

## Evaluation of Flukefinder® and coproantigen ELISA as alternatives to sedimentation for detecting *Fasciola hepatica* in sheep

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### Abstract

The goals of the current research were to assess the frequency of *Fasciola hepatica* infestation (FHI) in sheep with different diagnostic procedures. In addition, a comparison was made of the sensitivity, specificity, and accuracy of standard sedimentation (SS), (as a reference coprodiagnostic method) with the recently developed commercial kits (coproantigen enzyme linked immunosorbent assay [C Ag ELISA] and Flukefinder® [FF] instruments as methods for effective detection of FHI). A total of 92 faecal specimens were obtained from animals naturally infested with FH (46 ewes and rams each) in diverse regions of Mosul, Iraq. The frequencies of FHI were 15.2%, 16.3%, and 20.6% with (SS), (FF) and (C Ag ELISA), respectively. According to the statistical analysis, the diagnosis of helminthic infection in both FF and C Ag ELISA were equally accurate techniques (accuracy of FF = 96.7%, confidence interval [CI] = 90.8–99.3, and Kappa value [ $\kappa$ ] = 0.877), whereas the accuracy of C Ag ELISA = 94.6, CI = 87.8–0.78, and  $\kappa$  = 0.816). FF had higher specificity and positive predictive values, whereas C Ag ELISA had the highest sensitivity and negative predictive values. In conclusion, sheep in Mosul, Iraq, frequently have FHI. However, for the routine diagnosis (SS) of FHI, it is advised to employ either FF or C Ag ELISA as an alternative.

*Fasciolosis, ovine, liver fluke, small ruminants*

*Fasciola hepatica*, or the common liver fluke, is a significant problem from both an economic and medical aspect (Howell 2024; Hassan et al. 2025). Fascioliasis or liver rot in small ruminants impairs growth and causes a decrease in meat, milk, and wool production, as well as a high morbidity and mortality (Stuen and Ersdal 2022; Lan et al. 2024). The condemnation of ill sheep's livers, as well as the costs of anthelmintic medications, are also economically significant (Hayward et al. 2021). The illness occurs in areas with specific climatic conditions that are appropriate to the intermediate host snail's lifecycle (Bargues et al. 2021). *Fasciola hepatica* has an indirect life cycle. The Lymnaeidae species serves as intermediary hosts. The parasite grows in three stages within the snail: sporocyst, rediae, and cercariae, which take approximately 5–7 weeks to complete depending on temperature and environmental conditions. Cercariae emerge from the snail and infect aquatic plants as metacercariae. After being digested by ruminants, metacercariae excyst in the small intestine, penetrate the intestinal wall, and reach the liver via the peritoneum. Immature flukes spend 8–12 weeks (prepatent period) in the hepatic parenchyma before entering the bile ducts and maturing into adults (Stuen and Ersdal 2022; Hassan et al. 2025).

In recent years, there has been an increase in reports of emerging resistance of the parasite to various flukicidal agents (Novobilský et al. 2016; Kamaludeen et al. 2019). Resistance monitoring requires accurate coproscopical methods with a high sensitivity

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to reliably identify patent infestations and good accuracy to detection of trematode eggs shedding intensity in faeces (Sargison and Scott 2011). In addition, the number of eggs in faeces is generally lower than number of eggs shed by gastrointestinal nematodes and is frequently difficult to diagnose (Hendrix and Robinson 2023).

The ideal diagnostic approach for *F. hepatica* infestation in sheep involves a combination of serological and coprological methods, each with its own strong points and limitations. Although coprological procedures such as Kato-Katz, sedimentation, or formalin-ether concentration methods are the gold standard for trematode infection diagnosis (Calvopina et al. 2018), these procedures are only sensitive after 8 weeks post infestation (only during the patent period), while sheep have an especially long pre-patent phase (Olaogun et al. 2022; Hendrix and Robinson 2023). These limitations make it desirable to use better diagnostic techniques for early infection detection. However, more specific approaches, such as Mini-FLOTAC and Flukefinder®, are reliable diagnostic tools for these diseases in cattle (Bosco et al. 2023) and humans (Zárate-Rendón et al. 2019). The Flukefinder® is a commercial egg-detecting equipment that employs a modified sedimentation and fine filtering process.

