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Morphological and molecular diagnosis of Fasciola species and study the prevalence of fascioliasis in slaughtered cattle in Mosul Province-Iraq

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Abstract

Fasciola species cause a parasitic disease that poses a significant threat to the health and economic productivity of livestock worldwide, including Iraq. The study aimed to investigate fascioliasis and assess its prevalence in cattle slaughtered in Mosul abattoirs from August 2024 to the end of February 2025. Isolated flukes were collected from infected livers for morphological examination and molecular analysis, based on gene amplification, sequencing, and subsequent phylogenetic analysis. Out of 200 liver samples examined, an overall infection rate was 9.5%, which was higher in females with no significant difference at P-value < 0.05. The highest prevalence was observed in cattle over two years of age, with a statistically significant difference at P-value < 0.05. The greatest monthly infection rates were recorded in October and September; differences were not statistically significant difference at P-value < 0.05. Morphological evaluation using carmine staining confirmed the presence of two species: *Fasciola gigantica* and *Fasciola hepatica*. Moreover, the combined analysis of the 28S rRNA and 16S rRNA genes, along with morphological features proved effective in accurately identifying the species using conventional PCR. This approach revealed three specific fragments: 377 bp and 529 bp, indicative of *F. gigantica*, and 618 bp characteristic of *F. hepatica*. Molecular analysis showed 98–100% sequence similarity with known *Fasciola* species. Phylogenetic analysis revealed clear genetic differentiation between *F. gigantica* and *F. hepatica* and significant genetic diversity within *F. gigantica* populations, including newly classified Iraqi isolates (PV344638 and PV290745–PV290747). Additionally, two isolates from this study (PV335564 and PV335557) were closely related to known *F. hepatica* populations.

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Introduction

Fasciola, also known as liver flukes, belong to a group of parasitic flatworms that are among the most significant agents of fascioliasis a disease that severely impacts livestock health and production worldwide (1). Two main species are responsible for this infection: *F. hepatica* and *F. gigantica*, both of which commonly infect ruminants such as cattle, sheep, and goats. Fascioliasis has been reported across diverse regions, including Asia, Africa, Europe, the Americas, and Oceania (2). Besides ruminants, other animals like donkeys, horses, camels, and mules can also serve as definitive hosts (3,4). Due to its high prevalence in areas with large cattle populations and its potential for zoonotic transmission, fascioliasis is considered a major global health and agricultural concern (5).

Taxonomically, these flukes are part of the phylum Platyhelminthes, class Trematoda, order Digenea, and family Fasciolidae. The species *F. hepatica* was first documented by Linnaeus in 1758, while *F. gigantica* was described later by Cobbold in 1855 (6). In regions where the two species overlap, particularly in parts of Asia and Africa, hybrid forms with intermediate features and mixed genetic profiles have been observed (7).

The life cycle of *Fasciola* spp. involves aquatic snails from the Lymnaeidae family as intermediate hosts. These snails thrive in stagnant or marshy environments. Once inside the final host, the adult parasites settle in the bile ducts and begin producing eggs, these eggs are expelled in the feces, hatch in water, and release miracidia that infect snails, after undergoing several developmental stages inside the snail, the parasites emerge as cercariae, which then encyst on vegetation as metacercariae the infective stage. When ingested by a suitable host, these cysts release juvenile flukes that migrate through the liver before maturing in the bile ducts over the course of 8 to 10 weeks (8).

The severity of the disease varies depending on the number of ingested metacercariae, the species of host, and how advanced the infection is in the liver (9). During the early stages, migrating immature flukes can cause liver hemorrhage and tissue injury. As they reach the bile ducts, they begin feeding on blood and damage the mucosal lining using their spiny surfaces. In acute cases, the liver becomes swollen, fragile, and may develop fibrinous coatings. With chronic infection, tissue scarring, cirrhosis, and thickened or cystic bile ducts are often observed (10).

Accurately identifying *Fasciola* spp. is difficult through clinical symptoms, pathogenesis, or immunological methods alone. Serology does not distinguish between *F. hepatica* and *F. gigantica*, so identification typically relies on a combination of morphological analysis and molecular techniques. DNA-based methods, including gene sequencing and PCR, have greatly improved species resolution (11-13). PCR enables the selective amplification of genetic regions using primers that target conserved sequences (14,15). Ribosomal DNA markers like ITS1, ITS2, and 28S rDNA are widely used to distinguish the species (16,17), while the 16S rRNA gene though helpful may fall short in resolving differences between closely related flukes (18). ITS-2 spacer, situated between 5.8S and 28S regions, plays a key role in genetic confirmation (19). Moreover, mitochondrial DNA markers have proven useful for analyzing genetic variation and understanding evolutionary relationships within the genus (20,21).

