

Research Article

Prevalence of gastrointestinal parasites in captive exotic birds at central zoo, Bagmati, Nepal

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ABSTRACT

Gastrointestinal (GI) parasitic infection is among the major health problems affecting captive exotic birds, leading to poor growth, reduced reproductive performance, and even mortality. Central Zoo, Nepal, serves as a form of ex-situ conservation, and this study aimed to determine the general prevalence of helminth and protozoan gastrointestinal parasites in 16 different species of exotic avian fauna. Freshly defecated fecal samples were collected from 100 individuals, with five to ten individuals per species. We used iodine wet mount, flotation, and sedimentation methods to detect the protozoan and helminth samples, where five genera of parasites were detected at a 54% prevalence rate. The study results were that one protozoan genus, *Eimeria* sp. (37%), and four helminth genera. Among them, *Ascaridia* sp. (23%) showed the highest prevalence, followed by *Codiostomum* sp. (3%), *Trichostrongylus* sp. (10%), and *Strongyloides* sp. (7%). The prevalence of protozoan parasites (57) was higher than that of helminth parasites (43), and the difference was statistically insignificant ($\chi^2 = 0.197$, $p = 0.656$). Similarly, the difference in prevalence between single and mixed infections was found to be statistically significant ($\chi^2 = 4.481$, $p = 0.034$). The prevalence of protozoan parasites (57) was higher than that of helminth parasites (43), and the difference was statistically insignificant ($\chi^2 = 0.197$, $p = 0.656$). Similarly, the difference in prevalence between single and mixed infections was found to be statistically significant ($\chi^2 = 4.481$, $p = 0.034$). Double infections were the highest occurrence (76.19%) compared to the multiple infections (23.80%) among the mixed infections. Evidence-based deworming protocols were recommended to enhance healthy captive avian populations.

Keywords: Exotic, flotation, helminths, protozoan, sedimentation.

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INTRODUCTION

A zoological garden (zoo) represents an ex-situ form of conservation, where wild animals are kept in captivity for purposes such as exhibition, education, research, and the protection of endangered species (Thawait *et al.*, 2014; Mir *et al.*, 2016). Animals may be held in zoological gardens for part or all their lives in a captive environment (Lima *et al.*, 2016), which can be

highly effective and impactful for the breeding of both exotic and indigenous species. The animals in ex-situ site acquire specifically artificial habitat appropriate for the survival, growth, and breeding of each species (IUCN, 2014). As several species are homed at a defined area, and relatively available with smaller space, proper hygiene and health security becomes challenging in zoo (Knezevich, 2018). The scheduled cleaning process and maintenance of habitat may always be not enough to control the infections caused by protozoan, helminths, fungus, and viruses (Kramer *et al.*, 2024). Meanwhile, captivity is also known to facilitate the transmission of various parasitic diseases between exotic and local bird species (Ajibade *et al.*, 2010). Monitoring the prevalence of parasitic infection in captivity is essential for the formulation of appropriate management system.

In Nepal, Central Zoo is located at the middle of city, that covers an area of six hectares, providing shelter to 1164 individuals of 110 species (Aryal *et al.*, 2024). It has aimed to serve as centre for ex-situ wildlife research and conservation education. There are separate sections for birds, reptiles, mammals and fishes. As the diverse intestinal parasites are known to be highly prevalent in different birds of Nepal (Adhikari *et al.*, 2022), its status in birds of central zoo has limited data. Importantly, some of the avian parasites possesses potential risks to human health as zoonotic agents (Maske *et al.*, 1990; Adekunle & Olayide, 2008). Therefore, it is necessary to take appropriate precautions for the prevention of spread of zoonotic and epidemic diseases among captive avian fauna and associated human health (Lima *et al.*, 2016). The management practices influence the prevalence rate and transmission of different gastrointestinal nematodes and protozoan parasites in birds (Marietto *et al.*, 2009). These parasites are responsible for a range of clinical symptoms: reduced feed conversion efficiency, poor weight gain, decreased egg production, catarrh, anorexia, diarrhoea, intestinal obstruction, emaciation, anaemia, paralysis, poor feathering, and even mortality in captive birds (Jegade *et al.*, 2015). Nematodes and protozoan parasites are among the most significant gastrointestinal parasites, often contributing to multiple GI parasitic infections in birds (Radfar *et al.*, 2012). Thus, infections of gastrointestinal parasites pose a major threat to the conservation of endemic and endangered avian fauna (Grogan *et al.*, 2014).

