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Assessment of Anthelmintic Resistance in Cattle Parasite Populations

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Abstract | Globally, Gastrointestinal Nematodes (GINs) of cattle with anthelmintic resistance (AR) are increasingly recognized as a significant hindrance to livestock productivity, health, and sustainable parasite management. This study aimed to assess the prevalence, severity, and trends of anthelmintic resistance among cow parasite populations of four different farms. Faecal egg counts (FEC) techniques are employed to test 120 calves, of which 104 (86.7%) are GIN-infected. Three classes of the commonly used anthelmintic drugs—benzimidazoles (albendazole), macrocyclic lactones (ivermectin), and imidazothiazoles (Levamisole)—were tested for efficacy through the Faecal Egg Count Reduction Test (FECRT). Results showed potential resistance to imidazothiazoles but specific resistance to benzimidazoles and macrocyclic lactones. Haemonchus spp. were identified as the most prevalent genus in resistant populations in post-treatment larval cultures. Between farms, there was different medication effectiveness, which was likely due to differing deworming methods. The primary reasons for resistance were identified through a risk factor analysis, including underdosing, faecal egg testing, rotational deworming, and repeated use of the same anthelmintic. To prevent the dissemination of anthelmintic resistance and ensure effective parasite control in cattle, these results emphasize the critical need for instituting integrated parasite management controls, including targeted selection treatments and regular monitoring.

Keywords | Anthelmintic resistance, Gastrointestinal nematodes, Cattle, FECRT, Drug efficacy

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INTRODUCTION

Gastrointestinal nematodes (GINs) are a significant limitation to the health and productivity of cattle in both tropical and temperate livestock systems. Internal parasites cause impaired nutrient absorption, decreased feed efficiency (Avramenko *et al*., 2020), and compromised immune function, resulting in poor weight gain, reduced milk production, reproductive inefficiency (Ramos *et al*., 2016), and, under chronic infestation, mortality. Control of GIN infections has long been based on the regular use of anthelmintic drugs (Geurden *et al*., 2015). Nevertheless, their intensive and frequently uncontrolled use has led to the widespread development of anthelmintic resistance (AR), which is now acknowledged as an expanding risk to sustainable livestock production (Demeler *et al*., 2012).

Anthelmintic resistance involves the survival of drug-treated nematode parasites, which once responded to medications, and subsequently causes recurrent infections and impaired control measures (Demeler *et al*., 2010). All these are the main drivers behind anthelmintic resistance, including repeated re-use of one drug class in succession without rotation (Gasbarre, 2014), under-dosing resulting from improper estimation of weights, a failure to perform fecal egg count monitoring, and mass administration of all herd animals without identification based on their infection status (Beech *et al*., 2011). The outcome of these practices is a decreased efficacy of drugs, higher parasite control costs, and increased reliance on limited treatment options, particularly in areas where new anthelmintic molecules are not easily accessible (Baiak *et al*., 2018).

With this background, the present study aimed to evaluate the current situation of anthelmintic resistance in gastrointestinal nematodes of cattle on the selected farms (Hou *et al*., 2022). The investigation includes an extensive parasitological survey using faecal egg count methods for prevalence determination and the Faecal Egg Count Reduction Test (FECRT) for efficacy determination of the standard anthelmintics benzimidazoles (albendazole) (Nielsen *et al*., 2014), macrocyclic lactones (ivermectin), and imidazothiazoles (Levamisole). Additionally, post-treatment larval cultures were conducted to identify surviving nematode genera, providing insight into the species composition involved in resistance. A farm-level structured assessment was also implemented to detect management and drug-use practices responsible for the establishment of resistance.

By combining epidemiological information with laboratory results, this research aims to provide valuable insights into the incidence, severity, and drug resistance profiles of GIN infections (Charlier *et al*., 2022). The findings are hoped to guide evidence-based deworming strategies and promote the adoption of integrated parasite management practices towards reducing the development of anthelmintic resistance among cattle populations.

MATERIALS AND METHODS

The research was conducted at several cattle farms. 120 cattle were sampled randomly from parasitically infested herds with clinical evidence of parasitism, as indicated by diarrhoea, poor body weight gain, and rough coats. Both local and crossbred individuals of all age groups and physiological conditions were used in the research to provide diverse representation in the sampled population.

