



# An insight into the epidemiology of foodborne zoonotic fascioliasis in small ruminants in northwestern region of Bangladesh

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## Abstract

Fascioliasis is one of the most common foodborne zoonotic infection of ruminants in Bangladesh. To estimate the prevalence and associated risk factors of fascioliasis and extent of liver damage, 825 livers of sheep and goats were randomly inspected during onsite slaughterhouse visiting in Naogaon, Natore, Rajshahi and Joypurhat districts. The overall prevalence of fascioliasis was 25.09% and significantly ( $P=0.008$ ) higher in goats (26.11%) than sheep (24.00%). During gross inspection, *Fasciola* infected livers were increased in size, fibrosed, fatty, multiple white or reddish necrotic foci on the parietal surface, hard to cut, calcified, and numerous mature and immature flukes were also observed. In histoarchitecture, inflammatory cell infiltration in the hepatic parenchyma and periportal area, fibrous connective tissue proliferation around necrotic area, hyperplastic bile duct, congestion, and primary biliary cirrhosis were seen in acute and chronic fascioliasis. Epidemiological investigations revealed that fascioliasis was higher in goats than sheep. Age, sex, BCS and season were found to have statistically significant associations with fascioliasis in goats. In case of sheep, age (OR = 5.8671; 95% CI: 2.9482–11.6757,  $P < 0.0001$ ), sex (OR = 3.7317; 95% CI: 1.9052–7.3094,  $p < 0.0001$ ), BCS (OR = 6.0346; 95% CI: 1.7986–20.2472,  $p < .0001$ ), and season (OR = 8.2308; 95% CI: 3.9922–16.9693,  $p = < .0001$ ) were also found to have statistically significant associations with fascioliasis. Results of the study can help for molecular epidemiology of fascioliasis in small ruminants to plan fluke control programs for safe food production.

**Keywords** Fascioliasis · Slaughterhouse · Epidemiology · Sheep · Goat · Bangladesh

## Introduction

Fascioliasis is a foodborne zoonotic infection caused by *F. gigantica*, *F. hepatica* and their hybrid affecting a wide range of small and large ruminants along with human in tropical and sub-tropical countries (Hasan et al. 2022). The disease itself is an emerging public health and food safety issue (Mia et al. 2021). Approximately 2.4 million people

over 60 countries across the world account for fascioliasis and approximately 180 million people are laid on risk zone thus considered as a neglected tropical disease (Mehmood et al. 2017; Rahman et al. 2017). The disease causes huge economic losses in livestock industry by liver condemnation, reduced weight gain (up to 20%) and losing of quality and quantity (3–15% loss) of milk production, loss of draught power, reproductive failure and mortality (Aghayan et al. 2019; Khan et al. 2017; Mohanta et al. 2014; Opio et al. 2021). It has been estimated that more than 700 million domestic ruminants worldwide are in jeopardy and economic loss exceeds more than US\$ 3 billion per year (Spithill et al. 1999). The disease is characterized by both chronic and acute forms of liver lesion. On postmortem, gross pathology represents pale, firm and irregular outlined liver, several types of fibrosis, calcified and thickened bile duct, pipe stem liver and both adult and immature flukes (Howell et al. 2019). Histopathologically, lymphocytes, mononuclear cell infiltration, calcium deposition, hyperplastic bile duct,

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caseous necrosis, biliary cirrhosis, granuloma, congestion and so on (Sultana et al. 2022).

The incidence of fascioliasis has increased over the past two decades globally, possibly due to changes in farming practice, climate and development of anthelmintic resistance. Geographical feasibility, vulnerable climatic condition and availability of vector snail *Lymnaea auricularia var rufescence* make *F. gigantica* is one of the most endemic parasite in domestic ruminants in Bangladesh (Ahasan et al. 2016; Mohanta et al. 2014). Previous published reports revealed that the prevalence of fascioliasis is 21–53% in cattle (Rahman et al. 2017), 10–32% in goats (Al-Mamun et al. 2011b; Amin 2016; Islam and Ripa 2015; Rahman et al. 2017), 8.4–31% in sheep (Al-Mamun et al. 2011b; Amer et al. 2016; Amin 2016; Islam et al. 2014; Islam and Ripa 2015; Rahman et al. 2017) and 19 to 51% in buffaloes respectively. (Alim et al. 2005; Biswas et al. 2014; Roy et al. 2016; Saha et al. 2013). But mostly these reports were based on either fecal sample examination or passive surveillance data that lacking information about in-depth epidemiology and the extent of liver damage in sheep and goats as well as parasitic load in a single liver (Islam et al. 2016a; Shykat et al. 2022). In recent times a passive surveillance data based epidemiological study has been carried out in domestic ruminants where the hot spot, clusters and risk factors of fascioliasis are also identified in Bangladesh (Rahman et al. 2017). Abattoir survey provide an opportunity for inspection and evaluation of carcass fitness for human consumption as it allows checking the live animals on arrival as well as the carcasses and other parts such as organs of slaughtered animals. Abattoir surveys also play a significant role in the epidemiology of certain diseases as it incites true prevalence, provides necessary information for the scientific evaluation of pathological lesions of the respective diseases. Previous abattoir survey reported the prevalence of fascioliasis in slaughtered animals was 15–66% in cattle (Basak et al. 2011; Islam et al. 2016a), 3.8–22% in goats, 81% in sheep (Amin 2016) and 23–47% in buffaloes (Ahmedullah et al. 2007) in Bangladesh. The actual burden of fascioliasis including subclinical disease is likely much higher than that reported above. Fascioliasis still is a problem in livestock farming and development in Bangladesh. Recent studies revealed that development of anthelmintic resistance by *Fasciola* against major flukicidal drugs such as Triclabendazole (TCBZ), Nitroxinil (NTON) and Oxytoclozanide (OCZN) (Hasan et al. 2022) and existence of hybrid *Fasciola*, lack of strategic deworming, sanitation and hygiene, vulnerability to climate change such as increased rain fall, flood, seasonal disaster, lack of nutrition,, strategic failure of vector snail control and improper farm management regarding fascioliasis directly or indirectly influences fascioliasis here (Mohanta et al. 2014). Previous reports revealed that the animal populations of the northwest region of Bangladesh are in a high-risk zone for

