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Research Article

Prevalence of liver fluke infections in domestic animals from district Mardan. A retrospective population-based study from Pakistan

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Abstract

Fascioliasis is a major problem and poses serious threat to livestock resulting in mortality and economic losses to livestock industry. The liver flukes must be observed as an emergent health threat due to the increasing number of human cases. The present study was undertaken to ascertain the existence of liver flukes' infection among animals in Mardan. About 125 samples were examined, these fecal samples were then preserved in the plastic polythene bags having 10% formalin solution with proper labeling and were examined in laboratory under microscope for detection of liver fluke. The infection rate of Fascioliasis is 12% in bulls and 11% in male buffaloes, compared to 18% in cows and 15% in female buffaloes. Both genders graze in the same fields, exposing them equally to infective larvae. However, infection rates are slightly higher in females than males. The higher infection prevalence during the monsoon season (June, July, and August), was observed. This is likely due to heavy rainfall creating optimal temperatures and moisture levels that encourage parasite growth. High infection rates in calves and buffalo babies (19.05% and 23.08%, respectively) due to their weaker immunity, making them more susceptible to disease. It is concluded that heavy infection is present in Mardan. The infection rate was 15.2%. Fascioliasis infection rates are higher in females and peak during monsoon season, with young animals most susceptible due to weaker immunity. Recommendations include targeted deworming, enhanced monitoring during monsoons, improved pasture management, and immunity-boosting measures for young livestock.

Keywords: Fascioliasis, Liver flukes, Animals, Cattle, Pakistan

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Introduction

Fascioliasis is a parasitic infection spread by endoparasitic trematode of the genus *Fasciola*, that inhabit bile ducts of humans and animals [1]. The most common species is *Fasciola hepatica* and *Fasciola gigantica* primarily infect the hepatobiliary systems of cattle and sheep, posing a significant public health concern [2]. *Fasciola hepatica*, called sheep liver fluke, is parasitic flatworm belongs to phylum Platyhelminthes, class Trematoda, subclass Digenea, order Echinostomida, suborder Distomata, family Fasciolidae, and genus *Fasciola* [3]. It infects the liver of many mammals with humans. Liver flukes are widespread helminthic parasites that affect herbivores and are found in many parts of the world [4].

Liver flukes are emerging public health threat because of increasing cases [5]. Additionally, they cause severe diseases in livestock, leading to substantial economic losses from animal death, liver condemnation, and decreased meat, milk, and wool production, also costs related to cure and anthelmintics [6]. Fascioliasis is a zoonotic infection mainly transmitted through contaminated water and intermediate hosts, ultimately reaching humans [7]. Though found globally, it is more prevalent in regions where animal husbandry is common [8]. About 750 million people are considered at higher risk of infection from food-borne trematodes [9]. *F. hepatica* have complex life cycle [10]. The true economic burden may be underestimated, especially in less industrialized regions, due to indirect costs like labor loss and treatment. Globally, fascioliasis affects around 2.6 million people, underscoring its public health significance [11]. In cattle, fasciolosis often results in chronic, subclinical infections, leading to many untreated animals [12 - 19]. It causes notable losses in the livestock sector due to lowered productivity, liver rejection at slaughter, and diminished carcass value [12, 20]. For example, in Switzerland, the annual financial loss per infected cow can be as high as €376 [21]. In Denmark, between 2000 and 2003, bovine fasciolosis prevalence ranged from 12% to 24% at the herd level and 1.7% to 4.3% at the individual animal level, with associations noted with grazing patterns, wetland exposure, and soil type [22].

In Northern Ireland, field-based cattle farming practices expose animals to *F. hepatica* metacercariae through pasture grazing. Although usually chronic, fascioliasis-related mortality in cattle has been reported, with 2–9% of deaths investigated between 2010 and 2013 attributed to the disease [23]. Surveillance of fecal samples collected from 2012 to 2014 by the Agri-Food and Biosciences Institute revealed that 10 to 17% of samples found *F. hepatica* eggs. With the added challenges of climate change [24] and emerging anthelmintic resistance, liver fluke infections may pose an increasing threat to agriculture in Northern Ireland and beyond [25].

Numerous studies have used data to study infections level and spatial risk factors for parasitic infections [21, 26 - 31]. Northern Ireland has a strong record of using livestock surveillance data to model parasitic diseases like fascioliasis [32], However, recent analyses have been limited, missing opportunities to explore trends or test new epidemiological hypotheses. Conversely, in the Republic of Ireland, spatial modeling using bulk milk samples from dairy farms has effectively identified liver fluke distribution, clustering, and risk on a national level [33 - 35]. Similar analyses are lacking for Northern Ireland in recent years. In Pakistan, liver flukes are relatively prevalent. The primary definitive hosts include cattle, sheep, and goats, but other mammals, including humans, are also susceptible [36]. Fascioliasis poses a serious challenge to the development of the

cattle industry in Pakistan. Inappropriately, consistent and comprehensive data on fascioliasis within the country are lacking. Although some studies have been conducted in various regions [37]. There is a need for updated and localized research. The current study aims to assess the prevalence of liver fluke infections in domestic livestock in District Mardan, Khyber Pakhtunkhwa, Pakistan, and to investigate factors contributing to differential infection rates among animals.

Materials and Methods

In order to study the frequency of liver flukes in domestic animals (i.e., cows, bulls and buffaloes), the research study was carried out from April 2021 to August 2021.

Ethical consideration and permissions

The study was conducted by following the rules of declarations of Helsinki. Ethical permission was obtained from the university department. The consent to publish the data was obtained from the animal holders and the relevant university department.

