EISSN: 2231-0916 doi: 10.14202/vetworld.2026.5180-5196 OPEN ACCESS

Veterinary World

RESEARCH ARTICLE

From field efficacy to molecular signals: assessment of five anthelmintics and levamisole-associated screening in gastrointestinal strongyles of smallholder meat goats in Nakhon Ratchasima, Thailand

Pornphutthachat Sota[1], Urusa Thaenkham[2], Abigail Hui En Chan[2] and Pramote Paengkoum[1]

1. School of Animal Technology and Innovation, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima, Thailand.
2. Laboratory of Helminth Biodiversity and Drug Development, Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

ABSTRACT

Background and Aim: Anthelmintic resistance among gastrointestinal strongyle nematodes threatens goat productivity and sustainable parasite control. Field efficacy assessments combined with molecular surveillance may improve resistance monitoring, particularly where mixed nematode infections complicate interpretation. This study evaluated the *in vivo* efficacy of five anthelmintics against gastrointestinal strongyles in smallholder meat goats in Nakhon Ratchasima Province, Thailand, and investigated the relationship between farm-level levamisole efficacy and resistance-associated molecular signals.

Materials and Methods: A field study was conducted on 13 smallholder goat farms between November 2024 and March 2025. Of 738 goats screened, 423 met the inclusion criteria for paired fecal egg count reduction testing (FECRT). Five anthelmintic treatments representing three drug classes were evaluated: albendazole, fenbendazole, ivermectin–clorsulon, eprinomectin, and levamisole. Treatment allocation within farms was nonrandomized and constrained by the number of eligible goats available for treatment; consequently, not all five anthelmintics were evaluated on every farm. Paired fecal egg counts from Day 0 and Day 14 were used to calculate farm-level fecal egg count reduction percentages and 90% confidence intervals, and treatment responses were classified according to the 2023 World Association for the Advancement of Veterinary Parasitology guidelines. FECRT reflected the combined treatment response of mixed strongyle populations. Strongyle genera were identified using polymerase chain reaction assays targeting pooled egg samples, and levamisole-associated *Hco-acr-8* S168T allele signals were assessed qualitatively in *Haemonchus*-positive pooled egg DNA.

Results: FECRT classified all evaluated farms as resistant for fenbendazole (10/10), ivermectin–clorsulon (12/12), and eprinomectin (12/12). For albendazole, nine of 10 farms were classified as resistant and one as inconclusive. Levamisole showed comparatively favorable but variable efficacy, with four farms (30.8%) classified as susceptible, two (15.4%) as inconclusive, and seven (53.8%) as resistant. *Haemonchus* and *Trichostrongylus* were detected on all farms, whereas *Oesophagostomum* was detected on two farms. Susceptible-associated *Hco-acr-8* signals were detected on all 13 farms, while resistant-associated signals were detected on 11 farms, resulting in co-detection of both signals on 11 farms. The qualitative pooled-sample assay did not determine individual parasite genotypes or allele frequencies. Molecular allele signal patterns did not consistently correspond with phenotypic levamisole responses.

Conclusion: Reduced field efficacy of multiple anthelmintic classes was widespread across the surveyed farms. Levamisole remained effective on some farms but requires farm-specific efficacy confirmation. The incomplete correspondence between FECRT classifications and pooled molecular signals supports their complementary use in resistance surveillance; however, the molecular assay should not be interpreted as an independent predictor of farm-level levamisole resistance. Longitudinal investigations are needed to determine the predictive value of resistance-associated markers.

Keywords: anthelmintic efficacy, anthelmintic resistance, fecal egg count reduction test, gastrointestinal strongyle nematodes, goat production, levamisole resistance, molecular resistance markers, Thailand.

INTRODUCTION

Goat production is an important socioeconomic activity in tropical and subtropical regions, contributing to household income, food security, and rural livelihoods, particularly among low- and middle-income farming

Corresponding Author: Pornphutthachat Sota
E-mail: Pornphutthachat.so@g.sut.ac.th

Received: 16-07-2026, **Accepted:** 15-10-2026, **Published online:** 10-11-2026

Co-authors: UT: urusa.tha@mahidol.ac.th; AHEC: abigail.cha@mahidol.ac.th; PP: pramote@sut.ac.th

How to cite: Sota P, Thaenkham U, Chan AHE, Paengkoum P. From field efficacy to molecular signals: assessment of five anthelmintics and levamisole-associated screening in gastrointestinal strongyles of smallholder meat goats in Nakhon Ratchasima, Thailand. Vet World. 2026;19(11):5180-5196.

Copyright: Sota *et al*. This article is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by/4.0/).

households [1–3]. In Thailand, goat farming has expanded as an important livestock enterprise, with Nakhon Ratchasima Province representing a major goat-producing area in the northeastern region [4]. However, gastrointestinal nematode (GIN) infections remain a major constraint to goat productivity, health, and welfare. Strongyle nematodes, particularly *Haemonchus* spp. and *Trichostrongylus* spp., are associated with clinical and subclinical conditions, including anemia, gastroenteritis, impaired growth, poor body condition, and reduced production performance [5, 6]. Control of these infections has relied extensively on synthetic anthelmintics, particularly benzimidazoles, macrocyclic lactones, and imidazothiazoles, making sustained drug efficacy essential for productive and economically viable smallholder goat farming [7].

Anthelmintic resistance (AR) has emerged as a major challenge to GIN control in small ruminants worldwide, partly because of repeated, empirical, and potentially inappropriate anthelmintic administration [7]. Previous investigations in Thailand have documented reduced efficacy or resistance to commonly administered anthelmintics in goats, including albendazole, ivermectin-based treatments, eprinomectin, and levamisole [8–11]. Nevertheless, interpretation of AR under field conditions requires careful consideration because *in vivo* treatment responses may be influenced by several factors beyond genetically mediated parasite resistance. These include host physiology, pharmacokinetic variation, drug absorption and metabolism, dosing accuracy, infection intensity, route of administration, previous anthelmintic exposure, mixed parasite populations, and farm management practices [7, 12, 13]. Consequently, reduced treatment efficacy observed under field conditions does not necessarily establish the underlying mechanism of resistance.

The fecal egg count reduction test (FECRT) remains one of the most widely used practical approaches for evaluating *in vivo* anthelmintic efficacy under field conditions [14]. However, because strongyle-type eggs are generally assessed collectively, the FECRT reflects the combined treatment response of potentially mixed nematode populations and does not readily distinguish genus- or species-specific susceptibility. Recent investigations in Thailand have therefore integrated FECRT findings with nemabiome analysis, larval identification, or molecular screening of resistance-associated markers to strengthen AR surveillance in goat herds [11, 15]. These complementary approaches provide additional information on parasite composition and potential resistance-associated genetic variation, supporting a more comprehensive interpretation of field efficacy results.

Levamisole is of particular interest because previous studies in Thailand have indicated that its efficacy may be retained in some goat herds, whereas other larval-based and molecular investigations have identified evidence of levamisole-associated resistance in gastrointestinal strongyles [8, 9]. Molecular screening of resistance-associated alleles, including the S168T substitution associated with the *Hco-acr-8* gene in *Haemonchus*, may provide complementary information on potential resistance-related genetic variation. However, a resistance-associated molecular signal does not independently establish phenotypic resistance or predict treatment failure, particularly when analyses use pooled material from naturally mixed nematode infections. Accordingly, molecular findings should be interpreted alongside field efficacy data and information on the composition of the parasite population.

Despite increasing recognition of AR in gastrointestinal strongyle nematodes of goats, important knowledge gaps remain regarding the comparative field efficacy of different anthelmintic classes in smallholder meat goat production systems in northeastern Thailand. Existing studies have documented reduced susceptibility to commonly used anthelmintics and demonstrated the usefulness of combining phenotypic and molecular approaches [8–11, 15]. However, these studies have differed in geographic setting, the range of anthelmintics evaluated, phenotypic assays, and molecular targets. Localized evidence from Nakhon Ratchasima Province remains limited, particularly regarding comparative farm-level FECRT responses across benzimidazoles, macrocyclic lactone-based treatments, and levamisole under naturally occurring mixed strongyle infections. Farm-level differences in parasite populations, management practices, and previous anthelmintic exposure may influence treatment outcomes, emphasizing the need for region-specific efficacy assessments rather than relying exclusively on findings from other geographic areas or production systems.

An additional knowledge gap concerns the relationship between farm-level levamisole efficacy and molecular signals associated with levamisole resistance in *Haemonchus*-positive pooled egg samples. Although resistance-associated molecular markers may contribute to surveillance, their interpretation in naturally infected goat populations remains challenging because pooled samples may contain genetically heterogeneous parasites and multiple strongyle genera. Consequently, the extent to which qualitative detection of levamisole-associated *Hco-acr-8* S168T allele signals corresponds with farm-level FECRT classifications requires further investigation. Integrating comparative anthelmintic efficacy assessment, identification of major strongyle genera,

and targeted molecular screening may therefore provide a more informative basis for understanding treatment responses and identifying priorities for resistance surveillance in smallholder goat farms.

This study aimed to evaluate the *in vivo* efficacy of five anthelmintic treatments representing three major drug classes against gastrointestinal strongyle nematodes in naturally infected smallholder meat goats in Nakhon Ratchasima Province, Thailand. Specifically, the study assessed farm-level fecal egg count reduction (FECR) following treatment with albendazole and fenbendazole (benzimidazoles), ivermectin–clorsulon and eprinomectin (macrocyclic lactone-based treatments), and levamisole (an imidazothiazole). The study also aimed to identify the major gastrointestinal strongyle genera present in pooled egg samples to provide additional information on parasite composition within the surveyed farms.