fascioliasis. (Rahman et al. 2017). The area has a diversified geography covering both plain and low land, a number of rivers, small and large water bodies, marshy lands and country border altogether which is favorable and convenient for vector snail growth and reproduction, completing the life cycle of *Fasciola* and risk of zoonosis. Despite the wide prevalence of the malady and huge loss sustained from fascioliasis across the country, no epidemiological study based on either coprological study or slaughterhouse inspection in small ruminants have so far been undertaken in northwestern regions of this country. Therefore, the objectives of the study were to determine the prevalence of fascioliasis in small ruminants and associated risk factors by onsite local slaughter house carcass inspection and revealing the extent of liver damage by gross and histopathological changes in the liver within the study area to understand the epidemiology to ensure better livestock farming and safe food production.

## Materials and methodology

### Study area and sampling

The study was conducted in twenty selected slaughterhouse of four districts of north-western region of Bangladesh named Naogaon, Natore, Rajshahi and Joypurhat. Most of the regions of Naogaon and Natore are mainly plain land having water marsh while Rajshahi and Joypurhat are the parts of Barind tract.

### Study population

The study population includes sheep and goats brought to these selected slaughter house for slaughtering from November 2019 to October 2020 from various parts of the study area. Sheep and goats were classified based on their origin districts. Sheep and goats of various age groups and both sexes (male and female) were included in the study.

### Study design and sampling method

A cross-sectional study design was used to estimate the prevalence of fasciolosis in sheep and goats and assess the associated risk factors. A regular slaughterhouse visit was arranged for 5 days every month for 5 selected slaughterhouses of a particular district one after another during the study period.

### Sample size determination

The sample size was determined according to Thrusfield's (2018) formula by considering 50% expected prevalence in both sheep and goats and with 5% precision to have a larger

sample size by using the formula  $N = (Z)^2 P(1-P)/d^2$  (Thrusfield 2018). Sample size was 825 (400 sheep and 425 goats).

### Antemortem examination

During the ante mortem inspection, information regarding the species, sex, and origin of the animals were recorded. The age of each animal was confirmed as the physical appearance of body and examining the dental pad and incisor teeth according to (Ridler and West 2010).

### Post-mortem examination

On spot examination of livers for flukes and gross lesions was performed during post-mortem examinations, following standard meat inspection procedures; where the liver and gall bladder of individual sheep and goat was visually inspected, palpated, and incised (Oljira et al. 2022). Pathological lesions were recorded carefully. Infected liver samples of sheep and goat were then kept in plastic bags and transported to the laboratory of the Department of Parasitology, Bangladesh Agricultural University (BAU) by maintaining cool chain.

### Processing of tissue for histopathology

Infected liver tissue were cut into small pieces about 1 cubic cm in size and fixed in 10% buffered neutral formalin for histopathology. NBF-fixed tissues were dehydrated, embedded in paraffin, and sectioned at 3–4  $\mu\text{m}$  in thickness. The deparaffinized sections were stained with H & E staining according to (Luna 1968). To avoid personal variation and biases, all stained tissue sections were labeled anonymously and investigated by one individual.

### Photomicrographs

The histomorphological attributes of liver tissue sections photomicrographs were taken by photomicroscope (Model: CX41U-LH50HG, Olympus Corp., Tokyo, Japan).

### Data management and analysis

Animal's phenotypic parameter was transferred to an MS Excel spreadsheet (Microsoft Excel 2018, Microsoft Corp, Redmond, WA, USA) for processing. The data were then analyzed using IBM SPSS Statistics (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp). Variables such as study districts, sex, age, breed, BCS and season were categorized. The levels were for study districts "Naogaon", "Natore", "Rajshahi" and "Joypurhat"; sex "male and female"; age " $x \leq 2$  years", " $2 < x \leq 3$  years" and " $3 < x \leq 4$  years"; BCS

"poor", "medium" and "healthy"; season "winter (December–February)", "Pre-monsoon (March–May)", Monsoon (June–August)", "Post-monsoon (September–November)"; Chi square test was used for comparison of the prevalence rates of fasciolosis between different animal species, age and sex. Differences were considered significant when probability value ( $p$ )  $\leq 0.05$ . Initially a bivariable analysis between fascioliasis (positive, negative) and explanatory variables was performed using Pearson's chi-square test. Forward step-wise logistic regression was performed for the multivariable analysis, with an inclusion cutoff criterion of  $P \leq 0.2$ . Collinearity among explanatory variables was also checked by Pearson's chi-square test. If collinearity was detected, only one of the collinear variables was included in multivariable logistic regression model.

