Comparative Evaluation of Diagnostic Methods for Detection of *Fasciola hepatica* in Ruminant Faeces

Liliia Suliman

Independent project • 30 credits
Swedish University of Agricultural Sciences, SLU
Department of Animal Biosciences
Master's programme
Uppsala, 2026

Comparative Evaluation of Diagnostic Methods for Detection of *Fasciola hepatica* in Ruminant Faeces

Liliia Suliman

Supervisor:	**Giulio Grandi, Swedish University of Agricultural Sciences, Department of Animal Biosciences (HBIO)**
Assistant supervisor:	Johan Höglund, Swedish University of Agricultural Sciences, Department of Animal Biosciences (HBIO)
Examiner:	Sara Hägglund Valarcher, Swedish University of Agricultural Sciences, Department of Clinical Sciences (KV)

Credits:	30 credits
Level:	Second cycle, A2E
Course title:	Independent project in Animal Science
Course code:	EX0870
Programme/education:	Master's programme, Agriculture programme - Animal Science
Course coordinating dept:	Department of Animal Biosciences (HBIO), Swedish University of Agricultural Sciences (SLU)
Place of publication:	Uppsala
Year of publication:	2026
Copyright:	All featured images are used with permission from the copyright owner.
Keywords:	*Fasciola hepatica, Paramphistomum spp.,* liver fluke, rumen fluke, AI-based copromicroscopy, sedimentation, coproantigen-ELISA.

Swedish University of Agricultural Sciences
Faculty of Veterinary Parasitology
Department of Animal Biosciences

Abstract

Fasciolosis, caused by the liver fluke *Fasciola hepatica*, is a parasitic disease of major economic importance in cattle and sheep production worldwide. Accurate diagnosis is essential for effective control, yet the performance of available diagnostic methods varies considerably depending on infection intensity, host species, and practical circumstances.

This master's thesis compared three methods: i) traditional sedimentation, ii) AI-based copromicroscopy (OvaCyte™), and iii) a commercial coproantigen-ELISA test, for diagnosing *F. hepatica* in faecal samples of ruminants.

A total of 70 faecal samples were collected from livestock (47 from cattle and 23 from sheep) at four locations in Sweden. For 28 cattle and 23 sheep that were sampled at slaughter, infection status was first determined through visual liver inspection. Faecal samples were then analysed using all three methods. Detection rates, egg counts, coinfection with rumem fluke , time efficiency, and agreement between the different methods were evaluated.

Coproantigen-ELISA detected *F. hepatica* in the highest proportion of tested samples (49/70, 70.0%), followed by traditional sedimentation (45/70, 64.3%) and OvaCyte™ (37/70, 52.9%). In sheep, all three methods detected *F. hepatica* in all 23 samples (100%). In cattle, ELISA was positive in 26 of 47 samples (55.3%) , sedimentation in 22 of 47 samples (46.8%) , and OvaCyte™ in 14 of 47 samples (29.8%). OvaCyte™ recovered the highest total number of *F. hepatica* eggs (1,261 EPG) compared to sedimentation (1,146 eggs/5g). Rumen fluke infection was common in sheep (73.9% by sedimentation, 60.9% by OvaCyte™) but rare in cattle (2.1–4.3%). ELISA was the most time-efficient method (6 hours for 70 samples), followed by OvaCyte™ (12 to 15 minutes preparation plus 3 minutes reading time per sample) and sedimentation (45 minutes sedimentation plus 11 to 16 minutes reading time per sample). Pairwise agreement between methods was moderate (κ = 0.44–0.47), with the highest agreement observed between the microscopy-based detection methods.

No single diagnostic method is optimal for all situations. ELISA is most effective for herd level screening and early detection in cattle. Sedimentation remains a practical, low cost alternative for confirmatory diagnosis, particularly in resource limited settings. OvaCyte™ provides full automation, consistent results, and excellent performance in sheep, along with the unique ability to detect mixed infections with rumen flukes simultaneously. In sheep, OvaCyte™ is the preferred method due to its perfect detection rate, highest egg recovery, and ability to detect mixed infections with other flukes. For mixed farms, ELISA is recommended for cattle, but it is also suitable for sheep. When clinical suspicion is high or the consequences of missing an infection are severe, using multiple tests in parallel provides the most reliable diagnosis.

Keywords: Fasciola hepatica, Paramphistomum spp., liver fluke, rumen fluke, AI-based copromicroscopy, sedimentation, coproantigen-ELISA.

Table of Contents

List of tables

List of figures

Abbreviations

AI – Artificial Intelligence
Bio-X – Bio-X Diagnostics
ELISA – Enzyme-Linked Immunosorbent Assay
EPG – Eggs Per Gram
Fh – Fasciola hepatica
κ – Kappa (Cohen's kappa coefficient)
N – Negative (slaughter code)
OC – OvaCyte
OD – Optical Density
Ps-Paraphistomus spp.
P – Positive
TMB – Tetramethylbenzidine
QC – Quality Control

1. Introduction

1.1 Background

Parasitic infections remain among the most significant challenges facing livestock production systems globally, significantly impacting animal health, welfare, and agricultural productivity (Charlier et al., 2014). Fasciolosis is classified as an anthropozoonosis, affecting both animals and humans (Mas-Coma et al., 2009; Mehmood et al., 2017). The disease is primarily transmitted by herbivorous livestock through the excretion of parasite eggs in faeces (Caravedo and Cabada, 2020). Although ruminants are the main definitive hosts, *Fasciola hepatica* has also been reported in a wide range of other mammalian species, including equids (horses), rodents, lagomorphs, carnivores, and humans (Boray, 1969; Mas-Coma et al., 2009; Valero et al., 2002; Tanabe et al., 2024; Kronenberg et al., 2025).

Among the numerous parasitic infections of ruminants, fascioliasis, caused by the liver fluke *Fasciola hepatica*, is a large leaf-shaped flatworm found in temperate countries worldwide and mainly infecting sheep, cattle and other grazing animals (Mehmood et al., 2017). A major obstacle to efficient livestock production in both temperate and tropical regions, this parasite can reach prevalence rates exceeding 80% in cattle in some endemic areas (Beesley et al., 2018).

Fasciola hepatica, commonly known as the liver fluke, belongs to the class Trematoda within the phylum Platyhelminthes. Together with tropical counterpart *Fasciola gigantica*, it causes fasciolosis, a disease of significant veterinary importance (Dalton, 2021). The parasite has a complex, indirect life cycle that requires two hosts: a definitive mammalian host (typically ruminants) and an intermediate snail host (primarily lymnaeid species such as *Galba truncatula* in Europe) (Andrews, 1999, cited in Dalton, 2021; Vázquez Perera et al., 2019, Novobilský et.al, 2014) .

In addition to *F. hepatica*, faecal samples were examined for *Paramphistomum spp*. (rumen fluke). While adult rumen flukes are generally non-pathogenic, the immature stages cause severe inflammation of the small intestine, leading to diarrhea, weight loss, and death in young animals (Gordon et al., 2013). The primary rumen fluke species affecting European livestock is *Calicophron daubneyi* (Fenemore et al., 2021).

Over the past three decades, paramphistomosis has emerged as a significant parasitic disease across Europe (Gordon et al., 2013). *Calicophron daubneyi* has spread rapidly, reaching high prevalence in the United Kingdom, Ireland, France, Spain, and the Netherlands (Gordon et al., 2013).

Mixed infections with *F. hepatica* and rumen flukes are common, as both parasites share the same snail intermediate host, *Galba truncatula* (Jones et al., 2015). Diagnosis is based on egg detection using sedimentation, though eggs are morphologically similar to those of *F. hepatica* but are colourless rather than golden-yellow. Treatment options are limited, as triclabendazole – the drug of choice for *F. hepatica* – is ineffective against paramphistomes (Gordon et al., 2013).

1.2 Epidemiology and Global Distribution

Fasciolosis occurs worldwide and has been reported on all continents except Antarctica, with distribution patterns varying depending on climatic conditions, availability of intermediate hosts, and agricultural practices (Mehmood et al., 2017; Dalton, 2021). The epidemiology of the parasite is closely linked to environmental factors, particularly humidity and temperature, which influence both the free-living stages of the parasite and the population dynamics of its intermediate host (Beesley et al., 2018).

A global review by Mehmood et al. (2017) found that the prevalence of bovine fascioliasis varied significantly by geographic region, ranging from 1.2% to 91.0% in Africa, 3.0% to 66.7% in the Americas, 0.71% to 69.2% in Asia, 26.5% to 81.0% in Oceania, and 0.12% to 86.0% in Europe. A global meta-analysis by Lan et al. (2024), which reviewed 371 studies covering approximately 57.9 million cattle, estimated the pooled global prevalence of bovine fasciolosis at 17%, while ovine fasciolosis was estimated at 13% based on data from 37.4 million sheep (Lan et al., 2024). In sheep, fasciolosis is often more acute and severe than in cattle, reflecting the greater susceptibility of sheep to the pathogenic effects of migrating juvenile flukes (Boray, 1969; Behm and Sangster, 1999). More recent country-specific studies using sedimentation have reported prevalences of 29% in Nigerian cattle (Agbajelola et al., 2025), 17% in Chinese cattle (Lan et al., 2025), 25% in Iranian cattle (Hoseini et al., 2025), and up to 70% in highland Peru (Cueva-Rodriguez et al., 2024). In sheep, sedimentation-based studies have reported rates ranging from 13% in the Ethiopian highlands to 62% in parts of South America (Mehmood et al., 2017; Lan et al., 2024).

In Europe, the prevalence of *F. hepatica* detected by sedimentation varies considerably between countries. A study in Austria reported a prevalence of 28.5% in dairy cows (Schoiswohl, 2025), while a recent survey in north-western Spain found a prevalence of 51.2% in cattle (González-Warleta et al., 2025). In sheep from north-western Spain, prevalence as high as 51.2% has been reported using sedimentation (González-Warleta et al., 2025). In Poland, the prevalence detected in goats was very low (0.5%), reflecting the species' browsing behaviour

(Mickiewicz et al., 2024). In the United Kingdom, sedimentation-based surveys have reported ovine fasciolosis prevalence ranging from 5% to 30%, depending on region and season (Gordon et al., 2013; Jones et al., 2017).

According to a serological survey using ELISA, the prevalence of *F. hepatica* in Swedish cattle herds is 9.8%, although complete prevalence data based on faecal sediment analysis are still lacking (Novobilsky et al., 2015). Serological surveys in Swedish sheep have suggested that fasciolosis is present in many flocks, but specific sedimentation-based data are lacking (Novobilsky et al., 2015). A 2020 Swedish study reported within-herd *F. hepatica* prevalence ranging from 27% to 85% in dairy herds, with untreated infected cows showing significantly reduced milk yield (Novobilský et al., 2020). In Swedish sheep, comprehensive economic data are lacking; however, serological surveys suggest that fasciolosis is widespread, and production losses due to reduced weight gain and poor fertility are likely significant (Novobilský et al., 2015). Recent data from the FarmStat report further indicate that liver fluke infections continue to occur in Swedish sheep flocks, with regional variation and higher occurrence in south-western Sweden and Västra Götaland (Gård & Djurhälsan, 2025).

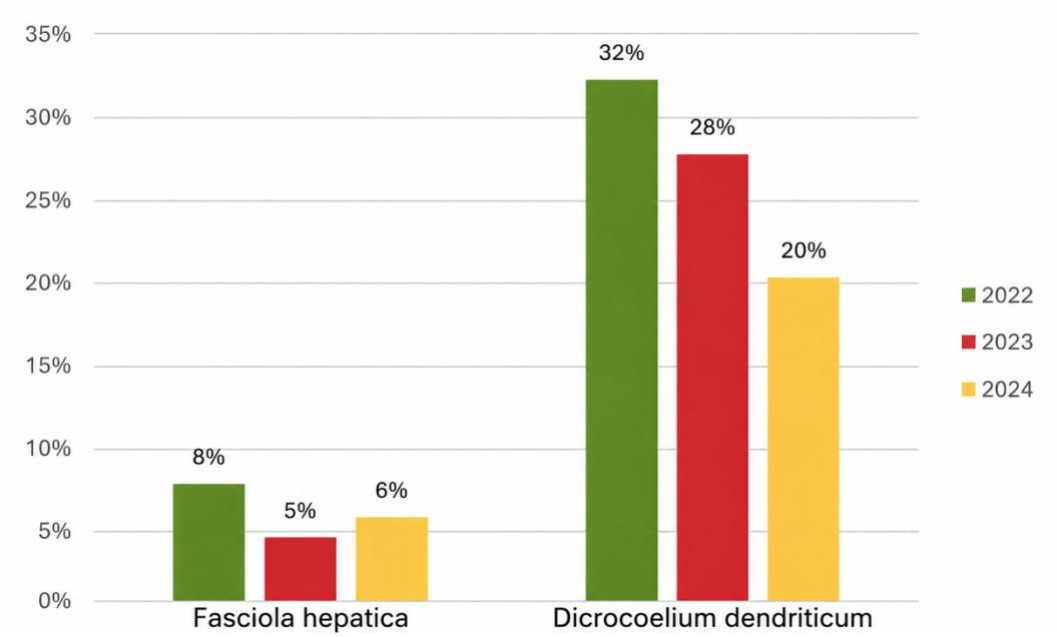

Figure 1. Prevalence of liver fluke findings in sheep herds across 2022–2024. (Gård & Djurhälsan,2025) 9140-FarmStat-rapporten-2024_SLUTVERSION-webb.pdf [2026-06-07].

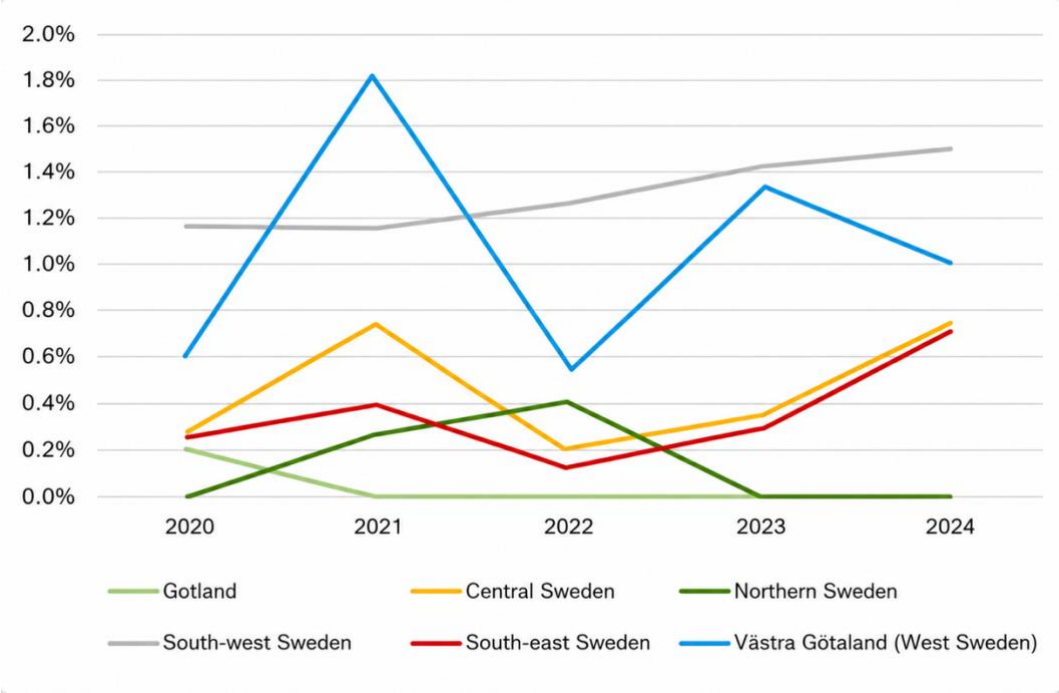

Figure 2. Regional differences for finding Code 80 (F. hepatica) in sheep 2020-2024 (Gård & Djurhälsan,2025) 9140-FarmStat-rapporten-2024_SLUTVERSION-webb.pdf [2026-06-07].

It is important to note that prevalence estimates are highly dependent on the diagnostic method used, with sedimentation generally having lower sensitivity than indirect serological tests due to the long pre-patent period and intermittent egg shedding (Charlier et al., 2008). Therefore, prevalence figures based on sedimentation likely underestimate the true infection rate. Furthermore, the sensitivity of sedimentation in sheep is generally higher than in cattle because sheep tend to shed more eggs per gram of faeces, reflecting their higher susceptibility and fluke burden (Boray, 1969). Climate change has been associated with the spread of fasciolosis to previously unaffected areas, as milder winters and increased precipitation create favourable conditions for disease transmission at higher latitudes and altitudes (Beesley et al., 2018).

1.3 Economic impact

The economic consequences of fasciolosis in livestock production are substantial and multifaceted. Globally, annual losses to the livestock industry are estimated to exceed 3 billion US dollars (Mehmood et al., 2017). These losses include direct impacts such as mortality, liver condemnation, reduced weight gain, decreased milk yield, and impaired reproduction, as well as indirect costs including anthelmintic treatments, veterinary services, and control measures (Charlier et al.,

2020). In sheep, economic losses are particularly severe due to higher mortality rates during acute fasciolosis, with reported case fatality rates of up to 10–20% in heavily infected flocks (Boray, 1969; Behm and Sangster, 1999).

In Europe, annual losses are estimated at €52 million in Switzerland (Schweizer et al., 2005), and £23 million in the United Kindom (UK) (Bennett and Ijpelaar, 2005). Ovine fasciolosis costs the UK sheep industry an estimated £10–15 million each year, mainly from liver condemnation, reduced weight gain, and mortality (Gordon et al., 2013). Selective flukicide treatment of non-lactating cows in Swedish dairy herds resulted in a significant increase in milk production of approximately 1 kg per cow per day in the subsequent lactation, highlighting the substantial production losses caused by subclinical infections (Novobilský et al., 2020). These Swedish findings align with the broader European context, where fasciolosis remains a major constraint to efficient cattle production despite advances in diagnostic methods (Charlier et al., 2013). In sheep, fasciolosis continues to cause significant economic losses across Europe, with prevalence rates in some regions exceeding 10% and annual liver condemnation costs amounting to millions of euros (Khoramian et al., 2024; Fennouh et al., 2025). A European-wide assessment estimated the combined annual cost of helminth infections, including fasciolosis, at €1.8 billion across 18 participating countries, with 81% attributed to production losses (Charlier et al., 2020). Ovine fasciolosis was estimated to account for approximately 15% of total helminth-related losses in sheep across Europe (Charlier et al., 2020).

