

Prevalence and risk factors analysis of fasciolid trematodes with phylogenetic analysis of *Fasciola gigantica* in small ruminants of Malakand Division, Pakistan

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Summary

Fasciolosis infects small ruminants, reduces productivity, and causes considerable economic losses to livestock producers. Despite its economic and veterinary importance, there is a lack of comprehensive studies on fasciolosis in the Malakand Division, Pakistan. Therefore, the study aimed to investigate the prevalence and risk factors associated with fasciolosis in small ruminants in Malakand Division. A total of 1307 rectal fecal samples of sheep and 1274 of goats were collected randomly. The collected fecal samples were examined for Fasciolid eggs using the standard sedimentation technique, followed by microscopic identification based on egg morphology and operculum characteristics. The overall prevalence of fasciolosis in sheep and goats was 3.9 % and 2.04 %, respectively. The present study showed significant differences based on the Chi-square test ($P < 0.05$) in the prevalence of fasciolosis among animals of different ages, health conditions, drinking water sources, treatment status, and across the four seasons of the study year. Fasciolosis was observed to be significantly higher in young sheep and goats (7.08 %, 2.88 %), in untreated (4.45 %, 2.27 %), emaciated (5.88 %, 5.15 %), stream water drinking (5.41 %, 2.51 %), and during the summer season (6.48 %, 2.98 %), respectively. Microscopic examination revealed the presence of two *Fasciola* species, *Fasciola hepatica* and *Fasciola gigantica*, in fecal samples. However, the internal transcribed spacer (ITS) gene amplification and sequencing were performed for *Fasciola gigantica* to confirm the species identity and explore genetic relationships. Phylogenetic analysis based on the ITS gene, using a neighbor-joining method implemented in MEGA 11 software, revealed a close genetic relationship between isolates from the Malakand Division and those from Egypt, Iran, Nigeria, and Saudi Arabia. The present study highlights the occurrence of *Fasciola* species in the area, underscoring age, health status, and treatment practices as key risk factors. These findings provide a foundation for the development of effective control strategies against fasciolosis in small ruminants in the Malakand Division.

Keywords: *Fasciola*; fasciolosis; prevalence; risk factors; small ruminants; Malakand Division

Introduction

Fasciolosis is one of the most neglected food- and water-borne zoonotic diseases of cattle, buffaloes, sheep, goats, horses, and

humans of all ages. It is the most important helminth infectious disease worldwide (Alkahtani *et al.*, 2024). Fasciolosis is caused by the ingestion of encysted metacercariae of digenetic fasciolid trematodes, *Fasciola hepatica* and *Fasciola gigantica* (Najib *et*

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al., 2020; Nkurunziza *et al.*, 2024). Fasciolid trematodes are commonly called flatworms or liver flukes, which belong to the family Fasciolidae of the class Trematoda of the phylum Platyhelminthes. The presence of cephalic cones and shoulders in the body of *Fasciola hepatica* and their absence in *Fasciola gigantica* are the two most common morphological characteristics that distinguish these two species (Ahmed *et al.*, 2005; Valino *et al.*, 2017). According to geographical distribution, there are some differences between the occurrence of *F. hepatica* and *F. gigantica* because *F. hepatica* is distributed across nearly all continents of the world, while *F. gigantica* is mostly specific to the parts of Asia and Africa, including Pakistan (Abdullah, 2023; Caravedo & Cabada, 2020; Tang *et al.*, 2021; Afshan *et al.*, 2014a). To complete their life cycle, fasciolids use two types of hosts: an intermediate host (specific host) and a definitive host (Ibrahim, 2017). Two species of snails, *Lymnaea natalensis* and *Lymnaea truncatula*, are the most common intermediate hosts of fasciolosis in rainy muddy areas. While cattle, buffalo, goats, sheep, and horses are the definitive hosts (Ulhaq *et al.*, 2022). During the transmission of the disease, buffaloes, sheep, goats, horses, and humans ingest the encysted metacercarial form of *Fasciola*, and the worm then migrates to the liver, where it causes liver damage; the mature worm then resides in the bile duct (Seldemir, 2000). After the parasite enters the liver, it causes an acute infection, and the incubation period lasts 2 – 4 weeks. When metacercariae migrate to the bile ducts and mature into adult flukes, chronic disease occurs, with an incubation period of 3 – 11 months (Phalee *et al.*, 2015). The acute form of fasciolosis includes fever, skin rashes, hair coat browning, liver cirrhosis, and reduced reproductive performance. In contrast, the chronic form is commonly associated with anemia, jaundice, and persistent hair discoloration (Mas-Coma *et al.*, 2009). Fasciolosis leads to significant weight loss and reduced meat and milk production in livestock, causing severe economic losses to the agricultural sector, particularly in developing countries.

