



## Research Article

# Avian Parasitology in Captive Psittaciformes: A Study of *Ascaridia* spp. Infections in Gujranwala, Punjab, Pakistan

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**Abstract** | Infections caused by *Ascaridia* spp. are commonly referred to as *ascaridiosis*. In parrots, diarrhea, weight loss, lethargy, intestinal blockage, and respiratory distress are examples of clinical signs of illness. In the present study, the prevalence and detection of *Ascaridia* spp. infection have been investigated in 110 parrots across nine species housed at the RASSA Bird Aviary and farm in Gujranwala, Punjab, Pakistan. The species examined included the Grey Parrot (*Psittacus erithacus*) (n=14), Cockatiel (*Nymphicus hollandicus*) (n=12), Senegal Parrot (*Poicephalus senegalus*) (n=12), Rump Parrot (*Psephotus haematonotus*) (n=14), Indian Ringneck Parrot (*Psittacula krameri*) (n=10), Lovebird (*Agapornis* spp.) (n=12), Sun Conure (*Aratinga solstitialis*) (n=10), Alexandrine Parrot (*Psittacula eupatria*) (n=14), and Australian Budgerigar (*Melopsittacus undulatus*) (n=12). In total, 45 out of 110 birds tested positive for *Ascaridia* spp., with a prevalence of 40.9%. Specifically, *Ascaridia galli* and *Ascaridia platyceri* were identified as the predominant parasites. The prevalence of *Ascaridia galli* was highest in Grey Parrots (21.4%), followed by Rump Parrots (21.4%), Alexandrine Parrots (14.3%), Sun Conures (10.0%), Indian Ringneck Parrots (10.0%), and Senegal Parrots (8.3%). No *Ascaridia galli* infections were detected in Cockatiels, Lovebirds, and Australian Budgerigars. The prevalence of *Ascaridia platyceri* was highest in Grey Parrots (50.0%), followed by Senegal Parrots (41.7%), Rump Parrots (28.6%), Alexandrine Parrots (28.6%), Sun Conures (30.0%), Cockatiels (25.0%), Lovebirds (25.0%), Australian Budgerigars (25.0%), and Indian Ringneck Parrots (20.0%). Notably, this study represents the first report of *Ascaridia platyceri* in Pakistan, highlighting its previously undocumented presence in the region. These findings emphasize the significant presence of *Ascaridia* spp. in captive parrots and highlight the need for effective management strategies to ensure their health and welfare.

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## Introduction

*Ascaridia* is a large nematode (roundworm) genus in the superfamily *Ascaridoidea* that parasitizes several different vertebrate hosts, such as birds

(Aldamigh *et al.*, 2024). There are a wide variety of species in the genus *Ascaridia* at least 15 have been recognized so far. Remarkably, five of these species show host selectivity and pathogenicity by favoring parrots (*Psittaciformes*) as hosts (Soulsby, 1982). Some

*Ascaridia* that are unique to parrots consist of *A. platyceri*, *A. nicobarensis*, *A. ornata*, *A. sergiomeirai*, and *A. hermaphrodita*. Parrots can still become infected with different *Ascaridia* species, though. Parrots are known to harbor *A. galli* and *A. columbae*, which are known to prey on gallinaceous and columbiform birds, respectively (Kajerová *et al.*, 2004). According to their morphology, *Ascaridia* are distinguished by their long, cylindrical bodies that are designed for parasitism in the gastrointestinal system of their hosts (Samour, 2016). *Ascaridia* spp. eggs have an elliptical form and a strong, thick shell, which helps them withstand harsh environments better (Zajac and Conboy, 2012).

