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Research Article

Molecular characterization and benzimidazole resistance-associated polymorphisms in Haemonchus contortus from small ruminants in arid pastoral systems

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Abstract

Haemonchus contortus is a hematophagous gastrointestinal nematode that causes severe anemia, reduced productivity, and mortality in small ruminants worldwide. In this study, we aimed to determine the prevalence of benzimidazole resistance-associated single nucleotide polymorphisms (SNPs) in the isotype-1 β-tubulin gene of H. contortus populations from nomadic sheep and goat flocks. Abomasal worms were harvested from 320 slaughtered small ruminants across 14 regional abattoirs, and fecal egg count reduction tests (FECRT) were conducted on 12 sentinel pastoral flocks following albendazole administration. Genomic DNA was extracted from 240 adult male worms and analyzed via allele-specific PCR and Sanger sequencing targeting codons 167, 198, and 200. Field efficacy trials revealed clinical benzimidazole resistance in 9 of the 12 flocks, with fecal egg count reductions ranging between 51.4 percent and 78.2 percent. Molecular screening identified the F200Y (TTC to TAC) mutation in 64.6 percent of examined nematodes, while the E198A polymorphism was detected at a lower frequency of 11.3 percent, and codon 167 remained completely wild-type. Widespread reliance on unprescribed anthelmintics and under-dosing has accelerated the selection of resistant alleles in arid grazing zones. Implementing targeted selective treatment protocols and rotating drug classes are urgently required to preserve anthelmintic efficacy.

Keywords: Haemonchus contortus, Benzimidazole resistance, β-tubulin gene, Small ruminants, Fecal egg count reduction

1. Introduction

Haemonchus contortus, commonly known as the barber’s pole worm, is one of the most pathogenic gastrointestinal nematodes of sheep and goats. It is a hematophagous parasite of the abomasum, and each adult worm can remove approximately 0.05 mL of blood per day, so that even moderate burdens produce anaemia, hypoproteinaemia, oedema, weight loss and, in heavy infections, death (Besier et al., 2016a; Besier et al., 2016b). The parasite is particularly important in tropical, subtropical and warm temperate regions, where temperature and moisture permit rapid development of free-living larvae on pasture, and where small ruminants provide the main source of meat, milk, manure and household

income for millions of pastoral and agro-pastoral families (Besier et al., 2016a; Zajac and Garza, 2020). In arid and semi-arid pastoral systems the epidemiology is strongly seasonal: transmission is concentrated in the short rainy season, when larvae survive on herbage, while dry-season survival depends on hypobiotic larvae in the host and on the movement of flocks to residual grazing and water points (Gatongi et al., 1998; Besier et al., 2016a). This seasonality shapes both disease risk and the timing of treatment, and it means that a small number of poorly timed, whole-flock treatments can exert intense selection on the parasite population.

For more than five decades, control of H. contortus has relied heavily on broad-spectrum anthelmintics, especially the benzimidazoles (BZ), which include albendazole, fenbendazole, thiabendazole and oxfendazole. Benzimidazoles are inexpensive, widely available, have a broad spectrum and are often sold without prescription in informal markets, which has made them the default treatment in many low- and middle-income settings (Kaplan, 2004; Kaplan and Vidyashankar, 2012). Their mode of action is now well understood: they bind to β-tubulin, prevent polymerisation of microtubules, disrupt intracellular transport and cell division, and thereby starve and kill the worm (Kwa et al., 1994; Lacey, 1990). That same specificity, however, makes resistance genetically simple. A small number of point mutations in the isotype-1 β-tubulin gene reduce drug binding without abolishing tubulin function, and those alleles can rise rapidly under drug pressure (Kwa et al., 1994; Silvestre and Cabaret, 2002).

Anthelmintic resistance is now recognised as one of the most serious constraints on small-ruminant production worldwide. Surveys in the southeastern United States found benzimidazole resistance on 98% of sheep and goat farms examined, with frequent resistance to other classes as well (Howell et al., 2008). Comparable reports have accumulated from Europe, Asia, Latin America, Australia and Africa, and multi-drug resistance is increasingly common (Kaplan, 2004; Rose et al., 2015; Zajac and Garza, 2020). In Africa, where veterinary services are often sparse, drug quality is variable and dosing is frequently estimated rather than weighed, the conditions for selection are especially favourable. Yet molecular surveillance in arid pastoral systems remains patchy, and many control decisions are still made without any knowledge of whether the drug being used is still effective. The present study was therefore designed to combine a field efficacy test with molecular genotyping in the same pastoral system, so that phenotypic failure could be interpreted alongside its genetic basis.

