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Original Article

Fasciola hepatica in Republic Democratic of Timor-Leste: The Prevalence and Risk Factors Associate with It

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Abstract

Objective: This study aimed to determine the prevalence of *Fasciola hepatica* in buffalo in Timor-Leste and the risk factors associated with this prevalence.
Methods: The method used in this study was multistage random sampling for sites selection and purposive sampling for samples collection. In total there were 486 samples collected from 6 villages from 3 municipalities representing 3 regions (East, central, and west) in Timor-Leste. Samples were collected directly with rectal exploration methods. The samples were examined using sedimentation method. For all risk factors identified, odds ratios (ORs) and their 95% CIs were calculated.
Results: The laboratory results indicated that the prevalence of *F. hepatica* was 17.1%. The Municipality of Manufahi had the highest rate with the prevalence of 25.3%, followed by Bobonaro and Baucau with 14.8% and 11.1%, respectively. Numerous risk variables for *F. hepatica* infestation were identified in this study, including water abundance, which favors snail presence in the environment, rearing techniques, and body condition scores.
Conclusions: According to this study, the overall prevalence of *F. hepatica* in the municipalities surveyed was 17.1% (95% CI: 13.8-20.7%). Manufahi Municipality had the highest prevalence rate at 25.3% (18.8-32.7), followed by Baucau at 11.1% (6.7-17.0) and Bobonaro at 14.8% (9.7-21.2).

Keywords: Prevalence, *Fasciola, Fasciola hepatica*, buffaloes

INTRODUCTION

Timor-Leste is considered as a small country located in Southeast Asia (between Australia and Indonesia) and its independence was just restored on May 20, 2002. The majority of the population of this country lives depending on the agricultural sector including the activity in animal husbandry. The animals raised are goats, sheep, pigs, poultry, Bali cattle and buffaloes. Buffaloes can be infected by the internal parasite. One of the internal parasites infected in animals is *Fasciola hepatica*. Fascioliasis is a very important disease to consider. First, it is a zoonotic disease and, second, it caused economic damage [1]. As a zoonotic disease, it is estimated that 2.4 to 17 million people are infected in 70 to 81 countries on all continents except Antarctica [2, 3]. It infects humans and

animals through consumption of infectious fluke larvae (metacercariae), which are found in contaminated water – typically prey (encysted) on aquatic plants or potentially floating in water – such as in swampy areas, ponds or flooded pastures [3].

An infestation of liver flukes results in financial loss. There were significant morbidity, mortality, and financial losses for the cattle sectors, which resulted in financial losses [4]. Around nine dollars are lost per animal in infected properties because of decreased weaning weight at slaughter, and it was estimated that the economic losses with the conviction of 250,000 livers infected by flukes cost U$ 140,000.00, or 15% of the livers inspected in official slaughterhouses in the state of Rio Grande do Sul [5]. Loss of weight, poor reproductive potential, and eventually death also result in financial loss [6]. Additionally, the cost of preventative treatment for buffaloes with anthelmintic medications like triclabendazole, rafoxanide, and/or albendazole also contributed to the economic loss [7, 6]

Additionally, lower animal production, slower growth rates, liver condemnation, lower pulling power, and excessive anthelmintic use all result in financial losses [6].

Fasciolosis is worldwide disease. There have been reports of it from numerous nations. The prevalence of *F. hepatica* in in Brazil, for example was reported to be between 20% to 28.37% [8] and it is similar to the prevalence of *F. hepatica* in India and Egypt, which ranges from 9.1% to 25.59%) [7, 9], Since there have been no reports of *F. hepatica* prevalence in Timor-Leste, the purpose of this study was to ascertain the prevalence of the disease in buffalo as well as the risk factors linked to it.

METHODS

This research was conducted following the approval of the Human Ethics and Animal Ethics Committees of The National Institute of Science and Technology (INCT). All procedures involving human participants and animals were reviewed and approved to ensure compliance with ethical standards and guidelines. The study adhered to the principles outlined by the relevant regulatory frameworks to safeguard the welfare of both human and animal subjects involved in the research.