Following the field efficacy assessment, levamisole was selected for targeted molecular investigation because it demonstrated comparatively favorable but variable efficacy across farms. The subsequent molecular analysis examined the relationship between farm-level levamisole FECRT classifications and qualitative detection of levamisole-associated *Hco-acr-8* S168T allele signals in pooled egg DNA from *Haemonchus*-positive samples. By integrating multi-class FECRT findings, genus-level strongyle detection, and resistance-associated molecular screening, this study sought to generate region-specific evidence to inform farm-level anthelmintic efficacy monitoring and support evidence-based gastrointestinal parasite management in smallholder meat goat production systems.

MATERIALS AND METHODS

Ethical approval

All animal handling, anthelmintic administration, and fecal sampling procedures were conducted under a research protocol reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Suranaree University of Technology (Approval No. COA.SUT-IACUC-013/2024). Farm owners provided both verbal and written informed consent for fecal sample collection. Animal handling procedures were performed in accordance with applicable guidelines for the care and use of animals for scientific purposes, with measures taken to minimize handling-related stress and discomfort. The five anthelmintic treatments were administered as part of the approved research protocol rather than routine farm management. All treatments were administered by a veterinarian or trained personnel under close veterinary supervision. Animals were monitored for adverse reactions during the study, and farm owners were instructed to report any abnormalities following treatment. No treatment-related adverse reactions were observed or reported.

Study period and location

The field study was conducted between November 2024 and March 2025 on smallholder meat goat farms in Muang and Dan Khun Thot districts of Nakhon Ratchasima Province, northeastern Thailand. The province was selected because it is an important goat-producing region. According to the Department of Livestock Development, Nakhon Ratchasima ranked second nationally in goat population in 2025, with 110,544 goats [4]. Field activities included farm recruitment, animal selection, anthelmintic administration, and paired fecal sample collection. Collected samples were transported to Suranaree University of Technology for parasitological and molecular analyses.

Study design

A nonrandomized, farm-based field study was conducted to evaluate the efficacy of five anthelmintic treatments against naturally occurring gastrointestinal strongyle infections in smallholder meat goats. Thirteen farms were recruited through a coordinator-assisted, eligibility-based approach in collaboration with local cooperative personnel. Farms were eligible if they maintained actively raised meat goats, were accessible for repeated visits, had sufficient animals available for paired fecal sampling, and had owners willing to participate. The number of participating farms was determined by field feasibility and the availability of eligible farms. Available farm records, farm-owner interviews, and on-farm verification of anthelmintic products were used to obtain information on previous anthelmintic use; however, the frequency, exact timing, and duration of previous treatments were not consistently available across farms and were therefore used only as contextual information rather than as variables in the efficacy analysis.

At the animal level, eligible goats were at least 3 months old, of either sex, naturally infected with GINs, and had not received anthelmintic treatment during the preceding 2 months. Animals with severe clinical illness or obvious injuries were excluded. Goats were enrolled if they were expected to remain available for paired fecal

sampling on Day 0 and Day 14. Each animal received only one assigned anthelmintic treatment. Treatment allocation was nonrandomized and depended on the number of eligible goats available within each farm; consequently, not all five treatments were evaluated on every farm. The study did not involve sequential or rotational anthelmintic administration.

Anthelmintic efficacy was evaluated using a paired pre- and post-treatment FECRT design. Only goats with complete paired fecal samples and pretreatment strongyle-type egg counts of at least 50 eggs per gram (EPG) were included in the efficacy analysis. Farm-level treatment responses were evaluated using FECR estimates and corresponding confidence intervals (CIs). In addition, pooled strongyle-type egg samples were subjected to molecular assays for genus-level parasite detection. Because levamisole demonstrated comparatively favorable field efficacy, *Haemonchus*-positive pooled egg DNA was subsequently screened for levamisole-associated *Hco-acr-8* S168T allele signals. Molecular findings were compared descriptively with farm-level levamisole FECRT classifications.

Study farms, animal selection, and fecal sample collection

Individual goats were identified using existing ear tags, where available, and were additionally marked with temporary study identification numbers using livestock marking spray. Study identification numbers, farm identification codes, ear tag numbers when available, and basic animal characteristics were recorded to ensure accurate matching of pretreatment and post-treatment samples.

Approximately 5 g of feces was collected directly from the rectum of each enrolled goat on Day 0, before anthelmintic administration, and again on Day 14 after treatment. Samples were transferred into labeled sterile plastic bags, maintained in a cool box at approximately 4 °C, and transported to Suranaree University of Technology within 3 h of collection. Each sample was processed for parasitological and molecular examinations. A 2-g aliquot was used for fecal egg counting, while a separate 2-g aliquot was used for flotation-based recovery of strongyle-type eggs for subsequent molecular analysis.

Anthelmintic treatments and FECRT

Five anthelmintic treatments representing three major drug classes were evaluated: albendazole, fenbendazole, ivermectin–clorsulon, eprinomectin, and levamisole. Albendazole and fenbendazole were administered orally at doses of 5.625 and 5 mg/kg body weight, respectively. The ivermectin–clorsulon formulation contained ivermectin at 10 mg/mL and clorsulon at 100 mg/mL and was administered by subcutaneous injection at doses equivalent to 0.2 mg/kg ivermectin and 2.0 mg/kg clorsulon. Eprinomectin was administered subcutaneously at 0.2 mg/kg body weight, whereas levamisole was administered subcutaneously at 5 mg/kg body weight. The selected doses were based on manufacturer product-label information and previous goat-specific FECRT investigations [8, 15, 16]. Albendazole and fenbendazole were used according to product-label recommendations for goats, whereas the ivermectin–clorsulon formulation, eprinomectin, and levamisole represented extra-label use in goats under veterinary supervision.

Detailed information on the commercial products, active ingredients, formulations, concentrations, manufacturers, batch or lot numbers, expiration dates, administered doses, and routes of administration is provided in Supplementary Table S1. These details are included to support methodological reproducibility and do not constitute an evaluation or comparison of commercial product quality.

Eligible goats within each farm were assigned to available treatment groups after Day 0 fecal sampling. Treatment allocation was not randomized and was constrained by the number of eligible animals available during the sampling period. Allocation was not stratified or matched according to age, sex, baseline EPG, body weight, or previous anthelmintic exposure. Body weight was used only for dose calculation, whereas previous anthelmintic exposure was considered an eligibility criterion. Each goat received a single assigned anthelmintic treatment, followed by fecal sampling on Day 14. Because animal availability differed among farms, not all treatments were assessed on every farm. The number of goats included in each farm–drug combination is presented in Table 1. Anthelmintic doses were calculated according to individual body weights measured before treatment. Administration was performed by a veterinarian or trained personnel under close veterinary supervision to ensure delivery of the intended dose.

Anthelmintic efficacy was assessed using paired FECRT measurements from individual fecal samples collected on Day 0 and Day 14. Strongyle-type eggs were identified morphologically using a modified formalin–ethyl acetate concentration technique (M-FECT) [17]. Briefly, 2 g of feces was homogenized in 10 mL of 10% formalin, filtered, and centrifuged at 1,500 rpm for 3 min. The sediment was resuspended in 7 mL of 10% formalin

and 3 mL of ethyl acetate, mixed, and centrifuged again at 1,500 rpm for 3 min. After removal of the upper layers, the remaining sediment was resuspended in 10% formalin at approximately twice the sediment volume. Because the recovered sediment volume varied among samples, the final suspension volume was not fixed. A 3-mL plastic transfer pipette of the same type was used throughout the study for dropwise examination. The total number of drops in each final suspension was counted directly rather than assuming a fixed drop volume. EPG was calculated as the mean number of eggs observed per examined drop multiplied by the total number of drops in the final suspension and divided by the 2-g fecal mass examined.

Table 1: Pre-treatment and post-treatment fecal egg counts of strongyle-type nematodes and numbers of goats included in the fecal egg count reduction test across farm–drug combinations.

