Occurrence and Genetic characterization of *Fasciola* spp. infections

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Abstract

Fasciolosis, caused by *Fasciola* species, is a significant parasitic disease affecting sheep, particularly in tropical and subtropical regions. This study aimed to assess the occurrence and molecular identification of *Fasciola* species in sheep from Patna, Bihar. A total of 445 sheep from various age groups, sexes, and body conditions were screened for fasciolosis through coprological examination. The overall occurrence rate was 25.16%, with a higher prevalence in females, sheep aged 1-3 years, and those with poor body condition. Molecular analysis of *Fasciola* samples revealed *Fasciola gigantica* as the only species present, identified through PCR amplification of the ITS-1 rRNA gene. Phylogenetic analysis showed high genetic similarity between Patna isolates and those from regions like Vietnam, Thailand, and Egypt. These findings highlight the importance of early detection and control measures for fasciolosis in sheep farming in Patna and can serve as a foundation for future control strategies in the region and state.

Keywords: Genetic characterization; Fasciola; Sheep

Introduction

Sheep play a vital role in India's rural economy, especially in arid, semi-arid, and mountainous regions, by providing wool, meat, milk, skins, and manure. Sheep are known for their versatility and adaptability making them integral to the livelihoods of millions nationwide. Sheep are susceptible to various parasitic diseases, including helminthic, protozoal, and ectoparasitic infections, with fasciolosis being a major health concern (Besana & Paller 2020). *Fasciola* is a flat and leaf-like helminthic parasite that affects both animals and humans. Fasciolosis is a major disease impacting sheep in tropical and subtropical areas. *Fasciola* is an important digenean trematode parasite, commonly called liver flukes. Fasciolosis is a zoonotic and parasitic disease caused by two species of flukes *Fasciola hepatica* and *Fasciola gigantica* (Tolan 2011). Fasciolosis is widespread in India caused by *F. gigantica* whereas *F. hepatica* is mainly reported in the temperate Himalayan region (Singh *et al*. 2021).

Fasciola has a complex life cycle that involves both free-living stages and an intermediate snail host. The epidemiology of fasciolosis is closely linked to the ecological characteristics of these snail intermediate hosts responsible for transmitting *Fasciola* species. Fasciolosis is influenced by multiple factors such as the grazing system, nutritional status, climate, ecosystem, and genetic factors. Pasture quality also influenced the number of infected animals by trematode. Sex, age and body condition score (BCS) were found to be the significant risk factors for the occurrence of fasciolosis (Hambal *et al*. 2020). Identification of closely related species is usually difficult to differentiate accurately between *Fasciola* spp. based on morphological criteria (Aryaeipour *et al*., 2014). Molecular marker such as internal transcribed spacers of ribosomal DNA (ITS-1) is generally used for molecular characterization of different species of *Fasciola* (Ichikawa and Itagaki 2010). This study attempts to know the occurrence and *Fasciola* sp. and use of molecular techniques for genetic characterization of the same.

Materials and Methods

A total of 445 sheep of different areas (Latitude: 25.6154; Longitude: 85.1010 P), age groups, sex and body condition score (BCS) were screened for the presence of fasciolosis. The selected sheep owners are well known for sheep rearing, especially as a primary source of their livelihood. Faecal samples of sheep were collected directly from the rectum and kept in labelled stoppered wide-mouthed bottles of 20-30 ml capacity. In cases when samples were to be examined later 70% ethanol was added to preserve the morphological characteristics of parasitic eggs. The faecal samples were examined using the direct, sedimentation and floatation techniques as described by Soulsby, 1982. Identification of the eggs was made based on morphological characteristics of the eggs.

The liver fluke samples were collected from the livers of five sheep that had been diagnosed with fasciolosis during post-mortem examinations in the Department of Veterinary Pathology, Bihar Veterinary College. The collected parasites were thoroughly rinsed in PBS (pH=7.4) to eliminate debris before being preserved in 70% ethanol for further analysis.

DNA Extraction

Genomic DNA was isolated from three different flukes using the NucleoSpin® Tissue Kit (Takara Bio, Japan) with slight modifications to the manufacturer's protocol. The isolated DNA samples were stored at -20°C until further molecular detection by PCR amplification.

