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Beneath the Feathers: Hidden Burden of *Serratospiculum* and Other Endoparasites in Falcons Raised in Captivity in Serbia

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Simple Summary

This study represents the first report on parasitic infections in falcons in Serbia, with emphasis on the pathogenic nematode *Serratospiculum* spp. More than half of the examined birds were infected with endoparasites, predominantly *Caryospora* spp. The prevalence of infections was significantly reduced in individuals maintained under preventive health management, including routine hygiene and deworming protocols. These results highlight the necessity of implementing preventive strategies as an essential component of falcon health management in captivity and falconry, particularly considering the clinical relevance of parasites such as *Serratospiculum* spp. that pose a severe respiratory threat.

Abstract

Parasitic infections represent an important health challenge in falcons (Falconidae), where they may negatively influence survival, reproduction, and performance. This study is the first investigation in Serbia assessing the prevalence and diversity of endoparasites in captive falcons, with special emphasis on *Serratospiculum* spp. A total of 145 live birds (hybrid falcon, *Falco peregrinus*, *F. cherrug*, *F. rusticolus*) and 1 dead individual were examined. Diagnostic procedures included necropsy, coprological analysis, and molecular detection. Endoparasites were confirmed in 55.2% of fecal samples. The most frequent were *Caryospora* spp. (41.4%), followed by *Porrocaecum* spp. (18.6%), *Capillaria* spp. (3.4%), and *Serratospiculum* spp. (2.8%). Statistically significant differences were observed between sexes, locations, and preventive health management. Falcons kept under preventive measures showed a markedly lower prevalence compared with those lacking such care. These findings highlight the persistent occurrence of endoparasites in Serbian falcons and underscore the importance of preventive programs. Regular deworming, strict hygiene, and vector control represent essential strategies for minimizing infections, particularly of *Serratospiculum* spp., which poses a serious respiratory health threat.

Keywords: Falconidae; *Serratospiculum* spp.; endoparasites; prevalence; prevention

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1. Introduction

The earliest traces of falconry date back to around 5000 B.C., originating from the archeological site of Tell Chuera in northeastern Syria. However, captive breeding techniques for

peregrine falcons were not developed until the 1960s [1]. Falcons, as top predators, possess unique morphological, physiological, and behavioral adaptations that make them highly efficient hunters; for example, the peregrine falcon has been measured to reach dive speeds of over 320 km/h (200 mph) [2].

Falconry birds may be susceptible to various diseases, including parasitic infections [3,4]. Ascarid infections are indicated as more pathogenic for young birds and a potential problem in breeding centers for birds of prey [4]. Another nematode infecting birds of prey are *Capillaria* spp., which can cause fatal infections in species such as Gyrfalcons (*Falco rusticolus*), Red-necked Falcons (*Falco chicquera*), and Peregrine Falcons (*Falco peregrinus*) [4]. Also, infections with coccidia are recognized as most common in captive falcons. In a study by Al-Kharkhi et al. [5], retrospective analysis of the fecal records revealed that 35.7% of the samples examined were positive for one or more endoparasites. The most common findings were oocysts of *Caryospora* spp., detected in 53.6% birds, while serratospiculiasis had a prevalence of 7.7%, with the highest rate of 10.8% in November, declining to 3.25% by April 2012.

Serratospiculiasis is a disease of air sacs caused by filariid nematodes of genus *Serratospiculum* and *Serratospiculoides*, the family Diplotriaenidae [6,7]. This disease has been reported mainly in falconiform birds, but it has been reported in another species [8]. These parasites have an indirect life cycle which includes arthropodes, primarily coprophagous beetles, as intermediate hosts. After the ingestion of infected arthropodes, larvae are released into the gastrointestinal tract of the bird. Larvae then migrate to the air sacs where they mature and form adults [9,10]. Adult parasites, larvae, and embryoted eggs that are present in air sacs cause tissue damage and inflammation. Secondary bacterial infections can lead to pneumonia, airsaculitis, and aspergillosis [11]. Clinical signs include lethargy, dyspnoea, and poor body conditions [10].

Serratospiculiasis is a common disease among captive falcons in the Arabic Penninsula, predominantly caused by *Serratospiculum seurati* [12,13]. In Europe, *Serratospiculum spp.* has been reported in Switzerland, Iceland, Italy, Poland, Germany, and Slovakia [13–17]. *Serratospiculum tendo* has been identified in free-living peregrine falcons in Germany, Poland, and Italy [14–18]. In addition to *S. tendo*, both *S. amaculata* and *S. guttatum* have been documented in European bird species, specifically in the Gyrfalcon and Great Tit (*Parus major*) [8,15].