Farm	Albendazole (Pre)	Albendazole (Post)	Fenbendazole (Pre)	Fenbendazole (Post)	Ivermectin–clorsulon (Pre)	Ivermectin–clorsulon (Post)	Eprinomectin (Pre)	Eprinomectin (Post)	Levamisole (Pre)	Levamisole (Post)
A	142.5 ± 68.7 (4)	11.4 ± 4.2	314.2 ± 384.9 (5)	77.2 ± 40.8	175.0 ± 97.6 (3)	116.0 ± 127.2	120.1 ± 78.3 (5)	28.6 ± 24.9	71.9 ± 26.1 (4)	10.8 ± 7.6
B	NA	NA	NA	NA	NA	NA	122.9 ± 38.4 (5)	46.2 ± 22.0	101.6 ± 24.2 (4)	5.0 ± 10.0
C	78.7 ± 11.9 (3)	41.2 ± 14.5	73.4 ± 22.4 (5)	58.1 ± 12.7	66.0 ± 26.0 (3)	25.0 ± 17.6	106.3 ± 55.4 (4)	63.6 ± 42.5	138.3 ± 38.5 (6)	22.6 ± 34.5
D	180.9 ± 119.7 (7)	436.5 ± 399.5	401.1 ± 259.7 (6)	735.5 ± 464.7	204.0 ± 61.8 (7)	656.6 ± 532.3	537.7 ± 400.2 (7)	279.2 ± 183.8	231.3 ± 273.5 (4)	61.5 ± 48.4
E	NA	NA	NA	NA	212.7 ± 208.7 (6)	50.5 ± 28.1	94.0 ± 65.7 (5)	40.3 ± 70.3	177.5 ± 110.5 (3)	65.2 ± 30.3
F	243.3 ± 163.1 (4)	145.5 ± 127.1	208.2 ± 222.7 (7)	190.3 ± 294.8	299.2 ± 294.5 (7)	191.3 ± 249.8	76.0 ± 23.4 (5)	53.1 ± 67.8	241.5 ± 257.5 (4)	8.8 ± 6.4
G	181.2 ± 179.0 (5)	60.3 ± 82.6	NA	NA	711.6 ± 953.5 (15)	1656.1 ± 1684.2	234.1 ± 94.3 (7)	119.1 ± 55.4	209.7 ± 193.5 (12)	2.9 ± 7.8
H	NA	NA	178.9 ± 79.5 (5)	114.4 ± 79.6	81.3 ± 8.3 (3)	9.3 ± 3.3	177.3 ± 147.7 (4)	35.1 ± 32.9	78.8 ± 19.2 (5)	5.5 ± 8.4
I	640.8 ± 761.6 (10)	316.5 ± 253.6	619.4 ± 526.1 (7)	496.1 ± 379.6	265.8 ± 373.0 (11)	148.9 ± 156.9	384.7 ± 395.5 (13)	197.7 ± 335.2	359.1 ± 313.3 (12)	13.9 ± 14.8
J	443.3 ± 309.9 (10)	164.0 ± 126.6	337.2 ± 257.1 (5)	128.6 ± 54.7	461.9 ± 601.7 (8)	141.3 ± 158.5	214.6 ± 213.1 (8)	120.3 ± 87.5	829.2 ± 864.9 (12)	6.4 ± 8.3
K	206.6 ± 261.2 (16)	465.7 ± 533.6	305.2 ± 234.4 (13)	623.8 ± 704.3	317.5 ± 249.1 (14)	364.0 ± 273.2	208.6 ± 185.2 (10)	265.0 ± 122.7	189.8 ± 159.7 (7)	30.2 ± 29.8
L	201.2 ± 243.2 (7)	42.6 ± 32.4	202.8 ± 152.8 (6)	106.5 ± 112.7	136.8 ± 84.6 (6)	34.3 ± 18.1	134.5 ± 93.7 (9)	70.7 ± 82.3	137.8 ± 90.4 (8)	3.9 ± 5.6
M	709.4 ± 977.6 (6)	107.2 ± 137.9	126.1 ± 88.9 (4)	221.0 ± 176.2	256.0 ± 268.5 (32)	230.2 ± 284.2	NA	NA	430.6 ± 413.2 (10)	14.8 ± 28.7
Total n	72	—	63	—	115	—	82	—	91	—

Values are presented as arithmetic mean ± standard deviation (SD) of eggs per gram (EPG) of feces. Pre = Pretreatment EPG; Post = Post-treatment EPG; n = Number of goats with paired Day 0 and Day 14 fecal samples included in the fecal egg count reduction test (FECRT); NA = Not assessed. Numbers in parentheses in the pretreatment columns indicate the number of goats included in each farm–drug combination. Total values represent the number of goats evaluated for each anthelmintic treatment.

Based on the recorded number of drops in each final suspension, the sample-specific conversion factor ranged from 3 to 36 EPG per detected egg, with a median of 11.5 EPG among goats included in the FECRT analysis. Samples were initially examined once. When no strongyle-type eggs were detected, two additional aliquots were examined before the sample was classified as negative.

All microscopic examinations were performed by the same trained and experienced observer, who was not blinded to sampling time or treatment allocation. No independent verification or routine duplicate counting was performed. The pretreatment threshold of ≥50 EPG was applied only during the final FECRT analysis and was not used for treatment allocation. Only goats with paired Day 0 and Day 14 fecal samples and pretreatment strongyle-type egg counts of at least 50 EPG were retained for analysis, consistent with the individual-animal eligibility criterion applied in previous field FECRT studies involving goats in Thailand [8, 10]. Arithmetic mean pretreatment and post-treatment EPG values were subsequently calculated for each farm–drug combination.

Farm-level FECR estimation and interpretation of anthelmintic efficacy

Farm-level FECR was calculated for each farm–drug combination using arithmetic mean pretreatment and post-treatment EPG values according to the following equation:

$$\text{FECR (\%)} = 100 \times [1 - (\text{mean post-treatment EPG}/\text{mean pretreatment EPG})]$$

The use of arithmetic means for group-level FECRT estimation followed the recommendations of the World

Association for the Advancement of Veterinary Parasitology (WAAVP) guideline [14].

The 90% CIs were estimated using R software version 4.5.2 and a paired nonparametric bootstrap procedure involving 5,000 resampling iterations within each farm–drug combination. Paired Day 0 and Day 14 observations from the same goat were resampled together to preserve the within-animal relationship between pretreatment and post-treatment measurements. Because all included goats had pretreatment counts of ≥50 EPG, zero pretreatment means did not occur during bootstrap resampling. The lower and upper 90% confidence limits were obtained from the 5th and 95th percentiles of the bootstrap FECR distribution, respectively.

Zero post-treatment EPG values were retained without adjustment, and negative FECR estimates were preserved as calculated. No parametric model was fitted to account for overdispersion; uncertainty was estimated using the paired nonparametric bootstrap procedure described above.

Anthelmintic efficacy was classified according to the updated WAAVP 2023 clinical protocol for ruminants [14]. Farm–drug combinations were classified as susceptible when the lower limit of the 90% CI was ≥90% and the upper limit was ≥99%. Resistance was assigned when the upper limit of the 90% CI was < 99%. Results that did not meet either classification criterion were considered inconclusive.

Recovery of strongyle-type eggs for molecular analysis and detection of strongyle genera

Strongyle-type eggs were recovered from fecal samples using a flotation technique adapted from Income *et al.* [18]. Briefly, fecal material was homogenized in saturated sodium chloride (NaCl) solution, and the resulting suspension was filtered using a sieve to recover the floating egg fraction. Recovered eggs were pooled at the farm-level, rinsed with distilled water, and preserved in 70% ethanol at −20 °C until molecular analysis.

Before DNA extraction, the recovered eggs were disrupted by homogenization with silica beads. Total genomic DNA was extracted using the Genomic DNA Mini Kit (Geneaid Biotech Ltd., Taipei, Taiwan), following the manufacturer's instructions.

Genus-level identification of strongyle nematodes was performed using nested polymerase chain reaction (PCR) assays targeting two genetic regions: the nuclear internal transcribed spacer 2 (ITS2) region and the mitochondrial 16S ribosomal RNA (rRNA) gene. The assays targeted three strongyle genera: *Haemonchus*, *Trichostrongylus*, and *Oesophagostomum*. Because the molecular assays were restricted to these genera, other strongyle genera potentially present in the pooled samples were not evaluated. Accordingly, molecular findings were interpreted qualitatively as the presence or absence of each targeted genus at the farm-level.

Primer sets and amplification strategies were adapted from previously described protocols by Income *et al.* [18] and Chan *et al.* [9, 19]. Primer sequences, expected amplicon sizes, and information on previously reported assay specificity are provided in Supplementary Table S2.

Nested PCR amplification was performed using a T100™ thermal cycler (Bio-Rad, California, USA) and 2× i-Taq™ Master Mix (iNtRON Biotechnology, Gyeonggi, South Korea), following the amplification and thermocycling procedures described by Chan *et al.* [9]. Following the first amplification round, PCR products were diluted tenfold with nuclease-free water and used as templates for separate second-round genus-specific PCR assays targeting *Haemonchus*, *Trichostrongylus*, and *Oesophagostomum*.

Sequence-confirmed reference DNA obtained from previously characterized samples reported by Chan *et al.* [9, 19] served as the positive control for each assay. Nuclease-free water was included as the no-template control (NTC). No separate DNA extraction-negative control was included. Assay performance was verified by the presence of an amplicon of the expected size in the positive control and the absence of amplification in the NTC.

Each farm-level pooled-sample was initially tested once for each targeted genus. Assays producing weak or ambiguous bands were repeated before final interpretation. Amplification products were visualized by electrophoresis on 1.5% agarose gels stained with SYBR™ Safe (Invitrogen, Massachusetts, USA).

Selection of levamisole for molecular investigation and detection of levamisole-associated allele signals

Levamisole was selected for additional molecular investigation because it demonstrated comparatively better overall efficacy across the surveyed farms than the other evaluated anthelmintics. Molecular screening was subsequently undertaken to compare farm-level levamisole FECRT classifications with the detection of levamisole-associated *Hco-acr-8* S168T allele signals in *Haemonchus*-positive pooled egg samples.

Farm-level pooled egg samples were prepared from post-treatment fecal collections. Approximately 8–10 goats per farm were sampled for strongyle-type egg recovery. Samples were selected pragmatically based on availability during farm sampling, generally from the first available goats sampled. Only samples yielding sufficient

numbers of strongyle-type eggs for subsequent DNA extraction contributed to the final farm-level pool. Eggs obtained from contributing samples were combined within each farm for qualitative molecular screening. The goats contributing to each pool were not restricted to those assigned to the levamisole treatment group and could include goats from other treatment groups.

