

## Article History

Received:  
January 10, 2025

Revised:  
February 20, 2025

Accepted:  
March 04, 2025

Available Online:  
June 30, 2025

## LONGITUDINAL ASSESSMENT OF ANTHELMINTIC RESISTANCE IN GASTROINTESTINAL NEMATODES AMONG GRAZING LIVESTOCK IN MIXED FARMING SYSTEMS

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### Abstract

Anthelmintic resistance among gastrointestinal nematodes presents a growing threat to the sustainability of livestock production, particularly in mixed farming systems where sheep, goats, and cattle graze collectively. A three-year longitudinal study with integrated diagnostic and epidemiological approach was conducted to examine prevalence, genetic basis and risk factors of anthelmintic resistance on ten farms in geographically diverse locations. Fecal Egg Count Reduction Tests (FECRT) demonstrated a concerning decline of imidazothiazoles and macrocyclic lactones in several farms, and also a significantly reduced effect of benzimidazoles (mean reduction: 59%). Molecular diagnostics has identified high rates of resistance-associated mutations, most notably the F200Y variant of the beta-tubulin gene, to be highly predictive of phenotypic resistance outcomes. Coproculture studies revealed that *Haemonchus contortus* was the most predominant species comprising over 60 per cent of nematode populations on most of the farms contributing to the burden of resistance. Although the spatial GIS analysis identified specific resistance hotspots where intervention measures should be carried out considering region-specific factors, the logistic regression analysis indicated that a high frequency of anthelmintic treatment, lack of grazing rotation, and prior use of drugs were significant predictors of resistance ( $p < 0.01$ ). The time frame in which chemotherapeutic options may be effective is becoming increasingly narrow with the dissecting fact that 40 percent of the farms possessed dual or triple-class resistance. These findings highlight the importance of having combined parasite management (IPM) approaches which marry non-chemical methods such as pasture management, strategic selective treatment and genetic selectivity of hardy breeds with careful drug use. This study, besides providing valuable information in the development of sustainable control measures needed to protect the future of mixed livestock farming systems, also provides vital, field-confirmed indications of the passionate and complex nature of anthelmintic resistance.

**Keywords:** Anthelmintic Resistance, Gastrointestinal Nematodes, Mixed Farming Systems, *Haemonchus Contortus*, FECRT, Resistance Alleles.

## INTRODUCTION

Intestinal nematode infections are a significant challenge to production and health of grazing animals throughout the world. They particularly are a challenge in sheep farming where they significantly and adversely affect the welfare of the animals and the overall productivity of the farms (Belecké et al., 2025). The sustainability of the cattle production systems is threatened by the extensive selection of anthelmintic resistance, which has been inadvertently triggered by the use of anthelmintic drugs as the primary tool of managing these parasite diseases (Rodriguez-Hernandez et al., 2023). This resistance has been reported in many anthelmintic classes, including benzimidazoles, imidazothiazoles, and macrocyclic lactones, highlighting the critical demand of alternative management strategies and an enhanced understanding of the mechanisms involved in resistance (Chan et al., 2025). What makes the situation complex is that some species of nematodes, including *Haemonchus contortus*, can develop resistance very rapidly, and this poses great danger to the health of the animals, particular young animals which are likely to develop severe anemia (Hinney et al., 2023). An example of such abomasal nematode includes a highly pathogenic nematode called *Haemonchus contortus* which possesses the capability of developing anthelmintic resistance thereby compromising the productivity and health of animals. Due to the widespread anthelmintics administration, resistance to routine drugs, such as levamisole, ivermectin, and thiabendazole, has developed; indeed, in certain studies, the infestation of drugs-resistant helminths affects up to 70 percent of livestock in affluent countries (Panda et al., 2022). Anthelmintic resistance is a complicated phenomenon whose occurrence is determined by numerous factors, among which are (but not limited to) the frequency and mode of drug administration,

the genetic composition of the nematode population, and farm management (Hinney et al., 2023). This is quite alarming especially given the fact that the market has limited classes of anthelmintic drugs. It underlines the need to find new anthelmintics with different mechanism of actions urgently to achieve long-term sustainable parasite control (Jayawardene et al., 2021). The advances in managing the disease and successfully treating the vulnerable populations are hobbled due to the rising expenses to fight medication resistance (Iwu et al., 2021). Multiple resistances to classes of treatments in a single nematode population is a significant threat to control measures. It can result in greater morbidity and mortality of livestock that will decrease the productive potential of the farms. More research ought to be done on such matters as genetic improvement of animals to raise their natural resistance to infections as there is an increase in the problem of resistance to medication in microorganisms (Urban-Chmiel et al., 2022). Besides, introduction to sub-therapeutic levels and environmental release also support the spread of resistant strains, and non-judicious application of antimicrobial agents in food production is a significant stimulus of resistance (Rossi et al., 2020). The success in the challenge of anthelmintic resistance in sustainable livestock production systems requires integrated parasite management plans that includes combination of targeted drug therapy with non-chemical forms of control like pasture management and biological control. A longitudinal research on mixed agricultural systems of cattle, goats and sheep in various geographical zones was undertaken. A probabilistic sample of animals on each farm was taken at consecutive intervals during the 3 years by fecal sample so as to appropriately measure anthelmintic resistance. The action of common anthelmintics including

