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Helminths of captive and free-ranging populations of the mountain gazelle (*Gazella gazella*): Evidence from faecal examination

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Funding information

Scientific and Technological Research Council of Türkiye (TÜBİTAK), Grant/Award Number: 123Z575

Abstract

Background: Understanding parasite diversity in wild and captive animal populations has critical implications for both individual animal health and ecosystem dynamics in a broader sense. In mountain gazelles (*Gazella gazella*), the gastrointestinal helminth community is poorly understood, limiting our efforts in the conservation of this endangered bovid species. This species has only two remaining populations in the world, including the isolated northernmost population in Türkiye.

Objectives: To identify and compare the diversity and prevalence of gastrointestinal helminths in captive and free-ranging populations of mountain gazelles in Hatay, Türkiye, and to assess potential zoonotic risks.

Methods: In total, 105 fresh faecal samples, 45 individual samples and 60 faecal samples, representing 16 pools, from both captive and free-ranging populations were collected and analysed using Fulleborn flotation, Benedek sedimentation and Bearman–Wetzel methods faecal flotation methods, including the McMaster technique to determine the severity of infection.

Results: We detected 12 helminth taxa in our examination of faecal samples, including gastrointestinal nematodes, lungworms and trematodes. Parasites from the Trichostrongyloidea family demonstrated variable hatching stages and rates, potentially influenced by ambient conditions. We also detected one protozoan among the samples. Our results revealed a higher diversity of parasites in free-ranging populations compared to captive ones.

Conclusions: This study underscores the necessity for regular parasitological surveillance in both captive and free-ranging wildlife populations for effective conservation management. It also contributes to the 'One Health' perspective by highlighting the potential zoonotic risks posed by parasites in wild ruminants. Our results have implications for the conservation and management of the mountain gazelle.

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KEYWORDS

faecal examination, Gazella gazella, gastrointestinal parasites, helminths, lungworms, the mountain gazelle

1 | INTRODUCTION

The management, conservation and health care of wildlife are essential across many disciplines (Mackenzie & Jeggo, 2019). Various factors threaten the lives of wild animals, including infections, parasitic diseases, poaching, habitat fragmentation, human activities and climate change (Muraleedharan, 2016). With the rapidly increasing human population, interactions between people and wild animals have become more prevalent (König et al., 2020), leading to a two-way exchange of parasites and an increase in their impact (Apio et al., 2013). Indeed, the majority of infectious diseases, which greatly affect public health, livestock and food safety, originate from wildlife (Idland et al., 2021; Mackenzie & Jeggo, 2019). Diseases such as parasitic infections can be transmitted directly or indirectly through contaminated water, soil, meat or faeces, affecting other wild animals' populations, domestic species or humans (Figueiredo et al., 2020).

Several serious diseases leading to morbidity and mortality are caused by gastrointestinal helminths, including trematodes, cestodes and nematodes (Cook. 1986). These diseases have a significant negative influence on domestic and wild animals as well as the environment in addition to humans (Thompson, 2013). The various transmission pathways for helminths make their prevalence high, creating an issue that requires attention (Shoop, 1991). It has also been shown that the dynamics of wild ungulate populations are adversely impacted by the rise in parasitic load, which refers the number or quantity of parasites carried by a single infected host organism (Albon et al., 2002; Gulland, 1992; Maublanc et al., 2009). The clinical manifestations of parasitic infections can vary depending on the region where they occur. These infections harm the host's physical condition, impair nutrient utilization, reduce reproductive success, may cause abortions, and increase the risk of congenital transmission. Lungworms, bacteria, viruses and stress factors play a significant role in pneumonia among wild sheep. The parasite Strongyloides spp. can trigger anorexia, dyspnea and diarrhoea in Boselaphus tragocamelus, Axis axis, Bos gaurus and Rusa unicolor (Gupta et al., 2011). The Haemoenchus spp. parasite can cause severe gastrointestinal ulcers and blood loss, making the host weak and more vulnerable to other illnesses in the populations of Odocoileus virginianus and Cervus nippon. It can also lead to significant mortality and morbidity (Bhat et al., 2022).