Sample collection

Fresh rectal faecal samples from selected cattle were obtained directly using sterile gloves and placed in hermetically sealed containers (Canton *et al*., 2017). The samples were labelled correctly and shipped refrigerated (4°C) to the laboratory. Processing was completed within 24 hours of sampling to maintain the integrity of the samples and prevent hatching or damage to helminth eggs.

Parasitological examination

The eggs per gram (EPG) of faeces was calculated using the Modified McMaster technique, which enables the quantitative estimation of nematode egg load. For the efficacy testing phase, only samples with a parasitic load of≥150 EPG were used, thereby limiting the analysis to animals with a high parasitic burd (Alani and Kawan, 2024; De Graef *et al*., 2013).

Anthelmintic treatment

The following anthelmintics available in the market were given orally to the 120 positive cattle, which were divided into four groups of 30 animals each randomly (Fissiha and Kinde, 2021):

Group I: Benzimidazoles (7.5 mg/kg body weight of albendazole, for instance).

Group II: Macrocyclic Lactones, like 0.2 mg/kg body weight of ivermectin

Group III: Imidazothiazoles (7.5 mg/kg body weight of Levamisole, for instance).

Group IV: Placebo control group receiving no treatment

All body weights of the cattle were measured accurately or estimated with a weight tape, and dosage was carefully calculated not to administer either too much or too little dosage.

Fecal egg count reduction test (FECRT)

Faecal samples from all the animals were also taken 14 days after treatment to evaluate the effectiveness of the treatment. The Faecal Egg Count Reduction (FECR) percentage was determined by the formula (Brown *et al*., 2022):

$$\mathrm{FECR}(\%)\left(\frac{=\ \text{Pre} - \text{treatment EPG} - \text{Post treatment EPG}}{\text{Pre treatment EPG}}\right)\times 100$$

Based on the WAAVP guidelines, efficacy below 95% with a lower 95% confidence limit below 90% was considered indicative of resistance to anthelmintics.

Larval identification and culture

For qualitative verification of the infecting genera of nematodes, faecal pools were cultured from each group (Fazzio *et al*., 2014). The cultures were incubated under optimal conditions for 7–10 days, allowing the third-stage larvae (L3) to develop. The L3 larvae were collected and identified using a compound microscope, based on their morphology (e.g., tail length of the sheath, head shape), utilizing standard taxonomic keys (das Neves *et al*., 2014).

RESULTS AND DISCUSSION

This section presents the results of a field study conducted to establish the prevalence of gastrointestinal nematodes (GINs) in cattle and the efficacy of traditional anthelmintic drugs. Faecal Egg Count Reduction Tests (FECRT), parasitological examinations (Nabukenya *et al.*, 2014), larval cultures, and data analysis specific to a particular farm are the foundation of the conclusions. In field conditions, the results provide valuable new insights into the prevalence of parasite infections and the emerging issue of anthelmintic resistance.

Prevalence of gastrointestinal nematodes (GINs)

One hundred twenty cattle from different farms were examined. A total of 104 animals (86.7%) were positive for gastrointestinal nematodes using the faecal examination.

Table 1: Prevalence and intensity of GIN infection across study sites.

Farm code	No. of cattle examined	No. positive for GIN	Prevalence (%)	Mean EPG (±SD)
Farm A	30	26	86.7	620 ± 145
Farm B	30	27	90.0	700 ± 180
Farm C	30	25	83.3	660 ± 170
Farm D	30	26	86.7	720 ± 200
Total	120	104	86.7	675 ± 174

As Table 1 illustrates, a total of 120 cattle from four farms were examined, with 104 animals (86.7%) testing positive for gastrointestinal nematodes. The rates of prevalence were relatively narrow, from 83.3% to 90.0%, between farms, which reflects the overall high infection rate (Leathwick and Besier, 2014). Farm B had the highest prevalence at 90.0%, whereas Farm C recorded the lowest at 83.3%. The mean EPG ranged between 620 ± 145 and 720 ± 200, with Farm D having the highest mean EPG, indicating a greater intensity of infection (George *et al.*, 2017). Regardless of minimal variations in infection and egg rates, all the farms had moderate to heavy parasitic loads, highlighting the pressing need for regular deworming practices, parasite control programs, and resistance surveillance to mitigate the effects of GINs in cattle.

