Infection rate and efficacy of anthelmintic drugs against helminthiasis in dairy animals of Faisalabad, Pakistan

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Original Article

ABSTRACT

Background: Gastrointestinal (GI) helminths represent a significant challenge in the livestock industry, leading to substantial economic losses due to high morbidity, mortality, reduced productivity, and increased treatment costs.

Objective: The present study aimed to accomplish three objectives; (1) assess the infection rate of GI helminths in cattle and buffalo, (2) identify the associated risk factors, (3) evaluate the comparative efficacy of commercially available anthelmintic drugs to address the emerging resistance observed on livestock farms in Faisalabad, Pakistan.

Material and Methods: Stool samples were collected using a simple random sampling technique. Data on various risk factors were gathered through a pre-designed questionnaire. The samples were microscopically examined following standard qualitative and quantitative stool examination protocols. Animals with positive infections were divided into four groups, to conduct a randomized controlled trial for comparative anthelmintic efficacy. Each group was assigned to receive Albendazole (G1), Levamisole (G2), Ivermectin (G3), and normal saline (G4).

Results: The results indicated that 57.03% of the samples tested positive for GI helminths, with a significantly higher infection rate in cattle (63.01%) compared to buffalo (38.04%) (P<0.05). A significant association was observed between different breed types, with Sahiwal cattle showing a higher infection rate (69.77%). In buffalo, however, there was no significant difference in infection. Age-wise analysis revealed that younger animals had higher infection rate. Conversely, younger animals exhibited a lower infection rate in buffalo than adults. The deworming status also had a significant impact on infection rates. In the comparative anthelmintic efficacy test, Albendazole demonstrated superior effectiveness to the other treatments.

Conclusion: Our results provided essential baseline data on the threat of GI helminthiasis and the factors affecting livestock production. It also highlighted the importance of using targeted or rotational anthelmintic drugs to prevent anthelmintic resistance in livestock populations.

Keywords: anthelminthic; buffalo, cattle; helminths; infection; resistance.

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INTRODUCTION

Pakistan's agriculture sector is a major driver of economic growth, accounting for over 21% of the country's gross domestic product (GDP), of which the livestock industry accounts for roughly 56.67%. This country's diverse geo-climatic conditions foster an extensive range of animal breeds, with cattle and buffalo being especially prominent, comprising around 26.3 million and 24.2 million, respectively. The increasing worldwide demand for dairy and meat products offers significant opportunities for investment and expansion in the livestock sector. Nevertheless, the sector has several hurdles that hinder its ability to achieve its full potential, including the morbid effects of GI parasites[1].

The GI parasites, especially helminths, including nematodes, trematodes, and cestodes, significantly

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challenge livestock economics and adversely affect animal health and production. These helminths cause significant mortality and morbidity, resulting in clinical manifestations such as anorexia, anemia, diarrhea, and dehydration[2]. The economic strain impacts rural farmers and risks the commercial dairy sector, resulting in reduced production, increased treatment costs, and, in extreme cases, livestock mortality. Young animals may experience mortality rates over 40%, losing between 6 and 13 kg annually[3]. The frequency of GI helminths in Pakistan is markedly higher than normal, further intensified by seasonal variations, climatic conditions, and livestock producers' lack of awareness about efficient management strategies.

Seasonal changes play a crucial role in the prevalence of GI helminthiasis, with higher rates observed during warmer, humid months that create optimal conditions for parasite transmission. For instance, a recent study showed that certain parasites thrive in specific climatic conditions, leading to increased infection rates in livestock[4]. Furthermore, the management practices employed by farmers, such as inadequate hygiene and irregular deworming schedules, contribute to the perpetuation of these infections. Farmers fail to recognize the signs of parasitic diseases, particularly subclinical cases, which can lead to significant economic losses that often go unreported. Several surveys identified a range of helminth species affecting ruminants in Pakistan, including *Trichostrongylus* spp., *Fasciola* spp., and *Haemonchus* spp.[5]. These infections can significantly diminish animal output by impairing weight gain, milk yield, and general health. The inappropriate application of anthelmintics raises concerns over the emergence of drug resistance, complicating control measures, and demanding alternate tactics for controlling parasite diseases.

Despite the critical importance of addressing GI helminthiasis in livestock, comprehensive studies on the prevalence of helminths and the effective use of anthelmintics at the farm level in Pakistan remain limited. The prevalence of GI helminths and the related risk factors have not been thoroughly studied in the dairy farms of the Faisalabad district, which houses a substantial segment of the nation's cattle population. Comprehending the dynamics of GI helminthic infections in this region is crucial for formulating effective management methods to improve cattle health and productivity. The present research aims to assess the infection rate of GI helminths in large ruminant populations at commercial farm level and identify the risk factors associated with their spread, in addition to anthelminthic resistance which has emerged as major threat to the livestock sector. No such practice was conducted at the dairy farm level in Pakistan. Consequently, anthelmintic efficacy trial against GI helminths was performed on a dairy farm in Faisalabad, Pakistan.

