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Strongyle nematode fauna in three ruminants in upper northern Thailand

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Abstract

Gastrointestinal parasites, particularly strongyle nematodes, pose a significant threat to the health of ruminants. Due to the technical limitations of microscopic and conventional PCR-based methods, the strongyle parasite fauna has not been well studied even in common livestock animals. The present study aimed to investigate the prevalence and diversity of strongyle nematodes in three ruminant species in northern Thailand using a nemabiome approach. Fecal samples were collected from buffaloes, cattle, and goats that were raised for meat and for dairy in four provinces in northern Thailand. Strongyle infections were determined using egg flotation and McMaster techniques followed by DNA metabarcoding for species identification. The results showed high prevalence of strongyles especially in goats raised for meat (88 %), and in goats raised for dairy (72 %). Significantly more goats and cattle raised for meat were strongyle egg-positive compared to their dairy counterparts. Notably, deworming frequency was not significantly associated with strongyle egg-positivity in all ruminant groups apart from dairy goats. Nemabiome analysis identified 11 strongyle species across seven genera. Among the ruminant hosts, beef cattle exhibited the highest strongyle richness. Additionally, the dominance of specific strongyle species influenced the differences observed in diversity indices. This research is the first to apply the nemabiome approach to assess strongyle nematode diversity in northern Thailand, providing valuable insights into nematode community compositions. These findings emphasize the importance of molecular techniques for parasite monitoring and the development of targeted control strategies.

Keywords: Buffalo, Cattle, Goat, Nemabiome, Northern Thailand, Strongyle nematodes, Strongyle diversity

Data availability

The raw sequence data have been deposited in the DNA Data Bank of the Japan Sequence Read Archive under the accession number DRA PRJDB19829.

51 **1 Introduction**

52 Gastrointestinal (GI) parasites, particularly strongyle nematodes, pose significant concerns for the
53 health and productivity of ruminants. The health impacts of GI parasites vary among ruminant
54 species. For instance, *Haemonchus contortus*, the barber's pole worm, is especially pathogenic to
55 goats, leading to severe anemia, weight loss, and mortality [1,2]. *Trichostrongylus* spp. affect both
56 cattle and goats, causing gastrointestinal disturbances and reduced nutrient absorption, which hinder
57 their growth and productivity [1,3]. Additionally, *Oesophagostomum* spp., although less prevalent, can
58 result in chronic diarrhea and weight loss in cattle [1]. These health challenges translate into
59 substantial economic losses for farmers; a recent systematic review estimated that GI parasites cause
60 an annual loss of US\$ 8.5 billion in the USA due to reduced weight gain and increased mortality rates
61 [4], highlighting the need for effective management strategies.

62 The prevalence of GI parasites, particularly strongyle nematodes, varies significantly among
63 ruminant species in Thailand, influenced by regional differences and management practices. A recent
64 study conducted in Mahasarakam detected strongyle eggs in 28 % of the tested buffaloes by
65 microscopic examination [5]. In beef cattle, the prevalence of strongyle eggs detected by microscopic
66 examination ranged from 27 % to 82 % across Thailand [6–9], while in dairy cattle the prevalence was
67 approximately 8 % in Nakhon Ratchasima in 2024 [10]. Goats exhibited higher prevalence of strongyle
68 eggs than cattle, with herd prevalence reaching up to 100 % in certain areas [9,11–13].

69 Previous reports on strongyle nematodes in Thailand relied primarily on traditional techniques,
70 including microscopic detection of eggs followed by larval culture [8,12,13]. These microscopic
71 techniques can lead to misidentification, particularly when species exhibit overlapping morphological
72 features. Although several studies have utilized molecular methods such as conventional polymerase
73 chain reaction (PCR) and Sanger sequencing [9,10,13,14], these methods could only identify select
74 strongyle genera, which potentially led to an underestimation of the prevalence and diversity of the
75 parasite fauna in these host animals.

The nemabiome analysis, a metabarcoding approach to sequence the internal transcribed spacer 2 (ITS-2) of ribosomal DNA (rDNA) of strongyle nematodes, represents a significant advancement in the accuracy and robustness of nematode species identification [15]. By utilizing high-throughput sequencing technologies, this method overcomes the shortcomings of conventional diagnostics, providing a more reliable and comprehensive understanding of nematode communities in various hosts, including cattle, sheep, goats, and horses [15–18]. This study aims to address the existing knowledge gap by conducting an in-depth analysis of the strongyle nematode fauna in buffaloes, cattle, and goats in upper northern Thailand.

2 Methodology

2.1 Fecal sampling from animals and questionnaire

To investigate the prevalence of strongyle nematodes, rectal fecal sampling was conducted randomly on 46 ruminant farms located in the provinces: Chiang Mai, Chiang Rai, Lamphun, and Lampang (Fig. 1). A total of 518 fecal samples were collected from beef buffaloes (*Bubalus bubalis*; 82 individuals from 10 farms), dairy buffaloes (*Bubalus bubalis*; 57 individuals from 2 farms), beef cattle (*Bos indicus*; 102 individuals from 18 farms), dairy cattle (*Bos taurus*; 40 individuals from 1 farm), meat goats (*Capra hircus*; 68 individuals from 10 farms), and dairy goats (*Capra hircus*; 169 individuals from 5 farms). The samples were collected between 2022 and 2024 from animals that had not been dewormed in the past two months (Table S1).

To aid in our understanding of the factors that might lead to increased prevalence of strongyle nematodes in ruminants, farm-level data were collected via a questionnaire. These data included the purpose of the animal in question (meat or dairy), the raising system (indoors, free-ranging, or free-roaming) and deworming practices.