Farm-level pooled DNA samples positive for *Haemonchus* in the genus-specific PCR assay were subjected to allele-specific conventional PCR to detect the levamisole-associated single-nucleotide polymorphism in the *Hco-acr-8* gene. The assay targeted the S168T substitution (AGC→ACC on the protein-coding strand) in the *Hco-acr-8* gene using separate amplification reactions for susceptible- and resistance-associated alleles [20].

The susceptible- and resistance-associated allele reactions were performed separately in a final volume of 20 µL containing 10 µL of 2× i-Taq™ PCR Master Mix (iNtRON Biotechnology, Gyeonggi, South Korea), 1 µL of the corresponding allele-specific forward primer (10 µM), 1 µL of the common reverse primer (10 µM), 3 µL of nuclease-free water, and 5 µL of genomic DNA template. Thermocycling conditions consisted of an initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 45 s, annealing at 56 °C for 30 s, and extension at 72 °C for 1 min. A final extension was performed at 72 °C for 10 min.

Amplification products were separated by electrophoresis on 1.5% agarose gels. The presence of an amplicon at the expected size of 184 bp was interpreted as positive amplification for the corresponding allele-associated signal. Positive and NTCs were included in each allele-specific PCR assay.

Gel bands were interpreted without knowledge of the corresponding farm-level FECRT classification. Weak or ambiguous bands were reassessed by repeating PCR amplification and gel electrophoresis before final interpretation. Band intensity was not used to estimate the relative abundance or proportion of susceptible- or resistance-associated alleles. Cross-amplification, analytical sensitivity, and the ability to detect minority alleles in mixed DNA were not independently evaluated in the present study; therefore, allele-specific amplification was interpreted qualitatively as signal presence or absence.

Farm-level molecular patterns were determined from the presence or absence of the expected allele-specific amplification products. Detection of only the susceptible-associated amplicon was classified as a susceptible-associated allele signal, whereas detection of only the resistant-associated amplicon was classified as a resistant-associated allele signal. Detection of both amplicons in the same pooled-sample was classified as co-detection of susceptible- and resistant-associated allele signals.

Because the assay was qualitative and performed using farm-level pooled egg DNA, the resulting molecular patterns were not interpreted as individual-parasite genotypes, allele frequencies, or estimates of relative allele proportions.

Statistical analysis

Pretreatment and post-treatment EPG values were summarized as arithmetic means for each farm–drug combination. Farm-level FECR percentages and corresponding 90% CIs were calculated and interpreted according to the WAAVP 2023 clinical protocol for ruminants [14].

Molecular detection results for the targeted strongyle genera and levamisole-associated *Hco-acr-8* allele signals were summarized as presence–absence data at the farm-level. Farm-level levamisole FECRT classifications were compared descriptively with the corresponding pooled *Hco-acr-8* allele signal patterns. No formal statistical test of association between phenotypic efficacy and molecular findings was performed.

RESULTS

Pre- and post-treatment fecal egg counts across the five anthelmintics

A total of 738 goats from 13 smallholder meat goat farms in Nakhon Ratchasima Province, Thailand, were initially screened. A review of available farm records indicated that ivermectin was routinely used as the primary anthelmintic on all 13 farms. Previous albendazole use was documented on some farms, whereas no previous levamisole use was recorded on any participating farm. Following application of the eligibility criteria, 423 goats were included in the FECR analysis. The remaining 315 goats were excluded because of pretreatment strongyle-type egg counts below 50 EPG (n = 301), missing Day 14 samples (n = 10), clinical illness (n = 3), or other reasons (n = 1). Levamisole was evaluated on all 13 farms, eprinomectin and ivermectin–clorsulon on 12 farms each, and albendazole and fenbendazole on 10 farms each. The numbers of goats included in each farm–drug combination are presented in Table 1.

Among the 423 goats included in the analysis, 396 (93.6%) were female and 27 (6.4%) were male. Most goats were Boer crossbreds (397/423, 93.9%), followed by Anglo-Nubian goats (22/423, 5.2%) and other breeds (4/423,

0.9%). Age ranged from 0.3 to 11 years, with a median of 2.0 years. Regarding age distribution, 24.8% were younger than 1 year, 18.0% were 1 to <2 years, 22.9% were 2 to <3 years, and 34.3% were at least 3 years old. Body weight ranged from 5 to 62 kg, with a median of 30 kg.

As shown in Table 1, the arithmetic mean ± standard deviation of pretreatment and post-treatment strongyle-type EPG values varied considerably among farm–drug combinations. Mean pretreatment EPG values ranged from 66.0 to 829.2, whereas mean post-treatment EPG values ranged from 2.9 to 1656.1. Some pretreatment group means were relatively close to the minimum inclusion threshold of 50 EPG, which may have contributed to greater uncertainty in the corresponding FECR estimates. Overall, the magnitude and direction of changes in egg counts between Day 0 and Day 14 differed substantially among farms and anthelmintic treatments.

Farm-level FECR evaluation and interpretation of anthelmintic efficacy

Farm-level FECR estimates, corresponding 90% CIs, and efficacy classifications for the five anthelmintic treatments are presented in Table 2. Considerable variation in treatment efficacy was observed among farms and across anthelmintic treatments. Levamisole demonstrated the most favorable overall efficacy profile, with FECR values ranging from 63.3% to 99.2%. In contrast, fenbendazole, ivermectin–clorsulon, and eprinomectin showed consistently poor efficacy across the evaluated farms.

According to the WAAVP 2023 clinical protocol, albendazole was classified as resistant on nine of the 10 evaluated farms and inconclusive on one farm (Table 2). Fenbendazole (10/10 farms), ivermectin–clorsulon (12/12 farms), and eprinomectin (12/12 farms) were classified as resistant on all farms where they were evaluated. In comparison, levamisole was classified as susceptible on four farms (30.8%), inconclusive on two farms (15.4%), and resistant on seven farms (53.8%).

Negative FECR values were recorded for several farm–drug combinations, indicating that mean post-treatment EPG values exceeded the corresponding pretreatment values (Table 2). The lowest FECR estimates were observed for ivermectin–clorsulon on Farms D (−221.9%) and G (−132.7%), albendazole on Farms D (−141.3%) and K (−125.4%), and fenbendazole on Farm K (−104.4%). Individual paired Day 0 and Day 14 EPG values for these five farm–drug combinations are illustrated in Supplementary Figure S1. Post-treatment EPG increased in most or all goats in four of these combinations, whereas the response to fenbendazole on Farm K showed greater variability among individual animals.

Because levamisole produced the highest number of susceptible farm-level classifications among the evaluated anthelmintics (Table 2), it was selected for further molecular investigation.

Table 2: Farm-level fecal egg count reduction estimates, 90% confidence intervals, and efficacy classifications for five anthelmintic treatments against gastrointestinal strongyle nematodes.

Farm	ALB FECR (%)	L90CI	U90CI	Status	FEN FECR (%)	L90CI	U90CI	Status	IV-CL FECR (%)	L90CI	U90CI	Status	EPM FECR (%)	L90CI	U90CI	Status	LVM FECR (%)	L90CI	U90CI	Status
A	92.0	90.8	92.7	R	75.4	−7.8	90.8	R	33.7	−15.9	77.1	R	76.2	50.4	91.3	R	85.0	71.5	94.1	R
B	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	62.4	49.5	73.7	R	95.1	87.4	100.0	I
C	47.7	37.1	58.2	R	20.8	−5.0	37.2	R	62.1	55.7	72.4	R	40.1	−23.6	70.2	R	83.7	63.8	96.2	R
D	−141.3	−357.6	−31.6	R	−83.4	−198.8	−15.7	R	−221.9	−405.3	−75.0	R	48.1	21.9	65.4	R	73.4	9.0	91.8	R
E	NA	NA	NA	NA	NA	NA	NA	NA	76.3	46.2	87.0	R	57.1	−18.0	94.7	R	63.3	33.0	79.7	R
F	40.2	33.2	53.5	R	8.6	−31.6	63.9	R	36.1	−5.9	74.4	R	30.1	−20.4	73.8	R	96.4	89.4	98.2	R
G	66.7	−4.7	100.0	I	NA	NA	NA	NA	−132.7	−362.7	−35.4	R	49.1	32.5	62.8	R	98.6	96.4	100.0	S
H	NA	NA	NA	NA	36.1	13.5	58.3	R	88.5	86.1	91.0	R	80.2	73.6	88.9	R	93.0	82.8	100.0	I
I	50.6	15.1	66.1	R	19.9	−40.9	55.5	R	44.0	−23.3	68.8	R	48.6	−15.0	80.2	R	96.1	92.6	98.0	R
J	63.0	52.4	72.1	R	61.9	20.3	78.8	R	69.4	52.7	76.7	R	44.0	5.9	64.0	R	99.2	98.3	99.7	S
K	−125.4	−280.4	−42.5	R	−104.4	−221.6	−17.0	R	−14.7	−40.3	4.9	R	−27.0	−112.2	17.1	R	84.1	69.2	92.1	R
L	78.8	59.9	86.5	R	47.5	38.7	65.4	R	74.9	57.9	85.6	R	47.5	29.6	67.4	R	97.2	93.4	99.4	S
M	84.9	59.4	95.8	R	−75.2	−322.2	27.9	R	10.1	−24.8	35.5	R	NA	NA	NA	NA	96.6	91.7	99.7	S

FECR = Fecal egg count reduction (%); L90CI = Lower limit of the 90% confidence interval; U90CI = Upper limit of the 90% confidence interval; R = Resistant; S = Susceptible; I = Inconclusive; NA = Not assessed; ALB = Albendazole; FEN = Fenbendazole; IV-CL = Ivermectin-Clorsulon; EPM = Eprinomectin; LVM = Levamisole. Negative FECR values indicate that the mean post-treatment eggs per gram of feces exceeded the corresponding pretreatment mean. Efficacy classifications were based on the World Association for the Advancement of Veterinary Parasitology 2023 clinical protocol.