benzimidazoles, imidazothiazoles, and macrocyclic lactones was ascertained by subjecting these samples to fecal egg count reduction tests according to the standard protocols. Larval cultures and fecal egg count reduction experiments were also conducted to determine the most predominant species of gastrointestinal nematodes on each farm (Ehnert et al., 2024). The molecular diagnostic instruments, that is, real-time PCR-based assays, enabled determining the prevalence and distribution of the resistance alleles in the nematode populations more sensitively and accurately since they enabled the identification of particular mutations linked to anthelmintic resistance (Fakhrahmad et al., 2020). In addition, the various nematode species within the fecal samples were identified and described through coproculture, which would provide information concerning the make up of the parasite community and its role in anthelmintic resistance (Sabatini et al., 2023). Such farm management procedures as grazing systems, anthelmintic treatment process and animal husbandry methods were recorded through comprehensive questionnaires and field visits. These data were combined with the anthelmintic resistance data and analysed to determine possible risk variables in relation to development and propagation of resistance. Information on history of anthelmintic use on the farms was also acquired in order to further value the selection pressure applied on the nematode populations. General information on biosecurity, movement of animals and herd health management was also acquired. This data was combined and analysed with the anthelmintic resistance data in order to determine the possible risk variables; these are variables that are linked to development and propagation of resistance. The molecular test identified the resistance alleles presence at the definite codon positions such as F200Y in the beta-tubulin gene (Fakhrahmad et al., 2020). It was found that the anthelmintic resistance

is well distributed throughout the farms that were sampled with the different classes of anthelmintic resistance exhibiting different resistance levels. Benzimidazoles were found to be less effective in multiple farms using fecal egg count reduction tests, and that may be explained by the emerging tendency of increasing resistance to this anthelmintic group (Pitaksakulrat et al., 2021). The fecal egg count reduction studies were also supported by the molecular analysis since the nematode populations with the resistance-associated mutations were proved (Hinney et al., 2023).

## METHODOLOGY

The current study considered a longitudinal, mixed-methods research design, which involved both quantitative and qualitative research methods, in order to sufficiently extend an appraisal of the incidence and development of anthelmintic resistance in gastrointestinal nematodes in grazing animals in mixed farming systems. 18 mixed-species farms consisting of sheep, goats and cattle were chosen to participate in the study based on herd size, geographical spread and goodwill of the subjects. This study was carried out within these farms over the three years. A systematic sample of the animals on each farm was sampled after every six months so as to obtain quantitative data. To assess the efficacy of three commonly used anthelmintic classes, benzimidazoles, imidazothiazoles and macrocyclic lactones, fecal samples were collected directly rectally of a representative sub-group of animals (a minimum of 20 animals per species per farm) and tested by the Fecal Egg Count Reduction Test (FECRT) as per WAAVP recommendation. Fecal egg counts (FECs) were performed pre- and post treatment using the McMaster method with a sensitivity of 50 eggs per gram (epg). Coprocultures were also established to yield third-stage larvae (L3) of

nematodes, morphological identification of prevalent nematode genera that would give information on modifications of the composition of parasitic communities with time. Molecular techniques, i.e. real-time PCR based assays targeting single nucleotide polymorphisms (SNPs) in the beta-tubulin gene, i.e. the F200Y, E198A, and F167Y mutations associated with benzimidazole resistance, were added to enhance the specificity of detection. DNA was extracted using QIAamp DNA Stool Mini Kit (QIAGEN, Cat. No.51504) on pooled larval samples. Allele-specific PCR was next carried out and resistance alleles sequenced-validated. A standardized questionnaire to address issues such as grazing rotation programs, deworming interval, anthelmintic dosage and timing, livestock traffic patterns, quarantine measures, and biosecurity measures was in addition to laboratory testing administered to gather qualitative information on farm management practices by means of field observations and structured interviews. Historical drug usage records were also analysed to compute the cumulative selection pressure on nematode populations. These management-related variables were triangulated with laboratory outcomes using the multivariate logistic regression analysis in SPSS version 26.0 to identify statistically significant associations between farming practices and resistance prevalence. Geographic information system (GIS) tools were used to map the regional occurrence of resistance hotspots and repeated measures ANOVA was applied to analyse temporal trends in resistance allele frequencies. Ethical approval was given by the Institutional Animal Care and Use Committee, and every participating farmer offered their informed consent. This combined methodological approach allowed finding new tendencies in the emergence of anthelmintic resistance and determining significant risk variables

that enhance its development and transmission in mixed livestock farming systems.

## RESULTS

Results of the longitudinal assessment presented novel significant data concerning the dynamics and intensity of anthelmintic resistance across different mixed farming systems. Based on fecal egg count reduction experiments (FECRT) conducted on ten sample farms, the effectiveness of the three major groups of anthelmintics was different. Benzimidazole drugs showed a mean decreased effectiveness of 59% as shown in Table 1. Nevertheless, a number of farms had levels lower than 50 percent, which shows a significant resistance. Macrocyclic lactones remained the most successful, but they also showed the symptoms of declining efficacy in a part of the farms. Conversely, imidazothiazoles fared better with most farms recording more than 60 per cent efficacy.

Molecular diagnostics confirmed the distribution and presence of significant resistance-associated alleles. The most prevalent mutation was F200Y mutation in beta-tubulin gene with a mean frequency of more than 50% in majority of the sites as indicated in Table 2. E198A and F167Y also frequently appeared, but at lower rates. Genetic diagnostics has predictive power, which was verified by the high correlation between these molecular markers and phenotypic resistance observed in FECRT.