PCR amplification of ITS-1 rRNA gene

The PCR amplification of the portion of ITS-1 rRNA gene of *Fasciola* spp. was carried out from DNA samples with the following primer pairs: Forward 5′- TTGCGCTGATTACGTCCCTG-3′ and Reverse 5′-TTGGCTGCGCTCTTCATCGAC -3′ (Itagaki *et al*. 2005). PCR reaction was performed following the reaction composition and reaction condition as specified by Itagaki *et al.*, (2005) with some minor modifications for amplification of an expected 600 bp product. The reaction was conducted in a 25 µl reaction following the specific PCR programme standardized for the ITS-1 region. The PCR programme comprised of initial denaturation of 94ºC for 5 minutes, followed by 37 cycles at 94ºC for 30 seconds (cyclic denaturation), 55ºC for 30 seconds (annealing) and 72ºC for 45 seconds (cyclic extension) and a final extension of 72ºC for 5 minutes. Analysis of results carried out by agar gel electrophoresis on 1.5% agarose gel, using a 100 bp DNA ladder (Takara Bio) revealed amplified products in high yield and quality when documented in a gel documentation system (VILBER).

Nucleotide sequencing and phylogenetic analysis

The amplified products were outsourced to Eurofins Genomic in Bangalore, Karnataka for sequencing. Sequencing of the ITS-1 amplicons was done with the same primers used during PCR amplification. The generated sequences were compared using BLASTn algorithm with the sequences available in the National Centre for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov) to identify the *Fasciola* species. Further, the sequences were used to construct a species-specific phylogenetic

Fig. 1. Collection of faecal samples

Fig. 2. Flukes sample

tree based on sequence homology with the published sequences originating from different countries using the neighbour-joining method using the MEGA 11 programme (Tamura *et al*., 2021).

Statistical analysis

Data obtained in all the experiments were analyzed using statistical software SPSS-29.0 (SPSS Corporation, USA). ANOVA (one-way) and chi-square tests were applied according to the data type.

Results and Discussion

Occurrence of fasciolosis

Out of 445 faecal samples, 112 were positive for egg of *Fasciola* spp. Coprological examination revealed the overall occurrence of fasciolosis was 25.16%. This finding highlights a substantial presence of *Fasciola* infection within the sheep population. Sex, age and body condition score (BCS) wise occurrence of fasciolosis in sheep presented in Table- 1**.** Abdullah *et al.* (2020) reported that 23.54% of sheep were positive for fasciolosis in Kashmir valley. Yemisrach and Mekonnen (2012) confirmed the presence of ovine fasciolosis through routine post-mortem examinations to be 28.7% in Debre Zeit town, Ethiopia. However, compared to the present findings, Bitew *et al.* (2010) recorded a comparatively higher infection rate, noting that 49% of the sheep population was positive for fasciolosis. Bogale *et al.* (2012) reported an overall occurrence of 43.75% for ovine fasciolosis. Hirpa *et al.* (2014) found 35.94% of sheep were tested positive for liver fluke infection.

Sex-wise, the occurrence of fasciolosis was significantly ($P<0.05$) higher in females (26.35%) than in male sheep (19.31%). Anjum *et al.* (2014) also reported a higher occurrence of fasciolosis in female sheep than in male sheep. Asrede and Shifaw (2015) also found a higher infection rate in females compared to male sheep in Ethiopia. The higher rate of fasciolosis in females may be attributed to prolonged rearing for milk production and physiological stresses like gestation and lactation. These conditions stress the animals, and inadequate food and water during these periods can lower their immune status, making females more susceptible to infection than males.

Age-wise occurrence of ovine fasciolosis was significantly ($p<0.05$) higher in the age group 1-3 years (40.36%) of fasciolosis than in the age group <1 years (9.87%). A similar finding was reported by Bogale *et al.* (2012) who documented a significant association of age with fasciolosis infection. Hirpa *et al.* (2014), ovine fasciolosis infection varies significantly ($P<0.05$) among different age groups. The variation in age-wise occurrence can be linked to the exposure of animals to the infection, as younger animals are primarily kept at home. In comparison, older and adult animals are taken out for grazing, which increases their risk of contracting infectious metacercaria. Additionally, lower occurrence in the old age group was due to self-cure phenomenon and acquired immunity which increased with age and might be high grazing activity in adult sheep.

The BCS-wise occurrence of ovine fasciolosis was significantly ($p<0.05$) more in animals with poor body condition (51.06%) followed by medium body condition (21.84%) while least in good body condition (10.61%). The higher occurrence of ovine fasciolosis in animals with poor body condition was also reported by Bitew *et al.* (2010). The difference in the occurrence of ovine fasciolosis based on body condition score can be attributed to better immunity in animals with good body conditions while those with poor body condition body scores are vulnerable to parasitic infection. It may also signify the importance of fasciolosis in causing weight loss and emaciation, a characteristic sign of the disease.