In Asia, China experiences annual losses exceeding 200 million US dollars (Lan et al., 2025). In sheep from China, prevalence studies have reported rates of 5–20%, with corresponding economic losses due to reduced meat production and liver condemnation (Lan et al., 2024).

In Australia, annual losses due to *F. hepatica* in livestock are estimated at 50–80 million Australian dollars, with an additional 10 million dollars spent on treatments (Tran et al., 2022). In sheep, the percentage of carcases with liver fluke nearly doubled between 2020/21 and 2022/23, from 0.5% to 0.9%, with approximately 15% of Australian flocks considered at high or moderate risk (Animal Health Australia, 2024).

In Russia, annual losses from fasciolosis in cattle are estimated at approximately 20 million US dollars, primarily due to liver condemnation and reduced productivity (Gorokhov et al., 2019). In sheep from southern Russia, prevalence varies from 45% to 80% in wetland pastures and reaches 70–83% in mountainous areas, with each infected animal losing 6.5–9.7 kg in body weight and up to 20% of its annual wool production (Kabardiev et al., 2024).

In the Americas, the United States (US) faces losses of approximately 50 million US dollars annually (UpToDate, 2025), while Argentina and Brazil report annual

losses of 80 million and 60 million US dollars, respectively (Strydom et al., 2023; Molento et al., 2018). In South America, ovine fasciolosis is particularly problematic in the Andean highlands, where prevalence in sheep can exceed 50%, results in substantial economic losses for smallholder farmers (Cueva-Rodriguez et al., 2024).

1.4 Problem Statement

Despite the availability of several diagnostic methods for detecting *Fasciola hepatica*, significant knowledge gaps remain regarding their relative performance in the field (Charlier et al., 2020). Direct comparative studies evaluating traditional sedimentation, AI-based copromicroscopy, and coproantigen ELISA on the same faecal samples are limited (Beesley et al., 2018; Felländer et al., 2026). In parallel, automated image-based diagnostic approaches, including artificial intelligence (AI), are being explored in parasitology, for example through systems such as VETSCAN IMAGYST, which uses digital image processing and algorithmic analysis to detect parasites (Nagamori et al., 2020). However, to date, no published studies have specifically evaluated AI-based systems for the detection of *F. hepatica*. This represents a critical knowledge gap and highlights the need to investigate whether such approaches could complement or enhance existing diagnostic methods in field-based surveillance.

This study addresses these knowledge gaps by comparing three diagnostic methods—conventional sedimentation, AI-based copromicroscopy (OvaCyte™ Fluke Plus), and a commercial coproantigen ELISA—using faecal samples from ruminants (cattle and sheep) in Sweden. Information on the presence of infection was obtained either by visual inspection at the slaughterhouse or from previous farm records of infection.

1.5 Aim and Objective

The primary aim of this master's thesis is to evaluate and compare the diagnostic performance of three methods for detecting *F. hepatica* infection in cattle faeces as described in section “Materials and Methods”.

Specific objectives:

1. Conduct on 47 faecal samples from cattle and 23 from sheep and record the presence and number of *F. hepatica* eggs, by:
    a. Traditional sedimentation;
    b. AI-based copromicroscopy using OvaCyte™ Fluke Plus;

c. Coproantigen ELISA (Bio K 201/2).

2. Compare the results obtained using the three diagnostic methods and assess their agreement.

3. Provide evidence-based recommendations for the choice of method in various diagnostic contexts.

1.6 Hypothesises

Null Hypothesis (H_0): There is no significant difference in sensitivity between traditional sedimentation, AI-based copromicroscopy (OvaCyte™), and coproantigen-ELISA for detecting *F. hepatica* infection in cattle.

Alternative Hypotheses:

Hypothesis$_1$: Coproantigen-ELISA demonstrates greater sensitivity than both egg-detection methods, particularly for detecting low-intensity infections and infections during the pre-patent period (Mezo et al., 2004; Charlier et al., 2008).

Hypothesis$_2$: AI-based copromicroscopy (OvaCyte™) shows sensitivity that is not inferior to traditional sedimentation for detecting *F. hepatica* eggs, while reduced analysis time. However, its relative sensitivity may vary from higher to lower depending on infection intensity.

Hypothesis$_3$: There is moderate to substantial agreement between traditional sedimentation and AI-based copromicroscopy, as both methods detect parasite eggs (Charlier et al., 2008).

Hypothesis$_4$: Traditional sedimentation detects more positive samples than AI-based copromicroscopy, particularly in samples with low egg counts.

Hypothesis$_5$: All three methods yield positive results in samples from animals known to have liver fluke infection based on visual observation at slaughter or veterinary communication.

1.7 Significance of the Study

This study adds to the evidence base for selecting diagnostic methods in veterinary practice. Understanding the strengths and limitations of each method enables more accurate interpretation of test results and more informed clinical decisions (Charlier et al., 2020). From a technological perspective, evaluating an automated AI-based system supports the development of an evidence base for new digital diagnostic methods in veterinary parasitology (Nagamori et al., 2020). From an agricultural perspective, improved diagnostics for fasciolosis support more

effective disease control, reduced production losses, and increased sustainability of livestock farms (Mehmood et al., 2017).

1.8 Scope and Delimitation

This study focuses on detecting *F. hepatica* in cattle and sheep faeces, using 70 faecal samples collected from four sources in Sweden (Figure 3):

i) Närkes slakteri, Gällersta (cattle, n=8),
ii) Bredaryd farm (cattle, n=19),
iii) Dahlberg slakteri, Brålanda (cattle, n=6),
iv) Varekils slakteri AB (cattle n=15; sheep n=23).

Reference information on infection status was obtained through visual inspection and communication with the farmer or veterinarians present at the slaughter.

Figure 3. Location of farm and slaughterhouses (Image created with Canva from the prompt" Map of Sweden with samples location", 2026).

2. Literature review

2.1 Biology and Morphology

Fasciola hepatica is a digenetic trematode belonging to the class Trematoda within the phylum Platyhelminthes (Dalton, 2021). The adult fluke is leaf shaped, flat, and measures 20–30 mm in length and 8–13 mm in width (Andrews, 1999). In sheep, adult flukes tend to grow larger than those in cattle, although egg production per fluke is lower (Valero et al., 2001). The anterior end features a conical projection bearing the oral sucker. The ventral sucker, is located mid-ventrally. The entire body surface is covered with tiny spines, which help the fluke attach to the bile ducts of its host, (Mas-Coma et al., 2009).

The fluke has a simple digestive system with a mouth, pharynx, oesophagus, and two highly branched tubes (caeca) that extend almost to the posterior end of the body (Andrews, 1999). It eliminates waste through a system of specialised flame cells. Its nervous system consists of a pair of cerebral ganglia and nerve cords running along the body (Dalton, 2021).

F. hepatica is hermaphroditic, meaning each individual possesses both male and female reproductive organs. The male system has two branched testes in the middle of the body, while the female system has a single branched ovary towards the anterior end, along with vitelline glands that produce yolk for the eggs (Mas-Coma et al., 2009). Flukes can self-fertilise or mate with each other (Andrews, 1999). Sheep do not develop acquired resistance to reinfection, whereas cattle develop resistance after 5–6 months of infection, contributing to higher fluke burdens in sheep (Behm and Sangster, 1999).

The eggs are oval, golden-yellow, and have a small lid (operculum). They measure about 130–150 μm in length and 70–90 μm in width (Andrews, 1999). Because *F. hepatica* is the same parasite species in both sheep and cattle, eggs from the two hosts are morphologically indistinguishable by routine microscopy (Valero et al., 2001). The eggs are passed out with the host's faeces and must develop in water before they hatch (Dalton, 2021).

2.2 Life cycle

The life cycle of *F. hepatica* (Figure 4) involves alternation of generations and a change of host species (Mas-Coma et al., 2009). The hermaphroditic adult stage is a generalist and parasitises a wide range of domestic and wild mammal, which serve as definitive hosts. In sheep, the acute phase of fasciolosis caused by

migrating juvenile flukes is more severe and often fatal, whereas cattle develop chronic, subclinical infections (Boray, 1969; Behm and Sangster, 1999).

Fertilised eggs are excreted in faeces and, under suitable conditions of warmth and moisture, develop into free-swimming miracidia, which penetrate a suitable snail intermediate host (Andrews, 1999). The same snail species, *Galba truncatula*, serves as the intermediate host for both cattle and sheep (Mas-Coma et al.). Inside the snail, the parasite undergoes asexual reproduction through sporocyst, redia, and cercaria stages. Mature cercariae emerge from the snail and encyst on aquatic vegetation as metacercariae, the infective stage for the definitive host (Dalton, 2021). After ingestion, metacercariae excyst in the small intestine, penetrate the intestinal wall, migrate through the peritoneal cavity, and burrow through the liver parenchyma before establishing in the bile ducts, where they mature into adult flukes and begin egg production (Mas-Coma et al., 2009). The prepatent period (time from infection to egg shedding) is 8–10 weeks in both cattle and sheep, but clinical signs appear earlier in sheep due to higher susceptibility to tissue damage (Boray, 1969; Andrews, 1999).

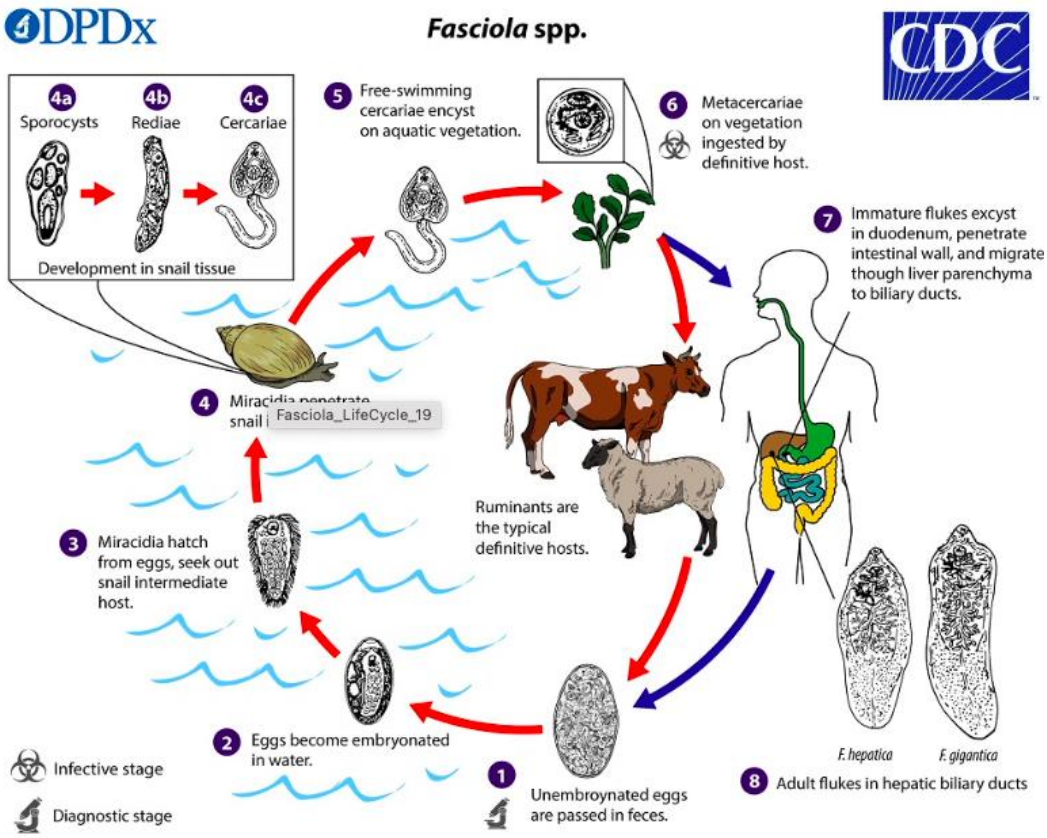

Figure 4. Life cycle of F.hepatica (CDC, 2019)
http://www.cdc.gov/dpdx/fascioliasis/index.html, [2026-03-08].

Skrjabin and Schulz (1935) proposed dividing the developmental cycle into four periods, which are described in Table S1.

2.3 Pathogenesis

The pathogenesis of fascioliasis is generally divided into two distinct phases: the acute migratory phase and the chronic biliary phase (Tanabe et al., 2024). The severity of both phases is highly dependent on the dose of metacercarial infection. During the acute phase, newly hatched larvae penetrate the intestinal wall, migrate through the abdominal cavity, and invade the liver parenchyma, causing extensive mechanical tissue destruction. Tissue damage occurs through three main mechanisms: the action of proteolytic enzymes secreted by the parasites, the host immune response to the parasite's excretory/secretory products and tegument, and the mechanical impact of the migrating parasites (Tanabe et al., 2024). In sheep, acute fasciolosis can cause sudden death due to massive liver haemorrhage, with mortality rates reaching 12% in heavily infected flocks (Nikpay et al., 2025). During the acute phase, infected sheep show significant elevation of liver enzymes (AST), reflecting extensive hepatic tissue damage (Nikpay et al., 2025).

The chronic phase begins when the flukes establish themselves in the bile ducts, typically 8–10 weeks after infection, causing progressive cholangitis, periductal fibrosis, and bile duct epithelial hyperplasia (Tanabe et al., 2024). Chronic infections are associated with progressive weight loss, reduced milk production, impaired fertility, and increased susceptibility to comorbidities (Charlier et al., 2013). In sheep, chronic fasciolosis leads to reduced wool quality and weight loss, with economic losses per infected animal being substantial (Khoramian et al., 2024; Mariko et al., 2025).

2.4 Diagnostic Techniques for Liver Fluke Detection

Accurate and timely diagnosis of *F. hepatica* infection is essential for effective disease management, guiding decisions on treatment, control strategies, and surveillance programmes (Charlier et al., 2008; Dalton, 2021). However, diagnosing fascioliasis presents several challenges. Clinical signs are nonspecific, and the relationship between fluke burden and production losses is complex, with even subclinical infections causing economically significant productivity losses (Charlier et al., 2009). In this thesis focus is on direct methods for the detection flukes in ruminant faeces.

In Swedish slaughterhouses, liver findings are classified using specific codes that provide valuable information about parasite status. Code 79/80 indicate that adult *F. hepatica* (large liver fluke) has been found in the liver at slaughter, confirming patent infection (Lindqvist, 2019).

While codes 83/84 (liver parasite infection) and 87/88 (other liver infections) were not classified as positive, they may reflect previous infections (Lindqvist Frisk, 2019). This conservative approach means that some low-intensity or larval infections may be misclassified as negative, but it increases the reliability of diagnostic accuracy estimates (Rapsch et al., 2006).

2.4.1 Traditional Diagnostic Approaches

Historically, direct diagnosis of fasciolosis on farms has relied heavily on detecting parasite eggs in stool samples using sedimentation methods (Rapsch et al., 2006). These methods exploit the differences in density between parasite eggs and faecal debris, allowing for the eggs to be concentrated and visualized under a microscope. The main advantage of sedimentation is its high specificity: when eggs are detected and correctly identified by experienced microscopists, a definitive diagnosis of overt infection can be made (Charlier et al., 2008; Rapsch et al., 2006).

Despite these advantages, sedimentation has several well-documented limitations. Its sensitivity varies widely, with reported values ranging from 43% to 81% depending on infection intensity, sample size, stage of infection, and examiner skill (Rapsch et al., 2006; Charlier et al., 2008; Mazeri et al., 2016). Additionally, sedimentation is time-consuming, requiring approximately 45 minutes of passive waiting for sedimentation steps plus active work time for each sample (Rapsch et al., 2006). During the prepatent period, that lasts 8-10 weeks after ingestion of metacercariae, infected animals do not lay eggs and invariably test negative by sedimentation (Andrews, 1999). Even during the patent period, egg laying may be intermittent, and animals with low fluke burdens may lay eggs in numbers below detection limits (Charlier et al., 2008).

2.4.2 Advances in Diagnostic Technologies

In response to the limitations of traditional copromicroscopy, two main categories of alternative diagnostic methods have emerged: immunodiagnostic tests and automated image analysis systems (Charlier et al., 2020).

Coproantigen ELISA detects parasite antigens—specifically, cathepsin L protease—excreted in the faeces of infected animals (Mezo et al., 2004). Unlike egg detection, coproantigen ELISA enables detection of infections during the prepatent period, with positive results as early as 4-5 weeks after infection, a correlation between antigen levels and fluke burden, and minimal inter-observer variability (Mezo et al., 2004; Charlier et al., 2008). The Bio K 201/2 *F. hepatica*

Antigen ELISA kit (Bio-X Diagnostics, Belgium) is specifically designed for this purpose and uses an indirect sandwich format with biotin-streptavidin amplification (Bio-X Diagnostics, 2025). Studies report sensitivities of 77-94% and specificities exceeding 95% (Mezo et al., 2004; Charlier et al., 2008; Mazeri et al., 2016).

AI-based copromicroscopy. Automated image-analysis systems are an emerging technology in diagnostic parasitology (Holmström et al., 2017). The OvaCyte™ Fluke Plus system combines digital microscopy with artificial intelligence algorithms to automatically identify and quantify parasite eggs (OvaCyte, 2025). The system captures multiple images per sample, processes them using deep learning algorithms trained on thousands of egg images, and provides quantitative results with minimal operator intervention. Evaluations in human parasitology have shown sensitivities comparable to those of expert microscopists with significantly reduced analysis time (Holmström et al., 2017).