The fascinating order of avian wonders known as Psittaciformes, which includes the majestic macaws and the endearing cockatiels, has a long history of being held in captivity and has developed a bond with humans (Eberhard, 2016). Psittaciformes, the family of birds generally referred to as parrots, includes more than 400 species, such as lorikeets, parakeets, cockatoos, and macaws. According to historical records, parrots were domesticated as pets by several ancient civilizations, such as the Greeks, Romans, and Egyptians (Collar, 1997). According to Pires (2012), the Psittaciformes trade is a significant market in terms of economics. Parrots support plant species dispersal and forest regeneration by eating fruits and excreting seeds in various places (Galetti, 1993).

Psittaciformes are particularly significant in Pakistan and the South Asian area. Native species are found in Pakistan including the three types of parakeets: Plum-headed (*Psittacula cyanocephala*), Rose-ringed (*Psittacula krameri*), and Alexandrine (*Psittacula eupatria*). These species are not only essential to the regional ecosystems but also hold significant cultural importance (Roberts, 1991). According to Bird Life International (2020), native parrots of Southeast Asia include the Red-breasted parakeet (*Psittacula alexandri*), Blossom-headed parakeet (*Psittacula roseata*), and Blue-rumped parrot (*Psittinus cyanurus*). Their high value in the pet trade contributes to the economic benefits of the communities where they are bred and sold (Pires, 2012). Understanding parasite illnesses like as *Ascaridia* spp. infections is crucial since Psittaciformes health is closely related to their survival and ecological roles (Anderson, 2000). The occurrence of gastrointestinal nematodes, particularly *Ascaridia* spp., in captive environments poses significant concerns for the welfare and health

of our avian companions. An infection of *Ascaridia* usually results in ascariidiosis. *Ascaridia* spp. infections affect more than only particular bird species such as chickens, pigeons, doves, and turkeys (Sadaf *et al.* 2021).

In Psittaciformes, *Ascaridia* are primarily transmitted through the ingestion of contaminated food or water, as well as through the birds grooming and feather maintenance activities (Soulsby, 1982). Clinical signs of infection in birds might include diarrhea, weight loss, lethargy, and in extreme cases, intestinal obstruction (Samour, 2016). According to Permin and Hansen (1998), a reliable method of diagnosing *Ascaridia* infections is to use microscopic methods, to look for eggs in fecal flotation. This technique enables early diagnosis and management by detecting infections even in asymptomatic birds (Soulsby, 1982). Fecal flotation technique help to recover parasite eggs, such as *Ascaridia* spp., from fecal samples (Höglund *et al.*, 2023). Microscopic investigation focuses on these catch to provide a thorough study and confirmation. When combined, these techniques serve as the first diagnosis' detection, rapidly spotting possible infestations and opening the door to more research. Effective detection methods for these parasite illnesses can lead to improved health management and conservation measures for Psittaciformes (Samour, 2016).

## Materials and Methods

### Study area

The study took place at the RASSA bird aviary and farm, located in Bakhtay Wala, Gujranwala, Punjab, Pakistan. Fecal samples were gathered from the birds and analysis was conducted at the biology department's lab located at the Gujranwala campus (VGJW01) of Virtual University of Pakistan.

### Study period

The study was conducted over a span of approximately four weeks, from April 18<sup>th</sup> to May 17<sup>th</sup>, 2024.

### Sample collection

Fecal samples were obtained from a total of 110 parrots representing nine species under the order Psittaciformes. These species included the Alexandrine Parakeet (*Psittacula eupatria*) mostly blue, white, yellow and spangle in color variation, African Grey Parrot (*Psittacus erithacus*), Sun Conure (*Aratinga solstitialis*), Senegal Parrot (*Poicephalus senegalus*),

Plum-headed Parakeet (*Psittacula cyanocephala*), Australian Budgerigar (*Melopsittacus undulatus*), Cockatiel (*Nymphicus hollandicus*), Indian Ringneck Parakeet (*Psittacula krameri*), Lovebirds (*Agapornis* spp.) and their variations. To collect the samples, the following steps were undertaken: The parrots were provided with clean food and water to avoid contamination. All parrots were kept in separate caged in pairing. Sticks were used to gather samples from the cage bottoms. Each sample was individually bagged in a polyethylene plastic clear bag labeled with key information of the species and gender of the parrots. Within an hour of collection, the samples were taken to laboratory for examination.