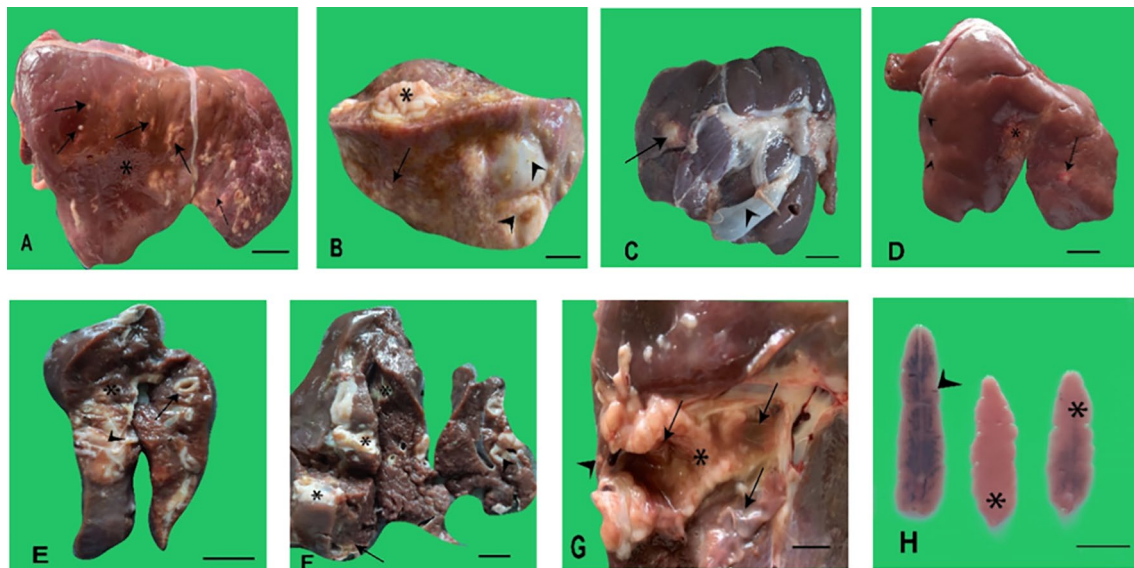
## Results

### Gross pathology of liver

Various gross pathological alterations were observed during onsite liver inspection. The *Fasciola* infected liver were increased in size (hepatomegaly), inflamed with firm and hard consistency and difficult to cut. There were multiple white necrotic foci found on the parietal surface with whitish or reddish discoloration throughout the capsule (Fig. 1A). The parietal surface of the liver had pale appearance that might be resulting from the extensive fibrous tissue proliferation and fatty change. There was also a fibrotic caseous nodule indicating chronic fascioliasis (Fig. 1B). The bile ducts were found obstructed with adult flukes (signs of obstructive jaundice). In some of the infected livers, distention of the gall bladder and the presence of the coagulative necrotic area were seen both on the visceral and parietal surfaces with a roughened and thick capsule (Fig. 1C and Fig. 1D). The bile ducts were hard and calcified which is characterized by distinct grating sound while sectioning and the appearance of pipe stem liver (Fig. 1E and Fig. 1F). Numerous twisted flukes, both mature and immature, were openly visible that caused complete obstruction of the biliary pathways (Fig. 1G and Fig. 1H).

### Histopathology

Varying degrees of microscopic alterations were observed in the histoarchitecture of liver of *Fasciola* infected goat and sheep livers that were largely dependent on the duration and intensity of the infection. Histopathological examination of acute fascioliasis revealed inflammatory cell infiltration in the hepatic parenchyma as well as around the necrotic foci that were bound by a fibrous connective tissue capsule (Fig. 2A and Fig. 2B). Congestion was mostly found in the



**Fig. 1** Gross lesions of the livers of *Fasciola* infected goats and sheep. **A** Small size multiple white necrotic foci (arrows) and hemorrhages (asterisks) were seen on the parietal surface of the liver in acute fascioliasis. **B** Fatty liver of sheep bearing caseous fibrous nodule (arrow head) and fibrous tissue proliferation (asterisk) along with necrotic caseous mass (arrow). **C** Goat liver with fibrous nodule (arrow) at visceral surface and distended gall bladder (arrow head) which contains more than 10 flukes. **D** Sheep liver with cirrhosis (asterisks) and caseous nodule on parietal surface. **E** and **F** Pipe-stem

liver of sheep and goat and fluke was found to escape following sectioning of the bile ducts of the liver (asterisk) in chronically *Fasciola* infection and hard calcareous mass (asterisks) and hyperplasia of bile duct opening (arrow). **G** Fibrous tissue proliferation around gall bladder (asterisk), numerous immature and adult liver flukes (arrow) and caseous necrotic nodule (arrowhead). **H** Flattened mature *F. gigantica* (arrow head) and immature *F. gigantica* flukes (asterisk) collected from gall bladder and bile ducts. Bar = 2.5 cm