Considering the threats wild ungulates face, such as climate change, habitat fragmentation and poaching, along with their expanding distribution and interactions with other species, understanding the helminths of wild ungulates is crucial. Previous studies emphasise the importance of regular monitoring of parasites and parasitic diseases in wild ungulate species (Akramova et al., 2020; Carrau et al., 2021; Davidson et al., 2015; Debeffe et al., 2014; Figueiredo et al., 2020; Hoberg et al., 2001; Idland et al., 2021; Jaiswal et al., 2014; Kapnisis et al., 2022; Kutz et al., 2004; Meister et al., 1993; Muraleedharan, 2016; Patra et al., 2022; Romero-Castañón et al., 2008).

The genus Gazella, a member of the Bovidae family, which is the largest and richest ungulate family in the order Artiodactyla (Groves, 1997), is distributed across a wide geography from Africa to North Asia, including southeastern Anatolia and the Arabian Peninsula, and has adapted to desert and semidesert climates (Lerp et al., 2013). Among other gazelle species, Gazella gazella (the mountain gazelle; TSN: 625093) is especially important because it is classified as 'Endangered' species by IUCN and its population has decreased over the past century anthropogenic activities, poaching, road kills and habitat degradation and fragmentation (Yom-Tov, 2021). Today, G. gazella is distributed in a limited area in Israel, Palestine, Jordan and Kırıkhan district of Hatay province in Türkiye (Mallon & Kingswood, 2001; Yom-Tov, 2021). Even though the Hatay population of mountain gazelle was once connected to the Israeli population, it became an isolated population with the extinction of the Syrian population (Kankılıç et al., 2012). The mountain gazelle (G. gazella) population has shown varying trends in different regions, reflecting both conservation successes and challenges. According to the initial data, the Hatay population of mountain gazelle consisted of approximately 200 individuals in 2008. The latest inventory studies, conducted by the Hatay Branch of Nature Conservation National Parks of Türkiye, reveal a count of 1331 individuals in 2023. This data shows that the mountain gazelle population has increased over the years, indicating that conservation efforts in the region have positively impacted the population size. In contrast, the Israeli population of mountain gazelle, which was around 10,000 individuals in the 1990s, has decreased by 50% over a decade, with the number of individuals declining to 2500 in 2015 (IUCN SSC Antelope Specialist Group, 2017; Saragusty et al., 2006).

There have not been many studies done on the helminths in the Gazella genus (Apio et al., 2013; Baghi et al., 2016; Casselino et al., 2001; Chertkova, 1971; Elsamı et al., 1981; Saud et al., 2012; Yılmaz, 2021; Zerek et al., 2022). Studies on species belonging to the genus Gazella have generally been carried out from the perspective of morphological identification of helminths, species conservation and wildlife management or pharmacology (Akramova et al., 2020; Eslami et al., 1980; Mohammed et al., 2007; Moreno Manas et al., 2019; Ortiz et al., 2006). As the potential competition and transmission of helminths between wild herbivores and livestock in shared grasslands could lead to local extinction of wild populations (Modabbernia et al., 2021), monitoring the parasitic fauna and implementing preventive measures for helminth contamination are necessary in wild ruminants. In this study, we examined the helminths of the mountain gazelle by analysing faecal samples in the northernmost Hatay population of the species.



FIGURE 1 A general scene from the study site (left) and the study species, Gazella gazella (right).

2 | MATERIALS AND METHODS

2.1 | Study site

The study was conducted in the Hatay Mountain Gazelle Wildlife Development Area, in Hatay province, Türkiye, near the Syrian border (36°32' N, 36°32' E; 200-450 m). The study site constitutes the only and the main range of the northernmost population of G. gazella (Kankılıç et al., 2012) and predominantly has grassland vegetation with several shrubland patches, extensive cropland areas and rocky hills (Figure 1). The site was declared as Wildlife Development Area in 2019, covering approximately 13,288 ha. Observations confirmed that mountain gazelles actively use this area throughout the year (T. Kankılıç, personal observation). Within the study site, there is the Hatay Mountain Gazelle Production Centre where captive mountain gazelles were found. This production centre shares a similar vegetation structure to the area where free-ranging individuals are found, characterised predominantly by grassland vegetation interspersed with patches of shrubs. Additional forage is supplied by animal caretakers for captive individuals, but no anthelmintics have been applied.