Sites Selection Methods

To determine the study area, the researcher used the multistage random sampling method. The selection of the research site was based on the multisage sampling method by dividing Timor-Leste into 3 regions. The 3 regions are: 1). West Region represented by Municipality of Bobonaro, Administrative Post of Cailaco, villages of Meligo and Atudara. 2). East Region, represented by Baucau Municipality, Administrative Post of Vemasse, villages of Vemasse tasi and Uato-Lari. 3). Central Region was represented by Manufahi Municipality, Same Administrative Post, villages of Betano and Daisua [10] (Figure 1).

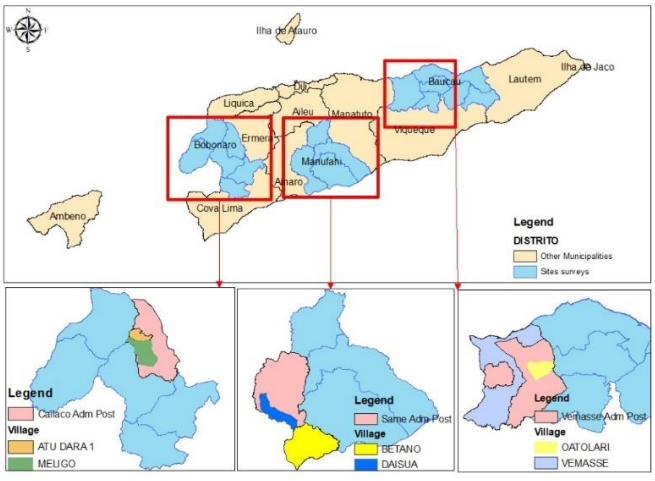

Figure 1. Site surveys (West region - Bobonaro, Central region - Manufahi and East region – Baucau)

The Study Samples

Purposive sampling method was used for the selection of the animal raised by farmers. The selection method mentioned was based on the specific criteria such as famers must have buffaloes. The faeces samples were to be collected from buffaloes of all ages (youngest to the oldest - between 6 months - 6 years), with various body condition Scores – BCS. The samples targeted both sexes, male and female buffaloes. To determine the age of the animal through the teeth, the method described by [11]. To determine the animal's body condition scores the method described by [12, 13]were used.

Sample size and collection

This study was designed based on its expected prevalence of 30%. The expected prevalence was based on research findings of who found that the prevalence of *F. hepatica* was 28,37 %. The level of confidence for this survey was set to be 95% and the desired absolute precision was 10% [14]. Based on these settings, 81 samples were to be collected from each village (Table 1), hence in six villages, a total of 486 faeces samples were collected. The fecal samples were preserved in 10% formalin prior to examination for *F. hepatica* eggs. To collect a buffalo's fecal sample, the following procedures were followed: first gloves were put on and then soaked with soap before a hand was inserted into the buffalo's rectum. Following that, fresh samples of feces were collected from the buffalo's rectum. Additionally, fresh samples were taken from the ground in case the selected buffalo defecated while being collected [4, 15]. A plastic container that has previously been labeled was used to pack the collected samples. After that, the samples were stored in a cool box and preserved with 10% formalin. The collected samples were then taken to the Veterinary Diagnostic Laboratory in Dili to be examined.

Table 1. The approximate sample size required to estimated prevalence in a large population with the desire fixed width confidence limits [14]

Expected Prevalence	**Level of confidence 95%**		
	Desired absolute precision		
	10%	5%	1%
10%	35	138	3457
20%	61	126	6147
30% ⟷	81	323	8067
40%	92	369	9220
50%	96	384	9604
60%	92	369	9220
70%	81	323	8067
80%	61	126	6147
90%	35	328	3457

Variables Observed in the research

The variables observed in this research were the prevalence of the *F. hepatica* in buffaloes based on the study site, breeding system, risk factors, body condition of the animals; presence of snails at the sites survey; and age group of buffaloes.

Data collection and sample collection methods

Data were collected by interviewing farmers. The interviews with farmers were conducted only after obtaining their informed consent. Each participant was provided with detailed information about the purpose, scope, and confidentiality of the study. They were given the opportunity to ask questions and

clarify any concerns before signing the consent form, ensuring their voluntary participation in the research. When the interview was over, the samples were then collected from famers' buffaloes. The samples were collected directly from buffaloes' rectums.