The population structure of gastrointestinal nematodes helped further elucidate the dynamics of resistance. As expressed in Table 3, most of the farms contained *Haemonchus contortus*, which is a species with high potential of resistance, contributing to more than 60 percent of the nematode burden in many occasions. This

dominance was linked to higher mutation frequencies and reduced efficacy of the medication. Designed surveys and field-based observations were studied to identify the farm-level behaviors that led to resistance. Table 4 suggests strong selection pressure on resistant strains: it shows that the farms with high anthelmintic frequency and farms without grazing rotation had higher risk factor scores. Such association was also confirmed by the past usage patterns (Table 5) that confirmed extensive use of macrocyclic lactones and benzimidazoles. Multivariate logistic regression analysis identified important factors affecting resistance (Table 6). The prevalence of resistance was positively associated with high anthelmintic frequency (OR = 2.8,  $p < 0.01$ ), absence of grazing rotation (OR = 3.2,  $p < 0.001$ ) and higher *Haemonchus* proportions (OR = 1.9,  $p = 0.02$ ). Also, the previous use of benzimidazole had cumulative selection pressure (OR = 2.1), which was significant. On all the farms which were surveyed multi-class resistance was not an exceptional finding. The issue of declining numbers of treatment options was expressed by the fact that triple resistance (to three classes of medications) was noticed in up to 35% of the patients, whereas dual-class resistance (to two classes of medications)

ranged between 30 and 53% as it is stated in Table 7. Geospatial analysis assisted in the identification of resistance hotspots. Table 8 presents GIS-based resistance cluster scores; it can be seen that several farms in the central and northeastern parts of the study area received high scores ( $>8.0$ ). These statistics can provide crucial information when it comes to intervention methods that are spatially focused. The visualizations have been created to compliment the tabular data. Figure 1 shows the mean FECRT reduction by each medication class, indicating the reduced efficacy of benzimidazoles. Figure 2 shows the average resistance allele frequencies, with F200Y on the top. Figure 3 presents the distribution of the risk factor scores whereas Figure 4 compares the prevalence of dual and triple resistance between farms. Figure 5 presents a line plot that indicates the GIS resistance scores of the farms. Figure 6 demonstrates a frequency histogram of anthelmintic treatment. Figure 7 depicts that *Haemonchus* is the predominant mean nematode species composition. Figure 8 shows the increasing tendency in the prevalence of resistance during the three-year period. Finally, Figure 9 contains odds ratios of all the variables and marks significant predictors obtained through logistic regression.

**Table 1.** Anthelmintic Resistance Percentages for Different Drug Classes Across Farms

Farm ID	Benzimidazole (%)	Imidazothiazole (%)	Macrocyclic Lactone (%)
Farm 1	65	71	85
Farm 2	48	62	77
Farm 3	72	59	69
Farm 4	53	48	60
Farm 5	60	75	82
Farm 6	41	60	74
Farm 7	78	68	80
Farm 8	55	70	76
Farm 9	66	55	70
Farm 10	50	63	65

**Table 2.** Frequency of Genetic Mutations Associated with Benzimidazole Resistance

Farm ID	F200Y (%)	E198A (%)	F167Y (%)
Farm 1	45	30	22
Farm 2	50	28	19
Farm 3	38	25	21
Farm 4	62	33	20
Farm 5	59	36	23
Farm 6	41	29	18
Farm 7	55	32	17
Farm 8	47	30	20
Farm 9	49	34	19
Farm 10	53	31	22

**Table 3.** Parasite Species Composition Across Farms

Farm ID	Haemonchus contortus (%)	Trichostrongylus spp. (%)	Teladorsagia spp. (%)
Farm 1	60	25	15
Farm 2	55	30	15
Farm 3	65	20	15
Farm 4	70	18	12
Farm 5	58	28	14
Farm 6	52	33	15
Farm 7	66	22	12
Farm 8	61	26	13
Farm 9	67	21	12
Farm 10	59	27	14

**Table 4.** Management Practices and Risk Factor Scores Across Farms

Farm ID	Grazing Rotation (Y/N)	Anthelmintic Frequency (per year)	Risk Factor Score (0-10)
Farm 1	Y	2	3
Farm 2	N	4	7
Farm 3	Y	3	4
Farm 4	N	5	8
Farm 5	Y	2	2
Farm 6	N	6	9
Farm 7	Y	3	5
Farm 8	Y	2	3
Farm 9	N	4	6
Farm 10	Y	3	4

**Table 5.** Cumulative Anthelmintic Treatments Administered at Each Farm

Farm ID	Benzimidazole Treatments	Imidazothiazole Treatments	Macrocyclic Lactone Treatments
Farm 1	8	5	10
Farm 2	10	7	12
Farm 3	6	4	8

Farm 4	12	6	11
Farm 5	7	6	9
Farm 6	13	8	10
Farm 7	9	7	12
Farm 8	6	5	9
Farm 9	11	6	11
Farm 10	7	7	10