Several commercial kits based on both coproantigens and antibody detection have been developed in accordance with the advantages of immunodiagnosics, particularly early detection and the ability to examine several specimens fast. Some of these kits include Ildana Biotech® (Dublin, Ireland), IDEXX® (Westbrook, USA), Svanova® (Uppsala, Sweden) and Bio-X® (Rue de la Caléstienne, Belgium). All of these kits are valuable instruments for monitoring anti *F. hepatica* antibodies by evaluating single bulk tank milk samples, and Bio-X® is effective for treatment management (Palmer et al. 2014; Munita et al. 2019). These tests allow for detection 2–4 weeks after infestation and well before the patent period; however, antibody levels do not immediately correlate to parasite numbers, and antibodies persist for weeks after the parasitic infection is paused, so they are not always suggestive of active infection.

The identification of *F. hepatica* antigens in faeces offers several advantages, including the ability to detect only actively metabolizing flukes. Kajugu et al. (2015) showed the diagnosis of fasciolosis should be based on consideration of both faecal eggs counting and coproantigen ELISA findings, to ensure optimum sensitivity for pre-patent and low-level infections. Although the results of the reported studies demonstrate that the coproantigen ELISA (Ag ELISA *Fasciola hepatica*, BIO K 201®) offers some advantages, including the ability to detect only actively metabolizing flukes, (accurate and reliable test), it does not provide conclusive evidence to support the discontinuation of the straightforward and reasonably priced faecal egg sedimentation test. Nevertheless, the coproantigen ELISA can be used as a suitable adjunctive test to aid in the diagnosis of *F. hepatica* in adult cattle and sheep (Palmer et al. 2014) and has the potential to be used as an early diagnostic test (Nur Hafizah et al. 2023). More research that directly compares the coproantigen ELISA to the faecal egg sedimentation test in various clinical settings and investigates various diagnostic methods is needed in order to draw conclusions about the more sensitive test.

The study aimed to estimate the frequency of *F. hepatica* infestation (FHI) in sheep of different ages, sexes, breeds and management. Furthermore, a comparison of the sensitivity and specificity of the methods was conducted to investigate whether the methods are able to distinguish between truly positive and truly negative samples.

### Material and Methods

This study was approved by the College of Veterinary Medicine Committee, University of Mosul, Iraq under the approval number: UM.VET.2024.050.

#### Animals

This study was applied to include 92 animals (46 rams and 46 ewes) of different ages (1–7 years), breeds, and management practices, presented at the Veterinary Teaching Hospital, University of Mosul, Iraq.

### Sample collection and examination

Twenty grams of faeces were collected directly from the rectum. The samples were labelled, placed in a cooler box and transported to the laboratory of clinical pathology at the College of Veterinary Medicine, University of Mosul in Iraq. Each sample was examined using standard sedimentation as described by Hendrix and Robinson (2023), Flukefinder® method (Flukefinder® Diagnostic System, Soda Springs, Idaho, USA), which is based on differential sieving followed by a sedimentation procedure (carried out according to the manufacturer's instructions). The study was used to detect the presence or absence of liver fluke eggs in faecal samples as described by Mungube et al. (2006). The eggs of *Fasciola* species appeared yellow against a blue background if present and to distinguish between eggs of *Fasciola* species and those of *Paramphistomum* species, methylene blue stain drop was added in which the eggs of *Fasciola* species should stain yellow while the eggs of *Paramphistomum* species stain blue as described by Berhe et al. (2009).

Monoscreen Ag ELISA *Fasciola hepatica* (BIO K 201, Bio-X Diagnostics S.A., 5580 Rochefort, Belgium), was used according to the manufacturer's instructions. The optical densities in the microwells were evaluated spectrophotometrically (Bio-Tek Instruments, Winooski, Vermont, USA, MicroQuant microplate reader), using a 450 nm filter. The intensity of the resulting blue colour is proportionate to the sample's coproantigen titre.