Samples Examination Method

The method used in the analysis of samples was the sedimentation method (Parfit and Banks modification). The first step of sample examination was the preparation of a clean and dry object glass and cover glass, then 2 grams of feces were weighed from each feces sample, 10 ml of water was added and mixed until it was homogenized, it was then transferred into a tube. Once it was transferred into a tube, after 10 minutes, its supernatant was sucked out using a Pasteur pipette. Afterwards, 3 drops of NaOH were added. Next 10 ml of water were added and let it settle for 10 minutes. After 10 minutes, the suspension was again sucked up using a pipette, then 2 drops of Methylene blue were added, afterwards a drop of suspension was drawn and was dropped into an object glass and covered with a cover glass. The sample was then examined under a microscope at 10x magnification to identify *F. hepatica*'s eggs. The eggs' that have been identified were photographed (if it is positive) using digital camera [15].

Data Analysis and map creation

According to [16, 14] the formula used to analyze prevalence is as follows:

$$\text{Prevalence} = \frac{\text{Number of cases}}{\text{Total of population at risk}} \text{ x } 100\% \quad (1)$$

Data analysis was performed using Microsoft Excel and SPSS Software version 26. First, the data were inserted into Microsoft Excel and later imported into SPSS Software version 26 for descriptive statistical analysis. To compare the relative odds of the occurrence of the outcome of interest (e.g. *F. hepatica* infestation), given exposure to the variable of interest (e.g. the presence of snails), Odds ratios are used with the interpretation as follows: OR=1 Exposure to risk factors does not affect odds of outcome; OR>1 Exposure to risk factors is associated with higher odds of outcome; OR>1 Exposure to risk factors is associated with lower odds of outcome [17]. For all risk factors identified, odds ratios (ORs) and their 95% CIs were computed. Since OR can only be computed for non-zero values, all cells in that computation were given a one if any of the cells contained a zero value. The Chi Square test ($\chi 2$), a non-parametric test, was also used to analyze the significance of the laboratory testing for *F. hepatica* for each risk factor. The 95% confidence interval was calculated using Woolf's method [18]

A map to show site surveys was created using QGIS and the shape file for map creation was derived from the United Nations Office for the Coordination of Humanitarian Affairs (OCHA) Timor Leste [19].

RESULTS

Six villages in three municipalities were selected to participate in this study. These villages include: Vemasse and Uato-Lari in the Vemasse administration post, Municipality of Baucau; Atudara and Meligo in the Cailaco administration post, Municipality of Bobonaro; and Betano and Daisua in the Same administration post, Municipality of Manufahi (see Figure 1).

Prevalence based on sites surveys

Overall, the prevalence of *F. hepatica* in this study was 17.1% (95% CI: 13.8-20.7%). However, *F. hepatica* prevalence varies by municipalities. Manufahi municipality had the greatest prevalence among the surveyed municipalities, at 25.3% (95% CI: 18.8-32.7%), followed by Bobonaro and Baucau with the prevalence of 14.8% (95% CI: 9.7-21.2) and 11.1% (95% CI: 6.7-17.0%), respectively (Table 2). The prevalence of *F. hepatica* infestation in different municipalities differ significantly, X^2 (2, N = 486) = 12.41, p = 0.002), At the village level, Vemasse had the lowest prevalence (9.9%, 95% CI: 4.4–18.5%)—and Daisua had the highest prevalence (25.9%, 95% CI: 16.8–36.9%), (Table 2) and the infestation of *F. hepatica* in different villages vary significantly, X^2 (5, N = 486) = 13.32, p = 0.021).