According to a survey conducted by the World Health Organization (WHO, 2021), fasciolosis affects at least 17 million individuals, and 180 million are at risk of fasciolosis in more than 70 countries worldwide. Developing and underdeveloped countries are at higher risk of fasciolosis-related consequences than developed countries (Sarkari *et al.*, 2018). In different areas of Pakistan, fasciolosis is one of the major diseases of small ruminants, including sheep and goats (Akhtar *et al.*, 2012). In the development of livestock production, fasciolosis is one of the major problems (Bilal *et al.*, 2009). The prevalence of fasciolosis was recorded as 1.48 times higher in cattle as compared to sheep and goats (Kruchynenko *et al.*, 2022). The overall prevalence of fasciolosis in cattle was 6.41 %, while in small ruminants, it was 2.03 % (Afshan *et al.*, 2014b; Afshan *et al.*, 2022). Different studies conducted on the epidemiology of fasciolosis among different animals show that risk factors like age, sex, breed, livestock management, and health conditions have a significant influence on the prevalence of fasciolosis (Nkurunziza *et al.*, 2024; Bulla-Castaneda *et al.*, 2023; Kurnianto *et al.*, 2022;

Sarwar *et al.*, 2025; Khan *et al.*, 2025b). Fasciolosis remains a major parasitic disease of small ruminants, particularly in endemic regions where its burden is high. Although comprehensive data on prevalence, associated risk factors, and phylogenetic analyses are well documented globally, such data remain limited in Pakistan, particularly in the Malakand Division. Therefore, this study aims to address this information gap by investigating the prevalence, phylogenetic analysis, and risk factors associated with fasciolosis in sheep and goats in Malakand Division, Pakistan. The findings of the current study would help inform the government's parasitic control authorities to improve diagnostic efforts and practices.

Materials and Methods

Study area

The study was conducted in the Malakand Division of Khyber Pakhtunkhwa (KP), Pakistan. Malakand Division is famous for livestock rearing and agricultural activities. Geographically, Malakand Division is situated in the northern part of the country, approximately 86.30 km (53.51 miles) by road from Peshawar, the capital of Khyber Pakhtunkhwa province of Pakistan. Its north latitude lies 35°29' – 59°99' N and its east longitude lies 72°00' – 00°0' E with 8.4 inches of annual rainfall. The area of Malakand Division is about 12,358 square miles (32,007 km²), including both plains and mountains, with an estimated population of 8.7 million. Maximum temperatures range from 16 – 32°C in June and July in the summer, and the minimum temperatures range from 11 – 2°C in January and February in the winter season (Fig. 1).



Fig. 1. The figure shows different locations of study area.

Coprological examination and assessment of risk factors

Collection and Processing of Fecal Samples

In the current prevalence study, fresh feces from small ruminants (1307 sheep and 1274 goats) were collected across eight district-wise localities in the study area over a full year, from January 2024 to December 2024, including all four seasons. Fresh fecal materials (10 – 15 g) were collected directly from the rectum of randomly selected small ruminants using gloved fingers. Then, feces were placed in collecting bottles containing 70 % ethanol. The collected bottles were labeled with host sex, age, location, and date (Raza *et al.*, 2014). To identify risk factors, the owners of small ruminants were asked about the host's gender, geographic location, grazing pattern, age, feeding type, health condition, prior anthelmintic treatment, and drinking water source. The collected fecal samples were brought to the parasitology laboratory in the Department of Zoology at the University of Malakand, Pakistan, for the diagnosis of Fasciolid trematode eggs.