Molecular identification of strongyle-type nematodes at the farm-level

Molecular examination of pooled strongyle-type egg samples demonstrated that *Haemonchus* spp. and *Trichostrongylus* spp. were detected on all 13 farms, whereas *Oesophagostomum* spp. were detected only on Farms I and L (Figure 1A). Thus, *Haemonchus* and *Trichostrongylus* were the most widely detected strongyle genera across the participating farms, as illustrated in Figure 1A.

doi: 10.14202/vetworld.2026.5180-5196

Farm-level levamisole phenotypic status and allele signal patterns

Farm-level levamisole FECRT classifications and corresponding qualitative *Hco-acr-8* S168T allele signal patterns are presented in Figure 1B. The susceptible-associated allele signal was detected on all 13 farms, whereas the resistant-associated allele signal was detected on 11 farms. Only Farms D and J showed the susceptible-associated allele signal without detection of the resistant-associated signal. The remaining 11 farms exhibited co-detection of susceptible- and resistant-associated allele signals (Figure 1B). Representative amplification patterns for the *Hco-acr-8* S168T marker are presented in Figure 2.

Comparison of molecular findings with farm-level levamisole FECRT classifications revealed variable correspondence between phenotypic responses and pooled allele signal patterns (Figure 1B). Farm J was classified as susceptible by FECRT and exhibited only the susceptible-associated allele signal. Farms B and H showed inconclusive FECRT classifications despite co-detection of susceptible- and resistant-associated signals. Six farms classified as resistant by FECRT also exhibited co-detection of both allele signals.

In contrast, Farms G, L, and M were classified as susceptible by FECRT despite co-detection of susceptible- and resistant-associated signals. Farm D showed the opposite pattern, with a resistant FECRT classification despite detection of only the susceptible-associated allele signal (Figure 1B). Overall, farm-level levamisole FECRT classifications did not consistently correspond with the qualitative *Hco-acr-8* allele signal patterns detected in *Haemonchus*-positive pooled egg DNA.

A Farm-level detection of targeted strongyle genera

Farm	*Haemonchus*	*Trichostrongylus*	*Oesophagostomum*
A	●	●	○
B	●	●	○
C	●	●	○
D	●	●	○
E	●	●	○
F	●	●	○
G	●	●	○
H	●	●	○
I	●	●	●
J	●	●	○
K	●	●	○
L	●	●	●
M	●	●	○

B Farm-level levamisole phenotypic status and *Hco-acr-8* allele signals

Farm	Phenotypic status	Susceptible-associated signal	Resistant-associated signal	Pooled allele-signal pattern
A	Resistant	Yes	Yes	Co-detection
B	Inconclusive	Yes	Yes	Co-detection
C	Resistant	Yes	Yes	Co-detection
D	Resistant	Yes	No	Susceptible-associated signal only
E	Resistant	Yes	Yes	Co-detection
F	Resistant	Yes	Yes	Co-detection
G	Susceptible	Yes	Yes	Co-detection
H	Inconclusive	Yes	Yes	Co-detection
I	Resistant	Yes	Yes	Co-detection
J	Susceptible	Yes	No	Susceptible-associated signal only
K	Resistant	Yes	Yes	Co-detection
L	Susceptible	Yes	Yes	Co-detection
M	Susceptible	Yes	Yes	Co-detection

Targeted strongyle genera
● = Detected ○ = Not detected

Levamisole phenotypic status
= Susceptible = Resistant = Inconclusive

Susceptible-associated signal
Yes = Detected

Resistant-associated signal
Yes = Detected No= Not detected

Levamisole-associated allele-signal pattern
= Susceptible-associated signal only
= Co-detection of susceptible- and resistant-associated signals

Figure 1: Farm-level detection of gastrointestinal strongyle genera and levamisole phenotypic efficacy classifications in relation to pooled *Hco-acr-8* S168T allele signal patterns. (A) Detection of the targeted strongyle genera *Haemonchus*, *Trichostrongylus*, and *Oesophagostomum* using polymerase chain reaction, presented as detected or not detected. (B) Farm-level levamisole fecal egg count reduction test classifications and corresponding qualitative *Hco-acr-8* S168T allele signal patterns detected in *Haemonchus*-positive pooled egg DNA. Susceptible-associated and resistant-associated signals indicate detection of the corresponding allele-specific PCR amplification products. Co-detection indicates the presence of both susceptible- and resistant-associated signals in the same farm-level pooled DNA sample. Because DNA was pooled at the farm-level, the observed patterns do not represent individual-parasite genotypes or allele frequencies.

DISCUSSION

Widespread reduction in anthelmintic efficacy across multiple drug classes

The present study demonstrated substantially reduced field efficacy of several commonly used anthelmintics against gastrointestinal strongyle nematodes in smallholder meat goats in Nakhon Ratchasima Province, Thailand.

Fenbendazole, ivermectin–clorsulon, and eprinomectin were classified as resistant on all evaluated farms, whereas albendazole was classified as resistant on nine farms and inconclusive on one farm (Table 2). These findings indicate that treatment options from two major anthelmintic classes, benzimidazoles and macrocyclic lactones, may be unreliable under the conditions investigated. However, inconclusive FECRT classifications represent uncertainty in efficacy rather than confirmation of either susceptibility or resistance.

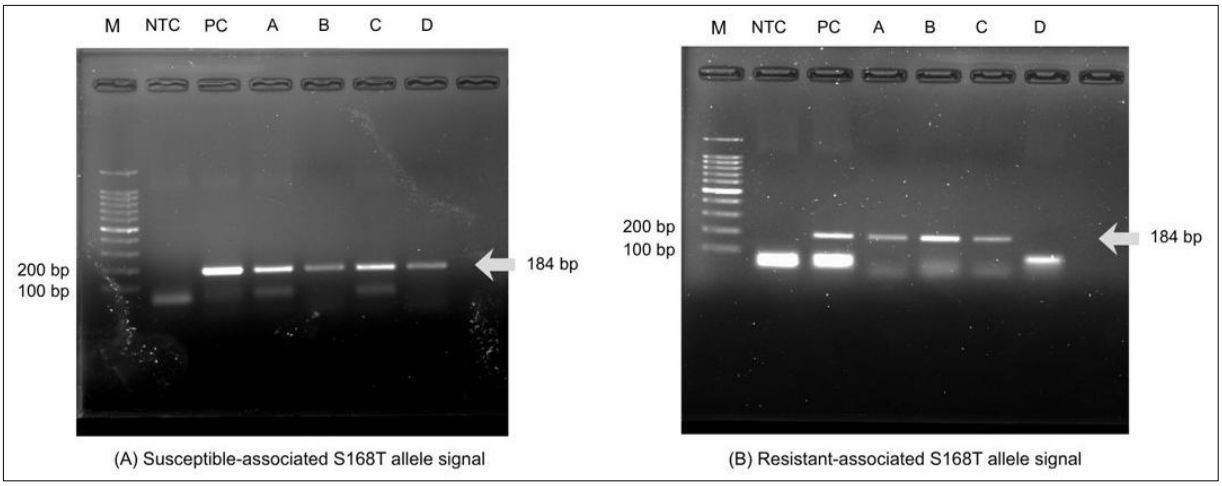


Figure 2: Representative allele-specific polymerase chain reaction amplification patterns for the *Hco-acr-8* S168T marker in farm-level pooled strongyle egg DNA. (A) Susceptible-associated allele signal. (B) Resistant-associated allele signal. The expected amplification product was 184 base pairs (bp), as indicated by the arrows. M = 100-bp DNA ladder; NTC = No-template control; PC = Positive control; lanes A–D correspond to Farms A–D, respectively. Detection of an amplification product at the expected size was interpreted as the presence of the corresponding allele-associated signal. Farms A–C illustrate co-detection of susceptible- and resistant-associated allele signals, whereas Farm D illustrates a susceptible-associated allele signal only. No resistant-associated allele signal-only pattern was observed among the study farms.

The observed reduction in efficacy has important implications for gastrointestinal parasite control in smallholder goat production systems. Nevertheless, field-based FECRT outcomes should be interpreted cautiously because several biological, pharmacological, and management-related factors may influence treatment responses. These include natural variation in egg shedding, reinfection pressure, relatively low pretreatment EPG values in some farm–drug combinations, host-related pharmacokinetic differences, drug formulation, dosing accuracy, and administration practices [7, 12, 13]. Although these factors may contribute to apparent treatment failure, the present study did not directly investigate their individual effects. Consequently, reduced FECR values should not be attributed exclusively to genetically mediated parasite resistance.

Among the five evaluated treatments, levamisole demonstrated the most favorable overall efficacy profile, although resistant or inconclusive FECRT classifications were recorded on several farms (Table 2). These findings suggest that levamisole may remain a treatment option on farms where its efficacy has been confirmed, but its comparatively better performance does not justify indiscriminate use.