Table 2. Laboratory test result of *F. hepatica* based on site surveys

Municipio	Posto	Suco	Laboratory test		Total	Prevalence	95%CI (%)
			Nagative	Positive			
Baucau	Vemasse	Vemasse	73	8	81	9.9%	4.4-18.5
		Uato-lari	71	10	81	12.3%	6.1-21.5
		Total	144	18	162	11.1%	6.7-17.0
Bobonaro	Cailaco	Atudara	71	10	81	12.3%	61-21.5
		Meligo	67	14	81	17.3%	9.8-27.3
		Total	138	24	162	14.8%	9.7-21.2
Manufahi	Same	Betano	61	20	81	24.7%	15.8-35.5
		Daisua	60	21	81	25.9%	16.8-36.9
		Total	121	41	162	25.3%	18.8-32.7
Grand total			403	83	486	17.1%	13.8-20.7

Risk factors associated with sites surveys

Odds ratio of risk factor associated with different municipalities having *F. hepatica* infestation is shown in Table 3.

Table 3. ORs of different municipalities of having *F. hepatica* infection

Factor	D+	D-	% +ve	OR	Lower 95% CI	Upper 95% CI
Baucau	18	144	11.11%	1.00*		
Bobonaro	24	138	14.81%	1.39	0.72	2.68
Manufahi	41	121	25.31%	2.71	1.48	4.96

*Comparartive fator (referent). D+ (Disease positive), D- (Disease negative), %+ve (Percent positive).

Risk factors associated with rearing system

Odds ratio of different rearing system can be seen in Table 5.

Table 5. ORs of different rearing system to the likelihood of having *F. hepatica* infection

Factor	D+	D-	% +ve	OR	Lower 95% CI	Upper 95% CI
Intensive*	13	25	34.21%	2.94	1.41	6.11
Extensive	19	90	17.43%	1.19	0.67	2.12
Semi-Intensive	51	288	15.04%	1.00**		

*Intensive rearing system here is referring to buffaloes that are locked/tied up in open without proper feeding, treatment and good hygiene, **Comparartive factor (referent). D+ (Disease positive), D- (Disease negative), %+ve (Percent positive).

Different rearing systems have varying risks of *F. hepatica* infestation (Table 5). When comparing intensive and semi-intensive rearing systems, it was discovered that the former was 2.94 times more likely to have an infection with the worm. Compared to the comparison factor, the extensive raising system was 1.19 times riskier. The probability of *Fasciola* infestation varies significantly depending on the rearing system, X^2 (2, N = 486) = 8.88, p = 0.012).

Prevalence of *F. hepatica* based on whether the buffalos are raised next to rice fields, rivers, or swamps.

During the survey, questions about a few risk variables were targeted. These refer to whether the buffalos are raised next to rice fields, rivers, or swamps areas. The results of this study indicated that the buffaloes raised near rivers and swamps had the highest prevalence based on these characteristics, at 31.00% (95% CI: 19.5-44.5%), followed by those raised near rivers at 17.10% (95% CI: 12.8-22.1%) and rice fields at 12.70% (95% CI: 7.8-19.1%), (Table 6) and these differences of *F. hepatica* prevalence from different field conditions differ significantly, X^2 (4, N = 486) = 11.89, p = 0.018).

Table 6. Prevalence of *F. hepatica* based on some risk factors

Description	Laboratory test		Total	Prevalence	95%CI
	Negative	Positive			
Rice field	131	19	150	12.70%	7.8-19.1%
River	223	46	269	17.10%	12.8-22.1%
Swamp	5	0	5	0.00%	0.0-52.2%
Rice field & Swamp	4	0	4	0.00%	0.0-60.2%
River & Swamp	40	18	58	31.00%	19.5-44.5%
Total	403	83	486	17.10%	13.8-20.7%

Risk factors associated with whether the buffalos are raised next to rice fields, rivers, or swamps.

According to Table 7, the probability of *Fasciola* infestation is 3.10 times higher for buffaloes that grazed close to river and swampy places than for those who were raised close to rice fields only (the referent factor). Buffaloes kept in both rice fields and swampy locations are the second most dangerous, with a risk that is 1.72 times higher than the comparison factor. Last but not least, buffaloes raised close to swampy areas are 1.38 times more likely to have a *F. hepatica* infestation than those reared in rice fields, and those that graze near rivers were the third most at risk (1.42 times). Raising buffaloes close to rice fields, rivers, or swampy areas increases their chance of contracting *F. hepatica* significantly, X^2 (4, N = 486) = 11.89, p = 0.018).