This research was conducted to assess the prevalence of *Fasciola* infection in cattle slaughtered in Mosul province-Iraq. It also aimed to identify the species; depending on morphological characteristics, molecular assays, and phylogenetic evaluation of the recovered flukes.

Materials and methods

Ethical approval

All approaches and procedures employed in this study adhered to the guidelines established by the Scientific Ethical Committee for Animal Experiments at the College of Veterinary Medicine, University of Mosul, UM.VET.2024.030.

Study Site and Sample Collection

This study was conducted at various locations in Mosul Province-Iraq, from August 2024 until the end of February 2025. Total of 200 liver samples from both infected and uninfected slaughtered cattle were analyzed, additionally recording animal sex, age, and species on standardized data sheets. Isolated flukes were obtained from various sources, including local abattoirs, butcher shops, and private veterinary practices around Mosul province and collected by gently squeezing the liver tissue to separate the flukes from the bile ducts and nearby tissues. Each isolated flukes were initially washed with distilled water to remove any remaining tissue and bile then classified according to its dimensions and gross identification. The adult flukes were preserved in 70% ethanol and stored in labeled containers for further morphological analysis and staining process. A collection of 1-4 flukes in each infected liver were stored at -20°C for molecular analysis. Following collection, the flukes were sent to the Department of Microbiology Laboratory, College of Veterinary Medicine- University of Mosul.

Morphological analysis

Morphological identification was performed using the authorized morphological keys based on studies of (22-24). The specimens were stained and subjected to comprehensive morphological analysis; macroscopic examination, utilizing body length-to-width ratio measurements, facilitated the distinction of fluke species, as demonstrated in Figure 2. The photos were taken with a Samsung Galaxy A51 FE digital camera (12-megapixel quality), utilizing a standard ruler to facilitate precise measurement of the parasites' dimensions.

Staining process

For the staining protocols, the samples preserved in 70% ethanol were transferred to prepared carmine stain solution and incubated for approximately 4 hours. Approximately 60 samples of perforated worms were stained during this process. After staining, the samples were immersed in 70% ethanol for 20 to 30 minutes to remove excess dye and then underwent sequential dehydration through acidic and basic ethanol treatments. The samples were then immersed in 70% ethanol for 10 minutes, followed by 95% ethanol for 20 minutes, and the process concluded with two or three exchanges of 100% ethanol, each lasting 20 minutes. For permanent fixation, the punctured worms were placed on glass slides with a drop of toluene to prevent drying, covered with a layer of DPX mounting medium (dibutyl phthalate polystyrene xylene), and covered with a plastic cover for microscopic analysis (25,26). The body structure identification was performed and marked based on study of (27).

Molecular study

Genomic DNA extraction and PCR amplification

Genomic DNA was extracted from roughly 20 mg of isolated fluke tissue employ the Primary Prep™ Genomic DNA Isolation Kit (Addbio, Korea), following the manufacturer's guidelines (28,29). The target regions of *Fasciola* spp. 16S rRNA and 28S rRNA genes were amplified via species-specific PCR with primers selected for *F. hepatica* and *F. gigantica* (30). Two sets of primers were utilized to amplify the 16S rRNA gene of *F. gigantica*, while a single set targeted the 28S rRNA gene of *F. hepatica* for molecular identification refer to table 1. PCR reactions were performed using a gradient thermal cycler following the cycling methods outlined in table 2. Four vials that include pure genomic DNA, stored at -20°C, were acquired for PCR production. The PCR thermal cycling parameters, modified from Mucheka and Lamb, commenced with an initial denaturation at 94°C for 5 minutes, succeeded by 35 cycles comprising denaturation at 94°C for 45 seconds, annealing at 56°C for 30 seconds, and extension at 72°C for 1 minute, culminating in a final extension at 72°C for 5 minutes as shown in table 3. PCR products were separated by electrophoresis on a 1.5% agarose gel, prepared by dissolving 1 g of agarose powder in 100 ml of TBE solution, with the incorporation of 3 µL of ethidium bromide for DNA visualization. Approximately 5 µL of each PCR result, together with a DNA ladder, were loaded into wells formed by sample combs. Electrophoresis was performed at 100 V and 3 W for approximately 40 minutes. The resulting DNA bands were observed via a UV transilluminator.