central veins and also in the sinusoids due to extravasation of blood from the blood vessels (Fig. 2B). The histopathological lesions of chronic fascioliasis were characterized by infiltration of fibroblasts admixed with lymphocytes and few mononuclear cells in the area previously migrated by young flukes. Classic features of *Fasciola*-infected liver like chronic inflammation, coagulative necrosis, inflammatory cell infiltration, and fibrosis were also evident (Fig. 2C). The infected livers had primary biliary cirrhosis with extensive proliferation of fibrous connective tissue surrounding the intra-hepatic bile ductules and peri portal infiltration of mononuclear inflammatory cells (Fig. 2D). The walls of the bile ducts were infiltrated with eosinophils, lymphocytes, and macrophages and thickened as a consequence of fibrous tissue proliferation (Fig. 2E and Fig. 2F). Liver cirrhosis was characterized by extensive fibrous connective tissue proliferation around the regenerative hepatic lobules that were infiltrated with inflammatory cells (Fig. 2G and Fig. 2H).

### Status of acute and chronic fascioliasis based on gross and histopathology

Based on the characteristic gross pathology for acute and chronic fascioliasis, the level of acute fascioliasis in goat and sheep were 13.52% and sheep 7.30% respectively whereas the level of chronic fascioliasis in goat and sheep were

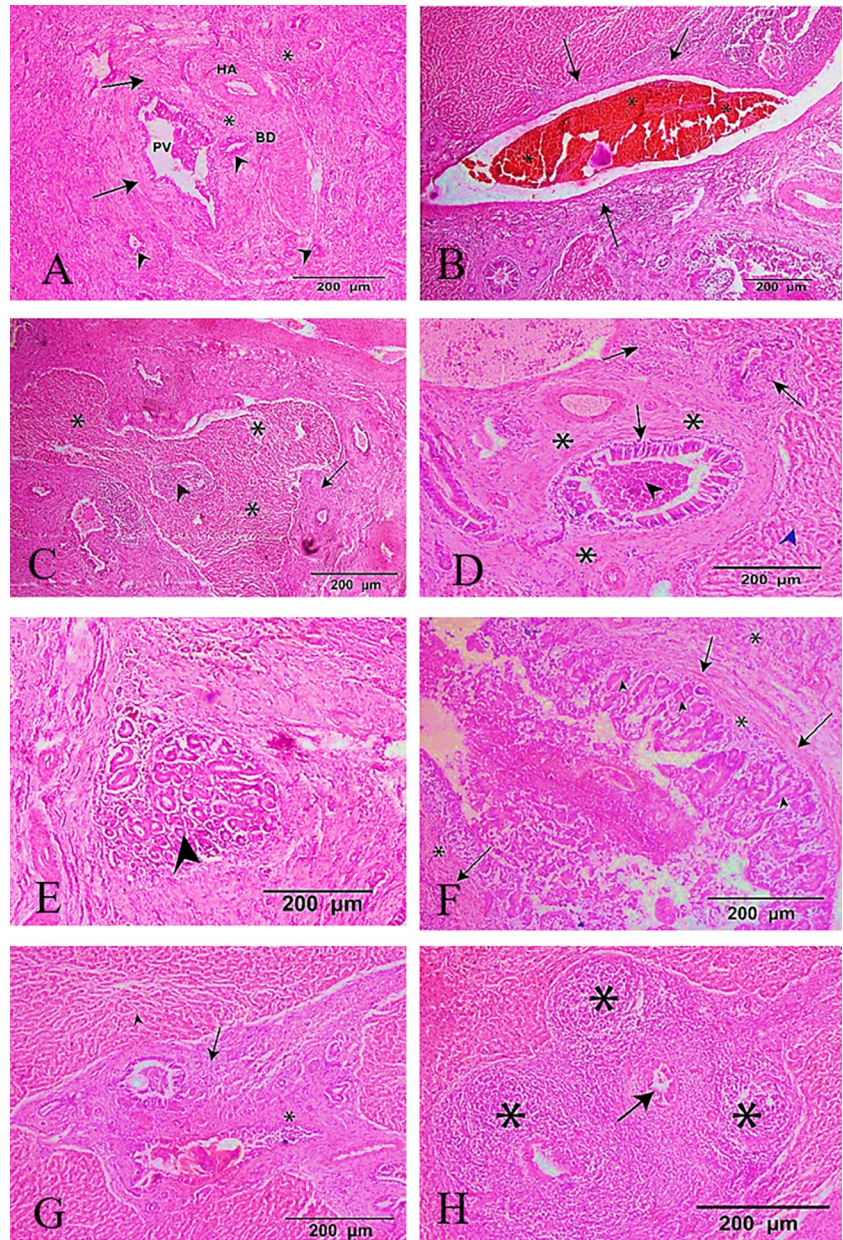
86.68% and 92.70% respectively (Table 2). There were statistically significant differences ( $p < 0.05$ ) in between sheep and goats in respect of acute fascioliasis but there were no statistically significant differences in case of chronic fascioliasis (Fig. 3A). The most common histopathological findings were liver cirrhosis (43.24% for goats; 53.93% for sheep), primary biliary cirrhosis (84.34% for goats; 82.22% for sheep), bile duct hyperplasia (94.79% for goats; 91.02% for sheep) and infiltration of mononuclear cells (59.34% for goats; 51.68% for sheep) in sheep and goat liver (Table 3). Microscopic observations revealed that there were significant differences in between sheep and goat regarding liver cirrhosis, portal fibrosis, pericellular fibrosis, congestion, adenomatous hyperplasia and fatty change (Fig. 3B).

