

## RESEARCH ARTICLE

F.U. Vet. J. Health Sci. 2025; 39 (3): 200 - 206 http://www.fusabil.org

# Prevalence, Diversity and Prophylactic Efficacy of Gastrointestinal Parasites in a Zoo in Ankara

This study aimed to evaluate the prevalence, taxonomic diversity, co-infection patterns, and outcomes of routine prophylactic practices for gastrointestinal parasites in avian and mammalian species housed in a zoo in Ankara. A total of 272 fresh fecal samples were collected and examined using flotation and sedimentation techniques. Associations with class and sex were analyzed using Chi-square/Fisher's exact tests and logistic regression. The overall positivity rate was 30.5% (95% Cl: 25.3-36.2); 35.1% in birds (95% Cl: 27.9-43.1) and 25.0% in mammals (95% Cl: 18.2-33.3). However, the effect of class was not confirmed in the multivariable model. The most frequent taxon was Eimeria spp. (15.4%), while strongylid-type eggs, Capillaria spp., Ascaridia spp., and Nematodirus spp. were detected at lower rates. Co-infections were common, with Eimeria spp. occupying a central position within the network. Routine antiparasitic treatments yielded marked benefits against nematode targets, with fecal negativity reaching 83.5% in treated animals, 82.9% with ivermectin, and 100% with selamectin in a small sample. Nevertheless, Eimeria burden persisted, highlighting the need for concurrent anticoccidial treatment and environmental interventions such as litter and moisture control. The findings support the critical importance of regular fecal parasitological surveillance tailored to taxonomic class and species, along with combination prophylaxis, for welfare improvement and infection control in zoo settings.

Key Words: Antiparasitic treatment, co-infection, gastrointestinal parasites, prevalence, zoo animals

## Ankara'daki Bir Hayvanat Bahçesinde Gastrointestinal Parazitlerin Yaygınlığı, Çeşitliliği ve Profilaksi Etkinliği

Bu çalışma, Ankara'da yer alan bir hayvanat bahçesinde barındırılan kanatlı ve memeli türlerinde gastrointestinal parazitlerin yaygınlığını, taksonomik çeşitliliğini, ko-enfeksiyon örüntülerini ve rutin profilaksi uygulamalarının sonuçlarını değerlendirmeyi amaçlamıştır. Toplam 272 bireyden alınan taze dışkı örnekleri flotasyon ve sedimentasyon yöntemleriyle incelenmiş, sınıf ve cinsiyetle ilişkiler ki-kare/Fisher testleri ve lojistik regresyonla analiz edilmistir. Genel pozitiflik %30.5 olarak saptanmış (95% GA: %25.3–%36.2); kuşlarda %35.1 (95% GA: %27.9–%43.1), memelilerde %25.0 (95% GA: %18.2–%33.3) bulunmuş, ancak sınıf etkisi çok değişkenli modelle doğrulanmamıştır. En sık takson *Eimeria* spp. (%15.4) olup, Strongylid tip yumurtalar, *Capillaria* Faculty of Veterinary Medicine, spp., Ascaridia spp. ve Nematodirus spp. daha düşük oranlarda izlenmiştir. Ko-enfeksiyonlar sık görülmüş ve ağın merkezinde Eimeria spp. yer almıştır. Rutin antiparaziter uygulamalar nematod hedeflerine karşı belirgin yarar sağlamış; tedavi alanlarda dışkı negatifliği %83.5'e yükselmiş, ivermektinle %82.9 ve küçük örneklemde selamektinle %100'e ulaşmıştır. Bununla birlikte Eimeria yükü devam etmiş, antikoksidiyal tedavi ve altlık-nem yönetimi gibi çevresel önlemlerin eş zamanlı gerekliliğini ortaya koymuştur. Bulgular, hayvanat bahçelerinde sınıfa ve türe özgü düzenli dışkı parazitolojik sürveyans ile kombinasyon profilaksisinin refah ve enfeksiyon kontrolü açısından kritik önemini desteklemektedir.

> Anahtar Kelimeler: Antiparaziter tedavi, gastrointestinal parazitler, hayvanat bahçesi hayvanları, ko-enfeksiyon, prevalans

### Introduction

Zoos alter the ecological and hygienic conditions of wild animals, thereby increasing their vulnerability to gastrointestinal parasitic infections. Both zoos and wildlife parks often exhibit high parasite prevalence rates among birds and mammals, underscoring the necessity of routine parasitological surveillance and targeted preventive measures (1, 2). For instance, an evaluation conducted in a Chinese zoo reported gastrointestinal parasites in 42.3% of sampled animals, with particularly high rates in non-primate mammals (50.0%), followed by primates (31.6%) and birds (26.3%) (1). Similar patterns have been reported across various settings, where captive mammals generally display a higher infection burden than avian species (2).