Differences in interspecific management practices may contribute to the observed variability in parasite prevalence among different avian species. Likewise, protozoans and helminths are other prevalent parasites responsible for different symptoms in birds (Khan *et al.*, 2019). In Nepal, limited studies on avian gastrointestinal parasites may hinder effective conservation efforts and contribute to population declines. Therefore, the present study aimed to assess the prevalence and diversity of gastrointestinal parasites in exotic bird species housed at the Central Zoo, Nepal.

MATERIALS AND METHODS

Study Area

The study area was located in the Central Zoo, Jawalakhel, Lalitpur. The primary objective of the zoo is a successfully breed exotic and endangered fauna and release the surviving individuals into the wild. This study was focused on these 16 exotic bird species, comprising a total of 190 individuals. Birds at the Central Zoo, Jawalakhel, are kept under semi-captive conditions in metal-mesh enclosures with shared feeding and watering facilities; thus, the area

was selected for the study. Also, the area primarily serves as a diverse collection of exotic bird species kept in close captivity, making it a representative and practical site to study the prevalence and risk factors of gastrointestinal parasitic infection in captive exotic birds.

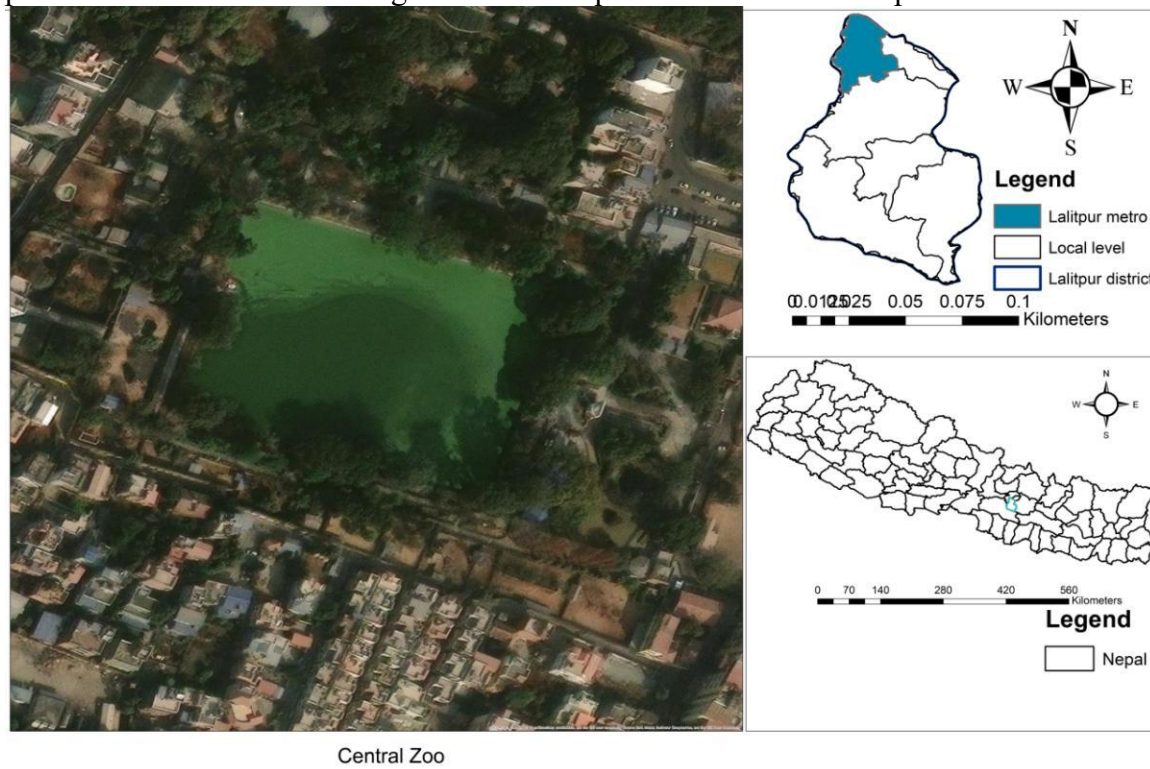


Figure 1: Map of the study Area Showing Central Zoo, Jawalakhel, Lalitpur

Sample Collection, Preservation, And Transportation

All procedures were conducted in accordance with the ethical guidelines for research involving animals, and permission was obtained from the Central Zoo authorities before the commencement of the study. The simple random sampling technique was employed for sample collection. The area of each cage was divided into 20 partitions, numbered in sequence, starting from the door or window in a clockwise direction. A total of 100 faecal samples (each weighing 3-5 grams) were collected non-invasively from pre-set sheets in clean, sterile vials early in the morning, before the routine cleaning of cages in the zoo. The number of samples per species ranged from 5 to 10, depending on the size of the cage and the number of blocks placed with sheets as per randomization.