MATERIAL AND METHODS

The study utilized two study types: a cohort analytical study and a randomized controlled trial. Both were conducted at the Molecular Parasitology Laboratory of the University of Agriculture, Faisalabad, Pakistan, from March to May 2023.

Study design: Stool samples were collected from two livestock breeding stations of domestic cattle and buffalo, and microscopically examined for GI helminths. A controlled randomized trial was conducted among the cattle infected with GI helminths in the livestock experimental station, University of Agriculture Faisalabad (main campus). Four groups received Albendazole, Levamisole, Ivermectin, and saline.

Study area: Faisalabad, Punjab, Pakistan, is the third largest metropolis in the country, divided into six tehsils: Faisalabad Saddar, Faisalabad City, Samundri, Tandlianwala, Chak Jhumra, and Jaranwala (Fig. 1). The region experiences four distinct seasons and is 184 m above sea level, approximately 30 km from the Chenab River. The average temperature ranges from 6°C to 41°C, with an annual rainfall of 256 mm and humidity levels between 60% and 66%[6].

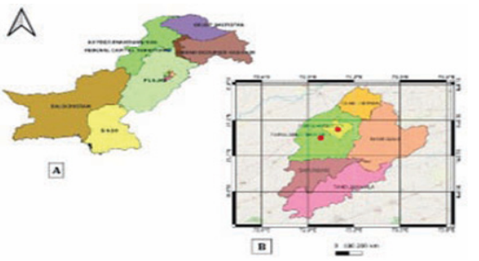

Fig. 1. Study area. **(A)** shows the Pakistan map and the Punjab province. **(B)** shows Faisalabad district. Red dots show the selected farms. Maps were generated using QGIS software.

Breeding sites: The livestock experimental station, University of Agriculture Faisalabad (Main Campus), and Proka Dairy farm, Ghulam Muhammadabad, Faisalabad were selected. Both breeding sites were strategically selected to maximize scientific and operational rigor. The first is typified as a commercial farm with structured management protocols. This deliberate contrast enabled comparative analysis of anthelmintic efficacy across dominant husbandry systems while controlling for extraneous variables. The second farm represents small-scale peri-urban dairy operations with high animal density, and limited sanitation access. It is worth mentioning that resource constraints and the need for consistent randomized controlled trial implementation (including farmer compliance, standardized sampling, and treatment monitoring) further necessitated focusing on these well-characterized sites with established high infection rate (>30% in pre-surveys) and prior collaboration agreements. Although other sites exist, selected farms

provided optimal heterogeneity in risk factors while ensuring methodological feasibility.

Stool examination: Three hundred and eighty-four faecal samples were randomly collected from cattle and buffalo at the two studied breeding sites over a three-month seasonal period (March-May, 2023). Samples were collected from animals of varying ages, including calves (1-3 m), heifers (6 m-2 y), and pregnant dry animals (over 2 y). Collected samples were transported to the Molecular Parasitology Laboratory at the University of Agriculture, Faisalabad for microscopic examination. Samples were examined using the direct smear method and flotation techniques (utilizing table salt) to identify the presence of GI helminth eggs[7].

Additionally, the McMaster egg counting method was employed to quantify eggs per gram (EPG), allowing for the assessment of infection intensity. Two grams of feces were combined with 28 ml of sedimentation solution (33% $ZnSO_4$). The mixture was sifted to eliminate big particles. Each chamber of McMaster was filled with a sample and analysed using a light microscope at 10X[8]. All eggs in each line were enumerated, and EPG was computed using the following formula: EPG = (N of eggs/N of chambers) X [(60 ml/5 g)/0.15 ml].

Comparative efficacy evaluation: A randomized controlled trial was conducted to assess the efficacy of anthelmintic drugs in animals infected with GI helminths. The selected administered anthelminthic drugs; were Albendazole (Albamax®) for G1, Levamisole (Nilverm®) for G2, and Ivermectin (Ivomac®) for G3, compared with saline for normal control G4. According to the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines, each group comprised 4 animals, and the treatment was allocated groupwise[9]. After treatment, faecal samples were collected again at 7-, 14-, and 21-days post-treatment to evaluate the fecal egg count reduction test (FECRT)[10]. The efficacy of each anthelmintic was calculated using the formula: Efficacy% = [(EPG before treatment - EPG after treatment)/EPG before treatment] x 100.

Statistical analysis: Statistical analysis was conducted using SPSS version 19.0 software (SPSS Inc., Chicago, IL, USA). Data were analysed employing descriptive statistics and analysis of variance (ANOVA), followed by Tukey's test for multiple comparisons. A significance level of $P<0.05$ was established for determining statistical significance.