Sedimentation technique and egg diagnosis

The sedimentation technique was used to detect Fasciolid trematode eggs because their eggs are larger than those of other parasites; therefore, they settle quickly in the test tube when centrifuged in a NaCl solution (Soulsby, 1982; Zajac & Conboy, 2012). In this technique, the first 3 grams of the fecal sample were dissolved in 20 ml of water, then filtered through a sieve several times. The filtrate was centrifuged for 3 – 5 min. Afterward, the supernatant was discarded, and the pellet was resuspended in normal saline. A drop of the mixture was placed on a glass slide, covered with a coverslip, and examined under the microscope at 4×, 10× and 40× for the presence of parasite eggs (Mumtaz *et al.*, 2024; Ruhoollah *et al.*, 2021; Soulsby, 1982).

Fasciolid eggs identification

The identification of Fasciolid eggs was based on observations of egg shape, coloration, contents, and operculum morphology, as described by Aghayan *et al.* (2019). The *Fasciola* egg is the largest helminth egg, oval in shape, with yellowish-brown to brown or golden-yellowish color, a thin shell, and an operculum at one end, as described by Hussein *et al.* (2010).

DNA extraction

DNA was extracted from individual *Fasciola gigantica* samples, which were obtained from selected slaughterhouses of the study area using a commercial DNA extraction kit (Qiagen, Hilden, Germany) following the manufacturer's instructions.

PCR amplification

For molecular characterization, DNA was extracted from adult *Fasciola* specimens, and the internal transcribed spacer region (ITS), a nuclear ribosomal DNA marker, was amplified using polymerase chain reaction (PCR) following the protocol of Mehmood *et al.* (2024) and Ruhoollah *et al.* (2025). ITS was selected for

molecular characterization because it is a well-established nuclear ribosomal DNA marker widely used for species-level identification, phylogenetic analysis, and taxonomy of fasciolid trematodes such as *Fasciola hepatica* and *Fasciola gigantica*. It is highly conserved within species and its use is supported by the availability of reference data in public databases (Mas-Coma *et al.*, 2009). The reaction mixture contained template DNA, forward primer (5' GCGACCTGAAATCTACTCTTACACAAGCG 3') and reverse primer (5' GACGTACGTATGGTCAAAGACCAGTT 3'), dNTPs, Taq polymerase, and buffer in a total volume of 25 µL. Thermo-cycling conditions included an initial denaturation at 96 °C for 8 minutes, followed by 30 cycles of 96 °C for 60 seconds, annealing at 54 °C for 60 seconds, extension at 72 °C for 60 seconds, and a final extension at 72 °C for 8 minutes. The amplified ITS gene PCR products were verified by agarose gel electrophoresis and visualized under UV illumination, as described by (Amor *et al.*, 2011; Ndosi *et al.*, 2023; Khan *et al.*, 2025a). The amplified ITS fragment was approximately 420 bp in length for both isolates (Fig. 2).

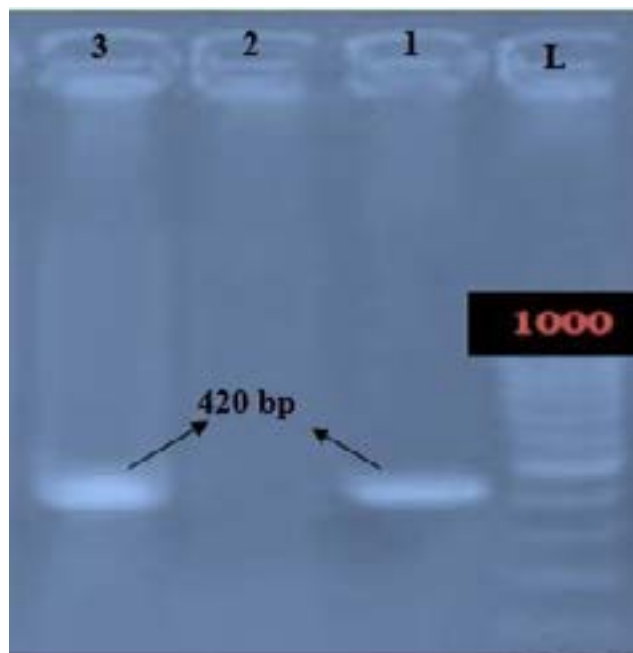


Fig. 2. Agarose gel electrophoresis showing PCR amplification of ITS region of *F. gigantica*.