The aim of this study was to assess the presence of endoparasites in falcons across Serbia and to evaluate key risk factors, including sex, age, type, location, and preventive measures.

2. Materials and Methods

2.1. Study Design

The study was conducted from March 2023 to August 2024 on 145 falcons raised in captivity in Belgrade Zoo Garden, Kanjiža, Pančevo, and Senta (Figure 1). In this study, we investigated 5 hybrids (gyr × peregrine), 23 gyrfalcons, 71 peregrine and 46 Saker Falcons (*Falco cherrug*), and 1 peregrine falcon that died during the sampling period. In all birds, parasitological and molecular detection of samples was performed. Also, the influence of various individual and environmental variables was investigated. The analysis of variables encompassed the following parameters: sex (male or female), age (juvenile, subadults, and adults), type (hybrid falcon, gyrfalcon, peregrine falcon, and saker falcon), location (Belgrade Zoo, Kanjiža, Pančevo, and Senta), and prevention (yes or no).



Figure 1. Map of Serbia with places where the survey was conducted. The map was generated by using QGIS v3.36 (Zürich, Switzerland) [19].

2.2. Parasitological Examination

A total of 145 fecal samples were collected from birds. The samples were stored at +4 °C in labeled disposable containers and transported to the Department of Parasitology at the Faculty of Veterinary Medicine, University of Belgrade, for parasitological analysis. For microscopic examination, samples were prepared using qualitative coprological diagnostic procedures—centrifugal flotation with saturated zinc sulfate solution (with a specific density of 1.18 at 20 °C). Each fecal sample was examined in duplicate under a light microscope (Olympus CX 23, Olympus, Tokyo, Japan) at magnifications of 100× and 400×. All eggs found were photographed and identified according to their morphological characteristics [4].

2.3. Post Mortem Examination

In March 2023, an adult female peregrine falcon that died in Belgrade Zoo Garden was examined. The bird was transported to the Faculty of Veterinary Medicine, Department of Forensic Medicine, University of Belgrade, where necropsy was performed. The recovered parasites were washed in saline solution and subsequently fixed in 70% ethanol.

2.4. Molecular Detection of Serratospiculum spp.

Molecular analyses were performed to detect DNA of *Serratospiculum* spp. From the dead bird, DNA was isolated from the parasite samples that were found in air sacs, using Thermo Scientific DNA Blood and Tissues Kit (Thermo Scientific, Paisley, UK) according to the manufacturer's recommendations. From the other live birds, feces was used as a material for DNA extraction. Before extraction, parasites were mixed up in TissueLyser with 200 µL elution buffer (Qiagen, Hilden, Germany). Extracted DNA samples were stored at –20 °C until use. All PCR amplifications were performed using a T100 BIO-RAD Thermal Cycler (Dubai Biotechnology and Research Park, Dubai, United Arab Emirates). PCR

reaction mix was prepared using KAPA Taq Polymerase. DNA amplifications were carried out in a final volume of 25 µL containing 2.5 µL of nuclease-free water, 1.25 µL of each primer, 10 µL of Taq-polymerase and 10 µL of DNA template. To detect *Serratospiculum* spp., all samples were subjected to PCR using forward and reverse set of primers D-1F (GCC-TATAATGGTGAAACCGCGAAC) and D-1R (CCGGTTCA AGCCACTGCGATTA) [20]. The thermal protocol involved 2 min of initial denaturation at 93 °C, followed by 45 cycles of denaturation (93 °C, 30 s), primer annealing (48 °C, 15 s), extension (72 °C, 45 s), and a final extension step at 72 °C for 5 min. PCR products were visualized with UV light after staining the 2% agarose gel with Midori Green Direct (Nippon Genetics, Tokyo, Japan). A commercial O'RangeRulerTM 100bp DNA Ladder (Thermo Fisher Scientific, Waltham, MA, USA) was used as size marker. Parasites found in the dead falcon served as positive control, while negative control was dH_2O.