Ethical considerations: All animal sample procedures adhered to the ethical guidelines established by the Institutional Animal Care and Use Committee (IACUC) at the University of Agriculture, Faisalabad. Informed consent was obtained from all participating farmers before sample collection.

RESULTS

Out of 384 samples examined, 204 were positive for GI helminthic infections (53.13%). Nematodes were the most prevalent, accounting for 35.68% of the total infections, followed by trematodes (13.80%) and cestodes (3.65%). Of the total samples analyzed, 292 were from cattle and 92 from buffalo. The GI helminthiasis was significantly higher in the cattle population (63.01%) compared to buffalo (38.04%).

Cattle: Infection rate of GI helminths was 62.98% in females, and 33.33% in males. Breed-wise analysis indicated that GI helminths were significantly higher in Sahiwal cattle (69.77%), compared to crossbreeds (38.96%). The age distribution of the 292 cattle samples showed that the highest infection rate was observed in the 0–1-y age group (82.22%), followed by the 1-2 y group (61.29%), and the adult group over 2 years (46%).

Buffalo: Out of 92 buffalo samples, 88 were female, and 4 were male. Infection rate was higher in females at 39.77%, compared to 25% in males. Among the different breeds, the Nili Ravi buffalo showed 42.59% infection rate, which is significantly higher than the 7.14% observed in the Brown buffalo. The highest infection rate (71.43%) was found in adult buffalo over 2 y of age. This was followed by the group aged 0-1 y (42.86%), and those aged 1-2 y (22.86%) (Fig. 2).

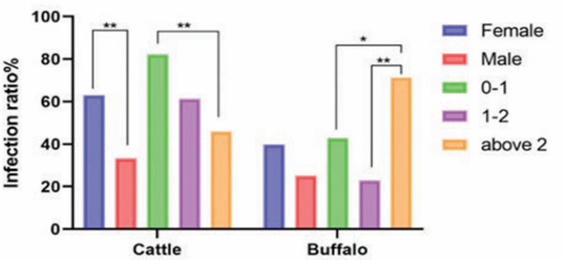

Fig. 2. Infection rates of GI helminths in cattle and buffalo.

Associated factor-wise infection rate: The analysis of deworming status indicated that non-dewormed animals had a significantly higher infection rate (71.79%), compared to dewormed animals (34%). The infection rate also varied according to the feeding system. The highest infection rate (72.22%) was seen in animals fed roughages, followed by those on concentrates and fodder (56.74%), and total mixed rations (TMR) (35.35%). Additionally, the type of housing floors influenced the infection rate. Animals on muddy floors had the highest infection rate (84.85%), followed by those on semi-concrete floors (54.82%), and concrete floors (47.37%). Statistical analysis revealed a significant association ($P<0.05$) between the type of flooring and helminthiasis. Overall, the results highlight the complex interplay of various factors that affect the prevalence of GI helminths in livestock (Fig. 3).

Comparative anthelminthic efficacy trial: All treatment groups exhibited significant reductions (P<0.05) in egg per gram (EPG) counts when compared to the control group. Albendazole showed the highest efficacy at 72.73%, followed by Ivermectin at 63.63%, and Levamisole at 36.36% (Fig. 4). By day 7, there was a significant difference (P<0.05) in efficacy between Albendazole and Levamisole; however, no significant differences were observed between Albendazole and Ivermectin, or between Levamisole and Ivermectin. This trend continued on days 14, 21, and 28. Albendazole outperformed the other treatments, indicating its superior effectiveness against GI helminthiasis in cattle.

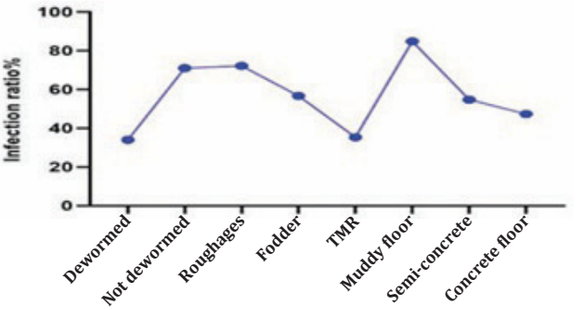

Fig. 3. Factors associated with GI helminthiasis.