Table 7. ORs of different pasture conditions where buffaloes are reared

Factor	D+	D-	% +ve	OR	Lower 95% CI	Upper 95% CI
Rice field	19	131	12.67%	1.00*		
River	46	223	17.10%	1.42	0.80	2.53
Swamp	1**	5	16.67%	1.38	0.15	12.45
Rice field & Swamp	1**	4	20.00%	1.72	0.18	16.25
River & Swamp	18	40	31.03%	3.10	1.49	6.47

*Comparartive fator (referent), **was 0 (no positive), a one was put to calculate OR because OR cannot be calculated when it is zero. D+ (Disease positive), D- (Disease negative), %+ve (Percent positive).

Prevalence based on Body Condition Score (BCS)

Additionally, the study noted various BCS for every buffalo that was sampled. The following Table (Table 8) shows that the highest prevalence was found in buffaloes with BCS of 1, which had a prevalence of 23.8% (95% CI: 8.2-47.2%), followed by BCS 2, which had a prevalence of 22.5% (95% CI: 13.9-33.25%) and BCS 3, which had a prevalence of 20.9% (95% CI: 14.8-28.2%), (Table 8). However, the differences of prevalence of *F. hepatica* infestation amongst different body condition scores (BCS) does not differ significantly, X^2 (4, N = 486) = 8.37, p = 0.079).

Risk factors associated with body conditions scores (BCS)

Odds Ratio of different body condition scores of tested buffaloes are shown in Table 9 bellow.

Table 9. Odds Ratio of different body condition scores of tested buffaloes

BCS	D+	D-	% +ve	OR	Lower 95% CI	Upper 95% CI
1	5	16	23.81%	2.40	0.81	7.18
2	18	62	22.50%	2.23	1.13	4.42
3	32	121	20.92%	2.04	1.14	3.65
4	23	177	11.50%	1.00*		
5	5	27	15.63%	1.43	0.50	4.07

*Comparartive fator (referent). D+ (Disease positive), D- (Disease negative), %+ve (Percent positive).

According to Table 9, buffaloes with a body condition score (BCS) of 1 are 2.40 times more likely to develop an infestation of *F. hepatica* than those with a BCS of 4 (the comparative factor). Furthermore, compared to the comparison factor, buffaloes with a BCS of 2 are 2.23 times more likely to have an infection with *F. hepatica*. In comparison to the comparative factor (BCS 4), buffaloes with a BCS of 3 are 2.04 times more likely to have an infestation with *F. hepatica*. Finally, the buffaloes with the BCS of 5 are 1.43 times more chance of being infected with *F. hepatica*. The risk of *F. hepatica* infestation among different body condition scores (BCS) of buffalo does not differ substantially, X^2 (4, N = 486) = 8.37, p = 0.079).

Prevalence based on the presence of an intermediate host (snails)

This survey also recorded the presence of snails at each research site since they are an intermediate host for fascioliasis. When snails were present, the prevalence was greater (17.4%, 95% CI: 14.1-21.3%) compared to areas without snails, which had a prevalence of 12.1%, 95% CI: 3.4-28.2%), (Table 10), however statistically, these differences are not significant, X^2 (1, N = 486) = 0.61, p = 0.43)

Table 10. Prevalence of *F. hepatica* based on the presence of snail around the site surveys

Snail presence	Laboratory test		Total	Prevalence	95%CI
	Negative	Positive			
No.	29	4	33	12.1%	3.4-28.2%
Yes	374	79	453	17.4%	14.1-21.3%
Total	403	83	486	17.1%	13.8-20.7%

Risk factors associated with the presence of intermediate host (snails)

Odds Ratio of buffaloes tested based on the presence of snails are shown in Table 11.

In comparison to farmers who did not record snails in their locations, those who reported snails near or around grazing ground are 1.53 times more likely to be infected. However, there is no significant difference in the risk of *F. hepatica* infestation between buffaloes with and without snails, X^2 (1, N = 486) = 0.61, p = 0.433).

Table 11. Odds Ratio of buffaloes tested based on the presence of snails

Factor (Snails' presence)	D+	D-	%+ve	OR	Lower 95% CI	Upper 95% CI
Yes	79	374	17.44%	1.53	0.52	4.48
No	4	29	12.12%	1.00*		

*Comparartive fator (referent). D+ (Disease positive), D- (Disease negative), %+ve (Percent positive).