Each sample was collected with a new, sterile polystyrene spatula specific to each bird species to avoid cross-contamination. The vials were then filled with a 2.5% potassium dichromate solution, and all collected samples were properly labeled. The vials were kept in an airtight, cool box, and the sampling procedure was repeated accordingly. The fecal samples of exotic birds were preserved in a 2.5% potassium dichromate solution to maintain protozoan morphology and to prevent the development of helminths eggs and larvae. The preserved samples were examined at the Central Department of Zoology laboratory for gastrointestinal parasites, including helminths and protozoa. Additionally, key informant interviews were conducted using a semi-structured questionnaire to assess captive bird management practices

at the Central Zoo.

Lab processing and identification

In the laboratory, the samples were examined microscopically using iodine wet mount, flotation, and sedimentation methods to detect eggs, oocysts, and cysts of Protozoan and helminth parasites (Hendrix, 2006; Christie *et al.*, 2011). For the validation and reliability of the test, all three methods were employed. For the wet mount, stool samples were carefully mixed using a glass rod. A single drop of the sample was placed on a glass slide, with or without iodine staining, covered with a coverslip, and examined under a microscope at total magnifications of 40X and 100X.

For the flotation technique, a high-specific gravity flotation medium (45% w/v NaCl) was used to isolate the products of endoparasites (Christie *et al.*, 2011). Approximately 2 grams of fecal sample were centrifuged at 2,000 rpm for 5 minutes using 4 mL of ethyl acetate and 10 ml of 10% formalin. The debris was removed, and the sediment was checked with a microscope. After that, centrifugation occurred on a glass slide with the staining of iodine solution in the sedimentation method. The slide was observed under a microscope at 40 × magnification. By using the ocular and stage micrometer, eggs, oocysts, and cysts were measured. Morphological characters were identified by using the literature and online resources (<https://www.cdc.gov/parasites/>) (Soulsby, 1982; Duszynski *et al.*, 2000; Zajac *et al.*, 2012).

Data Analysis

All data was entered in Microsoft Excel (Version 2011), the percentage prevalence was calculating using following formula;

$$\text{Prevalence (\%)} = \frac{\text{No. of positive samples}}{\text{No. of total samples}} \times 100 \dots\dots\dots \text{Eq.1}$$

The size of Oocyst of *Eimeria* spp., egg of *Ascaridia* sp., *Trichostrongylus* sp., *Strongyloides* sp. and *Codiotomum* sp. were analysed based on previously published literatures (Soulsby 1982; Duszynski *et al.*, 2000; Zajiac *et al.*, 2012).

