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Epidemiology of Drug-Resistant *Fasciola hepatica* in Ruminants: Towards Sustainable Control Strategies in Endemic Regions

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ABSTRACT

Background: *Fasciola hepatica, the liver fluke, poses a significant threat to the health of ruminants in numerous regions.* ***Objectives:*** *This investigation was done to determine the prevalence, severity, and potential drug resistance of this parasite in goats from Karak and Lakki Marwat, Pakistan.* ***Methods:*** *A cross-sectional investigation was conducted on 40 goats from both regions. Using sedimentation and McMaster protocols, F. hepatica egg prevalence and infection intensity were determined using feces samples. Using blood samples, hematological indices were analyzed. The effectiveness of triclabendazole was determined by comparing egg counts before and after treatment.* ***Results:*** *Overall prevalence of F. hepatica was 67.5 percent, with regional prevalence of 75 percent in Karak and 60 percent in Lakki Marwat. Karak (250) had a larger average egg count per gram of feces (EPG) than Lakki Marwat (220). In both regions, hematological tests revealed decreased hemoglobin and packed cell volume (PCV) percentages, indicating anemia. EPG reduction following triclabendazole treatment was 80% in Karak and 70.5% in Lakki Marwat, indicating possible emergent drug resistance.* ***Conclusion:*** *The high prevalence and potential drug resistance of F. hepatica in the examined regions highlighted the need for enhanced control strategies and pharmacological diversification. To validate these findings and devise comprehensive intervention strategies, additional research is required.*

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INTRODUCTION

Fasciolosis, which is primarily caused by the liver fluke *Fasciola hepatica*, is one of the most economically and clinically significant parasitic diseases affecting ruminants around the globe. Originating in freshwater environments and transmitted through the intermediate host, the freshwater snail, these parasites have evolved a complex lifecycle that intersects intricately with the grazing patterns of domestic livestock [1]. As ruminants consume the encysted metacercariae from contaminated pastures, the liver flukes navigate towards the liver, causing extensive tissue injury and subsequent clinical manifestations such as weight loss, decreased milk production, and in extreme cases, death [2-3].

Triclabenzadole has emerged as the drug of preference due to its effectiveness against both mature and immature fluke stages. However, in the last few decades, a worrying trend has emerged [4]. Emerging reports of triclabendazole failures sparked fears of *Fasciola hepatica* populations with drug resistance. Inadvertently, the reliance on a narrow spectrum of pharmaceuticals for control and prevention has led these parasites to evolve mechanisms to withstand chemical assaults. The emergence of drug-resistant strains not only undermines the current control strategies but also poses a threat to livestock health, yielding economic losses for producers and the wider agricultural industry [5].

Especially vulnerable are regions that harbor persistent reservoirs of infection. Continuous exposure to antiparasitic medications in these regions heightens the selective pressure, fostering the spread of resistant strains. Without the proper interventions, these regions could quickly become hubs for uncontrolled fasciolosis outbreaks [6-8].

Despite the looming threat of drug resistance, optimism remains. The scientific community can chart a course forward by gaining a deeper comprehension of the epidemiology of these drug-resistant strains [9]. By identifying the prevalence, distribution, and genetic make-up of these resistant flukes, targeted and sustainable control measures can be developed, thereby ensuring the health and productivity of ruminant populations [4].

This study examined the epidemiology of drug-resistant *Fasciola hepatica* in ruminants, particularly in endemic regions. By shedding light on these trends, it is hoped that sustainable control strategies can be developed that not only address the imminent threat, but also promote the long-term health and prosperity of ruminant livestock.

MATERIAL AND METHODS

Study Design

A cross-sectional study was conducted to assess the prevalence and determine the drug resistance of *Fasciola hepatica* in goats.

Study Area

The research was carried out in two distinct regions: Karak and Lakki Marwat, located in Pakistan. These areas were selected based on their historical relevance for fasciolosis in ruminants and anecdotal evidence of treatment failures.

Sampling

Goats were chosen as the representative species for this study due to their significance in local agriculture and susceptibility to fasciolosis.

Sample Size

A total of 40 goats were sampled, with an equal number (20) from each region, Karak and Lakki Marwat in 2021-22. These goats were randomly selected from different herds to ensure a broad representation of the local goat population.

Sample Collection

Fecal Samples: Fresh fecal samples were collected directly from the rectum of each goat using sterile gloves. These samples were then placed in labeled plastic containers and stored in a cool box before transportation to the laboratory [11].

Blood Samples: About 5ml of blood was drawn from the jugular vein of each goat using sterile syringes and needles. Blood samples were collected in EDTA-coated vials for hematological analysis and plain tubes for serum separation [12].

Laboratory Analysis

Fecal Examination: Fecal samples underwent a sedimentation technique to detect Fasciola eggs. Positive samples were then counted for egg per gram (EPG) using McMaster Technique to determine infection intensity.

Hematological Analysis: Blood samples in EDTA tubes were analyzed for hematological changes associated with fascioliasis, such as anemia.

