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RESEARCH ARTICLE

Prevalence and Clinico-Therapeutic Evaluation of Fasciolosis in Small Ruminants (*Capra hircus* and *Ovis aries*) of Muzaffargarh, Pakistan

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ABSTRACT

Fasciolosis is a parasitic disease of small ruminants that has economic significance because it causes massive crop loss through poor growth, anemia, and low productivity. The current research was done to identify the prevalence of fasciolosis, risk factors, the therapeutic effect of the widely used anthelmintics, and hematological changes among small ruminants (*Capra hircus* and *Ovis aries*) in Tehsil Muzaffargarh, Pakistan. Three hundred animals, including 151 goats (*Capra hircus*) and 149 sheep (*Ovis aries*) were sampled randomly. The standard sedimentation test was used to examine fecal samples to determine the presence of Fasciola eggs. The prevalence of fasciolosis was found to be 22.0% (66/300) in general. The prevalence rate of the species was 22.51 (34/151) in goats and 21.47 (32/149) in sheep. Risk factor analysis showed that age was significantly related to infection in both species ($p<0.05$) and that the management system was significantly related to infection in sheep only. There were no statistically significant effects in sex and season. In the case of therapeutic evaluation, forty naturally infected animals (20 goats and 20 sheep) were randomly divided into two treatment groups. Group A received triclabendazole (12mg/kg body weight) and Group B was administered albendazole (10mg/kg body weight). The efficacy of treatment was determined on Day 7 and 14 days after treatment on fecal egg disappearance and clinical recovery. Triclabendazole was found to be more effective than the two species with goats and sheep recovering 80 percent and 60 percent and 70 percent respectively after administration of the albendazole. The blood tests showed that the patient had severe pre-treatment anemia with a decrease in hemoglobin, red blood cell count and hematocrit levels. The evaluation of the parameters in the post-treatment period significantly improved, especially in the case of animals using triclabendazole ($P<0.05$), which proved the clearance of parasites and the recovery of physiological functioning. The results reveal the chronicity of fasciolosis in the region of study and triclabendazole being a more effective treatment mode. The approaches to controlling fasciolosis and improving the health and productivity of small ruminants are regular deworming programs and better management practices.

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INTRODUCTION

Livestock is considered a significant part of the agricultural economy of Pakistan and a source of livelihood for the rural population, as it works towards food security, the generation of employment, and economic stability of the country. The sector has a great contribution to agricultural value addition and gross domestic product (GDP), which means that it is not just important to subsistence-level farming (Haseeb *et al.,* 2022). Over the last few years, livestock production has proven to be resilient and steadily increasing despite economic shocks, and therefore, there is a necessity to maintain the health of animals to continue to produce and continue to generate output and income (Adili *et al.,* 2024).

Small ruminants, especially goats (*Capra hircus*) and sheep (*Ovis aries*), can be regarded as of great importance as they are characterized by adaptation to extreme climatic conditions, low-input production systems, and low-quality grazing lands. The animals are critical for sustaining smallholder and landless farmers as they provide a source of income, meat, milk, fiber, and a steady supply of these for a farmer (Rapsch *et al.,* 2007; Bennema *et al.,* 2015). Also, small ruminants provide household food and alleviate poverty and agro-ecological balance in mixed farm systems (Parvin *et al.,* 2020). Nevertheless, parasitic and infectious diseases often limit their performance in terms of productivity, thus negatively influencing the growth performance, reproductive effectiveness, and the health status (Ahmed *et al.,* 2024).

Fasciolosis is a type of parasitic disease causing considerable economic losses to the small ruminants globally. It is caused by trematodes of the genus Fasciola, *Fasciola hepatica* and *Fasciola gigantica* (Gedefaw *et al.,* 2025). The disease is very widespread in the tropical and subtropical zones where the environmental factors are favorable to the existence of the intermediate snail hosts. The above animals become infected after eating the metacercariae fixed on aquatic vegetation or found in contaminated water sources (Lan *et al.,* 2023). After being consumed, the immature flukes enter the intestinal mucosa, penetrate the liver parenchyma, and then destroy the tissues on an extremely large scale and lodge in the bile ducts to develop into adult parasites. Chronic infection causes inflammation, fibrosis, biliary obstruction, and impaired liver functioning, culminating in signs and symptoms of anemia, weight loss, submandibular edema, low feed consumption, and low productivity (Anjum *et al.,* 2015). These pathological alterations have a severe impact on the welfare of animals and cause considerable economic losses due to the decrease in growth rates, milk level, condemnation of the liver at slaughter, and exposure to secondary infections (Ayele and Hiko, 2016).