### Comparison between methods used in this study

Kappa statistics ( $\kappa$ ) were used to determine the strength of agreement between methods. If the  $\kappa$  value is  $> 0.75$  = excellent agreement,  $0.40-0.75$  = fair to good agreement,  $< 0.40$  = poor agreement (Fleiss et al. 2003). Moreover, the C Ag ELISA and FF method's sensitivity, and specificity were computed and compared to the SF technique (Baratloo et al. 2015).

### Statistical analysis

Chi Square ( $\chi^2$ ) test and odds ratio (OR) were used by IBM-SPSS Version 22 (Inc., Chicago, USA) to analyse the data in this study. Statistically significant data was determined at  $P \leq 0.05$ .

## Results

Depending on which laboratory procedures were applied, the percentage of positive samples varied, 14 (15.2%) were positive for faecal sedimentation, 15 (16.3%) for Flukefinder®, and 19 (20.6%) for the coproantigen enzyme linked immunosorbent test. The FF and coproantigen ELISA with faecal sedimentation did not differ significantly (Table 1).

Table 1. Comparison of the frequencies of *Fasciola hepatica* in sheep observed in the parasitological examination of 92 faecal samples using different tools.

| Tests                | Number of positive samples (%) | 95% Confidence interval | $\chi^2$ | P value    |
|----------------------|--------------------------------|-------------------------|----------|------------|
| Faecal sedimentation | 14 (15.2)                      | 8.3 to 25.5             |          | References |
| Flukefinder®         | 15 (16.3)                      | 9.1 to 26.9             | 0.04     | 0.80       |
| Coproantigen ELISA   | 19 (20.6)                      | 12.4 to 32.3            | 0.90     | 0.34       |

Table 2. Comparative estimation of faecal sedimentation test with Flukefinder® and coproantigen ELISA in detection of ova of *Fasciola hepatica* in faecal samples (n=92).

| Statistical parameters    | Faecal sedimentation test                    |                                                    |
|---------------------------|----------------------------------------------|----------------------------------------------------|
|                           | Flukefinder®<br>a = 13, b = 2, c = 1, d = 76 | Coproantigen ELISA<br>a = 14, b = 5, c = 0, d = 73 |
| Infection rate (%)        | 15.2                                         |                                                    |
| Accuracy (%)              | 96.7                                         | 94.6                                               |
| $\kappa$ value            | 0.877                                        | 0.816                                              |
| Sensitivity (%)           | 92.6                                         | 100.0                                              |
| Specificity (%)           | 97.4                                         | 93.6                                               |
| Positive predictive value | 86.7                                         | 73.7                                               |
| Negative predictive value | 98.7                                         | 100.0                                              |

a = Samples positive to test; b = samples positive to test but failing the gold standard test; c = samples negative to test, but positive to the gold standard test, d = samples negative to both tests.

After statistical analysis, both methods (FF and coproantigen ELISA produced favourable accuracy, but with varying degrees of precision. Excellent agreement ( $\kappa > 0.75$ ) was obtained between both tests compared to faecal sedimentation. Although C Ag ELISA had the best sensitivity and negative predictive values, FF had the greater specificity and positive predictive values (Table 2).

## Discussion

Ideally, *F. hepatica* illness should be detected early to avoid serious liver tissue damage and environmental contamination with fluke eggs, particularly those from treatment-resistant parasites. Estimating the infection rates of FHI in sheep using different diagnostic procedures was the goal of the study. In order to determine if the techniques can differentiate between truly positive and truly negative samples, a comparison of the methods' sensitivity and specificity was also carried out.