2.5 Comparative Studies on Diagnostic Methods

Several studies have compared diagnostic methods for detecting *F. hepatica*. Charlier et al. (2008) compared the sedimentation method and coproantigen ELISA, finding that coproantigen ELISA had significantly higher sensitivity (94% versus 43–64%). In sheep, similar trends have been observed: coproantigen ELISA demonstrated higher sensitivity (86–94%) compared to sedimentation (50–70%) for detecting *F. hepatica* in ovine faecal samples (Mezo et al., 2004; Rehbein et al., 2011).

Mazeri et al. (2016) evaluated five diagnostic tests using Bayesian analysis, reporting that the sensitivity of the sedimentation method varied by season (0.58–0.81), while the sensitivity of coproantigen ELISA remained stable (0.77). Seasonal variability in sedimentation sensitivity has also been documented in sheep, with lower detection rates during winter months when egg shedding is reduced (Gordon et al., 2013).

Palmer et al. (2014) reported a coproantigen ELISA sensitivity of 80% and specificity of 100%, compared with 70% and 80% for the sedimentation method.

In sheep, a study by Kajugu et al. (2015) reported that coproantigen ELISA had 100% specificity and showed no cross-reactivity with other parasites commonly found in sheep, including *Paramphistomum* spp.

However, only a limited number of studies have directly compared the traditional sedimentation method with automated AI-based systems for the detection of *F. hepatica* in cattle (Nagamori et al., 2020). Validation of AI-based copromicroscopy in sheep is even more limited, although recent studies have demonstrated promising results for automated detection of *F. hepatica* and

Calicophoron daubneyi eggs through deep learning approaches (Capuozzo et al., 2025).

2.6 Knowledge Gaps and Research Needs

Despite these advances, knowledge gaps remain regarding the comparative performance of traditional sedimentation, AI-based copromicroscopy, and coproantigen ELISA for detecting flukes using the same animal samples (Beesley et al., 2018). Field validation of AI-based systems is necessary before their widespread adoption (Nagamori et al., 2020). This study addresses these gaps by comparing the three methods using cattle samples from Sweden.

3. Materials and Methods

3.1 Study Design

This study was designed as a comparative diagnostic evaluation of three index tests: i) traditional sedimentation, ii) AI-based copromicroscopy (OvaCyte™ Fluke Plus), and iii) coproantigen-ELISA - for detecting *F. hepatica* in ruminant faecal samples. It is a diagnostic test validation study comparing these three index tests against a gold reference standard (liver pathology examination upon meat inspection).

The study followed STARD (Standards for Reporting Diagnostic Accuracy Studies) recommendations for study design and presentation of results where applicable (Bossuyt et al., 2015). Laboratory analyses were conducted in the Parasitology Laboratory at the Department of Animal Biosciences, Swedish University of Agricultural Sciences.

Samples were collected at four locations in Sweden (Figure 3) between A) Gällersta slaughterhouse, B) Bredaryd farm, C) Dahlberg slaughterhouse, and D) Varekils slaughterhouse between January and April 2026.

All slaughterhouse samples had liver inspection data available. The Bredaryd farm samples were collected directly from the farm and were not obtained from slaughter, but the farm had historical records of liver fluke problems.

For slaughterhouse samples (Gällersta, Dahlbergs, Varekils), the inclusion criterion was the availability of liver inspection data (slaughter code 80 or N). For farm samples (Bredaryd), the inclusion criterion was a documented history of repeated slaughter code 79/80 from previous meat inspections.

The study focused on comparing diagnostic methods across naturally infected animals regardless of production type or age, as these factors were not expected to affect the relative performance of the tests. No systematic data on age or production type were collected.

3.1.1 Sample Collection and Storage

All samples were transported to the laboratory under refrigeration (4°C) immediately after collection and processed according to the dates detailed in Table S2. OvaCyte™ analysis was performed within 24 hours for the first batch and within 3–8 days for subsequent samples. Traditional sedimentation was performed within 4–9 days of collection, depending on the batch. For coproantigen-ELISA, faecal samples were stored at −20°C and analysed in a single batch to ensure

consistency. Although prompt processing is ideal for optimal egg recovery, refrigeration at 4°C preserves egg morphology and antigen integrity for several days (Charlier et al., 2008) and freezing at −20°C maintains coproantigen stability for extended periods (Bio-X Diagnostics, 2025).

3.1.2 Sample identification

A total of 70 faecal samples were collected, each assigned a unique number (1-70) with corresponding animal identification numbers (Table S2).

For two animals from Bredaryd farm (samples 1383-7 and 1402-5), the individual faecal samples were too small to allow separate analysis by all three diagnostic methods. Therefore, these two samples were pooled (mixed together) to create a single representative sample (sample 19), which was then analysed by sedimentation, OvaCyte™, and ELISA.

3.1.3 Determination of Infection Status

The infection rate of the animals was determined as follows, depending on the source of the samples:

A) Gällersta slaughterhouse (1-8);

The infection status was determined by visual examination of the liver the slaughterhouse veterinarians. The following slaughterhouse codes were recorded:
- Slaughterhouse code "80" indicates the presence of trematodes in the liver and the culling of the liver due to visible invasion by flukes (Jordbruksverket, 2019). Samples with this code (samples 4, 5, 6, 8) showed visible signs of *F. hepatica* infection in the liver, including fibrosis of the bile ducts, thickening, or the presence of adult flukes.
- Slaughterhouse code "N" indicates no visible signs of infection. The samples with this code (samples 1, 2, 3, 7) contained a liver that appeared normal upon post-mortem inspection and was approved for human consumption.

Visual examination of the liver is a standard method used in slaughterhouses to detect fascioliasis and has been employed in numerous diagnostic studies (Rapsch et al., 2006; Charlier et al., 2008; Mazeri et al., 2016). Although this method can detect moderate to high-severity infections, it may not identify low-intensity infections when the number of flukes is insufficient to cause serious pathological changes (Rapsch et al., 2006).

B) Bredaryd farm (9-27);

For the Bredaryd farm samples, infection status was determined through verbal communication with the owner, who had participated in a previous liver fluke research project in collaboration with the Swedish University of Agricultural Sciences (SLU).

It should be noted that although infection at the farm level has been confirmed, the status of individual animals and the intensity of infection may vary. The detection rates observed in these groups probably reflect the known limitations of egg detection methods, including periodic egg laying, low fluke burden, and the presence of infections that occurred before patency (Rapsch et al., 2006; Charlier et al., 2008).

C) Dahlberg slaughterhouse (28-33);

In the samples collected at the Dahlberg slaughterhouse, the infectious status was determined by visual examination of the liver by slaughterhouse veterinarians during slaughter. All the samples from the Dahlberg farm had the slaughter code "80", indicating:

- visible signs of liver damage caused by *F. hepatica;*
- liver culling due to fluke infestation (Jordbruksverket, 2019).

As with the samples from Gällersta, visual inspection at slaughter provides convincing evidence of infection, but may not detect low-intensity infections (Rapsch et al., 2006). All six samples from Dahlbergs farm originated from animals with visibly infected livers, making this group particularly valuable for evaluating diagnostic test performance in confirmed moderate to severe infections.

D) Varekils slaughtherhouse (34-70).

As in the other slaughterhouses, samples with fluke positive status (code “79/80”) were confirmed by the slaughter veterinarian and collected from the intestines after slaughter. According to the veterinarian, all animals from which samples 34-70 were collected showed visible signs of *F. hepatica* infection in the liver at slaughter. The veterinarian confirmed the presence of adult flukes and/or typical pathological changes, including bile duct fibrosis and thickening, indicative of chronic fasciolosis (Shirai et al., 2006).

3.1.4 Sample processing and Aliquoting

Upon arrival at the laboratory, each faecal sample was thoroughly mixed and divided into three aliquots for parallel analysis:

- Aliquot A: for traditional sedimentation analysis (stored at 4°C, processed within 24 hours),
- Aliquot B: for the OvaCyte™ Fluke Plus assay (stored at 4°C, processed within 24-48 hours),
- Aliquot C: for coproantigen ELISA (stored at -20°C for batch analysis).

To ensure blinding of the results of other tests, each diagnostic test was preformed uisng sample coding (Rapsch et al., 2006).

3.2 AI-based Copromicroscopy Using OvaCyte™ Fluke Plus System

3.2.1 OvaCyte™ Fluke Plus System Overview

The OvaCyte™ Fluke Plus System is a specialised application of the standard OvaCyte platform, designed for the automated detection and quantification of liver flukes (*F. hepatica)* and rumen flukes (*Paraphistomum spp.)* in grazing animals. According to Telenostic (2026), the core OvaCyte technology is a "patent-protected, breakthrough parasite egg recovery platform" that uses artificial intelligence (AI) and digital analysis to automate copromicroscopy (the detection of parasite eggs in faeces) (Telenostic, 2026). The Fluke Plus system adapts this technology specifically for fluke eggs, which are denser than standard nematode eggs and therefore require a different flotation method (OvaCyte, 2025). The system provides a complete, point-of-care parasitic analysis, requiring approximately two minutes of user hands-on time for sample preparation and loading. After loading, the process is fully automated, and results are delivered directly to the device and a web application, facilitating targeted treatment decisions (Telenostic, 2026).

3.2.2 Samples preparation for AI-based copromicroscopy

Sample preparation for OvaCyte™ analysis (Figure 5) was performed according to the manufacturer's "Fluke Sample Preparation for *F. hepatica* & *Paramphistomum spp.*" protocol (OvaCyte, 2025), (Appendix 1).

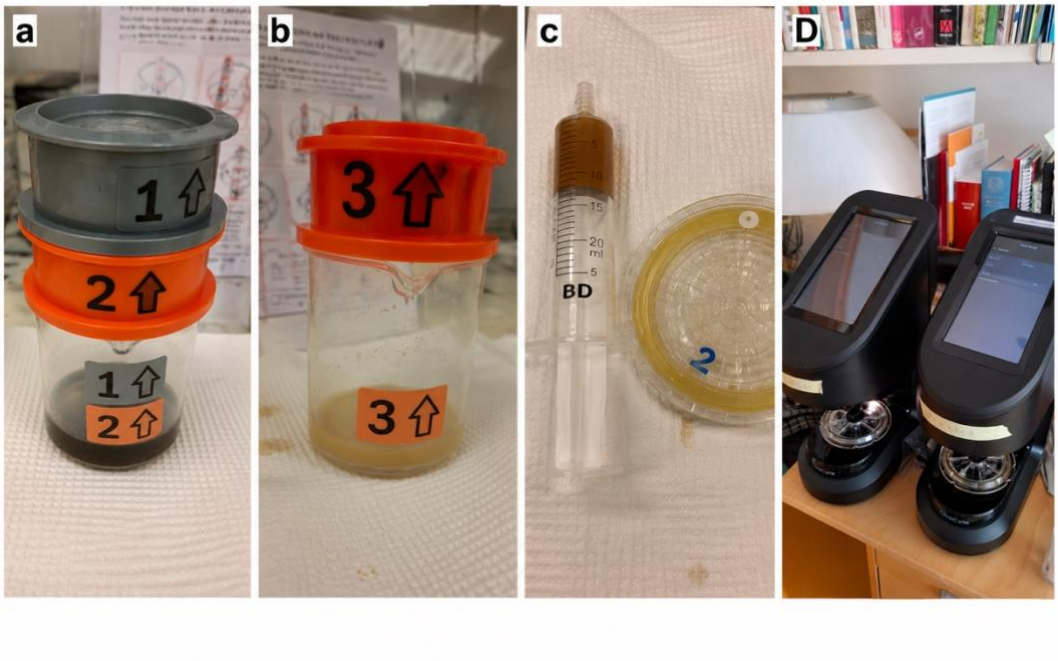

Figure 5. Sample preparetion for the OvaCyte Fluke Plus: a), b) filtration; c) filled cassette; d) analysing process [Suliman L,2026-01-23]

3.2.3 OvaCyte™ Analysis

OvaCyte™ analysis was performed on all samples within 1–8 days of collection, with analysis dates documented in the laboratory records (Table S3).

After the prepared sample filtrate is loaded into the OvaCyte cassette, the analysis is fully automated by the OvaCyte instrument and its artificial intelligence software (OvaCyte, 2025). According to Telenostic (2026), the system uses a "world-class image reference library" to provide reliable and accurate automated parasite identification. It performs a Faecal Egg Count (FEC) for a wide range of parasites, specifically including fluke eggs (*F.hepatica* and *Paraphistomum spp.*) when using the Fluke Plus protocol. The AI analyses digital images of the cassette chamber, identifying and counting parasite eggs based on their morphology. The results are then delivered directly to the device and web application" for seamless interpretation by the user, enabling rapid, targeted treatment decisions at the point of care (Telenostic, 2026).

3.3 Traditional Sedimentation

3.3.1 Traditional sedimentation Overview

Traditional sedimentation is a copromicroscopic technique used for the isolation and identification of high-density parasite eggs, particularly those of liver and rumen flukes. Unlike flotation methods, which use high-specific-gravity solutions to float lighter nematode eggs, sedimentation relies on gravity to allow denser eggs to sink to the bottom of a liquid medium, typically water (Rapsch et al., 2006).

The principle of this technique is that helminth eggs, especially those of trematodes (flukes), have a higher specific gravity than common flotation solutions. When a faecal suspension is left undisturbed, these dense eggs gradually settle to the bottom of the container, while lighter debris remains suspended or floats. The supernatant is then removed, and the sediment is examined microscopically for the presence of eggs (Charlier et al., 2008).

The advantages of this method are its simplicity and low cost, as it requires no specialised equipment or chemical flotation solutions (Centers for Disease Control and Prevention, 2019). It is effective for eggs that do not float well in standard flotation media and preserves egg morphology for accurate identification (Dryden, 2008). However, it is more time-consuming than flotation methods, requires examination of larger sediment volumes with more debris, and has lower sensitivity for low-intensity infections compared to centrifugation-based methods (Dryden et al., 2005; Nielsen, 2021).

3.3.2 Traditional sedimentation Procedure

Traditional sedimentation for detecting *F. hepatica* eggs (Figure 6) followed the protocol described by Rapsch et al. (2006) and Charlier et al. (2008), with modifications according to the standardised procedure at SLU (Appendix 2).

Sedimentation analysis was conducted over several sessions from January to April 2026 (Table S3).

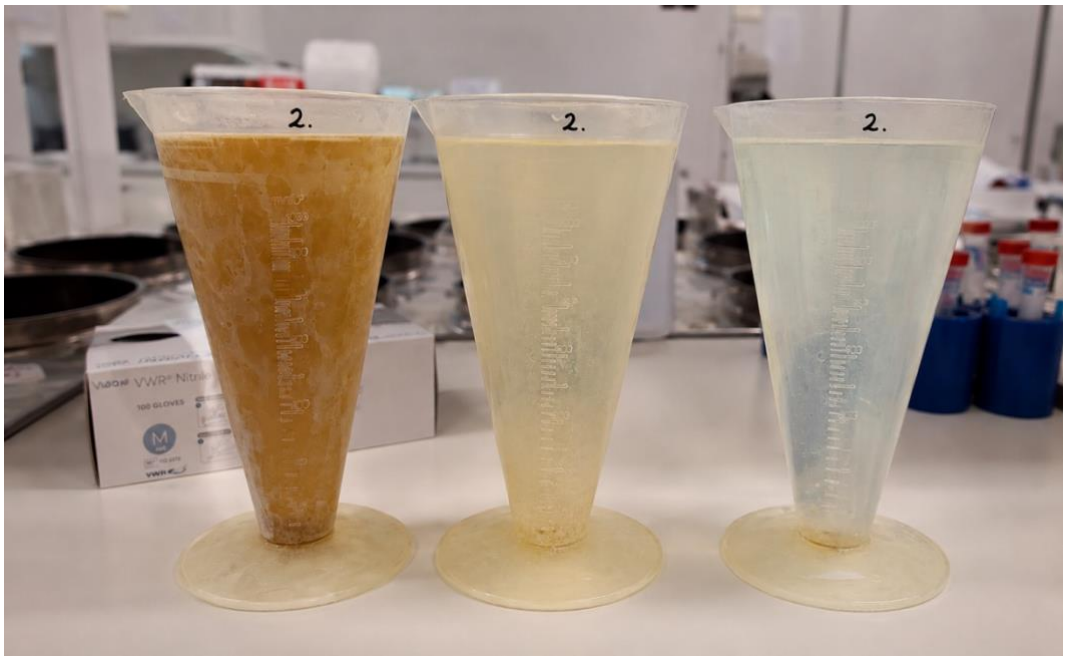

Figure 6. Traditional sedimentation for the detection of F. hepatica eggs, washing process [Suliman L,2026-01-26].

3.4 ELISA-coproantigen Test

All ELISA analyses were performed in April 2026 in a single batch for cattle and in 13 May 2026 for sheep samples.

3.4.1 Coproantigen-ELISA Principe

The coproantigen-ELISA was performed using the Bio-X Diagnostics *F. hepatica* Antigen ELISA kit (BIO K 201/2, Bio-X Diagnostics). This indirect sandwich ELISA (Figure S2) is designed to detect *F. hepatica* coproantigens in cattle and sheep faeces (Bio-X Diagnostics, 2025). The microplate wells are coated with a specific polyclonal antibody against *F. hepatica* (rows A, C, E, G) or with a non specific control antibody (rows B, D, F, H), (Bio-X Diagnostics, 2025). Coproantigens present in the faecal sample are captured by the specific antibody. After washing, a biotin-labelled monoclonal anti-*F. hepatica* antibody is added, followed by a peroxidase-labelled avidin conjugate. The enzyme converts the colourless tetramethylbenzidine substrate into a blue product, and the reaction is stopped with phosphoric acid, producing a yellow colour. The optical density is measured at 450 nm. The OD of the control wells is subtracted from the corresponding specific wells to correct for non-specific binding (Mezo et al., 2004; Bio-X Diagnostics, 2025).

3.4.2 Coproantigen-ELISA Procedure

The assay was performed according to the manufacturer's instructions (Bio-X Diagnostics, 2025) (Appendix 3).