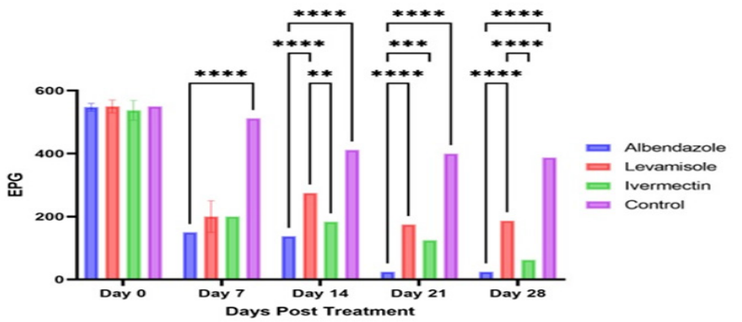

Fig. 4. Comparative efficacy of each treatment groups on day 0, 7, 14, 21, and 28. The highest mean post-treatment reduction in EPG on day 28 was noted in group A (Albendazole), followed by group C (Ivermectin), group B (Levamisole), and group D (normal saline).

DISCUSSION

Large ruminants are integral to agricultural economies, providing essential products such as milk and meat. However, the prevalence of GI helminthiasis significantly threatens livestock productivity and overall health. The current study highlights the impact of GI helminthiasis on livestock. Accordingly, 212 samples were collected from the Livestock Experimental Station at the University of Agriculture Faisalabad and 172 samples from Proka Farm. The findings revealed that the Livestock Experimental Station had a greater frequency of GI helminths than Proka Farm. This disparity may be ascribed to differences in management strategies or environmental factors between the two sites. The frequency among the tested population of Faisalabad, Punjab, Pakistan, was 57.03%. This result corresponds with previous research in Pakistan, that reported infection rates between 20% and 90%[11].

The identified parasite species, including *Ostertagia* spp., *Oesophagostomum* spp., *Trichostrongylus* spp., and *Fasciola* spp., align with those reported in the literature[12]. The variation in worm genera prevalence observed across different studies can be attributed to regional differences in climate, management practices, and environmental conditions. Presently, nematodes were the most prevalent group, accounting for 35.68% of the positive samples, followed by trematodes at 13.80% and cestodes at 3.65%. Notably, *Trichostrongylus* spp. was frequently identified, raising concerns about anthelmintic resistance. This finding aligns with previous research indicating that certain nematode species have resisted commonly used anthelmintics, complicating management strategies[13]. Additionally, the low prevalence of cestodes, particularly *Moniezia* spp., suggests a limited availability of intermediate hosts in the study area, which is further supported by earlier findings[14].

A gender-based analysis indicated that female cattle exhibited a higher prevalence of GI helminths at 62.98%, in contrast to 33.33% prevalence in males. This finding is consistent with Tuser *et al.*[14] who observed that females frequently exhibit a greater parasitic burden, likely attributable to immunocompromised conditions linked to reproduction. In our study, infection with GI helminths in buffalo was higher in females than males, a result that contrasted with Gunathilaka *et al.*[15]. This Discrepancy may arise from variations in management practices and environmental stressors that impact each gender differently.

Infection rates varied significantly among breeds, with Sahiwal cattle exhibiting a prevalence of 69.77%, in contrast to that of crossbreeds (38.98%). This indicates that Sahiwal cattle exhibit increased vulnerability to parasitic infections. This finding aligns with a previous study suggesting that indigenous breeds exhibit greater

resilience to parasitic infections than exotic breeds[16]. Additionally, significant age-related variations were observed in GI helminthic infections. Young cattle exhibited the highest infection rates (82.22%), whereas adult cattle demonstrated significantly lower rates (46%). The observed pattern indicates that younger animals exhibit reduced immune responses, increasing infection susceptibility[3]. The reduced prevalence of infections in adults may be ascribed to acquired immunity associated with prior parasite exposure.

The deworming status of animals significantly influenced the infection rate of GI helminths. In our study, its record in non-dewormed animals was 71.79% versus 34% in dewormed animals. This emphasizes the significance of regular deworming practices in managing parasitic infections and their essential role in controlling helminthiasis[17].

Seasonal analysis indicated that the infection rate for GI helminths was elevated in March and April (57.03%) relative to May and June (43.23%). This pattern may correlate with environmental factors that enhance parasite transmission in warmer months[18]. Monthly data revealed peaks in March and April, indicating that implementing effective management practices during these months may reduce the risk of infection[19].

Lastly, the efficacy of anthelmintic treatments was evaluated, with Albendazole demonstrating the highest effectiveness (72.73%), followed by Ivermectin (63.63%) and Levamisole (36.36%). Despite these successes, the emergence of anthelmintic resistance remains a pressing concern, necessitating ongoing evaluation of drug efficacy and the implementation of integrated parasite management strategies[20].

In conclusion, this study emphasizes the substantial impact of GI helminths on large ruminant health, and productivity. It highlights the need for comprehensive management strategies, including regular deworming, improved nutritional practices, and environmental management to reduce parasite exposure[21]. Continued research is essential to adapt treatment protocols and enhance livestock health outcomes in the face of evolving challenges posed by GI helminthiasis.

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Conflict of interest: All authors declare no conflict of interest.
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