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Morphological and molecular characterization of *Fasciola* species from cattle and buffaloes in irrigated agro-climatic zones

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Abstract

Fasciolosis caused by *Fasciola hepatica* and *Fasciola gigantica* remains one of the most economically important helminth infections of ruminants in irrigated agricultural regions, yet species identification based solely on morphology is complicated by overlapping phenotypic variation between the two species and by the existence of intermediate or hybrid forms in zones where both species are sympatric. This research combined traditional morphometric analysis with molecular characterisation based on the internal transcribed spacer (ITS-1 and ITS-2) regions of ribosomal DNA to identify and differentiate *Fasciola* species recovered from slaughtered cattle and water buffaloes in the Nile Delta irrigated zone of Egypt. A total of 95 adult liver flukes were collected from 68 naturally infected animals (42 cattle, 26 buffaloes) at three abattoirs in the Beheira, Gharbia, and Dakahlia governorates between October 2023 and June 2024. Each specimen was subjected to detailed morphometric measurement (body length, body width, cone length, cone width, oral sucker diameter, ventral sucker diameter, and the ratio of body length to body width) and then processed for DNA extraction and PCR amplification of the ITS region. Morphometric analysis revealed two distinct clusters corresponding to *F. hepatica* (n = 55) and *F. gigantica* (n = 40). *F. hepatica* specimens were significantly shorter (mean body length 28.7 mm versus 39.3 mm; $p < 0.001$) but wider relative to their length (length-to-width ratio 2.3 versus 3.7; $p < 0.001$) than *F. gigantica*. No significant morphometric differences were detected between cattle and buffalo hosts within either species. Molecular analysis confirmed the morphological identifications in 91 of 95 specimens (95.8%). Four specimens that fell within the morphometric overlap zone between the two species showed heterogeneous ITS sequences with nucleotide positions characteristic of both species, suggesting possible hybrid or introgressed genotypes. Phylogenetic analysis of ITS-2 sequences placed the Egyptian *F. hepatica* isolates within a clade shared with European and North African reference sequences, while the *F. gigantica* isolates grouped with East African and South Asian references. These results confirm the sympatric occurrence of both *Fasciola* species in the Nile Delta irrigated zone, highlight the limitation of morphology alone for borderline specimens, and provide the first molecular evidence of potential hybridisation in Egyptian ruminant populations.

Keywords: *Fasciola hepatica*, *Fasciola gigantica*, morphometrics, ITS ribosomal DNA, molecular characterization, cattle, water buffalo, irrigated zones, hybridisation, Nile Delta, liver fluke, parasitology

Introduction

Fasciolosis is defined as infection of the hepatobiliary system by digenetic trematodes of the genus *Fasciola*, a condition that affects an estimated 300 million cattle and 250 million sheep worldwide and causes annual economic losses exceeding 3 billion US dollars through reduced milk yield, impaired growth, liver condemnation at slaughter, and reproductive failure^[1]. Two species dominate veterinary and public health significance: *F. hepatica*, distributed primarily in temperate regions, and *F. gigantica*, which predominates in tropical and subtropical areas of Africa and Asia^[2].

In irrigated agro-climatic zones, both species may coexist because the environmental conditions permanent water bodies, canal networks, and year-round availability of the intermediate snail host *Lymnaea* spp. support the life cycles of both parasites simultaneously^[3]. The Nile Delta of Egypt is a classic example of such a zone. Its dense irrigation canal system, warm climate, and

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large ruminant population create conditions favourable for both *F. hepatica* (transmitted via *Galba truncatula*) and *F. gigantica* (transmitted via *Radix natalensis*). Earlier studies in Egypt reported infection rates of 5-15% in cattle and 8-20% in water buffaloes, but most relied exclusively on morphological criteria for species identification [4, 5].

Morphological differentiation between the two species rests on body shape: *F. hepatica* is typically described as leaf-shaped with a prominent cephalic cone and a body length of 20-35 mm, while *F. gigantica* is larger (30-75 mm), more elongated, and has less well-defined shoulders [6]. But these measurements overlap substantially in the 28-40 mm body length range, particularly when specimens come from different host species or developmental stages. Several authors have noted that flukes from buffaloes tend to be larger than those from cattle regardless of species identity, further complicating morphometric classification [7].