Efficacy of anthelmintics (FECRT results)

To assess the effectiveness of routinely applied anthelmintics and determine the presence of resistance (Rose *et al.*, 2015), the Faecal Egg Count Reduction Test (FECRT) was performed on 104 infested cattle. The cattle were divided into four groups at random, with each group receiving a different type of treatment, except for the control group. EPG post-treatment levels were compared with those pre-treatments to derive the faecal egg count reduction percentage (FECR%), which is used as a reference for determining anthelmintic resistance.

As shown in Table 2, all three treatment groups of anthelmintics exhibited different levels of efficacy against EPG counts. Group I, which received benzimidazoles, recorded an FECR of 82.9%, indicating confirmed resistance, as the reduction was less than the 95% level. Group II, which received macrocyclic lactones, also exhibited confirmed resistance with an FECR of 89.0% (Hodgkinson *et al.*, 2019). Group III, which was treated with imidazothiazoles, had an FECR of 94.8%, indicating suspected resistance as the result was on the borderline close to 95%. The control group (Group IV) exhibited a rise in EPG, validating the natural course of infection when left untreated. These results collectively indicate a broad and increasing resistance in GINs to several anthelmintic classes in the study area, underscoring the imperative need for strategic drug rotation, integrated parasite management, and ongoing resistance monitoring.

Larval culture findings post-treatment

Larval cultures were conducted using pooled faecal samples from each treatment group to identify the nematode taxa responsible for persistence after treatment (Ramos *et al.*, 2020). To allow for resistance patterns and to provide information on the dominant parasites capable of surviving anthelmintic treatment, this enabled the genus-level identification of infectious third-stage larvae (L3).

Table 2: FECRT results for different anthelmintic groups.

Treatment group	Drug class	Mean pre-treatment EPG	Mean post-treatment EPG	FECR (%)	95% confidence limit	Interpretation
Group I	Benzimidazoles	675	115	82.9	76	Confirmed resistance
Group II	Macrocyclic Lactones	690	76	89.0	84	Confirmed resistance
Group III	Imidazothiazoles	660	34	94.8	91	Suspected resistance
Group IV	Control	690	705	-2.2	-	No treatment

Table 3: Proportion of identified L3 larvae by genus post-treatment.

Nematode Genus	Group I (Albendazole)	Group II (Ivermectin)	Group III (Levamisole)	Control group
Haemonchus spp.	62%	57%	28%	68%
Trichostrongylus spp.	23%	28%	18%	20%
Oesophagostomum spp.	15%	15%	12%	12%

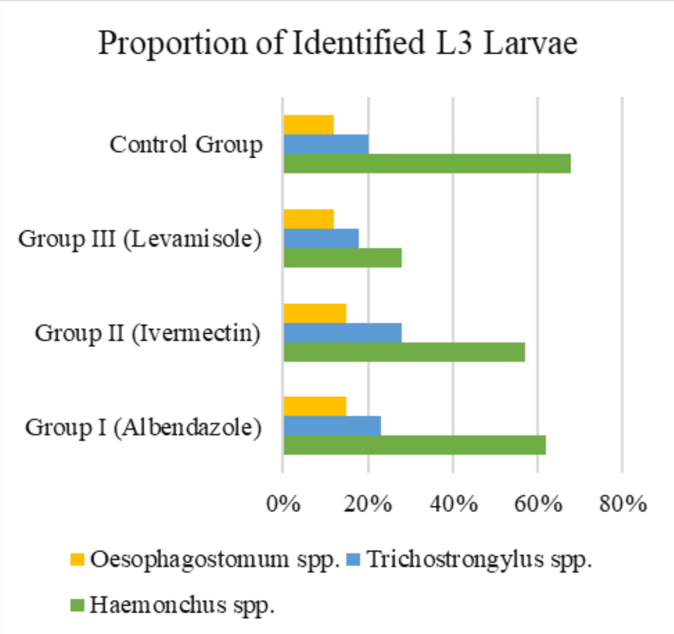

Figure 1: Proportion of identified L3 larvae by genus post-treatment.

