Prevalence of Gastrointestinal Nematodes and Eimeria species in Sheep in Northern Cyprus

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SUMMARY

This study was carried out to examine the prevalence of gastrointestinal nematodes and *Eimeria* occysts in sheep farms of Northern Cyprus. A total of 356 sheep faecal samples were randomly collected across sheep farms in suburban regions of Northern Cyprus. The feces were tested for parasites using centrifugal flotation method. Eggs of *Moniezia*, *Cooperia*, *Oesophagosuturom*, *Trichurus Haemonchus*, *Nematodirus*, *Trichostrongylus*, *Toxocara*, *Taeniidae*, *Spirocerca*, gastrointestinal nematode (GNI) genera and *Eimeria* oocysts were recovered from the samples. At least one parasite was present in 321 of the samples, amounting to 90.2% gastrointestinal parasite prevalence. The most encountered parasite in the study was *Haemonchus* with 45.5% prevalence followed by M*onieza* with 30.5% whereas *Taeniidae* was the least encountered (4.2%). As for the age of animals, 92.3 % of sheep under the age of one year were infected with parasites while 48.8% of animals above two years were infected. There was no significant relationship (P value=0.33) between the prevalence of gastrointestinal parasites and the sex of animals. Out of 356 studied sheep 110 (30.9%) were cross-infected with two parasite genera, and 15 (12.64%) were cross-infected with three parasite genera. The scanning electron microscopy (SEM) images confirmed the presence of *Cooperia*. GINs and *Eimeria* were extensively distributed and cross-infected in the sheep flocks leading to a higher risk of illness and mortality. This is the first report of the prevalence of gastrointestinal parasites across sheep farms in Northern Cyprus.

Keywords: Cyprus; *Eimeria*; GINs; Prevalence; Sheep

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INTRODUCTION

One of the mainstays of both smallholder and large-scale livestock farming businesses throughout the middle-east, Africa, Asia, and South America is sheep. More than two-thirds of the entire population of sheep and goats are found in the developing countries where they frequently offer key input to agribusiness ventures (Dawit et al., 2021). Gastrointestinal nematodiasis- a key parasitic infection in animals and humans, is generally disseminated globally (Han et al., 2017; Olaifa & Ozgor 2023) and it's the highest regular infection in sheep (Kordalis et al., 2019). Oftentimes, the septicity does not result in illness, but, on the other hand, adversely influences the production of animals (Mavrot et al., 2015; Kordalis et al. 2019). Numerous elements influence the infections of gastrointestinal nematodes in small ruminants. These factors comprise weather situations, farming operations, and the functional state of the animals. A Comprehensive understanding of epidemiology is essential for viable and standard control of gastrointestinal parasites in sheep (Chali and Hunde, 2021). In advanced countries, the utmost element of effect by the gastrointestinal nematode parasites is perhaps noticeable in the cost of control. Whereas their impact is higher in developing and underdeveloped countries owing to appropriate environmental dynamics for varied hosts and parasite species (Chali & Hunde, 2021).

Infections with *Eimeria spp* and gastrointestinal nematodes (GIN) continue to be major obstacles to sheep productivity in warming climes like the Mediterranean and surrounding areas, according to recent epidemiological surveys. For example, sheep in Ethiopia's tropical and semi-arid regions have exhibited a dry-season Eimeria oocyst prevalence of roughly 58%, with age and hygienic conditions being major risk factors (Lakew & Seyoum, 2016). Similar to this, pooled analyses in China show that several sheep breeds have incredibly high prevalence levels (sometimes over 95%), with dominating species like *E. ovinoidalis, E. crandallis,* and *E. ahsata* receiving a lot of media attention (Maurizio et al., 2024). This emphasises how common coccidial infections are at baseline in younger ruminants, particularly in lambs without acquired protection.