2.2 Fecal examination

Strongyle nematode eggs in fecal samples were examined through a centrifugal flotation technique. Ten grams of feces were mixed with distilled water, passed through a sieve, and centrifuged at $155 \times g$ for 10 min. The supernatant was discarded, and 14.5 ml of a saturated NaCl solution (specific gravity = 1.2) was used to resuspend the sediment. The suspension was then centrifuged at $155 \times g$ for 10 min. After centrifugation, drops of the saturated NaCl were added until a slightly inverted positive meniscus formed. A coverslip was then placed on top of the tube, and the sample was allowed to stand for 10 min. The coverslips were examined under a microscope to detect strongyle nematode eggs. Samples that were positive from the centrifugal flotation were subsequently analyzed using the modified McMaster technique [19]: two grams of feces were mixed with 58 ml of saturated NaCl solution and 0.15 ml of the suspension was loaded into each chamber of the McMaster slide. Strongyle eggs in both chambers of the McMaster slide were subsequently counted under a light microscope. Due to the limited amount of feces collected from goats, only the modified McMaster technique was used to detect and count strongyle eggs. As the modified McMaster technique has a sensitivity of >100 eggs per gram (EPG), we regarded $EPG \geq 100$ as an indicator of strongyle egg-positivity.

2.3 Genomic DNA extraction

Samples with $EPG \geq 200$ were randomly selected to identify strongyle nematode species by molecular analysis. For meat buffaloes, fecal samples with $EPG \geq 100$ were included in the molecular analysis due to the low number of positive samples. On the contrary, because many goats were positive, samples for genomic DNA extraction were intentionally selected to compare the differences between dairy and meat goats, as well as among different locations.

The parasite eggs of the selected samples were purified using the following protocols. One gram of feces was first mixed with 10 ml of water and filtered through a tea strainer. The filtrate was transferred to a 15 ml tube and centrifuged at $966 \times g$ for 5 min. After discarding the resultant supernatant, the sediment was transferred to a 1.5 ml tube and centrifuged at 13,000

×g for 10 min. The supernatant was removed using a micropipette, and the remaining sediment containing parasite eggs (approximately 250 mg) was used for DNA extraction with the DNeasy PowerSoil Pro Kit (Qiagen, Mo Bio Laboratories, Hilden, Germany) following the manufacturer's protocol.

2.4 DNA metabarcoding for strongyle nematode

PCR amplification of the ITS-2 rDNA locus of strongyle nematodes was performed on 73 DNA samples and 3 no-template negative controls using the primers tagged with an Illumina adapter: NC1_ON (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACGTCTGGTTCAGGGTTGTT-3') and NC2_ON (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTTAGTTTCTTTTCCTCCGCT-3'). The amplification reaction included 0.9 µl of each primer (10 µM), 15.0 µl of 2 × KAPA HiFi HotStart ReadyMix (Roche Molecular Systems, Pleasanton, California, USA), and 2.0 µl of template DNA in a reaction volume of 30.0 µl. The thermocycling conditions were: an initial denaturation at 95 °C for 2 min, followed by 30 cycles of 98 °C for 20 s, 62 °C for 15 s, and 72 °C for 15 s, with a final extension at 72 °C for 2 min. The PCR products were purified using AMPure XP beads (Beckman Coulter Inc., Brea, CA, USA) and then used as template for the index PCR using the Nextera XT index kit (Illumina, San Diego, CA, USA). After purification with AMPure XP beads, the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) was used to measure the DNA concentration of the indexed PCR products. The average length of the amplicons was measured using the High Sensitivity D5000 ScreenTape on a TapeStation system (Agilent Technologies, Santa Clara, CA, USA). The Illumina libraries were pooled with a concentration of 4 nM and sequenced on an Illumina MiSeq system using the MiSeq Reagent Kit v3 (600 cycles).

2.5 Bioinformatics analysis

The raw sequencing data were processed using the DADA2 plugin [20] in QIIME2 version 2024.5.0 [21] for demultiplexing, primer removal, denoising, and constructing the feature table.

The decontam R package [22] was employed to identify and remove potential contaminant amplicon sequence variants (ASVs) from the dataset, thereby minimizing PCR or sequencing errors. ASVs were clustered into operational taxonomic units (OTUs) with representative sequences for nemabiome analysis using *de novo* clustering commands based on a 97.0 % similarity threshold. The representative OTUs and feature table were exported for further analyses.

The taxonomic assignments of OTUs were performed using the IDTaxa function in the DECIPHER package [23] in R with a >60.0 % confidence score using the ITS-2 nemabiome database (www.nemabiome.ca) [24]. OTUs unclassified or identified only to the genus level by IDTaxa were further analyzed using standalone BLASTn against the ITS eukaryote sequence database [25]; OTUs that had >90.0 % identity and >80.0 % coverage to a nematode reference sequence received the taxonomic assignment [26]. OTUs that were not identified by either of the methods were labeled as unknown. All non-target sequences and OTUs with fewer reads than those in the negative control samples were excluded.

The following diversity indices were calculated using the diversity function from the vegan package [27]: the richness of strongyle taxa (the count of positive taxa in each sample), the Shannon index (a measure of species richness and evenness), and the inverse Simpson index (a measure highlighting the impact of dominance on diversity).

2.6 Statistical analysis

Descriptive statistics were used to summarize the variables from the questionnaire and the results of microscopic fecal examination. Univariate analysis of factors associated with strongyle egg positivity was conducted using a Chi-square test or Fisher's exact test (when $n \leq 5$). We considered a *p*-value of < 0.05 to indicate statistical significance.

Due to the high prevalence of strongyle nematodes in goats, we wanted to determine whether any farm-level factors, or any combination of farm-level factors could explain strongyle

infections. We explored two thresholds, an EPG ≥ 100 (McMaster technique sensitivity threshold), and an EPG ≥ 750 , which is the EPG threshold for targeted deworming in goats [28,29]. To investigate the potential effects of these factors on EPG thresholds, generalized linear binomial models were constructed with purpose (dairy or meat) and deworming frequency classification (once to twice a year; three or more times a year) as independent, and as interaction terms (i.e. variables). Model fit was evaluated using the deviance explained (1-residual deviance/null deviance); with values closer to 1 indicating that the model was able to explain a greater proportion of the variation in the observed data. The models were fitted in R.