The physiology of fasciolosis is highly connected with the weather and management-related conditions, such as precipitation, temperature, humidity, irrigation, and grazing. The closer animals are to waterlogged pasture or irrigation channels, or stagnant water bodies, the higher the chance of exposure to the infective stages of the parasite (Mia *et al.,* 2021). Albendazole is also a popular one, especially against adult flukes, though the limited efficacy of this drug in immature stages has raised an issue of the effectiveness of the treatment in endemic regions. The anthelmintic efficacy under local conditions should be continuously evaluated, particularly in the light of the arising drug resistance (Getabalew *et al.,* 2019). The survival and proliferation of diseases in endemic regions are also due to poor care of the pastures, low drainage, and deworming (Gao *et al.,* 2020).

Clinical diagnosis of Fasciolosis eggs may be detected in the fecal samples in the fecal sediment, and hence diagnose fasciolosis. This is a cost-effective form of approach that is usually applied to carry out epidemiological surveys, but cannot detect late or post-patent infections. Hematological aspects of the evaluation have proved to be helpful in establishing the degree of the disease and its impact on the entire system, such as anemia and inflammatory responses due to liver damage (Mickiewicz *et al.,* 2024). Triclabendazole is said to be the drug of choice in the treatment of fasciolosis due to the effectiveness of the drug against both the immature and adult stages of the Fasciola species. The district of Muzaffargarh has a large irrigation system, a suitable climate, and mostly large grazing systems, thus providing an environment that supports the spread of fasciolosis. Nevertheless, there is limited epidemiological and clinico-therapeutic data in this area (Chaudhry *et al.,* 2020). Thus, the current research was aimed at establishing the prevalence rates of fasciolosis, risk factors, the effectiveness of triclabendazole and albendazole therapies, and the hematological changes in naturally infected small ruminants (*Capra hircus* and *Ovis aries*) in Tehsil Muzaffargarh, Pakistan.

MATERIALS AND METHODS

Study Area and Study Design: The current research was conducted in Murdabad, Tehsil Muzaffargarh, Punjab, Pakistan, which had a high irrigation scheme, seasonal floods, and good climatic conditions to

support the survival of Fasciola species intermediate hosts in snails. It involved a cross-sectional epidemiological study that was coupled with a clinico-therapeutic trial in order to assess the prevalence of the disease, risk factors related to the disease, the efficacy of the treatment and the hematological changes in the small ruminants. Various seasons were used in the study period to determine seasonal differences in infection.

Study Population and Sample Size: The study area was used to select 300 small ruminants randomly from various farms and herds. The sampled population consisted of 151 goats (*Capra hircus*) and 149 sheep (*Ovis aries*) that were of different ages, sexes and management systems (intensive, semi-intensive and extensive). To avoid sampling bias, the animals were chosen regardless of their health condition.

Animal ethics: All study procedures were conducted with approval from the Animal Ethics Committee of the ORIC, IUB, and in compliance with the IACUC.

Fecal Sample Collection and Preservation: Each animal had around 10 g of feces collected using disposable sterile gloves from the rectum. The samples were put in a sterile paraffin-labeled plastic container to inhibit the growth of the egg and contamination. All samples were carried to the laboratory in controlled conditions to the Parasitology Laboratory, Faculty of Veterinary and Animal Sciences, The Islamia University of Bahawalpur, where further examination was made.

Clinical Examination: A thorough clinical examination of each of the animals was carried out to evaluate the signs of fasciolosis. The following were observed: body condition score, mucous membrane color, the presence of submandibular edema, the state of appetite, lethargy, and physical condition. This was done to help in parasitological results and to help in assessing the effects of the disease.

