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Contrasting Parasite Infection Patterns in Two Captive North American Wild Sheep Species: Seasonal Dynamics of Trichostrongyloids and Muellerius capillaris

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Abstract

Background

Gastrointestinal nematodes and lungworms are important parasites that can affect the health and population dynamics of free-ranging bighorn sheep (*Ovis canadensis*) and thinhorn sheep (*Ovis dalli*). However, information on the epidemiology of these helminths in captive North American wild sheep species remains limited. In this 12-month longitudinal study, we compared seasonal patterns of trichostrongyloid and *Muellerius capillaris* infections in both host species maintained under the same environmental and management conditions.

Results

A total of 117 individual faecal samples were examined monthly using the McMaster and modified Baermann techniques, supplemented by genus-specific multiplex qPCR. Clear differences were observed between the two host species. Despite identical housing, diet, and microclimatic conditions, trichostrongyloid faecal egg counts ($p = 0.044$) and *M. capillaris* larval counts ($p = 0.048$) were significantly higher in bighorn sheep than in thinhorn sheep. The two parasite groups also showed contrasting seasonal patterns. Trichostrongyloid egg counts were positively associated with temperature ($p < 0.001$), with pronounced summer peaks dominated by *Haemonchus* spp. (>80% of total parasite DNA). In contrast, *M. capillaris* larval shedding followed an inverse seasonal pattern, with the highest larval counts recorded during late autumn and winter ($p < 0.001$). Faecal egg counts were strongly correlated with parasite DNA quantified by qPCR (Spearman's $\rho = 0.79$, $p < 0.001$).

Conclusions

Our results indicate that trichostrongyloids and *M. capillaris* show contrasting seasonal infection patterns, supporting the need for parasite-specific monitoring throughout the year. The pronounced summer dominance of *Haemonchus* spp. suggests that captive conditions may favour its transmission and influence the composition of the gastrointestinal nematode community. Differences between the two host species and considerable individual variation in trichostrongyloid egg and *M. capillaris* larval shedding further indicate that host species and individual infection status should be considered when interpreting coprological findings. These results may contribute to better planning of parasite surveillance and timing of anthelmintic interventions in captive wild ruminants.

1. Introduction

The Bighorn sheep (*Ovis canadensis*) and Dall's sheep, also referred to as thinhorn sheep (*Ovis dalli*), are among the most emblematic wild ungulates of North America. Due to their conservation importance and documented population declines [1], both species have been the focus of considerable scientific attention. Much of the existing research has focused on free-ranging populations, where these species are exposed to a wide range of ecological and biological pressures. These include climate change [2], predation [3, 4], reduced genetic diversity [5], and the introduction of infectious diseases from other wild ruminant hosts as

well as from domestic small ruminants [6]. Parasitic infections represent a key component of disease ecology in wild sheep. Commonly reported endoparasites include small lungworms (family Protostrongylidae) *Protostrongylus* spp. [7, 8] and *Muellerius capillaris* [9], as well as a range of gastrointestinal (GI) nematodes within the superfamily Trichostrongyloidea (generally known as trichostrongyloids) [10, 11, 12].

Small lungworms (commonly referred to as protostrongylids) have been implicated as contributors to multifactorial pneumonia (often called bighorn sheep pneumonia complex) in wild species [8, 13]. A protostrongylid life cycle involves gastropod intermediate hosts, in which first-stage larvae (L1) develop to the infective third-stage larvae (L3). This development is strongly influenced by environmental and climatic conditions and takes several weeks. Infection of small ruminants takes place through the accidental ingestion of infected gastropods during grazing [14]. In wild North American sheep, infections with *Protostrongylus* spp. are usually more common, whereas *M. capillaris* is only rarely reported and does not appear to be a part of their native parasite fauna. Its presence in bighorn sheep has been linked to transmission from domestic small ruminants [6] with subsequent confirmation in free-ranging populations [9].

Among GI nematodes, trichostrongyloids are of particular concern due to their pathogenic potential [15, 16, 17]. Within this group, *Haemonchus* spp. are well recognised in domestic sheep as haematophagous parasites capable of causing marked anaemia and, under heavy burdens, mortality due to severe blood loss [18]. Trichostrongyloids infective larvae develop on pasture and are subsequently swallowed by grazing animals. The development and survival of larval stages are closely tied to environmental conditions. Temperature and humidity strongly influence viability of larvae; warm and moist conditions typical of rainy summers and mild spring or autumn periods are especially conducive to larval maturation and persistence. Vegetation further enhances survival by retaining surface moisture and providing protection from desiccation [19].

Although zoological institutions invest considerable effort in designing enclosures and husbandry practices that reflect species-specific ecological requirements, the inherent limitations of captivity cannot be eliminated. Zoo-housed ruminants are maintained under controlled but spatially limited conditions. In captivity, limited pasture rotation and inadequate dung removal may lead to repeated exposure to environments contaminated with infective parasite stages, potentially enhancing parasite transmission and the environmental persistence of parasitic stages [20] while captivity-associated stress may further modulate host susceptibility through effects on immune function [21]. These factors may also have implications for animal welfare, particularly when parasitic load increase. Therefore, effective control of parasitic infections represents an essential component of preventive health management and welfare assurance in zoological institutions. However, most available knowledge on both trichostrongyloids [11, 18, 22] and protostrongylids [9,23,24,] is derived from domestic small ruminants maintained under intensive farming conditions or free-ranging population. These studies have generated valuable epidemiological insights, yet comparable data for wild sheep species kept in a managed ex situ environment remain scarce.

Against this background our objectives were to: i) determine GI nematode and lungworm burden in a population of bighorn and thinhorn sheep maintained under zoo conditions, and ii) evaluate seasonal dynamic patterns of these helminth parasites over a one-year study period in relation to environmental and management-related factors.

2. Materials and methods

2.1 Study location and animals

The study was carried out at the Zoological and Botanical Garden of the City of Plzeň, located in the western part of the Czech Republic. The region is characterised by a temperate Central European climate with four distinct seasons, including two periods of increased precipitation typically occurring in late spring and early autumn, and comparatively drier conditions during summer and winter. Meteorological data at the study location were obtained from the Czech Hydrometeorological Institute using records from the Plzeň–Mikulka station (49.7645°N, 13.3787°E), located approximately 1.3 km from the study site.