2.5. Statistical Analyses

Results were analyzed using Graph Pad Prism software, version 9 (GraphPad, San Diego, CA, USA). Risk factors (sex, age, type, location, prevention) associated with parasitism were analyzed using the Chi-Square (χ^2) test. A 95% confidence interval was established in all tables, with statistical significance set at $p < 0.05$ and, $p < 0.01$, $p < 0.001$ levels.

3. Results

3.1. Prevalence of Endoparasites

In the total sample of 145 individuals, endoparasites (Figure 2) were detected in 80 birds (55.2%; 95% CI: 47.1–63.0) (Table 1). The most frequently recorded were *Caryospora* spp. (41.4%; 95% CI: 33.69–49.52), followed by *Porrocaecum* spp. (18.6%; 95% CI: 13.12–25.74) and *Capillaria* spp. (3.4%; 95% CI: 1.48–7.82). Overall, 4 of 145 (2.8%; 95% CI: 1.08–6.88) fecal samples were confirmed as positive for *Serratospiculum* spp. by PCR.

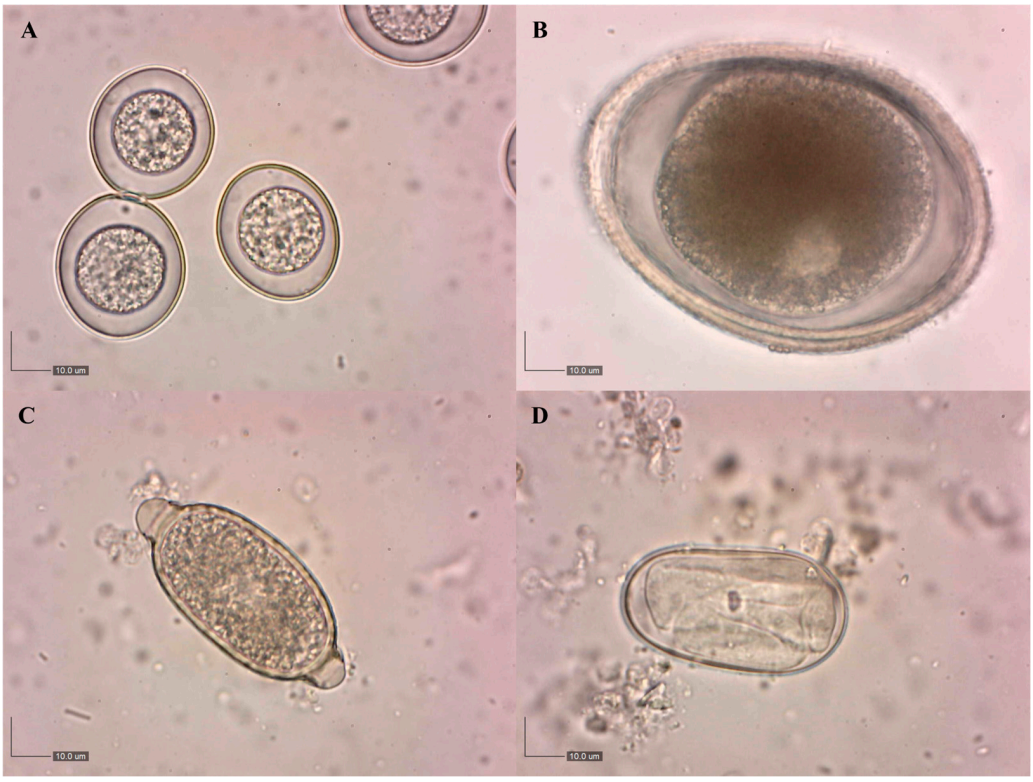


Figure 2. Parasitic elements detected in fecal samples, zinc sulfate flotation (×400): (**A**)—*Caryospora* spp. oocyst; (**B**)—*Porrocaecum* spp. egg; (**C**)—*Capillaria* spp. egg; (**D**)— *Serratospiculum* spp. egg.

Table 1. Prevalence of endoparasites detected in studied falcons.

Endoparasites	Positive Samples	*n* = 145 %	95% CI
Caryospora spp.	60	41.4	33.69–49.52
Porrocaecum spp.	27	18.6	13.12–25.74
Capillaria spp.	5	3.4	1.48–7.82
Serratospiculum spp.	4	2.8	1.08–6.88

Legend: *n*—number of tested birds; CI—confidence interval.

