Evidence of Anthelmintic Resistance in *Haemonchus contortus* Following Fenbendazole Treatment in Goats: A Field Case Study

Abstract

The emergence of anthelmintic resistance (AR) in gastrointestinal nematodes, particularly *Haemonchus contortus*, represents a serious threat to small ruminant production. This study reports a field case of suspected AR in goats following treatment with fenbendazole. Pre-treatment faecal examination revealed a high parasitic burden, with a mean faecal egg count (FEC) of 1820 ± 52.54 eggs per gram (EPG). Larval culture confirmed *Haemonchus spp*. as the predominant parasite.Post-treatment evaluation at 10–14 days showed a marked reduction in FEC to 122 ± 7.97 EPG, corresponding to a faecal egg count reduction (FECRT) of 93.33%, indicating partial drug efficacy. However, at 30 days post-treatment, FEC increased to 979 ± 32.36 EPG, suggesting rapid reinfection and/or survival of resistant parasite populations. Since the observed FECRT value falls below the accepted efficacy threshold of 95%, it is indicative of suspected resistance to fenbendazole. Statistical analysis revealed significant differences in FEC across sampling periods (Kruskal–Wallis $\chi^2 = 25.812$, df = 2, $p < 0.001$), with post hoc comparisons confirming significant variation between pre- and post-treatment counts. Despite normality indicated by Shapiro–Wilk test, FEC data were treated as non-parametric due to their over dispersed and skewed biological nature.In conclusion, the suboptimal FECRT (93.33%) combined with rapid resurgence of egg counts strongly suggests the presence of fenbendazole-resistant Haemonchus populations. These findings highlight the urgent need for routine resistance monitoring using FECRT and the adoption of integrated parasite management strategies to slow the progression of anthelmintic resistance in goat herds The emergence of anthelmintic resistance (AR) in gastrointestinal nematodes, particularly *Haemonchus contortus*, poses a significant threat to small ruminant production. This study documents a field case of suspected AR in goats following treatment with fenbendazole. Faecal samples were examined pre- and post-treatment using standard parasitological techniques. Initial post-treatment evaluation (10–14 days) showed absence of eggs; however, subsequent examination after one month revealed reappearance of Haemonchus eggs. These findings suggest either rapid reinfection or survival of resistant parasite populations. The study emphasizes the importance of faecal egg count reduction testing (FECRT) and integrated parasite management strategies to mitigate resistance development.

Keywords : *Haemonchus contortus*, goats, anthelmintic resistance, fenbendazole, reinfection, FECRT

Introduction

Gastrointestinal nematode infections remain a major constraint to goat production systems worldwide, particularly in tropical and subtropical regions. Among these, *Haemonchus contortus* is recognized as the most pathogenic species due to its hematophagous nature and high fecundity, leading to anemia, hypoproteinemia, and mortality in severe cases.Control of these parasites relies heavily on anthelmintics, including benzimidazoles, macrocyclic lactones, and imidazothiazoles (Edna *et al* 2025). However, the widespread and often indiscriminate use of these drugs has led to the development of anthelmintic resistance (AR), now reported globally (Besier,2012 and Gilleard,2013). Resistance to

benzimidazoles, such as fenbendazole, is particularly widespread in goat populations(Kaplan 2004 ,Sutherland, I.A. and Leathwick, D.M.,2011).The present study describes a field observation of recurrence of *H. contortus* infection following fenbendazole treatment, highlighting the potential emergence of AR and the need for sustainable parasite control practices(Coles, *et al.2006)*.