Table 1: Nucleotide primers used to amplify the 28S and 16S rRNA genes of *Fasciola* spp. (31-33)

Primers	Sequences 5'-3'	Target gene	Expected size (*bp*)
FGF FHGR	5'TGTTATGATTCATTGTTTGTAG3' 5'ATAAGAACCGACCTGGCTCAC3'	*F.gigantica*	529
FGIF FGIR	5'AGTCGTAACAAGGTTTCCGT3' 5' GTCCAGCCACAAGGACCG3'	*F.gigantica*	377
28F1 28R600	5'ACGTGATTACCCGCTGAACT3' 5'CTGAGAAAGTGCACTGACAAG3'	*F.hepatica*	618

Primers specific to *F. gigantica* include FGF (forward primers), FHGR (reverse primers), FGIF (forward primer), and FGIR (reverse primer). Primers specific to *F. hepatica* include 28F1 (forward primer) and 28R600 (reverse primer).

Table 2: Components for *Fasciola* species Subject to c-PCR (34,35)

reaction mixture's contents	The volume
Taq PCR PreMix	10 µl
Template DNA	4 µl
Forward primer (10 pmol/µl)	1 µl
Reverse primer (10 pmol/µl)	1 µl
Nuclease free water	4 µl
Total volume of Master-mix	20 µl

Sequencing and phylogenetic analysis

This investigation included six PCR products, two from *F. hepatica* and four from *F. gigantica* that were confirmed as positive via conventional PCR and subsequently sent to a commercial laboratory (Macrogen Inc., South Korea) for purification and Sanger sequencing. Sequence similarity was evaluated by contrasting the acquired sequences with previously published data accessible in the GenBank database utilising the BLAST program. The MEGA7 program (http://www.megasoftware.net, July 2016) was utilised for multiple sequence alignments, employing the MUSCLE technique to align our sequences with the reference 28S rRNA and 16S rRNA gene sequences of *F. hepatica* and *F. gigantica* obtained from GenBank. Phylogenetic trees were generated utilising three distinct methodologies: maximum likelihood, neighbor-joining, and the Tamura-Nei model. The reliability of the node was assessed using 1,000 bootstrap repetitions to guarantee a strong tree topology.

Table 3: PCR protocol for *Fasciola* species DNA amplification (34,35)

Phase	Temperature	Time	cycles
Initial denaturation of DNA	94 °C	5 minutes	1 X
Denaturation of DNA	94 °C	30 seconds	35 X
Annealing of Primers	56 °C	30 seconds	35 X
Extension	72 °C	1 minute	35 X
Final extension	72 °C	5 minutes	1 X

Statistical analysis

The Statistical Package for the Social Sciences (Sigma plot 14.0) software (36) was employed for data analysis. The chi-squared (χ^2) test was utilized to evaluate the association between variables; a p-value below 0.05 was considered significant, whereas a value above that threshold was judged non-significant.

Results

Prevalence of fascioliasis

The flukes were identified in calcified bile ducts as illustrated in Fugure (1). Results from this investigation revealed that 19 livers were infected with fascioliasis, resulting in an infection rate of 9.5%. Infection rate in male cattle was 8.47%, whereas in female cattle it was 17.3%, with no statistically significant difference between the sexes as described in table 4. The correlation between infection rate and cattle age indicates that the highest infection rate, 26%, was observed in cattle older than 2 years, in contrast to 4.5% in cattle equal and younger than 2 years old, demonstrating a statistically significant difference between the age groups, as illustrated in table 5.

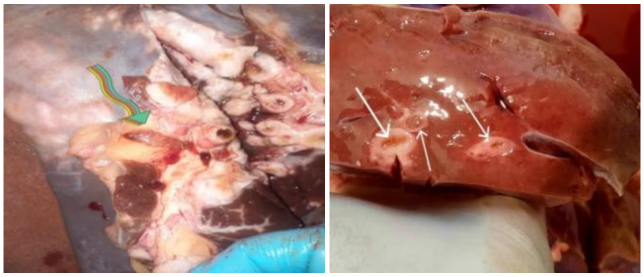

Figure 1: Indicates an infected liver incised with a blade to extract the flukes from the canaliculi of the bile ducts and surrounding tissues.

Table 4: Prevalence of fascioliasis in inspected liver of infected cattle according to sex

Sex	No. of inspected Livers	No. of Positive samples	Rate of infection %	P-value
Male	177	15	8.47 % a	
Female	23	4	17.3 % a	0.2262
Total	200	19	9.5 %	

Similar letters mean no significant differences at P<0.05.

In October and September, peak infection rates were reported at 16% and 15%, respectively, while August and February exhibited the lowest rates at 6.6% and 4%. Statistically, no significant differences were identified across the study months, as observed in table 6.