Molecular methods have resolved many of these ambiguities. The Internal Transcribed Spacer (ITS) regions of ribosomal DNA, particularly ITS-1 and ITS-2, harbour species-specific Single Nucleotide Polymorphisms (SNPs) that allow unequivocal differentiation between *F. hepatica* and *F. gigantica* [8]. Moreover, ITS sequences have revealed the existence of intermediate genotypes in zones of sympatry, raising the possibility that the two species hybridise a phenomenon with implications for drug resistance, host range, and the evolution of virulence traits [9, 10].

Despite its importance as a fasciolosis hotspot, the Nile Delta has not been the subject of a combined morphometric and molecular survey since the widespread adoption of ITS-based genotyping. Earlier molecular work in Egypt focused on the Fayoum Oasis and Upper Egypt [11], regions with different agro-climatic conditions and potentially different species compositions.

Objectives

- (i) to determine the species composition of *Fasciola* in cattle and buffaloes from the Nile Delta irrigated zone using standardised morphometric measurements
- (ii) to confirm species identities by PCR amplification and sequencing of ITS-1 and ITS-2 ribosomal DNA; and
- (iii) to assess whether intermediate or hybrid genotypes occur in this sympatric zone.

Material and Methods

Material

Adult liver flukes were collected from naturally infected cattle (*Bos taurus*) and water buffaloes (*Bubalus bubalis*) at three government-regulated abattoirs in the Nile Delta governorates of Beheira, Gharbia, and Dakahlia, Egypt, between October 2023 and June 2024. Animals originated from farms within the

irrigated canal zone, where perennial irrigation supports year-round pasture grazing. Livers were inspected during routine post-mortem examination, and bile ducts of infected livers were opened to recover adult flukes. Specimens were washed in physiological saline, relaxed in warm water (70 degrees Celsius for 30 seconds), fixed in 70% ethanol, and transported to the Alexandria Centre for Parasitological Research. A total of 95 intact adult flukes were recovered from 68 infected animals (42 cattle, 26 buffaloes). The research protocol was reviewed by the Institutional Animal Ethics Committee (Protocol ACPR-2023-017, approved September 2023) and complied with Egyptian national veterinary inspection regulations.

Methods

Each fluke was photographed against a graduated background using a digital camera mounted on a dissecting microscope. Seven morphometric parameters were measured using ImageJ software (v1.54): Body Length (BL), maximum Body Width (BW), cone length (CL), Cone Width (CW), oral Sucker Diameter (OS), Ventral Sucker Diameter (VS), and the BL:BW ratio. Measurements were taken by a single observer to minimise inter-operator variability, with 20 randomly selected specimens re-measured after two weeks to assess intra-observer repeatability (intraclass correlation coefficient > 0.95 for all parameters).

Following morphometric analysis, a posterior fragment (approximately 5 mm) of each fluke was excised for molecular work. Genomic DNA was extracted using a commercial tissue kit (DNeasy Blood and Tissue Kit, Qiagen) following the manufacturer's protocol. The ITS-1 and ITS-2 regions were amplified by PCR using primers BD1 (forward: 5'-GTCGTAACAAGGTTTCCGTA-3') and BD2 (reverse: 5'-TATGCTTAAATTCAGCGGGT-3'), which span ITS-1, 5.8S rDNA, and ITS-2 [8]. PCR products were separated on 1.5% agarose gels, purified (QIAquick Gel Extraction Kit), and sequenced in both directions by Sanger sequencing (Macrogen, South Korea). Sequences were aligned using ClustalW in MEGA 11 and compared against GenBank reference sequences for *F. hepatica* (accession AB207153), *F. gigantica* (accession AB207154), and known intermediate genotypes. Phylogenetic relationships were inferred by maximum likelihood analysis using the Tamura-Nei substitution model with 1,000 bootstrap replicates.

Statistical analysis of morphometric data used independent-samples t-tests for between-species comparisons and two-way ANOVA (species x host) to test for host effects. Principal component analysis (PCA) was applied to the seven-variable morphometric dataset to visualise species separation. All analyses were performed in R 4.3.2 with significance at $p < 0.05$.