Simultaneously, the transmission interval for important nematodes such as *Haemonchus contortus* and *Teladorsagia circumcincta* is being extended by climate change. The development of anthelmintic resistance (AR) in gastrointestinal nematodes adds still another level of complication. Resistance rates in examined flocks average 86% to benzimidazoles, 52% to moxidectin, and 48% to levamisole, according to a systematic analysis that covers small ruminants worldwide (Fissiha & Kinde 2021). The effectiveness of both benzimidazoles and macrocyclic lactones in standard protocols has been shown to be diminished, since field investigations conducted in areas such as northeastern Italy have verified extensive treatment failure against H. contortus on sheep farms (Maurizio et al., 2024). In other places, integrated evaluations contend that new drug development, behavioural changes among farmers and veterinarians, diagnostic advancements, and alternative tactics like plant-based management and immunisation are necessary for the worldwide control of GIN (Morgan & Van Dijk 2012).

It is conceivable that comparable patterns high baseline prevalence of Eimeria, prolonged GIN transmission seasons, and possible undetected resistance pressures exist locally given the geographic, climatic, and production realities of Northern Cyprus, including its semi-Mediterranean climate, variable farm systems, and combined age-sex management of flocks. However, there are still little data unique to a given region, and without official epidemiological studies, management recommendations are always conjectural.

A solid understanding of parasites on sheep farms and in different sheep farm locations is of high necessity for setting up active and viable control plans. This study is therefore aimed at investigating the incidence of gastrointestinal nematodes and *Eimeria* across Northern Cyprus sheep farms via their morphological features. Thus, the influence of animal sex and age on gastrointestinal parasitism needed to be clarified. Furthermore, scanning electron microscopy (SEM) is a technique used for the description of the surface structure and morphological properties of an organism. SEM was employed in this investigation to further confirm the presence of parasites in our study.

MATERIALS AND METHODS

Study area and sample collection protocol

Review of this study by the ethical committee of Cyprus International University was waived because samples were collected on commercial sheep farms outside the university and none of the procedures tampered with the welfare of animals as only fresh faecal samples were used for the study. All samples were collected across Northern Cyprus sheep farms. These farms raise sheep and goats largely for milk and meat production. Microscopic studies were carried out in the medical microbiology laboratory of Cyprus International University. Although not a very large country of 3,572 square miles, the island of Cyprus has a varied topography. It has two mountain ranges, one of which stretches along the Northern part of the island and reaches heights of over 1,000 m above sea level, and the other, which is larger, lies in the Southwestern part of the island and reaches a height of nearly 2,000 m above sea level. The climate of the island is Mediterranean with mild, wet winters and long, dry, hot summers. There is greater precipitation in the coastal areas and on the mountain ranges than on the central plain.

Figure1. Cyprus-Northern Cyprus Map

Sample collection

A total of 356 faecal samples were randomly collected from 356 sheep across the two sexes and different age groups. The samples were collected and kept in airtight containers. Upon arrival at the laboratory, each sample was mixed thoroughly and 5g was taken from each container for parasites identification.

Sample processing

Centrifugal faecal floatation technique was used for sample preparation according to the procedure of Segura *et al*., (2023) with slight modifications. The faecal sample was weighed and completely mixed with 20 ml of water, and the blend was strained using a tea strainer. The bottle in which the blending was carried out was rinsed with about 5 ml of water, strained then added to the initially strained liquid. The faecal remains on the sieve and is pushed till it's desiccated and cast off with the use of a not reusable glove and thrust through with a finger. The strained faeces-fluid blend was retained in a 15 ml centrifuge tips for centrifugation in a centrifuge at 2000 rpm for 3 minutes at 20^{o}C. The supernatant was discarded carefully to avoid the forfeiture of the fine material at the uppermost part of the residue. The centrifuge tubes were then filled with saturated NaCl solution and mixed the residue with the floatation medium using a stick or by gentle shaking then centrifuged at 3000 rpm for 3 minutes at 20^{o}C. The samples were recovered and kept at 4^{o}C until they were checked for the presence or absence of nematodes and E*imeria* oocysts.

Determination of nematode and Eimeria presence

To test for the absence or presence of nematodes egg or *Eimeria* oocysts in the faecal matter, some processed samples were collected, the disposable pipette was used to draw out two to three drops from the sediment in the test tube and was released on the microscopic glass slide then covered with cover slide and observed under a compound microscope. The samples were viewed under the microscope at 10x and 40x magnification, the microscope was connected to a visual software for effective observation and clarification.