Table 5: Prevalence of fascioliasis in inspected liver of infected cattle according to age

Age	No. of inspected Livers	No. of Positive samples	Rate of infection %	P-value
Cattle ≤ 2yrs.	154	7	4.5 % b	
Cattle > 2yrs.	46	12	26.08 % a	0.0002
Total	200	19	9.5 %	-

Different letters mean no significant differences at P<0.05.

Table 6: Prevalence of fascioliasis in inspected liver of infected cattle according to study months

Months of study	No. of examined samples	No. of Positive samples	Infection rate %
August	15	1	6.6 % a
September	20	3	15 % a
October	25	4	16 % a
November	45	5	11.1 % a
December	40	3	7.5 % a
January	30	2	6.6 % a
February	25	1	4 % a
Total	200	19	9.5 % a

Similar litters mean no significant differences at P<0.05.

The highest rate 42.10% with fascioliasis was found in samples with more than 10 worms per infected liver and there was no significant difference in severity of infection among infected cattle as observed in table 7.

Table 7: Prevalence of fascioliasis according to severity of infection and Fasciola In inspected liver of infected cattle

Severity of infection (No. of flukes / infected liver)	No. of Positive samples	Rate of infection %
Low (1-5)	4	21.05% a
Moderate (5-10)	7	36.84% a
High > (10)	8	42.10% a
Total	19	9.5%

Similar letters mean no significant differences at P<0.05.

Inspected livers of slaughtered cattle revealed the presence with two species of *Fasciola* were identified during the study period. Infection with *F. gigantica* was more prevalent at 63.15%, while *F. hepatica* was found at 36.48 %. No significant difference was observed by infection with both species diagnosed in the livers, as shown in table 8.

Table 8: Prevalence of fascioliasis in inspected liver of infected cattle according to identified species

Identified *Fasciola* spp.	No. of Positive samples	Infection rate %	P-value
F. hepatica	7	36.48 % a	0.347
F. gigantica	12	63.15 % a	
Total	19	9.5 %	-

Similar litters mean no significant differences at P<0.05.

Macroscopic and microscopic identification

Identification of Fasciola species traditionally, morphological approaches were the exclusive means of distinguishing Fasciola species. Mature *F. hepatica*, in contrast to *F. gigantica*, was smaller, had a broader shoulder, a diminished ventral sucker, and an expanded cephalic cone.

The morphological study categorized the specimens as *Fasciola gigantica* and *Fasciola hepatica*. Figures 2.A and 2.B illustrate the complete adult anatomies of each species, respectively. The flukes were identified from the affected livers of slaughtered cattle, with the results summarized in table 9 and figure 2.

Characteristics among 32 isolated flukes were identified for *F. gigantica* included its measurement, 27-42 mm in length and 5-11 mm in width, Fugure 2, A, while 26 isolated flukes were identified as *F. hepatica* ranged from 15-35 mm in length and 7-17 mm in width Fugure 2, B. The average body length to width, along with their corresponding standard deviations (SD) were 32.2 ± 4.5 mm in length and 8.2 ± 1.7 mm in width for *F. gigantica*, while the average 27.9 ± 5.4 mm in length and 11.9 ± 3.3 mm in width for *F. hepatica*, as observed in table 9.

Table 9: Measurement of Fasciola spp. dimension by ordinary ruler (mm)

Isolated flukes	No.	Average±SD (mm)		Range (mm)	
		length	width	length	width
Fasciola gigantica	32	32.2±4.5	8.2±1.7	27-42	5-11
Fasciola hepatica	26	27.9±5.4	11.9±3.3	15-35	7-17

Figure 2: A: *F. gigantica*. B: *F. hepatica* (both spp. were measured with ordinary ruler; fresh, without staining).

Furthermore, the morphological traits offered a dependable foundation for differentiating between the two species in the analyzed samples microscopically in Figure (3).

Molecular study

The amplification procedures employing specific primers (Macrogen Inc., South Korea) produced findings demonstrating that the initial PCR effectively amplified the 28S rRNA gene, resulting in a DNA band of approximately 618 bp, thereby confirming the presence of *Fasciola hepatica* (Figure 4A). In the second PCR experiment, specific primers for *Fasciola gigantica* were employed to amplify the 16S rRNA gene, yielding a distinct band at 377 bp (Figure 4B). The third PCR experiment, employing specific primers for *F. gigantica*, yielded a 16S rRNA gene product with a band size of 529 bp (Figure 4C).