## Epidemiology

### Descriptive statistics

A total of 825 livers have been investigated for the study in four districts of Rajshahi Division named Naogaon, Natore, Rajshahi, and Joypurhat. Out of the 825 samples (Table 1), most of the samples were taken from Naogaon district (35.25%,  $n = 290$ ) followed by Natore (23.03%,  $n = 190$ ), Rajshahi (21.21%,  $n = 175$ ) and Joypurhat

**Fig. 2** Histopathology of sheep and goat liver (H & E stained). **A** Infiltration of the inflammatory cell (asterisks) and a thin layer of connective tissue (arrow) around the portal area along with hyperplastic biliary duct (arrow head), **B** Extravasascular congestion at the portal area (asterisks) with infiltration of inflammatory cells (arrow), **C** Classical features of chronic inflammation (identify) in the *Fasciola* infected liver including coagulative necrosis (arrow head), infiltration of inflammatory cells (asterisks) and fibrosis (arrow), **D** Primary biliary cirrhosis showing extensive proliferation of fibrous connective tissue (asterisks) around the intra-hepatic bile ductules with periportal infiltration of mononuclear inflammatory cells (arrow), **E–F** Hyperplasia of bile ducts (arrow head) surrounded by a thin layer of fibrous connective tissue (arrow), **G** Extensive proliferation of fibrous connective tissue (asterisks) and infiltration of inflammatory cells into the sinusoidal spaces, **H** Liver cirrhosis with granulomatous nodules in chronic *Fasciola gigantica* infection



**Table 1** Prevalence of fascioliasis in sheep and goat

Species	Tested	Infected	Prevalence (%)	Odds ratio	95% CI
Sheep	400	96	24.00	Reference	–
Goat	425	111	26.11	1.1194	0.8166–1.5346
Total	825	207	25.09	–	–

(20.60%, n = 170). Samples were higher in amount for goat (51.51%, n = 425) than sheep (48.49%, n = 400). More liver were sampled from female (64.24%, n = 530) than male (35.75%, 295).

### Prevalence of fascioliasis in sheep and goat

The overall prevalence in sheep and goat tested was 25.09% (95% CI 22.17–28.20) (Table 1). The prevalence of fascioliasis was significantly ( $P=0.008$ ) higher in goats than sheep. The odds of positivity was 1.1194 times (95% CI: 0.8166–1.5346) higher in goats than sheep (Table 1).

### Prevalence of fascioliasis in slaughtered goats

Out of 425 slaughtered goats, 111 (26.11%) livers were found to contain immature and mature *Fasciola gigantica* (Table 1). At least five or more mature and immature flukes were showed during infected liver sectioning. The

**Table 2** Contingency tables and Pearson's chi-square test conducted to evaluate the association between explanatory variables and caprine fascioliasis in northwestern regions of Bangladesh, 2019–2020

Variables	Categories	Positive/Tested	Prevalence (%)	95% CI	P value
Districts	Naogaon	43/148	29.05	21.89–37.08	>0.05
	Natore	28/102	27.45	19.08–37.18	
	Rajshahi	21/89	23.60	15.24–33.78	
	Joypurhat	19/86	22.09	13.86–32.33	
Sex	Male	29/185	15.68	10.76–21.73	<0.05
	Female	82/240	34.17	28.19–40.54	
Age	≤2 years	26/93	27.96	19.14–38.22	<0.05
	2 < x ≤ 3 years	68/188	36.17	29.30–43.48	
	3 < x ≤ 4 years	17/144	11.81	7.03–18.23	
BCS	Poor	92/186	49.46	42.07–56.87	<0.05
	Medium	14/162	8.64	4.81–14.07	
	Healthy	5/77	6.49	2.14–14.51	
Season	Winter	11/120	9.17	4.67–15.81	<0.05
	Pre–monsoon	26/145	17.93	12.06–25.16	
	Monsoon	45/90	50.00	39.27–60.73	
	Post–monsoon	29/70	41.43	29.77–53.83	

**Table 3** Factors retained in a final multiple logistic regression model of the risk of caprine fascioliasis in northwestern regions of Bangladesh, 2019–2020

Risk factors	Categories	Odds ratio	95% CI	P value
Sex	Male	Reference	–	–
	Female	2.7918	1.7314–4.5017	.0001
Age	≤2 years	2.899	1.4699–5.7178	0.002
	2 < x ≤ 3 years	4.2333	2.3534–7.6151	<.0001
	3 < x ≤ 4 years	Reference	–	–
BCS	Poor	14.0936	5.4454–36.4765	<.0001
	Medium	1.3622	0.4723–3.9288	0.565
	Healthy	Reference	–	–
Season	Winter	Reference	–	–
	Pre-Monsoon	2.165	1.0213–4.5895	0.040
	Monsoon	9.9091	4.7036–20.8756	<.0001
	Post-Monsoon	7.0089	3.2083–15.3117	<.0001