#### Qualitative analysis of fecal samples

In order to do qualitative analysis on the samples, as mentioned in William (2001), a two-step approach was used. The method was based on direct microscopic examination and a simple fecal flotation technique.

#### Direct microscopic examination

The material used in direct microscopic examination, include fecal samples, glass slides and coverslips, toothpicks, a compound light microscope.

A 500mg fecal sample was carefully deposited on a clean glass slide with a toothpick. With the help of a toothpick, thoroughly mix the sample after adding one or two drops of water. To prevent air bubbles, a coverslip was carefully placed on top of the preparation. The prepared slide was examined under a microscope at 10x and 40x magnifications for *Ascaridia* spp. and other parasites.

#### Simple fecal flotation technique

According to Bowman (2014), simple flotation method was also used alongside direct microscopic examination to detect nematode eggs.

Simple fecal flotation method required Materials i.e., fecal samples, glass slides, coverslips, saturated sodium chloride solution, test tubes with racks, and a microscope.

1g of fecal sample was thoroughly mixed with a 10% sodium chloride solution. A cotton cloth was used to filter the mixture and transfer it to a test tube. More saline solution was poured into the tube to form a convex meniscus at the top. The test tube was covered with a coverslip and left undisturbed in the test tube

rack for 15 minutes. After the incubation period, the coverslip was gently removed from the test tube and placed on a fresh, sterilized glass microscope slide. The slide was then observed under the microscope at both 4x and 10x magnifications for the presence of nematode eggs.

## Results and Discussion

This study employed qualitative investigation (direct microscopic approach and simple flotation method) to explore the prevalence and identification of *Ascaridia* spp. infection in 110 Psittaciformes from nine distinct species, as indicated in Table 1. The combined use of direct microscopy and flotation techniques revealed infections caused by *Ascaridia* species, with the most common being *Ascaridia galli* and *Ascaridia platyceri*. In total, 45 out of 110 birds tested positive for *Ascaridia* spp with a prevalence of 40.9%. A breakdown of the overall prevalence by species is presented in Table 2.

**Table 1:** Parrot species and gender distribution of examined birds.

Common name	Scientific name	Total birds	Males	Females
Grey parrot	<i>Psittacus erithacus</i>	14	7	7
Cockatiel	<i>Nymphicus hollandicus</i>	12	6	6
Senegal parrot	<i>Poicephalus senegalus</i>	12	6	6
Rump parrot	<i>Psephotus haematonotus</i>	14	7	7
Indian ringneck parrot	<i>Psittacula krameri</i>	10	5	5
Love bird	<i>Agapornis</i> spp.	12	6	6
Sun conure	<i>Aratinga solstitialis</i>	10	5	5
Alexandrine parrot	<i>Psittacula eupatria</i>	14	7	7
Australian Budgerigars	<i>Melopsittacus undulatus</i>	12		6