Furthermore, studies have demonstrated that captive birds harbor a range of parasites such as Eimeria, ascarids, capillarids, and strongyles, with prevalence reaching approximately 31%, a rate markedly higher than that observed in free-ranging populations (3). Beyond identifying parasite presence, investigating co-infections and prevalence across taxonomic groups can provide critical insights into transmission dynamics and interdependence patterns in multi-host systems (4).

Halime KARA 1, a Mustafa GÜVEN 2, b Sami GÖKPINAR 3, c Nurperi KESKİN 4, d Zeynep Begüm BABACAN 3, e Ebubekir CEYLAN 5, f

- <sup>1</sup> Ankara Yıldırım Beyazıt University, Health Vocational School, Department of Veterinary, Ankara, Türkiye
- <sup>2</sup> İzmir Bakırçay University, Menemen Vocational School, Department of Veterinary, İzmir, Türkiye
- <sup>3</sup> Kırıkkale University, Faculty of Veterinary Medicine, Department of Parasitology, Kırıkkale, TÜRKİYE
- <sup>4</sup> Ankara University, Department of Graduate School of Health Sciences, Ankara, TÜRKİYE
- <sup>5</sup> Ankara University, Department of Internal Medicine, Ankara, TÜRKİYE
- a ORCID: 0000-0001-8202-5882
- b ORCID: 0000-0002-8097-0677
- ° ORCİD: 0000-0001-7071-869X
- d ORCID: 0009-0007-0023-0366
- e ORCID: 0000-0001-9053-8346
- f ORCID: 0000-0002-3993-3145

Received : 24.08.2025 : 11.10.2025 Accepted

## Correspondence

## Mustafa GÜVEN

İzmir Bakırçay University, Menemen Vocational School, Department of Veterinary İzmir, Türkiye

mustafa.guven@bakircay.edu.tr

Effective zoo veterinary medicine must therefore rely on systematic parasitological diagnostics and robust statistical analyses to guide prophylaxis and control strategies (5). Quantifying overall prevalence, identifying host-related risk factors, and profiling parasite diversity are pivotal steps in establishing evidence-based health protocols in husbandry contexts (6).

In this context, the present study aimed to systematically characterize the prevalence, taxonomic diversity, and co-infection patterns of gastrointestinal parasites in avian and mammalian species housed in a zoo in Ankara. Using fecal samples, we sought to calculate overall and class- or species-specific positivity rates, statistically evaluate infection risk factors, measure parasite diversity, and identify co-occurrence relationships among parasite taxa. The findings are expected to contribute to the design of preventive medicine and infection control programs in zoo settings.

#### **Materials and Methods**

Research and Publication Ethics: The protocol was reviewed by the Kırıkkale University Local Ethics Committee for Animal Experiments, which concluded that formal ethical approval was not required (Decision No: E.357539, dated 15 August 2025).

Study Area and Animal Material: This study was conducted at a zoo in Ankara. The study population included mammalian (Mammalia) and avian (Aves) species belonging to different families. Animals were housed under species-appropriate conditions and maintained in accordance with standard zoo husbandry protocols. Upon admission, all animals underwent quarantine procedures and antiparasitic treatments. Mammals received antiparasitic treatment twice a year, in spring and autumn. Birds were treated prophylactically admission; however, routine antiparasitic treatments were not performed thereafter in order to avoid stress. The most recent routine treatment was carried out one month prior to fecal sampling. The treatments applied included ivermectin for goats, llamas, camels, and deer; selamectin for rabbits; and a praziquantel, imidacloprid, and moxidectin combination for cats. No experimental infections were introduced during the study; all samples were collected as part of routine health monitoring and preventive veterinary care practices.

Sampling and Fecal Collection: Fresh fecal samples were collected from each individual, preferably in the morning. Samples were placed in plastic containers labeled with animal identification and collection date and transported to the laboratory under appropriate conditions. All samples were processed for parasitological examination as soon as possible after collection. To prevent cross-contamination between individuals, disposable gloves and sampling spoons were used (7).