Between May 2024 and May 2025, a longitudinal survey of parasitic infections was conducted in a Nearctic exposition of the zoo where two North American wild sheep species are displayed, specifically Sierra Nevada bighorn sheep (*Ovis canadensis californiana*) and thinhorn sheep (*Ovis dalli dalli*). The study included all adult individuals present in the exposition at the time of sampling. The bighorn sheep population (n = 4) consisted of two females, one imported from Royal Burgers' Zoo (the Netherlands) and the other from Prague Zoo (Czech Republic), while the remaining two individuals (one male and one female) were born at Zoo Plzeň. In contrast, the thinhorn sheep population (n = 5) consisted exclusively of individuals born at Zoo Plzeň, including three females and two males.

The diet of both species was comparable and divided into summer and winter-feeding regimes. The summer diet consisted of 0.2 kg of specialized granules (Zoofeed, Grazer Cu-controlled, Granovit AG, Kaiseraugst, Switzerland) suitable for herbivores (browsers and intermediate feeders), 0.1 kg of pressed oats, meadow hay ad libitum, wheat and oat straw ad libitum, and branches from willow, pine, poplar, aspen, and fruit trees for browsing. In winter, the diet remained largely unchanged, with the granule component increased to 0.3 kg.

The studied wild sheep species were maintained in spatially adjacent enclosures, separated by a glass barrier to prevent direct contact. The enclosures were situated on a sparsely grassed steep slope devoid of vegetation, where boulders created small rocky formations enabling the animals to exhibit natural behaviour.

Veterinary care was provided by two contracted veterinarians. During the study, bighorn sheep exhibiting clinical signs of illness and confirmed high trichostrongyloid burdens received two anthelmintic treatments (AHT) with levamisole plus praziquantel (First Drench®, Virbac, Australia), administered in June and August 2024.

2.2 Sample collection

Faecal sampling was performed at monthly intervals throughout the study period. Because rectal sampling was not feasible due to animal shyness and stress minimization, faecal samples were collected by zookeepers from each animal immediately after defecation whenever possible and always within maximum of 8 h after deposition to prevent environmental contamination and potential data bias. For each sampling day, at least 15 g of faeces were obtained from each animal. Following collection, samples were placed in plastic zip-lock bags with air removed prior to sealing, labelled with unique individual codes (IDs) assigned to each animal, stored at 4°C in a portable refrigerator, and transported to the parasitological laboratory at the Czech University of Life Sciences within 24 h.

2.3 Coprological analysis

Gastrointestinal nematode and lungworm burdens were assessed using individual faecal samples. Nematode egg output was quantified using the Concentration McMaster technique [25], with a detection limit of 20 eggs per gram (EPG). Although nematode eggs were identified to the genus level based on morphological and morphometric characteristics [26], trichostrongyloid eggs were pooled and recorded as strongyle-type due to the limited reliability of egg-based genus-level identification.

Protostrongylid larvae were recovered using a modified Baermann technique with a flask recovery apparatus according to McKenna [27]. Five grams of faecal pellets were wrapped in tissue paper and suspended in tap water. All recovered L1 larvae were identified to genus level based on morphological criteria [28] and expressed as larvae per gram of faeces (LPG).

Microscopic examination of eggs and L1 larvae was performed using an Olympus BX51 microscope, with morphometric analyses conducted using QuickPHOTO MICRO 3.2 software.

2.4 DNA extraction and multiplex real-time PCR analysis (qPCR)

Genomic DNA was extracted following the protocol of Reslová et al. [29]. After thawing, frozen faecal specimens were manually broken down within zip-lock bags, and a 1 g aliquot of each homogenized sample was processed using the Quick-DNA Fecal/Soil Microbe MiniPrep kit (Zymo Research, Irvine, CA, USA). For mechanical grinding, 800 µL of BashingBead Buffer and 3,200 µL of PBS were added to each tube and mixed thoroughly, after which 1,200 µL of the suspension was transferred to a ZR BashingBead Lysis Tube and homogenized for 90 s at 6,500 rpm on a MagNA Lyser Instrument (Roche, Basel, Switzerland). A negative isolation control (NIC) containing only buffers without faecal substrate was processed alongside every batch of 11 samples to monitor contamination, the remaining steps followed the kit guidelines, and each final DNA eluate (50 µL) was stored at − 20°C until analysis. The extracted DNA was then screened using a four-plex TaqMan real-time PCR assay [29] for the identification and semi-quantitative assessment of the trichostrongyloid genera, specifically *Haemonchus, Teladorsagia*, *Trichostrongylus*, *Nematodirus, Ashworthius* and *Chabertia* with the reaction mixture composition, primer and probe sequences, plasmid standard, and cycling conditions as described previously [29]. All samples were tested in duplicate on a QuantStudio™ 5 Real-Time PCR System (ThermoFisher Scientific, Waltham, MA, USA) in 96-well PCR plates, with a plasmid standard, a no-template control (NTC), and NICs included

on each plate and an internal amplification control (IAC) incorporated into every sample and control. Raw data were analysed using Design & Analysis software v2.8.0 (ThermoFisher Scientific), with quantification cycle (Cq) values determined using the passive reference dye set to 'None', and baseline-corrected normalized fluorescence (ΔRn) thresholds set to 8,000 for Standard (VIC), 8,000 for Haemonchus (VIC), 10,900 for Teladorsagia (FAM), 8,500 for Trichostrongylus (ROX), and 3,000 for IAC (Cy5). Results are presented as plasmid copy numbers corresponding to the specific DNA amount in the faecal sample.

2.5 Data analysis

Infection intensity of trichostrongyloids and protostrongylids was quantified as faecal egg counts and larval counts, expressed as eggs per gram (EPG) and larvae per gram (LPG), respectively. Molecular data were used to quantify the relative abundance of DNA from three detected trichostrongyloid genera (*Haemonchus*, *Teladorsagia*, and *Trichostrongylus*) based on qPCR results. Generalized linear mixed models (GLMMs) were used to assess the effects of host species and environmental conditions on seasonal variation in trichostrongyloid and protostrongylid infection intensity, and on the relative DNA quantities of the detected trichostrongyloid genera.