**Table 2:** Overall prevalence of *Ascaridia* spp. in parrots examined.

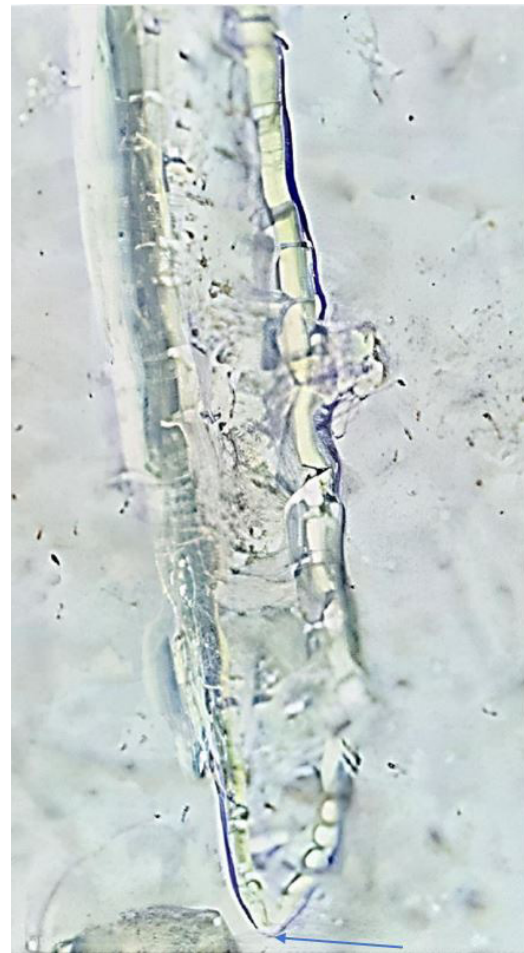
Common name	Total birds	Positive birds	Prevalence (%)
Grey parrot	14	10	71.4
Cockatiel	12	3	25.0
Senegal parrot	12	6	50.0
Rump parrot	14	7	50.0
Indian ringneck parrot	10	3	30.0
Love bird	12	3	25.0
Sun conure	10	4	40.0
Alexandrine parrot	14	6	42.9
Australian Budgerigars	12	3	25.0
Overall	110	45	40.9

**Table 3:** Prevalence of *Ascaridia galli*.

Species	Total birds	Positive birds	Prevalence (%)
Grey parrot	14	3	21.4
Cockatiel	12	0	0.0
Senegal parrot	12	1	8.3
Rump parrot	14	3	21.4
Indian ringneck parrot	10	1	10.0
Love bird	12	0	0.0
Sun conure	10	1	10.0
Alexandrine parrot	14	2	14.3
Australian Budgerigars	12	0	0.0
Overall	110	11	

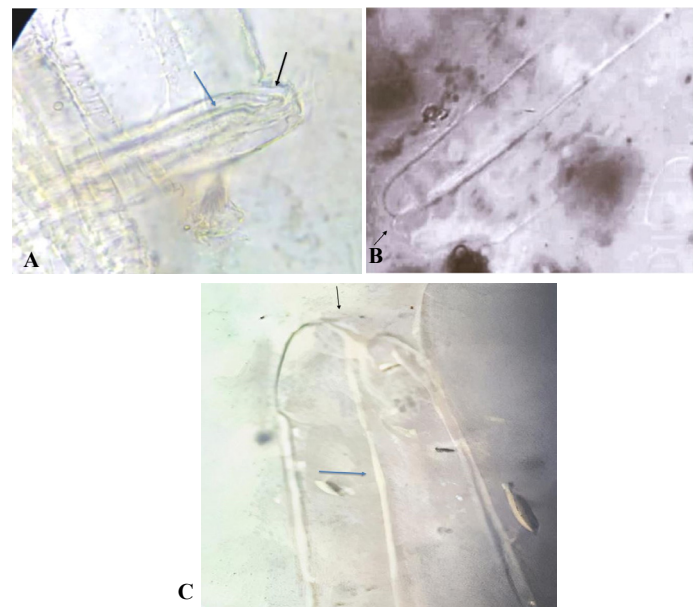
**Table 4:** Prevalence of *Ascaridia platyceri*.

Species	Total birds	Positive birds	Prevalence (%)
Grey parrot	14	7	50.0
Cockatiel	12	3	25.0
Senegal parrot	12	5	41.7
Rump parrot	14	4	28.6
Indian ringneck parrot	10	2	20.0
Love bird	12	3	25.0
Sun conure	10	3	30.0
Alexandrine parrot	14	4	28.6
Australian Budgerigars	12	3	25.0
Overall	110	34	30.9