Molecular identification of *Fasciola* species

The PCR amplicons of extracted DNA samples from *Fasciola* spp. showed single distinct band of 600 bp when compared with the DNA ladder in agarose gel (1.5%) (Fig. 3). The sequence for ITS-1 (600 bp) was subjected to the BLASTn programme of NCBI. The result depicted that our sequence included partial 18S,

Table 1: Occurrence of fasciolosis in sheep of Patna district, Patna according to sex, age and Body condition score (BCS)

Variables	Faecal Sample examined	No. of positive samples	Occurrence (%)	P-value
Sex				
Male	176	34	19.31	<0.05
Female	269	78	26.35	
Age Group				
<1 Years (Lamb)	81	8	9.87	<0.05
1-3 Years (Adult)	166	67	40.36	
>3 Years (Old)	198	37	18.68	
Body condition score (BCS)				
Poor	94	48	51.06	<0.05
Medium	238	52	21.84	
Good	113	12	10.61	

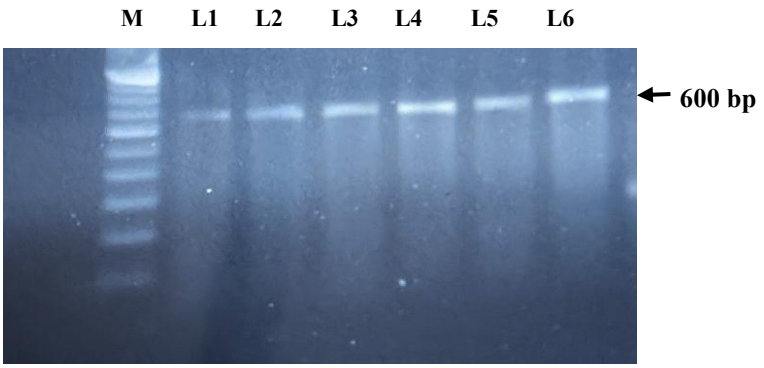

Fig. 3. Amplification of ITS-1 region of rDNA of *Fasciola* species
PCR-based amplification of ITS-1 gene, M: 100 bp DNA ladder, L1-L6: ITS-1 amplicons

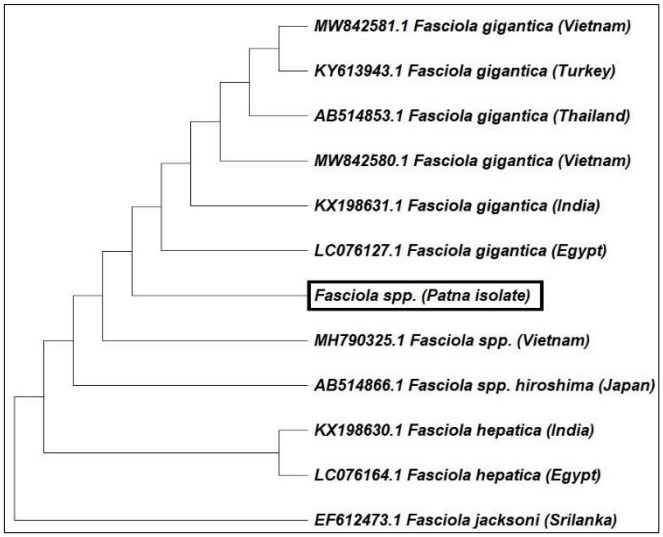

Fig. 4. Phylogenetic tree of *Fasciola* spp. (Patna isolate) based on ITS-1 gene sequence

complete ITS-1 and partial 5.8S of ribosomal DNA. The BLAST tool of NCBI revealed that flukes which had been collected from sheep were identified as *Fasciola gigantica*. To know the accuracy of divergence, the phylogenetic trees were constructed by the neighbour-joining method. The phylogenetic tree was generated using 13 sequences including the sample and *Fasciola jacksoni* as an outgroup. The evolutionary analyses were conducted using the MEGA 11 programme (Tamura *et al*., 2021). The present study revealed that isolates from Patna exhibited close genetic ties with those from different regions of the world. *Fasciola gigantica* sequences from Vietnam, Thailand, and Egypt clustered with Patna isolates, sharing 98.21% similarity. The phylogenetic tree obtained shows *Fasciola* spp. (Patna) isolate had maximum identity with existing *F.gigantica* (98.44%),

Fasciola spp. *hiroshima* (97.5%) and *Fasciola hepatica* (97.2%). The sequences clustered into three distinct clades. One clade comprised *F. hepatica* from India and Egypt. The other clade included the *F. gigantica* (Patna) isolate with all other *F. gigantica* isolates from different regions, including India. Another clade consists of *F. jacksoni* originating from Srilanka as an outgroup.

