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Original Article

A Coprological Investigation on Gastrointestinal Parasites of Wild Boars (*Sus scrofa*) from Hatay Province, Türkiye

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Abstract

Background: Research on disease surveillance in wildlife species is of fundamental importance for understanding the epidemiology of zoonotic diseases. **Methods:** Fecal samples were collected from 24 wild boars in the districts of Antakya, İskenderun, and Kırıkhan in Hatay province during the years 2018-2019. The Fülleborn flotation and Benedek sedimentation methods were applied to these samples. Additionally, feces were examined for the presence of *Cryptosporidium* spp. oocysts using Kinyoun acid-fast staining and nested PCR methods. **Results:** Microscopic examination revealed that 14 animals (58.33%) were infected with one or more parasite species. The prevalence rates of gastrointestinal parasites were as follows: *Eimeria* spp. 45.83%, *Metastrongylus* spp. 20.83%, *Ascaris suum* 20.83%, *Strongyloides* spp. 12.5%, and *Oesophagostomum* spp. 8.33%. Furthermore, no *Cryptosporidium* spp. was detected in any of the fecal samples. The infection rate was higher in male boars (64.70%) compared to females (42.85%). According to the results of Fisher's Exact Test, there was a statistically significant difference in parasite presence among the sampled districts, while no significant difference was found between parasite presence and sex. **Conclusions:** In conclusion, this study indicates that wild boar populations can serve as potential reservoirs of parasites for both animals and humans.

Keywords: *Ascaris suum, Cryptosporidium, Eimeria, Strongyloides*, Boar

1. Introduction

As most of the emerging zoonotic diseases originate from wildlife, studies on wildlife constitute a crucial component of infectious disease surveillance [1, 2]. Therefore, disease monitoring research, laboratory diagnostics, and molecular analyses in wild animal species are fundamental for understanding the epidemiology of zoonotic diseases and implementing health measures for humans and animals [3]. This is essential because the transmission of diseases between wild animals, domestic animals, and humans poses an increasing threat to human and animal health [4]. In this context, one of the significant wild animals for both human and animal health is the wild boar (*Sus scrofa*). The wild boar is a wild animal species native to Europe, Asia, and North Africa [5,6]. This species, which has one of the broadest geographical distributions of all terrestrial wild animal species [7], has seen a significant increase in population in Europe in recent years [8]. Taxonomically, the wild boar is classified in the order Artiodactyla (even-toed ungulates), suborder Nonruminantia, and family Suidae [9]. According to the International Union for Conservation of Nature (IUCN), it is listed as "Least Concern" (LC) on the Red List of Threatened Species [10]. Additionally, due to its

ability to cause significant damage to agricultural and natural habitats, threaten native species, and act as a source of infection for domestic animals and humans, it is categorized as one of the world's top 100 invasive species [11]. These animals can serve as reservoir hosts for various infectious agents, posing a disease source for wild animals, domestic animals, and humans. Consequently, they can play a role in the transmission of various viral, bacterial, and parasitic zoonotic diseases [12, 13, 14, 15]. Some parasitic zoonoses and domestic animal disease agents that may originate from wild boars include *A. suum*, *Babesia bigemina*, *Balantidium coli*, *Blastocystis* spp., *Cryptosporidium* spp., *Echinococcus multilocularis*, *Entamoeba histolytica*, *Enterocytozoon* spp., *Fascioloides magna*, *Giardia* spp., *Hepatozoon* spp., *Taenia solium*, *Theileria* spp., *Trichinella* spp., *Toxoplasma gondii*, *Sarcoptes scabiei*, and *Sarcocystis suihominis* [16, 17].

Cryptosporidium spp. is the fifth most common foodborne pathogen, with over eight million cases reported annually [18]. The infectious dose for cryptosporidiosis is extremely low, with just 10 oocysts sufficient to cause infection. Given the environmental resistance of *Cryptosporidium* spp. oocysts, the parasite has a high potential to spread to new hosts [19]. *Cryptosporidium* spp. can be transmitted either through the consumption of contaminated water and food or through direct contact with contaminated humans and animals. Pigs have been identified as potential environmental reservoirs, contaminating water and food sources not subjected to any disinfection process prior to human consumption [18, 20]. Among the reported *Cryptosporidium* species to date, *C. suis* and *C. scrofarum* are frequently detected in wild pigs and have also been found in humans, indicating the zoonotic potential of this parasite, which should not be overlooked [18].