Phylogenetic tree construction and sequencing

The amplicons were confirmed through gel electrophoresis. Two amplified DNA products (one each from goat and sheep samples) were randomly selected, purified, and sequenced at Macrogen Inc. in Seoul, South Korea. The sequences were assembled and aligned using ClustalW multiple alignment implemented in MEGA11. Sequence analysis was performed using BLASTn and the National Center for Biotechnology Information (NCBI) databases. A phylogenetic tree was constructed using a neighbor-joining method implemented in MEGA 11.

Table 1. Overall prevalence of Fascioliasis in sheep and goats.

S/No	Type of Host	Total Sample Examine	Total positive	Overall prevalence %
1	Sheep	1307	51	3.9 %
2	Goat	1274	26	2.04 %

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) version 22 was used for data analysis. Data were submitted, appropriate tests were performed for each parameter, and P-values < 0.05 were considered significant. Infection rate was calculated as the percentage of animals positive for Fasciolid trematodes within specific categories (age, sex, season, or management factors), while prevalence represents the overall proportion of infected animals in the total sampled population and for species-wise distribution of *Fasciola hepatica* and *Fasciola gigantica*. Both measurements were calculated as the number of positive samples divided by the total number of samples examined $\times 100$, and associations with risk factors were assessed using the Chi-square test at $P < 0.05$, as described by Irshad *et al.* (2023) and Hussain Shah *et al.* (2015).

Ethical Approval and/or Informed Consent

The study protocol (UOM/Admin/2024/501) was ethically approved by the Advanced Studies and Research Board, Malakand University, Lower Dir, Pakistan. All applicable national and institutional guidelines for the care and use of animals were followed during this research. Informed consent was obtained from the owners prior to sample collection.

Results and Discussion

Overall prevalence

A total of 77 (2.98 %) samples were positive for Fasciolid trematodes out of 2,581 fecal samples examined. The infection rate was higher in sheep, 3.9 % (51/1307), than in goats, 2.04 % (26/1274), indicating a comparatively lower occurrence of fasciolosis in goats (Table 1).

The overall prevalence of *F. hepatica* was higher than that of *F. gigantica*. In the case of sheep, 42 samples were positive for *F. hepatica* (3.21 %) and 9 for *F. gigantica* (0.68 %), while in goats, 19 were positive for *Fasciola hepatica* (1.49 %) and 7 for *Fasciola gigantica* (0.54 %) (Table 2).

The results of the present study are in line with the study conducted by Hussain Shah *et al.* (2015) in Peshawar, Khyber Pakhtunkhwa,

who reported the prevalence of trematodes (4.6 %) and (3.4 %) in goats and sheep, respectively. Similar findings were also reported by Haleem *et al.* (2016) in Mardan, Khyber Pakhtunkhwa, who reported 5.89 % *F. hepatica* and 3.83 % *F. gigantica* in sheep. In contrast to the current study, Ruhoollah *et al.* (2021) reported that the prevalence of *F. hepatica* was 13.58 % and 8.39 % in sheep and goats, respectively. This difference may be due to differences in sample size, study area, and diagnostic procedures. The study conducted by Rizwan *et al.* (2022) in Quetta, Pakistan, which is in contrast to the present study, reported that the prevalence of *F. hepatica* and *F. gigantica* in goats was 0.0698 % and 0.209 %, respectively. This difference may be due to the arid environment of the study area, where the intermediate hosts are negligible.

Risk factors Analysis influencing fasciolosis in small ruminants

Risk factors such as host gender, age, area, season, grazing system, health condition, source of drinking water, and treatment status of small ruminants were analyzed as variables (Table 3). Chi-square analysis showed that the prevalence of fasciolosis was statistically associated with the host gender, age, area, season, grazing system, health condition, source of drinking water, and treatment status of the host animal.

Sex-wise prevalence

To determine sex-wise prevalence, sheep and goats were categorized into two groups: males and females. Of the total animals evaluated, 526 were male sheep, 508 were male goats, 781 were female sheep, and 766 were female goats. The infection rate in male sheep was 4.372 % (23/526), higher than the rate in female sheep, which was 3.58 % (28/781). In goats, the infection rate in females was 2.088 % (16/766), compared to 1.96 % (10/508) in males. The prevalence rate according to the sex of sheep and goats in the study area was statistically nonsignificant ($P = 0.611$) (Table 2).