A negative binomial model (nbinom2) with a log link function was used to analyse coprological count data to account for overdispersion. Host species (bighorn sheep, BS; thinhorn sheep, TS) and mean temperature during the month preceding each sampling event were included as fixed effects, while individual identity (ID) was included as a random effect to account for repeated observations of the same animals (n = 9).

Relative DNA quantities were analysed using hurdle negative binomial GLMMs to account for zero-inflation. The hurdle model separated two processes: (i) the probability of parasite detection (presence/absence), modelled with a binomial distribution and logit link function, and (ii) the amount of parasite DNA in positive samples, modelled using a zero-truncated negative binomial GLMM. Both model components included host species and average temperature as fixed effects, and individual animal ID as a random effect.

The relationship between coprological infection intensity and molecular parasite load was assessed by correlating egg counts (EPG) with the sum of qPCR-quantified DNA of the three detected trichostrongyloid genera (*Haemonchus, Teladorsagia*, and *Trichostrongylus*) using Spearman's rank correlation coefficient.

Data visualisation was performed using the ggplot2 package. All models were fitted using the glmmTMB package in R (version 4.3.2) and model assumptions were assessed using the DHARMa package.

3. Results

A total of 117 faecal samples collected from nine individual animals were analysed using the McMaster method and qPCR. Seasonal patterns of trichostrongyloid and protostrongylid infections were evaluated in bighorn sheep (BS) and thinhorn sheep (TS).

3.1. Coprological findings

Strongylid-type eggs were detected in almost all samples, with prevalence of 92% in BS (95% CI = 81–98) and 87% in TS (95% CI = 76–94). The GLMM indicated that infection intensity differed significantly between host species, with BS consistently exhibiting higher faecal egg counts than TS ($p = 0.044$). Infection intensity was initially high in May 2024 (mean EPG = 4,400; range: 390–9,180) and decreased rapidly following the first anthelmintic treatment. In June, mean intensity declined to 460 (range: 220–620). Counts subsequently increased again, peaking in August 2024 (mean EPG = 9,075; range: 3,760–23,200). The second anthelmintic treatment administered at the end of August was followed by another marked decrease, with mean EPGs dropping to 70 in September (range: 40–100). A second, less pronounced peak occurred in May 2025 (mean EPG = 1,925; range: 280–3,640).

In contrast, untreated TS exhibited a more stable seasonal pattern with higher EPGs during spring and summer, reaching a maximum of 21,460 EPG in July 2024 followed by gradual decline during autumn (Fig. 1A).

The lowest egg counts in both host species were recorded in January 2025 (mean EPGs of 47; range: 0–60 and 53; range: 0–80). The model indicated a significant positive effect of mean monthly temperature on trichostrongyloid infection intensity ($p < 0.001$). The random effect of ID revealed between-individual variation (variance = 0.222), indicating consistent differences in infection intensity among animals (Table 1).

Table 1
Results of negative binomial generalized linear mixed models (GLMMs) assessing the effects of host species and temperature on trichostrongyloid faecal egg counts (EPG) and *Muellerius capillaris* larval counts (LPG).

	Factor	Estimate ± SE	z-value	*p*-value
Trichostrongyloids (EPG)	Host (BH)	0.948 ± 0.471	2.011	**0.044**
	Temperature	0.131 ± 0.025	5.276	**< 0.001**
	Random effect	Variance	SD	
	ID	0.2224	0.472	
M. capillaris (LPG)	Host (BH)	0.902 ± 0.457	1.976	**0.048**
	Temperature	-0.072 ± 0.014	-5.159	**< 0.001**
	Random effect	Variance	SD	
	ID	0.384	0.619	

Estimates, standard errors (SE), z-values, and *p*-values for fixed effects, along with variance and standard deviation (SD) for the random effect of individual animal identity (ID). Thinhorn sheep (TS) served as the reference level for the host species factor (bighorn sheep – BS). Temperature represents the mean monthly temperature preceding each sampling event. Statistically significant *p*-values are highlighted in bold.

Other GI nematodes were detected through coprological analysis, but generally at low intensity. In BS, *Capillaria* sp. was detected with a prevalence of 51% (CI = 37–65; mean EPG = 12), while in TS, the prevalence was 48% (CI = 35–61; mean EPG = 20). *Trichuris* sp. was detected in BS with a prevalence of 6% (CI = 1–16; mean EPG = 1) and in TS with a prevalence of 6% (CI = 2–15; mean EPG = 3).

Muellerius capillaris was the only lungworm taxon detected in the studied sheep, with larval counts significantly higher in BS than in TS ($p = 0.048$). In contrast to trichostrongyloid infections, infection intensity of *M. capillaris* showed a negative association with temperature during the month preceding sampling ($p < 0.001$) (Table 1). Peak larval counts were observed in BS (mean LPG = 453; range: 318–560) in December 2024 and in January in TS (mean LPG = 65; range: 2–172), whereas the lowest infection intensities occurred in August in TS (mean LPG = 22; range: 0–40) and in June in BS (mean LPG = 24; range: 21–38) as presented in Fig. 1B. Anthelmintic treatment was associated with reduction in *M. capillaris* infections; however, the effect was less pronounced than that observed for trichostrongyloids. Individual level variation was evident, as indicated by the random effect of ID (Table 1 and Fig. 1B).

3.2. Molecular detection and DNA quantification

The probability of detecting parasite DNA was significantly associated with host species, whereas environmental temperature did not serve as a significant predictor in the binary component of the GLMM (Table 2). For *Haemonchus* spp., BS was associated with a lower probability of occurence ($p = 0.037$). Conversely, a significant positive association was observed for *Trichostrongylus* spp., where host species (BS) increased the probability of sample positivity ($p = 0.040$). For *Teladorsagia* spp., no significant associations were found within the binary model ($p > 0.05$).

Table 2

Results of hurdle generalized linear mixed models (GLMMs) evaluating the probability of parasite DNA detection (binomial presence/absence component) and qPCR-quantified DNA levels in positive samples (zero-truncated negative binomial component) across three trichostrongyloid genera.