Prevalence based on age group

The age influences the prevalence of *F. hep*atica. Table 12 shows that the highest prevalence (25.3%, 95% CI: 17.1-35.0%) occurred at the age group of 1 to 12 months old, followed by those that are older than 3 years with the prevalence of 17.3% (95% CI: 12.4-23.1%). For some reason the prevalence of the age group of 13 to 24 months old were in the middle with the prevalence of 12.6% (95% CI: 6.5-21.5%), (Table 12). The differences of prevalence amongst age group is significant, X^2 (2, N = 486) = 6.30, p = 0.043).

Table 12. Prevalence of *F. hepatica* based on age group

Age group (months)	Laboratory test		Total	Prevalence	95%CI
	Negative	Positive			
1 -12 months	74	25	99	25.3%	17.1-35.0%
13 to 24	76	11	87	12.6%	6.5-21.5%
25 to 36	81	11	92	12.0%	6.1-20.4%
37 and older	172	36	208	17.3%	12.4-23.1%
Total	403	83	486	17.1%	13.8-20.7%

Risk factors associated with age group of buffaloes

Odds Ratio of buffaloes tested based on age group are shown in Table 13.

Table 13. Odds Ratio of buffaloes tested based on age group.

Factor	D+	D-	%+ve	OR	95% CI	
					Lower	Upper
1-12 months	25	74	25.25%	2.33	1.07	5.08
13-24 months	11	76	12.64%	1.00*		
>25 months	47	253	15.67%	1.28	0.63	2.60

*Comparartive fator (referent). D+ (Disease positive), D- (Disease negative), %+ve (Percent positive).

Buffaloes in the age group of 1–12 months have a 2.33-fold increased chance of contracting a *F. hepatica* infestation in comparison to those in the age group of 13–24 months. However, compared to the referent factor, the chance of having a *F. hepatica* infestation is 1.28 times higher for those older than 25 months. The risk of contracting an infestation with *F. hepatica* varies significantly by age group, X^2 (2, N = 486) = 6.30, p = 0.043).

DISCUSSION

The parasites *F. hepatica* is responsible for the disease called fascioliasis. Ingestion of encysted metacercariae of the liver fluke species *F. hepatica* is what causes the disease [20]. The life-cycle of liver flukes involves snails as an intermediate host.

Prevalence based on sites surveys

Municipalities had a significant differences of having *F. hepatica* infestation: X^2 (2, N = 486) = 12.41, p = 0.002) with buffalo in Manufahi and Bobonaro were more likely to be infested with *F. hepatica* compared to Baucau. Our data shows that Manufahi Municipality had the greatest prevalence (25.3%, 95% CI: 18.8-32.7%) in the current study (Table 2). Manufahi is by far 2.71 riskier (OR 2.71, 95% CI: 1.48 - 4.96), to have

Fasciola infestation compared to Baucau municipality (the referent factor) (Table 3). This could be due to the fact that Manufahi municipality experienced more rains at the time of sample collection, which helped the parasite and its intermediate host survive there better than the other two municipalities.

Bobonaro is the second most prevalent (14.8%, 95% CI: 9.7-21.2%) of *F. hepatica and it* is 1.39 riskier (OR 1.39, 95% CI: 0.72 - 2.68) to have *F. hepatica* infestation compared to Baucau. There was also rain in Bobonaro, at the time of sample collections compared to Baucau municipality. Rains change the environment's temperature and humidity, which favours the development of metacercariae from snails [21]. A dry climate, on the other hand, is unfavorable to the parasite and lowers its prevalence. According to a report, the summer months had the lowest occurrence of *F. hepatica* (10.4-12.8%) [22].

The overall prevalence of this parasite in buffaloes in this study was 17.1% (95% CI: 13.8-20.7%) with the prevalence at municipality level ranges from 14.8% (95% CI: 9.7-21.2%) to 25.3% (95% CI: 18.8-32.7%) and the prevalence of *F. hepatica* infestation in different municipalities differ significantly, X^2 (2, N = 486) = 12.41, p = 0.002). The finding of our prevalence study of *F. hepatica* of buffaloes in this study is not very different from other finding of prevalence study from other countries as presented from the table below (Table 14).