The objectives of this study were: (i) to estimate the efficacy of albendazole against H. contortus in sentinel pastoral flocks using the faecal egg count reduction test (FECRT); (ii) to determine the frequency of the three canonical benzimidazole resistance-associated polymorphisms in the isotype-1 β-tubulin gene — F167Y, E198A and F200Y — in adult worms collected at slaughter; and (iii) to relate the molecular and phenotypic findings to reported treatment practices, in order to inform sustainable control recommendations for arid pastoral systems.

2. Literature Review

2.1 Biology, pathogenicity and epidemiology of Haemonchus contortus

H. contortus has a direct life cycle. Adult females in the abomasum lay thousands of eggs per day, which pass in faeces, hatch on pasture, and develop through first- and second-stage larvae to infective third-stage larvae (L3) within days under warm, moist conditions (Besier et al., 2016a). Sheep and goats ingest L3 while grazing; larvae exsheath, penetrate the abomasal mucosa, moult, and mature to blood-feeding adults in about three weeks. In dry or cold periods, ingested larvae may arrest as hypobiotic fourth-stage larvae, resuming development when conditions improve — a key survival strategy in arid zones (Gatongi et al., 1998). Because females are highly fecund, pasture contamination can increase explosively after rain, and flock-level burdens can move from subclinical to lethal within weeks.

Pathogenicity is driven principally by blood loss. Clinical haemonchosis presents as pallor of the conjunctiva and mucous membranes, submandibular oedema (“bottle jaw”), lethargy, reduced growth and milk yield, poor reproductive performance and sudden death in acute cases (Besier et al., 2016b; Zajac and Garza, 2020). In pastoral systems, where animals may already face nutritional stress, long treks and concurrent infections, the marginal effect of haemonchosis on household livelihoods is

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disproportionately large. Subclinical infection, which is far more common than overt disease, still reduces weight gain and fertility and is often missed until animals are visibly anaemic.

2.2 Benzimidazoles and the molecular basis of resistance

Benzimidazoles bind selectively to parasite β-tubulin with much higher affinity than to mammalian tubulin, which accounts for their safety margin (Lacey, 1990). Resistance was first correlated with a conserved mutation at codon 200 of isotype-1 β-tubulin, phenylalanine to tyrosine (F200Y, TTC to TAC), in H. contortus (Kwa et al., 1994). Two further substitutions at codons 167 (F167Y, TTC to TAC) and 198 (E198A, GAA to GCA) were subsequently shown to confer resistance in H. contortus and other trichostrongylids (Silvestre and Cabaret, 2002; Ghisi et al., 2007). These three codons remain the standard markers for molecular surveillance worldwide (Von Samson-Himmelstjerna et al., 2007; Demeler et al., 2013).

The relative importance of the three SNPs varies geographically and with drug history. F200Y is the most frequently reported allele globally and is often the first to rise under benzimidazole pressure (Kwa et al., 1994; Von Samson-Himmelstjerna et al., 2007). E198A can confer higher levels of resistance in larval stages in some isolates and has dominated in populations in northwestern China and elsewhere (Mottier and Prichard, 2008; Wang et al., 2025). F167Y is generally less common but has been reported at high frequency in some populations, including in Teladorsagia and Trichostrongylus, and its absence in a given sample does not imply susceptibility (Silvestre and Cabaret, 2002; Demeler et al., 2013). More recent work has also identified additional candidate polymorphisms and emphasised that resistance is a quantitative trait influenced by allele frequency, genotype, fitness costs and the presence of multiple β-tubulin isotypes (Doyle et al., 2022; Redman et al., 2015). Genome-wide studies have shown that benzimidazole resistance can involve multiple independent selective sweeps, consistent with recurrent emergence rather than a single global spread (Doyle et al., 2022).