As shown in Table 3, *Haemonchus* spp. were the most dominant genus in all groups, particularly in Group I (Albendazole) and Group II (Ivermectin), where they comprised 62% and 57%, respectively, of the L3 larvae population. This finding aligns with the FECRT results, which demonstrate confirmed resistance in these groups (Jaeger and Carvalho-Costa, 2017). In Group III (Levamisole), where suspected resistance was observed, the presence of *Haemonchus* spp. had fallen to 28%, indicating a comparatively higher efficacy of this drug against the genus. The highest percentage of *Haemonchus* spp., namely 68%, was recorded in the control group, as expected due to its prevalence in untreated infections. The fact that *Trichostrongylus* spp. and *Oesophagostomum* spp. were still present in all groups at lower percentages further establishes the presence of mixed infections. These results validate that *Haemonchus* spp. plays an important part in anthelmintic resistance within the study location and emphasize the need for targeted control measures.

Farm-wise comparison of drug efficacy

To identify variability in resistance to anthelmintics under different management systems, farm-level faecal egg count reduction test (FECRT) values were compared. The comparison aims to establish the influence of deworming protocols, drug use frequency, and exposure to parasites on the efficacy of widely used anthelmintics.

Table 4: Farm-wise FECRT (%) for albendazole, ivermectin, and levamisole.

Farm code	Albendazole FECRT (%)	Ivermectin FECRT (%)	Levamisole FECRT (%)
Farm A	81.2	87.5	92.3
Farm B	84.6	89.1	95.5
Farm C	82.1	88.8	96.2
Farm D	83.9	90.5	94.8

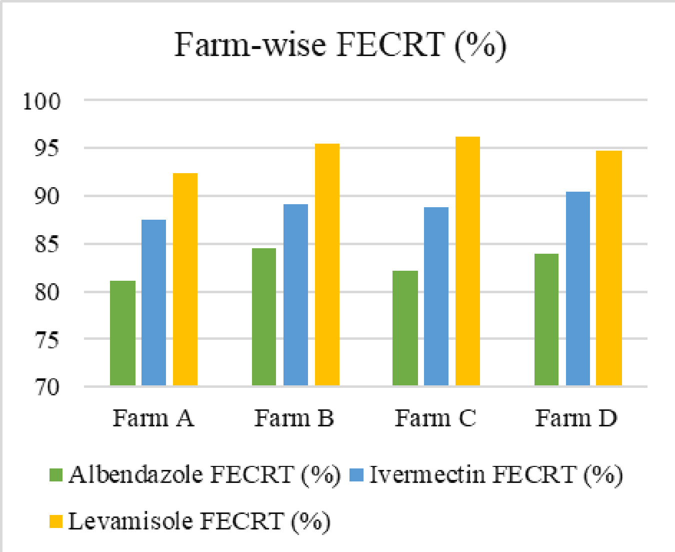

Figure 2: Farm-wise FECRT (%) for albendazole, ivermectin, and levamisole.

As indicated in Table 4, there was some variability in drug efficacy among the farms. Albendazole had the lowest FECRT values in all farms, ranging from 81.2% for Farm A to 84.6% for Farm B, further supporting evidence of established resistance (Sazmand *et al*., 2020). Ivermectin was slightly more effective, varying from 87.5% (Farm A) to 90.5% (Farm D), though still below the threshold of established resistance. Levamisole, however, showed the most excellent efficacy in all farms, with FECRT values of over 92%, reaching a maximum of 96.2% in Farm C, indicating only suspected resistance or reasonable control in such places (Sutherland and Leathwick, 2011). The differences in these values between farms indicate the influence of localized management practices and drug use history on resistance patterns, as well as the importance of customized parasite control measures at the farm level.

Identified risk factors accounting for resistance

To determine the underlying causes of anthelmintic resistance (Vineer *et al*., 2020), a systematic survey and observational checklist were used on all participating farms.

Table 5: Major risk factors identified in study farms.