The results of the current study align, to some extent, with those documented by Mumtaz *et al.* (2024); Ruhoollah *et al.* (2021); Durrani *et al.* (2017); Haleem *et al.* (2016); and Hussain Shah *et al.* (2015), who also reported variations in fasciolosis prevalence correlated with the sex of sheep and goats. These variations may

Table 2. Overall prevalence of Fasciolides trematodes species in sheep and goats.

Parasite species	Total samples of Sheep (1307)		Total samples of Goats (1274)	
	Positive samples	Prevalence %	Positive samples	Prevalence %
<i>F. hepatica</i>	42	3.21	19	1.49
<i>F. gigantica</i>	9	0.68	7	0.54

be linked to management practices, as female sheep are often confined, whereas male goats are typically allowed to graze freely. Moreover, variables including breed, species, and regional distribution may affect infection rates. In Bangladesh, Mazid *et al.* (2006) documented a greater incidence in female sheep and goats, ascribed to pregnancy and hormonal variations.

Age-wise prevalence

To find out the age-wise prevalence of infection, animals were classified as young (<1.5) and adults (>1.5) because this age approximates sexual maturity and the transition from immature to fully developed immune responses in small ruminants (Soulsby, 1982; Radostits *et al.* 2007). A total of 1307 (<1.5=268, >1.5=1039) fecal samples of sheep, while 1274 (<1.5=312, >1.5=962) fecal samples of goats were investigated. It was observed that the prevalence of fasciolosis in young sheep was 7.08 % (19/268), and in

goats was 2.88 % (9/312), which was higher than in adult sheep, 3.07 % (32/1039), and in goats, 1.76 % (17/962), respectively. Thus, age-wise prevalence showed that infection was higher in young animals than in older animals, with a statistically significant difference ($P = 0.003$). Our findings are similar to the results of Mumtaz *et al.* (2024) and Rashid *et al.* (2016), who reported that young animals were more infected than older ones. The results of Durrani *et al.* (2017) contradict those of the present study, which found that the prevalence of fasciolosis was higher in older animals as compared to young ones. The higher rate of fasciolosis in young small ruminants in the current study may be due to young animals often being allowed to graze freely, increasing their exposure to Fasciolid eggs. In contrast, adults are mostly kept captive, limiting their risk of infection. Secondly, it may be due to the weaker immune systems of young small ruminants compared with older animals.

Table 3. Shows the risk factors associated with the prevalence of fascioliasis.

Risk Factors	Variables	Sheep (1307)			Goats (1274)			P-value
		Sample	Positive	Prevalence %	Sample	Positive	Prevalence %	
Sex	Male	526	23	4.372	508	10	1.96	0.611
	Female	781	28	3.58	766	16	2.088	
Age	<1.5 years	268	19	7.08	312	9	2.88	0.003
	>1.5 years	1039	32	3.07	962	17	1.76	
Area	Dir (U)	200	23	11.5	250	10	4	0.97
	Dir (L)	445	20	4.49	395	7	1.77	
	Bajaur	101	1	0.99	114	2	1.75	
	Swat	100	1	1	100	1	1	
	Buner	150	2	1.33	150	2	1.33	
	Shangla	83	1	1.20	65	1	0.60	
	Malakand	150	2	1.33	109	2	1.83	
	Chitral	78	1	1.28	91	1	1.11	
Grazing system	Grazing	504	16	3.17	507	10	1.97	0.32
	Stall fed	803	35	4.35	767	16	2.08	
Health condition	Healthy	1018	34	3.33	1041	14	1.34	0.0001
	Emaciated	289	17	5.88	233	12	5.15	
Drinking water source	Open	427	19	4.44	412	10	2.42	0.039
	Bore	225	4	1.77	167	2	1.19	
	Stream	388	21	5.41	358	9	2.51	
	Tap water	267	7	2.62	337	5	1.48	
Season	Autumn	294	10	3.40	308	6	1.94	0.0004
	Winter	216	4	1.85	199	2	1.005	
	Spring	319	6	1.88	331	5	1.51	
	Summer	478	31	6.48	436	13	2.98	
Treatment	Treated	320	7	2.18	308	4	1.29	0.036
	Untreated	987	44	4.45	966	22	2.277	