To compare diversity indices across multiple groups and sampling locations, the Kruskal-Wallis test was employed, whereas the Mann-Whitney *U* test was utilized to compare diversity indices between the two category groups. Samples with incomplete data for specific factors were excluded from the analysis related to those factors to ensure the accuracy of the results. The analyses were performed using R version 4.4.1 (2024-06-14) for Windows (R Core Team, 2024).

3 Results

3.1 The strongyle parasites detected by microscopic fecal examination

Out of 281 fecal samples collected from beef buffalo, dairy buffalo, beef cattle, and dairy cattle, 115 tested positives for strongyle eggs using the flotation method (Table 1). Further examination using McMaster fecal egg counts detected strongyle eggs in 5 samples from beef buffaloes and 32 samples from beef cattle, respectively. No strongyle eggs were detected in dairy buffaloes or dairy cattle by McMaster. For meat and dairy goats, only McMaster fecal egg counts were performed due to the limited availability of fecal material. Out of 237 fecal samples, 60 (88 %) meat goats and 122 (72 %) dairy goats were positive for strongyle eggs. The mean EPG $\pm$ standard deviation for each host group was as follows: beef buffaloes (46 ± 52), beef cattle (170 ± 439), meat goats ($1,304 \pm 1,672$), and dairy goats ($930 \pm 1,645$). The EPG of the samples used for

the nemabiome analysis is shown in Fig. 2a. The microscopic fecal egg counts from each sampling location are provided in Table S2.

3.2 Factors associated with strongyle parasite infections

The presence of strongyle eggs was significantly associated with the rearing purpose of cattle ($p < 0.001$) and goats ($p = 0.013$), but not with buffaloes ($p = 0.337$) (Table 2). Specifically, beef cattle had significantly higher proportions of strongyle egg-positive samples than dairy cattle whereas dairy goats had significantly higher proportions of strongyle egg-positive samples compared to the meat goats (Table 2).

By province, the strongyle egg positivity in beef cattle differed significantly ($p = 0.001$). Pairwise comparisons between the three provinces, however, showed that a significant difference was found only between Chiang Mai and Chiang Rai ($p = 0.002$); there was no significant difference between Chiang Mai and Lamphun, and between Lamphun and Chiang Rai ($p > 0.05$). Likewise, for meat goats, there was no significant difference between goats from Chiang Mai and from Lampang ($p = 0.124$) (Table 2). Differences among provinces where the farms were located for buffaloes, dairy cattle, and dairy goats were not analyzed as all these farms were from Chiang Mai.

Differences in strongyle egg positivity among districts were observed for beef buffaloes and dairy goats, but not for dairy buffaloes and dairy cattle, as these two groups were from a single district. A significant difference in strongyle egg positivity was found among the four districts in Chiang Mai where beef buffaloes were raised ($p < 0.001$), with notable differences between Sankampaeng and Doisaket ($p < 0.001$), as well as Sankampaeng and Maeon ($p < 0.001$). Additionally, strongyle egg positivity in dairy goats varied by farm location, with dairy goats from Doisaket showing a higher probability of testing positive compared to those from Maeon ($p < 0.001$) (Table 2).

In beef buffaloes, free-roaming systems were likely associated with a lower strongyle-positive proportion compared to free-ranging systems ($p = 0.008$); however, we did not find any significant difference in strongyle egg positivity by raising system in beef cattle, and dairy goats ($p > 0.05$). Additionally, since all dairy buffaloes, dairy cattle, and meat goats were raised in a single system, these groups could not be analyzed.

Notably, we did not find any significant difference in deworming frequency among all ruminant groups, other than in dairy goats, where goats dewormed three or more times a year had significantly higher proportions of strongyle egg-positive samples compared to goats that were only dewormed once to twice a year. Furthermore, for dairy goats, those dewormed three or more times per year were significantly more likely to exceed the EPG threshold of 750 ($p = 0.001$) (Table S3), indicating that frequent deworming was associated with a higher proportion of samples exceeding the EPG threshold.

The model specified to investigate the effects of purpose (i.e. meat versus dairy) of goats and deworming frequency on strongyle egg-positivity ($EPG \geq 100$) explained 0.13 of the deviance in strongyle egg-positivity. In other words, only 13 % of the variation was accounted for by the model. On the other hand, the model specified to investigate whether these aforementioned factors affected the probability that a goat met the EPG threshold for targeted deworming ($EPG \geq 750$) explained 0.04 of the deviance; i.e., only 4 % of the variation was accounted for by the model.

3.3 Nemabiome analysis

A total of 16, 28, and 24 samples with $EPG \geq 200$ were randomly selected from beef cattle, and meat goats and dairy goats, respectively. For beef buffaloes, five samples with $EPG \geq 100$ were included in the analyses. The mean and standard deviation of reads per sample from the Illumina sequencing analysis was $24,184 \pm 18,073$. One buffalo sample was excluded due to a low read count (31 reads). After the denoising process, clustering, and exclusion of contaminants, 33

OTUs were taxonomically assigned, resulting in 11 species and one unclassified species from the genus *Trichostrongylus*. The list of strongyle nematodes detected is shown in Table 3.

3.4 The prevalence of strongyle nematode species across different hosts

Cooperia pectinata was detected in all samples from beef buffaloes and beef cattle, but was rarely found in meat and dairy goats. However, *Trichostrongylus colubriformis* and *Haemonchus contortus* were the two most prevalent nematode species in meat and dairy goats. Additionally, *H. contortus*, *Mecistocirrus digitatus* and *T. colubriformis* were positive in 50 % of beef buffalo samples. In beef cattle, *Oesophagostomum radiatum*, *Trichostrongylus axei*, *M. digitatus*, and *H. contortus* had a positive rate of 75 %, 69 %, 69 %, and 31 %, respectively. In addition, *Oesophagostomum columbianum* and *T. axei* were also observed in meat goats and dairy goats, respectively (Table 3).