At the level of overall infection (Table 2), prevalence was significantly higher ($p < 0.05$) in males (69.4%; 95% CI: 50.68–74.38) than in females (44.6%; 95% CI: 34.36–55.27). Age groups did not differ. Across falcon species, no difference was detected. Significant differences ($p < 0.001$) were observed with respect to location: 85.7% in Belgrade Zoo, 87.5% in Pančevo, 45.3% in Senta, while in Kanjiža, no positive findings were recorded. The application of preventive measures was associated ($p < 0.001$) with lower prevalence (51.5%; 95% CI: 43.11–59.79) compared with the non-prevention group (100.0%; 95% CI: 74.12–100.00).

Table 2. Risk factors associated with parasitic infections in studied falcons.

Variable	*N*	*n*	%	95% CI	χ^2	*p*
Sex						
Male	62	43	69.4	57.03–79.42	8.809	<0.05
Female	83	37	44.6	34.36–55.27		
Age						
<2 years	30	19	63.3	45.51–78.13	1.21	0.545
2–5 years	79	43	54.4	43.50–64.95		
>5 years	36	18	50.0	34.47–65.53		
Type						
Hybrid falcon	5	3	60.0	23.07–92.89	4.08	0.253
Gyrfalcon	23	17	73.9	53.53–87.45		
Peregrine falcon	71	37	52.1	39.93–64.05		
Saker falcon	46	23	50.0	36.12–63.88		
Location						
Belgrade Zoo	35	30	85.7	70.62–93.74	28.97	<0.001
Kanjiža	7	0	0.0	–		
Pančevo	8	7	87.5	52.91–99.36		
Senta	95	43	45.3	35.63–55.26		
Prevention						
Yes	134	69	51.5	43.11–59.79	9.671	<0.001
No	11	11	100.0	74.12–100.00		

Legend: *N*—number of tested birds; *n*—number of positive birds; CI—confidence interval.

The occurrence of *Caryospora* spp. was significantly higher ($p < 0.001$) in males (61.3%; 95% CI: 50.68–74.38) than in females (22.0%; 95% CI: 50.68–74.38). Age and species did not show statistically significant differences. With regard to prevention, 44.8% (95% CI: 36.62–53.22) of birds with implemented measures tested positive, significantly higher ($p < 0.001$) compared to birds without prevention (Table 3).

For infections with *Porrocaecum* spp., no differences were observed by sex or age. The highest prevalence was recorded in gyrfalcons (39.1%; 95% CI: 22.16–59.21), lower in peregrines (16.9%; 95% CI: 9.94–27.26), and in Saker falcons (13.0%; 95% CI: 6.19–25.67). Preventive measures were associated with lower prevalence (15.7%; 95% CI: 10.48–22.77) compared with the non-prevention group (54.5%; 95% CI: 28.01–78.73) (Table 4).

Table 3. Distribution of prevalences and risk factors associated with *Caryospora* spp. in studied falcons.

Variable	*N*	*n*	%	95% CI	χ^2	*p*
Sex						
Male	62	38	61.3	50.68–74.38	17.7	<0.001
Female	83	22	22.0	1.89–11.75		
Age						
<2 years	30	9	30.0	16.66–47.88		
2–5 years	79	38	48.1	37.43–58.95	3.485	0.1751
>5 years	36	13	36.1	22.48–52.42		
Type						
Hybrid falcon	5	3	60.0	23.07–92.90		
Gyrfalcon	23	13	56.5	36.81–74.36	3.588	0.3095
Peregrine falcon	71	27	38.0	27.63–49.66		
Saker falcon	46	17	37.0	24.52–51.40		
Preventive						
Yes	134	60	44.8	36.62–53.22	8.402	<0.001
No	11	0	0.0	–		

Legend: *N*—number of tested birds; *n*—number of positive birds; CI—confidence interval.

Table 4. Distribution of prevalences and risk factors associated with *Porrocaecum* spp. infection in studied falcons.

Variable	*N*	*n*	%	95% CI	χ^2	*p*
Sex						
Male	62	12	19.4	11.43–30.85	0.03852	0.8444
Female	83	15	18.1	11.27–27.70		
Age						
<2 years	30	8	26.7	14.18–44.49		
2–5 years	79	12	15.2	9.47–25.23	1.741	0.4187
>5 years	36	7	19.4	9.75–35.03		
Type						
Hybrid falcon	5	0	0	–		
Gyrfalcon	23	9	39.1	22.16–59.21	8.611	<0.05
Peregrine falcon	71	12	16.9	9.94–27.26		
Saker falcon	46	6	13.0	6.19–25.67		
Preventive						
Yes	134	21	15.7	10.48–22.77	10.14	<0.01
No	11	6	54.5	28.01–78.73		

Legend: *N*—number of tested birds; *n*—number of positive birds; CI—confidence interval.