Materials and Methods

The present investigation was carried out on ten goats (10) maintained under a semi-intensive management system. Under this system, animals were allowed to graze freely on natural pasture during the daytime and were confined in well-ventilated housing facilities during the night. Goats of varying age groups, including kids, growers, and adults, as well as both sexes, were included in the study to ensure representative sampling. The study was initiated following routine parasitological screening of the herd, which revealed the presence of gastrointestinal nematode infections. The animals comprised same age groups growers (6–12 months),and included both males and females to ensure representative sampling. The study followed a controlled experimental design. Initially, all animals were subjected to faecal examination for gastrointestinal nematode infection using standard parasitological techniques. Based on the faecal egg count (EPG), animals showing infection above a predetermined threshold (≥200 EPG) were selected. Based on the screening results, animals exhibiting evidence of parasitic infection were selected for detailed investigation. No prior anthelmintic treatment had been administered to the selected animals for at least 8–10 weeks before the commencement of the study to avoid interference with parasitological findings .Fresh faecal samples were collected per rectum using clean, disposable gloves to prevent contamination. Approximately 10–15 grams of faeces were collected from each animal and placed in sterile, labeled polyethylene containers indicating animal identification number, age, sex, and date of collection. The samples were immediately stored in insulated containers and transported to the laboratory under cool conditions. All samples were processed within 24 hours of collection to maintain sample integrity and prevent hatching of eggs.Quantitative estimation of faecal egg count was performed using the McMaster technique. Briefly, a known quantity of faeces (typically 2 grams) was thoroughly mixed with a flotation solution (saturated sodium chloride) to obtain a homogeneous suspension. The mixture was filtered through a sieve to remove debris, and the filtrate was used to fill both chambers of a McMaster counting slide. The slide was allowed to stand for a few minutes to permit flotation of eggs. Eggs within the engraved grid area were counted under a light microscope at 10× magnification. The number of eggs counted was multiplied by the appropriate factor to express the results as eggs per gram (EPG) of faeces. (Paul *et al*,2014) For species identification, pooled faecal samples from infected animals were subjected to larval culture. Approximately 10–15 grams of faeces were mixed with moistened sawdust to maintain optimal humidity and aeration. The mixture was incubated at 25–27°C for 7–10 days under controlled conditions, with periodic moistening to prevent desiccation. After incubation, third-stage larvae (L3) were recovered using the Baermann technique. The cultured material was placed on a sieve or cheesecloth suspended in a funnel containing lukewarm water. After 12–24 hours, larvae migrated into the water and were collected from the stem of the funnel. The recovered larvae were concentrated and examined microscopically(Florian and Lewis, 2014). Identification was carried out based on standard morphological characteristics such as sheath length,

tail morphology, and intestinal cells, confirming the predominance of *Haemonchus spp*. Following confirmation of infection, affected animals were treated with fenbendazole administered orally at the recommended therapeutic dose rate (generally 5–10 mg/kg body weight). The drug was given as a single dose using a dosing syringe, ensuring accurate dosage based on individual body weight. Post-treatment efficacy was assessed through repeated faecal examinations. Faecal samples were collected at two intervals: first between 10–14 days post-treatment and subsequently at 30 days post-treatment. Quantitative faecal egg counts were again performed using the McMaster technique. The efficacy of the anthelmintic treatment was evaluated by comparing pre- and post-treatment EPG values. Reduction in egg count was used as an indicator of drug efficacy, while the persistence or reappearance of eggs was interpreted as possible reinfection or development of anthelmintic resistance. Faecal egg count (FEC) data was assessed using the Shapiro–Wilk test. Although the results indicated no significant deviation from normality ($p > 0.05$), non-parametric methods were adopted due to the discrete and overdispersed nature of FEC data. Differences among groups were analyzed using the Kruskal–Wallis test. For multiple pairwise comparisons, the Wilcoxon rank-sum test was applied, with p-values adjusted using the Bonferroni correction to control for Type I error.Faecal Egg Count Reduction Test (FECRT) was performed to evaluate the efficacy of the anthelmintic treatment and to detect potential drug resistance, using the standard formula. All statistical analyses were conducted using R software.

Results

Pre-treatment faecal examination of the studied goats revealed a moderate to high level of gastrointestinal nematode infection. The mean faecal egg count (FEC) was recorded as 1820± 52.54 eggs per gram (EPG), indicating a substantial parasitic burden within the herd (Table 1). Qualitative and quantitative findings were further supported by larval culture, which confirmed the predominance of *Haemonchus spp*. among the recovered third-stage (L3) larvae, suggesting that this species was the principal contributor to the infection. Following oral administration of fenbendazole at the recommended therapeutic dose, a significant reduction in faecal egg count was observed during the first post-treatment evaluation. At 10–14 days post-treatment, the mean FEC decreased markedly to 122 ± 7.97 EPG, indicating a substantial reduction in parasite load and demonstrating an initial positive response to the anthelmintic treatment. However, during the second post-treatment evaluation conducted at 30 days, the mean FEC increased again to 979 ± 32.36EPG. This rise in egg count suggests re-establishment of infection within a relatively short period following treatment. The efficacy of fenbendazole was assessed using the Faecal Egg Count Reduction Test (FECRT), calculated to assess the efficacy of the anthelmintic treatment. The faecal egg count reduction was determined using the following formula

FECRT (%) =**[** {(Pre-treatment EPG) – (Post-treatment EPG)}/{Pre-treatment EPG}**] x{ 100**

The calculated reduction of 93.33% is below the generally accepted efficacy threshold of 95%, indicating suspected anthelmintic resistance to fenbendazole in the studied goat population. The subsequent increase in FEC at 30 days post-treatment further supports the possibility of survival of resistant parasite populations and/or rapid reinfection from contaminated grazing areas. It must be mentioned here that even though Shapiro result says