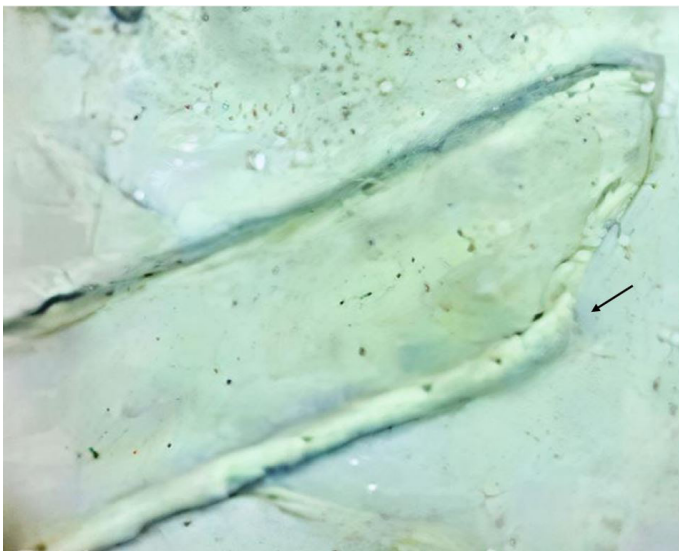


**Figure 1:** *Ascaridia platyceri* male posterior end lateral view. Blue arrow shows tail tip has a short conical protrusion at 10X.

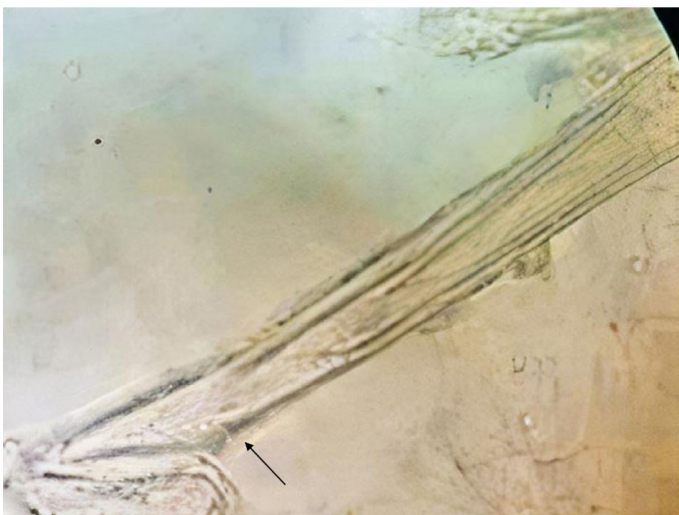
The prevalence varied among the species, with Grey Parrots (71.4%) having the highest infection rate, followed by Senegal Parrots (50%) and Rump Parrots (50%). Cockatiels (25%), Indian Ringnecks (30%), Lovebirds (25%), Sun Conures (40%), Alexandrine Parrots (42.9%), and black Australian Budgerigars (25%) showed lower infection rates. The infestation rates of *Ascaridia galli* are shown in Table 3, and the infestation rates of *Ascaridia platyceri* are shown in Table 4. The identification of *Ascaridia* spp. was based on the information provided by Kajerová *et al.* (2004), which helped identify two *Ascaridia* species: *Ascaridia platyceri* (Figures 1, 2) and *Ascaridia galli* (Figures 3, 4). The identification was made using microscopic examination with 4X and 10X lenses and compared with existing morphological descriptions from previous studies (Kajerová *et al.*, 2004). The ova of *Ascaridia galli* (Figure 5), identified through fecal flotation, exhibit an ellipsoidal shape with a thick, striated outer shell, facilitating their recognition and differentiation from other parasites.



**Figure 2:** *Ascaridia platyceri* anterior part lateral view. (A) Black arrow shows oral cavity is delineated by three lips, blue arrow shows esophagus at 10X. (B) Black arrow shows prominent three lips at oral opening at 10X. (C) Black arrow shows prominent lips, blue arrow shows esophagus at 10X.