The study site has a rich mammal community, as 23 mammal species were recorded in this area, including *Canis aureus* (golden jackal), *Canis lupus* (wolf) and *Vulpes vulpes* (red fox), which are classified as natural predators of mountain gazelle, and some others such as *Hyaena hyaena* (striped hyena), *Felis chaus* (jungle cat), *Felis silvestris* (wildcat), *Hystrix indica* (Indian porcupine), *Lepus europaeus* (wild rabbit) and *Meles meles* (European badger) (Akay et al., 2011; Çoğal & Sözen, 2017). In the study area, domestic sheep herds are also frequently observed throughout the year.

2.2 | Faecal sampling

Faecal sampling was carried out in April, July and September 2023, at several locations within the study area, between 07:00 and 19:00 local time (GMT+3). Faeces of both free-ranging and captive moun-

tain gazelles were sampled in the study. Before collecting the faecal samples of free-ranging mountain gazelles, individuals were watched with binoculars from a minimum distance of 500 m. The location where the group or the individual detected with binoculars was reached in the fastest way by vehicle and sometimes on foot. Then the observation point was visually examined and the freshest faecal samples were collected. As our observations suggest that G. gazella individuals either defecate in previously used spots or choose new locations, we collected fresh faeces from both new and previously defecated spots. Although faeces from both populations were collected as specified, due to the confined and fenced territory of the captive population, the average collection distance was 50 m in this population. Whether the sample was fresh or not was determined based on colour, hardness and moisture criteria. Fresh faeces are darker, softer and wetter than old faeces (Figure 2). Based on our previous observations, the freshest faeces have been identified due to their softness, shiny appearance and sometimes even the presence of mucus.

For each fresh faeces found in the field, approximately 10 g of faecal samples were taken and then put into plastic containers. The containers, including faecal samples, were then placed into a cooler (\sim 4°C) in the field. Containers, later, were transferred to the laboratory for further parasitological examinations.

2.3 | Coprological analysis

Flotation, sedimentation and Baermann-Wetzel were used for the detection of trematodes, cestodes and nematodes in faecal samples. Detections and measurements were performed using binocular light microscopes and photographs of detected parasites were taken by a digital microscope (Nikon Eclipse E600). Parasite detection was done by visual inspection and is based on reference books of Foreyt (2013), Taylor et al. (2015) and Zajac et al. (2021). The flotation technique is the best technique for the identification of nematode and cestode eggs and protozoan cysts from faecal samples, while the sedimentation technique works to select the trematode eggs, and



FIGURE 2 Faeces of *Gazella gazella*. Left: fresh faeces from a new defecation spot, Right: fresh and dry faeces together in a previously used spot.

Baermann-Wetzel is used to detect L1-stage lungworm larvae (Baermann, 1917; Figueiredo et al., 2020; Forrester & Lankester, 1997; Turner & Getz, 2010). Identification of eggs and larvae was conducted according to their morphological characteristics, as outlined in Foreyt (2013), Taylor et al. (2015) and Zajac et al. (2021) reference books. For example, the distinction between *Muellerius* spp. and *Neostrongylus* spp. from the *Metastrongylidae* family was based on their tail structures. The number of eggs per gram (EPG) was determined by egg counting technique McMaster, in faecal samples that were detected to be infected (Ezenwa, 2003; Nápravníková et al., 2019). For our study, all length measurements are given in micrometres (µm).

For the Fulleborn flotation procedure, approximately 2 g of faecal samples were placed into an examination container filled with a saturated saline solution. The mixture was homogenised by using a slide. Subsequently, the mixture was filtered through a strainer with a mesh size of $>300 \,\mu$ m into a second container. The strained mixture was filled with saturated saline solution, and three coverslips were placed on it. The mixture was allowed to stand for about 30 min. Afterwards, using forceps, the coverslips were taken out and positioned onto a slide. The slides were examined under a light microscope (Bowman, 2014; Taylor et al., 2015; Zajac et al., 2021).