Samples collection and preservation

About 125 fecal samples were collected from cattle having different sex and age. These fecal samples were taken from dairy farms & domestic animals of Maqam Chock, Lund Khwar and out skirts of Mardan. The collected fecal samples were then preserved in plastic polythene bags having 10% of Formalin Solution, which acted as preservative.

Labelling and storing samples

All the samples were accurately labeled according to the age, sex of the cattle, date and place from where the samples were collected & their details were mentioned on the polythene bags accordingly. These labeled samples were then brought to the laboratory and stored for the examination of liver fluke eggs.

Identification of bacteria by microscopy

In the laboratory, fecal samples were initially examined directly under a microscope. For samples in which liver fluke eggs were not detected through direct microscopy, sedimentation techniques were subsequently employed for further examination.

For sedimentation technique the following protocol was adopted.

For the sedimentation technique, 5 g of feces were soaked in 50 mL of distilled water (dH_2O) and mixed thoroughly. The mixture was filtered through a tea strainer into a plastic jar, then transferred to a 50 mL conical test tube. After settling for 5 minutes, the supernatant was transferred. The sediment was poured into a 10-15 mL conical tube, allowed sediment for another 5 minutes, and the supernatant was carefully removed.

Statistical analysis

The gender wise, age wise prevalence was observed during the study. A monthly data was used to organize this incident. The MS Excel 2010 software was used to estimate the prevalence [38].

Results and Discussion

About 125 samples were gathered from Maqam Chowk, Baghdada, Lund Khwar, and the outskirts of Mardan. The samples included 75 cows (both sexes) and 50 buffaloes (both sexes).

Gender wise prevalence

The infection in bulls and male buffaloes was found to be 12% and 11%, respectively, while the infection rate in

cows and female buffaloes was 18% and 15%, respectively. This difference may be attributed to the fact that both sexes graze together in the same fields, sharing equal chances of ingesting infective larvae. However, the prevalence was found slightly higher in female animals (cows and female buffaloes) compared to their male counterparts (bulls and male buffaloes), as shown in Table 1. This increased susceptibility in females may be due to a weakened immune system during physiological states such as gestation and lactation, which makes them more vulnerable to infections. Our findings are in agreement with [39 - 41].

Table 1: The gender wise prevalence of Liver flukes among different animals.

Gender	Examined Samples	Infected samples	Prevalence
Cow	50	9	18%
Bull	25	3	12%
Female Buffalo	39	6	15%
Male Buffalo	11	1	11%

Month wise prevalence

During the study, to assess the monthly variation in infection prevalence, it was observed that the rate was higher during the monsoon season-specifically in June, July, and August in Table 2. The likely reason for this increase is that heavy rainfall during the monsoon season creates favorable environmental conditions, such as optimal temperature and sufficient moisture, which promote the development and survival of parasites. Our findings are consistent with those of [42] who reported that parasitic infestation peaks during the rainy season. Similarly, our study aligns with the results of [43 - 45], who observed higher infestation rates during the summer months. Comparable observations were made by [46], who documented the highest prevalence during the monsoon season compared to winter.

Table 2: The month wise prevalence of Liver flukes among different animals

Month	Examined Samples	Infected samples	Prevalence
April	19	-	-
May	21	3	14.28%
June	17	4	23.53%
July	30	5	16.67%
August	38	7	18.42%

Age wise prevalence

Age is a crucial determinant in the prevalence of infection, as evidenced by the results of this study. As shown in Table 3, the infection rate was higher in both calves and buffalo calves, recorded at 19.05% and 23.08%, respectively. The primary reason for this increased infection rate is the lower immunity in young animals, making them more susceptible to disease. Our findings are in agreement with those of [47, 48], who also reported higher infestation rates in calves compared to older animals. Similarly, it has been noted that young animals are generally more vulnerable to parasitic infections than adults [49]. However, our results contrast with the findings of [50], who reported a higher prevalence of infestation in adult animals.

Table 3: The age wise prevalence of Liver flukes among different animals.

Age	Examined Samples	Infected samples	Prevalence
Cows (adults)	54	8	14.87%
Calves (Babies)	21	4	19.05%
Buffaloes (adults)	37	4	10.81%
Buffaloes (Babies)	13	3	23.08%

The age depends on frequency of animals is shown in Figure 1.

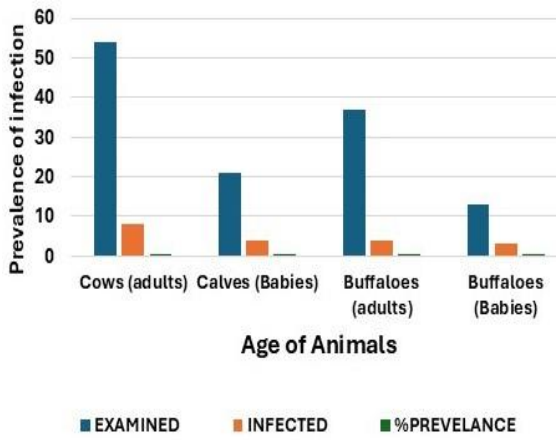

Figure 1: The age depends on the prevalence of animals.

Conclusion

The study concludes that fascioliasis infection rates are slightly higher in female cattle and buffaloes, with the highest prevalence observed during the monsoon season — likely due to favorable environmental conditions that support parasite development. Calves and young buffaloes are particularly vulnerable, primarily due to their underdeveloped immune systems. Based on these findings, it is recommended to implement targeted deworming programs, especially for female and young livestock; enhance surveillance and diagnostic efforts during the monsoon months; improve pasture management to minimize exposure to infective larvae; and explore strategies to strengthen immunity in young animals to reduce disease susceptibility.

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Authors contribution

All authors contributed equally to the manuscript.

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