3.4.3 Interpretation of Results

For each sample, the net optical density was calculated as:

Delta OD = OD (specific well) – OD (control well)

The same calculation was applied to the positive control. The sample-to-positive (S/P) ratio was then calculated as follows:

S/P (%) = (Delta OD sample / Delta OD positive) × 100

According to the manufacturer's quality control sheet (Bio-X Diagnostics, 2025), samples with an S/P ratio >8% are considered positive for *F. hepatica* coproantigens. Samples with an S/P ≤8% are considered negative. The same cut-off was applied to all samples.

3.5 Statistical Analysis

All statistical analyses were performed using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) and R version 4.2.1 (R Core Team, 2022). The primary objectives of the statistical analysis were: (1) to describe the detection rates of the three diagnostic methods, (2) to quantify the level of agreement between the methods, and (3) to visualise the comparative performance of each method.

Descriptive statistics were calculated for each diagnostic method, including the number and percentage of positive samples, total egg counts (for sedimentation and OvaCyte™), and the range of egg counts in positive samples. These statistics provide a straightforward summary of each method's performance in detection (Table S5).

To assess the level of agreement between the three diagnostic methods, Cohen's kappa coefficient (κ) was calculated for each pairwise comparison. Kappa measures the agreement between two raters (or two diagnostic tests) after correcting for agreement that would occur by chance alone. Values range from −1 (complete disagreement) to +1 (perfect agreement).

Kappa values were interpreted according to the scale proposed by Landis and Koch (1977) (Table S6).

For each pair of diagnostic methods, results were arranged in a 2 x 2 contingency table (MedCalc, 2025):

Table 1. Agreement Analysis (Cohen's Kappa).

	Test B Positive	**Test B Negative**
Test A Positive	a (both positive)	b (A only)
Test A Negative	c (b only)	d (both negative)

Cohen's kappa (κ) was calculated using the formula (Cohen, 1960):

$\kappa = (P_0 - P_e) / (1 - P_e)$, where:

- **P_0 = observed agreement = (a + d) / n**
- **P_e = chance agreement = [(a + b)(a + c) + (c + d)(b + d)] / n^2**

3.6 Ethical Consideration

This study did not involve any live animal procedures beyond routine veterinary practice. All samples were collected in accordance with Swedish animal welfare legislation and ethical guidelines for research.

All samples were anonymised, and animal identification numbers were used only for tracking during laboratory analysis. No personally identifiable information about animal owners or farm personnel was collected or stored. Data were stored securely on a password-protected university server with access restricted to the research team.

The study protocol was reviewed by the Swedish University of Agricultural Sciences, and formal ethical approval was unnecessary for this project, as confirmed in the signed project plan.

3.7 Risk assessment and Safety Measures

All faecal samples were handled in a Class 2 laboratory environment following standard biosafety level 2 procedures. Personal protective equipment was always worn, work surfaces were disinfected before and after use, and all biological waste was disposed of according to safety protocols. Supervisors ensured that safety routines were followed throughout the project.

4. Results

Table 2. Results for three methods for F. hepatica detection.

Sample location	OvaCyte™ Fluke Plus		Traditional Sedimentation		ELISA coproantigen	
	positive	negative	positive	negative	positive	negative
A	1	7	3	5	4	4
B	3	16	5	14	4	15
C	1	5	3	3	4	2
D	31	5	34	3	37	0
Total	37	33	45	25	49	21

AI-based copromicroscopy using the OvaCyte™ Fluke Plus system detected *F. hepatica* eggs in 37 of 70 samples (52.9%) and *Paramphistomum spp.* eggs in 16 of 70 samples (22,9%). Among cattle (n=47), OvaCyte™ detected *F. hepatica* in 14 samples (29.8%) and *Paramphistomum* in 2 samples (4.3%) (samples 37 and 47, one egg each, below detection limit). Among sheep (n=23), OvaCyte™ detected *F. hepatica* in all 23 samples (100%) and *Paramphistomum* in 14 samples (60.9%). Sample preparation following the manufacturer's Fluke Sample Preparation protocol required approximately 10–15 minutes per sample, including weighing, mixing, filtration, tapping, and addition of flotation fluid. Automatic scanning required an additional 2–3 minutes per sample.

Traditional sedimentation detected *F. hepatica* eggs in 45 of 70 samples (64.3%) and *Paramphistomum spp.* eggs in 17 of 70 samples (24,2%). Among cattle (n=47), sedimentation detected *F. hepatica* in 22 samples (46.8%) and *Paramphistomum* in 1 sample (2.1%) (sample 2, one egg). Among sheep (n=23), sedimentation detected *F. hepatica* in all 23 samples (100%) and *Paramphistomum spp*. in 16 samples (69,5%). The total work time for microscopy was approximately 11–16 minutes per sample, with an additional 45 minutes of waiting time for sedimentation per sample.

Coproantigen-ELISA detected *F. hepatica* antigens in 49 of 70 samples (70.0%). Among cattle (n=47), 26 samples (55.3%) were positive, with a mean S/P ratio of 25.9% in Gällersta, 23.0% in Bredaryd, 20.3% in Dahlbergs, and 67.8% in Varekils cattle. Among sheep (n=23), all 23 samples (100%) were positive, with a mean S/P ratio of 86.4% (range 63.5–108.9%).

The highest positivity rates were observed in Varekils slaughterhouse cattle (14/14, 100%) and Varekils sheep (23/23, 100%), while the lowest was in Bredaryd farm (4/19, 21.1%).

The total active work time for ELISA was approximately 6 hours for all 70 samples (including sample preparation, incubations, washes, and plate reading), making it efficient for batch processing compared to egg-detection methods (Bio-X Diagnostics, 2025).

ELISA is not designed to detect *Paramphistomum* and therefore gave no results for this parasite (Bio-X Diagnostics, 2025; Kajugu et al., 2015).

Hypothesis 1 was accepted, as ELISA demonstrated the highest overall detection rate (70.0%) and outperformed both egg-detection methods, particularly in cattle where it detected 55.3% of infections compared to 46.8% by sedimentation and 29.8% by OvaCyte™. Hypothesis 2 was partially rejected: in cattle, OvaCyte™ was inferior to sedimentation, but in sheep it performed equally well, and its speed advantages were confirmed.

4.1 AI-based copromicroscopy OvaCyte™ Fluke Plus results

Table 3. AI-OvaCyte™ Fluke Plus System Results.

Location	**Fasciola hepatica**	
	Positive	**Negative**
A (1-8 cattle)	1	7
B (9-27 cattle)	3	16
C (28-33 cattle)	1	5
D (34-47 cattle)	9	5
D (48-70 sheep)	23	0
TOTAL samples	**37**	**33**

***** *Paramphistomum spp. eggs were detected in two cattle samples (samples 37 and 47) with one egg each (both below the detection limit) and in 14 sheep samples (47 eggs); 16 positive samples total.*

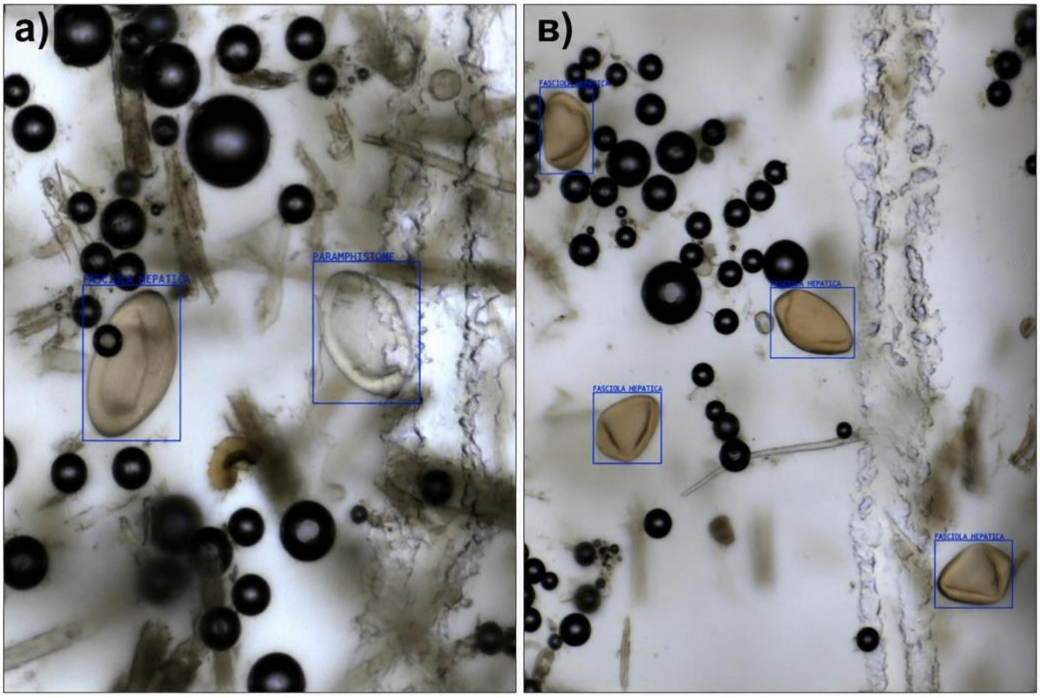

Figure 7. Detection by OvaCyte™ Fluke Plus in a:) F. hepatica and Paramphistomum spp. eggs in cattle [OvaCyte, 2026-04-01] and b) F. hepatica eggs in sheep [OvaCyte, 2026-04-20].

4.2 Traditional Sedimentation results

Table 4. Traditional sedimentation results.

Location	**Fasciola hepatica**	
	Positive	**Negative**
A (1-8 cattle)	3	5
B (9-27 cattle)	5	14
C (28-33 cattle)	3	3
D (34-47 cattle)	11	3
D (48-70 sheep)	23	0
TOTAL samples	**45**	**25**

* *Paramphistomum spp.* eggs were detected in one cattle sample (sample 2, one egg) and in 16 sheep samples (35 eggs); 17 positive samples total.

Figure 8. Fasciola hepatica egg detected by traditional sedimentation [Suliman L., 2026-04-21]

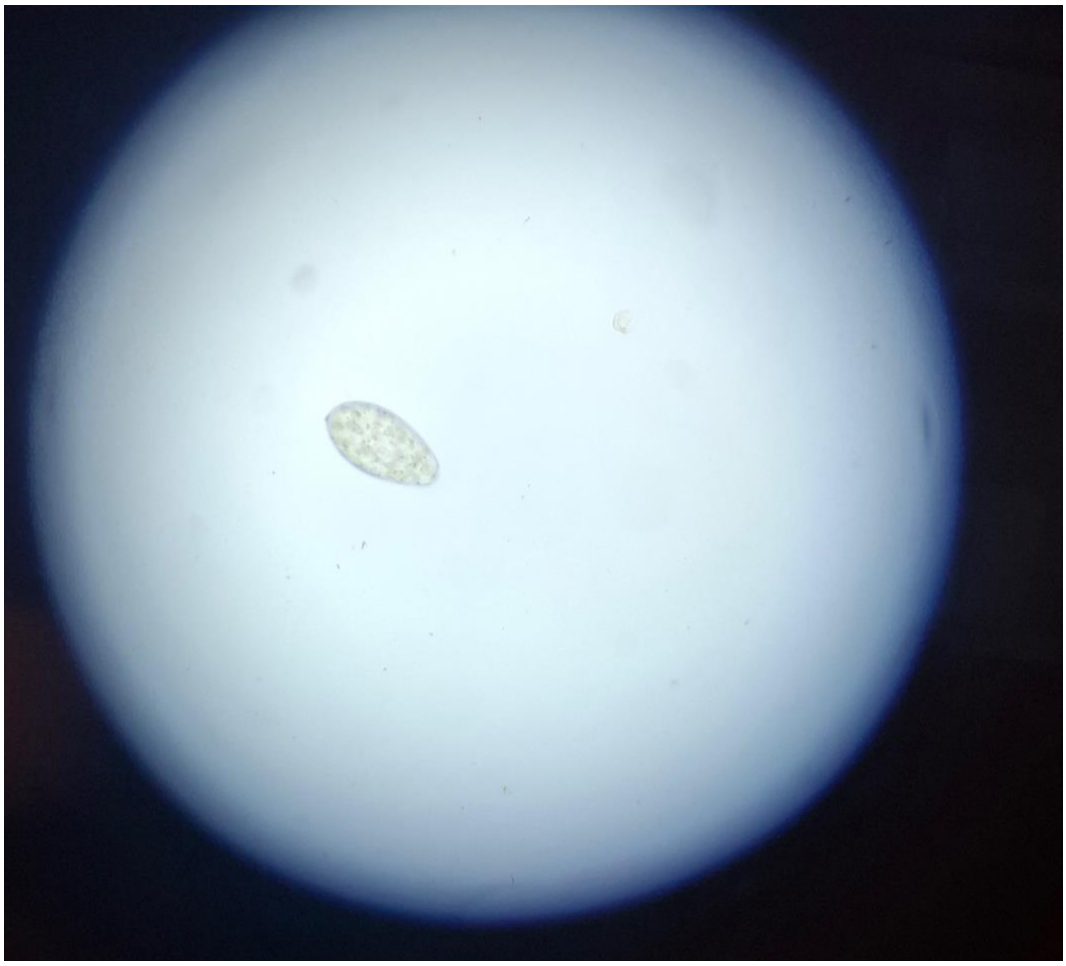

Figure 9. Paramphistomum spp. egg detected by traditional sedimentation [Suliman L., 2026-01-26].

4.3 ELISA-coproantigen results

Table 5. ELISA - coproantigen results.

Location	**Fh Positive**	**Fh Negative**	***Positivity rate (%)***	***Mean S/P ratio (%)***	***Range of S/P ratio (%)***
A (1-8 cattle)	4	4	50	25,9	15.4 – 46.7
B (9-27cattle)	4	15	21,1	23	9.8 – 54.6
C (28-33 cattle)	4	2	66,7	20,3	8.2 – 53.8
D (34-47 cattle)	14	0	100	67,8	27.8 – 94.6
D (48-70 sheep)	23	0	100	86,4	63.5 – 108.9
TOTAL	**49**	**21**	**70**	**65,5**	**8.2 – 108.9**

**Note: S/P ratio = (Delta OD sample / Delta OD positive) × 100. Cut-off for positivity: S/P >8% (Bio-X Diagnostics, 2025).*

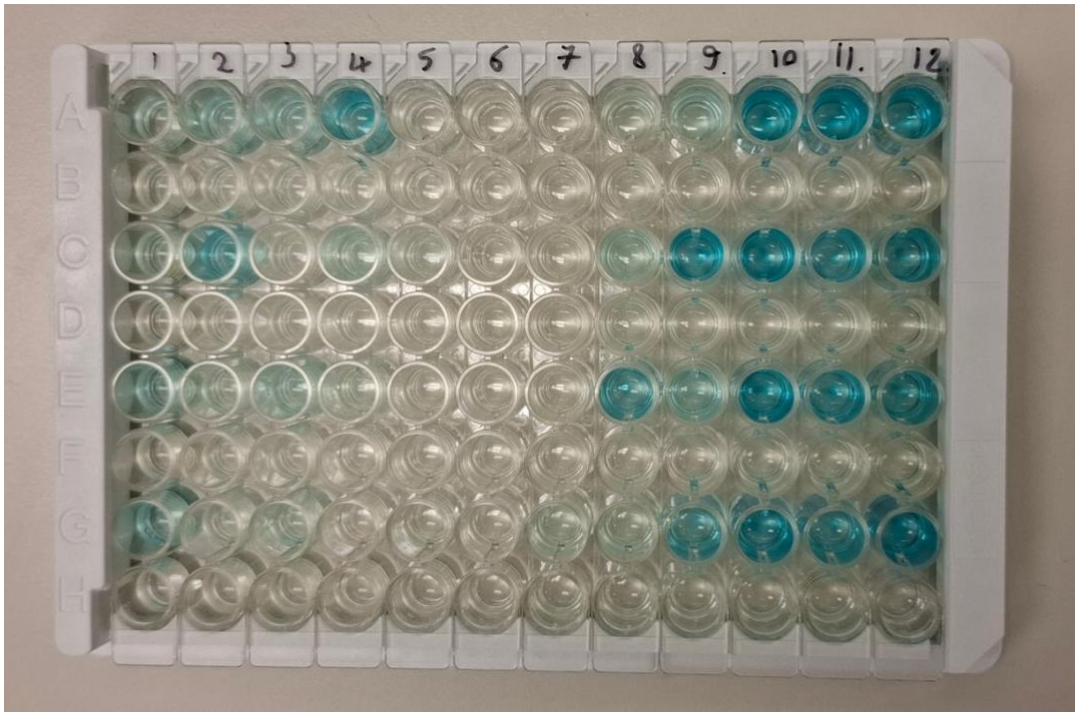

Figure 10. Presence of F. hepatica coproantigens (blue colour) in cattle faeces. [Suliman L., 2026-04-15].