For the Benedek sedimentation procedure, 3 g of faecal sample was mixed with tap water in the examination container. The mixture was homogenised with the slide and filtered into a 250 mL beaker with a strainer with a mesh size of >300 μ m. Tap water was added into the beaker. After 15-min waiting period, the supernatant was carefully poured off without moving the sediment at the bottom. Tap water was added again, and the same process was repeated three or four times until the supernatant became completely clear. Finally, without moving the sediment at the bottom with moving the sediment at the bottom.

sediment at the bottom was transferred to a Petri dish by gently shaking. A few drops of methylene blue are added to it and examined under a microscope (Acharya & Mishra, 2019; Adhikari et al., 2022; Becker et al., 2016; Taylor et al., 2015).

For the Baermann-Wetzel procedure, 5 g of faecal sample was taken into the gauze pad and wrapped. The faecal pouch was placed into the water completely. Migration of larvae was waited for 24 h. At the end of the 24-h period, after expelling the faecal pouch, the rubber by held in the middle of the hose, the liquid in the upper part was poured and the tube was removed. Third-fourth of the liquid at the top of the tube was drained and a drop of the residue was taken and a preparation was prepared for the microscope examination (Bowman, 2014).

For the McMaster procedure, 2 g of faecal sample was mixed well with 28 mL of flotation solution and strained through a strainer with a mesh size of >300 µm. Each chamber of McMaster slides is filled with the mixture by using a pipette immediately. When all the chambers of the slide were filled, it waited for 5 min. Then, the slide was transferred to the microscope for egg counts (Barda et al., 2014; Zajac et al., 2021). In terms of egg counts, the parasite eggs were counted in each chamber, and the total number of eggs was multiplied by 50 (Barda et al., 2014). We applied the McMaster procedure to only four faecal samples in which eggs were observed and where a sufficient amount of the sample remained after three different detection methods.

A total of 105 samples from captive and free-ranging mountain gazelle populations were analysed by conventional methods. Among 105 samples, 40 samples were collected from the captive mountain gazelle population and 65 samples were collected from the free-range mountain gazelle population. Each sample group was evaluated to represent the specific location from which it was collected. However, due

TABLE 1 Presence of helminth taxa in captive and free-ranging individuals of the studied Gazella gazella population.

		Captive			Free-ranging		
	Таха	Flotation	Sedimentation	Baerman- Wetzel	Flotation	Sedimentation	Baerman- Wetzel
Gastrointestinal nematodes	Nematodirus spp.*	+	+	_	_	+	_
	Skrjabinema spp.***	_	_	-	_	+	+
	Marshallagia spp.*	-	+	_	+	+	-
	Hookworm egg**	_	_	-	+	-	-
	Trichuris spp.*	-	+	_	_	+	-
	Toxocara spp.**	-	-	-	-	_	+
	Strongylidae spp.*	+	+	+	+	+	-
Lungworms	Protostrongylus spp.*	-	-	+	-	+	+
	Neostrongylus spp.**	-	-	-	-	+	+
	Cystocaulus spp.*	-	+	+	-	+	+
	Muellerius spp.***	-	-	-	-	+	-
Trematoda	Dicrocoelium spp.***	_	_	_	_	+	_

Note: Data was obtained using both individually examined and pooled samples. (+) and (-) signs refer to the presence and absence of corresponding taxon in captive or free-ranging individuals in our samples (* found in both pooled and individual samples; ** pooled samples only; *** individual samples only).

to insufficient quantities in some faecal samples, 16 pools were created for specific locations using the collected faeces from that location. A total of 45 individual samples and 60 faecal samples, constituting these 16 pools, were analysed. Finally, we conducted chi-square tests to compare the differences in occurrences between captive and free-ranging populations for each taxon and for all parasites using the MASS package (Venables & Ripley, 2002) implemented in the R environment (R Core Team, 2021).