Risk factor	Percentage of farms reporting issue
Frequent use of the same dewormer	100%
Under-dosing due to weight guess	75%
No faecal egg monitoring	100%
Lack of rotational deworming	100%
Treating all animals (non-selective)	90%

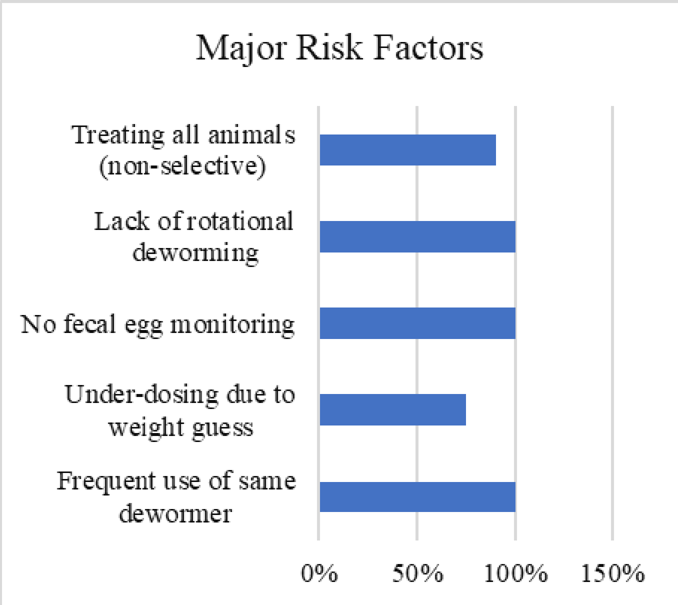

Figure 3: Major risk factors identified in study farms.

As indicated in Table 5, several key risk factors were consistently noted across the four study farms. Notably, 100% of the farms used the same dewormer repeatedly, did not perform faecal egg monitoring (Smout *et al.*, 2010), and did not perform rotational deworming, reflecting poor control measures for parasites and intense selection pressure for resistance. In addition, 75% of farms engaged in underdosing as a result of inaccurate animal weight estimation, a significant cause of subtherapeutic drug concentrations and the subsequent development of resistance. Moreover, 90% of farms administered drugs to all animals indiscriminately, not classifying heavily or lightly infected ones. These observations convincingly indicate that the extensive and frequent application of the same anthelmintics, unsound dosing habits (von Samson-Himmelstjerna, 2012), and improper monitoring practices of parasites are among the main reasons for the development and persistence of drug-resistant gastrointestinal nematodes.

CONCLUSIONS AND RECOMMENDATIONS

The research documents widespread gastrointestinal nematode infestations among cattle, supported by documented resistance to confirmed levels of benzimidazoles and macrocyclic lactones, as well as suspected resistance to imidazothiazoles. Dominance by *Haemonchus* spp. and endemic presence of fundamental risk factors, including everyday use and lack of rotation between drugs, under-dosing, and lack of monitoring, signify the imperative to transition away from the present deworming policy. To reduce the escalating issue of anthelmintic resistance, there is a proposal that cattle farms adopt integrated parasite management (IPM) techniques. These measures must include periodic faecal egg count monitoring, selective treatment of positives only rather than mass treatment of entire herds, dosing based on body weight, and strategic class rotation of anthelmintics. These also need education and training of the veterinarians and farmers on sustainable parasite management to help maintain the efficacy of the drugs and protect livestock health and productivity.

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NOVELTY STATEMENT

The present study offers an extensive and region-specific evaluation of anthelmintic resistance in gastrointestinal nematodes infesting cattle, integrating parasitological, clinical, and molecular methods. In contrast to earlier generalized descriptions, the study presents a detailed farm-wise comparison of drug efficacy, with an emphasis on the dominance of Haemonchus spp. following treatment, focusing on multi-drug resistance patterns. The combination of larval culture analysis with Faecal Egg Count Reduction Tests (FECRT) and on-ground risk factor identification e.g., dosing errors and failure to rotate deworming gives the research a practical, actionable aspect. The study's findings provide new insights into the dynamic resistance scenario and offer a valuable template for developing targeted, sustainable parasite control measures in cattle management systems.

AUTHOR'S CONTRIBUTION

All of the trials were designed by Turkan Ahmed Hama Hasan. Ibtisam Jassim Mohammed conducted all of the tests, gathered the data, and composed the manuscript draft. Batool Khalaf mohammed and Nibras Jamal helped with the data analysis that was done to prepare the work for submission to the journal. The final draft of the work was reviewed and approved by all authors for publication in the Journal of Animal and Health Production.

Ethical consideration

Not applicable.

Conflict of interest

The authors state that there is not conflict of notice.

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