Table 14. Prevalence of *F. hepatica* from different countries with different conditions

No	Prevalence	Sample size	Local condition	Country	Author/s
1	28.37%)	74 stool samples	Farm animals	Minas Gerais, Brazil	[8]
2	25.59 %	2704 Post-mortem examinations of liver	Slaughtered buffaloes	Punjab province, India	[25]
3	26.16%	1720 Faecal samples	Buffaloes at livestock farms	Punjab province, India	[25]
4	13.7%	8721 Faecal samples	Buffaloes in veterinary hospitals	Punjab province, India	[25]
5	10.50%	8783 Faecal samples	Household buffaloes	Punjab province, India	[25]
6	20.00%	105 livers of buffaloes	Buffaloes slaughtered at a meat packing plant	State of Rio Grande do Sul, Brazil	[5]
7	87.35%	767 Faecal samples	District farms	Guangxi, China	[23]
8	19.60%	3,356 Faecal samples	Buffaloes from 29 farms	Alexandria, Egypt	[7]
9	15.50 %	3,356 Faecal samples	Buffaloes from 29 farms	Beheira Egypt	[7]
10	9.10%,	3,356 Faecal samples	Buffaloes from 29 farms	Kafr el-Sheikh Egypt	[7]
11	16,8%	38,113	Buffaloes	Pakistan	[9]

According to the Table 14, the average prevalence from various agricultural conditions, from various samples, and various nations ranges from 9.1 to 87.35%, with an average prevalence of 27.42%.

The overall prevalence of this parasite in this study (17.1%, 95% CI: 13.8-20.7%), is lower compared to a study conducted in China's Guangxi with the prevalence ranges from 71.91 to 87.35% (n= 767) [23] but the prevalence of the current study is only slightly lower than the finding of *F. hepatica* in in Brazil with a prevalence rate between 20% to 28.37% [8] and it is similar to the prevalence of *F. hepatica* in India and Egypt, which ranges from 9.1% to 25.59%) [7, 9]. (see Table 14).

Prevalence based on rearing system

The prevalence of *F. hepatica* is influenced by the buffaloes' rearing practices. The buffaloes have a greater probability of contracting the disease under a rearing strategy that exposes them to the infection source, such as moist surroundings and the intermediate host (snails). One of the risk factors for Danish dairy cattle was identified to be grazing on wet pastures [24]. Buffaloes reared in areas with intensive rearing systems, can provide access to wet grass. In our study, we found that intensively reared buffaloes have higher prevalence (34.2%, 95% CI: 19.6-51.4%) compared to extensively reared buffaloes (4%, 95% CI: 10.8-25.9%) and semi-extensively reared buffaloes (15.0%, 95% CI: 11.4-19.3%) (Table 4). Furthermore it was discovered that the intensive rearing system was 2.94 times more likely to have an infection with the worm [OR 2.94 (1.41-6.11)] compared to the comparison factor, the extensive raising system was 1.19 times riskier [OR 1.19 (0.67- 2.12)] (see Table 5). The probability of *Fasciola* infestation varies significantly depending on the rearing system (X2 (d2, N = 486) = 8.88, p = 0.012). This is probably because farmers who reared buffaloes intensively fed them with grass and greens harvested from rice fields and nearby river that have been contaminated with metacercariae. Additionally, in this study, buffaloes kept in the yard but not given the necessary attention, food, or cleanliness are categorized as being in an intensive raising system. This could explain why intensive rearing systems are more common. Compared to semi-extensive rearing systems, extensive rearing systems are the second most prevalent and riskiest because they permit buffaloes to roam freely in swampy areas, leaving them open to environmental hazards such as muddy areas, wet grass, and snails.

Risk Factors Identified in site surveys

Some risk associated with *F. hepatica* infestation include the abundant of water, availability of snails in the environment, farming conditions and body condition scores (BCS). In the current study, the buffaloes that are raised near rivers and swamps had the highest prevalence, 31.00% (95% CI:19.5-44.5%) and this is similar to the finding that high prevalence of Fascioliasis (28.37%) found in farms that had ponds and flooded area [8].