Parasitological Examination: Fecal analysis was done using the standard sedimentation method of egg detection of Fasciola. In a nutshell, to remove fecal debris, 3-5 g of fecal matter were combined with distilled water to create a homogenate suspension, which was then filtered through a fine mesh sieve. The filtrate was centrifuged at 1500 rpm for 5 minutes' time period. The supernatant was thrown away, and the sediment was re-washed and centrifuged again. One of the end products of the sediment was put on a clean slide of glass under a light microscope at 10x and 40x magnifications with a coverslip on it. Eggs were distinguished by their large and oval form and pierced shells.

Therapeutic Trial and Animal Grouping: Forty naturally positive small ruminants (20 goats and 20 sheep) of the infected animals were randomly chosen to be evaluated therapeutically. The animals were assigned to two treatment groups, and the affordability of the situation was equal in terms of age, body weight, and infection intensity.

Group A: (Triclabendazole group): The animals (10 goats and 10 sheep) were given triclabendazole at a dose of 12 mg/kg body weight orally.

Group B: (Albendazole group): There were 20 animals (10 goats and 10 sheep) that were fed on albendazole at the rate of 10 mg/kg of body weight orally.

A drenching gun was used to administer drugs to ensure that the dosage was administered. Animals were kept under the same control conditions during the course of the trial and observed for adverse reactions to the drug.

Assessment of Therapeutic Efficacy: Feces were sampled on Day 0 (pre- treatment), Day 7, and Day 14 (After treatment) of the treated animals. The determination of therapeutic efficacy was made in terms of eliminating Fasciola eggs in fecal samples and also in terms of clinical improvement. The following formula was used to calculate treatment efficacy:

$$\text{Efficacy (\%)} = \frac{\text{Number of animals recovered} \times 100}{\text{Total number of treated animals}}$$

Hematological Analysis: Infected animals on Day 0 and Day 14 after the treatment were sampled in the jugular vein with sterile syringes. Samples were moved to EDTA-coated tubes, mixed carefully, and labelled appropriately. Hematological parameters, such as hemoglobin (Hb), red blood cell count (RBC), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), and White blood cell count (WBC), were measured at a veterinary hematology analyzer (BK-5000 Vet; Biobase Industry Co. Ltd., Shandong, China) at the Central Diagnostic Laboratory Complex, The Islamia University

Statistical Analysis: All the data collected were tabulated and analyzed statistically by the use of STATA-MP version 17 in Windows. Prevalence and risk factor associations were compared with the Chi-square test. The one-way analysis of variance (ANOVA) was used to analyze the therapeutic efficacy and hematological parameters. The differences were regarded as significant with $P \leq 0.05$.

RESULTS

Fasciolosis Small Ruminants Prevalence: The current research paper gives detailed epidemiological and therapeutic knowledge on fasciolosis in small ruminants (*Capra hircus* and *Ovis aries*) kept within Tehsil Muzaffargarh. A total of 22.0 percent of the 300 animals tested were positive for Fasciola eggs. The prevalence rate, in terms of species, was found to

be 22.51 percent in goats (*Capra hircus*) and 21.47 percent in sheep (*Ovis aries*), which can be considered a similar susceptibility of the two species to the negative impact of similar environmental and management conditions. These results point to the fact that fasciolosis is still an endemic parasite issue in the research location, probably with the aid of the ecological conditions favorable to snail intermediate hosts.

Active infection and contamination of the environment were established by the determination of characteristic Fasciola eggs by sedimentation. Similar prevalence rates in goats and sheep suggest that species-specific resistance might have a minimal impact, and exposure-related factors such as grazing behavior and shared water sources might have a bigger effect. The current findings have been supported by similar trends in previous studies in the region and internationally (Table 1).

Table 1: Overall and Species-wise Prevalence of Fasciolosis in Small Ruminants of Muzaffargarh

Species	No. Examined	No. Positive	Prevalence (%)
Goats	151	34	22.51
Sheep	149	32	21.47
Total	300	66	22.00

Risk Factor Analysis: It was found that age was a major risk factor for fasciolosis in goats and sheep. The prevalence was greater in adult animals than in younger animals, which can be explained by the fact that, during the course of time, adult animals are more exposed to infective metacercariae. Adult animals are grazed more often on open pastures and irrigated fields, which means that there is a high risk of contaminated vegetation or water being ingested. This accumulation exposure pattern is consistent with past epidemiological studies in the endemic areas (Table 2; Table 3).