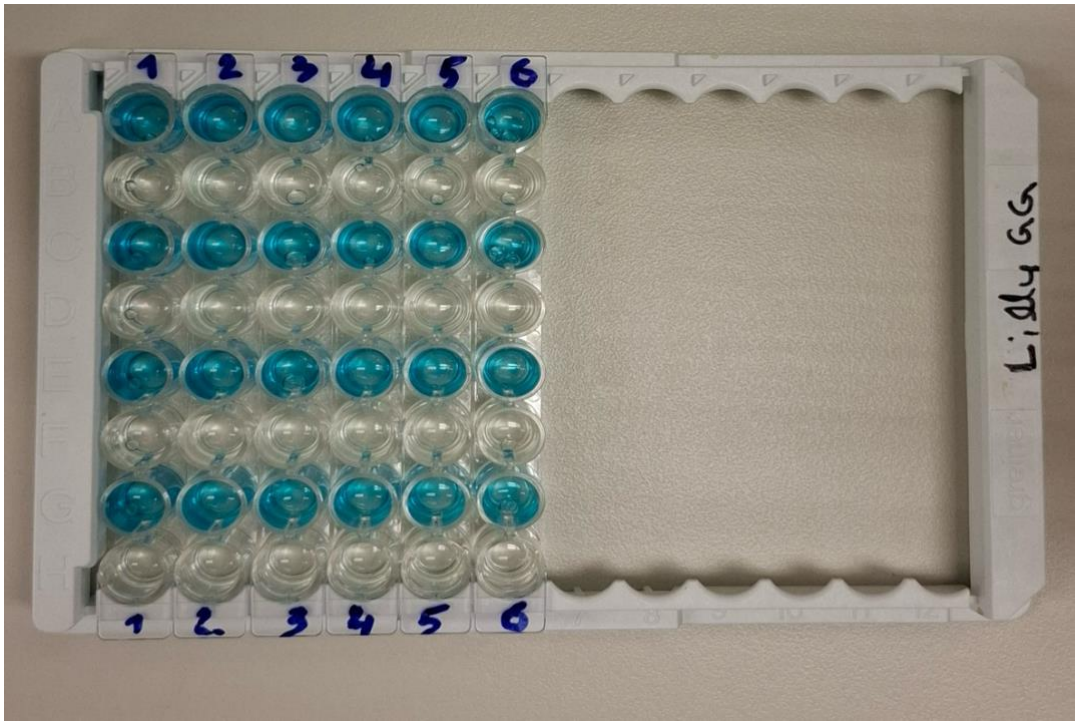

Figure 11. Presence of F. hepatica coproantigens (blue colour) in sheep faeces. [Suliman L., 2026-05-13].

4.4 Statistical Results

All statistical analyses were performed using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) and R version 4.2.1 (R Core Team, 2022). The primary objectives of the statistical analysis were: (1) to describe the detection rates of the three diagnostic methods (Figure 12), (2) to quantify the level of agreement between the methods (Figure 14; Table S7), and (3) to visualise the comparative performance of each method.

Descriptive statistics were calculated for each diagnostic method, including the number and percentage of positive samples, total egg counts (for sedimentation and OvaCyte™), and the range of egg counts in positive samples (Figure 13). These statistics provide a straightforward summary of each method's performance in detection (Table S8).

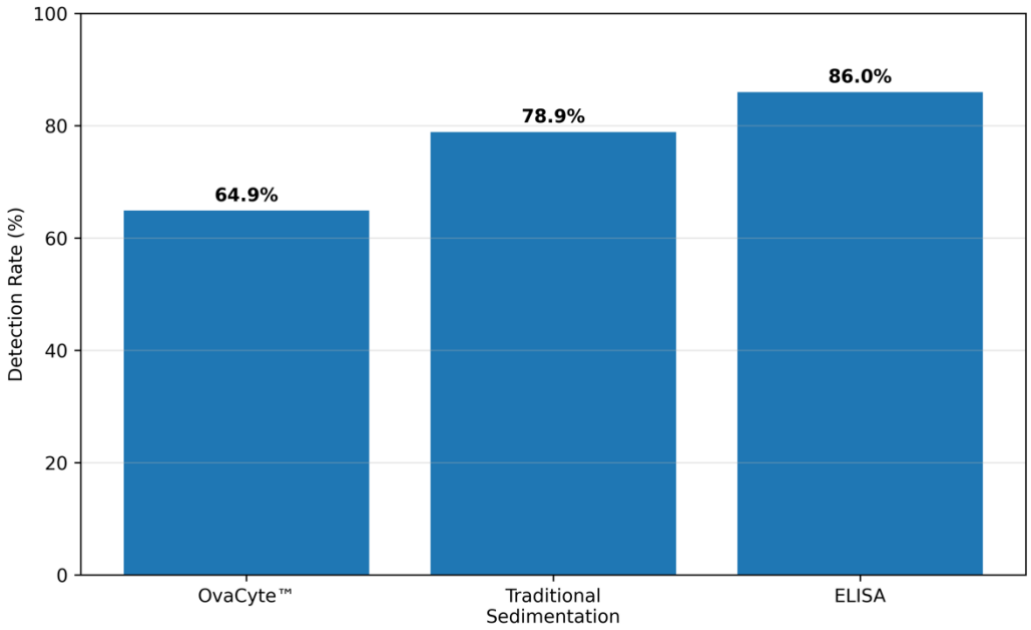

Figure 12. Detection rates of the three diagnostic methods among animals with confirmed or strongly suspected infection (R Core Team, 2022). https://www.R-project.org/ [2026-05-18].

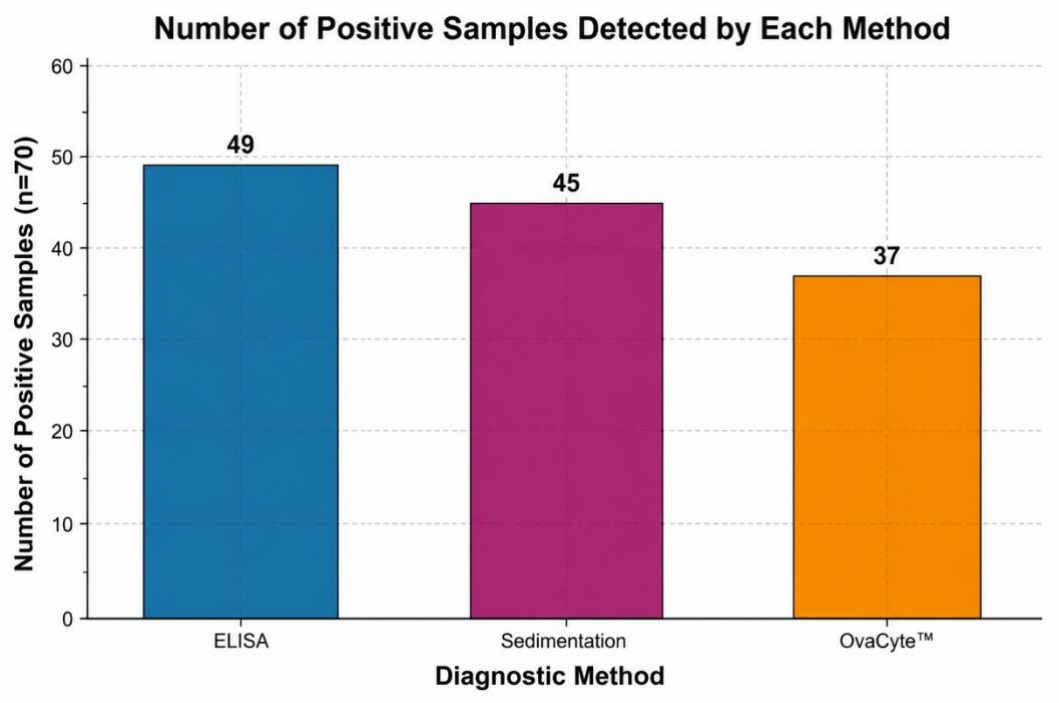

Figure 13. The range of egg counts in positive samples (R Core Team, 2022). https://www.R-project.org/ [2026-05-18].

Cohen's kappa was selected for the following reasons:

1. Binary outcome data – Each diagnostic test result is binary (positive/negative). Kappa is specifically designed for categorical (nominal) data (Cohen, 1960).
2. Correction for chance agreement – Simple percent agreement can be misleading when tests frequently agree on negative results. Kappa removes the component of agreement expected by chance, providing a more realistic measure of true agreement (Landis and Koch, 1977).
3. No gold standard required – Kappa measures agreement between tests without assuming one test is perfect, which is ideal when no true reference standard is available for all samples.
4. Moderate prevalence – The prevalence of *F. hepatica* infection ranged from 52.9% (OvaCyte™) to 70.0% (ELISA). Kappa performs reliably when prevalence is neither extremely high nor extremely low (Gwet, 2014).
5. Comparability with literature – Most comparative diagnostic studies in veterinary parasitology report kappa values, allowing direct comparison with previous work (Charlier et al., 2008; Mazeri et al., 2016).

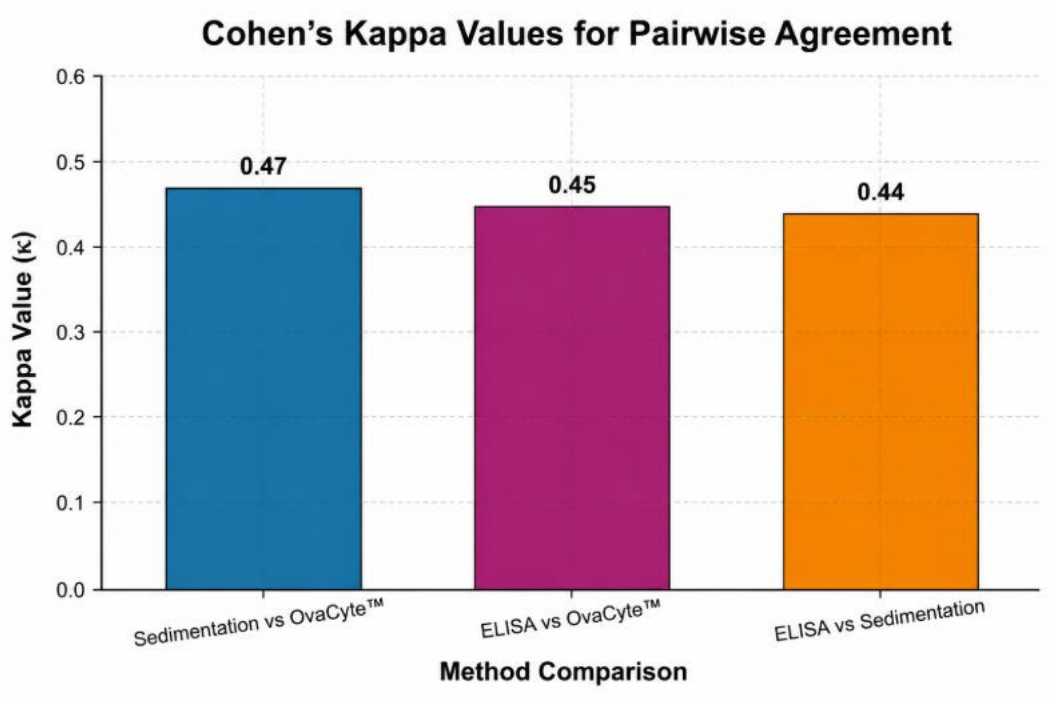

Figure 14. Level of agreement between the methods (R Core Team, 2022). https://www.R-project.org/ [2026-05-18].

All three pairwise comparisons showed *moderate agreement* (κ = 0.44–0.47), indicating that while the methods are correlated, they are not interchangeable. The highest agreement was observed between sedimentation and OvaCyte™ (κ = 0.47), which is expected as both are egg-detection methods based on similar biological principles (detection of *F. hepatica* eggs in faeces). The slightly lower agreement between ELISA and the two egg-detection methods (κ = 0.44–0.45) reflects the fundamental difference in what each method measures: ELISA detects parasite antigens (present even during pre-patent infections), whereas sedimentation and OvaCyte™ detect eggs (only present during patent infections).

4.5 Comparison of all results

OvaCyte™ detected *F. hepatica* in 52.9% of all samples and *Paramphistomum* in 22.9%. Traditional sedimentation detected *F. hepatica* in 64.3% and *Paramphistomum* in 24.3%. ELISA detected *F. hepatica* in 70.0% of all samples and is not applicable for *Paramphistomum*. In cattle, detection rates were 29.8% (OvaCyte™), 46.8% (sedimentation), and 55.3% (ELISA). In sheep, all three methods achieved 100% detection of *F. hepatica*. All methods performed perfectly in sheep, but sensitivity differed substantially in cattle. ELISA had the highest *F. hepatica* detection, while microscopy methods detected both parasites.

5. Discussion

This study compared three diagnostic methods for detecting *F. hepatica* in Swedish cattle and sheep: traditional sedimentation, AI-based copromicroscopy (OvaCyte™), and coproantigen ELISA (Bio-X). Each method showed distinct strengths and limitations depending on host species, infection intensity, and diagnostic purpose. Overall, ELISA detected the highest proportion of positive samples and was particularly effective compared to sedimentation and OvaCyte™ in cattle, while all three methods performed equally well (100%) in sheep. OvaCyte™ offered important advantages in automation and simultaneous detection of rumen flukes. Traditional sedimentation remained a practical and inexpensive confirmatory method, despite lower sensitivity and greater labour requirements..

5.1 Comparison between AI-based, Traditional sedimentation and ELISA test

5.1.1 Methodological consideration affecting OvaCyte™

OvaCyte™ detected 37 of 70 positive samples overall, with performance strongly influenced by host species and infection intensity. In sheep, the system detected all positive samples in this study, matching ELISA and sedimentation, and recovered the highest total number of *F. hepatica* eggs. This indicates excellent performance at moderate to high infection burdens. OvaCyte™ also uniquely enabled simultaneous detection of *Paramphistomum* spp., identifying 16 positive samples.

In cattle, however, OvaCyte™ detected fewer positive samples than sedimentation. Most missed samples had extremely low egg counts, suggesting reduced sensitivity at low infection intensities. This may reflect sampling error, as only a small proportion of the processed faecal material is ultimately analysed, increasing the probability that eggs are absent from the final aliquot (Mes, 2003). Multiple preparation steps may also contribute to mechanical egg loss during filtration and transfer (Rapsch et al., 2006; Barda et al., 2013). In addition, the AI algorithm was likely trained primarily on samples with clearly visible eggs, which may reduce sensitivity at very low egg counts.

Despite lower sensitivity in low-burden cattle infections, OvaCyte™ demonstrated several important strengths. Automation eliminated inter-observer variability, reduced passive processing time, and enabled simultaneous detection of

F. hepatica and *Paramphistomum* spp. without additional labour, making the system attractive for high-throughput laboratories and on-farm applications.

5.1.2 Methodological consideration affecting Sedimentation

Traditional sedimentation detected the second-highest number of positive samples and identified *Paramphistomum* spp. infections. This method remains advantageous as it is inexpensive, requires minimal equipment, and provides quantitative egg counts with high specificity.

However, sedimentation is labour-intensive and time-consuming, requiring multiple washing and decanting steps that may lead to egg loss and operator-dependent variation (Rapsch et al., 2006). The method also involves substantial passive waiting time (~ 45 minutes) in addition to 11-16 minutes of active work per sample, making it less suitable for high-throughput screening (Charlier et al., 2008). Furthermore, negative results cannot exclude infection, as low egg counts and intermittent shedding reduce sensitivity, particularly in cattle.

5.1.3 Methodological consideration affecting ELISA

ELISA differs fundamentally from the sedimentation and the AI-assisted method because it detects coproantigens rather than eggs. This offers several important advantages. First, ELISA enables prepatent detection, identifying infections as early as 4–5 weeks post-infection, before eggs appear in faeces (Charlier et al., 2008). All samples were collected during winter and early spring, several months after the end of the grazing season. Therefore, pre-patent infections – which occur within the first 8–10 weeks after infection – are unlikely to explain the ELISA-positive / egg-negative results observed in this study. Instead, these discordant results more likely reflect low-intensity chronic infections where egg counts were below the reliable detection threshold of the egg-detection methods (Rapsch et al., 2006). Second, ELISA is less affected by intermittent egg shedding because antigen excretion is generally more consistent than egg output, reducing the risk of false-negative results (Mezo et al., 2004). Third, ELISA provides objective results through spectrophotometric measurement, eliminating inter-observer variability. Finally, it was the most time-efficient method, requiring approximately six hours of active work for all 70 samples.

However, ELISA also has limitations. It requires laboratory infrastructure, including a plate reader, centrifuge, and pipettes, and has a higher cost per sample

than sedimentation. Unlike egg-based methods, ELISA does not provide quantitative egg counts and cannot assess infection intensity. In addition, it cannot detect *Paramphistomum* spp., as the antibodies are specific to *F. hepatica* (Bio-X Diagnostics, 2025; Kajugu et al., 2015).

5.2 Comparison with Previous Studies

The findings of this study are broadly consistent with previous studies comparing diagnostic methods for *F. hepatica* in ruminants. As previously reported, coproantigen ELISA detected more positive samples than sedimentation, particularly in cattle (Collyer, 2024; Dowling et al., 2024). Sedimentation sensitivity has also been shown to vary substantially depending on methodology and infection intensity (Godinho et al., 2025).

The low egg counts observed in many cattle samples likely contributed to the reduced performance of both egg-detection methods. Most positive cattle samples contained only 1–2 eggs per 5 g of faeces, which is close to the detection threshold of many coprological techniques (Rapsch et al., 2006). This explains why some cattle with visible liver flukes at slaughter tested negative by sedimentation and OvaCyte™ but positive by ELISA. In contrast, sheep samples contained substantially higher egg counts, allowing reliable detection by both egg-based methods.

Published data on AI-based copromicroscopy for *F. hepatica* remain limited. Previous studies have mainly focused on gastrointestinal nematodes or companion animal parasites rather than liver fluke in cattle (Nagamori et al., 2020; Elghryani et al., 2025). This study therefore provides one of the first evaluations of OvaCyte™ for naturally infected cattle and sheep, demonstrating strong performance in sheep but lower sensitivity in low-burden cattle infections.

5.3 Implication of Veterinary Practice

No single diagnostic method is optimal for all situations; method selection should depend on clinical purpose, available resources, and expected infection intensity (Charlier et al., 2008; Rapsch et al., 2006). ELISA is the most suitable method especially for early detection, particularly in cattle, due to its high detection rate. Sedimentation remains valuable as a low-cost confirmatory method with high specificity, especially where laboratory infrastructure is limited.

OvaCyte™ offers important advantages for automated, high-throughput diagnostics through standardised processing, elimination of inter-observer

variability, and simultaneous detection of *F. hepatica* and *Paramphistomum spp.* However, its lower sensitivity in low-burden cattle infections means that negative cattle results may require confirmation using another method.

For veterinary practice in Sweden, ELISA appears most appropriate for routine herd screening, while sedimentation remains useful for confirming infection in individual animals. OvaCyte™ may be particularly valuable in sheep and in settings where there is no staff trained in parasitology or directly on-farm. Because low egg counts and intermittent shedding can reduce the sensitivity of egg-based methods, combining ELISA with sedimentation or OvaCyte™ may provide the most reliable diagnosis in clinically important cases.