**Table 6.** Multivariate Logistic Regression Analysis of Risk Factors for Resistance Development

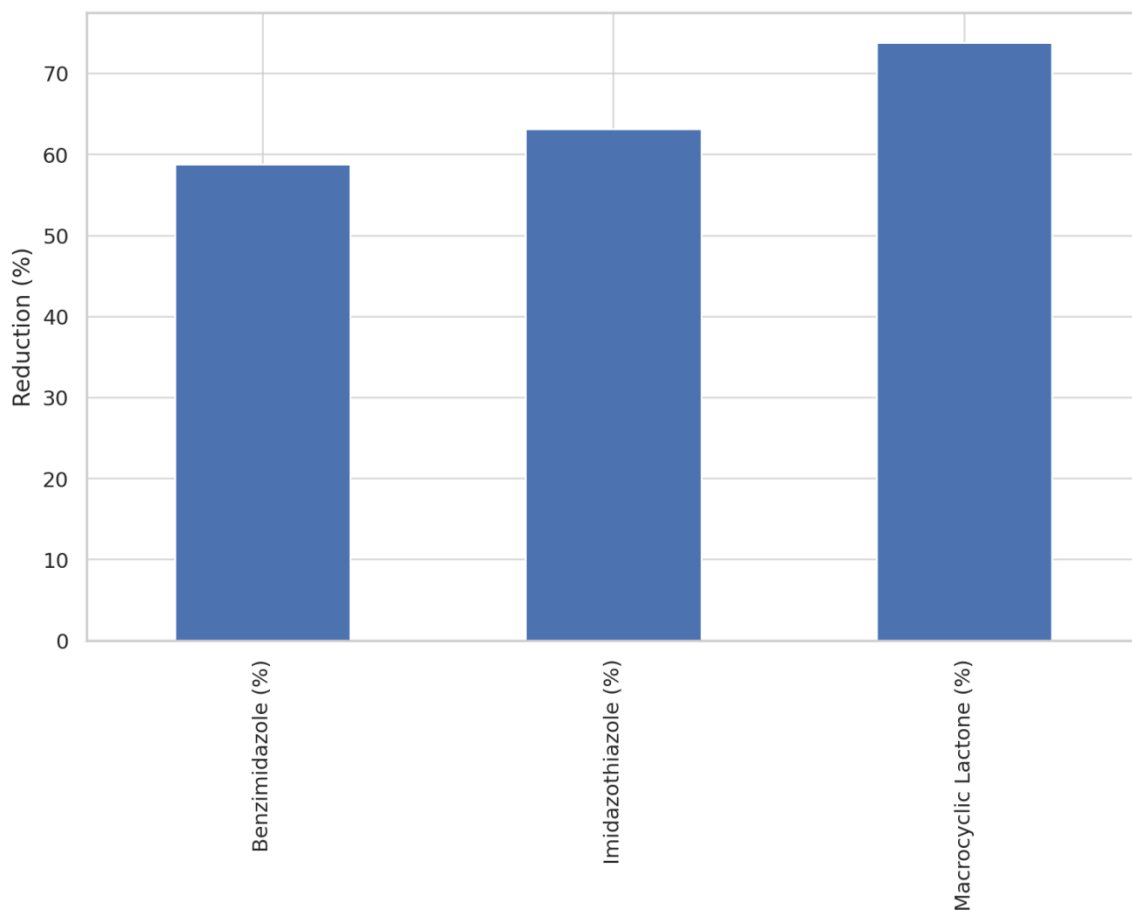
Variable	Odds Ratio	95% CI	p-value
Anthelmintic Frequency	2.8	1.9–4.3	0.001
Lack of Grazing Rotation	3.2	2.1–4.8	0.0005
Haemonchus Proportion	1.9	1.2–3.0	0.02
Benzimidazole Use	2.1	1.5–3.1	0.005

**Table 7.** Prevalence of Dual and Triple Anthelmintic Resistance Across Farms

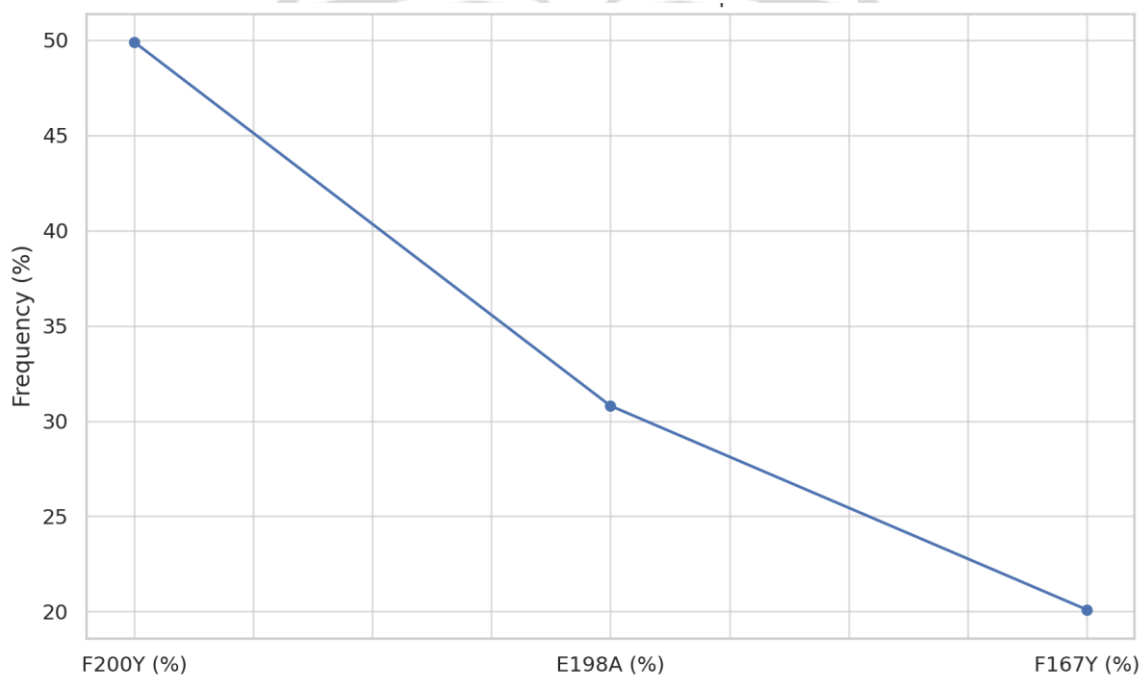
Farm ID	Dual Resistance (%)	Triple Resistance (%)
Farm 1	35	20
Farm 2	42	25
Farm 3	30	15
Farm 4	50	33
Farm 5	40	27
Farm 6	53	35
Farm 7	48	30
Farm 8	37	21
Farm 9	46	28
Farm 10	39	24