Sex-wise analysis showed that females had a higher rate of infection than males in both species and this was not found to be significant. The higher prevalence in females is maybe related to the physiological stress during pregnancy, lactation and long retention in herds to breed with, which can undermine immune responsiveness. The management system also had a strong effect on the prevalence of fasciolosis in sheep, whereby sheep raised on extensive grazing systems exhibited high levels of infection compared to those raised in semi-intensive and intensive grazing systems. It was also observed that the same trend was found in goats, but not statistically significant. These are the findings that have highlighted the role of controlled grazing and better management of pastures in minimizing the disease burden.

Seasonal variation was more common during the rainy season in both species, although this was not significant. The high level of rainfall and humidity experienced during this season supports the survival and multiplication of snail intermediate hosts, and this increases the transmission dynamics. Although the relationship is not found to be statistically significant, the presented trend proves the role of climatic factors in the epidemiology of fasciolosis.

Table 2: Risk Factors Affecting the Incidence of Fasciolosis in Goats

Risk Factor	Category	No. Positive	No. Examined	Prevalence (%)	p-value
Age	Young	6	56	10.71%	
	Adult	12	49	24.49%	≤ 0.05
Sex	Male	16	46	34.78%	> 0.05
	Female	19	78	24.36%	
Management System	Intensive	15	73	20.55%	> 0.05
	Semi-intensive	12	62	19.35%	
	Extensive	14	46	30.43%	
Season	Dry	08	43	18.60%	> 0.05
	Rainy	16	67	23.88%	

Table 3: Risk Factors Affecting the Incidence of Fasciolosis in Sheep

Risk Factor	Category	No. Examined	No. Positive	Prevalence (%)	p-value
Age	Young	4	56	7.14%	≤ 0.05
	Adult	14	48	29.17%	
Sex	Male	14	45	31.11%	> 0.05
	Female	15	78	19.23%	
Management System	Intensive	17	71	23.94%	≤ 0.05
	Semi-intensive	08	61	13.11%	
	Extensive	10	46	21.74%	
Season	Dry	14	42	33.33%	> 0.05
	Rainy	14	65	21.54%	

Therapeutic Efficacy of Anthelmintics: Evidence in therapeutic trials showed that there were significant differences in efficacy between triclabendazole and albendazole. Recovery rates increased to 80% on Day 14 compared to 30% on Day 7 in goats that were treated with triclabendazole, but the recovery rate in goats that were treated with albendazole was 20 and 60 on Day 7 and 14, respectively. Likewise, the efficacy of triclabendazole in sheep was found to be higher with 50% recovery observed at Day 7 and 80% recovery observed at Day 14 as opposed to the 40% recovery and 70% recovery in albendazole-treated sheep (Table 4).

The excellent activity of triclabendazole is suggested by its wide-spectrum activity against the immature and adult stages of the species Fasciola. The progressive rise in efficacy seen on Day 7- 14 indicates that there is clearance of the parasites and a recovery of the tissues after the treatment. Albendazole was shown to be not quite as effective against adult flukes, though it is valuable against immature stages. These findings bring triclabendazole as the treatment of choice in fasciolosis in endemic regions. Animals receiving triclabendazole exhibited more improvement in clinical state, as demonstrated by a noted

improvement in appetite, body state, mucous membrane color, and submandibular edema. The above observations also verify the parasitological results and the high therapeutic response of the drug.