RESULTS

In the present study, the prevalence of parasites was 54% (54 tested positives out of 100 fecal samples) for gastrointestinal (GI) parasites. There were parasites from five genera, with the highest prevalence rate for *Eimeria* sp. (37.03%), followed by *Ascaridia* sp. (29.62%), *Trichostrongylus* sp. (18.51%), *Strongyloides* sp. (9.25%), and *Codiotomum* sp. (5.55%) (Figure 2). The prevalence of helminths (67%) was higher than that of protozoa (37%). There was one genus from protozoa (*Emeria* spp.) and four from helminths (*Ascaridia* sp., *Trichostrongylus* sp., *Strongyloides* sp., and *Codiotomum* sp.).

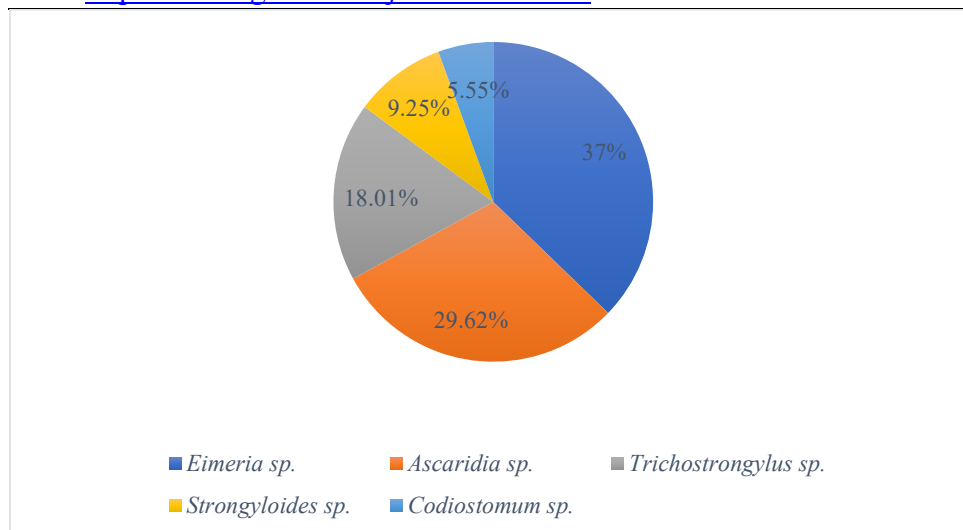


Figure 2: Prevalence of gastro intestinal parasites in exotic bird

Among the 16 exotic bird species sampled, the highest prevalence was observed in *Psittacus Erithacus* (100%) followed by *Cacatua moluccensis* (100%), *Struthio camelus*, (100%) *Syrmaticus reevesii* (100%), *Numida meleagris* (100%), *Dromiaius novaehollandiae* (75%), *Ara ararauna* (64%), *Nymphicus hollandicus* (60%), *Chrysolophus Pictus* (59%), *Melopsittacus undulates* (50%), *Lophura nycthemera*, (42.33%), *Chrysolophus amherstiae*, (40%), *Agapornis roseicollis* (40%) *Cacatua galerita* (33.33%) *Cacatua goffiniana* (33.33%) and *Lonchura oryziora* (33.33%) (Figure 3).