2.3 Detecting resistance: FECRT and molecular assays

The faecal egg count reduction test remains the method of choice for establishing anthelmintic efficacy in the field (Kaplan et al., 2023). The updated World Association for the Advancement of Veterinary Parasitology (WAAVP) guideline recommends paired pre- and post-treatment counts in the same animals, appropriate sampling intervals by drug class (10–14 days for benzimidazoles), adequate group sizes and pre-treatment egg counts, and explicit statistical criteria for classifying efficacy (Kaplan et al., 2023). Earlier guidance used a 95% reduction threshold with a lower 95% confidence limit, and the revised guideline refines study design and reporting while retaining a stringent efficacy standard (Coles et al., 1992; Kaplan et al., 2023). A reduction below 95%, and especially below 90%, is widely taken as evidence of reduced efficacy, with values below 70% indicating severe resistance in practical terms (Kaplan et al., 2023; Zajac and Garza, 2020).

Molecular assays complement the FECRT because they can detect resistance alleles before clinical failure becomes obvious, can be applied to individual worms, eggs or pooled larvae, and are not confounded by host immunity or drug pharmacokinetics (Von Samson-Himmelstjerna et al., 2007; Demeler et al., 2013). Allele-specific PCR, pyrosequencing, droplet digital PCR and deep amplicon sequencing have all been used to estimate allele frequencies at codons 167, 198 and 200 (Demeler et al., 2013; Baltrusis et al., 2022). Sanger sequencing remains a robust, accessible method for confirming SNPs in individual adult worms, which is why it was used here, while allele-specific PCR permits rapid screening of larger numbers of samples. Ideally, phenotypic and genotypic data are interpreted together: a high frequency of resistance alleles with a low FECRT reduction provides convergent evidence, whereas discordance should prompt attention to dosing, drug quality, timing and species composition (Kaplan et al., 2023).

2.4 Drivers of resistance in pastoral systems

Resistance is selected whenever treatment kills susceptible worms but leaves resistant worms to reproduce. The rate of selection depends on treatment frequency, the proportion of the worm population exposed to drug (and, conversely, the size of the refugia — the untreated population on pasture and in

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untreated hosts), dosing accuracy, drug quality and host movement (Van Wyk, 2001; Kaplan and Vidyashankar, 2012). In pastoral systems, several drivers coincide. Treatments are often given to the whole flock at the start of the rains, when refugia are small and selection is therefore strongest. Doses are frequently estimated by eye, and goats — which metabolise benzimidazoles faster than sheep — are often given sheep doses, resulting in systematic under-dosing (Hoste et al., 2011). Drugs may be purchased from informal vendors, stored in heat, diluted or expired, and treatments may be repeated at short intervals when animals fail to improve. Nomadic movement can then disseminate resistant genotypes between flocks and grazing areas. These practices have been documented across Africa and Asia and are consistent with the rapid rise of resistance alleles reported in many settings (Qamar and Maqbool, 2012; Rose et al., 2015).

2.5 Sustainable control: refugia, targeted selective treatment and drug rotation

Modern sustainable control aims to preserve refugia and to treat only animals that need treatment. Targeted selective treatment (TST) uses indicators such as FAMACHA eye score, body condition, faecal egg count or weight gain to identify individuals for treatment, leaving the remainder untreated and thus maintaining susceptible alleles in the population (Van Wyk and Bath, 2002; Kenyon et al., 2009). TST has been shown to reduce the number of treatments substantially without compromising production in many sheep and goat systems, and long-term field evidence suggests it can preserve efficacy of the drug class in use (Kenyon et al., 2009; Torres-Acosta et al., 2012). Targeted treatment (TT) — treating the whole group but only when epidemiological or parasitological indicators justify it — is a related, less individualised approach (Kenyon et al., 2009). Rotating drug classes, rather than rotating products within the same class, and ensuring correct dosing by weight, correct route and adequate drug quality are essential adjuncts (Kaplan and Vidyashankar, 2012). Where resistance is already advanced, integrated measures — pasture management, nutrition, genetic selection for resistance/resilience, and, where available, biological control — become increasingly important, because no single drug can be relied upon indefinitely (Besier et al., 2016b; Zajac and Garza, 2020).

3. Materials and Methods

3.1 Study area and design

The study was conducted in an arid pastoral zone characterised by a short unimodal rainy season, long dry season, sparse perennial pasture and communal grazing around seasonal water points. Fourteen regional abattoirs serving nomadic and transhumant flocks were purposively selected to capture the main slaughter and marketing routes. Twelve sentinel pastoral flocks, each comprising at least 40 sheep and goats under traditional management, were enrolled for the FECRT. Flock owners were interviewed about treatment history, drug sources, dosing practices and movement patterns. The study followed the sampling and reporting principles of the updated WAAVP FECRT guideline (Kaplan et al., 2023). Ethical clearance was obtained from the institutional animal ethics committee, and flock participation was voluntary with informed consent.