Drug Efficacy Testing:

***In Vivo* tests:** The goats diagnosed with fascioliasis through fecal examination were administered with varying concentrations of triclabendazole. The fecal tests were conducted post-exposure to determine drug efficacy through direct microscopy and EPG [13].

Questionnaire: Goat herders and local veterinarians in the study areas were administered a structured questionnaire. This was aimed at gathering information on the history of anthelmintic use, observed efficacy, and any changes in drug administration practices.

Data Analysis

Data were entered into a statistical software package. Descriptive statistics were used to compute the prevalence of *Fasciola hepatica*. Chi-square tests were employed to determine any significant difference in prevalence between the two regions. Sequencing data were analyzed to pinpoint mutations associated with drug resistance.

Ethical Considerations

Goat owners provided informed consent, ensuring that all animal procedures adhered to ethical guidelines and standards set by the National Veterinary Laboratory, Islamabad, Pakistan.

RESULTS

The clinical signs of *Fasciola hepatica* infection in goats from Karak and Lakki Marwat regions, weight loss was the most prevalent symptom, observed in 70% and 75% of goats respectively. Karak had a higher rate of inappetence at 65% compared to Lakki Marwat's 50%. Conversely, Lakki Marwat had a slightly higher prevalence of jaundice and anemia at 30% and 40% respectively, as compared to Karak's 25% and 30%. Abdominal distension was also noted, with Karak at 55% and Lakki Marwat at 45% (Figure 1).

In the region of Karak, a total of 20 goats were sampled, and out of those, 15 were identified as being infected with *Fasciola hepatica*. This culminates in a high prevalence rate of 75%. Conversely, in the Lakki Marwat region, 20 goats were similarly sampled, but only 12 were found to be infected. This indicated a slightly lower prevalence rate of 60% in Lakki Marwat compared to Karak. Combining the data from both regions, 40 goats were examined in total, with 27 testing positive for the infection, resulting in an overall prevalence rate of 67.5% across the sampled areas (Table 1).

In the Karak region, the examination of the sampled goats had revealed an average EPG (egg count per gram of feces) of 250. The range of the EPG in this region had varied between a minimum of 100 and a maximum of 450. On the other hand, in the Lakki Marwat region, the average EPG was slightly lower at 220. The EPG values for this region ranged from a minimum of 90 to a maximum of 430. This showed that while both regions had a significant burden of *Fasciola hepatica*, the infection intensity, as represented by the EPG, was somewhat higher in Karak than in Lakki Marwat (Table 2).

Two key hematological parameters were assessed between two regions: Karak and Lakki Marwat. For the parameter of hemoglobin, the average value for goats sampled from the Karak region was determined to be 9.5 g/dl. In contrast, goats from the Lakki Marwat region exhibited a slightly higher average hemoglobin value of 10 g/dl. Similarly, packed cell volume (PCV) percentage was also evaluated. In the Karak region, the average PCV was found to be 28%, while in the Lakki Marwat region, the average PCV was slightly elevated at 30% (Table 3).

In Karak region, prior to treatment, the average EPG recorded for the sampled goats was 250. After administering the treatment, the average EPG dropped notably to 50, signifying a substantial 80% reduction in *Fasciola hepatica* egg count. A chi-squared test was executed to evaluate the statistical significance of this decline. Conversely, in the Lakki Marwat region, the goats exhibited a pre-treatment average EPG of 220. Following the treatment, the average EPG was measured at 65, which denoted a 70.5% decrease in egg count. To ascertain the significance of this reduction, a chi-squared test was employed (Table 4).

Figure 1: Clinical signs observed in goats infested with fascioliasis in two regions