Widespread poor efficacy across multiple anthelmintic classes in Thai goat production

The reduced efficacy observed across multiple anthelmintic classes is consistent with previous investigations documenting diminished anthelmintic efficacy in GINs of goats in Thailand. Earlier studies have reported reduced treatment responses in goat populations from Sing Buri, Ratchaburi, Chiang Mai, Nakhon Si Thammarat, and northeastern Thailand [8–11, 15, 21]. Collectively, these reports indicate that reduced anthelmintic efficacy is not restricted to a single geographic region but represents a broader challenge for gastrointestinal parasite control in Thai goat production systems.

Recent studies in Thailand have also emphasized the value of integrating phenotypic efficacy testing with molecular or species-informed diagnostic approaches. Reduced treatment efficacy has been reported alongside resistance-associated molecular patterns or changes in post-treatment strongyle populations [9, 11, 21]. In this context, the present study adds farm-level evidence from Nakhon Ratchasima Province, where multiple anthelmintic treatments showed reduced efficacy under naturally occurring gastrointestinal strongyle infections.

These findings support replacing purely empirical deworming practices with farm-specific parasite control strategies informed by treatment-response monitoring. Periodic FECRT, accurate recording of anthelmintic administration and dosing histories, and selection of treatments according to documented farm-level efficacy may

improve the reliability of parasite control. Where feasible, genus- or species-resolved parasitological examinations and molecular screening for resistance-associated markers may provide complementary information for interpreting treatment responses.

Reduced efficacy of benzimidazoles and macrocyclic lactone-based treatments

The consistently poor responses to fenbendazole, ivermectin–clorsulon, and eprinomectin, together with the predominantly resistant classifications for albendazole, indicate substantially reduced field reliability of benzimidazole- and macrocyclic lactone-based treatments across the surveyed farms (Table 2). These findings are consistent with international evidence documenting reduced efficacy or resistance to these drug classes in GINs of goats across different production systems [7, 22, 23].

For benzimidazoles, previous molecular investigations have identified resistance-associated polymorphisms in the β-tubulin gene of important gastrointestinal strongyle nematodes, including *Haemonchus contortus* and *Trichostrongylus* spp. [16, 24, 25]. In Thailand, the F200Y substitution in the β-tubulin isotype 1 gene has been reported in *H. contortus* populations from goats, including populations sampled in Nakhon Ratchasima Province [26]. Additional field investigations have documented correspondence between low albendazole FECR values, persistence of *Haemonchus* and *Trichostrongylus* larvae after treatment, and elevated frequencies of F200Y resistance-associated alleles [11].

Although these previous findings provide a relevant biological context for the reduced efficacy of albendazole and fenbendazole observed in the present study, benzimidazole-associated β-tubulin mutations were not examined in the current parasite samples. Therefore, the present results cannot independently establish whether the observed treatment responses were attributable to the F200Y substitution or other resistance mechanisms.

The poor responses to ivermectin–clorsulon and eprinomectin similarly indicate reduced efficacy of the evaluated macrocyclic lactone-based treatments under the study conditions. However, the ivermectin–clorsulon formulation contained two active ingredients, and the study did not independently evaluate their contributions to treatment efficacy. Accordingly, the findings should be interpreted as responses to the administered formulation rather than as an isolated assessment of ivermectin.

Negative FECR values were recorded in several farm–drug combinations, particularly for ivermectin–clorsulon, albendazole, and fenbendazole (Table 2). These values indicate that arithmetic mean post-treatment egg counts exceeded the corresponding pretreatment values, as illustrated by the individual paired observations in Supplementary Figure S1. Negative FECR estimates should not be interpreted as evidence of a particular biological mechanism. Natural variation in egg shedding, reinfection pressure, and relatively low pretreatment egg counts may influence FECRT outcomes [14]. In addition, drug formulation, pharmacokinetic variability, and dosing or administration factors may affect apparent treatment efficacy [5, 7, 13]. Because these potential influences were not independently quantified, their contributions to the observed negative FECR values remain uncertain.

Levamisole efficacy and discordance between phenotypic and molecular findings

Levamisole demonstrated the most favorable phenotypic efficacy profile among the five evaluated anthelmintics. However, seven of the 13 farms (53.8%) were classified as resistant, four (30.8%) as susceptible, and two (15.4%) as inconclusive (Table 2). Therefore, despite comparatively better efficacy, levamisole cannot be considered uniformly reliable across the surveyed farms.

Previous investigations have similarly reported that levamisole may retain greater efficacy than benzimidazoles or macrocyclic lactones in certain goat populations, although levamisole resistance has also been documented in Thailand and other countries [8, 9, 21, 27, 28]. Additional *in vivo* FECRT evidence has demonstrated variation in levamisole efficacy among ruminant populations, including resistant strongyle populations in goats despite increased dosing [29]. These findings reinforce the need to confirm levamisole efficacy at the farm-level rather than assuming susceptibility based on its comparatively favorable performance elsewhere.

The molecular investigation provided additional information on levamisole-associated allele signals in *Haemonchus*-positive pooled egg samples. Susceptible-associated *Hco-acr-8* S168T signals were detected on all 13 farms, whereas resistant-associated signals were detected on 11 farms (Figure 1B). Consequently, co-detection of susceptible- and resistance-associated signals was the predominant molecular pattern. Representative allele-specific amplification results are presented in Figure 2.

Importantly, the molecular patterns did not consistently correspond with farm-level levamisole FECRT classifications. Farms G, L, and M were classified as susceptible despite co-detection of susceptible- and

resistance-associated signals. Conversely, Farm D was classified as resistant by FECRT but showed only the susceptible-associated signal (Figure 1B). These findings demonstrate that qualitative detection of the investigated *Hco-acr-8* allele signals did not uniformly distinguish susceptible from resistant farm-level treatment responses.

Levamisole resistance has been associated with genetic variation affecting nicotinic acetylcholine receptor-related genes and ACR-8-associated pathways [30–32]. However, previous investigations have indicated that the predictive relationship between the *Hco-acr-8* S168T marker and phenotypic levamisole resistance may vary among parasite populations [20, 31]. The discordant findings observed in the present study are consistent with the need for caution when interpreting this marker as an independent indicator of treatment failure. The *Hco-acr-8* S168T marker was not independently validated specifically for the parasite populations investigated in this study or as a predictive marker under the exact assay conditions used here.

Several possible explanations warrant consideration. First, FECRT based on strongyle-type eggs reflects the combined treatment-response of naturally occurring mixed nematode populations. Because *Haemonchus* and *Trichostrongylus* were detected on all farms (Figure 1A), the farm-level phenotypic response may have included contributions from nematodes not specifically assessed by the *Hco-acr-8* assay. Second, levamisole resistance may involve additional receptor-associated loci, altered gene expression, or other mechanisms not captured by the targeted S168T assay [31, 32]. However, these possibilities were not directly examined and cannot be established from the present findings.

Furthermore, molecular screening was qualitative and performed using farm-level pooled egg DNA. Co-detection of susceptible- and resistance-associated amplification products therefore indicates the presence of both molecular signals within the pooled material but does not establish individual-parasite heterozygosity, allele frequency, or the relative abundance of resistance-associated variants. The observed discordance may reflect the combined effects of mixed strongyle infections, pooled-sample analysis, qualitative molecular detection, and incomplete coverage of potential resistance mechanisms.

Taken together, these findings indicate that FECRT and targeted *Hco-acr-8* S168T screening provide complementary but noninterchangeable information. FECRT evaluates the overall *in vivo* treatment-response at the farm-level, whereas allele-specific PCR provides targeted information on the presence of a levamisole-associated molecular signal in *Haemonchus*-positive pooled egg DNA. The combined interpretation of these approaches may strengthen resistance surveillance, although the clinical predictive value of the investigated marker requires further validation.

Farm-level context and implications for sustainable parasite control

The observed treatment responses should also be considered in relation to the documented anthelmintic use histories of the participating farms. Previous anthelmintic-use history indicated that ivermectin was routinely used as the principal deworming drug on all 13 farms, whereas albendazole had been administered on some farms. No previous levamisole use was reported by farm owners or identified during on-farm verification. However, because complete longitudinal treatment histories were not available, unrecorded historical exposure, particularly before animals entered the participating farms, cannot be completely excluded.

Repeated ivermectin administration provides relevant management context for the reduced efficacy of the evaluated macrocyclic lactone-based treatments. Similarly, previous albendazole exposure may be relevant to interpreting the poor responses to benzimidazoles. Nevertheless, the study did not establish a causal relationship between historical drug exposure and the observed FECRT classifications. Other biological, pharmacological, and farm management factors may also have influenced treatment responses [7].

The lack of documented prior levamisole administration may explain its comparatively retained efficacy on some farms. However, resistant or inconclusive levamisole responses were still observed on nine farms (Table 2), indicating that documented treatment history alone did not explain the variation in efficacy.

The introduction of breeding or replacement animals from external sources has been identified as a potential pathway for introducing resistant parasite populations into livestock farms [20, 21, 23, 33, 34]. Although the present study did not evaluate animal movement, this mechanism may be relevant when considering resistance-associated molecular signals on farms without recorded exposure to a particular anthelmintic.

The detection of resistance-associated *Hco-acr-8* signals on some farms classified as phenotypically susceptible also highlights the limitations of interpreting molecular findings independently of treatment efficacy. Such signals may warrant further investigation, but their detection does not establish that phenotypic resistance

will subsequently develop. Longitudinal monitoring and quantitative molecular analysis are needed to determine whether particular allele signal patterns are associated with changes in levamisole efficacy over time.