Factor		Presence/Absence of parasite DNA			DNA level in positive samples		
		Estimate ± SE	z-value	*p*-value	Estimate ± SE	z-value	*p*-value
Haemonchus spp.	Host (BH)	-1.344 ± 0.643	-2.090	**0.037**	0.950 ± 0.326	2.92	**0.004**
	Temperature	0.058 ± 0.037	1.579	0.114	0.115 ± 0.024	4.75	**< 0.001**
	Random effect	Variance	SD		Variance	SD	
	ID	0.2669	0.516		2.895e-08	0.00017	
Teladorsagia spp.	Host (BH)	1.224 ± 1.005	1.217	0.224	1.211 ± 0.243	4.99	**< 0.001**
	Temperature	-0.001 ± 0.040	-0.043	0.966	0.038 ± 0.018	2.14	**0.033**
	Random effect	Variance	SD		Variance	SD	
	ID	1.172	1.082		6.657e-09	8.159e-05	
Trichostrongylus spp.	Host (BH)	2.223 ± 2.050	2.050	**0.040**	0.768 ± 0.338	2.27	**0.023**
	Temperature	0.057 ± 0.043	1.328	0.184	0.066 ± 0.021	3.15	**0.002**
	Random effect	Variance	SD		Variance	SD	
	ID	1	1		0.07101	0.2665	

Estimates, standard errors (SE), z-values, and *p*-values for fixed effects, alongside variance and standard deviation (SD) for the random effect of individual identity (ID). Thinhorn sheep (TS) served as the reference category for host species (bighorn sheep – BS). Temperature represents the mean monthly temperature preceding sampling. Statistically significant *p*-values are shown in bold.

Quantitative analysis of DNA levels in positive samples revealed that temperature was a highly significant positive predictor across all three trichostrongyloid genera. The strongest association was observed for *Haemonchus* spp. ($p < 0.001$), followed by *Trichostrongylus* spp. ($p = 0.002$) and *Teladorsagia* spp. ($p = 0.033$). The host species was also associated with variations in DNA quantities, with the most pronounced effect recorded for *Teladorsagia* sp. ($p < 0.001$), as summarized in Table 2.

The results for the random effects indicate considerable differences in probability of parasite DNA detection among individuals, particularly for *Teladorsagia* spp., where the random intercept variance was the highest (variance = 1.172; Table 2). Conversely, in the quantitative DNA level models for *Haemonchus* spp. and *Trichostrongylus* spp., the variance attributed to the random effect of host ID was negligible. Trichostrongyloid egg counts (EPG), as determined by the McMaster method, were significantly and positively correlated with the total relative quantity of trichostrongyloid DNA measured by qPCR (Spearman's $\rho = 0.79$, $p < 0.001$; Fig. 2).

3.3 Seasonal dynamics and host-specific variation in trichostrongyloid burden

Longitudinal monitoring of molecular data revealed a highly synchronized seasonal pattern of trichostrongyloid infection across both host groups, though with significant differences in the magnitude of the parasite burden (Fig. 3A; 3B). In both hosts, qPCR plasmid copies numbers remained at or near the limit of detection during the winter months (November 2024 to March 2025). A marked increase in infection intensity was observed starting in late spring, with peaks consistently dominated by *Haemonchus* spp. However, the magnitude of infection in BS was substantially higher; peak DNA levels in July 2024 exhibited an approximately five-fold increase in plasmid copies compared to those recorded in TS during the same period (Fig. 4).

Community composition also exhibited seasonal succession. While *Haemonchus* spp. was the overwhelmingly dominant genus during summer peaks, accounting for over 80% of the total qPCR plasmid copy numbers, *Trichostrongylus* spp. showed a notable presence during the transitional spring and autumn periods. Specifically, in BS, a prominent peak of *Trichostrongylus* spp. plasmid copies was recorded in May 2024, which was absent in TS. In contrast, *Teladorsagia* spp. remained a minor and relatively stable component of the trichostrongyloid community in both hosts throughout the year, showing minimal response to the temperature fluctuations.

4. Discussion

This study provides the first longitudinal assessment of trichostrongyloid and *M. capillaris* dynamics in captive populations of two North American wild sheep species maintained under identical environmental and husbandry conditions. By minimizing variation in diet, climate, and management, the study offers a rare opportunity to evaluate the effects of seasonality and host species on parasite infection patterns. Three principal findings emerged: (i) trichostrongyloid faecal egg counts exhibited pronounced warm-season peaks, (ii) *Muellerius capillaris* displayed an inverse seasonal pattern with maximum larval shedding during colder months, and (iii) bighorn sheep consistently harboured higher parasite burdens than thinhorn sheep, despite the two anthelmintic treatments.

The captive setting likely influenced transmission dynamics in our study. The repeated use of relatively small enclosures may promote a build-up of infective stages in the environment, a pattern consistent with the persistently high nematode burdens reported in captive wild ruminants [20] and increase transmission

pressure compared with free-ranging populations. Nevertheless, the seasonal patterns observed in current study closely reflected known ecological constraints on parasite development, suggesting that climatic drivers remained important despite the artificial environment. Stable year-round nutrition may also have altered host–parasite interactions in our study, as adequate nutrition can increase host tolerance to parasitic infection [30], potentially allowing animals to tolerate infection levels that might have resulted in clinical disease or reduced survival under natural conditions [31].

4.1. Trichostrongyloid infections

Trichostrongyloid infections were detected throughout the year in both host species, but displayed pronounced seasonality, with the lowest EPG values and parasite DNA quantities recorded during winter and peak values observed during summer. This pattern is consistent with the temperature-dependent development [19, 32] and overwinter survival of free-living larval stages in upper soil layers [33, 34]. Our findings also agree with observations from domestic and wild ruminants in temperate regions [12, 19, 35].

The spring increase likely reflected both transmission from overwintering larvae and increasing environmental contamination. Periparturient relaxation of immunity may have further contributed to increased egg shedding around lambing [36, 37]. In our study bighorn sheep lambed primarily in early to mid-May, whereas thinhorn sheep lambed from late May to early June, which may partly explain the earlier and more pronounced spring peak observed in bighorn sheep. Similar periparturient increases in strongyle egg shedding have previously been reported in captive wild ruminants, including mohor gazelles and elands [38, 39].