Wild boars, whose hunting is permitted during specific periods, can be found almost everywhere in Türkiye, except for a few provinces in Central and Eastern Anatolia characterized by vast steppes and plains [21, 22]. Despite their presence across nearly all ecosystems in Türkiye, there are limited studies on the prevalence of gastrointestinal parasites in wild boars [23, 24, 25, 26]. Moreover, no studies have been found regarding the parasites of wild boars in Hatay province. This study aims to determine the prevalence of gastrointestinal parasites in wild boars hunted in Hatay province.

2. Materials and Methods

2.1 Study Area

Located at the southernmost point of Türkiye, bordering Syria, Hatay province is surrounded by the Orontes River, the Amik Plain, the Amanos Mountains, the Samandağ coast, and the Gulf of İskenderun. Due to its combination of terrestrial and marine ecosystems, it is one of the most unique areas in Türkiye in terms of biodiversity and ecological significance. The region, rich in endemic and other wild animal species [27], is also situated on one of the world's most critical migratory bird routes [28]. The most commonly encountered wild animal in Hatay province is the wild boar [29]. In this study, wild boars were obtained from the districts of Antakya, İskenderun, and Kırıkhan in Hatay province (Fig. 1).

2.2 Collection and Examination of Fecal Samples

The material for this study consisted of fecal samples collected from 24 adult (>12 months) wild boars (seven females, 17 males) hunted in the mountainous regions of the Antakya, İskenderun, and Kırıkhan districts of Hatay province between 2018 and 2019. Specifically, the samples were collected from 10 boars in Antakya, four in İskenderun, and 10 in Kırıkhan. These wild boars were legally hunted by hunters during the permitted hunting period. Within 24 hours following the hunt, approximately 10-20 grams of fresh fecal samples were taken from the rectum of each animal using disposable gloves. The samples were then placed in sterile, leak-proof plastic containers labeled with protocol numbers and transported to the laboratory under a cold chain, where they were examined for parasites within 24-48 hours. Each fecal sample was analyzed for the presence of nematode larvae, cestode eggs, and coccidian protozoan oocysts using the Fülleborn flotation method, and for trematode eggs using the Benedek sedimentation technique. The samples were examined under a light microscope [30]. The identified parasitic forms (eggs/cysts/oocysts/larvae) were diagnosed with the aid of relevant literature [30] and photographed. Additionally, the presence of *Cryptosporidium* spp.