3 | RESULTS

In total, we detected 12 helminth taxa in our examination of faecal samples of G. gazella using by few techniques (Tables 1 and 2; Figures 2–5). The identification keys for these taxa were provided in Table S1. Among all faecal samples we examined, more than 60% contained helminth eggs (specifically for gastrointestinal nematodes and trematodes) or larvae (for lungworms). Out of 45 individually examined samples, we detected helminths in 26, including 11 out of 20 from the captive population and 15 out of 25 from the free-ranging population. The occurrence frequency of parasites in these samples was not significantly different between captive and free-ranging populations $(\chi^2 = 0.001, p > 0.05)$. Additionally, most of the pooled samples (14 out of 16) also contained helminths. Helminth taxa richness was notably higher in the free-ranging population compared to the captive one (12 vs. 6; Table 1). Specifically, eggs of Dicrocoelium spp., Skrjabinema spp. and Toxocara spp., along with larvae of Muellerius spp. and Neostrongylus spp., were only observed in the free-ranging population but not in the captive one (Table 1). Among the individually examined faecal samples, the most frequent taxa were the lungworm Cystocaulus spp. (17.7%; Table 2; Figure 4), and the gastrointestinal nematodes Nematodirus spp. and Trichuris spp., both of which were found in 15.5% of the samples

(Table 2; Figure 3). Nematodirus spp. was the most frequently observed parasite in samples from the captive population with none detected in the free-ranging populations; this difference was significant (p = 0.005; Table 2). Although some taxa showed higher frequency of occurrence in the free-ranging population compared to the captive one, and vice versa, none of these differences were statistically significant (p > 0.05, Table 2). For the record, we also found a coccidian protozoan in one faecal sample. Furthermore, among the four faecal samples analysed using the McMaster method, we detected just two eggs in one sample and the EPG estimated as 100.

4 DISCUSSION

Our study revealed that the northernmost population of the mountain gazelle, *G. gazella*, possess several helminth taxa, including gastrointestinal nematodes, lungworms and trematodes. Our results also suggest that helminths in free-ranging individuals of the mountain gazelle are more diverse compared to captive individuals.

Various research has been conducted to identify the helminth fauna of different *Gazella* species, primarily focused on captive populations with a lesser emphasis on free-ranging populations. In studies of captive *Gazella* subgutturosa (TSN: 625101), numerous helminths have been revealed, including *Marshallagia* marshalli, *Camelostrongylus* mentulatus, Ostertagia spp., *Trichostrongylus* spp. and Nematodirus spp. (Baghi et al., 2016; Elsami et al., 1981; Eslami et al., 1980; Modabbernia et al., 2021). Similarly, studies on captive populations of other *Gazella* species like *G. dama* mhorr (TSN: 898925), *G. cuvieri* (TSN: 625090), *G. dorcas* neglecta (TSN: 898645) and *Gazella* leptoceros (TSN: 625095) have identified helminths, such as Nematodirus spathiger, Trichostrongylus vitrinus and Ostertagia ostertagi (Abaigar et al., 1995; Goossens et al., 2005; Ortiz et al., 2001). In captive populations

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TABLE 2 Occurrence frequency of helminth taxa in captive and free-ranging individuals of the studied Gazella gazella population.

	Occurrence fre	quency (% of samples)	Statistics		
Таха	Captive	Free-ranging	Total	χ^2	р
Gastrointestinal nematodes					
Nematodirus spp.	35.0	0.0	15.5	7.87	0.005
Skrjabinema spp.	0.0	16.0	8.8	1.81	>0.05
Marshallagia spp.	5.0	12.0	8.8	0.09	>0.05
Trichuris spp.	15.0	16.0	15.5	~0	>0.05
Strongylidae spp.	10.0	0.0	4.4	0.79	>0.05
Lungworms					
Protostrongylus spp.	10.0	8.0	8.8	~0	>0.05
Cystocaulus spp.	10.0	24.0	17.7	0.69	>0.05
Muellerius spp.	0.0	8.0	4.4	0.32	>0.05
Trematoda					
Dicrocoelium spp.	0.0	4.0	2.2	~0	>0.05

Note: Data is obtained only using individually examined samples. The statistics section presents the results of a chi-square test conducted to compare the differences in occurrences between captive and free-ranging populations for each taxon.