The summer peak coincided with environmental conditions favourable for larval development [40] and was accompanied by a marked shift in parasite community composition. Molecular analyses demonstrated that *Haemonchus* spp. accounted for more than 80% of total trichostrongyloid DNA during peak summer transmission, whereas its contribution declined to almost zero between November and February. Althought after the drug administration to BSs between May and June, a decrease in *Hoemonchus* spp. DNA plasmid copy numbers was observed in the treated animals during the following month, accompanied by a reduction in trichostrongyloid EPGs. However, by July, parasite levels had increased again. A similar rapid recurrence of pasture contamination was observed in captive fallow deer one month after treatment with a combination of rafoxanide and mebendazole against *Ashworthius sidemi* [41]. In contrast, following the second dose, administered to BSs between August and September, no substantial recurrence was observed, possibly due to the combined effects of anthelmintic treatment and less favourable environmental conditions [18]. A comparable warm-season increase in strongyle transmission has been reported in free-ranging Rocky Mountain bighorn sheep, where egg counts peaked during late gestation, summer, and the rut [12]. However, the overwhelming dominance of *Haemonchus* spp. observed in the present study contrasts with northern wild populations. In free ranging thinhorn sheep from the Mackenzie Mountains, gastrointestinal helminth communities were dominated by *Marshallagia marshalli*, while *Haemonchus* spp. was not detected [35]. The present findings therefore illustrate how captivity can reshape parasite communities even in host species whose natural ranges would not support transmission of certain parasite taxa.

A strong positive correlation between trichostrongyloid EPG and qPCR-derived parasite DNA supported the consistency between molecular and coprological measures of infection intensity. However, molecular analyses additionally revealed seasonal shifts in parasite community composition that would have remained undetected using conventional coprological approaches alone. Besides the summer dominance of *Haemonchus* spp., *Trichostrongylus* spp. was most abundant during spring and autumn. The relatively stable contribution of *Teladorsagia* spp. throughout the year may reflect the greater ability of this genus to persist under cooler conditions, including survival of free-living larval stages over winter [42]. In addition, the lower reproductive output of *Teladorsagia* spp. females compared with *Haemonchus* spp. [43] may cause its relatively limited contribution to faecal egg counts despite its persistence within the parasite community. These findings closely match the ecological characteristics of the respective genera [19] and demonstrate the value of molecular surveillance in captive wildlife populations.

4.2. Lungworm infections

In contrast to trichostrongyloid, *M. capillaris* followed an opposite seasonal pattern, with larval shedding peaking during late autumn and winter and declining during summer. This inverse relationship may indicate that transmission is driven primarily by the ecology of gastropod intermediate hosts [44], rather than by direct climatic effects on parasite stages.

Because infection requires the ingestion of infected snails or slugs and the prepatent period extends over several weeks [14, 45], larval shedding reflects transmission events that occurred earlier in the season. Increased gastropod activity during cooler and more humid periods probably results in greater exposure of sheep to infective larvae, which subsequently manifests as elevated larval excretion during autumn and winter. The persistence of low-level shedding throughout the year is likely further supported by the long lifespan of both infected gastropods and adult parasites within the host [6]. The observed pattern is broadly consistent with previous studies of both domestic sheep and free-ranging wild sheep infected with protostrongylids, despite differences in the timing of peak larval excretion among geographic regions [24, 46, 47]. Such variation is expected because gastropod activity is highly sensitive to local climatic conditions. Nevertheless, the consistency of the overall seasonal trend across studies suggests that intermediate-host ecology is the principal determinant of protostrongylid transmission dynamics.

Together, these findings emphasize that trichostrongyloids and protostrongylids respond to fundamentally different ecological drivers. Whereas trichostrongyloid transmission appears to be governed largely by environmental conditions affecting free-living larval stages, *M. capillaris* transmission is mediated indirectly through the ecology of gastropod hosts, whose activity and availability are also shaped by the surrounding environment. Consequently, optimal surveillance and control strategies are likely to differ between the two parasite groups.

4.3. Host species differences

Across all parasitological indicators, bighorn sheep exhibited consistently higher infection levels than thinhorn sheep. The difference was evident not only for trichostrongyloid EPG but also for DNA quantities of all three trichostrongyloid genera and for lungworm LPG. Because both species experienced

comparable environmental exposure and management conditions, differential exposure alone is unlikely to explain the observed pattern. One possible explanation lies in the contrasting demographic histories of the two species. Historical population declines and genetic bottlenecks have been documented in bighorn sheep [5, 48, 49], whereas thinhorn sheep generally maintained larger effective population sizes across their natural range [50]. Previous work demonstrated that lower heterozygosity in wild bighorn sheep is associated with increased lungworm burdens [51], supporting the hypothesis that population history may continue to influence parasite susceptibility.

However, interpretation of these differences requires caution because both study populations have been maintained in captivity for multiple generations. Long-term breeding within relatively small captive populations may influence immune competence through inbreeding and genetic drift [52, 53]. Consequently, the observed interspecific differences probably reflect a combination of evolutionary history, captive population management, and contemporary host immune responses.

Substantial variation among individuals further highlights the importance of host-level factors. Several animals contributed disproportionately to environmental contamination, consistent with the widely recognised phenomenon of “super-shedders” [54, 55]. The observation that the highest individual egg output was recorded in a thinhorn sheep male, despite higher average burdens in bighorn sheep at the population level, illustrates the importance of individual-level heterogeneity. Such animals may have a disproportionate epidemiological impact and could represent priority targets for parasite monitoring and control programmes in zoological collections.

Several limitations should be considered when interpreting our results. The study included a relatively small number of animals (n = 9), although these represented the entire adult population of both species kept at Zoo Plzeň during the study period. This limits the generalisation of our findings to other captive populations. Although both species were maintained under comparable conditions, minor differences between enclosures could not be fully controlled. Despite these limitations, repeated individual sampling over the complete annual cycle allowed us to characterise seasonal parasite patterns in both host species under largely comparable environmental and management conditions.

5. Conclusions

This study demonstrates that parasite dynamics in captive North American wild sheep are shaped by a combination of environmental seasonality and host-related factors. Trichostrongyloid infections peaked during warmer months and were characterised by a seasonal dominance of *Haemonchus* spp., whereas *Muellerius capillaris* exhibited an inverse seasonal pattern linked to the ecology of gastropod intermediate hosts. Bighorn sheep consistently carried higher parasite burdens than thinhorn sheep across all monitored parasite groups, suggesting species-specific differences in susceptibility, while marked inter-individual variation indicated that a small number of hosts may contribute disproportionately to parasite transmission.