5.4 Study Limitations

This study has several limitations that should be considered when interpreting the results: i) the samples from the Bredaryd farm had no gold standard (visual liver inspection), ii) no negative sheep faecal samples were included and iii) the reference standard relied on routine visual liver inspection at slaughter rather than systematic dissection, so true infection status may have been misclassified.

Information on animal age and production type (e.g., dairy vs. beef) was not available for most samples. While these factors may influence infection intensity and egg shedding patterns, they were not the focus of this diagnostic method comparison study. Future studies could investigate whether production type or age affects test performance.

Differences in infection egg-excretion intensity between cattle and sheep also complicated comparisons between methods, as sheep generally had much higher egg counts than cattle. Furthermore, the diagnostic methods differed substantially in principle and sample preparation, which may partly explain differences in performance. Both egg-detection methods were strongly affected by low egg counts. In contrast, ELISA is validated for both cattle and sheep but requires more advanced laboratory equipment, samples must be provessed in batch and this method can only detect *F. hepatica* but not rumen flukes.

Additional limitations include the lack of a gold standard for *Paramphistomum* detection, sampling from a limited number of Swedish farms and slaughterhouses, and collection of samples at a single time point, preventing assessment of intermittent egg shedding and temporal variation. Although efforts were made to minimise observer bias, complete blinding was not possible for all samples.

6. Conclusion

Sheep exhibited substantially higher egg counts and detection rates than cattle, consistent with their known greater susceptibility to *F. hepatica* infection. Consequently, OvaCyte™ performed particularly well in sheep, whereas ELISA remained the most effective method for routine cattle screening.

Paramphistomum infections were common in sheep but rare in cattle, and OvaCyte™ offered the additional advantage of automated dual-parasite detection without extra labour. Regarding efficiency, ELISA required the least active work time, OvaCyte™ enabled efficient automated processing. Sedimentation was the most time-consuming method.

Overall, the study demonstrates that diagnostic choice should depend on host species, clinical purpose, and available resources. For maximum diagnostic reliability, particularly where missing infections may have important clinical or economic consequences, combining multiple diagnostic methods is recommended. Future optimisation of AI-based diagnostic systems may further improve sensitivity in low-burden infections and strengthen their role in automated parasite surveillance in livestock production.

References

Aftab, A., Raina, O. K., Maxton, A., & Masih, S. A. (2024). Advances in diagnostic approaches to *Fasciola* infection in animals and humans: An overviews. *Journal of helminthology*, *98*, e12. https://doi.org/10.1017/S0022149X23000950

Agbajelola, V. I., & Agbajelola, B. S. (2025). A Systematic Review and Meta-analysis on the Prevalence of Bovine Fascioliasis in Nigeria. *Acta parasitologica*, *70*(1), 57. https://doi.org/10.1007/s11686-025-00991-7

Andrews, S.J. (1999) The Life Cycle of F. hepatica. 3rd Edition, CABI Publishing, Wallingford, 1-30.

Andrews, S.J. (1999) The life cycle of Fasciola hepatica, in Dalton, J.P. (ed.) *Fasciolosis*. Wallingford: CAB International, pp. 1-29.

Barda, B. D., Rinaldi, L., Ianniello, D., Zepherine, H., Salvo, F., Sadutshang, T., Cringoli, G., Clementi, M., & Albonico, M. (2013). Mini-FLOTAC, an innovative direct diagnostic technique for intestinal parasitic infections: experience from the field. *PLoS neglected tropical diseases*, *7*(8), e2344. https://doi.org/10.1371/journal.pntd.0002344

Behm, C.A., Sangster, N.C., (1999). Pathology, pathophysiology and clinical aspects. In: Dalton, J.P. (Ed.), Fasciolosis. CAB International Publishing, Wallingford, pp. 185–224.

Bennett, R. and IJpelaar, J. (2005), Updated Estimates of the Costs Associated with Thirty Four Endemic Livestock Diseases in Great Britain: A Note. Journal of Agricultural Economics, 56: 135-144. https://doi.org/10.1111/j.1477-9552.2005.tb00126.x

Bio-X Diagnostics (2025) *Fasciola hepatica ELISA kit for antigenic diagnosis: Indirect Sandwich test for faeces*. BIO K 201/2 instruction manual. Rochefort, Belgium: Bio-X Diagnostics.

Boray J. C. (1969). Experimental fascioliasis in Australia. *Advances in parasitology*, *7*, 95–210. https://doi.org/10.1016/s0065-308x(08)60435-2

Bossuyt, P. M., Reitsma, J. B., Bruns, D. E., Gatsonis, C. A., Glasziou, P. P., Irwig, L., Lijmer, J. G., Moher, D., Rennie, D., de Vet, H. C., Kressel, H. Y., Rifai, N., Golub, R. M., Altman, D. G., Hooft, L., Korevaar, D. A., Cohen, J. F., & STARD Group (2015). STARD 2015: an updated list of essential items for reporting diagnostic accuracy studies. *BMJ (Clinical research ed.)*, *351*, h5527. https://doi.org/10.1136/bmj.h5527

Beesley, N. J., Caminade, C., Charlier, J., Flynn, R. J., Hodgkinson, J. E., Martinez-Moreno, A., Martinez-Valladares, M., Perez, J., Rinaldi, L., & Williams, D. J. L. (2018). Fasciola and fasciolosis in ruminants in Europe: Identifying research needs. *Transboundary and emerging diseases*, *65 Suppl 1*(Suppl 1), 199–216. https://doi.org/10.1111/tbed.12682

Capuozzo, Salvatore & Maurelli, Maria & Marrone, Stefano & Celano, Biase & Martone, Giuseppe & Vitiello, Paola & Hammami, Ines & Bosco, Antonio & Ciuca, Lavinia

& Cringoli, Giuseppe & Sansone, Carlo & Rinaldi, Laura. (2025). A Dedicated Deep Learning Workflow for Automatic Fasciola hepatica and Calicophoron daubneyi Egg Detection using the Kubic FLOTAC Microscope. International Journal for Parasitology. 56. https://doi.org/10.1016/j.ijpara.2025.08.007

Caravedo, M. A., & Cabada, M. M. (2020). Human Fascioliasis: Current Epidemiological Status and Strategies for Diagnosis, Treatment, and Control. *Research and reports in tropical medicine*, *11*, 149–158. https://doi.org/10.2147/RRTM.S237461

Centers for Disease Control and Prevention (CDC) (2019). *Stool Specimens – Specimen Processing: Concentration Procedures (Sedimentation Techniques)*. Available at: https://www.cdc.gov/dpdx/diagnosticprocedures/stool/specimenproc.html [Accessed: 2026-04-07]

Charlier, J., De Meulemeester, L., Claerebout, E., Williams, D., & Vercruysse, J. (2008). Qualitative and quantitative evaluation of coprological and serological techniques for the diagnosis of fasciolosis in cattle. *Veterinary parasitology*, *153*(1-2), 44–51. https://doi.org/10.1016/j.vetpar.2008.01.035

Charlier, J., De Cat, A., Forbes, A., & Vercruysse, J. (2009). Measurement of antibodies to gastrointestinal nematodes and liver fluke in meat juice of beef cattle and associations with carcass parameters. *Veterinary parasitology*, *166*(3-4), 235–240. https://doi.org/10.1016/j.vetpar.2009.09.040

Charlier, J., Vercruysse, J., Morgan, E.R., Dijk, J.V., & Williams, D. (2013). Recent advances in the diagnosis, impact on production and prediction of Fasciola hepatica in cattle. *Parasitology, 141*, 326 - 335.

Charlier, J., Vercruysse, J., Morgan, E., van Dijk, J., & Williams, D. J. (2014). Recent advances in the diagnosis, impact on production and prediction of Fasciola hepatica in cattle. *Parasitology*, *141*(3), 326–335. https://doi.org/10.1017/S0031182013001662

Charlier, J., Rinaldi, L., Musella, V., Ploeger, H. W., Chartier, C., Vineer, H. R., Hinney, B., von Samson-Himmelstjerna, G., Băcescu, B., Mickiewicz, M., Mateus, T. L., Martinez-Valladares, M., Quealy, S., Azaizeh, H., Sekovska, B., Akkari, H., Petkevicius, S., Hektoen, L., Höglund, J., Morgan, E. R., … Claerebout, E. (2020). Initial assessment of the economic burden of major parasitic helminth infections to the ruminant livestock industry in Europe. *Preventive veterinary medicine*, *182*, 105103. https://doi.org/10.1016/j.prevetmed.2020.105103

Cohen, J. (1960) A Coefficient of Agreement for Nominal Scales. Educational and Psychological Measurement, 20, 37-46. http://dx.doi.org/10.1177/001316446002000104

Collyer J. (2024). *Fasciola hepatica*: can the coproantigen ELISA replace the faecal egg sedimentation test?. *Veterinary evidence*, *9*(4), vetevid-09-4-698. https://doi.org/10.18849/ve.v9i4.698

Cueva-Rodríguez, M., Torrel, T., Hobán, C., Alvarez-García, W., Mejía, F., & Vargas-Rocha, L. (2024). Prevalence of Fasciola hepatica and Calicophoron spp. in

extensively reared cattle in the Florida district (Amazonas), Peru. *Revista Mexicana De Ciencias Pecuarias*, *15*(2), 376-392. https://doi.org/10.22319/rmcp.v15i2.6496

Dalton, J.P. (ed.) (2021) *Fasciolosis*. 2nd edn. Wallingford: CAB International.

Dowling, A., Laven, R.A., Lawrence, K.E. and Howell, A. (2024) 'The use of a Bayesian latent class model to estimate the test characteristics of three liver fluke diagnostic tests under New Zealand field conditions', *Veterinary Parasitology*, 332, p. 110305. http://doi.org/10.1016/j.vetpar.2024.110305

Dryden, M.W., (2008). Clinical diagnostic parasitology: The inefficiency of common techniques (Proceedings). *dvm360*. Available at: https://www.dvm360.com/view/clinical-diagnostic-parasitology-inefficiency-common-techniques-proceedings [Accessed: 2026-04-07]

Dryden, M. W., Payne, P. A., Ridley, R., & Smith, V. (2005). Comparison of common fecal flotation techniques for the recovery of parasite eggs and oocysts. *Veterinary therapeutics : research in applied veterinary medicine*, *6*(1), 15–28.

Elghryani, N., Lahan, G., Bor Gohain, J., Collins, E., McOwan, T. and de Waal, T. (2026) 'Evaluation of OvaCyte for the detection of gastrointestinal parasites in ovine and bovine animals: comparison with traditional flotation techniques', *Parasite*, 33. https://doi.org/10.1051/parasite/2026002

Felländer, M., Grandi, G., Juremalm, M., Ljungström, B., Gustafsson, K., & Höglund, J. (2026). *Three ways to find a fluke: Evaluating diagnostic methods for Fasciola hepatica surveillance in Swedish sheep*. *Veterinary Parasitology: Regional Studies and Reports, 70*, 101469. https://doi.org/10.1016/j.vprsr.2026.101469

Fenemore, C., Floyd, T., & Mitchell, S. (2021). Rumen Fluke in Great Britain. *Journal of comparative pathology*, *184*, 31–36. https://doi.org/10.1016/j.jcpa.2021.01.012

Fox, N. J., White, P. C., McClean, C. J., Marion, G., Evans, A., & Hutchings, M. R. (2011). Predicting impacts of climate change on Fasciola hepatica risk. *PloS one*, *6*(1), e16126. https://doi.org/10.1371/journal.pone.0016126

Godinho, N.M., Spíndola, C.Z., Lavina, M.S., Chryssafidis, A.L. and de Moura, A.B. (2025). Contribution to an efficient diagnosis of Fasciola hepatica infection in naturally infected cattle', *Veterinary Parasitology: Regional Studies and Reports*, 47, p. 101456. https://doi.org/10.1016/j.vprsr.2025.101301

Gordon, D. K., Roberts, L. C., Lean, N., Zadoks, R. N., Sargison, N. D., & Skuce, P. J. (2013). Identification of the rumen fluke, Calicophoron daubneyi, in GB livestock: possible implications for liver fluke diagnosis. *Veterinary parasitology*, *195*(1-2), 65–71. https://doi.org/10.1016/j.vetpar.2013.01.014

Gorokhov, V.V., Kuchin, A.V., Sokolova, O.V. (2019) 'Epidemiology and economic impact of fasciolosis in the Russian Federation', Russian Journal of Parasitology, 13(4), pp. 56-64.

Gorokhov, V. V., Molchanov, I. A., Maĭsheva-Kolesnikova, M. A., & Gorokhova, E. V. (2011). *Meditsinskaia parazitologiia i parazitarnye bolezni*, (3), 55–59.

Gwet, K.L. (2014) Handbook of Inter-Rater Reliability: The Definitive Guide to Measuring the Extent of Agreement among Raters. Advanced Analytics, LLC.

Gård & Djurhälsan (2025) FarmStat-rapporten 2024. Skara: Gård & Djurhälsan Sverige AB.Available at: https://www.gardochdjurhalsan.se/wp-content/uploads/2024/01/9140-FarmStat-rapporten-2024_SLUTVERSION-webb.pdf [Accessed: 2026-06-07]

Holmström, O., Linder, N., Ngasala, B., Mårtensson, A., Linder, E., Lundin, M., Moilanen, H., Suutala, A., Diwan, V., & Lundin, J. (2017). Point-of-care mobile digital microscopy and deep learning for the detection of soil-transmitted helminths and Schistosoma haematobium. *Global health action*, *10*(sup3), 1337325. https://doi.org/10.1080/16549716.2017.1337325

Hoseini, F. N., Mohammadi, M., Pirestani, M., Aligolzadeh, A., Modarresnia, L., Pal, M., Zeinali, A., Eslahi, A. V., & Badri, M. (2025). Helminth infections in slaughtered livestock of Qazvin Province, Iran: implications for food safety and public health. *Irish veterinary journal*, *79*(1), 8. https://doi.org/10.1186/s13620-025-00325-z

Ismail, A., Abdelsalam, M. A., Shahin, M. H., Ahmed, Y., Bahcecioglu, I. H., Yalniz, M., & Tawheed, A. (2025). Hepatobiliary fascioliasis: A neglected re-emerging threat, its diagnostic and management challenges. *World journal of gastrointestinal pathophysiology*, *16*(2), 107599. https://doi.org/10.4291/wjgp.v16.i2.107599

Issia, L., Pietrokovsky, S., Sousa-Figueiredo, J., Stothard, J. R., & Wisnivesky-Colli, C. (2009). Fasciola hepatica infections in livestock flock, guanacos and coypus in two wildlife reserves in Argentina. *Veterinary parasitology*, *165*(3-4), 341–344. https://doi.org/10.1016/j.vetpar.2009.07.011

Jones, R. A., Williams, H. W., Dalesman, S., & Brophy, P. M. (2015). Confirmation of Galba truncatula as an intermediate host snail for Calicophoron daubneyi in Great Britain, with evidence of alternative snail species hosting Fasciola hepatica. *Parasites & vectors*, *8*, 656. https://doi.org/10.1186/s13071-015-1271-x

Jones, R. A., Williams, H. W., Dalesman, S., Ayodeji, S., Thomas, R. K., & Brophy, P. M. (2017). The prevalence and development of digenean parasites within their intermediate snail host, Galba truncatula, in a geographic area where the presence of Calicophoron daubneyi has recently been confirmed. *Veterinary parasitology*, *240*, 68–74. https://doi.org/10.1016/j.vetpar.2017.03.021

Jordbruksverket (2019) *Statens jordbruksverks föreskrifter om köttkontroll m.m.* SJVFH 2019:37, Saknr L150. Jönköping: Jordbruksverket. https://jordbruksverket.se/ [Accessed: 2026-04-07]

Kabardiev, S.S., Bittirov, A.M., Karpushchenko, K.A. and Shapiev, B.I. (2024) 'Epizootiological analysis of fasciolosis in ruminants during transhumance grazing in the subalpine zone of Kabardino-Balkaria', *Perm Agrarian Journal*, (47), pp. 98-103. http://doi: 10.47737/2307-2873_2024_47_98

Kajugu, P. E., Hanna, R. E., Edgar, H. W., McMahon, C., Cooper, M., Gordon, A., Barley, J. P., Malone, F. E., Brennan, G. P., & Fairweather, I. (2015). Fasciola hepatica: Specificity of a coproantigen ELISA test for diagnosis of fasciolosis in faecal samples from cattle and sheep concurrently infected with gastrointestinal nematodes, coccidians and/or rumen flukes (paramphistomes), under field conditions.

Veterinary parasitology, *212*(3-4), 181–187. https://doi.org/10.1016/j.vetpar.2015.07.018

Kaplan R. M. (2001). Fasciola hepatica: a review of the economic impact in cattle and considerations for control. *Veterinary therapeutics: research in applied veterinary medicine*, *2*(1), 40–50.

Khoramian, H., Arbabi, M., Osqoi, M.M., Delavari, M., Hooshyar, H. and Asgari, M. (2024)'Prevalence of ruminants fascioliasis and their economic effects in Kashan, center of Iran', *FAO AGRIS*. Available at: https://agris.fao.org [Accessed: 2026-05-03].