3.5 Strongyle nematode composition and diversity

The composition of strongyle parasite communities in each host is depicted in Fig. 2. In beef buffaloes, *C. pectinata* was dominant. In beef cattle, *C. pectinata* and *M. digitatus* collectively accounted for more than 80 % of the parasite fauna. The parasite communities in meat and dairy goats were dominated by two species: *T. colubriformis* and *H. contortus*, with sporadic occurrence of *O. columbianum* in meat goats (Table S4).

Alpha diversity indices were calculated to assess the variety and abundance of strongyle nematode species in each sample, allowing for the comparisons of nematode communities among ruminant hosts. The Kruskal-Wallis and Dunn's tests identified significant differences in richness, and Shannon and Simpson indices across the three ruminant hosts (Fig. 3a). Beef cattle exhibited the highest richness ($\chi^2 = 19.51$, $p < 0.001$) and Shannon diversity ($\chi^2 = 18.07$, $p < 0.001$), while dairy goats showed the lowest values. Additionally, Simpson diversity was significantly different between animals ($\chi^2 = 16.43$, $p < 0.001$), with meat goats displaying higher

indices than dairy goats. The Mann-Whitney U test revealed no significant differences in alpha diversity measures between beef cattle samples from the two sampling locations (Doitao and Li district) (Richness: $p = 0.603$; Shannon: $p = 0.273$; Simpson: $p = 0.561$) (Fig. 3b). Similarly, no significant differences were observed across the three sampling locations (Maechaem, Maeon, and Doisaket) in meat goats, as indicated by the Kruskal-Wallis test (Richness: $p = 0.103$; Shannon: $p = 0.625$; Simpson: $p = 0.677$) (Fig. 3c). In contrast, nematode communities in dairy goats differed significantly between Doisaket and Maeon. Richness was significantly higher in Doisaket ($W = 108.5$, $p = 0.031$), and both Shannon ($W = 112$, $p = 0.020$) and Simpson indices ($W = 113$, $p = 0.018$) were also significantly greater in Doisaket compared to Maeon (Fig. 3d).

4 Discussions

The present study reported the prevalence of strongyle nematodes in three ruminant hosts across four provinces in northern Thailand. A high prevalence of strongyle parasites was observed in goats, which is comparable to previous studies conducted in the lower northern region of Thailand (76.8 %) [13] and the southern region of Thailand [11]. Furthermore, our results align with previous reports from the northeastern part of the country, which found that beef cattle had higher parasite prevalence compared to dairy cattle [10], and that beef cattle had higher parasite prevalence than beef buffaloes [5].

A statistically significant association was observed between location and strongyle egg positivity in dairy goats in two districts, Doisaket and Maeon. However, this may be confounded by other factors such as the deworming practices, which showed significant differences in our Chi-square and Fisher's exact tests (Table 2). Indeed, nearly all farms in Doisaket and Maeon followed a similar raising system (free-range grazing), except for one farm in Doisaket that always kept goats indoors. One farm in Doisaket also raised both meat and dairy goats whereas all other farms in Doisaket and all farms in Maeon raised goats strictly for dairy. Another differentiating factor between the two locations was the deworming frequency. In Doisaket, two farms dewormed their goats three times or more per year

whereas three farms in Maeon dewormed their goats only twice per year. The potential confounding effect of deworming practices suggests that location alone may not fully explain the observed differences in strongyle egg positivity between the two districts.

Indeed, although we attempted to fit a generalized linear model to explain strongyle egg-positivity, our model accounted for 13% of the variation, suggesting that much of the variation was unexplained. Further analysis is needed to determine which other human and/or natural factors may contribute to strongyle egg-positivity in these ruminants. For beef cattle, the parasite prevalence was not significantly different between Chiang Mai (Doitao) and Lamphun (Li) nor between Lamphun and Chiang Rai (Maesuai), but was significantly different between Chiang Mai and Chiang Rai (Table 2). A study conducted in 1998 documented variations in nematode egg counts in cattle across provinces in Thailand, including Lampang and Chiang Rai, suggesting that local climatic conditions significantly influence nematode populations [8]. Our study echoes this finding.

The factors associated with the strongyle infection results align with a previous study in Kanchanaburi, which found an association between GI parasite infection and the raising purpose (meat and dairy goats, as well as beef and dairy cattle), noting that farm management intensity and deworming frequency played significant roles [9]. Previous recommendations for targeted selective deworming in goats under tropical conditions suggest using an EPG threshold of ≥ 750 to reduce the frequent use of anthelmintics[28,29]. However, in our study, this EPG cutoff was significantly associated with categorical deworming frequencies (1-2 times vs. three times or more per year) within the dairy goat group. Interestingly, strongyle positivity was higher in goats dewormed three times or more annually. These findings highlight the need for further investigation into the appropriate EPG thresholds and the impact of strongyle prevalence in this area. Additionally, a previous study found that deworming program was associated with the prevalence of GI parasites in beef cattle, with twice-yearly deworming resulting in higher prevalence compared to deworming every 3 months [7]. In our study, however, most beef cattle farms practiced deworming only once to twice per year, and

therefore, the effect of the deworming program on the parasite prevalence in cattle was not evaluated.

Due to the morphological similarities of strongyle parasite eggs among species, the identification of strongyle species has traditionally relied on the morphological identification of larvae cultured from eggs. Previous studies have employed this larval culture technique to identify the prevalent strongyle species in Thailand [8,13]. This method, however, is time-consuming and requires expert taxonomic skills, limiting the extent of its application. Although PCR-based methods offer an alternative, they are unable to detect non-target species and resolve mixed infections, preventing comprehensive parasite detection. As a result, previous molecular studies on the identification of strongyle species in Thailand have only identified a limited number of species, primarily because only certain species-specific primers were used [9,10]. Subsequently, the diversity of strongyle parasite fauna has not been well studied, even in common livestock animals, until recent years. Thanks to the advancement of high-throughput sequencing technologies, an increasing number of studies have utilized a metabarcoding approach to reveal the strongyle parasite fauna in various livestock animals such as horses [17], cattle [30], sheep [31], and goats [32] .