Infections with *Capillaria* spp. were rare and limited to females (6.0%; 95% CI: 2.60–13.39) and to the group younger than 2 years (16.7%; 95% CI: 7.38–33.57), with absence of positives in older categories ($p < 0.001$). Differences by species/type did not reach statistical significance; positive findings were recorded only in peregrine falcons (7.0%; 95% CI: 3.05–15.45), exclusively in those without prevention (45.5%; 95% CI: 21.27–71.99) (Table 5).

Serratospiculum spp. were recorded with low overall frequency; no significant differences were established by sex, age, or species. Significant differences ($p < 0.001$) were confirmed in birds without preventive measures. Positive findings were noted exclusively in birds without prevention (36.4%; 95% CI: 21.27–71.99) (Table 6).

Table 5. Distribution of prevalences and risk factors associated with *Capillaria* spp. infection in studied falcons.

Variable	*N*	*n*	%	95% CI	χ^2	*p*
Sex						
Male	62	0	0.0	–	3.868	<0.05
Female	83	5	6.0	2.60–13.39		
Age						
<2 years	30	5	16.7	7.38–33.57	19.85	<0.001
2–5 years	79	0	0.0	–		
>5 years	36	0	0.0	–		
Type						
Hybrid falcon	5	0	0	–	5.397	0.1449
Gyrfalcon	23	0	0.0	–		
Peregrine falcon	71	5	7.0	3.05–15.45		
Saker falcon	46	0	0.0	–		
Preventive						
Yes	134	0	0.0	–	63.08	<0.001
No	11	5	45.5	21.27–71.99		

Legend: *N*—number of tested birds; *n*—number of positive birds; CI—confidence interval.

Table 6. Distribution of prevalences and risk factors associated with *Serratospiculum* spp. infection in studied falcons.

Variable	*N*	*n*	%	95% CI	χ^2	*p*
Sex						
Male	62	0	0	–	3.073	0.0796
Female	83	4	4.8	1.89–11.75		
Age						
<2 years	30	2	6.7	1.18–21.32	4.999	0.0821
2–5 years	79	0	0	–		
>5 years	36	2	5.6	0.99–18.14		
Type						
Hybrid falcon	5	0	0	–	4.528	0.2098
Gyrfalcon	23	2	8.7	1.55–26.80		
Peregrine falcon	71	2	2.8	0.49–9.45		
Saker falcon	46	0	0	–		
Preventive						
Yes	134	0	0	–	63.08	<0.001
No	11	4	36.4	21.27–71.99		

Legend: *N*—number of tested birds; *n*—number of positive birds; CI—confidence interval.

3.2. Necropsy

The bird was in a poor body condition (body score 3/5) considering the absence of fat tissue. The feathers were dry and few wing feathers were broken. During the necropsy, the trachea, lungs, air sacs, kidneys, spleen, liver, heart, gallbladder, and the whole digestive tract (esophagus, proventriculus, ventriculus, intestines) were examined. Adult nematode specimens were found in the air sacs and counted (51 adult worms was found). Nematodes were elongated, thin and yellow in color. Identification of parasites was carried out using the morphological criteria for *Serratospiculum* species described by Skrjabin [21], with particular emphasis on the characteristic lengths of the small and large male spicules.