Parasitological Examination Methods: To detect gastrointestinal parasites, Fülleborn flotation and sedimentation techniques were employed (8). In the

flotation method, a saturated sodium chloride (NaCl) solution was used to separate helminth eggs and protozoan oocysts based on density differences. The sedimentation technique was applied particularly for the detection of high-density trematode eggs. All preparations were examined under a light microscope using ×10 and ×40 objectives. Parasite eggs and/or oocysts were identified according to their morphological characteristics (9).

Statistical Analyses: Comparisons of categorical variables were conducted to assess differences in parasite positivity rates between birds and mammals, as well as between sexes. The Chi-square test was used for contingency tables, and Fisher's exact test was applied to 2×2 tables when expected frequencies were low. Logistic regression analysis was performed with parasite positivity status as the dependent variable, and sex and class as independent variables. To account for potential correlations among individuals of the same species, cluster-robust standard errors were calculated at the species level.

Parasite diversity within each species was quantified using Shannon and Simpson diversity indices. Co-infection patterns were evaluated by determining the frequency of concurrent detection of multiple parasite taxa within the same individual, and a co-infection matrix was generated. Statistical significance was set at a two-sided p-value of <0.05.

All analyses were conducted using IBM SPSS Statistics version 23.0, with an alpha level of 0.05. Descriptive statistics summarized parasite positivity by species, taxonomic class, and sex. Prevalence estimates were calculated with 95% confidence intervals using the Wilson method (10).

Limitations: This study does not have an experimental design. Therefore, researchers have no control over sample selection and size. The data were obtained from all individuals present in a single zoo, which, by the nature of the study, constitutes a census. Consequently, a conventional sample size calculation was not applied. Instead, all individuals available in the study were evaluated, and distributions across categories such as species, class, and sex were compared observationally. In this context, it should be noted that statistical power is limited, particularly in small subgroups. Analyses conducted for subgroups with very low sample sizes are presented descriptively, not inferentially.

### Results

The overall positivity rate was determined to be 30.5%, with a 95% confidence interval (CI) of 25.3-36.2. Among the positive samples, 62.7% contained a single parasite genus, while 37.3% harbored at least two genera concurrently. In birds, the positivity rate was 35.1% (95% CI: 27.9-43.1), whereas in mammals it was 25.0% (95% CI: 18.2-33.3) (Table 1). Comparison of birds and mammals using the Chi-square test yielded  $\chi^2=2.81$ ; p=0.094, indicating that the difference was not

statistically significant. *Eimeria* spp. was the most frequently observed parasite in birds, whereas strongylid-type eggs and *Capillaria* spp. were more common in mammals.

Among individuals with available sex information, the prevalence of infection was 36.8% in males (95% CI: 28.9–45.5) and 25.2% in females (95% CI: 18.8–32.8). The difference was not statistically significant (p=0.052), although a slightly higher positivity rate was observed in males compared to females (Chi-square test,  $\chi^2$ =3.78; p=0.052) (Table 2).

In the multivariable logistic regression model, the odds ratio (OR) for female sex was 0.55 (95% CI: 0.30–1.01; p=0.052), and for the mammalian class it was 0.58 (95% CI: 0.19–1.78; p=0.339). Although both variables showed a tendency towards reduced odds of positivity, neither reached statistical significance (Tables 3 and 4).

Regarding parasite distribution, *Eimeria* spp. was the most frequently detected genus (15.4%), followed by strongylid-type eggs (7.7%), *Capillaria* spp. (6.2%), *Ascaridia* spp. (5.9%), and *Nematodirus* spp. (2.9%). The prevalence of all other genera remained below 1% (Table 5, Figure 1).

**Table 1.** Overall and class-specific prevalence of gastrointestinal parasites with 95% confidence intervals (Wilson method)

Category	N	Positive	Prevalence	95% CI (Lower)	95% CI (Upper)
Overall	272	83	30.5%	25.3%	36.2%
Birds	148	52	35.1%	27.9%	43.1%
Mammals	124	31	25.0%	18.2%	33.3%

Note: Comparison between birds and mammals was not statistically significant ( $\chi^2 = 2.81$ ; p=0.094).

**Table 2.** Prevalence of gastrointestinal parasites by sex with 95% confidence intervals (Wilson method)

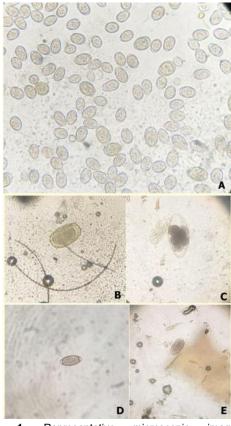
Sex	N	Positive	Prevalence	95% CI (Lower)	95% CI (Upper)
Female	147	37	25.2%	18.8%	32.8%
Male	125	46	36.8%	28.9%	45.5%

Note: Difference between sexes approached statistical significance ( $\chi^2 = 3.78$ ; p=0.052).