highest number of liver fluke were observed in a single liver was 125. The average number of fluke per liver was 13.50. In bivariable analysis, study districts, sex, age, season and BCS were significantly associated with fascioliasis (Table 2). Five variables were significantly associated with fascioliasis in multiple logistic regression (Table 3). The odds of infected with fascioliasis was 2.7918 times (95% CI: 1.7314–4.5017,  $P < 0.05$ ) higher in female goats than male goat. The prevalence of fascioliasis was significantly ( $P < 0.001$ ) higher in ≤2 years old (OR: 2.899, 95% CI: 1.4699–5.7178) and 2 < x ≤ 3 years old goats (OR: 4.2333, 95% CI: 2.3534–7.6151) than 3 < x ≤ 4 years old goats. Prevalence of fascioliasis was significantly associated with body condition scoring. The risk of fascioliasis in goats having

poor body condition had 14.0936 times higher (95% CI, 5.4454–36.4765,  $P = 0.001$ ) than healthy goats. Seasonal variation also played a key part in the prevalence of fascioliasis. Goats were infected 9.9091 times higher in monsoon (95% CI: 4.7036–20.8756,  $P < 0.001$ ), 7.0089 times higher in post-monsoon (95% CI: 3.2083–15.3117,  $P < 0.001$ ), 2.165 times higher in pre-monsoon (95% CI: 1.0213–4.5895,  $P = 0.040$ ) than winter season.

### Prevalence of fascioliasis in slaughtered sheep

A total of 96 livers (24.00%) were infected out of 400 slaughtered sheep (Table 1). The load of immature and mature *Fasciola* in a single infected liver were from 3 to 52. Average number of flukes load per liver was 8.33. In bivariable analysis, study districts, sex, age, season and BCS were significantly associated with ovine fascioliasis (Table 4). Five variables were significantly associated with fascioliasis in multiple logistic regression (Table 5). The odds of infected with fascioliasis was 3.7317 times (95% CI: 1.7314–4.5017,  $P < 0.05$ ) higher in female sheep than male sheep. The prevalence of fascioliasis was significantly ( $P < 0.001$ ) higher in ≤2 years old (OR: 3.5245, 95% CI: 1.6004–7.7618) and 2 < x ≤ 3 years old sheep (OR: 5.8671, 95% CI: 2.9482–11.6757) than 3 < x ≤ 4 years old sheep. Prevalence of fascioliasis was significantly associated with body condition scoring. The risk of fascioliasis in sheep having poor body condition was 6.0346 times higher (95% CI, 1.7986–20.2472,  $P = 0.001$ ) than healthy sheep. Seasonal variation also played a key part in the prevalence of fascioliasis. Sheep were infected 8.2308 times higher in monsoon (95% CI: 3.9922–16.9693,  $P < 0.001$ ), 4.2943 times higher

**Table 4** Contingency tables and Pearson's chi-square test conducted to evaluate the association between explanatory variables and ovine fascioliasis in northwestern regions of Bangladesh, 2019–2020

Variables	Categories	Positive/Tested	Prevalence (%)	95% CI	<i>P</i> value
Districts	Naogaon	35/142	24.65	17.81–32.58	> 0.05
	Natore	22/88	25.00	16.38–35.37	
	Rajshahi	23/86	26.74	17.77–37.38	
	Joypurhat	16/84	19.05	11.30–29.08	
Sex	Male	11/110	10.00	5.10–17.19	< 0.05
	Female	85/290	29.31	24.13–34.91	
Age	≤ 2 years	21/86	24.42	15.80–34.87	< 0.05
	2 < x ≤ 3 years	64/183	34.97	28.09–42.36	
	3 < x ≤ 4 years	11/131	8.40	4.27–14.53	
BCS	Poor	82/236	34.75	28.69–41.20	< 0.05
	Medium	11/ 127	8.66	4.40–14.97	
	Healthy	3/ 37	8.11	1.70–21.91	
Season	Winter	13/120	10.83	5.90–17.81	< 0.05
	Pre–Monsoon	19/130	14.62	9.03–21.88	
	Monsoon	40/80	50.00	38.60–61.40	
	Post–Monsoon	24/ 70	34.29	23.35–46.60	

**Table 5** Factors retained in a final multiple logistic regression model of the risk of ovine fascioliasis in northwestern regions of Bangladesh, 2019–2020