Season-wise prevalence

In the current study, it was found that the prevalence of fasciolosis was higher in sheep, 6.48 % (31/478), and goats, 2.98 % (13/436), in summer, and the lowest prevalence in sheep, 1.005 % (2/199), and in goats, 1.85 % (4/216), was recorded in winter. In spring, the prevalence in sheep and goats was 1.88 % (6/316) and 1.51 % (5/331), respectively, while in autumn, the prevalence was 3.40 % (10/294) and 1.94 % (6/308), respectively. The results were statistically significant ($P = 0.0004$) (Table 3). The findings of this study are similar to those of Ruhoollah *et al.* (2021). They also observed the highest prevalence of fasciolosis in small ruminants during summer. The findings of Ayele *et al.* (2018) and Durrani *et al.* (2017) are in contrast to the current study, as they reported the highest prevalence in sheep and goats in autumn, whereas Ashoor & Wakid (2023) reported the highest prevalence in spring. In the current study, the highest prevalence of infection in summer may be due to high temperatures and moisture during the Monsoon season, thereby increasing fluke ova hatching and the encystation of Fasciolid cercariae, ultimately increasing the population of intermediate host snails. Consequently, increased cercarial production and encystation on vegetation enhance the transmission risk of fasciolosis in grazing small ruminants (Mas-Coma *et al.*, 2005). In the winter season, the prevalence of fasciolosis was lowest due to cool, dry conditions that reduced the population of intermediate hosts.

Body condition-wise prevalence

The health status of the host animals in the current study was also considered an important parameter for the prevalence of fasciolosis. In the present study, sheep and goats were categorized into two groups, i.e., normal and emaciated. In the current study, the prevalence of fasciolosis was high in emaciated sheep, 5.88 % (17/289), and goats, 5.15 % (12/233), while the prevalence of fasciolosis in normal sheep and goats was 3.33 % (34/1018) and 1.34 % (14/1041), respectively. The results were statistically significant ($P = 0.0001$) (Table 3). These findings are similar to the findings of Ayele *et al.* (2018). They also reported a higher prevalence of fasciolosis in sheep and goats with poor body condition than in healthy animals. However, the results of Dabasa *et al.* (2017) are in contrast to those of the current study, which reported a high prevalence in normal sheep and goats. The high infection rate among emaciated animals in the current study may be due to a weakened immune system.

Area-wise prevalence

In the study area, which includes eight distinct locations, the highest prevalence of fasciolosis in sheep was observed in Dir Upper (11.5 %), followed by Dir Lower (4.49 %), Buner and Malakand (1.33 %), Chitral (1.28 %), Shangla (1.20 %), Swat (1 %), and the lowest prevalence in Bajaur (0.99 %). On the other hand, the highest prevalence in goats was also recorded in Dir Upper (4 %), followed by Malakand (1.83 %), Dir Lower (1.77 %), Bajaur

(1.75 %), Buner (1.33 %), Chitral (1.11 %), Swat (1 %), and the lowest prevalence in Shangla (0.60 %). No statistically significant ($P = 0.97$) difference in prevalence was observed among various regions of the study area (Table 3). The findings of Tasleem *et al.* (2023) align with those of the present investigation, since they observed varying prevalence rates of fasciolosis infection in livestock across different sites within the study area. The variation in fasciolosis prevalence among the research locations was attributable to factors such as differing grazing systems, feeding environments, management practices, and owner care behaviors.

Grazing system-wise prevalence

The prevalence of fasciolosis was higher in stall-fed sheep (4.35 %) and goats (2.08 %) compared to grazing sheep (3.17 %) and goats (1.97 %), with a p-value of 0.32, indicating statistical insignificance (Table 3). The findings of the present study differ from those of Mohammed *et al.* (2023), who observed that goats kept on open grazing had higher infection rates than those in stalled feeders. The elevated occurrence of fasciolosis in stall-fed small ruminants may result from inadequate cleanliness in stalls or the harvesting of plants and grasses from regions inhabited by metacercariae, such as wetlands and marshes.

Treatment-wise prevalence

The prevalence of fasciolosis was significantly higher in untreated sheep (4.45 %) and goats (2.27 %) compared to treated sheep (2.18 %) and goats (1.29 %), with a p-value of 0.036 indicating statistical significance (Table 3). The findings of our study align with those of Mohammed *et al.* (2023), who similarly report a higher prevalence of fasciolosis in untreated sheep and goats than in treated animals, attributable to the administration of anthelmintic medications. The elevated infection rate in untreated animals within the study area may result from inadequate treatment procedures, diminished attention, and a negligent attitude among owners towards small ruminants.