Tubes of suspension containing GIT parasites were randomly selected to further confirm the presence of parasites in the samples. About half of the suspension from each selected tube was processed for scanning electron microscopy (SEM) study. Processing involved the fixing of the parasite suspension in each selected tube for about 48 hours in 10% (v/v) neutral formal saline at room temperature (18-22^{O}C), followed by drying it up in rising grades of alcohol, and then suspending the eggs in isoamyl acetate. Subsequently, the eggs were dried with a dryer and exposed to gold in a Quorum Technologies SC7640 sputter coater. The specimens were then observed by SEM (T-330A; JEOL, Tokyo, Japan).

Data analysis

Statically analysis was carried out employing SPSS software for Windows version 22.0 (SPSS Inc., Chicago, USA). Chi square test was carried out to determine the relationship between variables and relationship between variables were assumed to be real when the probability of finding the observed difference by chance was less than 5% (P < 0.05). The complete prevalence was estimated as a percentage of the number of sheep infested in the entire number of sheep investigated.

RESULTS

Sample characteristics

A total of 356 faecal samples were analysed for the presence of gastrointestinal parasites. Of these, 120 samples (33.7%) were obtained from male sheep, while 236 samples (66.3%) were from females. With regard to age distribution, 65 sheep (18.3%) were less than one year old, 170 (47.8%) were between 1–2 years, and 121 (34.0%) were older than two years (Table 1).

Table 1. Prevalence of gastrointestinal parasites according to age among Northern Cyprus sheep

Age	Number examined	Positive	Prevalence (%)	χ2	P-value
Under 1 year	65	60	92.3		
1–2 years	170	154	90.6	36.7	0.009
Above 2 years	121	59	48.8		

Overall Prevalence of Gastrointestinal Parasites

At least one gastrointestinal parasite was detected in 321 out of 356 samples, giving an overall parasite prevalence of 90.2%. Identification of endoparasites was based on the morphological characteristics of eggs using the centrifugal faecal flotation technique followed by light microscopy examination (Table 2).

Distribution of Gastrointestinal Nematodes and Eimeria spp.

Table 2. Prevalence of GIT nematodes and *Eimeria* in Northern Cyprus sheep farms

Nematode genera	Number infected	Prevalence (%)
Haemonchus	162	45.5
Nematodirus	95	26.7
Trichostrongylus	60	16.9
Monieza	107	30.5
Trichurus	42	11.8
Toxocara	20	5.6
Oesophagosuturom	17	4.8
Other *strongyles*	60	16.9
Taeniidae	15	4.2
Cooperia	32	9.0
Eimeria oocyst	127	35.7
Spirocerca	55	15.4

Eggs of gastrointestinal nematodes (GIN) belonging to the genera *Moniezia, Cooperia, Oesophagostomum, Trichuris, Haemonchus, Nematodirus, Trichostrongylus, Toxocara, Taeniidae, Spirocerca*, and other strongyle-type eggs were recovered.

Eimeria oocysts were detected in 127 samples (35.0%) of the total examined population. Among the gastrointestinal nematodes, *Haemonchus* spp. were the most frequently encountered, identified in 162 samples, corresponding to a 45.5% prevalence (Table 2).

Age-related Prevalence of Infection

Analysis of infection prevalence by age showed that younger animals were more susceptible to gastrointestinal parasite infections compared to older sheep. Animals below two years of age exhibited infection rates exceeding 90% (Table 3). Statistical analysis demonstrated a significant association between age and parasite prevalence (Chi-square = 36.7; p = 0.009), indicating that age is a significant risk factor for gastrointestinal parasitic infections (P < 0.05).

Table 3 Prevalence of gastrointestinal parasites according to age among Northern Cyprus sheep

Age	Number examined	Positive	Prevalence	χ2	P-value
Under 1 year	65	60	92.3 %		
1–2 years	170	154	90.6%	36.7	0.009
Above 2 years	121	59	48.8%		

Pattern of Single and Multiple Infections

Out of the 356 studied sheep, 110 (30.9%) were cross infected with two parasites, 15 of the examined samples (12.64%) were infected with three gastrointestinal parasites, 196 (55.1%) were infected with one gastrointestinal parasite while 35 (9.8%) returned a negative result for the incidence of at least one nematode species or *Eimeria* oocyst (Table 4).