**Table 3.** Chi-square/Fisher's exact test results for class and sex in relation to parasite positivity

Test	Chi- square	df	p- value	Note
Class (Birds vs. Mammals) ~ Positivity	2.81	1	0.094	Chi-square test
Sex (Female vs. Male) ~ Positivity	3.78	1	0.052	Chi-square test

Note: Both comparisons approached statistical significance but did not reach the conventional threshold (p<0.05).



**Figure 1.** Representative microscopic images of gastrointestinal parasites detected in zoo animals. (A) *Eimeria* spp. Oocysts, (B) *Ascaridia spp.* egg, (C) *Nematodiruss* spp. egg, (D) *Capillaria* spp. egg, (E) Strongyle-type egg

**Table 4.** Logistic regression analysis of sex and class as predictors of parasite positivity

Variable	Coefficient	<i>p</i> - value	OR	95% CI (Lower)	95% CI (Upper)
Intercept	-0.274	0.356	0.76	0.43	1.36
Sex (Female)	-0.606	0.052	0.55	0.30	1.01
Class (Mammals)	-0.550	0.339	0.58	0.19	1.78

Note: Female sex and mammalian class showed a trend toward reduced odds of positivity, but neither reached statistical significance (p>0.05).

**Table 5.** Prevalence of the eight most common gastrointestinal parasite taxa with 95% confidence intervals (Wilson method)

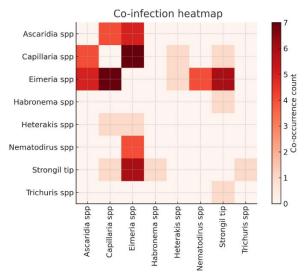
		,			
Category	N	Positive	Prevalence	95% CI (Lower)	95% CI (Upper)
Eimeria spp.	42	272	15.4%	11.6%	20.2%
Strongylid type	21	272	7.7%	5.1%	11.5%
Capillaria spp.	17	272	6.3%	3.9%	9.8%
Ascaridia spp.	16	272	5.9%	3.7%	9.3%
Nematodirus spp.	8	272	2.9%	1.5%	5.7%
Cystoisospora spp.	2	272	0.7%	0.2%	2.6%
Trichuris spp.	2	272	0.7%	0.2%	2.6%
Habronema spp.	1	272	0.4%	0.1%	2.1%

**Table 6.** Prevalence of gastrointestinal parasites in species with ≥5 samples, with 95% confidence intervals (Wilson method)

•	,				
Species	N	Positive	Prevalence	95% CI (Lower)	95% CI (Upper)
Peacock	6	6	100.0%	60.9%	100.0%
Angora Goat	14	13	92.9%	68.5%	98.7%
Light Brahma	6	5	83.3%	43.6%	97.0%
Plymouth Rock	6	4	66.7%	30.0%	90.3%
Brahma	6	4	66.7%	30.0%	90.3%
Ayam Cemani	5	3	60.0%	23.1%	88.2%
Mallard Duck	7	4	57.1%	25.0%	84.2%
Quail	7	4	57.1%	25.0%	84.2%
Denizli Chicken	6	3	50.0%	18.8%	81.2%
Egyptian Goose	7	3	42.9%	15.8%	74.9%
Fayoumi	6	2	33.3%	9.7%	70.0%
Pygmy Goat	14	4	28.6%	11.7%	54.6%
Pony	13	3	23.1%	8.2%	50.3%
Turkey	6	1	16.7%	3.0%	56.4%
Ankara Pigeon	6	1	16.7%	3.0%	56.4%
Rosecomb Chicken	6	1	16.7%	3.0%	56.4%
Pekin Duck	7	1	14.3%	2.6%	51.3%
Ruddy Shelduck	7	1	14.3%	2.6%	51.3%
Flamingo	7	1	14.3%	2.6%	51.3%
Turkish Angora Cat	20	2	10.0%	2.8%	30.1%
Fallow Deer	14	1	7.1%	1.3%	31.5%
Red Deer	6	0	0.0%	0.0%	39.0%
Bantam Cochin Chicken	5	0	0.0%	0.0%	43.4%
Guinea Fowl	6	0	0.0%	0.0%	39.0%
White Fallow Deer	15	0	0.0%	0.0%	20.4%
Zibrit Chicken	6	0	0.0%	0.0%	39.0%
İspenç Chicken	6	0	0.0%	0.0%	39.0%