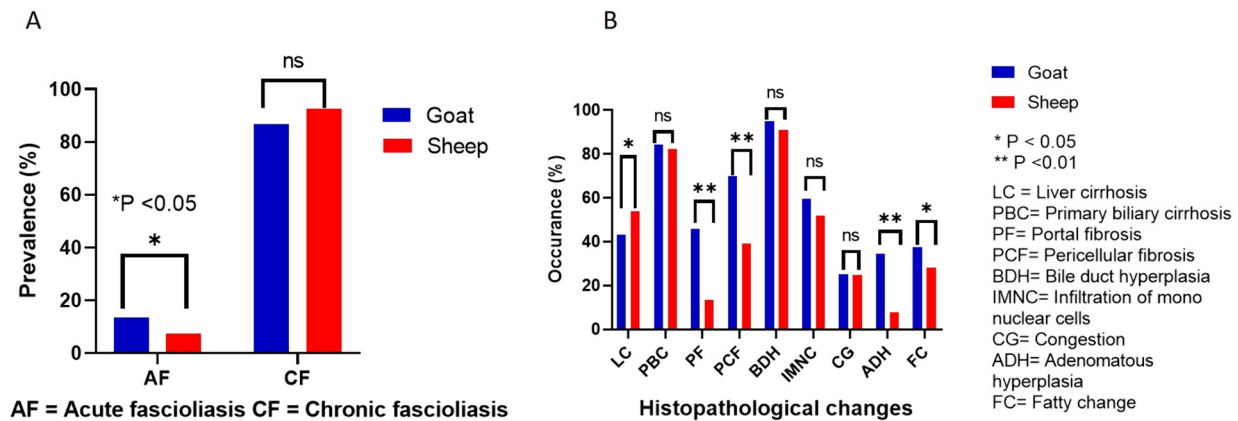
Risk factors	Categories	Odds ratio	95% CI	<i>P</i> value
Sex	Male	Reference	–	–
	Female	3.7317	1.9052–7.3094	< .0001
Age	≤ 2 years	3.5245	1.6004–7.7618	0.001
	2 < x ≤ 3 years	5.8671	2.9482–11.6757	< .0001
	3 < x ≤ 4 years	Reference	–	–
BCS	Poor	6.0346	1.7986–20.2472	0.001
	Medium	1.0747	0.2835–4.0742	0.006
	Healthy	Reference	–	–
Season	Winter	Reference	–	–
	Pre–Monsoon	1.4089	0.663–2.9939	0.371
	Monsoon	8.2308	3.9922–16.9693	< .0001
	Post–Monsoon	4.2943	2.0118–9.1667	< .0001

in post-monsoon (95% CI: 2.0118–9.1667, *P* < 0.001) than winter season.

## Discussions

A total of 825 livers of slaughtered sheep and goats were examined and confirmed fascioliasis by visualizing *F. gigantica* at necropsy in four different districts named Naogaon, Natore, Rajshahi and Joypurhat of northwestern region of Bangladesh. Diagnosing *F. gigantica* by visualization of the fluke at necropsy is considered the gold standard with 100% specificity and sensitivity. The overall prevalence of fascioliasis was 25.09%. The present findings is much higher

compared to previous reports of Bangladesh (Islam et al. 2016b; Islam and Ripa 2015; Mazid et al. 2006; Talukder et al. 2010). The possible reasons behind higher prevalence of fascioliasis in slaughtered sheep and goat of the study area might be diverse geoclimatic and land covering, presence of a good number of water channels, rivers and marshy lands which favor the vector snail's growth and multiplication. The prevalence of fascioliasis was found to be 26.11% in goats and 24.00% in sheep. These estimates are extremely high compared to previous reports from Bangladesh based on slaughter house inspection (Islam et al. 2016b; Islam and Ripa 2015; Talukder et al. 2010) but consistent to previous reports from Bangladesh based on coprology and passive surveillance data (Islam et al. 2014; Rahman et al. 2017; Sangma et al. 2012). By observing the characteristic gross pathology assigned to differentiate acute and chronic fascioliasis, the level of acute fascioliasis in goat and sheep were 13.52% and sheep 7.30% respectively whereas the level of chronic fascioliasis in goat and sheep were 86.68% and 92.70% respectively. Previously published reports from Bangladesh support the current findings of the present findings (Sultana et al. 2022). The reason behind high level of chronic fascioliasis in sheep and goats might be long time grazing in contaminated pasture and constant picking up infection, strategic failure of deworming and development of anthelmintic resistance against common flukicidal drugs (Hasan et al. 2022). In chronic fascioliasis, livers were enlarged, pale, hard to cut, calcified, pipe stem appearance of bile duct, bile duct hyperplasia, parietal surface had the extensive fibrous tissue proliferation and caseous necrotic nodule. These findings are consistent with previous report (Dharanesha et al. 2015; Talukder et al. 2010). Histopathologically, in acute fascioliasis, infected livers



**Fig. 3** Prevalence of acute and chronic fascioliasis in sheep and goats along with histopathological lesions occurrences in sheep and goat in chronic fascioliasis

showed migratory tracts of immature flukes and hemorrhages accompanied by infiltrates of neutrophils and mononuclear cells, both in goats and sheep. The results showed that the most affected sites were portal area especially bile ducts, where the inflammatory reaction started then extended to other parts of hepatic tissue which is consistent with previous report described for acute fascioliasis (Al-Sabaawy and Al-Sadi 2021). In chronic fascioliasis, there was biliary and portal cirrhosis due to proliferation of fibrous connective tissue accompanied by infiltration of mononuclear cells, thickening and hyperplastic changes in the lining epithelium of the bile duct. This may be due to the response of macrophages and lymphocytes in the necrotic areas during the later stages of fascioliasis and the merging fibrous tissues into the healing sites (El-Dakhly et al. 2008; Sayed et al. 2008). Distended bile ducts showed biliary and portal cirrhosis, thickening of bile ducts, calcification of bile ducts and fibrosis in bile ducts, characteristics of chronic fascioliasis (Al-Mahmood and Al-Sabaawy 2019).

All investigated topographic zones had a high rate of infection ranging from 22.09% to 29.05% in goats and 19.05% to 24.65% in sheep respectively. The prevalence estimated for caprine fascioliasis was much higher compared to previously published report from Bangladesh (Abbas et al. 2020; Islam and Ripa 2015; Talukder et al. 2010). The prevalence of ovine fascioliasis recorded in this study was extremely low compared to previously published reports from Bangladesh (Mazid et al. 2006). The number of sheep livers examined from four districts and the sample size was also large, whereas previous reports were only from Mymensingh district and their sample size was small. Prevalence variation for caprine and ovine fascioliasis in all four studied districts might be due to variation in geo-climatic conditions, rainfall, availability of water channels and appropriate snail vector. The prevalence of fascioliasis was significantly higher ( $P < 0.000$ ) in goats than sheep.