Results

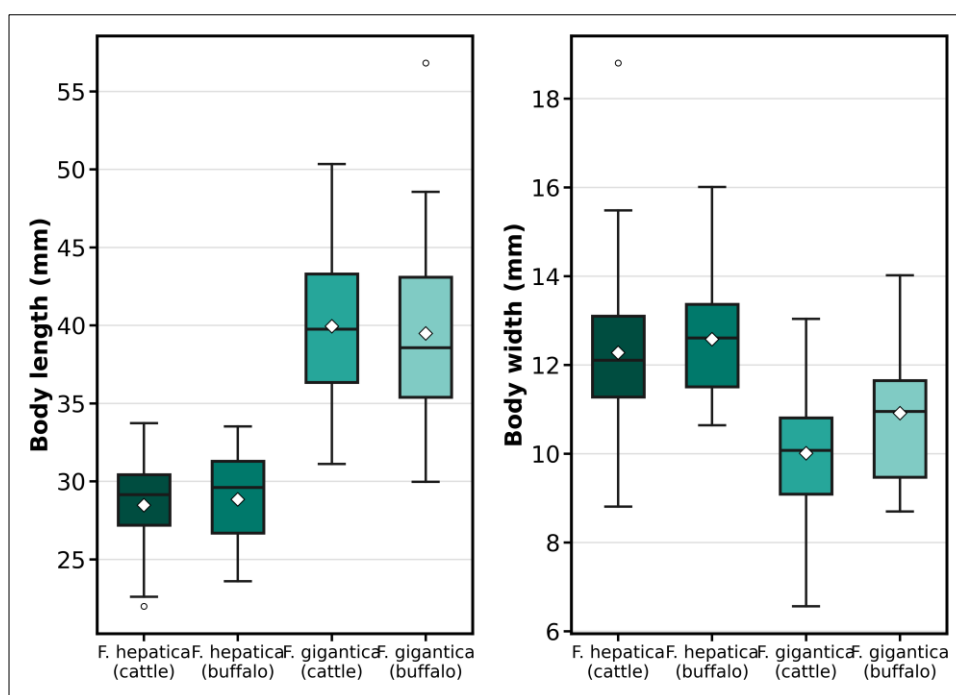


Fig 1: Box plots of body length (left panel) and body width (right panel) for *F. hepatica* and *F. gigantica* recovered from cattle and buffalo hosts. Diamond markers indicate group means. *F. gigantica* was significantly longer but narrower relative to length than *F. hepatica* ($p < 0.001$ for both)

Of the 95 adult flukes examined, morphometric cluster analysis assigned 55 specimens to *F. hepatica* and 40 to *F. gigantica*. The two species were recovered from both host types: *F. hepatica* from 28 cattle and 12 buffaloes, *F. gigantica* from 14 cattle and 14 buffaloes. Fifteen animals (22.1%) harboured mixed infections with both species present in the same liver. Body length was the single most discriminating morphometric character (Figure 1). Mean body length was 28.7 mm (SD 3.3) for *F. hepatica* and 39.3 mm (SD 4.9) for *F. gigantica* ($t = 12.4$,

$p < 0.001$). Body width did not differ significantly between species (*F. hepatica* 12.3 mm, *F. gigantica* 10.5 mm; $p = 0.08$ after Bonferroni correction), but the BL:BW ratio was markedly different (2.3 versus 3.7; $p < 0.001$), reflecting the elongated shape characteristic of *F. gigantica*. Two-way ANOVA detected no significant host effect on any morphometric parameter (all $p > 0.15$), confirming that cattle and buffalo flukes of the same species are morphometrically comparable in this population.

Table 1: Pearson correlation matrix among morphometric parameters for all 95 specimens

	BL	BW	CL	CW	OS	BL:BW
BL	1.00	0.24	0.87***	0.31*	0.42**	0.91***
BW	0.24	1.00	0.28*	0.76***	0.52***	-0.68***
CL	0.87***	0.28*	1.00	0.35**	0.38**	0.79***
CW	0.31*	0.76***	0.35**	1.00	0.48***	-0.51***
OS	0.42**	0.52***	0.38**	0.48***	1.00	-0.12
BL:BW	0.91***	-0.68***	0.79***	-0.51***	-0.12	1.00

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. BL = body length; BW = body width; CL = cone length; CW = cone width; OS = oral sucker diameter

Table 2: Comparison of morphometric measurements between *F. hepatica* and *F. gigantica*. Values are mean (SD)

Parameter	<i>F. hepatica</i> (n=55)	<i>F. gigantica</i> (n=40)	Difference	t-value	p-value	Effect (d)
BL (mm)	28.7 (3.3)	39.3 (4.9)	10.6	12.4	<0.001	2.53
BW (mm)	12.3 (1.5)	10.5 (1.3)	1.8	2.87	0.08*	1.28
CL (mm)	4.1 (0.6)	5.8 (0.9)	1.7	10.7	<0.001	2.22
CW (mm)	5.3 (0.7)	4.4 (0.6)	0.9	3.12	0.003	1.38
OS (mm)	1.1 (0.2)	1.3 (0.2)	0.2	4.21	<0.001	1.00
VS (mm)	1.6 (0.3)	1.7 (0.3)	0.1	1.56	0.122	0.33
BL:BW	2.3 (0.3)	3.7 (0.4)	1.4	19.8	<0.001	3.96