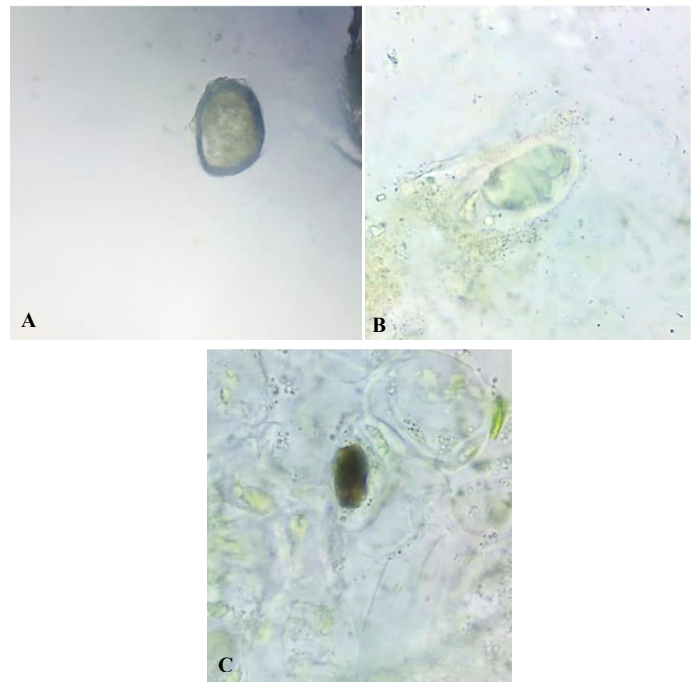


**Figure 3:** *Ascaridia galli* male lateral view of posterior end. Black arrow shows precloacal sucker at 10X.



**Figure 4:** *Ascaridia galli* female posterior end. Black arrow shows vulval opening at 4X.

This research investigated the prevalence and detection of infections with *Ascaridia* spp. in Psittaciformes in captivity within Gujranwala, Punjab, Pakistan. Two *Ascaridia* species, *A. galli* and *A. platyceri*, were identified as the predominant parasites based on morphological characteristics. Some *Ascaridia* species exhibit a preference for Psittaciformes, but the genus has a broader host range as documented by Aldamigh *et al.* (2024), Kajerová *et al.* (2004), with *Ascaridia platyceri* being one of them, *A. gallican* also infect Psittaciformes if kept near gallinaceous birds (Galosi *et al.*, 2015). The prevalence of *Ascaridia* spp. varied among different Psittaciformes species (Table 2). Grey Parrots showed the highest infection rate (71.4%), followed by Senegal Parrots and Rump Parrots (50% each). Cockatiels, Indian Ringneck Parrots, Love Birds, Sun Conures, Alexandrine Parrots, and



**Figure 5:** *Ascaridia galli* eggs (A) damaged intact egg at 10X. (B) showing thick shell, yellowish in color, oval to subspherical at 10X. (C) showing thick and smooth shell, oval to subspherical at 10X.

Australian Budgerigars exhibited lower infection rates ranging from 25% to 42.9%. *Ascaridia galli* infections were notably prevalent in Grey Parrots (21.4%), while *Ascaridia platyceri* infections were prominent in Grey Parrots (50%) and Senegal Parrots (41.7%). Our findings on the prevalence and identification of *Ascaridia* spp. infections in Psittaciformes are consistent with prior research confirming the significant presence of *Ascaridia* spp. in Psittaciformes across various regions conducted in this field. In Pakistan, a study conducted in Gujranwala and Jhang, Punjab, showed a prevalence of 33.93% for *Ascaridia* species in captive parrots. Another study by Khan *et al.* (2010) in Lahore Zoo, Punjab, Pakistan, reported 26.14% prevalence of *Ascaridia galli* in lovebirds, ringneck parrots, cockatiels, Australian Budgerigars, Alexandrine parrots, bluefronted amazons, eclectus parrots, and grey parrots belonging to the order Psittaciformes. In Chattogram, Bangladesh, a study found a prevalence of 9.09% of *Ascaridia* spp. in cockatiels, budgerigars, cockatoos, macaws, grey parrots, lovebirds, lorries, and rosellas belonging to the order Psittaciformes as pet birds (Bayzid *et al.*, 2022). A study from the state of Bagmati, Kathmandu Valley, Nepal, found that in Psittaciformes, the prevalence of *Ascaridia* species was the highest among the various gastrointestinal parasites identified. This study included captive-bred