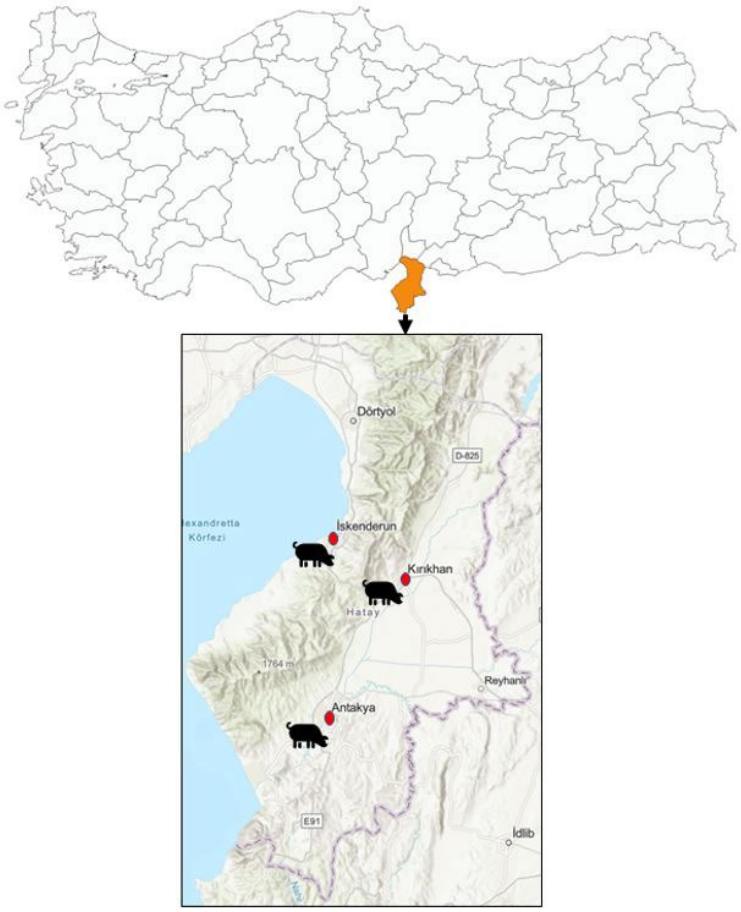

Figure 1. Hatay province, the area where the study was conducted

oocysts in the fecal samples was investigated using Kinyoun acid-fast staining [31] and nested PCR methods [32].

2.3 Molecular Investigation of *Cryptosporidium* spp.

2.3.1 DNA Extraction

For DNA isolation from the fecal samples, the GeneMATRIX® Stool DNA Purification Kit was used following the kit's procedure. The isolated DNA samples were stored at -20°C until the PCR reactions were conducted.

2.3.2 PCR Assay

The primers used for the nested PCR reaction were defined by Xiao *et al*. [32]. In the first PCR reaction, primers 5′-TTCTAGAGCTAATACATGCG-3′ and 5′-CCCATTTCCTTCGAAACAGGA-3′ were used to amplify a 1325 bp DNA fragment of the SSU rRNA gene region of *Cryptosporidium* species. In the second PCR stage, primers 5′-GGAAGGGTTGTATTTATTAGATAAAG-3′ and 5′-AAGGAGTAAGGAACAACCTCCA-3′ were used to amplify an 824-864 bp gene fragment. For both reactions, the mixture was prepared using 4 µL of 5x FIREPol® Master Mix (12.5 mM MgCl2), 0.5 µL each of forward and reverse primers, 5 µL of DNA, and 10 µL of nuclease-free water. In the second PCR reaction, instead of 5 µL of DNA, 5 µL of the first PCR reaction product was used. The first PCR reaction consisted of an initial denaturation at 95°C for 5 minutes, followed by 25 cycles of 95°C for 30 seconds, 58°C for 1 minute, and 72°C for 1 minute, with a final extension at 72°C for 5 minutes. The second PCR reaction was performed under the same conditions, but with 35 cycles. Genomic DNA obtained from the feces of a calf infected with *Cryptosporidium* spp. was used as a positive control, while distilled water was used as a negative control. The PCR products were run on

a 1% agarose gel at 120 V for 45 minutes. The gel was then visualized under UV light.

2.4 Statistical Analysis

Statistical analyses were performed using the SPSS 23.0 software package (Version 23.0 Armonk, NY: IBM Corp.). The Chi-Square test was utilized to investigate the significance of differences in the frequency of parasitic infections concerning the genders of the wild boars and the districts where sampling was conducted. Differences were considered significant at the $p<0.05$ level.

3. Results

Microscopic examination revealed that out of 24 wild boars (seven females, 17 males), 14 (58.33%) were found to be infected with one or more parasite species. A total of five parasites were identified, including one coccidian oocyst and four nematode eggs (Fig. 2). The prevalence of parasites detected in three districts of Hatay province is shown in Table 1. The highest prevalence among parasites was observed in *Eimeria* spp. (45.83%), followed by *Metastrongylus* spp. and *A. suum* with equal prevalences (20.83%), *Strongyloides* spp. (12.5%), and *Oesophagostomum* spp. (8.33%). Additionally, *Cryptosporidium* spp. was not detected in any of the fecal samples (Fig. 3) subjected to Kinyoun acid-fast staining and nested PCR methods (Table 1). The number of wild boars infected with multiple parasites was higher than those infected with a single parasite. Accordingly, 28.57% (4/14) of wild boars were infected with a single parasite, 57.14% (8/14) with two parasites, and 14.28% (2/14) with three parasites (Table 2). The rate of parasite infection was higher in male boars (64.70%) compared to females (42.85%). Parasites were found in wild boars in all districts. The highest percentage of infected boars was recorded in the Kırıkhan district (100%), followed by İskenderun (50%) and Antakya (20%) (Table 3). According to the Fisher's Exact Test, a statistically significant difference was observed among the districts sampled in terms of parasite presence, while no significant difference was found between parasite presence and sex.