**Table 7.** Co-infection pairs and their frequencies among positive individuals

Parasite pair	Count	% of positives
Capillaria spp. + Eimeria spp.	7	8.4%
Eimeria spp. + Strongylid type	6	7.2%
Ascaridia spp. + Eimeria spp.	5	6.0%
Eimeria spp. + Nematodirus spp.	4	4.8%
Ascaridia spp. + Capillaria spp.	4	4.8%
Capillaria spp. + Heterakis spp.	1	1.2%
Eimeria spp. + Heterakis spp.	1	1.2%
Capillaria spp. + Strongylid type	1	1.2%
Habronema spp. + Strongylid type	1	1.2%
Strongylid type + Trichuris spp.	1	1.2%



**Figure 2.** Co-infection heatmap illustrating pairwise associations among parasite taxa detected in zoo animals

**Table 8.** Parasite taxon richness, Shannon, and Simpson diversity indices by host species

Species	N	Positive	Taxon Richness	Shannon Index (H)	Simpson Index (1–D)
Partridge	1	1	3	1.099	0.667
Angora Goat	14	13	3	1.079	0.653
Quail	7	4	3	1.079	0.653
Peacock	6	6	3	1.058	0.639
Pygmy Goat	14	4	3	1.040	0.625
Light Brahma Chicken	6	5	3	1.011	0.611
Ankara Pigeon	6	1	2	0.693	0.500
Guinea Pig	4	2	2	0.693	0.500
Plymouth Rock Chicken	6	4	2	0.693	0.500
Mallard Duck	7	4	2	0.693	0.500
Spider Monkey	1	1	2	0.693	0.500
Ayam Cemani Chicken	5	3	2	0.637	0.444
Fayoumi Chicken	6	2	2	0.637	0.444
Angora Rabbit	4	3	2	0.562	0.375
Denizli Chicken	6	3	2	0.562	0.375
Pony	13	3	2	0.562	0.375

Table 9. Efficacy of routine antiparasitic treatments and residual prevalence of parasite taxa

Antiparasitic treatment	N	Negative (Cure)	% Cure (95% CI)	Eimeria spp.	Strongylid type	Capillaria spp.	Ascaridia spp.	Nematodirus spp.
Selamectin	4	4	100.0% (51.0–100.0%)	0.0%	0.0%	0.0%	0.0%	0.0%
Praziquantel + Imidacloprid + Moxidectin	11	9	81.8% (52.3–94.9%)	18.2%	0.0%	0.0%	0.0%	0.0%
Ivermectin	70	58	82.9% (72.4–89.9%)	8.6%	2.9%	2.9%	2.9%	1.4%
No routine treatment	187	118	63.1% (56.0–69.7%)	18.2%	10.2%	8.0%	7.5%	3,7%

At the species level (≥5 samples), the highest positivity rates were recorded in peafowl (6/6; 100%), Angora goats (13/14; 92.9%), and Light Brahma chickens (5/6; 83.3%). Detailed species-specific prevalence estimates are provided in Table 6, highlighting that smaller sample sizes were associated with wider confidence intervals and increased uncertainty.

The overall co-infection rate among positive individuals was 27.7%, with at least two parasite taxa present. The most frequent co-occurring pairs were Eimeria spp. + Capillaria spp. (n=7), Eimeria spp. + strongylid-type eggs (n=6), and Eimeria spp. + Ascaridia spp. (n=5) (Table 7). The co-infection heatmap demonstrated that Eimeria spp. occupied a central position in the network, clustering particularly with Capillaria spp. and strongylid-type eggs. Additional but less pronounced associations were observed with Ascaridia spp. and Nematodirus spp. (Figure 2).

Analysis of parasite diversity by host species (genus richness and Shannon/Simpson indices) indicated that Angora goats, quail, peafowl, pygmy goats, and Light Brahma chickens exhibited the highest richness, with at least three genera per host species. The Shannon index in this group ranged between 1.01 and 1.08 (Table 8). These findings suggest that certain species not only exhibited higher prevalence but also harbored a broader spectrum of parasite taxa.

Of the 272 animals, 85 had received at least one antiparasitic regimen, while 187 had not been treated. Across the full sample, overall fecal negativity was 69.5%. In treated animals, negativity reached 83.5% (95% CI: 74.2–89.9). Among routine treatments, the ivermectin group (n=70) achieved 82.9% negativity (95% CI: 72.4–89.9), the praziquantel + imidacloprid + moxidectin combination group (n=11) 81.8% (95% CI: 52.3–94.9), and the selamectin group (n=4) 100%. In untreated animals (n=187), negativity was 63.1% (95% CI: 56.0–69.7).