FIGURE 3 Helminth eggs obtained from faecal samples in *Gazella gazella*: (a) *Marshallagia* spp.; (b) *Nematodirus* spp.; (c) *Skrjabinema* spp.; (d) *Trichuris* spp. All photos are taken at 40× magnification (Scale bar: 100 µm). Identifications are based on Taylor et al. (2015) and Zajac et al. (2021).

of G. subgutturosa marica (TSN: 898657) and G. gazella in Saudi Arabia, the following helminths were identified: *Haemonchus contortus*, C. mentulatus, *Trichostrongylus probolurus*, N. spathiger and *Trichuris* spp. (Mohammed et al., 2007). Additional gastrointestinal nematodes such as *Teladorsagia* spp. (Yilmaz, 2021), *Nematodirus* spp., *Ostertagia* spp. and *Trichostrongylus* spp. (Altas & Iriadam, 2004) were also detected in G. subgutturosa. Contrarily, in free-ranging populations of *G. subgutturosa*, helminths like *Dicrocoelium dendriticum*, *M. marshalli*, *Skrjabinema ovis* and *Trichuris* spp. have been found (Akramova et al., 2020; Asadov, 1957). *Trichuris* spp. and strongyle-type found in captive populations of *G. leptoceros* in Belgium (Goossens et al., 2005). Moreover, in Farasan Islands (Saudi Arabia), strongyle-type eggs were identified in free-ranging *G. gazella farasani* (TSN: 898651) (Apio et al., 2013).



FIGURE 4 Helminth L1 larvae stages obtained from faecal samples in *Gazella gazella*: (a) *Cystocaulus* spp.; (b) *Muellerius* spp.; (c) *Protostrongylus* spp. All photos are taken at 40× magnification (Scale bar: 100 µm). Identifications are based on Taylor et al. (2015) and Zajac et al. (2021).



FIGURE 5 Helminth L1 larvae stage and egg obtained from faecal samples: (a) *Neostrongylus* spp.; (b) *Toxocara* spp. All photos are taken at 40× magnification (Scale bar: 100 µm). Identifications are based on Taylor et al. (2015) and Zajac et al. (2021).

After the rediscovery of the mountain gazelle population in Hatay, Türkiye in 2008 (Kankılıç et al., 2012), a few studies have been conducted on this population of the species, including a species action plan for the conservation of species (Akman et al., 2017) and phylogeographic and genetic studies (İlaslan, 2019; Saatoğlu et al., 2019). However, parasites of G. gazella have drawn little attention. Only Zerek et al. (2022) recently investigated the parasites in captive mountain gazelles in Hatay, Türkiye, using faecal examination. They detected four gastrointestinal helminths (Nematodirus spp., Marshallagia spp., Trichostrongylus spp. and Dictyocaulus filaria). In our study, we also detected two of these taxa (Nematodirus and Marshallagia) in our examination, but we also detected five other parasites in the captive population. Our results based on the faecal examination of faeces of captive and native individuals suggest that the Hatay population of G. gazella harbours a greater diversity of parasites, particularly helminths. But according to Zerek et al. (2022) as well as our own research, the

rate of parasitic infection in the population of mountain gazelles in Hatay, Türkiye, is still quite low. The reliability of our results is further strong by the use of the McMaster method, a well-established technique for quantifying parasitic infection rates (Zajac et al., 2021), which revealed only a small number of parasitic eggs in the examined faecal samples.

Parasites we identified in faecal samples of *G. gazella* are partly in accordance with previous studies on other *Gazella* species. Parasite studies in *Gazella* species have mostly been conducted in captive populations due to time constraints or the extreme difficulty or ease of collecting faecal samples. Therefore, our comparison on the results obtained from free-ranging individuals was rather limited. However, our data of captive populations is comparable with previous studies as we identified *Protostrongylus* spp., *Nematodirus* spp., *Trichuris* spp., *Strongylidae* spp., *Marshallagia* spp. and *Cystocaulus* spp., among frequently detected parasites in captive populations of *Gazella* species.

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In the family Trichostrongyloidea, most species typically exhibit first-stage larvae (L1) hatching from eggs. This process is expedited under warm and humid conditions, which potentially explains the hatching of larvae from strongylid-type eggs in our study, particularly given the hot climatic conditions prevalent during the July sampling period (Ortiz et al., 2006). However, it is noteworthy that certain genera like *Marshallagia* and *Nematodirus* deviate from this norm. In *Marshallagia*, second-stage larvae (L2) hatch, whereas in *Nematodirus*, it is the third-stage larvae (L3) that emerge from eggs. Consequently, these genera exhibit slower larval development and hatching rates compared to other Trichostrongyloidea species. This variation in larval development could elucidate why our study frequently identified *Nematodirus* spp. and *Marshallagia* spp., whereas strongylid-type eggs were comparatively rare.