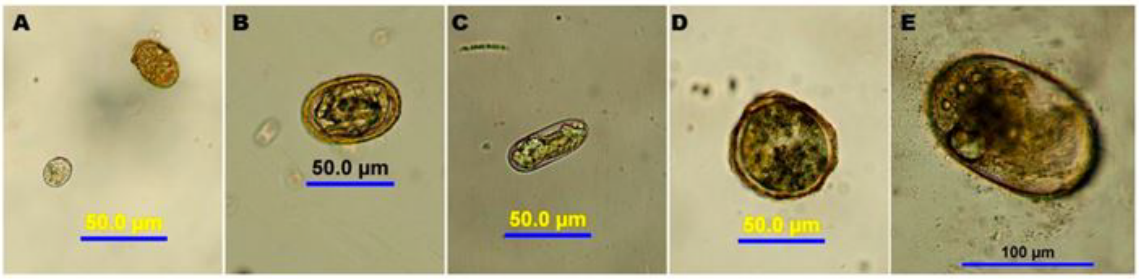

Figure 2. Parasites detected in the study. The photomicrographs of the images were taken under 400× magnifcation. **A.** *Eimeria* spp. oocyst **B.** *Metastrongylus* spp. egg, **C.** *Strongyloides* spp. egg, **D.** *Ascaris suum* egg, **E.** *Oesophagostomum* spp. egg

Table 1. Parasites Detected in Wild Boars in Hatay Province and Their Prevalence

Parasites	Number of infected wild boars in districts			Prevalence (%)
	Antakya	İskenderun	Kırıkhan	
Eimeria spp.	-	1	10	45.83
Metastrongylus spp.	2	1	2	20.83
Ascaris suum	2	-	3	20.83
Strongyloides spp.	-	-	3	12.5
Oesophagostomum spp.	-	1	1	8.33
Cryptosporidium spp.	-	-	-	-

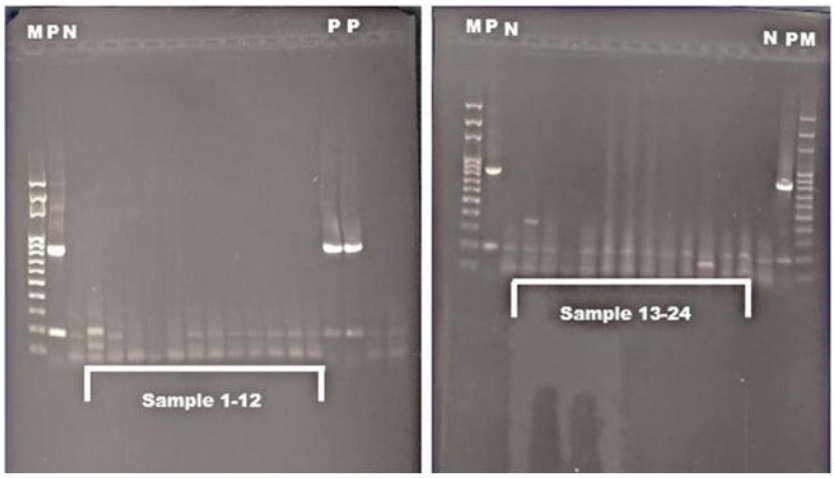

Figure 3. The gel image of the PCR product of the *Cryptosporidium* spp. M: Marker, P: Positive control, N: Negative control