Residual positivity rates varied across treatments. In the ivermectin group, residual nematode positivity was low (strongylid-type 2.9%, *Capillaria* spp. 2.9%, *Ascaridia* spp. 2.9%, *Nematodirus* spp. 1.4%), whereas *Eimeria* spp. persisted at 8.6%. In the praziquantel + imidacloprid + moxidectin group, nematodes were fully suppressed (0.0%), but *Eimeria* spp. remained at 18.2%. In the selamectin group, no residual positivity was

detected for any taxon. In untreated animals, residual prevalence was substantially higher: *Eimeria* spp. 18.2%, strongylid-type 10.2%, *Capillaria* spp. 8.0%, *Ascaridia* spp. 7.5%, and *Nematodirus* spp. 3.7% (Table 9).

Overall, parasite positivity in the study population was moderate, with a trend towards higher rates in birds and in males, although class and sex effects were not statistically confirmed in the multivariable model. *Eimeria* spp. emerged as the dominant parasite, frequently present in co-infections and central to the co-occurrence network. Routine antiparasitic treatments demonstrated clear efficacy against nematodes, whereas *Eimeria* burden persisted, underscoring the need for integrated anticoccidial and environmental control strategies.

### **Discussion**

This study demonstrated a moderate prevalence of gastrointestinal parasites among both avian and mammalian species in a zoo in Ankara, with parasite community structure varying substantially according to host class and species. The overall prevalence observed was lower than the 68.3% reported in the Rio de Janeiro Zoo (11) and the 42.3% reported in a Chinese zoo (1), yet it remains consistent with the range frequently reported in captive populations. Such variability across institutions is commonly attributed to differences in management practices, housing conditions, diet, and prophylactic protocols (2).

In this study, prevalence was higher in birds than in mammals, a finding that partly contrasts with the tenyear monitoring of two institutions in Spain, where Esteban-Sánchez et al. (2) reported higher prevalence among mammals. However, that study included mammalian sample sizes 4.5 times greater than avian samples, which may explain the divergence. Local husbandry factors such as flooring type, moisture, food and water hygiene, prophylactic regimens, and stocking density likely account for such discrepancies. Indeed, the literature emphasizes that environmental and management-related factors often exert a stronger influence than host class when comparing institutions (2, 12). Logistic regression in our dataset indicated a higher risk of infection in males, a finding consistent with observations in other captive animal studies where sexrelated differences in behavior and social stress potentially influence exposure risk (13).

From a taxonomic perspective, the dominance of *Eimeria* spp. aligns with patterns frequently observed in captive avian systems as well as broader poultry husbandry contexts (1). Eimeria oocysts can remain viable for months or even years in moist environments. Thanks to their multi-layered cyst walls, Eimeria oocysts are resistant to many disinfectants. This contributes to an increase in Eimeria load in bird enclosures (14, 15). This finding underscores the necessity of integrating environmental interventions (e.g., litter drying, bedding management) with pharmacological control strategies. Furthermore, it highlights the importance of considering parasite communities collectively, rather than focusing on single taxa in control programs.

The co-infection analysis revealed frequent co-occurrence of *Eimeria* spp. with *Capillaria* spp. and strongylid-type eggs, whereas associations with *Nematodirus* and *Trichuris* were less common. However, it must be emphasized that co-infection heatmaps are correlation-based and do not establish mechanistic interactions. As highlighted in parasite community ecology, co-occurrence alone cannot be interpreted as causality without support from mechanistic modeling or longitudinal sampling (16). Future work should validate these clusters through repeated sampling and multi-level joint species modeling approaches.

Diversity analysis indicated Shannon index values ranging from ~1.0–1.08 and Simpson (1–D) indices suggestive of moderate diversity. These values were higher than those reported in plains bison populations, where individual-level Shannon diversity was ~0.45 and herd-level ~0.75 using nemabiome methods (17). They were also comparable to values typically reported for fish parasite communities, where Shannon indices are often ≤1 (18). Collectively, these comparisons suggest that zoo populations host parasite communities characterized by balanced presence of a few dominant taxa, rather than high richness.

Species-level patterns of high prevalence were consistent with prior reports. Husbandry-related factors such as ground-level feeding, open water sources, heavy bedding use, and group housing conditions, despite frequent disinfection, can facilitate accumulation of oocysts and thus increase risk (14, 19). In addition, host factors such as diet and age have been associated with increased positivity in avian species, supporting the rationale for risk-based surveillance and prophylaxis tailored at class- or order-specific levels (19).