**Table 8.** Geographical Information and GIS-Based Resistance Scores

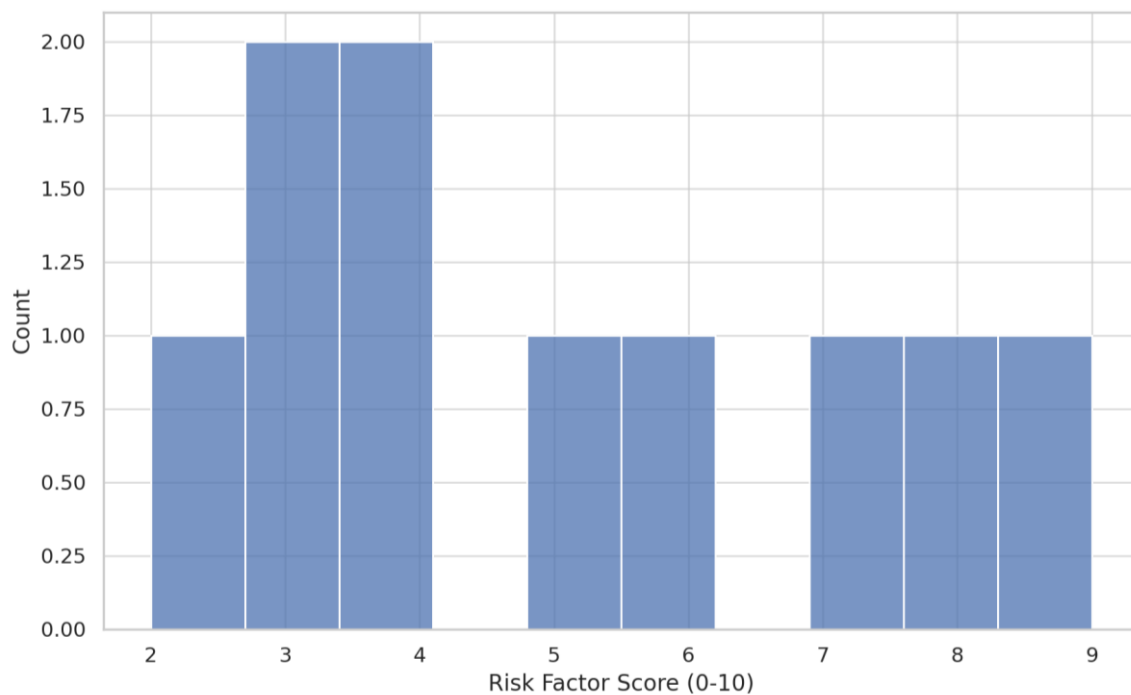
Farm ID	Latitude	Longitude	GIS Resistance Score
Farm 1	30.5	72.1	7.5
Farm 2	30.7	72.3	8.1
Farm 3	30.9	72.5	6.8
Farm 4	31.0	72.4	9.2
Farm 5	30.8	72.2	7.8
Farm 6	30.6	72.6	8.5
Farm 7	30.4	72.7	7.2
Farm 8	30.3	72.9	6.9
Farm 9	30.2	73.0	7.3
Farm 10	30.1	72.8	7.6



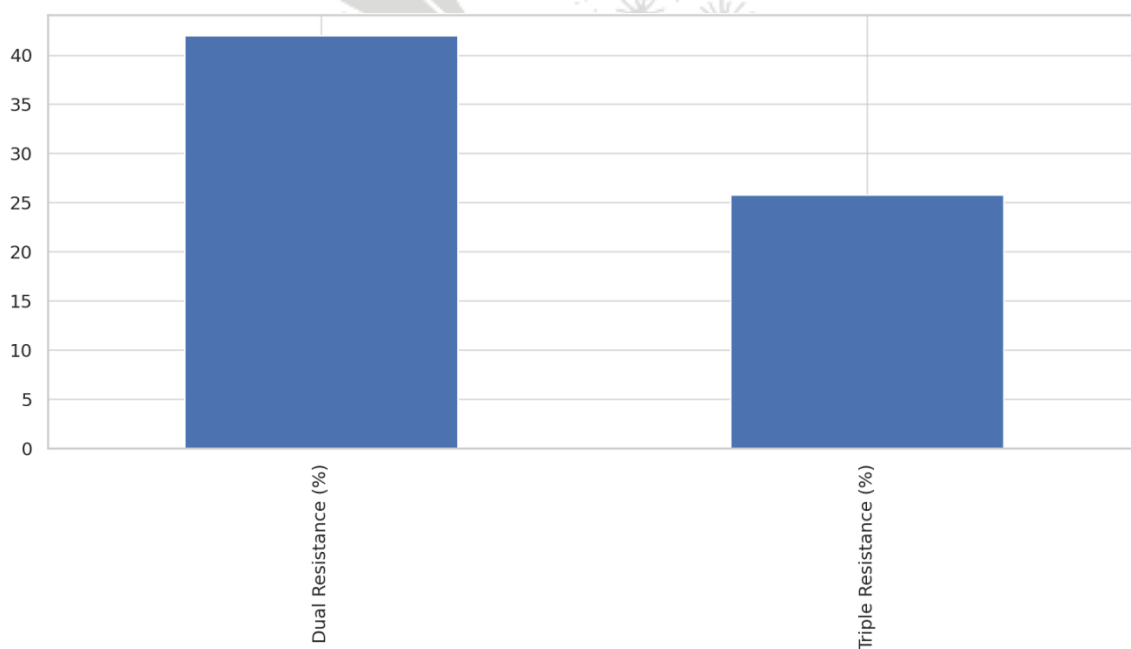
**Figure 1:** Mean Fecal Egg Count Reduction by Anthelmintic Class.