Table 2. Parasites Detected in Wild Boars in Hatay Province and Their Prevalence

Parasites detected in per wild boar	Number of infected wild boars
Eimeria spp.	3
Eimeria spp. + *Metastrongylus* spp.	1
Eimeria spp. + *Strongyloides* spp.	1
Eimeria spp. + *Ascaris suum*	3
Eimeria spp. + *Oesophagostomum* spp.	1
Eimeria spp. + *Strongyloides* spp. + *Oesophagostomum* spp.	1
Eimeria spp. + *Strongyloides* spp. + *Metastrongylus* spp.	1
Metastrongylus spp.	1
Ascaris suum + *Metastrongylus* spp.	2
	Total: 14

Table 3. Prevalence of Parasitic Infections in Wild Boars Based on Gender and Sampling Districts

Variable	Examined	Infected	%	P-value
Sex				$p > 0.05$
Female	7	3	42.85	
Male	17	11	64.70	
Total	24	14	58.83	
District				$p < 0.05$
Antakya	10	2	20	
İskenderun	4	2	50	
Kırıkhan	10	10	100	

4. Discussion

Wild boar is a wildlife animal widely hunted for food and sport purposes worldwide [33]. However, hunting activities carried out without proper hygiene measures during the processing of meat and offal pose a potential risk of transmitting various pathogens, including parasites, from these animals to humans and other domestic animals. In this regard, research is being conducted in many countries around the world to determine the risks of wild boar populations transmitting pathogenic parasites. In addition, DNA metabarcoding has been used in recent years as a valuable analysis method to identify gastrointestinal parasite species in wild animals, thereby helping to prevent the transmission of disease-causing zoonotic parasite species to humans and domestic animals

[34]. Studies have reported that wild boars harbor various parasites of significant importance to public health and veterinary medicine [35]. Therefore, there is a need for studies in Türkiye, as in the rest of the world, to reveal data on the parasitic fauna of wild boars. In Türkiye, which is a largely Muslim country, zoonotic parasites such as *Trichinella* are seen in sporadic cases due to the strict prohibition of consuming pork and wild boar meat according to Islamic regulations [36]. However, since Hatay is a province with various beliefs, a segment of the population can consume pork.

Although ectoparasites such as Ixodid ticks [16, 37] and protozoans like *Babesia* spp. [37] and *T. gondii* [38] have been detected in wild boars in Türkiye, the majority of reported parasites belong to the group of helminths. These include *T. spiralis* [39, 40, 41, 42], *A. suum* [43], *Echinococcus multilocularis* [44], *Echinococcus granulosus* [45], and *M. pudendotectus* [46]. However, most of these studies that focused on a single parasite are in the form of case reports. Consequently, research on the presence or prevalence of multiple helminth parasites in wild boars in Türkiye is limited [23, 24, 25, 26]. In studies conducted by Merdivenci [23, 24] on wild boars, eight species were reported, including *M. apri*, *Taenia hydatigena*, *Gongylonema pulchrum*, *Trichuris suis*, *Fasciola hepatica*, *A. suum*, *O. dentatum*, and *Haemonchus contortus*. Senlik *et al.* [25] identified 12 species in wild boars from Bursa province, including *M. apri*, *Metastrongylus salmi*, *Metastrongylus pudendotectus*, *Dicrocoelium dendriticum*, *Globocephalus urosubulatus*, *Macracanthorhynchus hirudinaceus*, *Gongylonema pulchrum*, *Physocephalus sexalatus*, *Trichuris suis*, *Ascarops strongylina*, *Hyostrongylus rubidus*, and *T. hydatigena*, during necropsy. Another study conducted on 30 wild boars in the Burdur region reported the presence of *T. suis* and *Dicrocoelium dendriticum* helminth species [26]. In the present study sampling wild boars from Antakya, İskenderun, and Kırıkhan districts of Hatay province, a total of 5 parasites were found in fecal samples of 24 wild boars, including *Eimeria* spp. among protozoans and *Metastrongylus* spp., *A. suum*, *Strongyloides* spp., and *Oesophagostomum* spp. among helminths. Additionally, *Cryptosporidium* spp. was not detected in any sample.