These findings have direct implications for parasite management in zoological collections. Surveillance efforts should focus on late spring and summer for trichostrongyloids and on late autumn and winter for protostrongylids and should incorporate molecular tools capable of detecting changes in parasite community composition. Importantly, the results support targeted anthelmintic treatment based on coprological examination, allowing treatment to be directed towards infected individuals and timed according to parasite prevalence and intensity, rather than relying on routine, non-targeted treatment. More broadly, the results demonstrate how captivity can modify parasite epidemiology by altering transmission opportunities and parasite community structure. Future studies integrating parasitological, immunological, and genetic approaches may help clarify the mechanisms underlying interspecific differences in susceptibility and improve evidence-based parasite management in captive wild ungulates.

Abbreviations

AHT
Anthelmintic treatment
BS
Bighorn sheep
CI
Confidence interval
EPG
Eggs per gram
GI
Gastrointestinal
GLMM
Generalized linear mixed model
ID
Identity
L1
First-stage larvae
LPG
Larvae per gram
qPCR
Quantitative polymerase chain reaction
TS
Thinhorn sheep

Declarations

Ethics approval and consent to participate

Ethical approval was not required for this study, as all faecal samples were collected non-invasively by zookeepers from the ground after spontaneous defecation, without handling or otherwise disturbing the

animals. Anthelmintic treatments were administered by the attending veterinarian as part of routine veterinary care, at the request of the ungulate curator, and independently of the present study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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Author Contribution

AK was the major contributor in writing the manuscript, NR provided the molecular analyses and contributed to writing the manuscript, JM provided the data analyses, prepared visualisations, AN contributed to the writing the manuscript, JV study coordinator and supervisor, provided advice on data interpretation.

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Availability of data and materials

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Figures

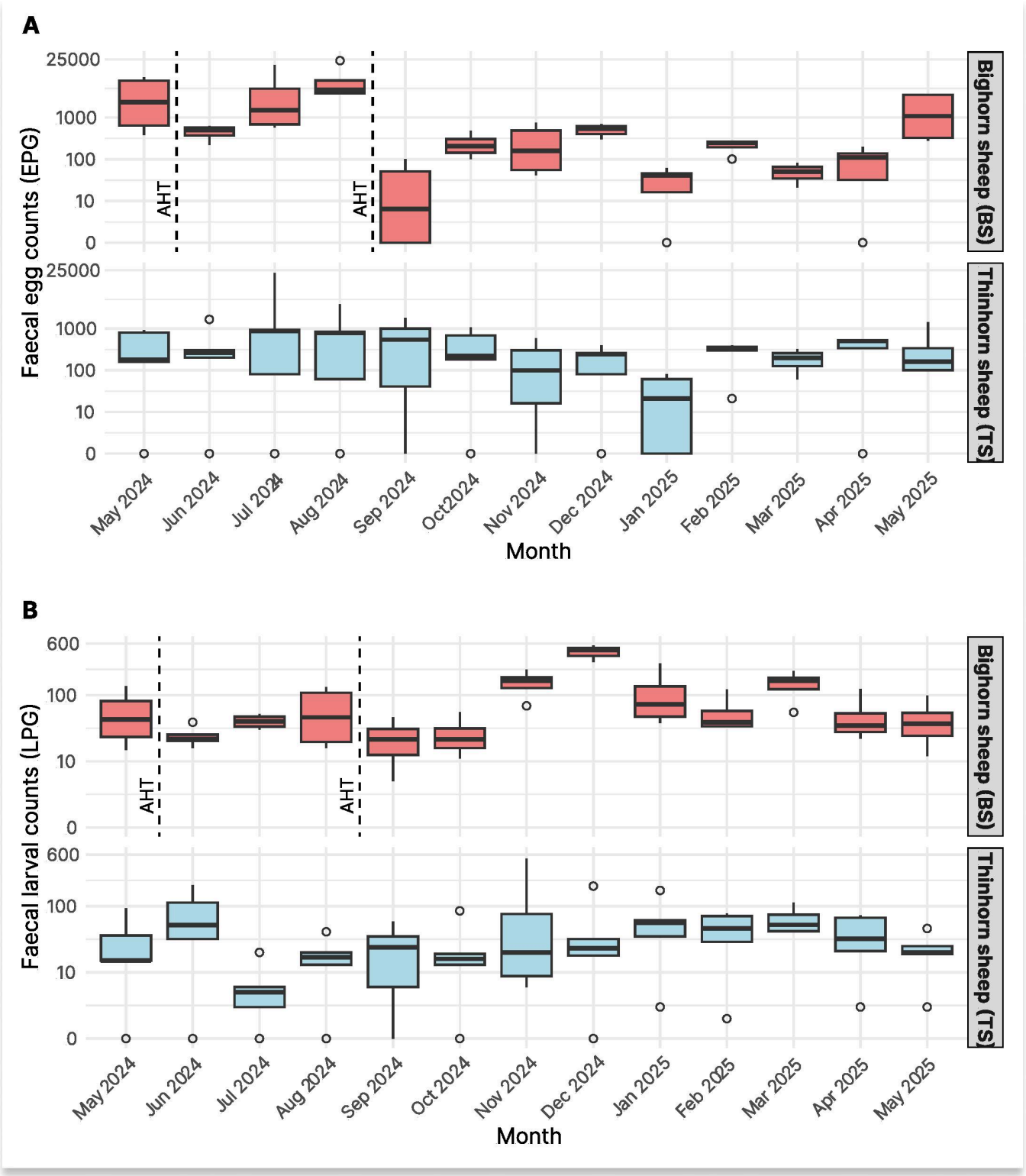


Figure 1

Seasonal dynamics of helminth shedding in captive bighorn sheep and thinhorn sheep. (**A**) Trichostrongyloid faecal egg counts expressed as eggs per gram of faeces (EPG) and (**B**) Faecal larval counts of *Muellerius capillaris* expressed as larvae per gram of faeces (LPG). Boxplots display the median (horizontal line), interquartile range (box), whiskers (minimum and maximum values within 1.5 times the

IQR), and potential outliers (individual points). Vertical dashed lines indicate targeted anthelmintic treatments (AHT) administered to bighorn sheep. Y-axes are presented on a $\log_{10}$ scale.