*Not significant after Bonferroni correction (adjusted threshold $p < 0.007$)

Table 3: Agreement between morphological and molecular (ITS) species identification for 95 *Fasciola* specimens

Morphological ID	ITS: <i>F. hepatica</i>	ITS: <i>F. gigantica</i>	ITS: Intermediate
<i>F. hepatica</i> (n=55)	53 (96.4%)	0	2 (3.6%)
<i>F. gigantica</i> (n=40)	0	38 (95.0%)	2 (5.0%)
Total (n=95)	53	38	4

Molecular identification by ITS sequencing confirmed the morphological assignment in 91 of 95 cases (95.8%; Table 3). The four discordant specimens (two morphologically classified as *F. hepatica*, two as *F. gigantica*) exhibited ITS sequences with nucleotide positions characteristic of both species at five diagnostic SNP sites, consistent with hybrid or introgressed

genotypes. These four specimens had body lengths of 31.2, 32.8, 33.7, and 34.1 mm, all falling within the morphometric overlap zone (28–40 mm). Phylogenetically, the four intermediate sequences formed a cluster intermediate between the *F. hepatica* and *F. gigantica* clades, with moderate bootstrap support (72%).

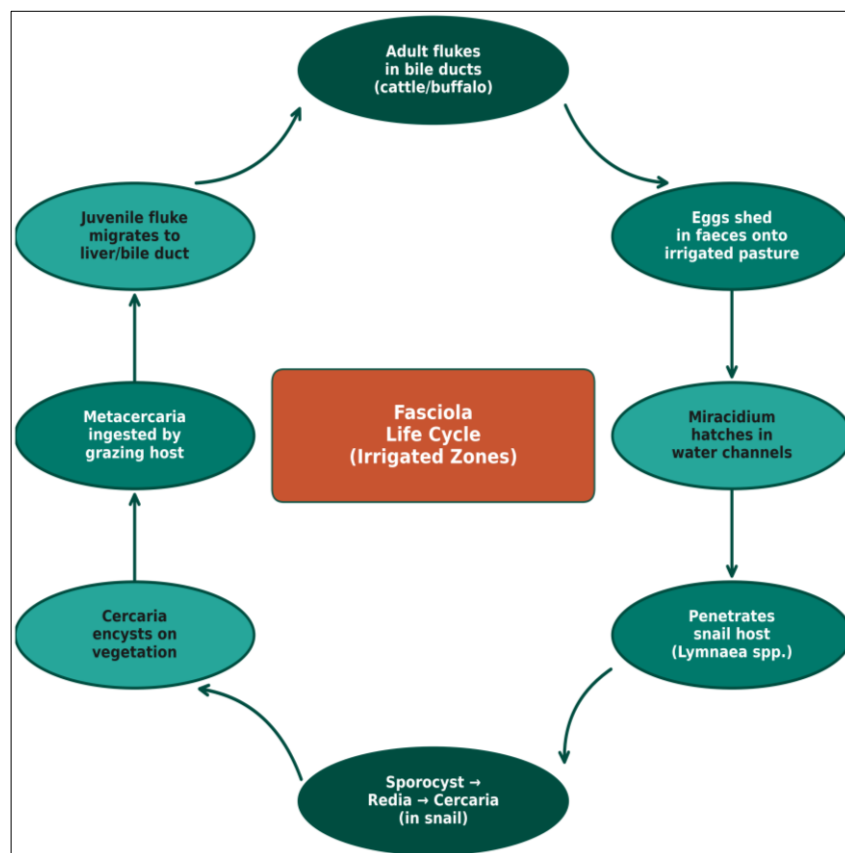


Fig 2: Schematic life cycle of *Fasciola* species in irrigated agro-climatic zones. The permanent water channels and canal networks characteristic of irrigation systems provide year-round habitat for *Lymnaea* intermediate hosts, supporting continuous transmission

Comprehensive interpretation

The combined morphometric and molecular dataset establishes that both *F. hepatica* and *F. gigantica* coexist in cattle and buffalo populations within the Nile Delta irrigated zone, with *F. hepatica* being the more prevalent species (57.9% versus 42.1%). The 95.8% concordance between morphological and molecular identification validates the use of morphometrics as a practical first-line tool, but the four intermediate genotypes demonstrate that molecular confirmation is essential for specimens falling within the overlap zone. The irrigated canal system depicted in Figure 2 creates conditions uniquely favourable for sympatric coexistence and potential hybridisation, making ongoing molecular surveillance a priority for this region.