The increased negativity rate (83.5%) observed among treated animals compared to untreated counterparts highlights the clinical utility of macrocyclic lactones in zoo settings, particularly against gastrointestinal nematodes. Macrocyclic lactones such as ivermectin, moxidectin, and selamectin act on glutamate-gated chloride channels and are recognized as broad-spectrum endectocides effective against nematodes. Differences in potency and resistance

patterns among compounds within this class have been documented (20, 21). The low residual prevalence of strongylid-type eggs, *Capillaria* spp., *Ascaridia* spp., and *Nematodirus* spp. observed in ivermectin-treated animals is consistent with this pharmacological spectrum. Likewise, the praziquantel + imidacloprid + moxidectin regimen (used in cats) nearly eliminated nematodes, in agreement with documented endo-/ectoparasitic efficacy of topical imidacloprid 10% + moxidectin 2.5% formulations (21, 22). The persistence of *Eimeria* spp. under this regimen was expected, as praziquantel targets cestodes and trematodes, while macrocyclic lactones are ineffective against coccidia (23).

The continued detection of *Eimeria* spp. despite routine deworming confirms that anthelmintic regimens are ineffective against coccidiosis and emphasizes the need for anticoccidial interventions. Given the resilience of *Eimeria* oocysts, effective control requires integrated pharmacological and environmental strategies (24; 25). Triazine derivatives such as toltrazuril have been shown to significantly reduce lesion scores and oocyst shedding, and when appropriately timed, can prevent recurrence (26, 27). Thus, rational zoo parasite control protocols should combine macrocyclic lactones for nematodes with species-appropriate anticoccidials and robust litter/moisture management.

Overall, the treatment program provided clear clinical benefit against nematodes but failed to suppress *Eimeria* burden due to both pharmacological limitations and environmental persistence. Integrated strategies combining species-targeted anticoccidials, litter/moisture control, and improved hygiene represent the most sustainable approach for long-term control and welfare improvement in zoo settings (24-26).

From a public health perspective, this study draws attention to the potential risk of zoonotic protozoa such as *Giardia*. Several zoos have reported zoonotic *G. duodenalis* assemblages (A/B) in mammals and birds, and molecular typing has been recommended to support source attribution. Because microscopy may lack sensitivity during low-shedding phases, periodic molecular surveillance of high-risk species would be advisable (2, 28).

Methodologically, the flotation and sedimentation approaches employed here are practical routine techniques for zoo surveillance. However, sensitivity may be limited for some taxa beyond trematodes, and intermittent egg/oocyst shedding may yield false negatives in single samples. The literature suggests that advanced quantitative methods such as Mini-FLOTAC/FLOTAC and serial sampling improve detection performance (7; 8; 19). The use of Wilson's method for prevalence confidence intervals in this study is also advantageous, as it provides more reliable coverage for small sample sizes, thus increasing the robustness of our estimates (10).