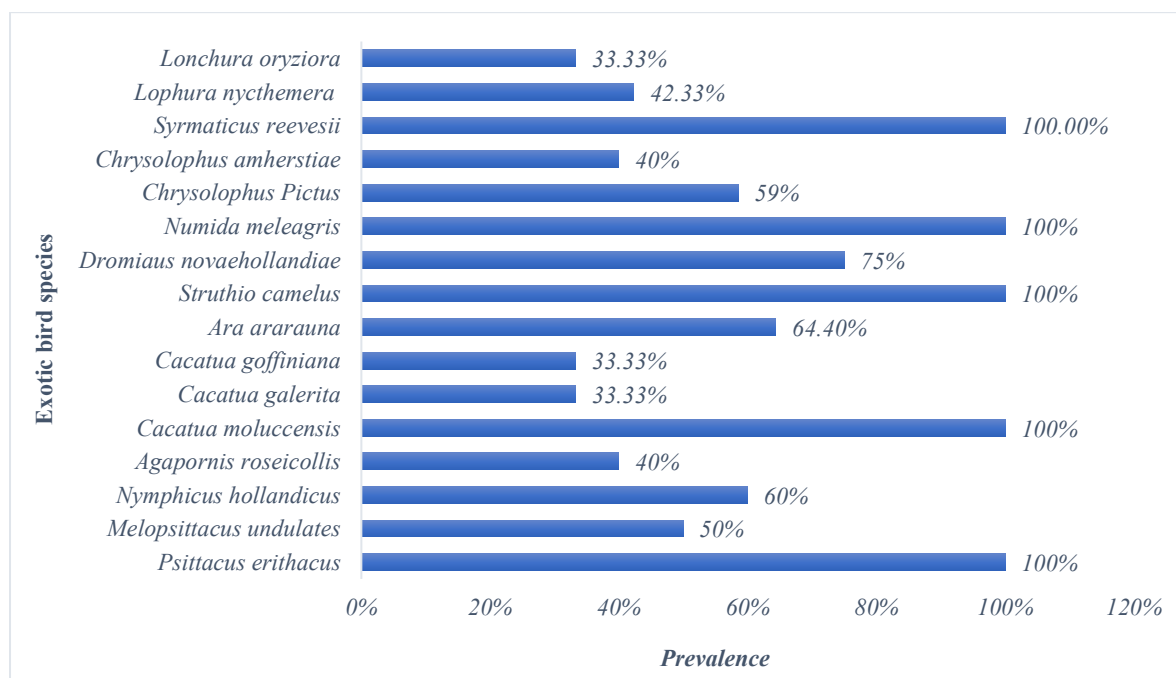
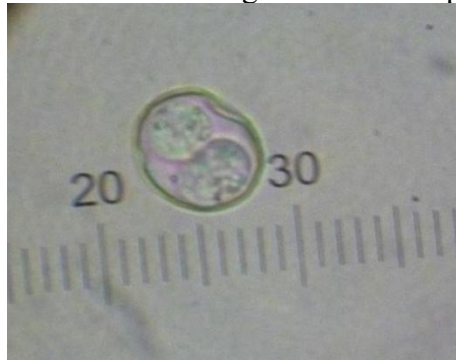


Figure 3: Exotic bird species wise general prevalence of GI parasites

Emus and ostriches exhibited multiple parasitic infections, while Galliformes showed the

highest prevalence of *Ascaridia* species (Figure 2). In contrast, Passeriformes had the lowest infection rate (Annex 1). Lovebirds demonstrated a 40% prevalence rate, which was higher compared to Java Sparrows, Sulphur-Crested Cockatoos, and White-Crested Cockatoos, each of which exhibited a 33.33% prevalence (Figure 3). In total, 16 exotic bird species in captivity were screened for gastrointestinal parasites (Annex 1).



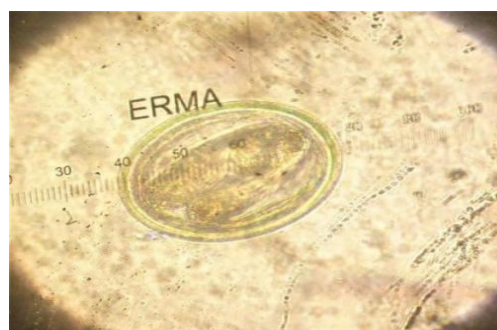
(a)



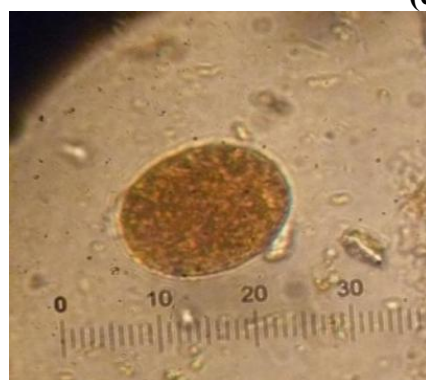
(b)



(c)



(d)



(e)

Figure 4: GI parasites identified in exotic birds. (a) Oocyst of *Eimeria* sp. (20.7x18 μm), (b) *Ascaridia* sp. egg (78x54 μm), (c) *Trichostrongylus* sp. egg (115-62.4 μm), (d) *Strongyloides* sp. egg (71x66 μm), (e) *Codiostomum* sp. egg (38x29 μm).