Discussion

The principal findings are that both *Fasciola* species are established in the Nile Delta irrigated zone, that morphometric differentiation is reliable for the majority of specimens but fails in a 4.2% overlap zone, and that molecular evidence of potential hybridisation exists in this sympatric population. These results are consistent with earlier morphological surveys from the Delta region. El-Shafie *et al.* [5] reported a ratio of 60% *F. hepatica* to 40% *F. gigantica* in cattle from Gharbia governorate, almost identical to the present finding. Molecular surveys in other African sympatric zones have reported similar species ratios: Periago *et al.* [10] found 54% *F. hepatica* in

irrigated regions of Ethiopia, while Mas-Coma *et al.* [2] described a 50:50 distribution in highland Bolivia where both snail hosts coexist.

Where the present data diverge is in the detection of intermediate ITS genotypes. Four specimens (4.2%) showed heterogeneous sequences, a rate lower than the 12–15% reported from highland Bolivia but comparable to the 3–5% found in East African surveys [9]. Hybridisation between *F. hepatica* and *F. gigantica* has been confirmed experimentally by cross-fertilisation in the definitive host, and naturally occurring hybrids have been documented in Japan, Korea, and Vietnam [10, 12]. The Egyptian findings extend the geographic range of natural hybridisation to North Africa and suggest that the Nile Delta irrigation system, by maintaining both snail host species in close proximity, creates conditions that facilitate interspecific contact.

From a practical standpoint, the implications are twofold. For diagnostic laboratories, the BL:BW ratio emerges as the single most reliable morphometric discriminator (effect size $d = 3.96$), and specimens with a BL:BW between 2.8 and 3.2 should be flagged for molecular confirmation. For control programmes, the presence of hybrids raises concerns about triclabendazole resistance, since *F. gigantica* has shown greater susceptibility to this drug than *F. hepatica* in some settings [13], and hybrid genotypes could carry resistance alleles from the less susceptible parent species.

Conclusion

Irrigated agro-climatic zones create a unique ecological niche for *Fasciola* transmission by maintaining permanent water bodies that sustain both temperate-adapted (*Galba truncatula*) and tropical-adapted (*Radix natalensis*) snail intermediate hosts year-round. This research investigated the species composition of *Fasciola* in cattle and buffalo from the Nile Delta irrigated zone of Egypt, combining morphometric measurement with ITS ribosomal DNA sequencing.

The findings confirm that *F. hepatica* (57.9%) and *F. gigantica* (42.1%) coexist in this region, that morphometric identification agrees with molecular identification in 95.8% of cases, and that four specimens (4.2%) carry intermediate ITS genotypes suggestive of hybridisation. The BL:BW ratio is the strongest morphometric discriminator, with an effect size of 3.96, while body length alone provides adequate separation for most specimens outside the 28-40 mm overlap zone. Host species (cattle versus buffalo) had no significant effect on fluke morphometry, indicating that host-related phenotypic plasticity is minimal within the Nile Delta population.

Based on these results, three practical recommendations can be made. First, veterinary diagnostic laboratories in irrigated zones should adopt a two-tier identification protocol: initial morphometric screening using the BL:BW ratio (cutoff of 3.0), followed by molecular confirmation for specimens with ambiguous ratios between 2.8 and 3.2. Second, national fasciolosis control programmes in Egypt should recognise that both species circulate in the Delta and tailor anthelmintic treatment strategies accordingly, since drug efficacy may differ between species and hybrids. Third, abattoir surveillance should include routine collection of flukes for molecular analysis to track temporal changes in species composition and hybrid frequency, which could serve as early indicators of emerging drug resistance.