#### References

- Cai W, Zhu Y, Wang F, Feng, et al. Prevalence of gastrointestinal parasites in zoo animals and phylogenetic characterization of Toxascaris leonina (Linstow, 1902) and Baylisascaris transfuga (Rudolphi, 1819) in Jiangsu Province, Eastern China. Animals 2024; 14(3): 375.
- Esteban-Sánchez L, García-Rodríguez JJ, García-García J, et al. Wild animals in captivity: An analysis of parasite biodiversity and transmission among animals at two zoological institutions with different typologies. Animals 2024; 14(5): 813.
- Carrera-Játiva PD, Morgan ER, Barrows M, Wronski T. Gastrointestinal parasites in captive and freeranging birds and potential cross-transmission in a zoo environment. J Zoo Wildl Med 2018; 49(1): 116-128.
- White LA, Forester JD, Craft ME. Using contact networks to explore mechanisms of parasite transmission in wildlife. Biol Rev 2017; 92(1): 389-409.
- Nath TC, Eom KS, Choe S, et al. Insight into one health approach: endoparasite infections in captive wildlife in Bangladesh. Pathogens 2021; 10(2): 250.
- Maurizio A, Frangipane di Regalbono A, Cassini R. Quantitative monitoring of selected groups of parasites in domestic ruminants: A comparative review. Pathogens 2021; 10(9): 1173.
- Zajac AM, Conboy GA, Little SE, Reichard MV. Veterinary Clinical Parasitology. 9th Edition, Hoboken, NJ: John Wiley & Sons; 2021.
- Taylor MA, Coop RL, Wall RL. Laboratory Diagnosis of Parasitism. In: Veterinary Parasitology. Chichester: John Wiley & Sons; 2015: 259-312.
- Anh NTL, Phuong NT, Ha GH, et al. Evaluation of techniques for detection of small trematode eggs in faeces of domestic animals. Veterinary Parasitology 2008; 156(3-4): 346-349.
- Newcombe RG. Two-sided confidence intervals for the single proportion: Comparison of seven methods. Stat Med 1998; 17(8): 857-872.
- Barbosa ADS, Pinheiro JL, Dos Santos CR, et al. Gastrointestinal parasites in captive animals at the Rio de Janeiro Zoo. Acta Parasitologica 2020; 65(1): 237-249.
- Ahmad K, Wahid Ullah QA, Adeel M, Fahad S. A cross-sectional study of prevalence of gastrointestinal parasites in captive wild animals in Pakistan zoological gardens. World 2024; 14(2): 234-241.
- 13. Dhakal P, Sharma HP, Shah R, Thapa PJ, Pokheral CP. Copromicroscopic study of gastrointestinal parasites in captive mammals at Central Zoo, Lalitpur, Nepal. Vet Med Sci 2023; 9(1): 457-464.
- Papini R, Girivetto M, Marangi M, Mancianti F, Giangaspero A. Endoparasite infections in pet and zoo birds in Italy. Sci World J 2012; 2012(1): 253127.

- Gao Y, Sun P, Hu D, et al. Advancements in understanding chicken coccidiosis: From Eimeria biology to innovative control strategies. One Health Advances 2024; 2(1): 6.
- Pedersen AB, Fenton A. Emphasizing the ecology in parasite community ecology. Trends Ecol Evol 2007; 22(3): 133-139.
- 17. Avramenko RW, Bras A, Redman EM, et al. High species diversity of trichostrongyle parasite communities within and between Western Canadian commercial and conservation bison herds revealed by nemabiome metabarcoding. Parasites & Vectors 2018; 11(1): 299.
- Montes MM, Martorelli SR. An ecological and comparative analysis of parasites in juvenile Mugil liza (Pisces, Mugilidae) from two sites in Samborombón Bay, Argentina. Iheringia Ser Zool 2015; 105(4): 403-410.
- Allievi C, Zanzani SA, Bottura F, Manfredi MT. Investigating endoparasites in captive birds of prey in Italy. Animals 2024; 14(24): 3579.
- Mwacalimba K, Sheehy J, Adolph C, et al. A review of moxidectin vs. other macrocyclic lactones for prevention of heartworm disease in dogs with an appraisal of two commercial formulations. Frontiers in Veterinary Science 2024; 11: 1377718.
- 21. Bowman DD. Heartworms, macrocyclic lactones, and the specter of resistance to prevention in the United States. Parasites Vectors 2012; 5(1): 138.
- Genchi M, Vismarra A, Lucchetti C, et al. Efficacy of imidacloprid 10%/moxidectin 2.5% spot on (Advocate®, Advantage Multi®) and doxycycline for the treatment of natural Dirofilaria immitis infections in dogs. Veterinary Parasitology 2019; 273: 11-16.
- 23. Chai JY. Praziquantel treatment in trematode and cestode infections: An update. Infect Chemother 2013; 45(1): 32.
- López-Osorio S, Chaparro-Gutiérrez JJ, Gómez-Osorio LM. Overview of poultry Eimeria life cycle and host-parasite interactions. Front Vet Sci 2020; 7: 384.
- Zhao D, Suo J, Liang L, et al. Innovative prevention and control of coccidiosis: targeting sporogony for new control agent development. Poultry Science 2024; 103(12): 104246
- Mathis GF, Froyman R, Irion T, Kennedy T. Coccidiosis control with toltrazuril in conjunction with anticoccidial medicated or nonmedicated feed. Avian Dis 2003; 47(2): 463-469.
- Alnassan AA, Shehata AA, Kotsch M, et al. Efficacy of early treatment with toltrazuril in prevention of coccidiosis and necrotic enteritis in chickens. Avian Pathology 2013; 42(5): 482-490.
- 28. Chen J, Zhou L, Cao W, et al. Prevalence and assemblage identified of *Giardia duodenalis* in zoo and farmed Asiatic black bears (Ursus thibetanus) from the Heilongjiang and Fujian Provinces of China. Parasite 2024; 31: 50.