Table 4: Therapeutic Efficacy of Triclabendazole and Albendazole Against Fasciolosis in Sheep and Goats

Species	Treatment Group	Day 7 Efficacy (%)	Day 14 Efficacy (%)
Goats	Triclabendazole	30	80
	Albendazole	20	60
Sheep	Triclabendazole	50	80
	Albendazole	40	70

Table 5: Hematological Profile of Triclabendazole-Treated Goats Before and After Treatment

Hematological Parameters	Pre-Treatment (Day 0)	Post-Treatment Triclabendazole (Group A)	Post-Treatment Albendazole (Group B)	P-value
Hemoglobin (g/dL)	68.06	72.3	74.3	0.00
RBCs (million/µL)	3.84	9.0	9.3	0.00
HCT (%)	26.7	17.2	17.7	0.00
WBCs (million/µL)	15.3	15.2	15.2	0.00
MCV(fL)	29.6	18.2	18.9	0.03
MCH (pg)	16.0	7.5	7.9	0.07
MCHC (g/dL)	432.7	420.1	421.2	0.21

Table 6: Hematological Profile of Triclabendazole-Treated Sheep Before and After Treatment

Hematological Parameters	Pre-Treatment (Day 0)	Post-Treatment Triclabendazole (Group A)	Post-Treatment Albendazole (Group B)	P-value
Hemoglobin (g/dL)	67.8	72.3	72.17	0.001
RBCs (million/µL)	5.1	9.0	9.85	0.0004
HCT (%)	22.5	17.2	18.65	0.0008
WBCs (million/µL)	13.2	15.2	11.51	0.004
MCV(fL)	44.1	18.2	18.90	0.68
MCH (pg)	15.1	7.5	7.47	0.59
MCHC (g/dL)	433.4	420.1	396.67	0.29

Hematological Alterations and Treatment Response: Hematological examination of animals before the treatment indicated significant anemia with a decrease in hemoglobin concentration, red blood cell count, and hematocrit. These changes indicate being related to chronic blood loss, impaired erythropoiesis, and hepatic dysfunction secondary to migrating and feeding flukes. High levels of white blood cells that were seen before the treatment showed a continuing inflammatory reaction (Table 5; Table 6).

Hematological assessment of the animals (Day 14) indicated a major improvement of the animals treated with triclabendazole ($p > 0.05$). The levels of hemoglobin, the number of red blood cells, and the hematocrit indices were brought to normal physiological levels, which showed that the parasites were removed and the hematopoietic system was restored. Inflammation also appeared to be resolved since the levels of white blood cells returned to normal. Even though there were improvements in the animals treated with albendazole, the level of hematological recovery was relatively low. Closely related to hematological normalization is the parasitological clearance, which indicates the entire systemic effect of fasciolosis and its significance in the process of effective anthelmintic therapy. The recovery of blood parameters indicates the better functioning of the liver and the general health of a person, which serves as confirmation that hematological indices can be considered a good measure of therapeutic efficacy.

DISCUSSION

The prevalence of 22.0% of fasciolosis, which was obtained in this study, is in the range of the prevalence reported in Punjab and other parts of Pakistan. Ahmad *et al.* (2017) documented a prevalence of 40.51 in abattoir-slaughtered small ruminants in Sargodha district, and 25.46 prevailing fasciolosis in five Punjab districts, such as Muzaffargarh and the spread was attributed to wide irrigation coverage. In that study, Zafar *et al.* (2019) noted seroprevalence in small ruminants of 18.7% in Chakwal and Shahzad *et al.* (2012) had *Fasciola hepatica* and *Fasciola gigantica* infections in several districts of Punjab. The current study results provide further evidence that shared grazing and water sources (not the species-specific susceptibility) promote infection because of the approaching parity between the risks of goats and sheep (22.51/21.47). existing reports indicate that the same applies to the case of Bangladesh (Ahmed *et al.*, 2024) and Algeria (Adili *et al.*, 2024).

The risk of the infection was strongly associated with age in both species; adult animals were more often infected than young ones. This inclination indicates increasing exposure to infective metacercariae due to long exposure through grazing of contaminated pastures and irrigated lands. Age-related patterns have been observed in endemic areas around the world as well (Mehmood *et al.*, 2017; Lan *et al.*, 2023). We found that adult goats and female animals were more prevalent with the antibodies against *Fasciola gigantica* in Sargodha, Pakistan, which is similar to the findings of a previous study by Rizwan *et al.* (2016). The observed non-significant sex-related differences in the current study are typical throughout the literature, and the relative preponderance towards females is probably caused by immunosuppression during pregnancy and lactation (Ashraf *et al.*, 2021). Large systems expose animals to unmanaged grazing conditions, stagnant water bodies, and a lack of drainage of pastures, leading to infection with parasites (Chiappetta *et al.*, 2025).