(Denwood *et. al*. 2019) the data to be normal (Table no. 2) , but faecal (faecal egg count) data are biologically non-normal as such faecal egg data counts are not continuous, over – dispersed and typically skewed in parasitology studies. So Kruskal–Wallis test was preferred instead of ANOVA (Denwood *et. al.* 2019, Denwood *et. al*. 2017 and Paul *et. al*. 2014).Kruskal-Wallis result showed chi-squared = 25.812 at df = 2 and the p-value was 2.483e-06*** which was highly significant (p< 0.001). For post Hoc comparison -Pairwise comparisons was done using Wilcoxon rank sum test with Bonferroni's model (Table No. 3) Faecal Egg Count Reduction Test (Kaplan *et. al*. 2023**,** Dobson *et. al.* 2011, Levecke *et. al.* 2012) was found to be 93.3% for post-treatment faecal egg count at 10-14 days and because it falls in 90–95% range, it is suggestive of anthelmintic resistance.

Discussion

Pre-treatment faecal examination revealed a moderate to high gastrointestinal nematode burden, with a mean FEC of 1820± 52.54 EPG (Table 1). Larval culture confirmed the predominance of *Haemonchus spp*. among recovered L3 larvae.Following treatment with Fenbendazole, the mean FEC declined to 122 ± 7.97 EPG at 14 days post-treatment, corresponding to a faecal egg count reduction (FECR) of 93.3%. Although this indicates a substantial initial reduction in parasite burden, the FECR did not reach the ≥95% threshold recommended by the World Association for the Advancement of Veterinary Parasitology, thereby raising suspicion of reduced drug efficacy. At Day 30 post-treatment, the mean FEC increased to 979 ± 32.36EPG, with a corresponding FECR of 47.2%. This marked decline in efficacy over time may be attributed to either reinfection or anthelmintic resistance, which can be differentiated based on epidemiological and parasitological considerations. Reinfection is typically associated with continued exposure to infective larvae on pasture, particularly under semi-intensive systems, and is characterized by a gradual rise in FEC following an initially effective treatment. However, the rapid rebound in egg counts within 30 days, coupled with a suboptimal initial FECR (<95%), suggests that a proportion of the parasite population may have survived treatment. Such survival is indicative of suspected anthelmintic resistance, especially in *Haemonchus spp.,* which is known for its high biotic potential and propensity to develop resistance. Therefore, while reinfection cannot be completely ruled out due to grazing exposure, the combined evidence incomplete initial reduction and rapid recovery of egg counts more strongly supports the presence of emerging resistance to fenbendazole in the studied herd.

The findings of the present investigation provide important insights into the emerging challenge of anthelmintic resistance in small ruminant production systems, particularly under semi-intensive management conditions. The reduced efficacy of fenbendazole observed in this study against gastrointestinal nematodes (Edna 2025), especially *Haemonchus contortus*, is indicative of a shifting parasitic landscape where conventional control measures are gradually losing their effectiveness. Although a noticeable reduction in faecal egg count (FEC) was recorded at 10–14 days post-treatment, the failure to achieve the recommended ≥95% reduction threshold clearly signals a deviation from optimal drug performance. This level of reduction, while suggestive of partial efficacy, is insufficient to ensure effective parasite control and strongly points toward the presence of resistant worm populations

within the herd. The temporal pattern of FEC observed in the study further strengthens this concern. The initial decline in egg counts followed by a significant resurgence at 30 days post-treatment reflects a complex interplay between drug efficacy, parasite biology, and environmental exposure. One plausible explanation for this rebound is rapid reinfection due to continuous grazing on contaminated pastures. In semi-intensive systems, animals are frequently exposed to infective third-stage larvae (L3) present on herbage, which facilitates quick re-establishment of infection even after deworming. However, the magnitude of the increase in FEC within such a short period cannot be solely attributed to reinfection. It also suggests that a proportion of the parasite population survived the initial treatment, indicating the presence of resistant individuals that were able to persist and subsequently reproduce within the host. *Haemonchus contortus*, the predominant parasite implicated in this study, is widely recognized for its high pathogenic potential and extraordinary ability to develop resistance to anthelmintics. Its biological characteristics—including a short prepatent period, high fecundity, and substantial genetic variability—enable rapid selection and propagation of resistant strains under drug pressure. Resistance to benzimidazole compounds such as fenbendazole has been extensively reported worldwide and is primarily associated with single nucleotide polymorphisms in the β-tubulin gene of the parasite. These mutations alter the binding affinity of the drug to its target site, thereby reducing its efficacy and allowing resistant worms to survive therapeutic doses that would otherwise be lethal.