By utilizing DNA metabarcoding, our study provides a detailed and comprehensive overview of the nematode community in each host species. In cattle, our results indicate a broad range of strongyle genera (Table 3), which are largely consistent with previous reports. A study conducted in Thailand using larval culture with morphological identification detected *Trichostrongylus*, *Cooperia*, *Oesophagostomum*, and *Bunostomum* spp.[8]. Similarly, studies utilizing molecular techniques with species-specific primers identified *M. digitatus* [14], *H. contortus* [10], *Haemonchus*, *Cooperia*, *Oesophagostomum*, and *Trichostrongylus* spp. [9], findings that align with our study. A study on dairy and beef cattle in northeastern Thailand, however, reported *Ostertagia ostertagi* as the most prevalent parasite [10], a species that was not detected in our study, suggesting potential regional variation in nematode occurrence and/or prevalence.

In goats, our study identified *Haemonchus*, *Trichostrongylus*, *Cooperia*, and *Oesophagostomum* spp., which aligns with a previous study conducted in Thailand that utilized species-specific PCR [9]. Additionally, our findings are consistent with Wuthijaree et al. (2022) who reported the prevalence of *H. contortus* and *Trichostrongylus* spp. through microscopic larval identification. Interestingly, the study by Wuthijaree et al. (2022) also detected species that were not observed in our study: *Teladorsagia* spp., *Bunostomum trigoncephalum*, *Chabertia ovina*, *Cooperia curticei*, and *Cooperia oncophora* [13]. These differences emphasize the need for continued surveillance to better characterize strongyle nematode diversity across different geographical regions.

Our analysis of beef cattle samples identified *Bunostomum plebotomum* and *M. digitatus*, which may have a significant impact on cattle health [33,34]. Since the fecal samples were collected from apparently healthy individuals in this study, the clinical impact of these infections remains unclear. Our findings support earlier reports of coinfection of multiple parasite species in both goats and cattle. Molecular identification of strongyle nematodes from western Thailand revealed that the co-occurrence of *Haemonchus* spp. and *Trichostrongylus* spp. was the most common in goats whereas *Cooperia* spp. followed by *Trichostrongylus* spp. were the most prevalent in cattle [9]. These findings are consistent with our findings. These differences underscore the need for localized studies to guide effective parasite control and prevention strategies.

We detected *Cyathostomum catinatum*, an equid cyathostomin, in one beef cattle and two meat goat samples (Table 3). A previous study also reported detecting of equid cyathostomins in sheep through a nemabiome study, with the authors acknowledging the possibility of contamination at the DNA or parasitological level [35]. Additionally, classical nemabiome studies, which rely on DNA extracted from strongyle larvae, exclude species with a prevalence below 0.05 % in their bioinformatics analyses, as such low counts typically correspond to fewer than one worm per sample, suggesting contamination or misidentification of reads [15,30]. Our study utilized coproDNA, which does not have a defined exclusion threshold. Therefore, the identification of *C. catinatum* may be due

to sequencing errors, as suggested in previous studies. Future research is needed to establish exclusion thresholds for coproDNA samples.

The differences observed among host groups in all three diversity indices suggest varying patterns of species abundance, evenness, and dominance. The identified parasite species were ruminant-host-specific, as described in the literature. *Haemonchus contortus* was present in all host species, whereas *M. digitatus* was exclusive to large ruminants (buffaloes and cattle) and absent in goats (Table 3). Additionally, *T. colubriformis* was the dominant species in dairy goats. These host-specific patterns contributed to shaping the diversity of the strongyle nematode fauna and accounted for the observed differences in diversity across ruminant hosts. However, due to the limited sample size, the factors associated with the diversity, as identified through microscopic examination, could not be used to further explain the variations in nematode community structure.

The nemabiome approach has proven effective in large-scale surveillance and epidemiological research, particularly for understanding the abundance and variation of GI nematodes on sheep farms across the United Kingdom [31]. Subsequent application of the nemabiome approach has examined how nematode species adapt to naïve small ruminant hosts and how species dominance shifts after anthelmintic treatment [32]. This method provides detailed data on individual parasite species, complementing fecal egg count information and offering insights into the development of anthelmintic resistance [30]. Our study is the first to apply the nemabiome approach to investigate strongyle species in ruminants in northern Thailand, providing comprehensive data on the species present in this region which could be utilized for the effective parasite monitoring and control strategies.

During the bioinformatics analysis, taxonomic assignments of OTUs were performed using the ITS-2 nemabiome and ITS eukaryote sequence databases. However, some species known to be present in Thailand were not detected. To ensure the accuracy of our findings, we reviewed the reference database and assessed whether strongyle taxa previously reported in Thailand or neighboring countries were represented. For instance, *Chabertia* spp., which have been reported in goats in the

Philippines through larval culture [36], as well as *Strongyloides* spp., *Trichuris* spp. and *Nematodirus* spp., whose eggs can be morphologically distinguished from other strongyle eggs, were all present in the reference dataset. Despite this, our nemabiome analysis detected several unknown OTUs that could not be assigned to any currently available strongyle taxa in the ITS-2 reference database. Species such as *Sarwaria bubalis*, which is known to occur in Asia, including Vietnam [37], and *Paracooperia nodulosa*, which has been reported in domestic goats in Malaysia [38], may not be well-represented in existing sequence databases. Similar cases may exist for other strongyle species, further emphasizing the limitations of current reference datasets.

This limitation underscores the critical need to expand regional reference databases to include strongyle species endemic to Thailand and surrounding countries. The absence or underrepresentation of regionally relevant taxa in publicly available databases may hinder accurate taxonomic assignments and biodiversity assessments. To address this issue, we explicitly discuss the validation challenges encountered in our study and emphasize the necessity of incorporating morphologically validated sequences from Thai ruminants into reference databases to improve the accuracy of future studies.

In conclusion, our findings underscore the usefulness of DNA metabarcoding techniques like nemabiome to enhance our understanding of parasite communities in livestock. The detailed species-level resolution offered by nemabiome data is extremely useful for tracking parasite populations and informing more precise control strategies. As strongyle nematode infections continue to pose significant challenges to livestock health, particularly in regions like northern Thailand, future studies should focus on expanding our understanding of regional variations in parasite species composition. Additionally, continued efforts to assess the clinical impact of these parasites will be essential in developing sustainable management practices and mitigating the growing threat of anthelmintic resistance.