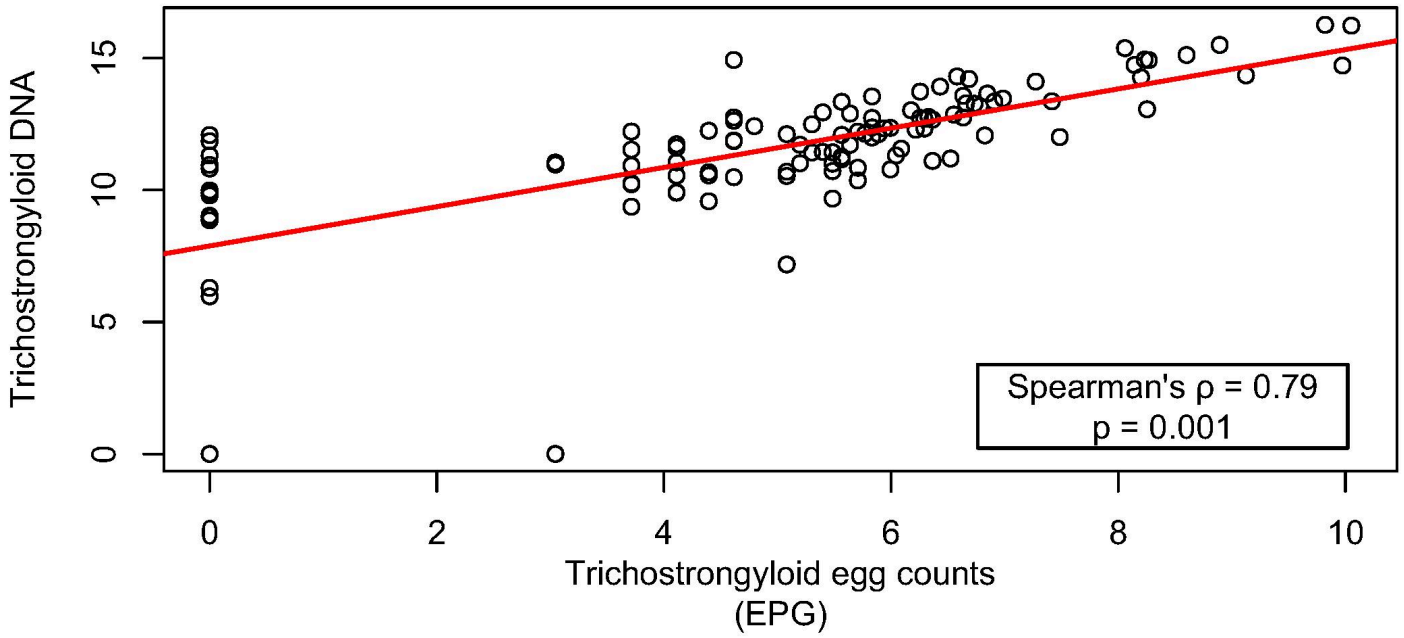


Figure 2

Correlation between coprological and molecular measures of trichostrongyloid infection intensity in captive wild sheep. The scatter plot displays the relationship between faecal egg counts (EPG) determined by the McMaster technique and total trichostrongyloid DNA levels (sum of *Haemonchus*, *Teladorsagia*, and *Trichostrongylus*plasmid copy numbers) quantified by multiplex qPCR across all evaluated faecal samples. Data on both y-axes are log-transformed.