Among the 54 positive samples, protozoan infections accounted for 57 % of cases and helminth infections for 43 % (Figure 5). The difference between protozoan and helminth prevalence was not statistically significant ($\chi^2 = 0.197$, $p = 0.656$).

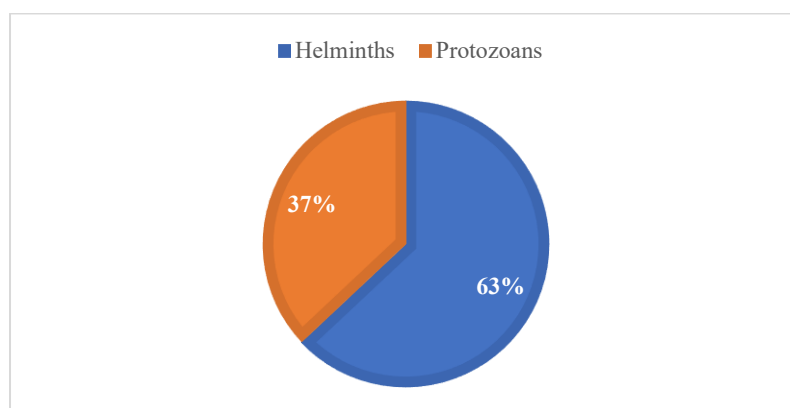


Figure 5: Prevalence of different helminths and protozoan parasites

Solitary infections (single-parasite infections) were significantly more frequent than mixed infections ($\chi^2 = 4.481$, $p = 0.034$) (Figure 6a). Among the positive samples, double infections comprised 76.2%, while multiple (more than two parasite genera) infections comprised 23.8% (Figure 6b). The difference between double and multiple infections was statistically significant ($\chi^2 = 9.523$, $p = 0.002$). Cestode and trematode parasites were not detected in any sample.