Our detection of *Toxocara* spp. in the faecal samples was an unexpected finding that raises several questions. Notably, *Toxocara vitulorum* is the only species within this genus known to infect cattle, zebu cattle and buffaloes (Akyol, 1993; Çelik et al., 2022), yet there exists no prior record of any *Toxocara* species in *Gazella* populations. Given the limitations of species-level identification in our study, two hypotheses emerge. The first is that the *Toxocara* spp. could indeed be *T. vitulorum*, although this would be a novel finding for the *Gazella* species. The second possibility is that our sample was contaminated, potentially by sympatric wild carnivores or shepherd dogs that cohabit the study area with free-ranging mountain gazelles. Due to these uncertainties, the specific identity of the *Toxocara* spp. detected in *G. gazella* faeces remains inconclusive.

In our study, the free-ranging population exhibited a twofold increase in parasite taxa richness compared to the captive population. Although this difference could be attributed to the larger size of the free-ranging population (>1000 individuals) vs. the captive population (50 individuals), as well as the extensive habitat available to the former (>10,000 ha), another plausible explanation is the shared habitat with other wild and domestic animals. For example, during the dry months of summer, the gathering of gazelles and other animals in areas where water is available increases the frequency of transmission of some parasites. Such coexistence could facilitate the transfer of parasites between different species. Notably, the diversity of parasite taxa found in our captive population is relatively higher than what has been reported in previous studies based on faecal examination for captive populations of other Gazella species globally (Abaigar et al., 1995; Anah et al., 2019; Baghi et al., 2016; Goossens et al., 2005). Even when taking into account the more extensive study period and finer taxonomic resolution of Ortiz et al. (2006), our findings still reveal greater diversity at the genus level. This finding is especially intriguing because, aside from local rodent populations and the occasional intrusion of red foxes (as reported by the study team), the captive population is largely isolated from contact with other wildlife or domestic animals. Additionally, because human visitors frequently access the area set aside for the captive population, there is a chance that these visits could unintentionally introduce parasitic eggs into the captive environment. These eggs can be carried on visitors' shoes contaminated by walking on soil or faeces that are already contaminated. Moreover, during occasional

direct interactions between visitors and the captive population, transmission could also occur through contact with contaminated clothing or hands.

The parasites we detected have impact on well-being and welfare of the species. The gastrointestinal tract of animals contains a variety of helminth parasites responsible for subclinical and clinical parasitism. Gastrointestinal system parasites can cause visible results such as decreased appetite and subsequent weight loss in wild and domestic ruminants. In cases where the parasite load is heavier, clinical symptoms such as diarrhoea, anaemia, submandibular oedema and weight loss may occur. The lungworms especially cause symptoms in lungs and bronchioles (Jenkins et al., 2007; Zanet et al., 2021). There are studies about the severe clinical symptoms and the importance of the nematodes on animal health and models (Liatis et al., 2017, Lloyd, 2003, Otranto & Deplazes, 2019). As parasitic infections have a direct impact on the well-being and overall fitness of wildlife species, comprehensive analyses of such parasites, particularly in wild animals like mountain gazelles, are critically important for effective conservation and management. These studies also contribute to the 'One Health' perspective, encompassing not only environmental health factors like soil quality, water sources and vegetation, all of which can influence the life cycle of parasites but also includes considerations regarding the health of wildlife and potential for zoonotic transmission to humans and domestic animals. This is an especially concerning issue, as many parasites prevalent in wildlife can cross species barriers to infect humans and domestic animals (Liatis et al., 2017). For example, although the main host of a parasite taxon we found in G. gazella faecal samples, specifically Dicrocoelium spp., is ruminants, they can also infect humans and carnivores through contamination, but intermediate hosts are needed. Moreover, if the identified *Toxocara* spp. eggs belong to carnivores (T. canis, T. cati and T. malaysiensis), they can lead to visceral larva migrans, ocular larva migrans, neurotoxocariasis and covert and cutaneous toxocariasis in humans. An intermediate host is not required for these infections; direct transmission can occur through faecal contamination (García-Rubio et al., 2023). However, when the eggs are from G. gazella, the role of T. vitulorum in human toxocariasis remains an unresolved question. Additionally, inhaling airborne Toxocara spp. eggs can lead to allergic and respiratory issues (Sultan et al., 2015). Our study serves as a complementary step in uncovering zoonotic helminth species in the endangered mountain gazelle. However, further research, ideally encompassing different populations and incorporating long-term monitoring, will be essential for constructing a complete profile of parasitic infections in this species.