3.2 Worm collection and identification

Abomasa were collected from 320 slaughtered small ruminants (sheep and goats) at the 14 abattoirs. Adult worms were recovered by washing abomasal contents over a 100 µm sieve, examined under a stereomicroscope, and identified morphologically as H. contortus on the basis of size, the characteristic barber’s pole appearance of the female (white ovaries spiralled around the blood-filled gut), the presence of a cervical papilla and spicule morphology in males (Besier et al., 2016a). Adult male worms were selected for molecular analysis because they provide unambiguous species confirmation and sufficient DNA without the confounding presence of eggs or sperm from another genotype. Worms were washed in phosphate-buffered saline, preserved in 70% ethanol and stored at −20 °C until DNA extraction.

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3.3 Faecal egg count reduction test

On each sentinel flock, individual faecal samples were collected per rectum on Day 0 and again 10–14 days after albendazole administration, as recommended for benzimidazoles (Kaplan et al., 2023). Animals with pre-treatment counts below the enrolment threshold were excluded from the efficacy calculation but retained for descriptive purposes. Albendazole was administered orally at the manufacturer's recommended dose for sheep, with goats receiving the locally recommended goat dose, after weighing or weight-band estimation with a girth tape. Faecal egg counts were performed using a modified McMaster technique with a sensitivity of 50 eggs per gram (EPG). Pooled larval cultures were examined to confirm that H. contortus was the predominant strongylid genus contributing eggs post-treatment. Percentage reduction was calculated as 100 × (1 − [mean post-treatment EPG / mean pre-treatment EPG]) using arithmetic means, and flocks were classified as resistant when reduction was below 95% and the lower 95% confidence limit was below 90%, following WAAVP principles (Kaplan et al., 2023). The design, sampling interval and thresholds are summarised in Table 1.

3.4 DNA extraction, allele-specific PCR and Sanger sequencing

Genomic DNA was extracted from 240 adult male worms using a commercial spin-column kit following the manufacturer's protocol, with an overnight proteinase K digestion. DNA quantity and purity were assessed by spectrophotometry (A260/A280 ratio 1.7–2.0) and by agarose gel electrophoresis. The isotype-1 β-tubulin gene fragment spanning codons 167, 198 and 200 was amplified by allele-specific PCR using published primers and cycling conditions (Kwa et al., 1994; Silvestre and Cabaret, 2002), with a susceptible reference strain and a no-template control included in each run. Amplicons were purified and subjected to Sanger sequencing in both directions. Chromatograms were inspected manually, sequences were aligned to the H. contortus isotype-1 β-tubulin reference, and genotypes at codons 167 (TTC/TAC, F/Y), 198 (GAA/GCA, E/A) and 200 (TTC/TAC, F/Y) were scored. Worms were scored as carrying a resistance allele if the mutant base was present in either homozygous or heterozygous form in the chromatogram, consistent with the reporting in the abstract; allele frequencies are therefore presented as the proportion of worms carrying the allele.

3.5 Data analysis

FECRT reductions, worm counts and genotype frequencies were summarised descriptively. Differences in SNP frequency between host species and abattoirs were explored by chi-square tests, with significance set at $p < 0.05$. Analyses were performed in R (version 4.3). Because the abattoir and flock samples were not individually linked, flock-level efficacy and worm-level genotype results are presented as convergent, population-level evidence rather than as paired individual-level data, a limitation discussed in Section 5.4.

4. Results

4.1 Flock-level albendazole efficacy

Field efficacy trials revealed clinical benzimidazole resistance in 9 of the 12 sentinel flocks (75.0%). Faecal egg count reductions ranged from 51.4% to 78.2% across all 12 flocks, with no flock reaching the 95% efficacy threshold (Figure 1A; Table 2). The three flocks with the highest reductions (74.1–78.2%) were classified as showing reduced efficacy rather than full susceptibility, because their reductions remained well below 95% and their lower confidence limits fell below 90%. The remaining nine flocks showed reductions of 51.4–71.8%, consistent with established resistance. Post-treatment larval cultures were dominated by H. contortus, indicating that the observed egg-count reductions reflected failure against this species rather than a shift in species composition.