Phylogenetic tree

The phylogenetic analysis, which used partial sequences of the 28S ribosomal RNA gene and the 16S ribosomal RNA gene, showed a distinct clustering between the isolates of *Fasciola gigantica* and *Fasciola hepatica*, with *Taenia solium* serving as an outgroup to root the tree. All the main nodes achieved a re-branching value of 100%, indicating high confidence in the branching patterns. *F. gigantica* isolates formed two strongly supported subclades. The original branch included isolates from China, Vietnam, Iran, and Egypt, including sequences MW842574, MW001204, and others. Two isolates of the F. *gigantica* from our study (PV344638 and PV290745-PV290747) clustered between these two branches, showing significant genetic similarity with previously documented sequences from China and Egypt. The sequences of *F. hepatica* formed a distinct monophyletic branch, except for *F. gigantica*, including isolates from Bulgaria (HM369292), Greece (HM369163, HM369231 and HM369258), South Africa (ON682164), and Iraq (OR676758). Two isolates from this study (PV335564 and PV335557) showed a close evolutionary relationship with the *F. hepatica* group, demonstrating a significant similarity in genetic sequences with global isolates, especially those found in Greece and Bulgaria. A group of *F. gigantica* isolates, including sequences from Sudan and Egypt, along with three local isolates, formed a distinct cluster, indicating that *F. gigantica* has had a unique evolutionary path in Africa and the Middle East. The phylogenetic tree clearly shows the genetic divergence between *F. hepatica* and *F. gigantica*, while also displaying the genetic variation within *F. gigantica* groups from different geographical regions, including the newly found isolates from Iraq (Table 10, Figure 5).

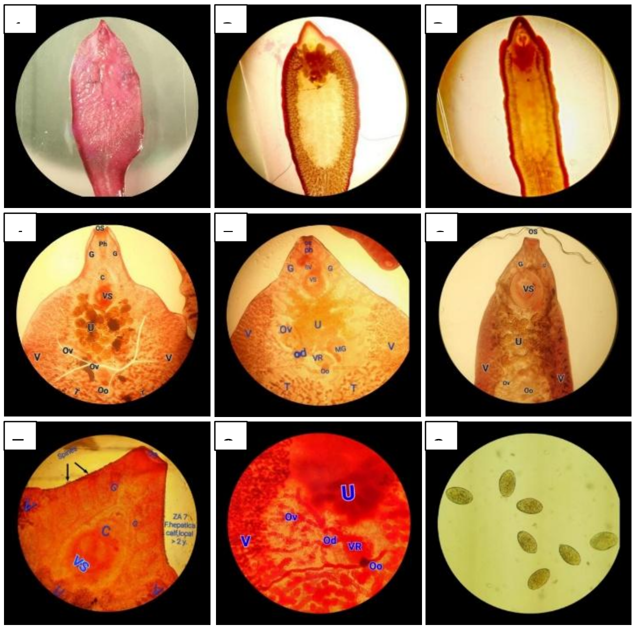

Figure 3: Several isolated *Fasciola* spp. entirely were mounted with acetocarmine stain, later examined with Dissecting and Light Microscope. Each representing photomicrographs depicting several structures of *Fasciola* species, including both fresh and fixed samples mounted. 1: an adult *Fasciola* species; 2: an adult *Fasciola hepatica*. 3: an adult *Fasciola gigantica*; 4, 5, 6 anteriar part under (D.M. 4X) & 7, 8 anteriar part under (L.M. 4X): illustrating the anatomical structures of *Fasciola* species; 9: eggs obtained from *Fasciola gigantica* under (L.M.) 10X. Two main gut caeca (G) extended from the pharynx (Ph) behind the oral sucker (OS), with the side branches of the gut (G) located above the reproductive organs. The cirrus (C) is positioned anterior to the ventral sucker (VS), also referred to as the acetabulum (A). Vitelline follicles (V) are distributed in two extensive lateral regions, whereas the two testes (T) exhibit significant branching and are in the medial section of the body. The vitelline reservoir (VR) near the back end of the Mehlis' gland complex (MG) comes from two main vitelline ducts, one on each side. The oviduct (od) carries eggs from the ovary (Ov) on the left side of the fluke to the ootype (Oo), where each egg connects with about 30 vitelline cells, leading to the creation of an eggshell around the group of cells. Fresh eggs are transported from the posterior, whereas mature eggs advance towards the gonopore, temporarily residing in the proximal coils of the uterus (U).