Eimeria spp. can cause significant health problems in young or weaned piglets. In severe infections, piglets may experience bloody diarrhea, loss of appetite, weight loss, lethargy, and even death [47]. *Eimeria* oocysts are resistant to environmental conditions and can persist in the environment, thus maintaining their ability to cause infection under suitable climatic conditions. Therefore, *Eimeria* is a commonly reported protozoan in wild boars worldwide. However, its presence has not been reported in wild boars in Türkiye in studies conducted until now. In this study, *Eimeria*, which was found to be the most prevalent parasite at rates of 12.5% as a single infection and 33.33% in mixed infections, with an overall prevalence of 45.83%, is reported in wild boars in Türkiye for the first time to our knowledge. There are studies reporting *Eimeria* spp. as the most prevalent parasite in wild boars worldwide, as in this study [35, 47, 48]. The prevalence of this parasite has been found to be 92.3% in Denmark [48], 75.6% in Mexico [35], 58.8% and 39.3% in two studies conducted in Poland [47, 49], 33.33% in Italy [50], 7.5% in Bulgaria [51], and 3% in Russia [52]. Climate differences, the age of the studied wild boars, and the size of the areas they inhabit are considered as reasons for these differences in prevalence.

Metastrongylus species are lungworms found in the respiratory tract of pigs. Acquired immunity develops in adults against *Metastrongylus* parasites, which are particularly common in young pigs. In wild boars, *Metastrongylus* spp. can cause destruction of interstitial tissues, lung obstruction, shortness of breath, progressive weight loss, and even fatal bronchopneumonia [14]. Additionally, some *Metastrongylus* species with zoonotic potential (*M. elongatus, M. salmi*) can rarely cause metastrongylosis in humans. Severe lung distress with bilateral pleural effusion, bloody, heavy phlegm cough, fatigue, and progressive shortness of breath are clinical symptoms observed in humans [53]. Due to their use of various worm species as intermediate hosts in their life cycles, these nematodes have a wide geographical distribution and are among the most common parasites of wild boars [52]. Indeed, studies conducted in different countries, including Türkiye, have reported the prevalence of this nematode in wild boars ranging from 13.6% to 85% [12, 25, 51, 52, 54, 55]. In these studies, it has been the most commonly detected parasite among nematodes. In this study, *Metastrongylus* spp., detected with equal prevalence as *A. suum* (20.83%), has been one of the two most prevalent nematodes, consistent with the aforementioned literature.

Ascaris suum is one of the common intestinal parasites of both domestic and wild pigs, with a worldwide distribution. Infections caused by this nematode can negatively affect the health and weight gain of wild boars. Wild boars play a role in transmitting this parasite to both domestic pigs and humans by serving as reservoirs. The eggs of this ascarid species, which are highly resistant to harsh environmental conditions such as freezing and extreme heat, can survive in the soil for up to 10 years. In this regard, the presence of wild boars in certain areas significantly increases the likelihood of spreading *A. suum* infections [56]. The prevalence of this species in wild boars has been reported to range from 3% to 88% in studies conducted in some countries [48, 51, 52, 53, 57, 58, 59]. *Ascaris suum*, which shows variability in frequency of occurrence in different geographic locations and population densities [60], was detected at a rate of 20.83% in this study. The presence of this parasite in a wild boar was reported in a case study in Türkiye [43], while its prevalence in domestic pigs was reported to be 4.1% [61]. To our knowledge, the prevalence of *A. suum* parasite in wild boars has been demonstrated for the first time in this study in Türkiye.

Strongyloides spp. and *Oesophagostomum* spp. are the least detected helminths in this study, with the rates of 12.5% and 8.33%, respectively. These nematodes have been reported in wild boars in various studies worldwide [35, 51, 62]. In Türkiye, *O. dentatum* has been reported by Merdivenci [23, 24]. To our knowledge, the presence of *Strongyloides* spp. in wild boars has been demonstrated for the first time in this study in Türkiye.