Table 4. Poly-parasitism of gastrointestinal nematodes in Northern Cyprus sheep farms

Load	One	Two	Three	Non-infected
Number	196	110	15	35
Per cent	55.1	30.9	12.64	9.8

Sex-related Distribution of Gastrointestinal Parasites

With regards to animal sex, observations revealed that all the parasites encountered were found in all the faecal samples taken from the male animals except for *Oesophagosuturom* while three of the 12 encountered parasites namely *Trichostrongylus, Oesophagosuturom* and *Trichurus* were not found in any of the female animals across the farms. The total number of samples collected from male animals tested during the study was 120 and 102 were positive for at least one endoparasite while 219 out of 236 samples taken from the female animals gave a positive result accounting for 92.8% prevalence therefore a higher prevalence of major gastrointestinal parasite infection was observed in female animals though the p-value (0.33) suggests that the relationship between the sex and prevalence is not statistically significant (*P* >0.05) (Table 5).

Table 5. Prevalence of GIT parasites according to the animal sex

Sex	Number examined	Number positive	Prevalence (%)	χ2	*P*-value
Male	120	102	85.0	0.182	0.33
Female	236	219	92.8		

Scanning Electron Microscopy (SEM) Findings

Figure 1 (a) and (b) revealed the mobility patterns of nematode on the slide used for the SEM study. The results of the characteristics of furrows left by the larvae and morphological identification were following that of Inês et al. (2011) when the culture of *S. stercoralis* and hookworm larvae were placed in an agar. Figure c and d also reveals the scanning electron micrographs of the infective larva of *Cooperia spp*. The head end of the larva was funnelled fashioned demeanour along with a projectile-like structure directed anteriorly. Crosswise striations were seen on the body and sheath. The tail was modest with a spoon-like structure at the tip. The tail-end shows the spoon-like structure at the tip of the sheath.

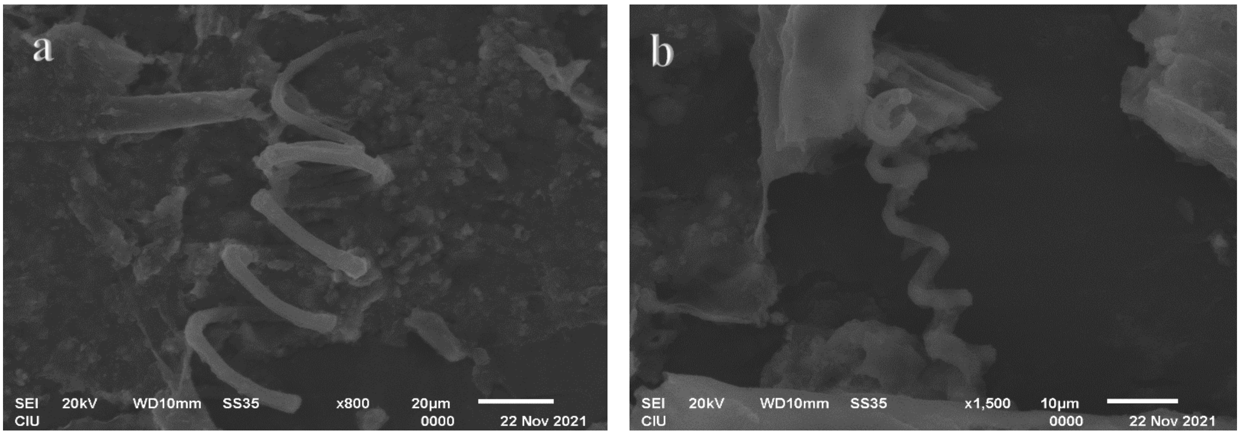

Figure 2 Characteristic tracks or furrows left by the crawling of GIN species

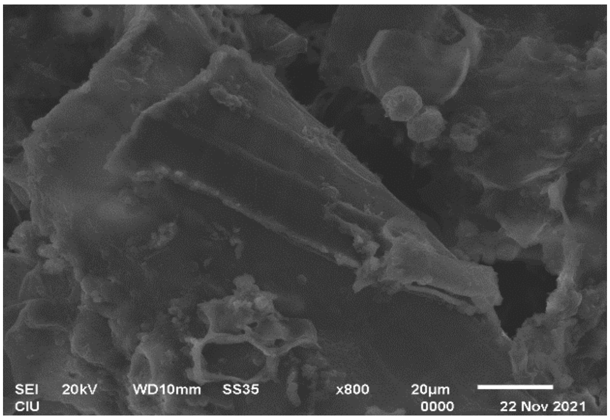

Figure 3 The head end of the larva with a loose sheath and a typical long arrow-like projection