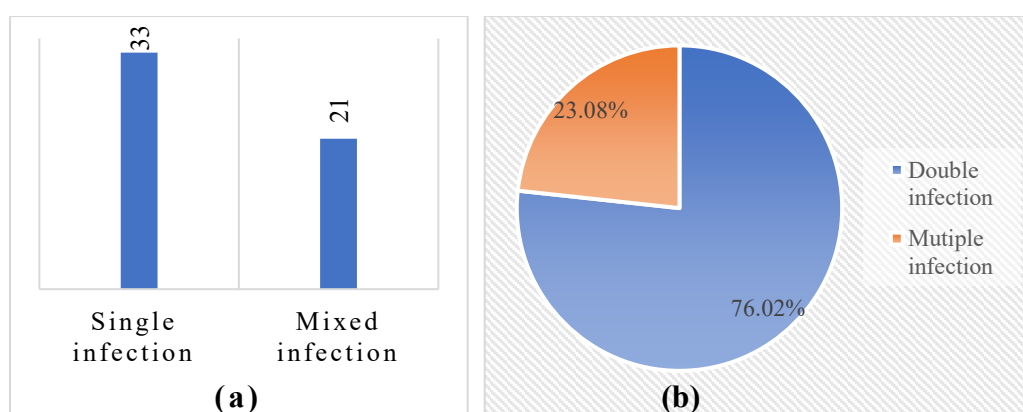


Figure 6: (a) Prevalence of infections; (b) Concurrency of parasite infection

DISCUSSIONS

The bird species are captivated and managed inside the zoo, where gastrointestinal infection could be of major concern to conservationists. To monitor gastrointestinal infections in captive exotic bird species, the genetic diversity and prevalence rates of gastrointestinal (GI) parasites were analyzed. The result showed a high prevalence rate (54%) among the tested samples of exotic bird species. It indicates a higher incidence of gastrointestinal infections in exotic birds at the central zoo in Nepal. The proper implementation of a management schedule and good monitoring of health status are inevitable for hygiene and lower infection. It seems to be a lack of proper management and health monitoring of captive birds in zoos that led to the higher infection rate than previously known reports for birds in open territories (Goldova *et al.*, 2006 and Patel *et al.*, 2000). This rate of parasitic infection is evidently higher, though it may have different routes of infection. Along with habitat cleaning, food quality and environmental interaction with visitors could be of another significance that influenced the rate of infection

The zoo is under clear and well-designed management practices, but the higher prevalence of parasitic infection suggests that gastrointestinal disease is not only dependent on cleaning and disinfecting practices but also on some other factors that influence it. Other factors could be their physical inactivity, interaction with infected air or food, and poor appetite (Ostrzyżek *et al.*, 2023). Comparatively poor management was a strong influence for GI parasites in captive birds (Parasni *et al.*, 2003; Reissing *et al.*, 2001; Pavlović *et al.*, 2003; Papini *et al.*, 2012; Edosomwan and Ogbonnia, 2014). However, the results were similar the earlier study (Ibrahim *et al.*, 2006; Eslami *et al.*, 2007). It was concluded that there was only one protozoan parasite, *Eimeria* oocyst. The high prevalence of *Eimeria* *sp.* may be attributed to contamination of food and water by fecal matter or contact with free-ranging birds. During the postmortem examination, infected birds showed a behavior of weakness, poor appetite, ruffled feathers, and bloody diarrhea (Dingle & Shanawany, 1999). The study found that helminth infections occurring in the zoo had *Ascaridia* *sp.*, *Trichostrongylus* *sp.*, *Strongyloides* *sp.*, and *Codiostomum* *sp.* Even though this endoparasite's host is pigeons (Momin *et al.*, 2006; Cordon *et al.*, 2009; Lima *et al.*, 2016). Among them, *Ascaridia* *sp.* was the most dominant gastrointestinal parasite (Attah *et al.*, 2013). *Eimeria*, *Ascaridia*, *Strongylus*, and *Codiostomum* *spp.* were common parasites reported by Parsani *et al.* (2003), Ibrahim *et al.* (2006), and Teixeira *et al.* (2012). Similarly, infection rates of 100 % were determined in captive African Grey Parrots, Guinea Fowls, Reeves's Pheasants, and Salmon-Crested Cockatoos.

CONCLUSION

The prevalence of protozoan parasites (57) was higher than that of helminth parasites (43), and the difference was statistically insignificant ($\chi^2 = 0.197$, $p = 0.656$). Similarly, the difference in prevalence between single and mixed infections was found to be statistically significant ($\chi^2 = 4.481$, $p = 0.034$). Among the mixed infections, double infections had the highest occurrence (76.19%) compared to the multiple infections (23.80%). The managed zoo is not only responsible for securing the health and survival of bird species but also for other associated factors that directly relate to feeding habits and the gastrointestinal tract. The risk of gastrointestinal infection with multiple parasite species was prevalent among exotic birds in the zoo. Integrated species management systems, regular monitoring of food quality, visitor interaction, maintaining internal health status, and air-transmitted infections through open air are major concerns for captive birds.

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Author's contribution

Research designed and concept by A.B. & R.G, data collection and analyzed A.B & K.C and first draft of the manuscript prepared by A.B., K.C. & A.K.S. Reviewed and edited by A.K.S & K.C.; Supervision: R.G. & Final draft prepared and finalized by all authors.

Conflicts of Interest

The author has no relevant financial or non-financial interests to disclose.

Ethics Approval Statement

Ethical approval for this study was obtained from the relevant institutional authority, and permission was granted by the management of Central Zoo, Bagmati, Nepal. The study involved non-invasive fecal sample collection only, and no harm or distress was caused to the birds.

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