Kronenberg, P. A., Fouché, N., Sekiya, M., Liechti, P., Frey, C. F., Mulcahy, G., & Eichenberger, R. M. (2025). A serological test based on mutated recombinant Fasciola hepatica cathepsin L protease for the diagnosis of equine fasciolosis. *Veterinary parasitology*, *340*, 110592. https://doi.org/10.1016/j.vetpar.2025.110592

Lan, Z., Zhang, X. H., Xing, J. L., Zhang, A. H., Wang, H. R., Zhang, X. C., Gao, J. F., & Wang, C. R. (2024). Global prevalence of liver disease in human and domestic animals caused by Fasciola: A systematic review and meta-analysis. *Journal of global health*, *14*, 04223. https://doi.org/10.7189/jogh.14.04223

Lan, Z., Zhang, Y. X., Zhang, A. H., Wang, Y. Y., Qiu, H. Y., Gao, J. F., Cheng, G. F., Elsheikha, H., & Wang, C. R. (2025). Prevalence, risk factors, and regional insights of bovine fasciolosis in China: A systematic review and meta-analysis. *Acta tropica*, *263*, 107570. https://doi.org/10.1016/j.actatropica.2025.107570

Landis, J. R., & Koch, G. G. (1977). The measurement of observer agreement for categorical data. *Biometrics*, *33*(1), 159–174.

Lindqvist Frisk, K. (2019) *Har dina djur stora leverflundror?* Gård & Djurhälsan. Available at: https://www.gardochdjurhalsan.se/har-dina-djur-stora-leverflundror/ [Accessed: 2026-04-24].

MARIKO, I., Yaro, A. S., & Tembely, S. (2025). Prevalences and Evaluation of Liver Loss Due to Fasciolisis in Sheep and Goats Slaughtered on the Gao Slaughtering Area in Mali. https://doi.org/10.21203/rs.3.rs-7836703/v1

Mas-Coma, S., Valero, M. A., & Bargues, M. D. (2009). Chapter 2. Fasciola, lymnaeids and human fascioliasis, with a global overview on disease transmission, epidemiology, evolutionary genetics, molecular epidemiology and control. *Advances in parasitology*, *69*, 41–146. https://doi.org/10.1016/S0065-308X(09)69002-3

Mazeri S, Sargison N, Kelly RF, Bronsvoort BMd, Handel I (2016) Evaluation of the Performance of Five Diagnostic Tests for *Fasciola hepatica* Infection in Naturally Infected Cattle Using a Bayesian No Gold Standard Approach. PLOS ONE 11(8): e0161621. https://doi.org/10.1371/journal.pone.0161621

MedCalc (2025) *Diagnostic test evaluation calculator*. Available at: https://www.medcalc.org/en/calc/diagnostic_test.php [Accessed: 23 April 2026).

Mehmood, K., Zhang, H., Sabir, A. J., Abbas, R. Z., Ijaz, M., Durrani, A. Z., Saleem, M. H., Ur Rehman, M., Iqbal, M. K., Wang, Y., Ahmad, H. I., Abbas, T., Hussain, R., Ghori, M. T., Ali, S., Khan, A. U., & Li, J. (2017). A review on epidemiology, global prevalence and economical losses of fasciolosis in ruminants. *Microbial pathogenesis*, *109*, 253–262. https://doi.org/10.1016/j.micpath.2017.06.006

Mes T. H. (2003). Technical variability and required sample size of helminth egg isolation procedures. *Veterinary parasitology*, *115*(4), 311–320. https://doi.org/10.1016/s0304-4017(03)00219-x

Mezo, M., González-Warleta, M., Carro, C., & Ubeira, F. M. (2004). An ultrasensitive capture ELISA for detection of Fasciola hepatica coproantigens in sheep and cattle using a new monoclonal antibody (MM3). *The Journal of parasitology*, *90*(4), 845–852. https://doi.org/10.1645/GE-192R

Mickiewicz, Marcin & Nowek, Zofia & Czopowicz, Michał & Moroz-Fik, Agata & Biernacka, Kinga & Potârniche, Adrian-Valentin & Szalus, Olga & Górski, Paweł & Nalbert, Tomasz & Buczek, Krzysztof & Mālniece, Aija & Markowska-Daniel, Iwona & Kaba, Jarosław. (2024). The herd-level prevalence of Fasciola hepatica infection in the goat population of Poland. Journal of Veterinary Research. 68. https://doi.org/10.2478/jvetres-2024-0044.

Microsoft Corporation (2026) *Microsoft Excel*. Version 2026. Redmond, WA: Microsoft Corporation.

Molento, M. B., Bennema, S., Bertot, J., Pritsch, I. C., & Arenal, A. (2018). Bovine fascioliasis in Brazil: Economic impact and forecasting. *Veterinary parasitology, regional studies and reports*, *12*, 1–3. https://doi.org/10.1016/j.vprsr.2017.12.004

Nagamori, Y., Hall Sedlak, R., DeRosa, A., Pullins, A., Cree, T., Loenser, M., Larson, B. S., Smith, R. B., & Goldstein, R. (2020). Evaluation of the VETSCAN IMAGYST: an in-clinic canine and feline fecal parasite detection system integrated with a deep learning algorithm. *Parasites & vectors*, *13*(1), 346. https://doi.org/10.1186/s13071-020-04215-x

Nielsen M. K. (2021). What makes a good fecal egg count technique?. *Veterinary parasitology*, *296*, 109509. https://doi.org/10.1016/j.vetpar.2021.109509

Nikpay, Ali & Ahmadiara, Emad & Araghi, Atefeh. (2025). Acute Fascioliasis as a Leading Cause of Mortality in Free-Ranging Flocks of Domestic Sheep in Babol Countryside, Mazandaran Prov- ince, Iran: A Case Report. Iranian Journal of Parasitology. 20. 608-614. doi**:** https://doi.org/10.18502/ijpa.v20i4.20470

Novobilský, A., Engström, A., Sollenberg, S., Gustafsson, K., Morrison, D. A., & Höglund, J. (2014). Transmission patterns of *Fasciola hepatica* to ruminants in Sweden. *Veterinary Parasitology, 203*(3–4), 276–286. https://doi.org/10.1016/j.vetpar.2014.04.015

Novobilský, A., Novák, J., Björkman, C. *et al.* Impact of meteorological and environmental factors on the spatial distribution of *Fasciola hepatica* in beef cattle herds in Sweden. *BMC Vet Res* 11, 128 (2015). https://doi.org/10.1186/s12917-015-0447-0

Novobilský, A., Sollenberg, S., & Höglund, J. (2015). Distribution of Fasciola hepatica in Swedish dairy cattle and associations with pasture management factors. *Geospatial health*, *9*(2), 293–300. https://doi.org/10.4081/gh.2015.351

Novobilský, A., Rustas, B. O., Grandi, G., Högberg, N., & Höglund, J. (2020). Selective flukicide treatment of non-lactating cows and the corresponding production impact of Fasciola hepatica in dairy herds in Sweden. *Veterinary parasitology*, *283*, 109180. https://doi.org/10.1016/j.vetpar.2020.109180

OvaCyte (2025) *OvaCyte Equine and Large Animal Standard FEC Preparation Procedure and Fluke Sample Preparation for Fasciola hepatica & Paramphistomum spp*. Instruction manuals. Lund, Sweden: OvaCyte AB.

Palmer, D. G., Lyon, J., Palmer, M. A., & Forshaw, D. (2014). Evaluation of a copro-antigen ELISA to detect Fasciola hepatica infection in sheep, cattle and horses. *Australian veterinary journal*, *92*(9), 357–361. https://doi.org/10.1111/avj.12224

R Core Team (2022) *R: A language and environment for statistical computing*. Version 4.2.1. Vienna, Austria: R Foundation for Statistical Computing. Available at: https://www.R-project.org/ [Accessed: 2026-05-18]

Rapsch, C., Schweizer, G., Grimm, F., Kohler, L., Bauer, C., Deplazes, P., Braun, U., & Torgerson, P. R. (2006). Estimating the true prevalence of Fasciola hepatica in cattle slaughtered in Switzerland in the absence of an absolute diagnostic test. *International journal for parasitology*, *36*(10-11), 1153–1158. https://doi.org/10.1016/j.ijpara.2006.06.001

Rehbein, S., Bussmann, M., Visser, M. and Winter, R. (2011) 'Efficacy of a new fasciolicide (compound 113) against Fasciola hepatica in sheep', *Veterinary Parasitology*, 182(2-4), pp. 278-284.

Rivière, L., Rousset, E., Jourdain, E., Delignette-Muller, M. L., & Lurier, T. (2025). Harmonisation of the diagnostic performances of serological ELISA tests for C. burnetii in ruminants in the absence of a gold standard: Optimal cut-offs and performances reassessment. *Preventive veterinary medicine*, *239*, 106509. https://doi.org/10.1016/j.prevetmed.2025.106509

Rojas-Moncada, J., Torrel-Pajares, T., & Vargas-Rocha, L. (2024). Validation of the natural sedimentation technique in the diagnosis of chronic fasciolosis. *Parasitology international*, *101*, 102889. https://doi.org/10.1016/j.parint.2024.102889

Schoiswohl, J. (2025) *Prävalenzstudie zu Endoparasiten beim Rind* [Prevalence study on endoparasites in cattle]. Research project. Irdning-Donnersbachtal: HBLFA Raumberg-Gumpenstein. Available at: https://raumberg-gumpenstein.at/en/research/current-research/successful-project-completion-prevalence-study-on-endoparasites-in-cattle.html [Accessed 2026-04-26].

Schweizer, G., Braun, U., Deplazes, P., & Torgerson, P. R. (2005). Estimating the financial losses due to bovine fasciolosis in Switzerland. *The Veterinary record*, *157*(7), 188–193. https://doi.org/10.1136/vr.157.7.188

Shumakovich, E.E. (1957) 'Development of Fasciola hepatica larvae in snails', *Trudy Gel'mintologicheskoy Laboratorii*, 8, pp. 45-52.

Shirai, W., Sato, T., Shibuya, H., Naito, K., & Tsukise, A. (2006). Anatomicopathological study of vascular and biliary systems using cast samples of Fasciola-infected bovine livers. *Journal of veterinary medicine. A, Physiology, pathology, clinical medicine*, *53*(5), 239–245. https://doi.org/10.1111/j.1439-0442.2006.00821.x

Skrjabin, K.I. and Schulz, R.S. (1935) *Fascioliasis of Domestic Animals and Measures for Its Control*. Moscow: Uch. komb. NKZ USSR

Strydom, T., Lavan, R. P., Torres, S., & Heaney, K. (2023). The Economic Impact of Parasitism from Nematodes, Trematodes and Ticks on Beef Cattle Production. *Animals : an open access journal from MDPI*, *13*(10), 1599. https://doi.org/10.3390/ani13101599

Suliman, L. (2026). Collection of photographs [Figure 5, 6, 8, 9, 10, 11]. Department of Animal Biosciences, Swedish University of Agricultural Sciences.

Tanabe, M. B., Caravedo, M. A., Clinton White, A., Jr, & Cabada, M. M. (2024). An Update on the Pathogenesis of Fascioliasis: What Do We Know?. *Research and reports in tropical medicine*, *15*, 13–24. https://doi.org/10.2147/RRTM.S397138

Taylor, E.L. (1930) 'The longevity of Fasciola hepatica in the host', *Veterinary Record*, 10(3), pp. 71-72.

Telenostic, 2026. *About OvaCyte*. Available at: https://www.telenostic.com/about-ovacyte/- [Accessed: 2026-04-07].

Tran, L., Toet, H., & Beddoe, T. (2022). Environmental detection of *Fasciola hepatica* by loop-mediated isothermal amplification. *PeerJ*, *10*, e13778. https://doi.org/10.7717/peerj.13778

United States Department of Agriculture (USDA) (2020) *Fasciola hepatica: Economic impact on the US cattle industry*. Animal and Plant Health Inspection Service Report. Washington, DC: USDA.

UpToDate (2025) 'Liver flukes: Fascioliasis', *UpToDate*. Available at: https://www.uptodate.com/contents/liver-flukes-fascioliasis, [Accessed: 2026-03-25].

Valero, M. A., Darce, N. A., Panova, M., & Mas-Coma, S. (2001). Relationships between host species and morphometric patterns in Fasciola hepatica adults and eggs from the northern Bolivian Altiplano hyperendemic region. *Veterinary parasitology*, *102*(1-2), 85–100. https://doi.org/10.1016/s0304-4017(01)00499-x

Valero, M. A., Panova, M., Comes, A. M., Fons, R., & Mas-Coma, S. (2002). Patterns in size and shedding of Fasciola hepatica eggs by naturally and experimentally infected murid rodents. *The Journal of parasitology*, *88*(2), 308–313. https://doi.org/10.1645/0022-3395(2002)088[0308:PISASO]2.0.CO;2

Vázquez Perera, Antonio & Alda, Pilar & Lounnas, Manon & Sabourin, Emeline & Alba Menéndez, Annia & Pointier, J. & Hurtrez-Boussès, Sylvie. (2019). Lymnaeid snails hosts of Fasciola hepatica and Fasciola gigantica (Trematoda: Digenea): A worldwide review. CAB Reviews Perspectives in Agriculture Veterinary Science Nutrition and Natural Resources. 13. 1-15. http://doi.org/10.1079/PAVSNNR201813062

Walsh, T. R., Angell, J. W., Bentham, A., Bentley, E., Clough, H., Graham-Brown, J., Howell, A., Steinke, S., Wakeham, A., & Williams, D. J. L. (2026). Evaluation of a lateral flow test to diagnose liver fluke in cattle and sheep. *International journal for parasitology*, *56*(5), 104752. https://doi.org/10.1016/j.ijpara.2025.11.004

Ward, E. (2025) 'Sedimentation techniques for liver fluke egg detection: a comprehensive guide for biomedical research', *Parasite Study*, 29 November. Available at: http://www.parasitestudy.com/posts/sedimentation-techniques-for-liver-fluke-egg-detection-a-comprehensive-guide-for-biomedical-research [Accessed: 2026-05-17].

Popular science summary

Three Ways to Catch a Liver Fluke – Which Test Works Best?

The liver fluke is a small, leaf-shaped parasite that lives in the bile ducts of cattle and sheep. This leads to weight loss, reduced milk production and liver damage, costing farmers millions of dollars annually. To combat the disease, it is first necessary to determine which animals are infected. But how do you choose the right test?

In this project, I compared three diagnostic methods using faecal samples from 47 cattle and 23 sheep in Sweden.

The old method – sedimentation is like panning for gold. You mix faeces with water, let the heavy fluke eggs sink to the bottom, and look for them under a microscope. It is cheap and simple, but slow and requires a trained eye. A single negative test does not mean the animal is free from fluke – eggs are not shed every day.

The modern method – ELISA detects fluke proteins (antigens) in the faeces. It can find infections several weeks before eggs appear, and it is fast enough to process 70 samples in six hours. It is the most sensitive test and the best choice for routine herd screening.

The futuristic method – OvaCyte™ uses artificial intelligence. A machine scans the sample, recognises fluke eggs, and counts them automatically. It is consistent, removes human error, and can also detect rumen fluke (another parasite) at the same time. It worked perfectly in sheep (100% detection) but struggled with low-level infections in cattle.

So which test should you choose? If you want to screen a whole herd for early infection, pick ELISA. If you need to confirm a sick animal on a budget, sedimentation still works. If you run a busy laboratory and process hundreds of samples, OvaCyte™ saves time and reduces mistakes.

No test is perfect. But by understanding their strengths and weaknesses, farmers and veterinarians can make smarter decisions – and that means healthier animals and lower losses.

Acknowledgements

First and foremost, I would like to express my deepest and most sincere gratitude to my main supervisor, **Giulio Grandi**, and my assistant supervisor, **Johan Höglund**. Both have been invaluable throughout this project, and I cannot thank them enough for their exceptional guidance, patience, and support. Their deep expertise in veterinary parasitology, combined with their willingness to share knowledge and provide constructive feedback at every stage, has been absolutely essential to the completion of this thesis. I am truly fortunate to have had such dedicated and inspiring supervisors.

I also wish to thank my examiner, **Sara Hägglund Valarcher,** for her thorough review and valuable suggestions.

I would like to acknowledge the staff at the Parasitology Laboratory, Department of Animal Biosciences (HBIO), Swedish University of Agricultural Sciences, for their technical assistance and for providing a welcoming working environment.

I am grateful to the slaughterhouses – Örebro Slakteri, Dahlbergs Slakteri, and Varekils Slakteri – as well as Bredaryd farm, for generously providing faecal samples and for their cooperation throughout the sampling process. Special thanks to the slaughterhouse veterinarians for sharing their inspection data.

I also wish to thank the OvaCyte team for their technical support with the automated analysis system.

Writing tools.
The preparation of this thesis was supported by digital writing tools. Grammarly was used for grammar, spelling, and clarity corrections. Generative AI models assisted with language refinement, structural organisation, and phrasing suggestions.

This use of digital tools complies with the Swedish University of Agricultural Sciences guidelines on generative AI and digital support tools. The tools were employed solely to improve the readability and presentation of the work. The author retains full responsibility for all scientific content, interpretations, and conclusions, and guarantees the accuracy and integrity of the material presented.

Appendix 1

Samples preparation for the OvaCyte™ Fluke Plus Copromicroscopy (OvaCyte, 2025).

Sample procedure:

1. Weigh sample: Mix the faecal sample thoroughly. Weigh 5 g (± 0.3 g) of faeces into a sample pot.
2. Add tap water: Top up the pot with water until just below the 50 ml mark and mix well.
3. Assemble filter stack: Place the grey filter on top of the coral filter, aligning arrows 1 and 2 so they point upwards.
4. First pour: Pour half of the prepared sample through the filter stack.
5. First tap: Tap the filters approximately 30 times, or until the liquid has passed through.
6. Second pour & tap: Pour the remaining sample through and repeat the tapping process (approximately 30 times).
7. Rinse: Fill the sample pot with water up to the 30 ml mark, pour through the filter stack, and tap. Repeat this rinse step once more. Ensure no excess fluid remains on the filter.
8. Invert filter stack: Invert the coral filter stack upside down onto a 500 ml beaker so that arrow 3 is pointing upwards (OvaCyte, 2025).
9. Add flotation fluid: Extract 15 ml of fluke flotation fluid into the provided syringe.
10. Flood the mesh: While holding the filter stack and beaker together, flood the top of the (now inverted) mesh with the flotation fluid. Repeat the tapping process, paying special attention to releasing any remaining droplets on the underside of the mesh.
11. Fill cassette: Fill the OvaCyte™ cassette from the resulting filtrate as normal, referring to the standard procedure described on page 1 of the Equine and Large Animal Standard FEC Preparation Procedure.