Ethic statement

The use of animals in this study was approved by the Animal Care and Use Committee of the Faculty of Veterinary Medicine, Chaing Mai University (approval number R21/2564 and S16/2567) in accordance to the Ethical Principles and Guidelines for the Use of Animal for Scientific purpose, the National Research Council of Thailand.

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CRedit authorship contribution statement

Thanakorn Rompo: Writing – original draft, Visualization, Investigation, Data curation. **Naoki Hayashi:** Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Ernest Teo:** Writing – review & editing, Formal analysis. **Tawatchai Singhla:** Investigation. Chakorn Kunkaew: Investigation. **Duangthatai Sripakdee:** Investigation. **Boondarika Nambooppha:** Investigation. **Saruda Wanganurakkul:** Investigation. **Kanthanis Limwibulpong:** Investigation. **Kanyatip Sangarun:** Investigation. **Napatsorn Suwongsaksri:** Investigation. **Saravalee Suphakarn:** Investigation. **Chanakan Chotiphutthikul:** Investigation. **Yuto Matsui:** Investigation. **Takao Irie:** Investigation. **Ayako Yoshida:** Investigation. **Lerdchai Chintapitaksakul:** Supervision. **Naoaki Misawa:** Supervision, Project administration, Funding acquisition. **Nariaki Nonaka:** Supervision, Project administration, Funding acquisition, Conceptualization. **Ryo Nakao:** Writing – review & editing, Supervision, Methodology, Data curation. **Saruda Tiwananthagorn:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

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Appendix A Supplementary data

Supplementary data to this article can be found online at

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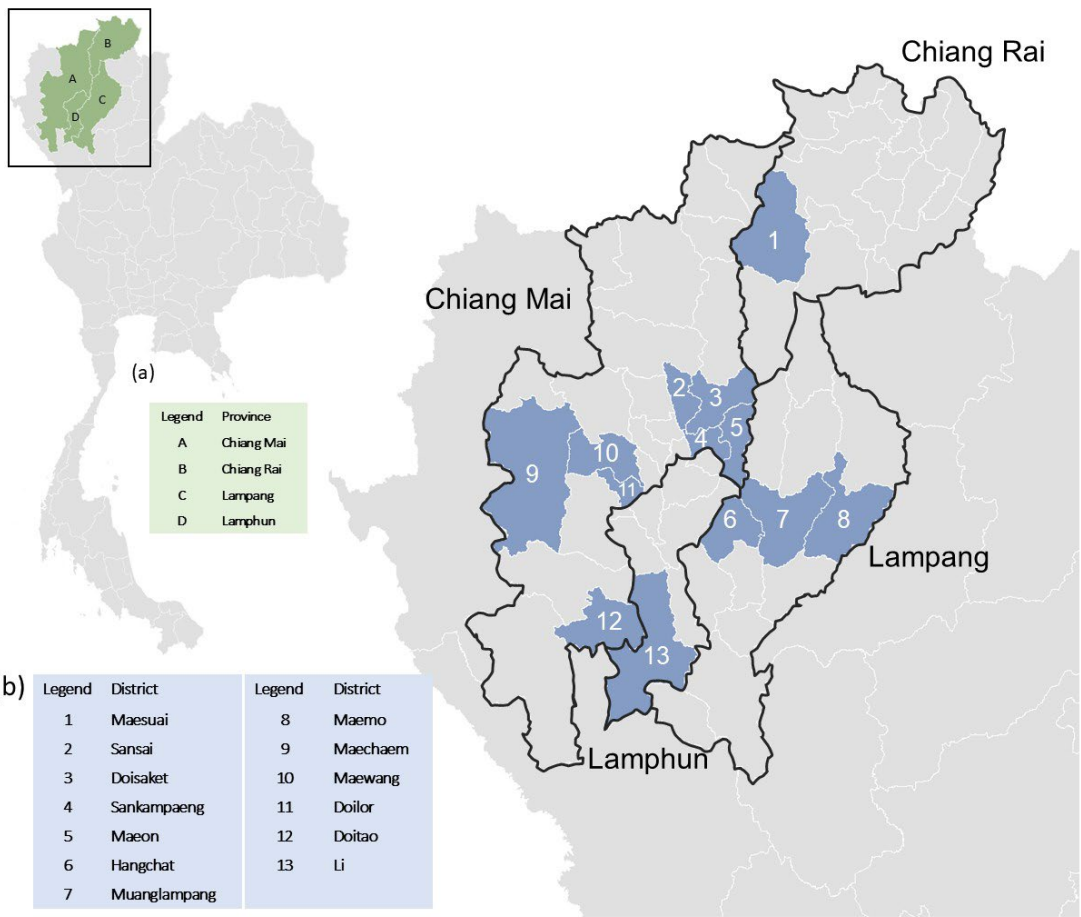
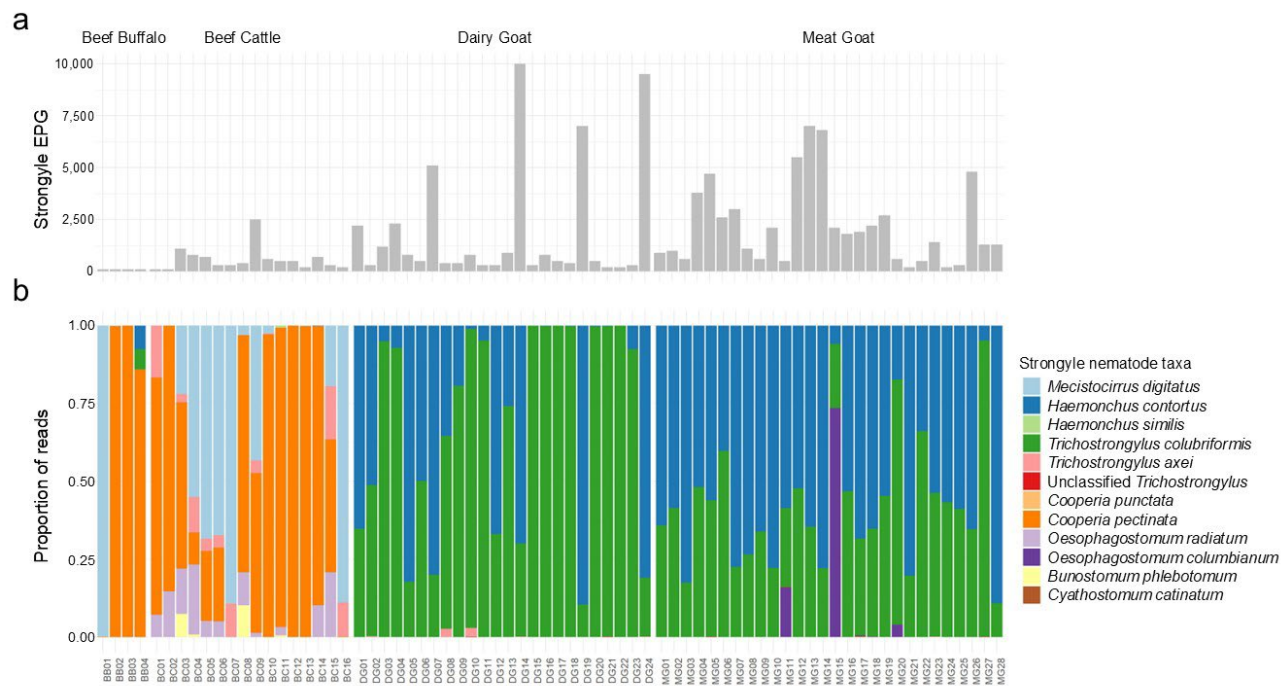