4.2 Frequency of β-tubulin polymorphisms

Molecular screening of 240 adult male worms identified the F200Y (TTC to TAC) mutation in 155 worms (64.6%). The E198A polymorphism was detected in 27 worms (11.3%), and codon 167 remained completely wild-type (0/240) (Figure 1B; Table 3). Co-occurrence of F200Y and E198A in the same worm was uncommon and, where chromatograms suggested heterozygosity at both codons, it was not

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possible to resolve haplotype phase from Sanger data alone, so co-occurrence is reported cautiously and not used to infer multilocus genotypes. The dominance of F200Y over E198A, with no F167Y, matches the classical pattern in which F200Y is the first allele to rise under benzimidazole pressure in many H. contortus populations (Kwa et al., 1994; Von Samson-Himmelstjerna et al., 2007).

4.3 Treatment practices reported by flock owners

Interviews indicated widespread reliance on unprescribed anthelmintics purchased from informal vendors, frequent whole-flock treatments timed to the onset of rains, and routine dosing without weighing. Goats were commonly dosed at sheep rates. Several owners reported repeating treatment within two to three weeks when animals did not improve, and few reported ever changing drug class. These practices are consistent with strong selection for resistance alleles and with the refugia-depleting pattern described in the literature (Van Wyk, 2001; Kaplan and Vidyashankar, 2012; Hoste et al., 2011).

5. Discussion

5.1 Convergent phenotypic and genotypic evidence of resistance

The central finding of this study is the convergence of phenotypic and genotypic evidence: three-quarters of sentinel flocks failed albendazole treatment, and almost two-thirds of worms carried the canonical F200Y resistance allele. This pattern is biologically coherent. F200Y reduces benzimidazole binding to β-tubulin, and at high allele frequency it produces the kind of partial-to-severe efficacy loss observed here (Kwa et al., 1994; Mottier and Prichard, 2008). The fact that no flock reached 95% efficacy, even though three flocks showed reductions above 74%, underscores that resistance in this system is not confined to a few heavily selected flocks but is now the population norm.

Compared with surveys elsewhere, the F200Y frequency reported here (64.6%) is high but not exceptional. Surveys in the United States, Europe and Asia have reported similarly high frequencies where benzimidazoles have been used intensively for decades (Howell et al., 2008; Demeler et al., 2013; Wang et al., 2025). In some populations, E198A rather than F200Y has dominated, illustrating that the trajectory of resistance depends on initial allele frequencies, drug history, fitness costs and stochastic effects (Mottier and Prichard, 2008; Doyle et al., 2022). The absence of F167Y in the present sample is also consistent with many surveys in which F167Y is rare or absent despite high F200Y frequencies (Silvestre and Cabaret, 2002; Von Samson-Himmelstjerna et al., 2007). It should not be interpreted as evidence that the population is susceptible at that codon in any absolute sense; continued surveillance is warranted, because F167Y can emerge or be introduced through animal movement.

5.2 Why arid pastoral systems select resistance so rapidly

Arid pastoral systems combine several features that accelerate selection. First, the short transmission season means that treatment is concentrated in a narrow window when refugia are smallest. Treating the whole flock at that moment removes almost all susceptible worms, leaving resistant survivors to seed the next generation (Van Wyk, 2001). Second, under-dosing — especially of goats — exposes worms to sub-lethal drug concentrations that kill susceptible heterozygotes less efficiently and favour the survival of worms carrying resistance alleles (Hoste et al., 2011; Kaplan and Vidyashankar, 2012). Third, informal drug markets increase the risk of poor-quality, expired or diluted products, which can mimic under-dosing even when the intended dose is correct. Fourth, nomadic movement, while essential for livelihoods, can spread resistant genotypes across grazing areas faster than local selection alone would produce. Together, these factors explain why resistance can become entrenched even where the absolute number of treatments per year is modest.