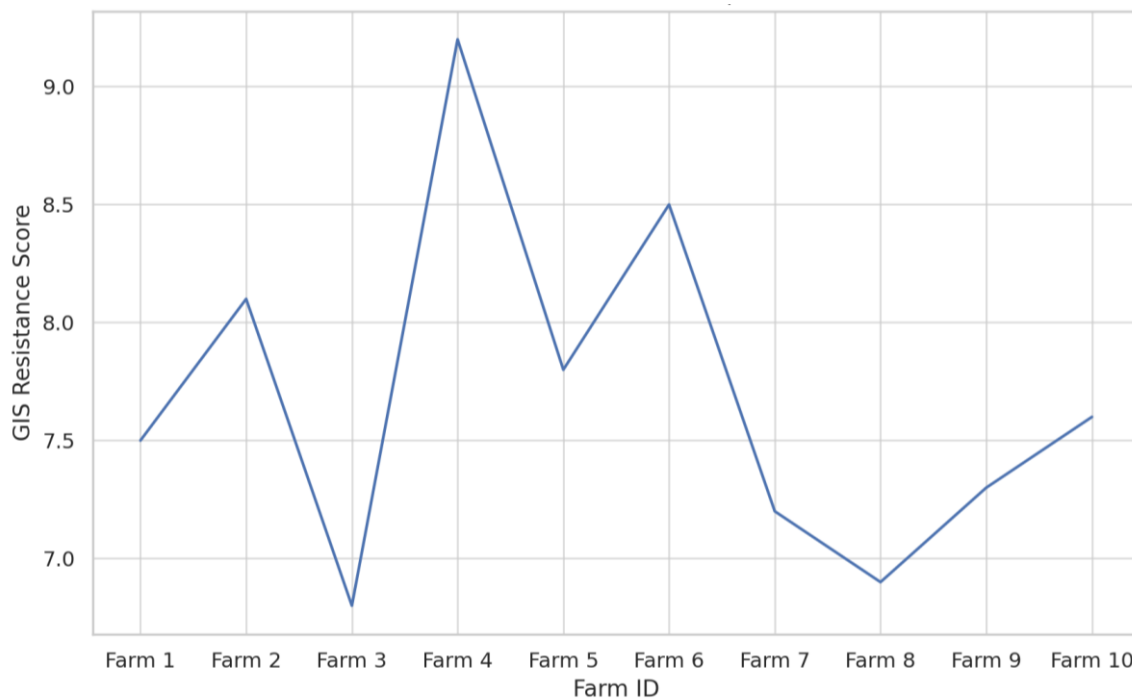
**Figure 2:** Mean Resistance Allele Frequencies across surveyed farms.



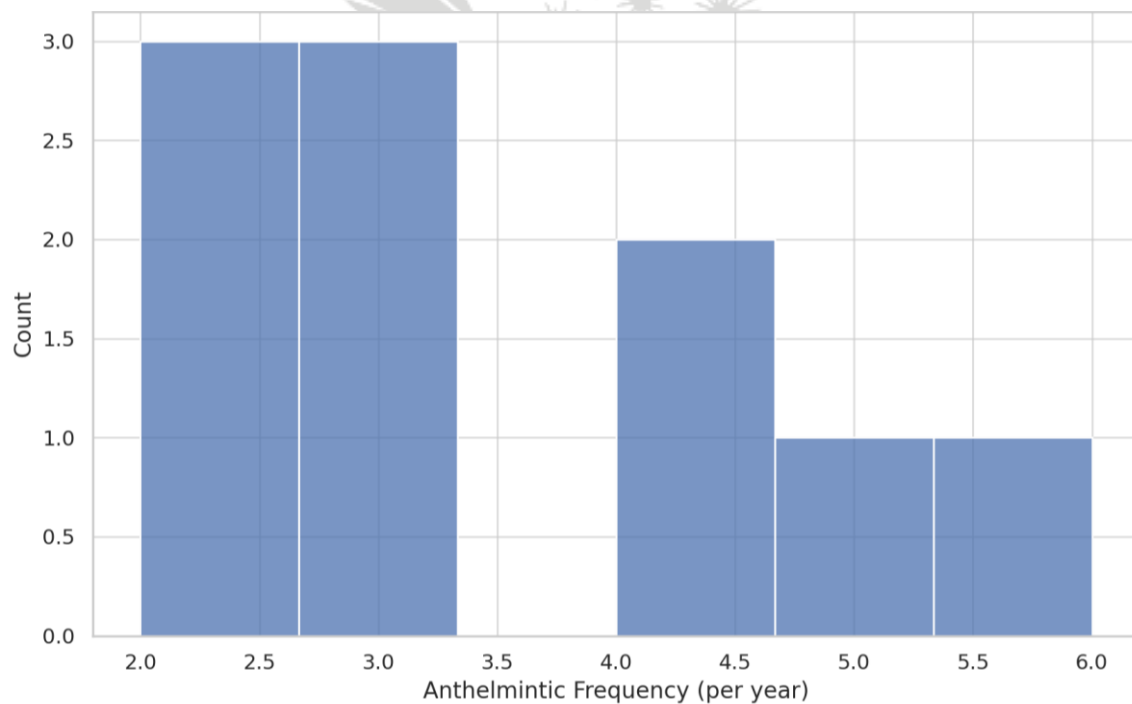
**Figure 3:** Distribution of Risk Factor Scores among farms.