From a practical perspective, the findings support a transition from routine empirical whole-herd deworming toward integrated, evidence-based parasite control. Appropriate strategies include accurate body weight-based dosing, avoidance of unnecessary repeated treatments, periodic FECRT monitoring, targeted selective treatment to maintain parasite refugia, and reassessment of anthelmintic efficacy when treatment failure is suspected [14, 35].

Where reduced or discordant treatment responses are identified, genus- or species-resolved parasitological examinations and molecular screening for resistance-associated markers may provide complementary information [10, 12, 22]. However, molecular screening should not replace direct assessment of treatment efficacy unless the predictive performance of the marker has been adequately established.

Nonchemical parasite management approaches may also be considered as components of integrated control programs. Previous investigations have reported anthelmintic or egg-reducing effects associated with *Azadirachta indica* supplementation and combinations of *Areca catechu* and *Piper betle* extracts in goats or gastrointestinal strongyle nematodes [36, 37]. Nevertheless, these interventions require appropriate validation and should be regarded as potential adjuncts rather than established substitutes for effective anthelmintic treatment.

Study limitations

Several limitations should be considered when interpreting the findings. First, FECRT was performed using strongyle-type fecal egg counts obtained from naturally infected goats. Although this approach reflects practical farm-level treatment responses, it does not distinguish the susceptibility of individual strongyle genera or species. Because *Haemonchus* and *Trichostrongylus* were detected across all participating farms (Figure 1A), the FECR estimates represent the combined response of potentially mixed nematode populations, without resolving the relative contribution of each genus.

Second, inconclusive FECRT classifications should be interpreted as uncertainty in efficacy rather than as a separate category of intermediate resistance. Under CI-based interpretation, inconclusive classifications arise when the available evidence does not support a definitive susceptible or resistant classification, particularly when FECR estimates approach decision thresholds or CIs are wide [14, 38, 39].

Treatment allocation was nonrandomized and depended on the availability of eligible goats within individual farms. A minimum pretreatment egg count of 50 EPG, relatively low baseline egg counts in some farm–drug combinations, small treatment groups, and natural variation in egg shedding may have influenced the precision of FECR estimates. Differences in pretreatment parasite burdens and group sizes should therefore be considered when comparing treatment responses among farm–drug combinations (Tables 1 and 2). No untreated control group was included; therefore, natural temporal variation in fecal egg counts over the 14-day interval could not be independently distinguished from treatment-related changes.

The numbers of evaluated goats and farm–drug combinations also differed among treatments, limiting direct comparisons of efficacy across drug classes. In addition, previous anthelmintic exposure was reconstructed from available farm records, which may not fully reflect treatments administered before animals entered the participating farms.

Third, the molecular investigation used qualitative allele-specific PCR on farm-level pooled egg DNA rather than individual-worm genotyping or quantitative assessment of allele frequencies. In addition, the farm-level pooled egg samples used for molecular screening were obtained from post-treatment collections using pragmatic sample selection and were not restricted to goats included in the corresponding levamisole FECRT groups; therefore, the molecular findings should be interpreted as qualitative farm-level signals rather than as direct treatment-specific or individual-level correlates of FECRT responses. Only the *Hco-acr-8* S168T locus was evaluated for levamisole-associated molecular variation. Consequently, the molecular findings cannot establish individual genotypes, quantify resistance-associated alleles, or exclude other genetic mechanisms contributing to reduced levamisole efficacy.

The targeted molecular assay also focused on *Haemonchus*-positive samples, whereas FECRT reflected the combined response of mixed strongyle populations. Resistance mechanisms in other nematode genera were therefore not assessed. In addition, potential pharmacological and field-related influences, including drug storage conditions, formulation differences, administration practices, plasma drug concentrations, and host pharmacokinetics, were not independently evaluated.

Finally, the study was conducted over a single field investigation period and did not include longitudinal follow-up to determine whether resistance-associated molecular signals preceded subsequent changes in phenotypic efficacy. The findings should therefore be generalized cautiously beyond the sampled smallholder goat farms in Nakhon Ratchasima Province.

CONCLUSION

This study demonstrated substantially reduced field efficacy of multiple anthelmintic treatments against gastrointestinal strongyle nematodes in smallholder meat goats in Nakhon Ratchasima Province, Thailand. Fenbendazole, ivermectin–clorsulon, and eprinomectin were classified as resistant on all evaluated farms, whereas albendazole was classified as resistant on nine farms and inconclusive on one farm. Levamisole showed comparatively better efficacy, with susceptible classifications on four farms, inconclusive classifications on two farms, and resistant classifications on seven farms. Molecular screening detected *Haemonchus* and *Trichostrongylus* on all 13 farms and *Oesophagostomum* on two farms. The susceptible-associated *Hco-acr-8* S168T allele signal was detected on all farms, whereas the resistant-associated signal was detected on 11 farms. However, these qualitative molecular patterns did not consistently correspond with farm-level levamisole FECRT classifications.

The findings highlight the need for farm-specific anthelmintic selection rather than routine empirical deworming. Levamisole may remain a treatment option where its efficacy has been confirmed, but its variable performance does not support indiscriminate use. Periodic FECRT monitoring, accurate body weight-based dosing, appropriate treatment records, and integrated parasite management strategies are essential for improving GIN control. Molecular screening may complement phenotypic efficacy assessment but should not independently determine treatment selection.

The principal strength of this study was the integration of field-based efficacy assessment across five anthelmintic treatments representing three major drug classes with genus-level molecular detection and targeted levamisole-associated allele screening. The inclusion of 423 goats from 13 smallholder farms provided region-specific evidence of variation in treatment responses under naturally occurring gastrointestinal strongyle infections. The use of paired fecal samples, farm-level FECR estimates, and CI-based efficacy classifications supported a systematic evaluation of anthelmintic performance.

Future investigations should include larger, geographically diverse goat populations, standardized treatment-group allocation, and longitudinal monitoring of anthelmintic efficacy. Species-resolved parasitological and molecular analyses, individual-parasite genotyping, and quantitative assessment of resistance-associated alleles are needed to clarify the relationship between molecular markers and phenotypic treatment responses. Further validation of *Hco-acr-8* S168T and additional levamisole-associated markers may improve their usefulness for resistance surveillance. Studies evaluating farm management practices, anthelmintic exposure histories, and integrated parasite control strategies would also support the development of more sustainable treatment programs.

The widespread reduction in anthelmintic efficacy observed across the surveyed farms underscores the need for evidence-based gastrointestinal parasite control in smallholder meat goats. Although levamisole demonstrated comparatively better efficacy, its variable farm-level performance and the incomplete correspondence between FECRT classifications and *Hco-acr-8* allele signal patterns emphasize that molecular detection cannot replace direct assessment of treatment response. Integrating routine efficacy monitoring with targeted molecular surveillance and sustainable parasite management offers a practical approach to improving anthelmintic stewardship in goat production systems.

DATA AVAILABILITY

The supplementary materials supporting this study, including Supplementary Tables S1–S2 and Supplementary Figure S1, have been deposited in the Zenodo repository and are available at https://doi.org/10.5281/zenodo.23099123.

GENERATIVE AI DECLARATION

The authors declare that generative artificial intelligence (AI) tools were used solely to improve the language, grammar, and readability of the manuscript. All scientific content, data analyses, interpretation of results, and

conclusions were developed and verified by the authors. The authors take full responsibility for the accuracy, integrity, and originality of the manuscript. No AI tool was listed as an author.

AUTHORS' CONTRIBUTIONS

PS: Conceptualization, methodology, investigation, data curation, formal analysis, visualization, software, writing – original draft, and writing – review and editing. UT: Supervision, methodology, validation, and writing – review and editing. AHEC: Methodology, investigation, validation, and writing – review and editing. PP: Supervision, validation, investigation, and writing – review and editing. All authors have read and approved the final version of the manuscript.

ACKNOWLEDGMENTS

The authors thank the participating goat farmers for their cooperation and assistance during farm visits and sample collection. The authors also thank Mr. Jirayu Yuyen for his leading role in fecal sample processing and microscopic parasitological examinations, as well as the field staff, laboratory personnel, and students who assisted with farm coordination, sample collection, and laboratory procedures. This research was supported by Suranaree University of Technology through the Research Brotherhood Scholarship (Grant No. BRO3-303-67-12-03).

COMPETING INTERESTS

The authors declare that they have no competing interests.

PUBLISHER'S NOTE

Veterinary World remains neutral with regard to jurisdictional claims in the published institutional affiliations.