In our study, we also discovered that when snails were present in an area, the prevalence was greater (17.4%, 95% CI: 14.1-21.3%) and are 1.53 times more likely to be infected. compared to areas without snails, which had a prevalence of 12.1% (3.4-28.2%) (see Table 10 and 11). Even though the prevalence vary, but this differences are not significant, X^2 (1, N = 486) = 0.61, p = 0.43). This is in agreement with the finding of [8] who discovered that the high prevalence (28.37%) seen in the buffaloes was caused by the existence of an intermediate host (*Lymnaea molluscs*). Farming condition influences the prevalence rate of *F. hepatica*. Infection rate fascioliasis in Punjab, Pakistan, for example was found to vary in different farming conditions [25]. These authors found that the prevalence of Fascioliasis was 25.59% in slaughtered buffaloes, 26.16% in buffaloes at livestock farms, 13.7% in veterinary hospitals and 10.5% in household buffaloes [25]. Poorest body conditions scores (body condition score of 1) contribute to more infection.

One of the factors that affect the prevalence of *F. hepatica* is body condition scores (BCS) (see Figure 2). It is reported that BCS had significant ($p < 0.01–0.001$) influence on the prevalence of fascioliasis [22]. In our study, we discovered that those who have very poor BCS (BCS 1) have the highest prevalence, 23.8% (95% CI: 8.2-47.2%), this was followed by BCS 2 and 3 with the prevalence of 22.5% (95% CI: 13.9-33.25) and 20.9% (95% CI: 14.8-28.2%) respectively (Table 8) and *the lower the BCS, the more risk of having F. hepatica infestation*. For example buffaloes with a body condition score (BCS) of 1 are 2.40 times riskier to have *F. hepatica* infestation compare with BCS 4 (see Table 8 and 9). However, statistically, there is no significant variation in the prevalence of *F. hepatica* infestation across body condition scores (BCS) (X^2 (4, N = 486) = 8.37, p = 0.079).

Figure 2. Body condition scores (BCS) observed during the survey

Prevalence based on age group

This study found that younger animals had a higher prevalence of *F. hepatica* than adults did. As an illustration, the prevalence of buffaloes under the age of one year was 25.3% (95% CI: 17.1-35.0%), while that of those over three years was 17.3% (95% CI: 12.4-23.1%) (see Table 12). Similarly, buffaloes in the age group of 1–12 months have a 2.33 fold increased chance of contracting a *F. hepatica* infestation compare with buffaloes in the age group of 13–24 months (see Table 12 and 13). Statistically, the prevalence varies significantly by age group X^2 (2, N = 486) = 6.30, p = 0.043). Younger animals frequently exhibit this since their immune is still forming and they are at a higher risk of contracting an infection. Similar findings were found in Pakistan, where calves of the buffalo and cow with the highest prevalence were those between the ages of 1 and 6 months (86.67%, 69.05%) as compared to those between the ages of 7 and 12 months (60%, 42.10%) [26].

Due to some reasons, in our study, buffaloes older than three years old (37 months or older) had a slightly higher prevalence of infection (17.3%, 95% CI: 12.4-23.1%) than those younger than two years (13 to 24 months), with a prevalence of 12.6% (95% CI: 6.5-21.5%) (Table 12). Animals older than two years were affected significantly more frequently than those younger than two years, according to a similar discovery published by [25]. These authors reported that the greater occurrence in older animals may be brought on by a reduction of immunity as a result of environmental influences.

CONCLUSION

This research showed that *F. hepatica* is present in the surveyed municipalities with an overall prevalence of 17.1% (95% CI: 13.8-20.7%). In terms of prevalence, Manufahi Municipality had the highest rate with 25.3% (18.8-32.7), followed by Bobonaro and Baucau with 14.8% (9.7-21.2) and 11,1% (6.7-17.0), respectively.

Some risk factors identified include young age, the presence of snails, poor body condition score and being raised next to rice fields, rivers, or swampy area.

CONFLICT OF INTEREST

We (authors) state that the content addressed in this work has no conflict of interest with any financial entity.

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