Water source-wise prevalence

The prevalence of fasciolosis in drinking water sources was highest in sheep (5.41 %) and goats (2.51 %) using stream water, followed by sheep (4.44 %) and goats (2.42 %) drinking open water, and finally sheep (2.62 %) and goats (1.48 %) using tap water. The lowest infection rates were seen in sheep (1.77 %) and goats (1.19 %) consuming bore water, with a p-value of 0.036, indicating statistical significance (Table 3). The results of the current investigation parallel the findings of Ruhoollah *et al.* (2021), who reported a high incidence of Fasciolid in sheep and goats that drink from open water sources. The elevated incidence of fasciolosis in animals that consume stream and open water may be attributed to the higher levels of pollution and contamination in these water sources. However, bore and tap water sources are often safe, clean, and fit for consumption.

Phylogenetic tree

After DNA extraction and ITS gene amplification, two representative PCR products, one from sheep and one from goat isolates, were selected for sequencing analysis. Following sequencing, the phylogenetic tree was constructed using the Neighbor-Joining method, comparing the two isolates from the present study with 11 published *Fasciola gigantica* sequences from Bangladesh, Iran, Nigeria, Vietnam, India, Egypt, Saudi Arabia, and Thailand. The phylogenetic analysis demonstrated that the first isolate (Malakand-1) was closely related to an Iranian isolate (KM085323), supported by a bootstrap value of (93 %) indicating a strong genetic relationship. Malakand-1 also showed close association with isolates from Saudi Arabia and Egypt, suggesting genetic similarity among geographically related Asian and Middle Eastern isolates.

The second isolate (Malakand-2) was close to isolates from Nigeria and Saudi Arabia, but this node received weak bootstrap support (4 %), indicating an unclear relationship. The whole tree shows both genetic similarities and differences between *F. gigantica* isolates from different places.

The similarity of the Malakand-1 isolate to Iranian and Middle Eastern genomes indicates a genetic connection between these regions, possibly due to livestock movements or historical transmission pathways. The distinctive location of Malakand-2, however lacking robust proof, may indicate the presence of multiple *F. gigantica* lineages in the vicinity. The results also indicate that additional molecular investigations using additional genetic markers and larger sample sizes are recommended to better understand the population structure and evolutionary relationships of the fasciolid trematodes (Fig 3).

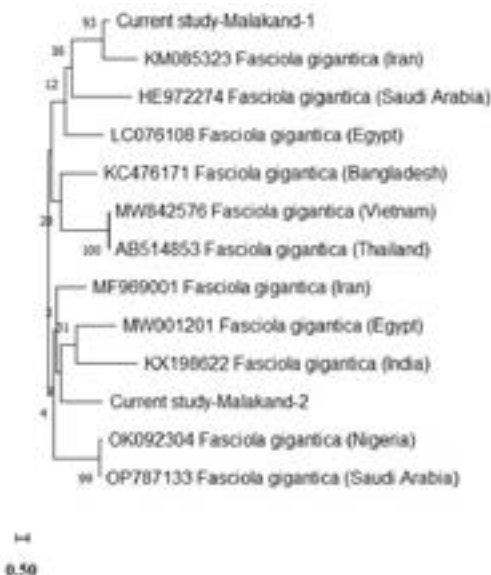


Fig. 3. Phylogenetic tree of *F. gigantica* in goats and sheep utilising the ITS marker. The generated tree depicts the genetic relationships among the isolates of the present investigation and reference sequences from various regions. Bootstrap values are provided at branch points indicating the confidence levels of the groups.

Conclusion

The current study demonstrated a moderate prevalence of fasciolosis in sheep and goats in Malakand Division, Pakistan. It was significantly associated with prior anthelmintic treatment, host age, drinking water quality, feeding pattern, and health status. Molecular analysis additionally confirmed that the species was *F. gigantica* and showed that it was closely related to Egyptian, Iranian, Nigerian, and Saudi Arabian isolates. The high bootstrap support in phylogenetic analysis indicates that the local isolates are genetically similar, warranting continued molecular monitoring. Effective control measures, such as the periodic use of anthelmintics and fasciolicides, are recommended to maximize benefits in small ruminants and prevent economic losses.

Conflict of Interest

The authors state no conflict of interest related to this study.

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