In this study, we analysed a total of 105 samples. Due to the scarcity of samples during certain study periods, we resorted to pooling based on geographic origins to mitigate this limitation. Through our analysis, we identified a total of 12 taxa from both pooled and individually examined specimens. It is conceivable that with a more robust sample size, permitting individual analysis of each specimen, we could have uncovered a broader spectrum of taxa. Despite these constraints, our research marks a significant milestone in the study of gastrointestinal helminths in endangered mountain gazelles, representing the most exhaustive study on this subject to date. It is

important to note that the methodological approach adopted, which leveraged nearly the entirety of our sample collection for helminth detection techniques, resulted in a limited number of samples being assessed using the McMaster method. This study underscores the critical balance between methodological rigor and the challenges posed by limited sample availability, highlighting areas for future improvement and research in the conservation of this vulnerable species.

AUTHOR CONTRIBUTIONS

Study concept and design: Mina Cansu Karaer, Tolga Kankılıç, Çağatay Tavşanoğlu and Hıfsı Oğuz Sarımehmetoğlu. Funding acquisition: Mina Cansu Karaer, Tolga Kankılıç and Çağatay Tavşanoğlu. Collection of samples in the field: Mina Cansu Karaer, Tolga Kankılıç and Çağatay Tavşanoğlu. Acquisition of laboratory data: Mina Cansu Karaer, Hande İrem Sönmez, Elif Madak and Hıfsı Oğuz Sarımehmetoğlu. Supervision: Tolga Kankılıç, Çağatay Tavşanoğlu and Hıfsı Oğuz Sarımehmetoğlu. Writing – original draft preparation: Mina Cansu Karaer. Writing – review & editing: Mina Cansu Karaer, Hande İrem Sönmez, Elif Madak, Tolga Kankılıç, Çağatay Tavşanoğlu and Hıfsı Oğuz Sarımehmetoğlu.

ACKNOWLEDGMENTS

We thank the Provincial Directorate of Nature Conservation and National Parks of Hatay for permissions and accommodation in the field, Kırıkhan district governor Fikret Dağ and the Hatay Nature Conservation Association, especially for Abdullah Öğünç, for their logistic support. We also thank two anonymous reviewers for constructive comments on the manuscript. This study is funded by the Scientific and Technological Research Council of Türkiye (TÜBİTAK) [Project no: 123Z575]. Parasitological examinations were performed in the Helminthology laboratory, Department of Parasitology, Faculty of Veterinary Medicine, Ankara University, Ankara, Türkiye. The fieldwork of the study was conducted with the permission of the General Directorate of Nature Conservation and National Parks of Türkiye (no: 8037146, date: 08.12.2022). This study is a part of the Ph.D. dissertation of the first author (Institute of Science, Hacettepe University, Türkiye).

CONFLICT OF INTEREST STATEMENT

No potential conflict of interest was reported by the authors.

DATA AVAILABILITY STATEMENT

All data supporting this research are presented in this manuscript.

ETHICS STATEMENT

The present study did not involve any human subject. Faeces collection in the field was conducted without any contact with the subject animal.

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PEER REVIEW

The peer review history for this article is available at https:// www.webofscience.com/api/gateway/wos/peer-review/10.1002/ vms3.1429.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Karaer, M. C., Sönmez, H. İ., Madak, E., Kankılıç, T., Tavşanoğlu, Ç., & Sarımehmetoğlu, H. O. (2024). Helminths of captive and free-ranging populations of the mountain gazelle (*Gazella gazella*): Evidence from faecal examination. *Veterinary Medicine and Science*, 10, e1429. https://doi.org/10.1002/vms3.1429