In the present work, the infection rate of FHI in Mosul city was 15.2%. These results are higher than the other recent studies that reported *F. hepatica* in Iraq. Mikaeel (2020) stated that the prevalence of FHI in in Duhok, Kurdistan Region, Iraq was 4.2% and 5.6% in serum and milk samples, respectively, using indirect ELISA technique. Also, prevalence of FH in Al-Najaf Al-Ashraf provinces was 0.67 % in slaughtered sheep (Al-Alo et al. 2019). The geographical distribution of *F. hepatica* in sheep populations is extensive, affecting regions across the globe, particularly in areas conducive to the life cycle of its intermediate host, freshwater snails. This distribution is influenced by environmental factors, livestock management practices, and the presence of suitable habitats for snail populations (Modabbernia et al. 2024).

Furthermore, frequencies of FHI in our work vary depending on the laboratory diagnostic methods employed. A 15.2%, 16.3%, and 20.6% of faecal samples were positive using (SS), (FF), and (C Ag ELISA), respectively. Different studies have utilized various techniques, revealed distinct prevalence rates and highlighted the importance of method selection in epidemiological assessments. Hammami et al. (2024) noted that the prevalence of FHI differed according to the type of laboratory tests, as the highest prevalence was in the bile examination (16.78%), followed by the liver dissection 11.28%, and the lowest prevalence was recorded in the faecal examination 9.12%. In Colombia, seroprevalence was found to be 46.5% in sheep, indicating a high exposure rate, while coprological prevalence was 47.7%, showing a strong correlation between serological and faecal tests (Delgado et al. 2023).

Based on the  $\kappa$  value, excellent agreement was obtained between both tests (FF and C Ag ELISA) compared to faecal sedimentation, and C Ag ELISA had the higher sensitivity and negative predictive values, FF had the greater specificity and positive predictive values.

This indicates that the FF and C Ag ELISA are suitable for detecting FHI in sheep. The faecal sedimentation is the most widely used method for diagnosing FHI because it is easy and inexpensive to run and does not require sophisticated laboratory equipment (Hendrix and Robinson 2023). However, the method is generally considered to have limitations regarding examination of faecal samples with low egg counts (Conceição et al. 2002; Becker et al. 2016; Ploeger et al. 2017; Alstedt et al. 2022). Egg shedding by *F. hepatica* as well as by other liver flukes is known to be intermittent and thus, egg per grams might show considerable variation over time (Düwel and Reisenleiter 1990; Andrews et al. 2021). Even without the use of methylene blue which is frequently used in sedimentation protocols to increase visibility by counter-staining plant particles in a blue colour, the primary use of Flukefinder® showed promise, since every single *F. hepatica* egg was visible in the sediment. Large amounts of coarse faecal material in the sediment often make it difficult to see the eggs during conventional sedimentation, and there is a significant chance that individual eggs will be missed during microscopical examination.

In addition, the traditional sedimentation method can investigate up to 10 g of faeces every process, while the Flukefinder® method only permits a sample size of 2 g of faeces.

The Flukefinder® technique has shown high sensitivity in detecting fluke eggs, particularly at lower infection levels, making it a valuable tool alongside other methods like Mini-FLOTAC and sedimentation techniques (Bosco et al. 2023). Kurnianto et al. (2022) reported that Flukefinder® appears to be the most accurate approach for detecting *Fasciola* eggs, with 80% sensitivity and 90% specificity compared to sedimentation and 100% in artificially spiked stool samples. Compared to Kato-Katz and Mini-FLOTAC® in humans, Flukefinder® observed fewer eggs and had less variability (Zárate-Rendón et al. 2019). Both Flukefinder® and the modified Flukefinder® methods were superior to sedimentation in terms of egg count data (Kahl et al. 2023).

The coproantigen ELISA detects excretory/secretory antigens secreted by live adult and late-immature *Fasciola* into faeces. The MM3-Copro ELISA (Bio-X Diagnostics, Rochefort, Belgium) has been shown to detect 100% of sheep with one fluke and 100% of cattle with two flukes (Mezo et al. 2004). The detection of *F. hepatica* antigens in faeces has been shown to have high sensitivity and specificity (Takeuchi-Storm et al. 2017). We conclude that FF and C Ag ELISA serve as an excellent tool to augment microscopy as the gold standard for FHI diagnosis.

#### Conflict of interest

All authors declare no conflicts of interest.

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