REFERENCES

1. Hegde NG. Goat development: an opportunity to strengthen rural economy in Asia and Africa. Asian J Res Anim Vet Sci. 2020.
2. Liang JB, Paengkoum P. Current status, challenges and the way forward for dairy goat production in Asia – conference summary of dairy goats in Asia. Asian-Australas J Anim Sci. 2019;32:1233.
3. Windsor PA, Nampanya S, Putthana V, Keonam K, Johnson K, Bush RD, *et al*. The endoparasitism challenge in developing countries as goat raising develops from smallholder to commercial production systems: a study from Laos. Vet Parasitol. 2018;251:95–100.
4. Department of Livestock Development (DLD). Livestock report in Thailand 2025 [Internet]. Bangkok: Information and Communication Technology, Department of Livestock Development, Thailand; 2025 [cited 28 Oct 2025]. Available: https://datahub.dld.go.th/datahub/index.php/data-service/book/livestock-book/2568.
5. Zajac AM, Garza J. Biology, epidemiology, and control of gastrointestinal nematodes of small ruminants. Vet Clin North Am Food Anim Pract. 2020;36:73–87.
6. Emery DL, Hunt PW, Le Jambre LF. *Haemonchus contortus*: the then and now, and where to from here? Int J Parasitol. 2016;46:755–769.
7. Baudinette E, O'Handley R, Trengove C. Anthelmintic resistance of gastrointestinal nematodes in goats: a systematic review and meta-analysis. Vet Parasitol. 2022;312:109809.
8. Ratanapob N, Thuamsuwan N, Thongyuan S. Anthelmintic resistance status of goat gastrointestinal nematodes in Sing Buri Province, Thailand. Vet World. 2022;15:83.
9. Chan AHE, Kaenkaew C, Pakdee W, Sungpradit S, Thaenkham U. Emergence of dual drug-resistant strongylids in goats: first phenotypic and genotypic evidence from Ratchaburi Province, central Thailand. BMC Vet Res. 2025;21:1–12.
10. Rerkyusuke S, Lamul P, Thipphayathon C, Kanawan K, Porntrakulpipat S. Caprine roundworm nematode resistance to macrocyclic lactones in northeastern Thailand. Vet Integr Sci. 2023;21:623–634.
11. Sontigun N, Sansamur C, Klong-Klaew T, Mektrirat R, Kaewthamasorn M, Fungwithaya P. Field-based and molecular evaluation of anthelmintic resistance in gastrointestinal strongyle nematodes of meat goats in southern Thailand. Vet World. 2025;18:2467.

12. Jackson F, Varady M, Bartley DJ. Managing anthelmintic resistance in goats—can we learn lessons from sheep? Small Rumin Res. 2012;103:3–9.
13. Lifschitz A, Lanusse C, Alvarez L. Host pharmacokinetics and drug accumulation of anthelmintics within target helminth parasites of ruminants. N Z Vet J. 2017;65:176–184.
14. Kaplan RM, Denwood MJ, Nielsen MK, Thamsborg SM, Torgerson PR, Gilleard JS, *et al*. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guideline for diagnosing anthelmintic resistance using the faecal egg count reduction test in ruminants, horses and swine. Vet Parasitol. 2023;318:109936.
15. Rompo T, Hayashi N, Hoketsu T, Teo E, Nonaka N, Namboopha B, *et al*. Effectiveness of eprinomectin, albendazole and their combination therapy against strongyle nematode in dairy goats: a clinical field study using nemabiome-integrated approach in Thailand. Curr Res Parasitol Vector Borne Dis. 2026;9:100345.
16. Guinda EFX, Afonso SMS, Fiedler S, Morgan ER, Ramünke S, Borchert M, *et al*. Efficacy of fenbendazole against gastrointestinal nematodes in naturally infected goats in Maputo Province, Mozambique using *in vivo*, *in vitro* and molecular assessment. Int J Parasitol Drugs Drug Resist. 2025;27:100572.
17. Sota P, Andityas M, Suyapoh W, Tangkawattana S. Comparative epidemiology of gastrointestinal parasites in cattle and buffalo under smallholder systems in Thailand. Vet Parasitol. 2026;345:110801.
18. Income N, Tongshoob J, Taksinoros S, Adisakwattana P, Rotejanaprasert C, Maneekan P, *et al*. Helminth infections in cattle and goats in Kanchanaburi, Thailand, with focus on strongyle nematode infections. Vet Sci. 2021;8:324.
19. Chan AHE, Chaisiri K, Morand S, Saralamba N, Thaenkham U. Evaluation and utility of mitochondrial ribosomal genes for molecular systematics of parasitic nematodes. Parasit Vectors. 2020;13:364.
20. Baltrušis P, Halvarsson P, Charvet CL, Höglund J. The presence and relative frequency detection of the levamisole-resistance-associated S168T substitution in *hco-acr-8* in *Haemonchus contortus*. Int J Parasitol Drugs Drug Resist. 2023;21:91–95.
21. Chan AHE, Thaenkham U, Kaenkaew C, Pakdee W, Sungpradit S. Hybridization, high genetic diversity, and molecular evidence of drug resistance to albendazole and levamisole in *Haemonchus* populations infecting goats in Thailand. BMC Vet Res. 2025;21:679.
22. Mohammedsalih KM, Khalafalla A, Bashar A, Abakar A, Hessain A, Juma FR, *et al*. Epidemiology of strongyle nematode infections and first report of benzimidazole resistance in *Haemonchus contortus* in goats in South Darfur State, Sudan. BMC Vet Res. 2019;15:184.
23. Mickiewicz M, Czopowicz M, Moroz A, Szaluś-Jordanow O, Górski P, Várady M, *et al*. Development of resistance to eprinomectin in gastrointestinal nematodes in a goat herd with pre-existing resistance to benzimidazoles. Pol J Vet Sci. 2019;22:753–760.
24. Williamson SM, Storey B, Howell S, Harper KM, Kaplan RM, Wolstenholme AJ. Candidate anthelmintic resistance-associated gene expression and sequence polymorphisms in a triple-resistant field isolate of *Haemonchus contortus*. Mol Biochem Parasitol. 2011;180:99–105.
25. Mottier MDL, Prichard RK. Genetic analysis of a relationship between macrocyclic lactone and benzimidazole anthelmintic selection on *Haemonchus contortus*. Pharmacogenet Genomics. 2008;18:129–140.
26. Pitaksakulrat O, Chaiyasaeng M, Artchayasawat A, Eamudomkarn C, Thongsahuan S, Boonmars T. The first molecular identification of benzimidazole resistance in *Haemonchus contortus* from goats in Thailand. Vet World. 2021;14:764.
27. Mickiewicz M, Czopowicz M, Moroz A, Potărniche AV, Szaluś-Jordanow O, Spinu M, *et al*. Prevalence of anthelmintic resistance of gastrointestinal nematodes in Polish goat herds assessed by the larval development test. BMC Vet Res. 2021;17:19.
28. Mortensen LL, Williamson LH, Terrill TH, Kircher RA, Larsen M, Kaplan RM. Evaluation of prevalence and clinical implications of anthelmintic resistance in gastrointestinal nematodes in goats. J Am Vet Med Assoc. 2003;223:495–500.
29. Mohammedsalih KM, Ibrahim AIY, Juma FR, Abdalmalaik AAH, Bashar A, von Samson-Himmelstjerna G, *et al*. Variability in the anthelmintic efficacy of levamisole against gastrointestinal nematodes of cattle, sheep and goats in South Darfur, Sudan. BMC Vet Res. 2026;22:128.
30. Antonopoulos A, Charvet CL, Maitland K, Doyle SR, Neveu C, Laing R. Functional validation of novel levamisole resistance marker S168T in *Haemonchus contortus*. Int J Parasitol Drugs Drug Resist. 2024;24:100524.
31. Rodrigues JFV, Monteiro JP, Almeida TM, Molento MB. A systematic review of the molecular mechanisms related to anthelmintic resistance in *Haemonchus contortus*: a contemporary narrative. Vet Parasitol. 2025; 334:110394.

32. Sarai RS, Kopp SR, Coleman GT, Kotze AC. Acetylcholine receptor subunit and P-glycoprotein transcription patterns in levamisole-susceptible and -resistant *Haemonchus contortus*. Int J Parasitol Drugs Drug Resist. 2013;3:51–58.
33. Francis EK, Antonopoulos A, Westman ME, McKay-Demeler J, Laing R, Šlapeta J. A mixed amplicon metabarcoding and sequencing approach for surveillance of drug resistance to levamisole and benzimidazole in *Haemonchus* spp. Int J Parasitol. 2024;54:55–64.
34. Beleckė A, Kupčinskas T, Stadalienė I, Höglund J, Thamsborg SM, Stuen S, *et al*. Anthelmintic resistance in small ruminants in the Nordic-Baltic region. Acta Vet Scand. 2021;63:18.
35. Kenyon F, Greer AW, Coles GC, Cringoli G, Papadopoulos E, Cabaret J, *et al*. The role of targeted selective treatments in the development of refugia-based approaches to the control of gastrointestinal nematodes of small ruminants. Vet Parasitol. 2009;164:3–11.
36. Sungpradit S, Leesombun A, Chanakarn C, Nakthong C, Boonmasawai S. Anthelmintic effects of *Areca catechu* L. (Arecaceae) and *Piper betle* L. (Piperaceae) combination on adult *Haemonchus* spp.: a scanning electron microscopy study. BMC Vet Res. 2025;21:491.
37. Archa P, Sota P, Paengkoum P. Impact of dietary purple neem (*Azadirachta indica*) leaves on growth, ability to neutralize free radicals and helminth control in meat goats. Front Vet Sci. 2026;13:1769618.
38. Levecke B, Dobson RJ, Speybroeck N, Vercruysse J, Charlier J. Novel insights in the faecal egg count reduction test for monitoring drug efficacy against gastrointestinal nematodes of veterinary importance. Vet Parasitol. 2012;188:391–396.
39. Denwood MJ, Kaplan RM, McKendrick IJ, Thamsborg SM, Nielsen MK, Levecke B. A statistical framework for calculating prospective sample sizes and classifying efficacy results for faecal egg count reduction tests in ruminants, horses and swine. Vet Parasitol. 2023;314:109867.

****