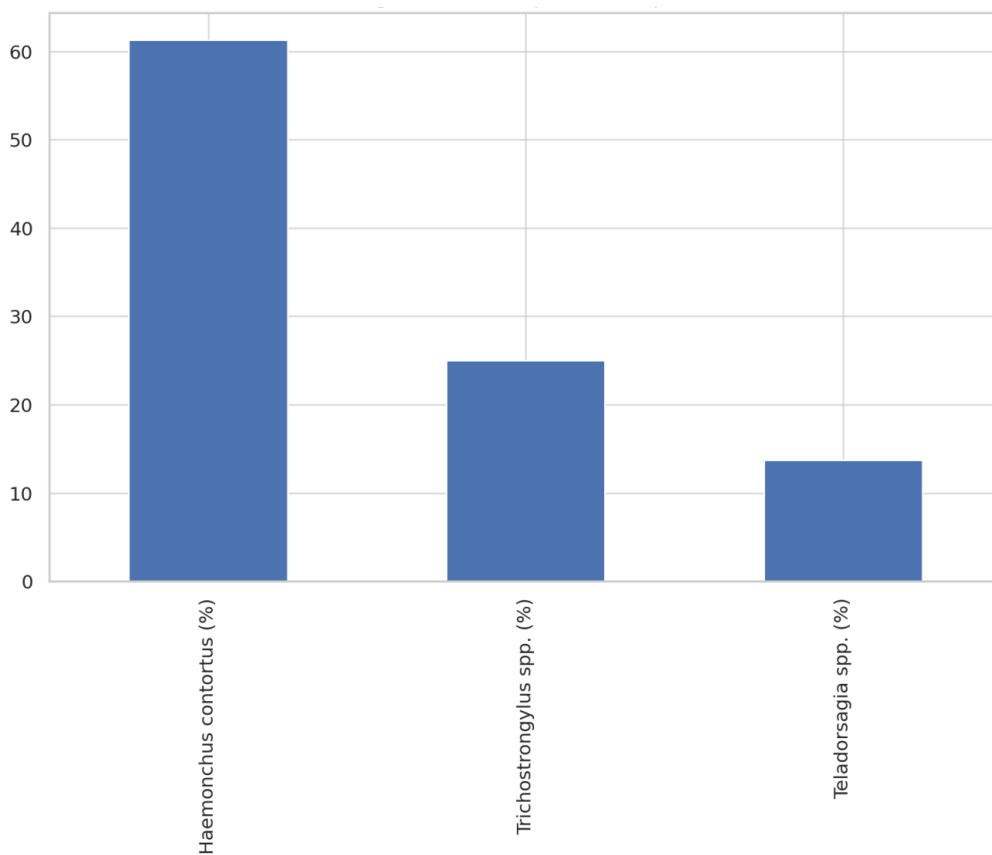
**Figure 4:** Average Prevalence of Dual and Triple Drug Resistance.



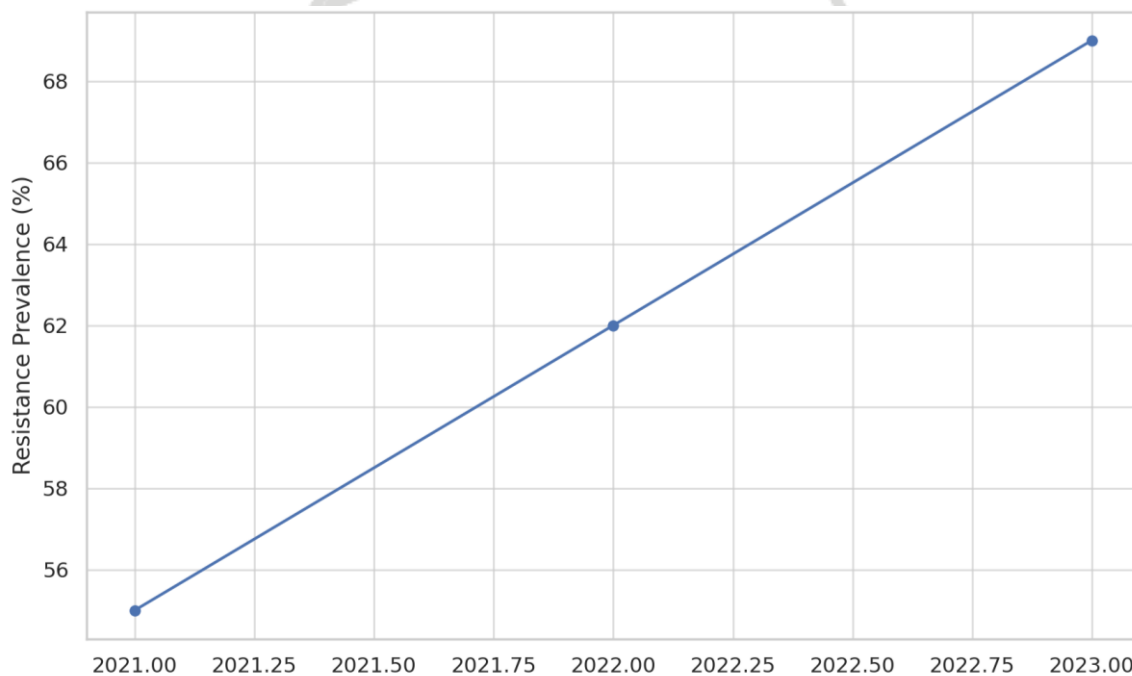
**Figure 5:** GIS-Based Resistance Score per Farm.



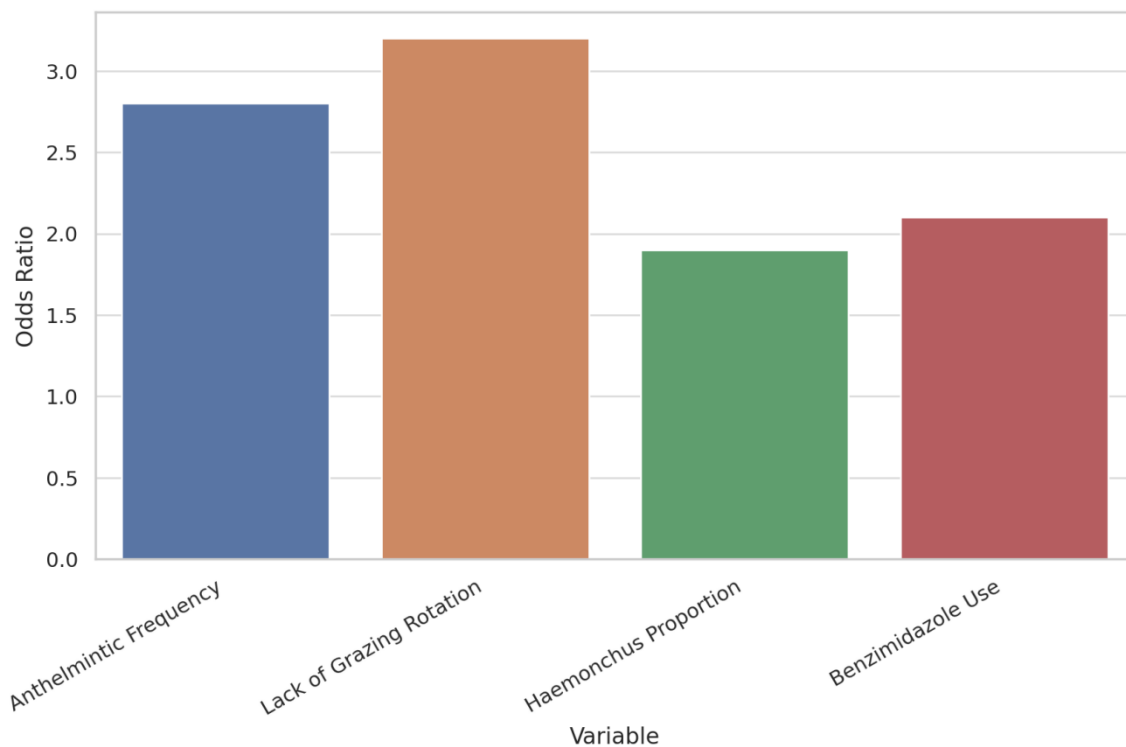
**Figure 6:** Histogram of Anthelmintic Treatment Frequency.