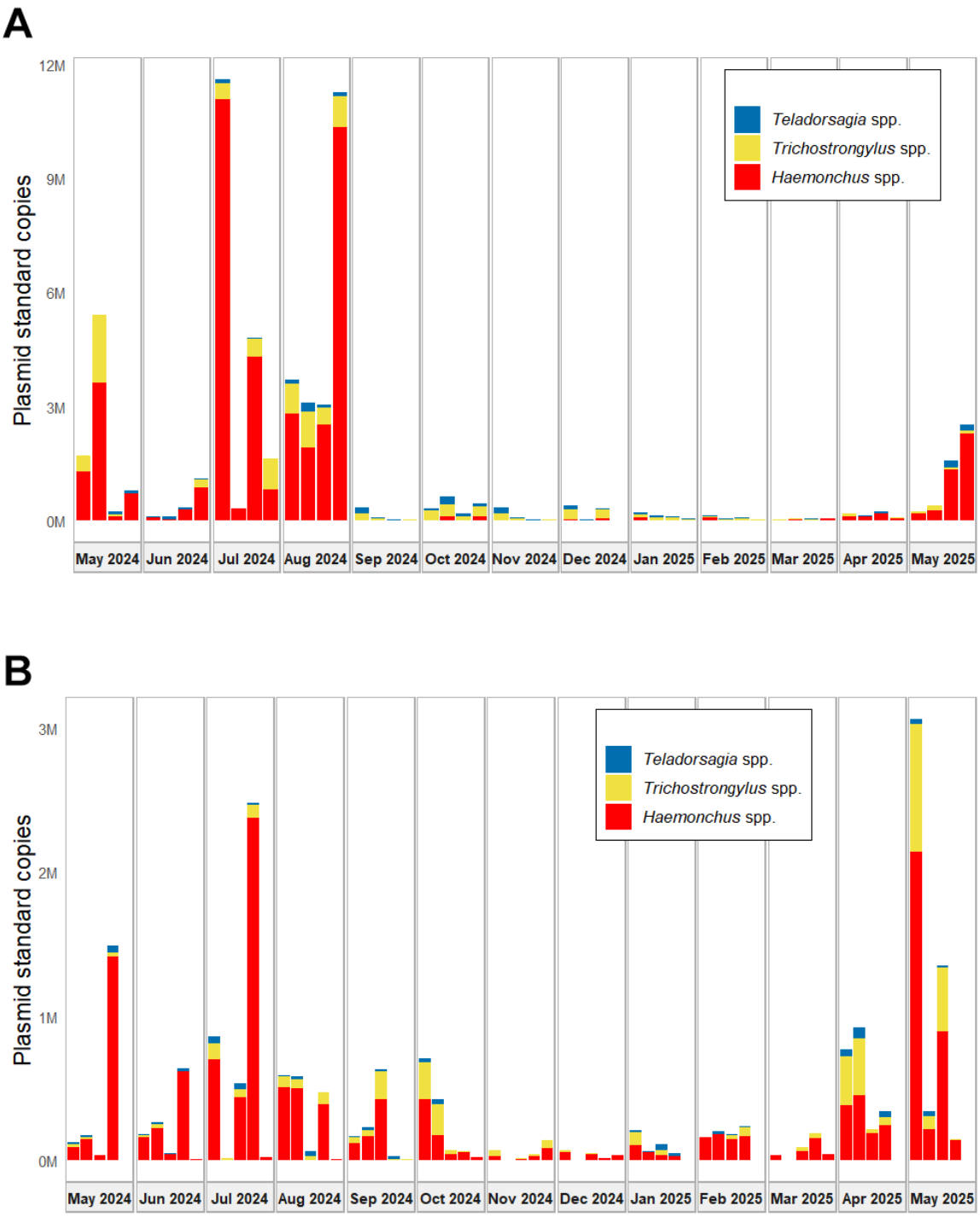


Figure 3

Seasonal dynamics and trichostrongyloid genera composition in captive **(A)** bighorn sheep (n = 4) and **(B)** thinhorn sheep (n = 5) within the study period. Stacked bars represent qPCR-quantified DNA levels (plasmid standard copy numbers) of *Haemonchus*spp., *Trichostrongylus* spp., and *Teladorsagia* spp. for each individual animal sampled within the respective month. Note the different scaling of the y-axes between panels A (0–12M) and B (0–3M).

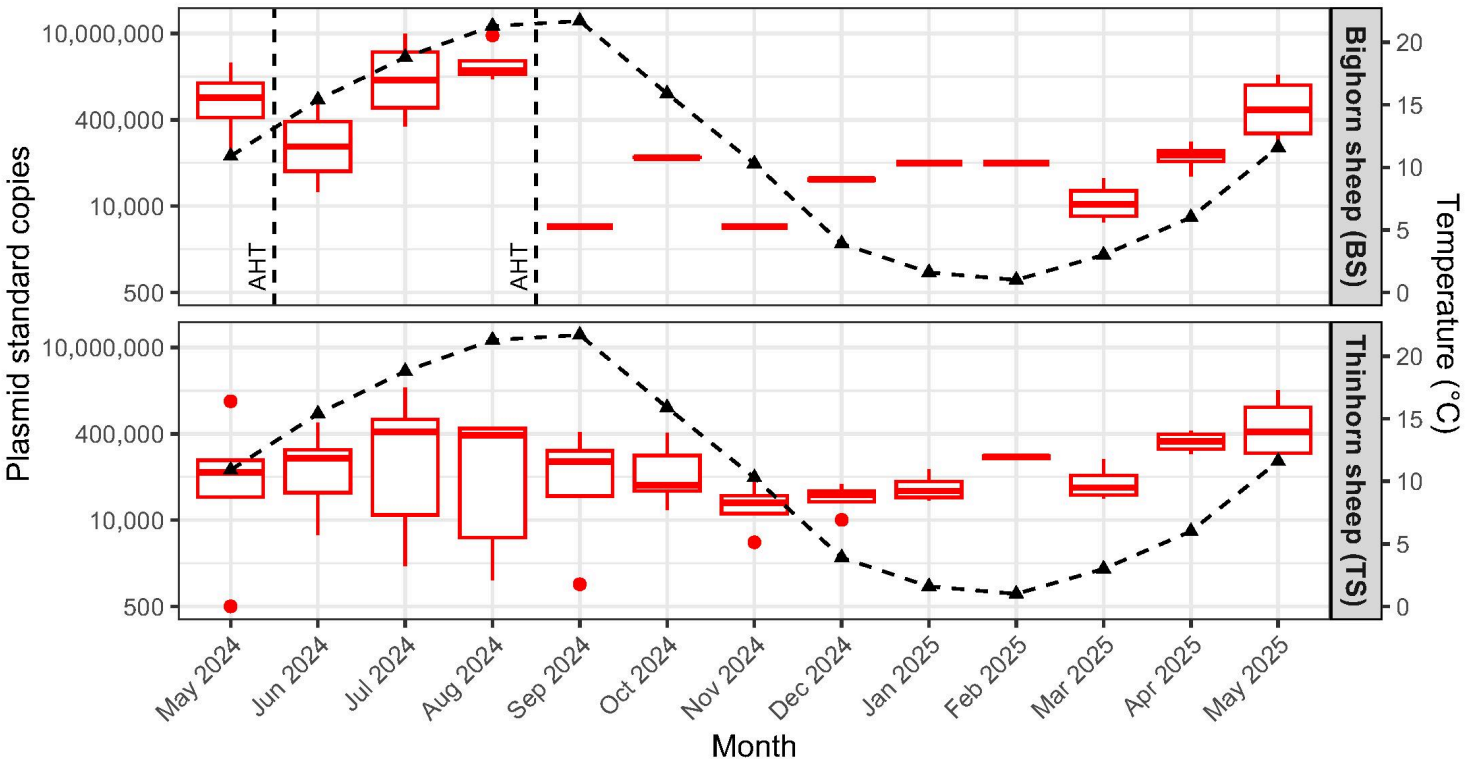


Figure 4

Seasonal dynamics of *Haemonchus* spp. DNA levels in relation to temperature in captive bighorn and thinhorn sheep. Red boxplots represent *Haemonchus* spp. DNA levels (expressed as plasmid standard copies) in bighorn sheep and thinhorn sheep. Boxplots display the median, interquartile range (IQR), and whiskers, with potential outliers shown as red dots. Black triangles, connected by dashed lines, represent the mean ambient temperature (°C) during the month preceding each sampling event and are plotted on the secondary y-axis (right). Vertical dashed lines indicate targeted anthelmintic treatments (AHT) administered to bighorn sheep. The primary y-axes are presented on a $\log_{10}$ scale.