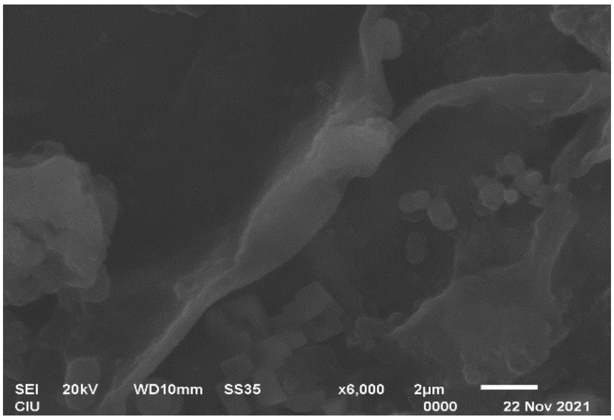

Figure 4 The tail-end spoon-like structure at the slope of the sheath

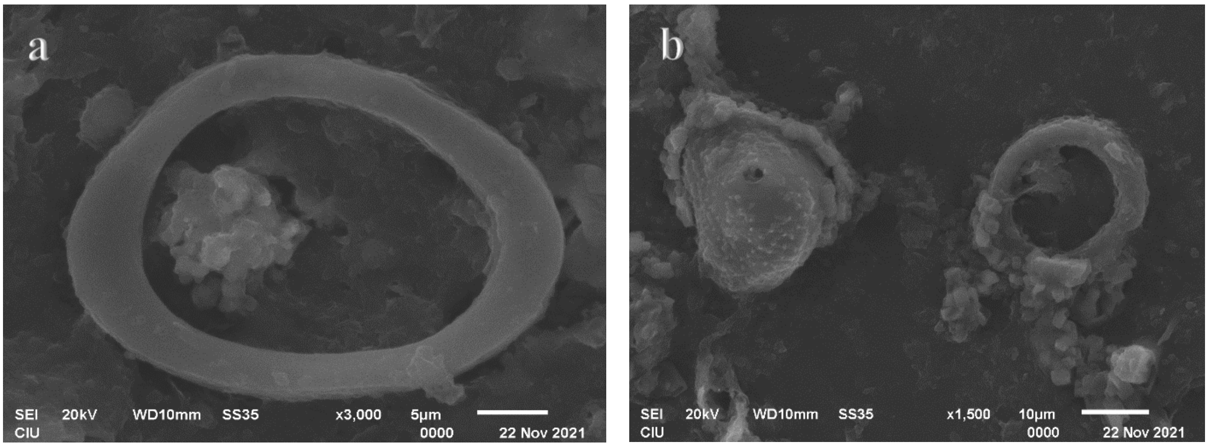

Figure 5 Egg cuticles of nematode parasites of sheep

DISCUSSION

The present study revealed the detail of the gastrointestinal parasitism status across sheep farms in the Northern region of Cyprus. Gastrointestinal Nematode parasites, such as *H. contortus* and *Nematodirus spp* minimize sheep production, as a result of the uninterrupted effects of their blood nurturing pattern, while the pathogenic impacts of most gastrointestinal parasitic nematodes is as a result of host distinctive and adaptive immune responses (Greer, 2008) injuring the absorptive coating of the gastrointestinal tract. The resulting pathophysiological impacts of these undertakings are ineffective feed exploitation, prompting a situation of relative protein shortage, fluid and electrolyte or macroelement disparities and anaemia, giving rise to medical symptoms, such as deprived weight increases, and lowered appetite, diarrhoea and death (Sinclair et al., 2016).