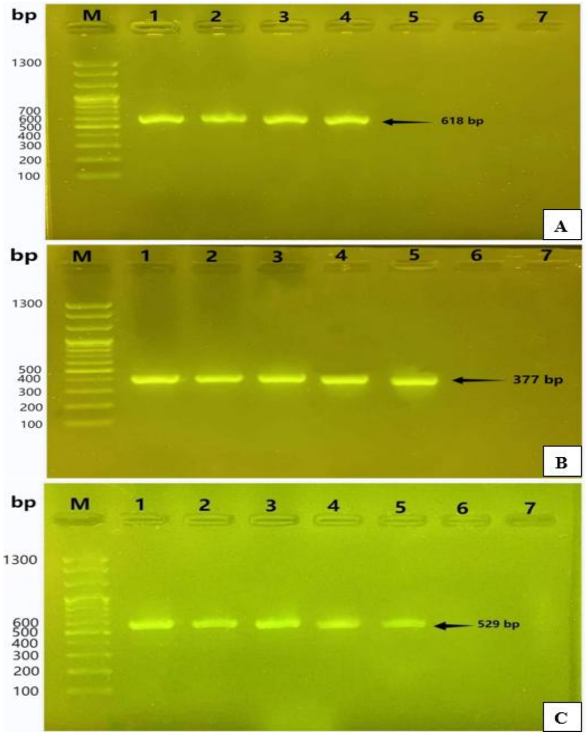

Figure 4: Results of PCR amplification by electrophoresis, 2% agarose gel and 3 µl of Green Safe dye: (A) The 28S rRNA gene shows a band size of 618 bp (Lanes 1–4: *Fasciola hepatica* samples; Lanes 5–7: negative controls); (B) The 16S rRNA gene shows a band size of 377 bp (Lanes 1–5: *Fasciola gigantica* samples; Lanes 6–7: negative controls); and (C) The 16S rRNA gene shows a band size of 529 bp (Lanes 1–5: *Fasciola gigantica* samples; Lanes 6–7: negative controls). Lane M: 100 base pair DNA ladder.

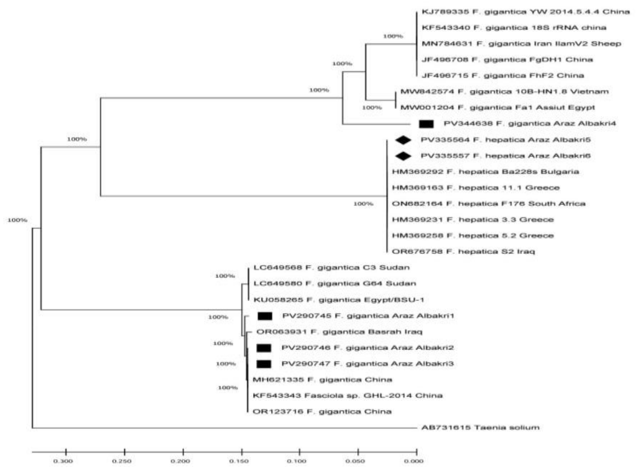

Figure 5: Phylogenetic relationship with global isolates based on 28S rRNA and 16S rRNA genes sequences of *F. hepatica* and *F. gigantica* isolated from the liver of cattle in Iraq.

Table 10: Degree of similarity between the global genetic sequences included in the Gene Bank and the genetic sequences of the isolates *Fasciola* species

Isolates	bp	Accession NO.	Country	Identity
F. gigantica isolate Araz_Albakri1	514	PV290745	Iraq, China, Egypt, Sudan	98-100%
F. gigantica isolate Araz_Albakri2	574	PV290746		
F. gigantica isolate Araz_Albakri3	573	PV290747		
F. gigantica isolate Araz_Albakri4	377	PV344638	China, Vietnam, Iran, Egypt	98-100%
F. hepatica isolate Araz_Albakri5	562	PV335564	Iraq, Bulgaria, Greece, South Africa	98-100%
F. hepatica isolate Araz_Albakri6	560	PV335557		

Discussion

Fascioliasis remains a great challenge to both animal and human health across global infection with significant economic threat through reduced livestock productivity, condemned livers, and treatment burdens a reality particularly in developing nations (8,37). While sporadic cases in Iraq are documented, our understanding suffers from a scarcity of comprehensive studies integrating robust prevalence data with precise molecular characterization of the causative *Fasciola* species.