Appendix 2

Traditional Sedimentation Procedure.

1. Sample weighing: 5 g of faeces was weighed into a clean beaker.
2. Initial mixing: 1000 ml of tap water was added, and the sample was mixed thoroughly with a stirring rod until a homogeneous suspension was achieved.
3. Sieving: The suspension was poured through a tea strainer (mesh size approximately 250 µm) into a sedimentation cone, using additional water to rinse the beaker and strainer.
4. First sedimentation: The suspension was left to sediment undisturbed for 30 minutes.
5. First decanting: The supernatant was carefully decanted, leaving approximately 10 ml of sediment.
6. Washing: Water was added to the 1000 ml mark, and the sediment was gently resuspended.
7. Second sedimentation: The suspension was left to sediment for a further 15 minutes.
8. Washing: Water was added to the 1000 ml mark, and the sediment was gently resuspended.
9. Third sedimentation: The suspension was left to sediment for a further 15 minutes.
10. Final decanting: The supernatant was carefully decanted, leaving approximately 5-10 ml of sediment.
11. Transfer: The sediment was poured into a petri dish, rinsing the cone with a small amount of water to recover all material.
12. Microscopic examination: The sediment was examined systematically under a stereomicroscope at 40× magnification.
13. Egg identification: *F. hepatica* eggs were identified based on morphological characteristics:

-Size: 130-150 µm × 70-90 µm
-Shape: Oval, operculated
-Colour: Yellowish-brown
-Contents: Undeveloped embryo surrounded by yolk cells (Andrews, 1999).

Recording: Presence or absence of *F. hepatica* eggs was recorded as "P" (positive) or "N" (negative). Where present, eggs were counted and recorded in the "Fh ägg i sediment" column.

Appendix 3

Procedure for ELISA-test.

Manufacturer's instructions (Bio-X Diagnostics, 2025):

Sample preparation:

- For cattle samples - 2 g of faeces, for sheep – 0,5 g of faeces, was mixed with 2 ml of dilution buffer (1:1 w/v dilution),
- Centrifugation: Samples were centrifuged at 1,000 × g for 10 minutes.
- Supernatant collection: Supernatants were collected for analysis.

Note: Coproantigen-ELISA results are pending and will be incorporated in subsequent analyses. The methodology is presented here according to the study protocol.

1. Incubation 1: Add 100µl of each sample to paired wells (specific and control). Cover and incubate for 2 hours at 21°C ± 3°C on a shaker.
2. Washing: Wash the plate three times with 300µl washing solution per well.
3. Conjugate 1: Add 100µl of diluted biotin-linked anti-Fasciola antibody to each well. Incubate for 1 hour at 21°C ± 3°C. Wash three times.
4. Conjugate 2: Add 100µl of diluted peroxidase-coupled avidin to each well. Incubate for 1 hour at 21°C ± 3°C. Wash three times.
5. Development: Add 100µl TMB chromogen (must be colourless). Incubate for 10 minutes at 21°C ± 3°C protected from light.
6. Stop: Add 50 µl stop solution (1 M phosphoric acid). Blue turns yellow.
7. Reading: Measure optical density (OD) at 450 nm.

Supplementary tables

Table S1. Life Cycle Periods of F. hepatica.

Period	Stage	Location	Duration	Key Features	References
Embryogony	Egg to miracidium	External environment (water, vegetation)	Varies with temperature	Egg size: 0.12–0.15 mm; golden-yellow; operculated; contains fertilised egg cell and 28–30 yolk cells	Skrjabin and Schulz, 1935; Andrews, 1999
Partheno gony	Miracidium to cercaria	Intermediate host (snail, e.g., Galba truncatula)	1.5–2 months (summer conditions)	Asexual reproduction; sporocyst → redia → daughter redia → cercaria; >400 cercariae per snail	Shumakovich, 1957; Mas-Coma et al., 2009
Cystogony	Cercaria to metacercaria	External environment (water, vegetation)	15–30 minutes (encystment)	Cercaria sheds tail; forms protective cyst (metacercaria); survives: hay 2–6 months, water up to 1 year	Skrjabin and Schulz, 1935; Andrews, 1999; Dalton, 2021
Maritogony	Metacercaria to adult fluke	Definitive host (ruminants, humans)	2–4 months to maturity; adult lives 4–5 years (up to 10 years)	Excystation in small intestine; migration through peritoneum and liver parenchyma; establishment in bile ducts; produces ~3,500 eggs/day	Taylor, 1930; Andrews, 1999; Mas-Coma et al., 2009

Table S2. Sample collection overview with sources and dates.

Sample No.	Animal ID	Source	Collection Date	Slaughter Code
1	7054	A	2026-01-22	N
2	2938	A	2026-01-22	N
3	8941	A	2026-01-22	N
4	3196	A	2026-01-22	79/80
5	80491	A	2026-01-22	79/80
6	24051	A	2026-01-22	79/80
7	11197	A	2026-01-22	N
8	6033	A	2026-01-22	79/80
9	0964-5	B	2026-02-24	NA
10	1174-9	B	2026-02-24	NA
11	1204-5	B	2026-02-24	NA
12	1235-9	B	2026-02-24	NA
13	1275-5	B	2026-02-24	NA
14	1359-7	B	2026-02-24	NA
15	1368-8	B	2026-02-24	NA
16	1369-6	B	2026-02-24	NA
17	1379-5	B	2026-02-24	NA
18	1382-9	B	2026-02-24	NA
19	1383-7 + 1402-5 (pooled)	B	2026-02-24	NA
20	1384-5	B	2026-02-24	NA

21	1393-6	B	2026-02-24	NA
22	1394-4	B	2026-02-24	NA
23	1396-9	B	2026-02-24	NA
24	1397-7	B	2026-02-24	NA
25	1398-5	B	2026-02-24	NA
26	1404-1	B	2026-02-24	NA
27	1407-4	B	2026-02-24	NA
28	9226-5 SE023663	C	2026-02-27	79/80
29	0068-8 SE204424	C	2026-02-27	79/80
30	0516-1 SE062667	C	2026-02-27	79/80
31	9210-9 SE023663	C	2026-02-27	79/80
32	0470-1 SE006596	C	2026-02-27	79/80
33	2306-2 SE023663	C	2026-02-27	79/80
34	919	D	2026-03-24	79/80
35	920	D	2026-03-24	79/80
36	923	D	2026-03-24	79/80
37	919	D	2026-03-24	79/80
38	919b	D	2026-03-24	79/80
39	919c	D	2026-03-24	79/80
40	919d	D	2026-03-24	79/80
41	919c	D	2026-03-24	79/80

42	919+920a	D	2026-03-24	79/80
43	919+920b	D	2026-03-24	79/80
44	919+920c	D	2026-03-24	79/80
45	919+923a	D	2026-03-24	79/80
46	919+923b	D	2026-03-24	79/80
47	919+923c	D	2026-03-24	79/80
48	Får 1	D	2026-04-15	79/80
49	Får 2	D	2026-04-15	79/80
50	Får 3	D	2026-04-15	79/80
51	Får 4	D	2026-04-15	79/80
52	Får 5	D	2026-04-15	79/80
53	Får 6	D	2026-04-15	79/80
54	Får 7	D	2026-04-15	79/80
55	Får 8	D	2026-04-15	79/80
56	Får 9	D	2026-04-26	79/80
57	Får 10	D	2026-04-26	79/80

58	Får 11	D	2026-04-26	79/80
59	Får 12	D	2026-04-26	79/80
60	Får 13	D	2026-04-26	79/80
61	Får 14	D	2026-04-26	79/80
62	Får 15	D	2026-04-26	79/80
63	Får 16	D	2026-04-26	79/80
64	Får 17	D	2026-04-26	79/80
65	Får 18	D	2026-04-26	79/80
66	Får 19	D	2026-04-26	79/80
67	Får 20	D	2026-04-26	79/80
68	Får 21	D	2026-04-26	79/80
69	Får 22	D	2026-04-26	79/80
70	Får 23	D	2026-04-26	79/80

Table S3. Samples processing schedule.

Sample No.	Location	OvaCyte™ Date	Sedimentation Date	ELISA Date
1-8	A	2026-01-23	2026-01-26	2026-04-15
9-27	B	2026-03-02 – 2026-03-05	2026-03-02 – 2026-03-05	2026-04-15
28-33	C	2026-03-05	2026-03-06 – 2026-03-09	2026-04-15
34-47	D	2026-03-26 – 2026-04-01	2026-03-26 – 2026-04-01	2026-04-15
48-55	D	2026-04-20	2026-04-20 – 2026-04-21	2026-05-13
56-70	D	2026-04-26	2026-05-04 – 2026-05-05	2026-05-13

Table S4. Results from three methods.

Sample ID	Location	OvaCyte™	Sedimentation	ELISA (S/P ratio value,%)
1	A	N	N	N
2	A	N	1 Ps	N
3	A	N	N	18.34
4	A	1 Fh	1 Fh	23.28
5	A	N	1 Fh	15.40
6	A	N	1 Fh	46.70
7	A	N	N	N
8	A	N	N	N
9	B	N	N	15.17

10	B	N	N	N
11	B	N	N	12.30
12	B	N	N	N
13	B	2 Fh	1 Fh	54.63
14	B	2 Fh	N	9.77
15	B	N	3 Fh	N
16	B	N	2 Fh	N
17	B	N	N	N
18	B	N	N	N
19	B	N	N	N
20	B	1 Fh	1 Fh	N
21	B	N	N	N
22	B	N	N	N
23	B	N	N	N
24	B	N	1 Fh	N
25	B	N	N	N
26	B	N	N	N
27	B	N	N	N
28	C	1 Fh	2 Fh	N
29	C	N	N	N
30	C	N	N	8.20
31	C	N	N	53.80
32	C	N	1 Fh	9.38

33	C	N	2 Fh	9.74
34	D	2 Fh	13 Fh	80.33
35	D	N	N	27.84
36	D	N	N	49.89
37	D	1 Fh, 1 Ps	2 Fh	81.63
38	D	1 Fh	1 Fh	68.54
39	D	3 Fh	3 Fh	94.56
40	D	1 Fh	4 Fh	87.25
41	D	1 Fh	1 Fh	77.30
42	D	1 Fh	2 Fh	57.66
43	D	N	3 Fh	70.47
44	D	1 Fh	N	60.46
45	D	1 Fh	8 Fh	60.86
46	D	N	7 Fh	65.96
47	D	2 Fh, 1 Ps	2 Fh	65.91
48	D	45 Fh, 11 Ps	73 Fh, 4 Ps	125,8
49	D	75 Fh, 3 Ps	37 Fh, 2 Ps	123,5
50	D	166 Fh, 1 Ps	105 Fh, 1 Ps	126,4
51	D	12 Fh, 3 Ps	10 Fh	131,0
52	D	128 Fh, 1 Ps	126 Fh, 1 Ps	140,5
53	D	143 Fh, 4 Ps	99 Fh, 2 Ps	116,6

54	D	47 Fh, 6 Ps	36 Fh, 1 Ps	137,7
55	D	55 Fh, 2 Ps	36 Fh, 1 Ps	116,7
56	D	20 Fh, 5 Ps	54 Fh, 4 Ps	123,5
57	D	90 Fh, 1 Ps	56 Fh	121,6
58	D	43 Fh	30 Fh	132,8
59	D	44 Fh	68 Fh, 1 Ps	118,9
60	D	30 Fh	36 Fh, 2 Ps	121,9
61	D	65 Fh, 1 Ps	52 Fh, 3 Ps	122,7
62	D	34 Fh, 2 Ps	34 Fh, 3 Ps	127,1
63	D	32 Fh, 4 Ps	58 Fh, 4 Ps	118,3
64	D	31 Fh	37 Fh, 1 Ps	123,6
65	D	34 Fh	61 Fh, 3 Ps	121,2
66	D	60 Fh, 3 Ps	21 Fh	109,9
67	D	58 Fh	14 Fh, 1 Ps	107,6
68	D	8 Fh	19 Fh	133,4
69	D	11 Fh	13 Fh	128,5

70	D	9 Fh	9 Fh	116,4
	TOTAL	**37 positive: 1261 eggs Fh, 49 eggs Ps**	**46 positive: 1146 eggs Fh, 35 egg Ps**	**49 positive: 8.2 - 140,5 %**

Table S5. Descriptive statistics for each diagnostic method.

Method	**Positive samples (n)**	**Detection rate (%)**	**Total *F. hepatica* eggs detected**	**Range of eggs (positive samples)**
OvaCyte™	37	52,9	1261	1-166
Traditional sedimentation	45	64,3	1146	1-126
ELISA	49	70	Not applicable	8.2–140.5% (S/P)

Table S6. Scale proposed by Landis and Koch.

Kappa value (κ)	**Strength of agreement**
< 0.00	Poor
0.00 – 0.20	Slight
0.21 – 0.40	Fair
0.41 – 0.60	Moderate
0.61 – 0.80	Substantial
0.81 – 1.00	Almost perfect

Table S7. Pairwise agreement between diagnostic methods (Cohen's kappa).

Comparison	**Both positive (a)**	**Both negative (d)**	**Observed agreement (%)**	**Kappa (κ)**	**Strength of agreement**
ELISA vs Sedimentation	31	16	67,1	**0.44**	Moderate
ELISA vs OvaCyte™	30	18	68,6	**0.45**	Moderate
Sedimentation vs OvaCyte™	29	21	71,4	**0.47**	Moderate

Note: Calculations are based on binary outcomes (positive/negative for F. hepatica).

Table S8. Comparison of diagnostic method performance.

Parameter	**Sedimentation**	**OvaCyte™**	**ELISA**
F. hepatica positive samples	45 (64.3%)	37 (52.9%)	49 (70.0%)
Paramphistomum positive samples	17 (24.3%)	16 (22.9%)	Not applicable
Total *F. hepatica* eggs	1146	1261	Not applicable
Total *Paramphistomum* eggs	35	49	Not applicable
Cattle *F. hepatica* detection (n=47)	22 (46.8%)	15 (31.9%)	26 (55.3%)
Sheep *F. hepatica* detection (n=23)	23 (100%)	22 (95.7%)	23 (100%)
Cattle *Paramphistomum* (n=47)	1 (2.1%)	2 (4.3%)	Not applicable
Sheep *Paramphistomum* (n=23)	16 (69.6%)	14 (60.9%)	Not applicable

**Note: One sheep sample was negative for Paramphistomum by OvaCyte™ but positive by sedimentation, accounting for the difference in counts*

Supplementary figures.

Figure S1. OvaCyte™ Fluke Plus sample preparation procedure, pdf-manual instruction (OvaCyte, 2025)

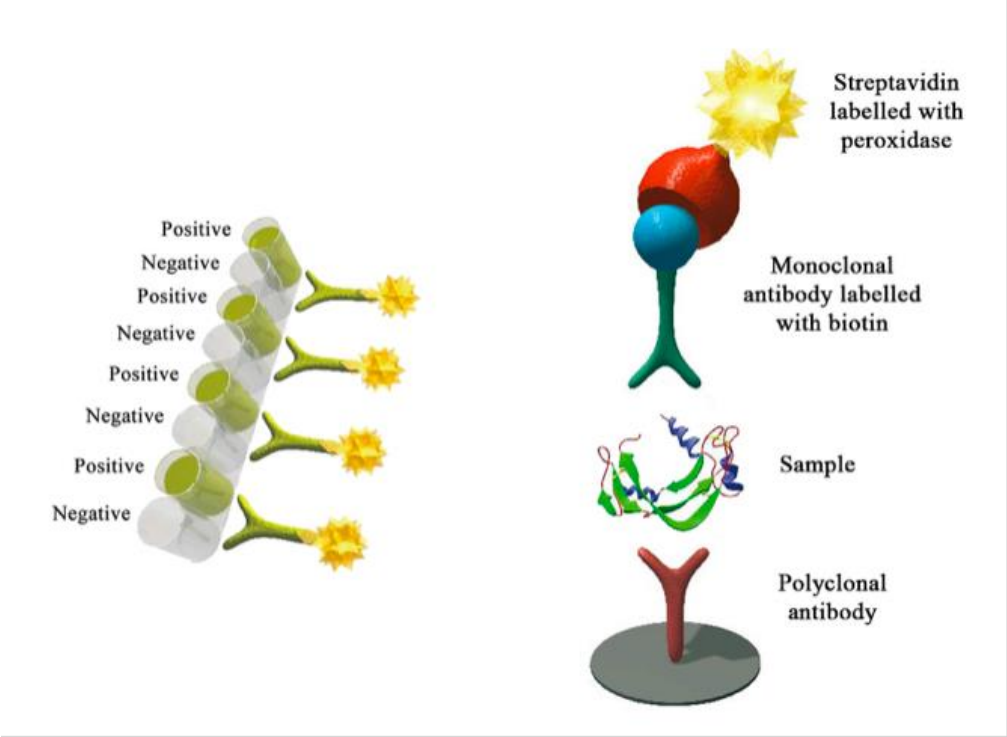

Figure S2. Coproantigen-ELISA principle, BIO K 201 - Monoscreen AgELISA Fasciola hepatica / indirect sandwich, double wells, pdf manual instruction (Bio-X Diagnostics, 2025).

Publishing and archiving

Approved students' theses at SLU can be published online. As a student you own the copyright to your work and in such cases, you need to approve the publication. In connection with your approval of publication, SLU will process your personal data (name) to make the work searchable on the internet. You can revoke your consent at any time by contacting the library.

Even if you choose not to publish the work or if you revoke your approval, the thesis will be archived digitally according to archive legislation.

You will find links to SLU's publication agreement and SLU's processing of personal data and your rights on this page:

- https://libanswers.slu.se/en/faq/228318

☒ YES, I, Liliia Suliman, have read and agree to the agreement for publication and the personal data processing that takes place in connection with this.

☐ NO, I/we do not give my/our permission to publish the full text of this work. However, the work will be uploaded for archiving and the metadata and summary will be visible and searchable.