The present study reported the occurrence of fasciolosis in sheep of Patna, Bihar. The overall occurrence rate was found to be 25.16% in the Patna district. *Fasciola gigantica* was found to be the only *Fasciola* species infecting sheep by molecular identification in the study area. Sex, age and body condition score (BCS) wise study showed the highest occurrence of ovine fasciolosis in female sheep, in sheep of age group 1-3 years of age and in poor body condition score of sheep, respectively. However, further studies can be carried out to assess the actual economic impact of the fasciolosis in sheep in the study area. Altogether, the present study provides baseline data on the occurrence of fasciolosis in sheep in Patna, Bihar which can be enormously helpful in formulating control strategies against fasciolosis in sheep in the study area as well as in the whole State.

Conclusion

The study found a 25.16% occurrence of fasciolosis in sheep from Patna, Bihar, with *Fasciola gigantica* identified as the sole species infecting the animals. Factors such as sex, age, and body condition significantly influenced infection rates, with females, sheep aged 1-3 years, and those in poor condition being most affected. The findings provide valuable baseline data, highlighting the need for targeted control strategies to mitigate fasciolosis' impact on sheep farming in the region.

Conflict of Interest - The authors declare that they have no conflict of interest.

References

1) Abdullah I, Tak H and Ganie S A. 2020. Prevalence of fasciolosis and dicrocoeliosis in sheep slaughtered in Kashmir Valley. International Journal of Scientific and Technology Research. 9 (1): 570-573.
2) Anjum R, Khan M N, Sajid M S and Javed M T. 2014. Frequency distribution of fasciolosis in small ruminants population at district Sargodha. Global Veterinaria. 12: 26-32.
3) Aryaeipour M, Rouhani S, Bandehpour M, Mirahmadi H, Kazemi B and Rokni M B. 2014. Genotyping and phylogenetic analysis of *Fasciola* spp. isolated from sheep and cattle using PCR-RFLP in Ardabil Province, Northwestern Iran. Iranian Journal of Public Health. 43(10): 1364-1371.
4) Asrede T and Shifaw A. 2015. Coprological Study on the Prevalence of Ovine Fasciolosis in Debre Birhan Agricultural Research Center, Ethiopia. European Journal of Biological Sciences. 7(3): 103-107.
5) Besana C.M., Paller V.G.V. 2020. Evaluation of Selected Slaughterhouses and Parasites of Slaughtered Livestock in Cotabato Province, Mindanao, Philippines. Journal of Livestock Science 11: 67-76 doi. 10.33259/JLivestSci.2020.67-76
6) Bitew M, Ibrahim N and Abdela N. 2010. Study on the prevalence of ovine fasciolosis in and around Dawa-Cheffa, Kemissie. African Journal of Agricultural Research. 5(21): 2981-2985.
7) Bogale B, Keno D and Chanie M. 2012. Ovine Fasciolosis: Episode and Major Determinants in Haru District, Western Ethiopia. Acta Parasitologica Globalis. 3 (1): 07-11.
8) Hambal M, Ayuni R, Vanda H, Amiruddin A and Athailla F. 2020. Occurrence of *Fasciola gigantica* and *Paramphistomum* spp. infection in Aceh Cattle. E3S Web of Conferences. 151: 01025.
9) Hirpa E, Getachew F, Amante M and Abdata D. 2014. Ovine fasciolosis prevalence in Hidebu Abote Woreda, North Shoa, Ethiopia. American-Eurasian Journal of Scientific Research. 9(4): 82-86.
10) Ichikawa M and Itagaki T. 2010. Discrimination of ITS-1 types of *Fasciola* spp. based on PCR-RFLP method. Parasitology Research. 106: 757-761.
11) Itagaki T, Kikawa M, Terasaki K, Shibahara T and Fukuda K. 2005. Molecular characterization of parthenogenic *Fasciola* sp. in Korea on the basis of DNA sequences of ribosomal ITS1 and mitochondrial NDI gene. Journal of Veterinary Medical Science. 67(11): 1115-1118.
12) Singh D K, Singh V K, Singh R N and Kumar P. 2021. Fasciolosis: Causes, Challenges and Controls. Pp-27-41. Springer Nature Singapore Pte Ltd.
13) Soulsby E J L. 1982. Helminths, Arthropods and Protozoa of Domesticated Animals. 7th Edition. Pp-40-52,766-767. London: Bailliere Tindall.
14) Tamura K, Stecher G and Kumar S. 2021. MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution. 38(7): 3022-3027.
15) Tolan R W. 2011. Fascioliasis Due to *Fasciola hepatica* and *Fasciola gigantica* Infection: An Update on This "Neglected" Neglected Tropical Disease. Laboratory Medicine. 42: 107-116.
16) Yemisrach A and Mekonnen A. 2012. An abattoir study on the prevalence of fasciolosis in cattle, sheep and goats in Debre Zeit town, Ethiopia. Global Veterinaria. 8(3): 308-314.