4. Discussion

The results of this study indicate that parasitic infections in falcons remain a significant concern, both in terms of animal health and in the context of managing populations in captivity and falconry centers. The overall prevalence of endoparasites was 55.2%, confirming that these pathogens are persistently present, with four groups standing out as the most important—*Caryospora* spp., *Porrocaecum* spp., *Capillaria* spp., and *Serratospiculum* spp. The most frequent finding in our investigation was *Caryospora* spp., detected in 41.4% of individuals. This high prevalence aligns with previous studies, although some have reported lower values. For example, in Saudi Arabia, prevalence was 10.7% [22], while Germany reported 12% [23], the UK 24% [24], the UAE 27% [25] and the USA 31% [26]. Recent studies in Italy reported prevalence of 28.6% [27], while in Poland it was 4% [28]. Even when considering the highest reported prevalence of 40% in a specific clinical series in Saudi Arabia [29], our findings are slightly higher, suggesting the influence of local risk factors. One of the key factors explaining such variation is the genetic background and species identity of the host. Mateuta et al. [25] demonstrated that gyr × peregrine hybrids and pure gyrfalcons exhibited significantly higher prevalence rates (25–27%) compared with peregrines (20%) and sakers (16.5%), whereas kestrels (*Falco tinnunculus*) showed almost no infection (< 1%). Our observation of higher prevalence in males also points toward the potential influence of biological and management-related factors. The role of the environment should not be underestimated. Juárez et al. [30] emphasized that *Caryospora* oocysts can persist in water and substrates, with significantly higher contamination observed in enclosed chambers compared with open aviaries. This is highly relevant to our findings, as differences in prevalence among locations, along with the observed association with preventive measures, underline the importance of hygiene and biosecurity. The wide distribution of this parasite has also been documented in wild raptors, highlighting the risk of introducing infections into falconry centers [31].

The second most common parasite identified was *Porrocaecum* spp., found in 18.6% of falcons examined. In a Polish falconry population, *Porrocaecum* was the dominant parasite, with a prevalence of 27% [28]. The authors indicate the importance of diet—particularly feeding raw prey—and exposure to contaminated soil as key drivers of transmission. Because *Porrocaecum* spp. require intermediate hosts such as earthworms, and small mammals may serve as paratenic hosts [32,33], it is evident that feeding practices and environmental exposure strongly shape prevalence patterns. The clinical importance of these parasites should not be underestimated [34] due to intestinal obstructions and severe cachexia. Our findings, showing a link between reduced prevalence and the implementation of preventive measures, underscore the value of strict biosecurity protocols and careful food source control in minimizing infection risks.

Capillaria spp. were less common, with a prevalence of 3.4%. Interestingly, infections were recorded only in females and in birds under two years of age, all of which belonged to the group without preventive care. These findings highlight younger birds as a particularly vulnerable category. Anderson [35] noted that certain *Capillaria* species have a direct life cycle, whereas others require intermediate hosts (e.g., earthworms), thereby increasing the likelihood of infection in environments where birds have contact with soil and invertebrates. The clinical significance of *Capillaria* spp. has been documented through reports of severe gastrointestinal disturbances in raptors. Tarello [36] described a favorable therapeutic response to ivermectin in falconids with marked capillariasis. In our sample, although rare, *Capillaria* infection was clearly associated with age and the absence of preventive measures, emphasizing once more the importance of controlled feeding and biosecurity.

The least frequently detected parasite in this study was *Serratospiculum* spp., with a prevalence of 2.8%. Although this percentage appears relatively low, it is noteworthy

that positive cases were recorded exclusively among birds without preventive care, again highlighting the strong role of management practices. The literature reports higher prevalence rates in certain contexts [10–13]. The pathogenic potential of *Serratospiculum* spp. is considerable, as it can cause severe respiratory symptoms, airsacculitis, granulomatous lesions, and, in more severe cases, even mortality. Similarly to other studies, infections in several birds were associated with pneumonia, airsacculitis, and early aspergillosis lesions, with gross examination revealing unilateral or bilateral lung congestion and pale yellow to green fluid in the air sacs [11]. The life cycle of these nematodes involves arthropods as intermediate hosts, most often beetles, and studies further confirmed the importance of this transmission pathway in birds of prey [11].

5. Conclusions

The results of this study clearly demonstrate the importance of preventive measures in controlling parasitic infections in falcons raised in captivity. Such measures are not only statistically associated with lower prevalence but also represent a practical cornerstone in reducing risk, particularly in the case of nematodes with complex life cycles. The environment clearly serves as the primary reservoir for coccidia, while diet and intermediate hosts play a decisive role in the maintenance and transmission of nematodes. The observed sex-related differences in prevalence, particularly with *Caryospora* spp., require further investigation, as the existing literature has not consistently confirmed such patterns. These findings emphasize the necessity not only of regular deworming but also of implementing measures to control potential vectors in the environment.

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Institutional Review Board Statement: Approval (No. 02-11/2023) of this study was obtained from the Ethical Commission for the Protection of Animal Welfare, Faculty of Veterinary Medicine, University of Belgrade.

Informed Consent Statement: Written informed consent was obtained from the owner of the animals involved in this study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

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