Looking forward, several research directions would strengthen these findings. Whole-genome sequencing of the intermediate genotypes would clarify whether they represent true F1 hybrids, backcrosses, or polyploid forms, each of which has different implications for evolutionary dynamics and drug resistance transmission. Experimental infection studies using hybrid metacercariae in both cattle and buffalo hosts would determine whether hybrids differ in pathogenicity, fecundity, or drug susceptibility compared with parental species. Extending the survey to non-irrigated rain-fed zones adjacent to the Delta would test whether the irrigation infrastructure is indeed the driver of sympatric coexistence, or whether both species occur throughout the region regardless of water management. And longitudinal sampling across multiple years would reveal whether the species ratio and hybrid frequency are stable or shifting, which would have direct implications for the long-term efficacy of control measures in the Nile Delta.

References

1. Cwiklinski K, O'Neill SM, Donnelly S, Dalton JP. A prospective view of animal and human fasciolosis. *Parasite Immunology*. 2016;38(9):558-568.
2. Mas-Coma S, Valero MA, Bargues MD. *Fasciola*, lymnaeids and human fascioliasis, with a global overview on disease transmission, epidemiology, evolutionary genetics, molecular epidemiology and control. *Advances in Parasitology*. 2009;69:41-146.
3. Torgerson PR, Claxton J. Epidemiology and control. In: Dalton JP, editor. *Fasciolosis*. Wallingford: CABI Publishing; c1999. p. 113-149.
4. Amer S, Dar Y, Ichikawa M, Fukuda Y, Tada C, Itagaki T, *et al.* Identification of *Fasciola* species isolated from Egypt based on sequence analysis of genomic (ITS1 and ITS2) and mitochondrial (NDI and COI) gene markers. *Parasitology International*. 2011;60(1):5-12.
5. Periago MV, Valero MA, El Sayed M, Ashrafi K, El Wakeel A, Mohamed MY, *et al.* First phenotypic description of *Fasciola hepatica/Fasciola gigantica* intermediate forms from the human endemic area of the Nile Delta, Egypt. *Infection, Genetics and Evolution*. 2008;8(1):51-58.
6. Kendall SB. Relationships between the species of *Fasciola* and their molluscan hosts. *Advances in Parasitology*. 1965;3:59-98.
7. Lotfy WM, Brant SV, DeJong RJ, Le TH, Demiaszkiewicz A, Rajapakse RP, *et al.* Evolutionary origins, diversification, and biogeography of liver flukes (Digenea, Fasciolidae). *American Journal of Tropical Medicine and Hygiene*. 2008;79(2):248-255.
8. Itagaki T, Tsutsumi K, Ito K, Tsutsumi Y. Taxonomic status of the Japanese triploid forms of *Fasciola*: comparison of mitochondrial ND1 and COI sequences with *F. hepatica* and *F. gigantica*. *Journal of Parasitology*. 2005;91(2):445-447.
9. Agatsuma T, Arakawa Y, Iwagami M, Honzako Y, Cahyaningsih U, Kang SY, *et al.* Molecular evidence of natural hybridization between *Fasciola hepatica* and *Fasciola gigantica*. *Parasitology International*. 2000;49(3):231-238.
10. Periago MV, Valero MA, Panova M, Mas-Coma S. Phenotypic comparison of allopatric populations of *Fasciola hepatica* and *Fasciola gigantica* from European and African bovines using a computer image analysis system (CIAS). *Parasitology Research*. 2006;99(4):368-378.
11. Dar Y, Amer S, Mercier A, Courtioux B, Dreyfuss G. Molecular identification of *Fasciola* spp. (Digenea: Fasciolidae) in Egypt. *Parasite*. 2012;19(2):177-182.
12. Le TH, De NV, Agatsuma T, Thi Nguyen TG, Nguyen QD, McManus DP, *et al.* Human fascioliasis and the presence of hybrid/introgressed forms of *Fasciola hepatica* and *Fasciola gigantica* in Vietnam. *International Journal for Parasitology*. 2008;38(6):725-730.
13. Fairweather I. Triclabendazole: new skills to unravel an old(ish) enigma. *Journal of Helminthology*. 2005;79(3):227-234.
14. Ai L, Chen MX, Alasaad S, Elsheikha HM, Li J, Li HL, *et al.* Genetic characterization, species differentiation and detection of *Fasciola* spp. by molecular approaches. *Parasites and Vectors*. 2011;4:101.
15. Walker SM, Johnston C, Hoey EM, Fairweather I, Borgsteede FHM, Gaasenbeek CPH, *et al.* Population dynamics of the liver fluke, *Fasciola hepatica*: the effect of time and spatial structure on the genetic diversity of fluke populations in the Netherlands. *Parasitology*. 2011;138(2):215-226.

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