Our investigation in Mosul province revealed a bovine fascioliasis prevalence of 9.5%. The study results sits within the broader spectrum observed across Iraq, where reported rates in cattle and sheep fluctuate markedly, ranging from 4% to over 39% in diverse governorates including Baghdad, Basra, Nineveh, Wasit, DhiQar, and Sulaimani (38). Factors like humidity, rainfall patterns, optimal temperature ranges (approximately 10-16°C), and inconsistencies in control programs like anthelmintic treatment schedules are main factors that fluctuate the prevalence (2). Consequently, regions with lower snail densities naturally exhibit reduced infection rates as highlighted by Ibrahim (39) and Mas-Coma *et al.* (6). Significant disparities emerge even between tropical zones with differing seasonal dynamics-extended dry periods versus those with minimal aridity. Our findings in Mosul also appear notably lower than rates documented in neighboring countries like Iran 66.6% in cattle (40) and Turkey 31.4% in sheep (41), underscoring potential regional epidemiological differences.

In this study the prevalence of Fascioliasis in cattle revealed an infection rate of 9.5% in Mosul city which was compatible with Barznji (42), which was 9.22% in Sulaimani abattoir, while more than studies conducted by Eman (43) in the Mosul abattoir, which was 6.79%.

Our data indicated a clear sex bias, with females exhibiting nearly double the infection rate of males (17.3% vs. 8.47%). This aligns with findings by Barznji (42) and Ismael and Chalabi (44), though contrasts with reports by Khan and Maqbool (45) and Nerway *et al.* (46). This female predisposition likely stems from fundamental husbandry practices. Female cattle, particularly dairy breeds, are typically retained longer within herds, accumulating exposure over extended lifespans before slaughter. Moreover, they often experience more frequent and prolonged pasturing the primary risk environment compared to males raised primarily for meat. Age also proved a critical determinant. Prevalence surged dramatically from 4.5% in cattle ≤ 2 years to 26% in older animals (>2 years). This pattern echoes observation by Al-Delemi (47), Barznji (42), and Abass *et al.* (48), though diverges from Belay *et al.* (49). The high prevalence in adult likely results from their sustained grazing activity, often near water sources (springs, streams, rivers) where metacercariae encyst on vegetation (50,51). Additionally, the chronic stage of fascioliasis means infections acquired earlier often manifest pathogenesis more severely in older animals, as noted by Al-Barwary (52). The prevalence demonstrated a distinct seasonal peak in our study, rising through September (15%), October (16%), and November (11%). This autumnal surge parallels observations by Koyee (53) and Albishtue *et al.* (54). However, contrasting patterns exist: Barznji (42) reported higher winter rates (Dec.-Feb.), while Hussain and Zghair (55) noted peaks in Spring Mars to April. Reconciling these discrepancies requires careful consideration. Geographic variation in climate, snail ecology, study duration, sampling intensity, and livestock management systems (e.g., indoor vs. outdoor feeding prevalence) are all plausible contributors (2). Furthermore, the timing of liver inspections relative to initial metacercarial ingestion is crucial. Given the 3-4 months maturation period of adult flukes (56), infections detected in Autumn often originate from metacercarial uptake occurring in late Spring or early Summer. Differences in whether studies primarily examined locally raised versus imported cattle at slaughter could also influence observed seasonal trends.

Morphological assessment, examining features like body dimensions, shape, and cephalic cone structure in adult flukes (22), remains indispensable, especially in field settings or regions with limited molecular resources. Our morphometric analysis yielded average sizes of (27.9 ± 5.4 x 11.9 ± 3.3) mm for *F. hepatica* and (32.2 ± 4.5 x 8.2 ± 1.7) mm for *F. gigantica*. These measurements align reasonably well with some studies (57,58) for *F. gigantica* but show expected variation compared to others (42). Such variation underscores the importance of context but confirms morphology's utility in distinguishing these species reliably when applied systematically (59,60).

To provide definitive identification and address potential limitations of morphology alone, we employed molecular characterization targeting the 28S rRNA and 16S rRNA genes. This approach is well-established in the literature (references 41-46 per your numbering). While the 16S rRNA gene is valuable in broader phylogenetic analyses, its resolution for separating closely related species like *F. hepatica* and *F. gigantica* can be insufficient alone. Therefore, it is often used synergistically with other markers (e.g., ITS-1, COI). Crucially, the nuclear 28S rRNA gene has proven highly effective for precise species discrimination, genotyping, and elucidating phylogenetic relationships within *Fasciola* species (14,61-66). However, according to the alignment of field isolates in different genomes, four species of Fasciola belong to *Fasciola gigantica*, whereas two species belong to *Fasciola hepatica*. Different sequences, *Fasciola gigantica* was from China, Egypt and Sudan while and *Fasciola hepatica* was from South Africa, Greece and Bulgaria, suggesting different places of parasite infection. In addition to the genetic relationship between the sequences in the current study and other countries, this may also be related to the legitimate and illegal trafficking of exported animals from different countries. The study results are compatible with most studies in North of Iraq like the studies of Othman and Hamm (67) found both *Fasciola* species, as reported by other researchers, such as Rekani and Mero (68), Mohammed *et al.* (69) and Raouf *et al.* (70) in the Sulaymaniyah Governorate, Simsek *et al.* (71) in Turkey, Shafiei *et al.* (72) and Sedighe *et al.* (73) in Iran, and Alkahtani *et al.* (74) in Saudi Arabia.