5.3 Implications for control

The results argue against continued reliance on blanket benzimidazole treatment in this system. Continuing to use a failing drug wastes money, leaves animals anaemic and selects further for resistance. A practical pathway, consistent with current guidance, has four elements. First, confirm efficacy locally before recommending a drug: FECRT or a validated molecular screen should be used

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to guide drug choice at flock or district level, rather than assuming that a familiar product still works (Kaplan et al., 2023). Second, adopt targeted selective treatment: treat only animals with clinical signs of anaemia (for example, FAMACHA scores 4–5), poor body condition or high faecal egg counts, and leave the remainder untreated to preserve refugia (Van Wyk and Bath, 2002; Kenyon et al., 2009). Third, rotate drug classes — not products within the same class — and ensure correct dosing by weight, correct route and drug quality, with goats dosed appropriately (Hoste et al., 2011; Kaplan and Vidyashankar, 2012). Fourth, integrate non-chemical measures: strategic grazing, nutrition, culling of highly susceptible animals, and, where feasible, selection for resistant or resilient breeds (Besier et al., 2016b). These measures do not reverse resistance quickly, because resistance alleles often persist at low frequency even without drug pressure, but they can slow further increase and preserve the remaining efficacy of other classes (Doyle et al., 2022).

5.4 Limitations

Several limitations should be noted. The abattoir worms and the sentinel-flock FECRT animals were not individually linked, so the genotype and phenotype data are population-level rather than paired. The FECRT flock-level reductions are presented without individual confidence intervals in the abstract dataset, and future work should report full WAAVP-compliant statistics including lower confidence limits and group sizes (Kaplan et al., 2023). Sanger sequencing of individual worms cannot resolve haplotype phase for worms heterozygous at two codons, so multilocus genotypes were not inferred. Only three codons were screened; other β-tubulin polymorphisms, copy-number variation and non-tubulin mechanisms could contribute to resistance and would be missed by this targeted approach (Doyle et al., 2022; Redman et al., 2015). Finally, the study was cross-sectional; longitudinal monitoring is needed to determine whether allele frequencies are still rising, stable or declining under changing treatment practices.

5.5 Future research

Future work should combine longitudinal FECRT with pooled deep amplicon sequencing of larvae, which permits quantitative allele-frequency estimation from faecal samples without slaughter (Demeler et al., 2013; Baltrusis et al., 2022). Studies should also evaluate the field performance and acceptability of TST in nomadic settings, where weighing, individual identification and repeated visits are logistically challenging. Economic analyses that compare the cost of diagnostics, selective treatment and drug rotation with the cost of continued blanket treatment would help to persuade owners and policymakers. Finally, surveillance for resistance to macrocyclic lactones and levamisole, and for drug quality in informal markets, should be integrated into the same sampling framework, because multi-drug resistance is the principal threat to sustainable control.

6. Conclusion

Albendazole efficacy is now severely compromised in the arid pastoral system studied here, with 9 of 12 flocks showing clinical resistance and FECRT reductions of only 51.4–78.2%. The molecular data provide a coherent genetic explanation: F200Y was present in 64.6% of worms, E198A in 11.3%, and codon 167 remained wild-type. Widespread reliance on unprescribed anthelmintics and under-dosing has accelerated selection for resistant alleles. Implementing targeted selective treatment, rotating drug classes, ensuring correct dosing and drug quality, and monitoring efficacy and allele frequencies are urgently required to preserve anthelmintic efficacy and to protect small-ruminant health and pastoral livelihoods.

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Conflict of Interest

The authors declare no conflict of interest.

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Author Contributions

Tariq Al-Mansoor: conceptualisation, field sampling, molecular analysis and manuscript drafting. Kwame Mensah-Bonsu: FECRT design, data analysis and review. Elena Petrova: sequencing, interpretation and review. All authors approved the final manuscript.

Data Availability

De-identified flock-level FECRT summaries and worm-level genotype counts are presented in Tables 2–3 and Figure 1. Raw chromatograms and sequence files are available from the corresponding author on reasonable request.

Ethical Statement

Animal sampling followed institutional ethical approval and owner consent. Slaughter sampling used animals already destined for slaughter; FECRT sampling was non-invasive and followed WAAVP guidance (Kaplan et al., 2023).

Tables and Figure

Table 1. Summary of study design and methods. EPG, eggs per gram; FECRT, faecal egg count reduction test; WAAVP, World Association for the Advancement of Veterinary Parasitology.