Conclusion

The findings of the present investigation provide significant and timely insights into the growing concern of anthelmintic resistance within small ruminant production systems, particularly those operating under semi-intensive management conditions. The observed reduced efficacy of fenbendazole against gastrointestinal nematodes, with *Haemonchus contortus* as the predominant species, reflects an evolving parasitic scenario where traditional chemotherapeutic interventions are progressively losing their reliability. This trend is alarming given the heavy dependence of goat farming systems on benzimidazole class drugs for parasite control. Although a measurable decline in faecal egg count (FEC) was recorded between 10 and 14 days post-treatment, the inability to achieve the recommended ≥95% reduction threshold, as stipulated in standard faecal egg count reduction tests (FECRT), clearly indicates suboptimal drug performance. A reduction of 93.33%, while appearing close to the threshold, cannot be considered satisfactory from a parasitological or clinical standpoint. Such marginal shortfalls often represent the early stages of resistance development, where susceptible worms are largely eliminated but resistant genotypes survive and begin to dominate the population. Therefore, the present findings should be interpreted not as an isolated inefficiency but as an early warning signal of emerging resistance within the herd.

In conclusion, the present study highlights the emerging challenge of anthelmintic resistance in goat production systems and underscores the limitations of conventional control strategies. The reduced efficacy of fenbendazole against *Haemonchus contortus* serves as a critical reminder of the need for judicious drug use and integrated management approaches. Proactive measures adopted at this stage can play a pivotal role in preserving the efficacy of existing anthelmintics and ensuring the long-term sustainability of small ruminant farming

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI TECHNOLOGIES SUCH AS LARGE LANGUAGE Models ,etc have been used during the writing or editing of manuscripts. This explaination will include the name.version. model and source of the generative AI technology and as well as all input prompts provided to the generative AI technology.

Details of the AI usage are given below

1. **ChatGPT**

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Figure 1. **Box plot of Faecal egg count Vs pre treatment and post treatment using fenbendazole**

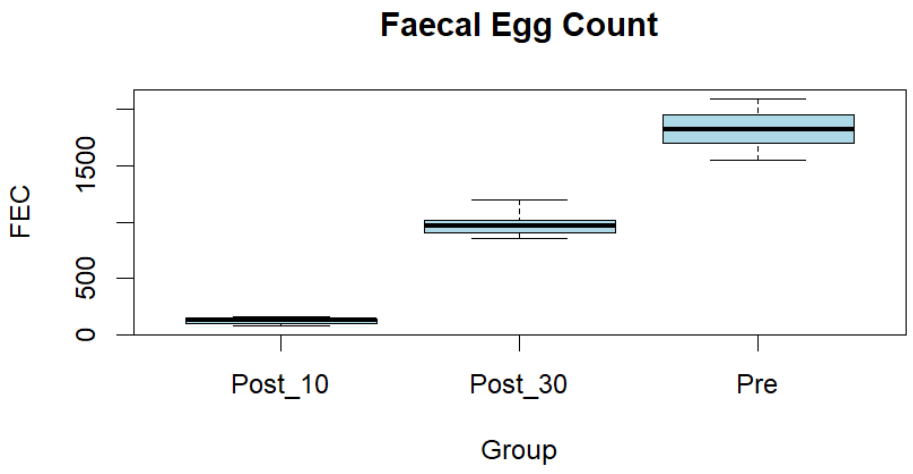

Table 1 Effect of Fenbendazole treatment on fecal egg count (EPG) in goats.

Treatment stage	Mean EPG (±SE)
Day 0 (pretreatment)	1820± 52.54

Day 14 (post-treatment)	122 ± 7.97
Day 30 (post-treatment)	979 ± 32.36

Fecal egg count reduction (FECR) = 93.33% (Day 14)

Fecal egg count reduction (FECR) = 47.22 % (Day 30)

Table No. 2. Group wise Shapiro-Wilk normality test

Group	P-value
Pre-treatment	0.983
Post-treatment(10-14 days)	0.8285
Post-treatment (30-days)	0.5208

Table No. 3. Kruskal wallis test and Post hoc comparison using Wilcoxon rank sum test

Kruskal Wallis test	**Post hoc Comparison**	**p-value**	**Interpretation**
chi-squared = 25.812***; p-value = 2.483e-06***	Post_10 vs Post_30	0.00054	• All p-values < 0.05 • Highly significant difference between all the groups
	Pre vs Post_10	0.00054	
	Pre vs Post_30	0.00055	