In terms of animal sex, observations showed the occurrence of GIT parasites affecting both sex groups almost equally. This is a result of equivalent exposure of both sexes, and they are as well from the same agroecology. The effect of sex on the vulnerability of sheep to infections could be ascribed to innate tendency and distinctive proneness due to hormonal regulation. The existing gender-wise remarks are in agreement with the finding of Salehi et al. (2022) where no significant difference observed between the infection level in females and males, with 56.9% and 43.94% rates of infection, respectively. Immune function may be influenced by hormonal and physiological variations, although exposure and management are the main causes of infection (Wesołowska, 2022). To further understand risk variables, sampling in Northern Cyprus should be stratified by age and production system in future studies.

Recent epidemiological investigations in various production systems also support the significant incidence of *Eimeria* oocysts on sheep ranches. In lambs, intestinal lesions, diarrhea, dehydration, and decreased weight gain are specifically linked to *Eimeria ovinoidalis* and *Eimeria crandallis* (Athanasiou *et al*., 2023). Etsay *et al.* (2020) reported the prevalence of sheep and goat coccidiosis (87.31% and 85.03% respectively) in different districts of the Tigray region in Ethiopia while the high prevalence of *Eimeria* oocyst was also recorded in Egypt in the work of Mohamaden *et al.* (2018). Our findings are consistence with the work of Höglund *et al.* (2020) which revealed that 13 out of 20 lambs sampled tested positive for *Moniezia expansa* as well as *Trichostrongylus axei been the least occurrence on the farm.* Animal management procedures and environmental cleanliness have a significant impact on these protozoan diseases, which have been found to be more common in systems that are restricted or under strict supervision (Cao *et al.*, 2023). It is probable that the management techniques used in this study, which involved confining sheep in pens and sharing feeding and watering locations, promoted oocyst accumulation and horizontal transmission within flocks.

Among the GIT nematodes, the one with the highest prevalence is *Haemonchus* amounting to 45.5% of the animals tested across the farms followed by *Moniezia* 30.5% followed by the eggs of *Nematodirus* 26.7%. The findings of this study are in agreement with the results of Chali & Hunde (2021) who reported the most prevalence of *Haemonchus* (20.8%) followed by *Trichostrongylus* (13.0%) and *Nematodirus* (10.2%) respectively. Similarly, these discoveries are the same of Lateef *et al.* (2005) who recorded the maximum incidence of *Haemonchus* (61.5%) followed by *Trichostrongylus* (46.1%) and *Ostertagia* (33.0%). Nginyi *et al.* (2001) detailed the genera of nematodes *Haemonchus* and *Trichostrongylus* (33.0%) and (29.0%), as the most prevalent respectively. *Haemonchus* being the most prevalent nematode in the present investigation might as well be due to its biotic likelihood which vindicated its high prevalence (Nginyi *et al.*, 2001; Chali and Hunde, 2021). *Haemonchus* is certainly one of the most pathogenic GIN in ruminants responsible for the pervasive illness and death of sheep and goats and consequently deserves distinct consideration in gastrointestinal parasite control plans (Tariq *et al.*, 2008; Mederos *et al.*, 2010; Sinclair *et al.*, 2016; Kuma *et al*., 2019; Costa-Junior *et al.*, 2021).

The results of this study disagree with the findings of Zeryehun (2006) who documented genera-wise occurrence in sheep for strongyles type eggs, *Strongyloides, Trichuris* as (39.8%), (17.5%) and (7.8%), in that order. Kantzoura *et al.* (2013) also reported prevalence in sheep for *Nematodirus* at (1.1%) and *Trichuris* at (2.9%). Mushtaq & Tasawar (2011) similarly recorded the prevalence of *Haemonchus* and *Trichuris* (6.5%) and (5.7%), respectively. While Al-Shaibani et al. (2008) recorded a higher prevalence of *Haemonchus* (24.6%) which was observed to be the leading gastrointestinal nematode parasite with *Trichostrongylus* (18.0%) being the next most prevalent species.

