cattle).

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