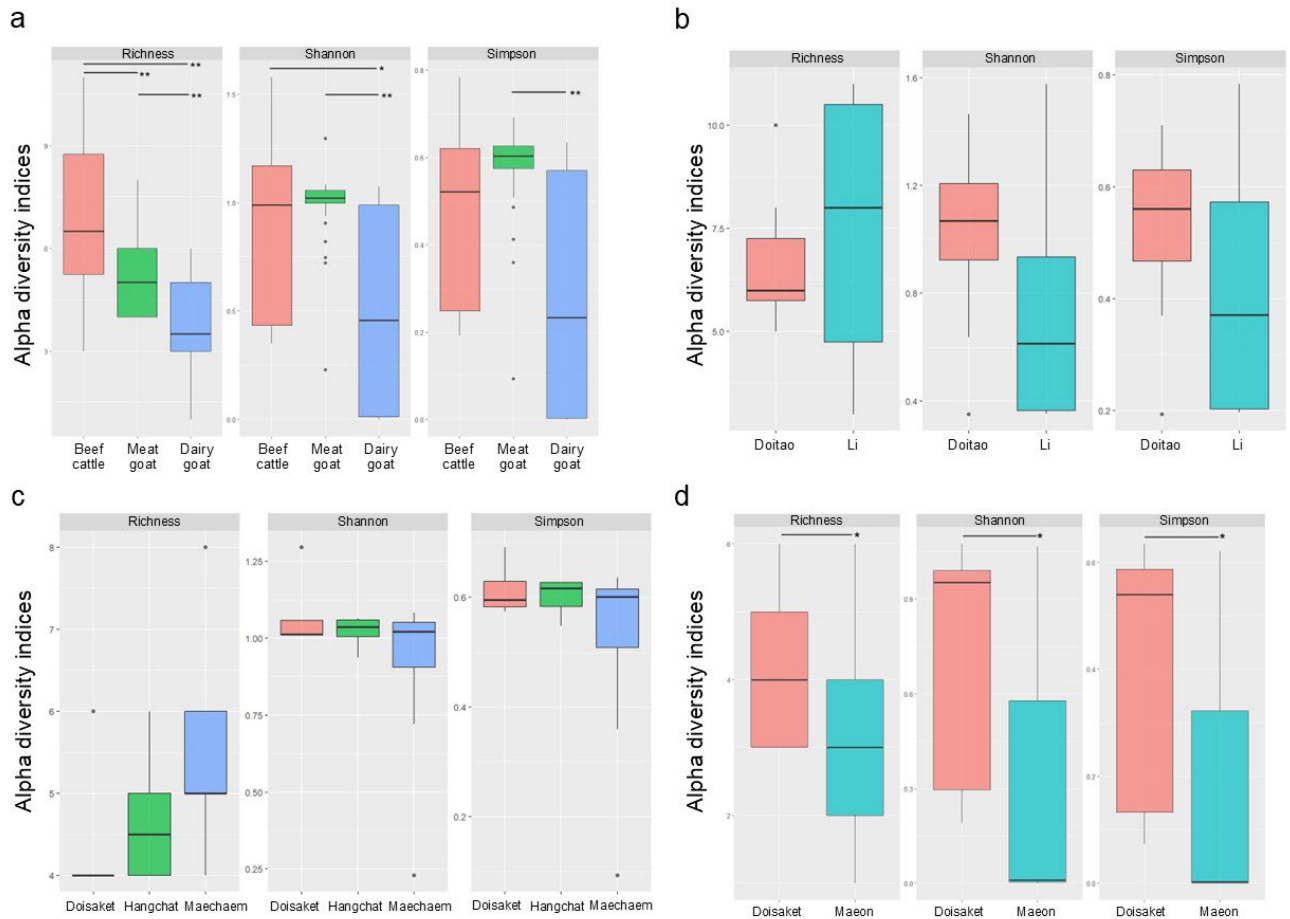
Figure 1. The sampling sites across the upper northern Thailand. (a) The sampling sites (green) at the provincial level. The names of the provinces are displayed in capital letters on the legend. (b) A closer view of the sampling sites (blue) at the district level. The names of the districts are displayed in number on the legend.