Goats were 1.1194 times higher (95% CI: 0.8166–1.5346) odds of positivity than sheep. This difference might be due to more grazing by goats compared to sheep in the study area. It was observed that sheep that were slaughtered, often reared in semi-intensive and sometimes intensive ways by farmer. That might be the possible cause of low prevalence in respect to goats. (Rahman et al. 2017). The odds of fascioliasis positivity in female goats and sheep was 2.7918 times (95% CI: 1.7314–4.5017,  $P < 0.05$ ) and 3.7317 times (95% CI: 1.7314–4.5017,  $P < 0.05$ ) respectively than male goats and sheep which is higher compared to the level of odds obtained in previously published reports (Aktaruz-zaman et al. 2013; Al-Mamun et al. 2011a; Hossain et al. 2011; Islam and Ripa 2015; Sangma et al. 2012). The exact reason for such a difference is unknown but might be associated with the physiological stresses such as pregnancy and lactation. It is evident that pregnancy greatly influence both innate and adaptive immunity (Mandonnet et al. 2005). Age specific variation was also determined. It was observed that animals aged between and  $2 < x \leq 3$  years having higher odds (OR: 4.2333 and OR: 5.8671 for goat and sheep respectively) than young ( $\leq 2$  years) and old animals ( $3 < x \leq 4$  years). Sheep and goat at this age frequently graze pastures and have longer exposure time, which may increase the likelihood of infection with *Fasciola metacercaria*. Additionally, the low prevalence in older sheep and goat can be attributed to the high immunogenicity of the parasite, which aids in the stimulation of acquired immunity in older animals. This finding is in line with the findings reported from different parts of Bangladesh (Al-Mamun et al. 2011a; Alim et al. 2004; Hossain et al. 2011; Islam and Ripa 2015; Karim et al. 2015; Rahman et al. 2017), India (Khanjari et al. 2014), Pakistan (Ruhoollah et al. 2021) and Turkey (Çelik and Aslan Çelik 2018). This study also revealed that there was a significant association between body condition score and fascioliasis in sheep and goats. Animals having a



poor body condition at time of slaughter were most affected by *Fasciola* spp. And animals having poor body condition had higher odds (OR: 14.0936 in goats and OR: 6.0346 in sheep) than healthy animals to fascioliasis. Many studies showed a positive association between fasciolosis and BCS (Kantzoura et al. 2011; Karim et al. 2015). In multiple logistic regression, seasonal variation had a profound impact on fascioliasis. Studies performed in monsoon and post-monsoon, animals had higher odds to positivity than winter and pre-monsoon. Goats were infected 9.9091 times higher in monsoon, 7.0089 times higher in post-monsoon, 2.165 times higher in pre-monsoon than winter season while sheep were infected 8.2308 times higher in monsoon, 4.2943 times higher in post-monsoon than winter season. This could be attributed to the fact that the snail, which serves as the intermediate host, abounds in rainy season. In Bangladesh winter approaches in November and goes in February. That times generally there is a lack of pasturing of animals and often farmers practiced dry lot feeding. As well as there is hibernation of snail vectors in winter. Generally, monsoon comprises June, July and August which is basically rainy season in Bangladesh. That's why infection rate was significantly higher in monsoon and post-monsoon. And this findings is consistent with the previous reports (Rahman et al. 2017). To the best of knowledge, described the status of fascioliasis for the first time in terms of epidemiology and histopathology in small ruminants by direct slaughterhouse inspection in northwestern region of Bangladesh. Because the risk factors identified in this study are unalterable, that should be referred as "risk indicators." These risk indicators could aid in the regular monitoring of this disease in the context of Bangladesh, with the goal of developing livestock and safe food production. One disadvantage of this study was its cross-sectional design, which was implemented at a single time point. Furthermore, animal trade and movement are common within the study area, but only recorded the slaughtered animals last reported location prior to transportation to the abattoir. The sample size calculated in this study was based on a 50% prevalence, but this study estimated a 25.09% prevalence.

## Conclusions

This study revealed that the prevalence of fascioliasis in slaughtered sheep and goats were generally high in the local abattoirs of northwestern region compared to other published reports from Bangladesh. This suggests that snail vectors and infective stage metacercaria are widely dispersed in the environment and thus represent a risk to public health in Bangladesh. Gross and histopathological alterations revealed an extensive damage in liver which leads to mandatory liver condemnation. Thus, it causes

serious economic losses of livestock producer and seller. The risk indicators could aid in the regular monitoring of this disease in the context of Bangladesh. Additional studies should be carried out to assess the risk for human fascioliasis.

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**Data availability** Data, photographs, coordinates, and analysis will be made available to readers upon request.

## Declarations

**Conflict of interest** The authors declare that they have no conflict of interests related to this work. They are solely accountable for the content and writing of the report.

**Ethical approval** We guarantee that this manuscript is original, does not infringe on any copyright or other proprietary right of any third party, is not under consideration by another journal and has not been previously published.

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