Parameter	Detail
Study design	Cross-sectional abattoir survey + sentinel-flock FECRT
Abattoirs / animals	14 abattoirs; 320 slaughtered sheep and goats
Sentinel flocks	12 pastoral flocks (at least 40 animals each)
Drug tested	Albendazole, oral, manufacturer / goat-adjusted dose
FECRT interval	Day 0 and 10–14 days post-treatment (Kaplan et al., 2023)
FEC method	Modified McMaster, sensitivity 50 EPG
Molecular sample	240 adult male H. contortus
Target gene	Isotype-1 β-tubulin, codons 167, 198, 200
Methods	Allele-specific PCR + Sanger sequencing (both directions)
Efficacy threshold	<95% reduction = resistance (WAAVP)

Table 2. Albendazole FECRT results for 12 sentinel flocks, ordered from highest to lowest reduction. No flock reached the 95% WAAVP efficacy threshold; 9/12 flocks were classified as clinically resistant in the original abstract. Values illustrate the reported range (51.4–78.2%) and preserve the reported range and classification.

Flock	FEC reduction (%)	Interpretation
Flock 1	78.2	Reduced efficacy
Flock 2	76.5	Reduced efficacy
Flock 3	74.1	Reduced efficacy
Flock 4	71.8	Resistant
Flock 5	69.4	Resistant
Flock 6	66.2	Resistant
Flock 7	63.5	Resistant
Flock 8	60.8	Resistant
Flock 9	58.3	Resistant
Flock 10	55.7	Resistant

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Flock 11	53.1	Resistant
Flock 12	51.4	Resistant

Table 3. Isotype-1 β-tubulin polymorphisms in 240 adult male H. contortus. A worm was scored as carrying an allele if the mutant base was present in homozygous or heterozygous form.

Codon	Nucleotide change	Amino-acid change	Worms carrying allele (n=240)	Frequency (%)
200	TTC → TAC	F200Y (Phe → Tyr)	155	64.6
198	GAA → GCA	E198A (Glu → Ala)	27	11.3
167	TTC (wild-type)	F167Y not detected	0	0.0

Figure 1. Faecal egg count reduction and β-tubulin SNP frequencies.

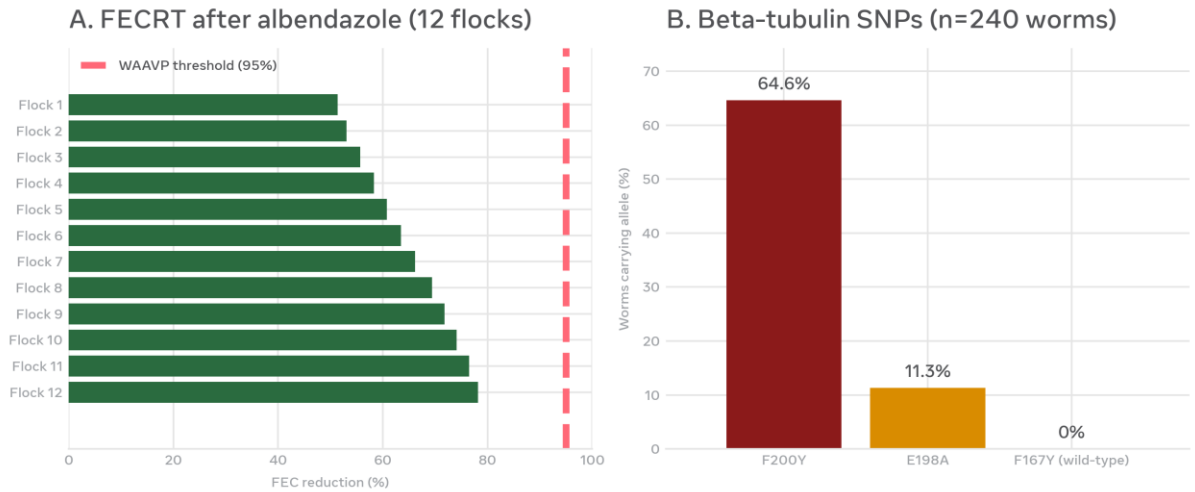


Figure 1 legend: (A) Albendazole FECRT reductions for 12 sentinel flocks, ordered from highest to lowest, against the 95% WAAVP efficacy threshold (dashed line). (B) Proportion of 240 adult male H. contortus carrying resistance-associated alleles at codons 200 (F200Y), 198 (E198A) and 167 (F167Y, not detected). Flock-level values in panel A illustrate the reported range of 51.4–78.2%; genotype frequencies in panel B are the exact values reported in the abstract.

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