12

13

14 **Figure 2.** Strongyle egg counts and the composition of grouped operational taxonomic unit (OTUs) of individual
 15 samples. (a) Eggs per gram (EPG) determined by the modified McMaster technique. (b) Proportion of sequencing
 16 reads assigned to each OTUs.



17

18 **Figure 3.** Alpha diversity indices (Richness, Shannon, and Simpson) of nematode communities. Comparison of the
 19 indices from: (a) three ruminant hosts: beef cattle, meat goats, and dairy goats; (b) beef cattle across sampling
 20 locations; (c) meat goat across sampling locations; and (d) dairy goat across sampling locations. Statistically
 21 significant differences are indicated as $p < 0.05$ (*) and $p < 0.001$ (**).

1 Table 1. Results of microscopic examination of strongyle nematode eggs in ruminants.

2

Ruminant host		No. samples collected	No. samples microscopically positive (%)	No. samples positive by the centrifugal flotation	No. samples positive by the McMaster	Average EPG (±SD)	No. samples used for nemabiome analysis
Buffalo	Beef	82	11 (13)	11	5	46 ± 52	5
	Dairy	57	12 (21)	12	0	NC	0
Cattle	Beef	102	87 (85)	87	32	170 ± 439	16
	Dairy	40	5 (13)	5	0	NC	0
Goat	Meat	68	60 (88)	NT	60	1,304 ± 1,672	28
	Dairy	169	122 (72)	NT	122	930 ± 1,645	24
Total		518	297 (57)		219	742 ± 1,441	73

3 EPG, Eggs per gram of feces. NT, not tested. NC, not calculated.

4 Table 2. Association between strongyle egg-positivity in ruminants and the variables obtained from the
5 questionnaire.
6

Factor / Ruminant host		No. strongyle egg negative	No. strongyle egg positive	p-value
Rearing purpose				
Buffalo	Beef	71	11	$p = 0.337$
	Dairy	45	12	
Cattle	Beef	15	87	$p < 0.0001$
	Dairy	35	5	
Goat	Meat	8	60	$p = 0.013$
	Dairy	47	122	
Farm location by province†				
Beef cattle	Chiang Mai (Doitao) [D]	10	20	$p = 0.001$ (Pairwise comparisons: D-L, $p = 0.118$; L-M, $p = 0.212$; D-M, $p = 0.002$)
	Lamphun (Li) [L]	5	37	
	Chiang Rai (Maesuai) [M]	0	30	
Meat goat	Chiang Mai	3	43	$p = 0.124$
	Lampang	5	17	
Farm location by district				
Beef buffalo	Maewang [1]	5	1	$p < 0.0001$ (Pairwise comparisons: 1-2, $p = 1.00$; 1-3, $p = 1.00$; 1-4, $p = .090$; 2-3, $p = 1.00$; 2-4; $p < 0.001$; 3-4; $p < 0.001$)
	Doisaket [2]	19	1	
	Maeon [3]	47	4	
	Sankampaeng [4]	0	5	
Dairy goat	Doisaket	8	68	$p < 0.0001$
	Maeon	39	54	
Raising system				
Beef buffalo	Free-ranging	6	5	$p = 0.008$
	Free-roaming	60	5	
Dairy buffalo	Indoor	1	22	NA
Beef cattle	Indoor	0	2	$p = 1.0$
	Free-roaming	14	54	
Dairy cattle	Free-ranging	35	5	NA
Meat goat	Free-ranging	8	60	NA
Dairy goat	Indoor	2	15	$p = .193$
	Free-ranging	45	107	
Deworming frequency per year				
Beef buffalo	Not received	2	0	$p = 1.0$
	1-2 times	64	10	
Dairy buffalo	N/A			
Beef cattle	Not received [N]	2	9	$p = 0.864$ (Pairwise comparisons: N-2, $p = 1.00$; N-3, $p = 1.00$; 2-3, $p = 0.572$)
	1-2 times [2]	12	43	
	3 times or more [3]	0	4	
Dairy cattle	1-2 times	35	5	NA
Meat goat	1-2 times	5	33	$p = 0.931$
	3 times or more	2	21	
Dairy goat	1-2 times	39	54	$p < 0.0001$
	3 times or more	8	68	

†All beef buffaloes (*n* districts = 3), dairy buffaloes (1), dairy cattle (1), and dairy goats (2) were from Chiang Mai. NA, not applicable.

8 Table 3. The list of detected strongyle nematodes with their prevalence in each ruminant.
9

Assigned taxon		No. OTUs	No. samples positive for each taxon (%)			
Genus	Species		Beef buffalo (n = 4)*	Beef cattle (n = 16)	Meat goat (n = 28)	Dairy goat (n = 24)
<i>Bunostomum</i>	<i>B. phlebotomum</i>	1	0	5 (31)	0	0
<i>Cooperia</i>	<i>C. pectinata</i>	6	4 (100)	16 (100)	2 (7)	0
	<i>C. punctata</i>	4	0	3 (19)	0	0
<i>Cyathostomum</i>	<i>C. catinatum</i>	1	0	1 (6)	2 (7)	0
<i>Haemonchus</i>	<i>H. contortus</i>	4	2 (50)	5 (31)	28 (100)	21 (88)
	<i>H. similis</i>	1	0	3 (19)	0	0
<i>Mecistocirrus</i>	<i>M. digitatus</i>	8	2 (50)	11 (69)	0	0
<i>Oesophagostomum</i>	<i>O. columbianum</i>	1	0	0	8 (29)	0
	<i>O. radiatum</i>	1	0	12 (75)	0	1 (4)
<i>Trichostrongylus</i>	<i>T. axei</i>	1	0	11 (69)	1 (4)	7 (29)
	<i>T. colubriformis</i>	4	2 (50)	0	28 (100)	24 (100)
	Unclassified <i>Trichostrongylus</i> sp.	1	0	0	11(39)	10 (42)

10 *The number in the parentheses indicates the number of the samples tested by a nemabiome analysis.