Chali & Hunde (2021) pointed out that the dissimilarities in the incidence of diverse gastrointestinal nematode parasites in the current and other investigations done in different localities could be a result of diverse climatic conditions such as temperature and humidity, ecologies, and pastures. Radostits *et al*. (1994) indicated that warm and wet weather offers promising circumstances for the transformation of eggs to larvae in the bulk of helminths which is what was observed in this study, the samples were taken at the onset of the winter season when the weather was warm and wet; conversely, the locations having severe summer and dry winter compact the parasitic load on the indigenous animals (Chali & Hunde, 2021). In arid and hot zones, apart from in flooded or other lastingly wet fallow, the spread of parasites is constrained to the wet season and the only avenue of carry-over of contamination from one rainy season to the other is via animals concealing grown-up worms and/or arrested larvae (Mohammed *et al.,* 2015). The persistence and diffusion of a free existing phase of nematode parasites are affected by micro-climatic conditions within the faecal pellets and herbage (Mohammed *et al.*, 2016).

The current research suggests to emphasize the necessity of integrated parasite control methods on Northern Cyprus sheep farms. *Haemonchus contortus* predominance in this study is in line with findings in other Mediterranean and subtropical settings, where warm, humid weather promotes larval survival and spread (Rose *et al.*, 2016). Because of its nature, the blood-feeding abomasal nematode *H. contortus* is one of the most harmful parasites in small ruminants, sometimes causing anaemia, hypoproteinemia, and in extreme situations, death (Kenyon *et al*., 2009). This emphasizes how its presence has an economic impact because infected animals have low growth, decreased reproductive performance, and decreased feed conversion efficiency, all of which affect farm profitability (Charlier *et al.,* 2020).

A sustainable method of reducing parasite burdens is provided by integrated parasite management (IPM) techniques. These include genetic selection for animals resistant to parasites, dietary supplements to boost host immunity; the use of alternative control measures such bioactive forages, and the selective treatment of heavily infected individuals as opposed to blanket dosage (Hoste *et al*., 2022). Reducing stocking density and enhancing hygiene in restricted housing are also essential for preventing the spread of Eimeria. In other Mediterranean systems, it has been demonstrated that educating farmers about these measures greatly lowers the prevalence of parasites and increases flock productivity (Vande Velde *et al.,* 2018).

It also noticed that in this study that animals were packed together in the same sheep pen, they were as well sedentary and they were under firm confinement in the process of feeding which leads to a high risk of helminth contamination. Gastrointestinal Nematode parasites, such as *H. contortus* and *Nematodirus spp* minimize sheep production, as a result of the uninterrupted effects of their blood nurturing pattern, while the diseases causing impacts of most gastrointestinal parasitic nematodes is as a result of host distinctive and adaptive immune responses (Greer, 2008) injuring the absorptive coating of the gastrointestinal tract. The resulting pathophysiological impacts of these undertakings are ineffective feed exploitation, prompting a situation of relative protein shortage, fluid and electrolyte or macroelement disparities and anaemia, giving rise to medical symptoms, such as deprived weight increases, lowered appetite, diarrhoea and death (Sinclair *et al*. 2016). Polyparasitism, as observed in this study, is common in small ruminant production systems and often results in additive or synergistic negative effects on animal health (Höglund *et al*., 2020) as well as the recurring contact with infections of both GINs and *Eimeria*. Mixed infections of *H. contortus*, *Nematodirus*, and *Eimeria* can exacerbate clinical signs, including diarrhea and weight loss, and complicate treatment strategies. This further supports the need for a holistic approach that integrates pasture management, improved housing hygiene, and strategic anthelmintic use rather than reliance on single interventions. The SEM images of *Cooperia spp* are in agreement with that of Thilakan & Sathianesan (2007). Figure e and f show the outer shape of nematode eggs found in the faecal sample analysed to further confirm the presence of GIN encountered in the microscopic analysis.

CONCLUSIONS

In summary, this investigation showed that both GINs and *Eimeria* as lone or simultaneous infections are dominant in sheep farms in Cyprus. Younger animals are likely to be more infected with GIT parasites while the sex of animals does not affect GIT parasites infection. The parasitic weight constitutes a pronounced financial influence and limitation to the sheep venture in attaining improved and viable productions. Consequently, these results recommend a need for well-harmonized, healthy regulation of sheep farms by veterinarians and the spreading of awareness on GINs and *Eimeria* to breeders and farmers to lessen the incidence of infections.

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CONFLICT OF INTEREST DECLARATION

The authors declare none.

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