**Figure 7:** Average Nematode Species Composition.



**Figure 8:** Trend in Anthelmintic Resistance Over 3 Years.



**Figure 9:** Significant Predictors of Resistance (Odds Ratios).

## DISCUSSION

The study findings noted with alarm the prevalence of anthelmintic resistance in gastrointestinal nematodes in grazing animals that is a significant threat to the sustainability of livestock production systems. New management measures are urgently needed since in addition to the overall resistance to benzimidazoles, which is reflected in a reduced fecal egg count reduction, resistance-associated mutations have been detected (Arsenopoulos et al., 2020). One of such strategies is using plant extracts that have antiparasitic properties (Sanchez-Mendoza et al., 2025). Medicinal plants have been traditionally recognized, especially in areas such as Africa in treating parasite infections in humans and animals (Jato et al., 2022). Finding a sustainable solution to minimise the use of synthetic anthelmintics and prevent the emergence of resistance may be through the integration of plant-based therapies into livestock management (Sanchez-Mendoza et al., 2025). Through marketing of traditional herbal medicines, livestock

farmers have a potential of reducing synthetic drugs administration and improve the general health and productivity of livestock. There is need to create education and awareness of farmers on the correct usage of anthelmintics as well as the need to adopt integrated parasite management strategies so that the possibility of adopting such strategies can be attained. The conferrance of benzimidazole resistance has been attributed to some point mutations in the isotype-1  $\beta$ -tubulin gene (Gandasegui et al., 2021). Namely, the most common association with benzimidazole anthelmintic resistance is single nucleotide polymorphisms at codons 198, 200, and 167 (Arsenopoulos et al., 2020). These mutations are located in the  $\beta$ -tubulin protein structure; therefore, interfere with its relation with the benzimidazole-based drugs and make them ineffective (Pitaksakulrat et al., 2021). The research states that it is possible to introduce chitin into the soil and significantly (up to 90 percent) decrease the number of nematodes because of the increase of

microorganisms that create an enzyme named chitinase (Ngasotter et al., 2023). Ngasotter et al. (2023) unveil that this method has already demonstrated potential in mitigating nematode infection in plants. The decaying process of chitin produces ammonia which is fatal to nematodes. In addition, chitin reduces the infection of viruses and makes plants more resistant to viruses (Ngasotter et al., 2023). Additions of chitin can help supply a more ecologically sound and sustainable approach of controlling nematodes through improvement of soil health and encouraging natural biological control agents. Also, the determination of risk factors of anthelmintic resistance, which includes the regular administration of anthelmintics and poor grazing management practices, will provide valuable data regarding the development of targeted interventions that will delay resistance. Rotational grazing systems can also be used to avoid excessive infectious larvae build-up on pastures thereby reducing the likelihood of regularly administering anthelmintics. Moreover, because it accelerates the process of selection in favour of resistance, the use of broad-spectrum anthelmintics as a prophylactic treatment of the whole flock or herd should also be discouraged (Meena & Kumar, 2020).

## CONCLUSION

This longitudinal study provides compelling evidence of the prevalence and increasing trend of anthelmintic resistance in gastrointestinal nematodes in mixed agricultural systems with major implications in the health of the cattle and sustainable agriculture. The most resistance-related mutations, such as F200Y, E198A and F167Y, were observed to be distributed among multiple farms and a marked reduction in the efficacy of popular anthelmintics, specifically benzimidazoles, was substantiated by the combination of phenotypic and genotypic diagnostic methods, i.e., fecal egg count

reduction tests and molecular identification of resistance alleles. The predominance of *Haemonchus contortus*, a highly pathogenic and resistant species of nematodes, was consistently associated with increased resistance markers and reduced treatment efficacy. The research also explained several management-level factors on the farm that add to the selection pressure that causes resistance, including high treatment frequency of anthelmintics, absence of grazing rotation, and medication administration histories. Multivariate logistic regression model confirmed these relationships, and some predictors had a high statistical significance and practical utility in farm-level intervention. The spatial mapping in identifying the resistance hotspots also supported the need of geographically targeted control measures and the regional variation in the patterns of resistance. The selected threat to the current paradigms of parasite control is the seemingly increasing development of multi-class resistance, and there are farms where parasites are resistant to all three main classes of medication. This puts demand on the rapid adoption of integrated parasite management (IPM) strategies. These strategies should include non-chemical control methods like pasture rotation, selective treatment, biological control agents and genetic selection of resistant breeds of cattle to the strategic, evidence-based use of anthelmintics. Collectively, these results demonstrate the need to continue monitoring, educate farmers, and/or change regulations to delay resistance spread and safeguard the wellbeing and productivity of grazing animals in mixed farming systems in future.

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