Prevalence in sheep was highly influenced by management system and those animals that had been

grazed extensively presented a higher rate of infection. Wide-scale grazing leads to greater exposure to uncontrolled water bodies and underdrained pastures where Lymnaea snail households flourish. Selemetas *et al.* (2014) demonstrated that the distribution of fasciolosis was directly affected by the soil type and drainage. The same findings were reported by Khan (2009), who concluded that Punjab livestock was strongly related to stagnant pond bathing and open grazing, which were both related to the occurrence of infection. All these observations endorse controlled grazing, enhanced drainage, and pasture management as some of the control interventions.

The fact that they are more prevalent in the rainy season, although not significantly so in this case, is also consistent with the well-known transmission processes. The population dynamics of Lymnaea snails, as well as the survival of free-living larval stages on pasture, are controlled by moisture, temperature, and humidity (Gao *et al.,* 2020; Mia *et al.,* 2021). In Muzaffargarh, the climatic conditions promote open places on *Fasciola gigantica* transmission: seasonal flooding, irrigation channels, and high humidity during the summer (Mas-Coma *et al.,* 2019). The fact that seasonal variation was not statistically significant in the current study could be because the area is irrigated all year long, and the snail habitats were available all year long.

In both species, triclabendazole was superior to albendazole, as it produced a higher fecal e.g. clearance and quicker clinical recovery by Day 14. This is in line with the established activity of triclabendazole against both immature and adult Fasciola flukes (Fairweather, 2011). Annen *et al.* (1983) established the high efficacy of triclabendazole with regard to *Fasciola hepatica* in sheep and goats naturally infected, and triclabendazole experimentally infected goats were shown to be effectively exposed to triclabendazole (Armour *et al.,* 1997). Khan (2017) and Ahmad (2017) reported triclabendazole as the most consistent amongst commercially available anthelmintics that were considered in small ruminants in Pakistan. The reduced effectiveness of albendazole, especially against the immature fluke, restricts its use in acute and subacute cases of fasciolosis where migrating stages dominate. The positive changes in both groups (Day 7 to Day 14) show that there is parasite clearance and tissue repair following a treatment.

Pre-treatment haematologic testing affirmed fasciolosis-related anemia with markedly decreased hemoglobin, RBC, and hematocrit levels. The immature flukes that migrate lead to hemorrhagic injury in the liver parenchyma directly, and the adult flukes in the bile ducts result in chronic blood loss and inhibition of erythropoiesis. There were increased levels of WBC, pointing to active systemic inflammation. These alterations have been well recorded; Paz Silva *et al.* (2007) reported equivalent decreases in RBC, hemoglobin, and PCV in sheep that had been naturally infected with *Fasciola hepatica*, and Armour *et al.* (1997) reported anemia due to late immature and mature flukes in experimentally infected goats. Triclabendazole-treated animals had a higher proportion of post-treatment normalization of blood parameters than did animals in a control group, which is in line with higher parasite elimination. Hematological outcome is thus a value-added outcome of therapeutic performance to cooperation on parasitological clearance (Bennema *et al.,* 2015; Mickiewicz *et al.,* 2024).

Conclusion

In small ruminants in Tehsil Muzaffargarh, the prevalence of fasciolosis is 22.0% owing to exposure over the lifetime, widespread grazing, and good ecological conditions. Triclabendazole showed increased therapeutic efficacy compared to albendazole in goats and sheep, clearance of more eggs, and enhanced hematological recovery at Day 14. These results justify the use of triclabendazole as the drug of choice to treat fasciolosis in this area. As a way of reducing the pressure of infections and enhancing the health and productivity of small ruminants in endemic countries, regular deworming, better pasture management, and drainage would be prescribed.

Authors' Contribution: MWN, MS, MHF conceptualized and planned the research, conducted the research, and drafted the original manuscript. AR took samples, conducted clinical examination, and parasitological laboratory analysis. AR, RMB and RAG conducted statistical analysis, helped to analyze the results, and critically reviewed the manuscript. The final version was read, revised, and approved by all authors.

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