Cryptosporidium spp. is one of the most important protozoans with zoonotic potential that acts as a host in wild boars [18]. The prevalence of this protozoan in wild boars has been reported as 1.4% in Portugal [17], 13.3% in Central Europe [20], 8.2% [63] and 21.7% [18] in two studies in Spain, and 25% in Sweden (20). However, *Cryptosporidium* was not detected in any of the 24 wild boars in this study, which differs from the aforementioned studies. This difference could be due to the relatively low number of pigs examined in this study compared to the literature. Another reason could be that all the boars examined in this study were adults, which could be attributed to the factor of age. Indeed, studies have been reported that age is one of the most important factors related to prevalence, and the prevalence of *Cryptosporidium* is higher in piglets and youngsters than in adults [18]. Although *Cryptosporidium* was reported at a rate of 8.8% (21/238) in a study conducted on pig farms in Türkiye [61], its presence has not been reported in wild boars. This was the first effort to detect *Cryptosporidium* in the wild boar fecal sample in this region by both morphological and molecular methods, however the results were negative. The use of these complementary methods aimed to maximize the reliability and accuracy of the findings. While morphological examination facilitates the visualization and identification of oocysts based on their structural features, molecular methods provide enhanced sensitivity and specificity, particularly for detecting low-level infections or identifying species/genotypes. Despite this comprehensive approach, no *Cryptosporidium* was detected in the samples analyzed in this study.

In the current study, a significant difference was found in terms of the presence of parasites among the sampled districts according to Fisher's Exact Test, while no significant difference was found between the presence of parasites and gender. In the Kırıkhan district, parasites were detected in all wild boars, with the highest prevalence rate (100%), followed by İskenderun (50%) and Antakya (20%), respectively. This situation in the Kırıkhan district can be explained by the more favorable environment for the survival and development of parasites. Additionally, there are studies indicating both statistically significant differences in parasite prevalence or presence between male and female wild boars [35, 52], as well as studies reporting no statistically significant difference, similar to this study [58, 59, 63].

The small sample size and the lack of molecular investigation of parasites other than *Cryptosporidium* are the limiting factors of this study. The reason for the small sample size of this study is that wild boars are difficult to catch but can only be obtained after hunting. It has been reported that to obtain positivity in the PCR method, 20 *Cryptosporidium* oocysts are required per gram of stool, while in microscopic methods, 100,000 to 500,000 *Cryptosporidium* oocysts are needed per gram of stool [64], Therefore, when

there are a small number of oocysts in the stool, the likelihood of obtaining false-negative results using microscopic methods is high. To prevent this, microscopic and molecular diagnostic methods have been used together only for *Cryptosporidium*. In future studies, if possible, increasing the sample size and examining parasites with zoonotic potential both microscopically and molecularly should not be ignored.

5. Conclusions

In conclusion, this study has demonstrated that wild boar populations serve as potential reservoirs of parasites for both animals and humans. Because the prevalence of nematodes with zoonotic potential (20.83%), *Metastrongylus* spp. and *A. suum* in this study is at a level that should not be underestimated. In addition, the pathogenic species of *Eimeria* protozoa (45.83%), the most common parasite found in wild boars in this study, may pose a serious health threat to both farm pigs and livestock. When wild boars venture from their natural habitats into public areas, they contaminate the environment with their feces, inevitably facilitating contact between the zoonotic pathogenic parasites they carry and humans. To prevent the spread of these zoonotic parasites, it is crucial for veterinarians to raise awareness among the public, particularly hunters, about the importance of this issue. Furthermore, conducting studies to determine the parasitic fauna of wild boars in different regions of Türkiye in the future would be beneficial.

Availability of Data and Materials

All of the data generated and analyzed during this study are included in this published manuscript.

Author Contributions

Conceptualization, A.Z., O.C., and İ.E.; Investigation, O.C. and İ.E.; Methodology, O.C. and İ.E.; Formal analysis, O.C.; Writing - Original draft preparation, A.Z.; Visualization, O.C.; Writing - Review and editing, A.Z., O.C., and İ.E.; Collection, recording and arranging of the samples, S.A.Ş.

Ethics Approval

This study was approved by Hatay Mustafa Kemal University Animal Experiments Local Ethics Committee (Decision number: 2024/03-04).

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Conflict of Interest

No conflict of interest was declared by the authors.

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