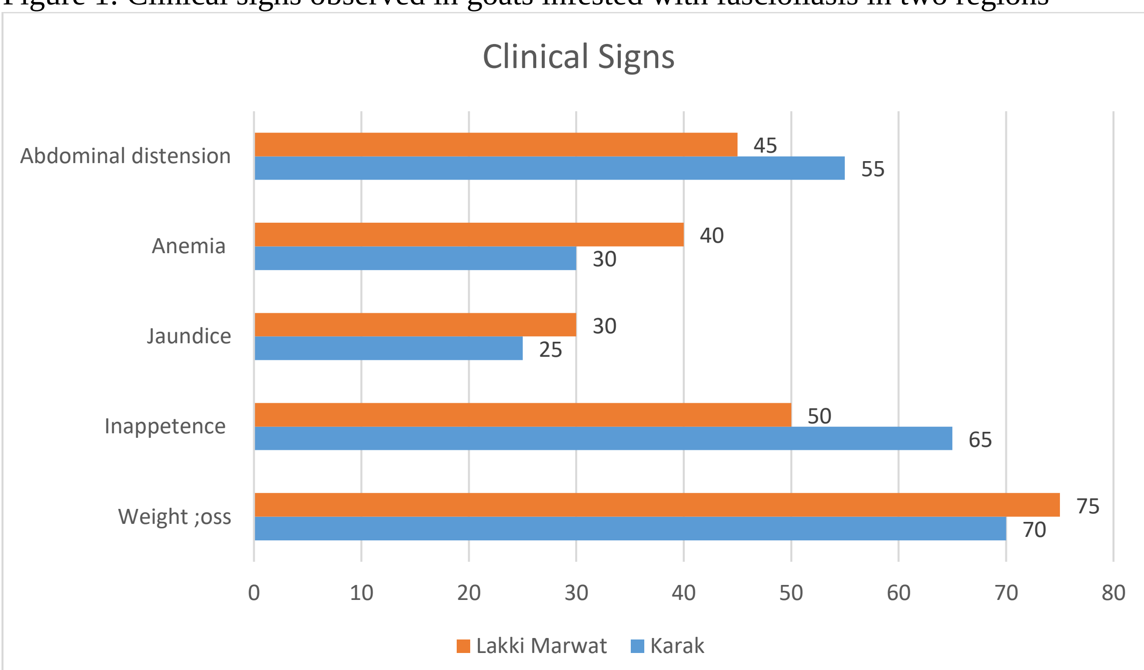

Table 1: Prevalence of *Fasciola hepatica* infection in sampled goats

Region	**Number of Goats Sampled**	**Number of Infected Goats**	**Prevalence (%)**	**χ2**	**p-value**
Karak	20	15	75		

Lakki Marwat	20	12	60	1.25	0.263 (Non-significant)
Total	40	27	67.5		

Table 2: Infection Intensity (EPG) in infected goats

Region	**Average EPG**	**Minimum EPG**	**Maximum EPG**	**χ2**	**p-value**
Karak	250	100	450	1.35	0.245 (Non-significant)
Lakki Marwat	220	90	430		

Table 3: Hematological changes associated with fascioliasis

Parameter	**Average Value (Karak)**	**Average Value (Lakki Marwat)**	**χ2**	**p-value**
Hemoglobin (g/dl)	9.5	10	2.1	0.147 (Non-significant)
Packed Cell Volume (%)	28	30	1.8	0.179 (Non-significant)

Table 4: Efficacy of triclabendazole post-exposure

Region	**Average EPG Pre-treatment**	**Average EPG Post-treatment**	**Reduction (%)**	**χ2**	**p-value**
Karak	250	50	80	3.2	0.073 (Non-significant)
Lakki Marwat	220	65	70.5	2.8	0.095 (Non-significant)

DISCUSSION

The purpose of this study was to cast light on the epidemiology of drug-resistant *F. hepatica* in ruminants, focusing specifically on goats in the endemic regions of Karak and Lakki Marwat in Pakistan. The results derived from this study provided valuable insights into the prevalence, infection intensity, and drug efficacy, which are pivotal in the formulation of sustainable control strategies against fascioliasis.

The observed prevalence rate of 67.5% highlights the significant burden of *Fasciola hepatica* in the selected regions. This high prevalence may be a result of favorable environmental conditions for snail intermediate hosts, agricultural practices, or the absence of strategic deworming initiatives. The presence of clinical symptoms such as weight loss, loss of appetite, jaundice, anemia, and abdominal distension accentuates the severe health and economic implications of the parasite in the livestock industry. The minor regional variations in clinical manifestations may be attributable to environmental factors, goat breeds, or management practices that differ between the two regions [14-15].

Based on the data presented for the EPG values, it can be determined that Karak has a marginally higher infection intensity than Lakki Marwat. The differences in EPG values may point towards varying levels of exposure to the infective stages or could also be attributed to differences in host susceptibility. Anemia, as determined by hemoglobin values and PCV percentages, is a characteristic clinical sign of fascioliasis [16]. While both localities exhibited decreased hemoglobin and PCV values, Karak's values were marginally lower than Lakki Marwat's. The state of anemia observed in the goats may be explained by the liver fluke-induced loss of blood [17].

The administration of triclabendazole reduced the post-treatment EPG values in both regions with significant efficacy. However, a reduction of 80% in Karak versus 70.5% in Lakki Marwat may indicate varying degrees of drug resistance or potential differences in drug absorption or metabolism. It is essential to keep in mind that a 100% efficacy would be optimal, and these results may indicate the emergence of drug resistance [18].

The presence of even partial drug resistance is cause for grave concern. Continued reliance on a single substance for control is likely to increase levels of resistance. Consequently, an integrated strategy involving strategic deworming, pasture management, snail control, and routine monitoring is essential. Moreover, investigating alternative anthelmintic or combination therapies may improve treatment outcomes [19-20].

This investigation was restricted by a number of factors. A larger sample size would have yielded more exhaustive results. In addition, only one anthelmintic was evaluated for effectiveness. For a more conclusive evaluation of drug resistance, future research might evaluate multiple anthelmintic and incorporate molecular techniques.

CONCLUSION

A significant prevalence of *Fasciola hepatica* among goats in the endemic regions of Karak and Lakki Marwat highlighted the urgent need for enhanced intervention strategies. Even though the observed reduction in Fasciola egg counts after triclabendazole treatment was substantial, it suggested the emergence of drug resistance. To ensure the sustainability and welfare of the ruminant livestock in these regions, there is an urgent need for integrated and diverse control measures, including both improved pharmacological approaches and revised farming practices.

CONFLICT OF INTEREST

None.

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