Conclusion

The study results indicated a higher prevalence of fascioliasis compared to previous studies referred as endemic in Iraq due to its ideal climate and continued livestock imports. The prevalence was higher in females than in males as well as in old cattle was higher than in younger ones, as noticed by liver examination. Both common species are present in Iraq, with *Fasciola gigantica* being the most common and more accurately by molecular study results confirm that the isolates were from Iraq and imported livestock.

routine fecal egg detection are essential periodically to detect the spread of chronic fascioliasis, furthermore epidemiological study is recommended to evaluate fascioliasis in definitive and intermediate hosts and importantly caution should be exercised because the disease is zoonotic, and its high prevalence poses a risk to humans, extensively In the field of animal health, veterinarians, farmers, rural communities, meat inspectors, authorities, and legislators must work together to control the disease. Molecular studies are essential to identify and classify *Fasciola* species and their origin, to prevent and control the disease additionally with specific anthelmintics, as the intermediate form is resistant to most anthelmintics.

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Conflict of interest

The authors affirm that the paper has no conflicts of interest.

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التشخيص المظهري والجزيئي لأنواع ديدان المسطحة الكبدية مع دراسة انتشار داء حلزون الكبد في الأبقار المذبوحة في منطقة الموصل- العراق

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الخلاصة

تُعد أنواع *Fasciola* من الطفيليات المهمة التي تُسبب مرض الفاشيولا، وهو مرض طفيلي يشكل تهديدًا كبيرًا على صحة وإنتاجية المجترات في جميع أنحاء العالم، بما في ذلك العراق. هدفت هذه الدراسة إلى تقصي داء الفاشيولا وتقدير معدل انتشاره في الأبقار المذبوحة في مجازر مدينة الموصل خلال الفترة من آب ٢٠٢٤ وحتى نهاية شباط ٢٠٢٥. تم جمع الديدان الكبدية من الأكباد المصابة لإجراء الفحص المظهري والتحليل الجزيئي، اعتمادًا على تضخيم الجينات وتسلسلها، ومن ثم تحليل العلاقات الوراثية. من بين ٢٠٠ عينة كبدية تم فحصها، بلغ معدل الإصابة الكلي ٩,٥%. كانت الإصابة أعلى في الإناث، لكنها لم تكن ذات دلالة إحصائية. وسُجلت أعلى نسبة إصابة في الأبقار التي يزيد عمرها عن سنتين، مع وجود فروق ذات دلالة إحصائية. كما كانت معدلات الإصابة الشهرية الأعلى في شهري أيلول وتشرين الأول، دون أن تصل هذه الفروق إلى مستوى الدلالة الإحصائية. أكد الفحص المظهري باستخدام صبغة الكارمين وجود نوعين من الطفيلي: *Fasciola gigantica* و*Fasciola hepatica.* أظهرت التحاليل الجزيئية المعتمدة على تضخيم جيني 28S rRNA و16S rRNA، إلى جانب الخصائص الشكلية، فعالية عالية في التحديد الدقيق للأنواع باستخدام تقنية PCR التقليدية. وتم الكشف عن ثلاثة حزم مميزة: ٣٧٧ زوج قاعدي و ٥٢٩ زوج قاعدي دالة على *F. gigantica* ، و٦١٨ زوج قاعدي خاصة بـ *F. hepatica*. أظهر تحليل التسلسل الجيني تطابقًا تراوح بين ٩٨–١٠٠% مع سلالات معروفة من أنواع الفاشيولا. وكشفت الدراسة عن وجود تمايز وراثي واضح بين نوعي *F. gigantica* و*F. hepatica*، إلى جانب تنوع جيني ملحوظ ضمن تجمعات *F. gigantica*، شمل عزلات عراقية جديدة PV344638 وPV290745–PV290747 بالإضافة إلى ذلك، أظهرت عزلتان من الدراسة الحالية PV335564 وPV335557 تقاربًا وراثيًا كبيرًا مع سلالات معروفة من *F. hepatica*.