exotic birds from 16 different species (Chokhal *et al.*, 2023). The *Ascaridia* species showed a prevalence rate of 23% across all examined samples. This indicates that *Ascaridia* species are particularly common among parrots and other birds in the Psittaciformes order, making it the most prevalent helminth parasite found in this group. In the Alipore Zoological Garden, India, 176 out of 392 birds tested positive for parasitic infections. Among Psittaciformes (including grey parrots, blue and yellow macaws, red and blue macaws, hill mynas, bare-eyed cockatoos, cockatiels, Greater Sulphur-crested cockatoos, smaller Sulphur-crested cockatoos, Goffin's cockatoos, Moluccan cockatoos, and citron-crested cockatoos), 48 out of 122 birds tested positive for *Ascaridia* spp., with a prevalence of 23.98%. *Ascaridia* spp. ova with prevalence 32.9% were found to be the most often observed gastrointestinal parasite infection in caged birds (Mondal and Manna, 2019).

In Chennai, India, the analysis of the 44 Psittaciformes samples was conducted by Prathipa *et al.* (2013), who found a prevalence of 11.20% for *Ascaridia* spp. with 5 out of 44 birds testing positive. In Nigeria, 2 out of 18 birds of three species (Senegal Parrot, Grey Parrot, Rose-ringed Parakeet) from the order Psittaciformes tested positive for *Ascaridia* spp. with a prevalence of 20.0%, indicating the second highest infection rate (Otegbade and Morenikeji, 2013). Research conducted in Italy by Papini *et al.* (2012) found that 6.8% of pet and zoo birds in the country were infected with *Ascaridia* spp. In Serbia, the prevalence of *Ascaridia* spp. in zoo birds ranged from 10.25% to 10.78% (Ilić *et al.*, 2018). In the Czech Republic, researchers discovered new cases of the nematode parasite *Ascaridia platyceri* in parrots. The parasite was discovered in 38 of these birds. Nine of the 15 distinct species of Psittaciform birds that had the parasite were novel hosts for *A. platyceri*, according to research conducted by Kajerová *et al.* (2004). Research carried out in northern Brazil by Lima *et al.* (2016) discovered that 40% of the parrots analyzed had *Ascaridia* spp. infection. *Ascaridia* infections have been shown to cause serious, frequently fatal diseases in captive psittacine, resulting in severe damage to the intestines and liver. These results emphasize the significance of effective management and preventative steps to mitigate the negative health effects of these parasites of captive birds (Greiner and Ritchie, 1994; Kajerová *et al.*, 2004). Infections caused by *Ascaridia* spp., commonly referred to as *ascaridiosis*. *Ascaridia*

spp. is the largest nematodes found in birds, often reside in small, harmless amounts in their tiny intestines. Even though ascariidiosis is often minor and may not manifest any clinical signs (Höglund *et al.*, 2023). Severe infestations can cause a number of extremely dangerous health problems. Among these are anemia, a severe inflammatory reaction, and maybe even death Fagerholm and Overstreet (2009). Interestingly, none of the birds examined in this study displayed visible signs of *Ascaridia* spp. infection, even though they carried the parasite. Furthermore, when foraging on contaminated surfaces or substrates in their environment, parrots may inadvertently eat infected eggs, which can contribute to the spread of *Ascaridia* spp. and *Ascaridia* spp. infections in parrots can cause symptoms including weight loss, lethargy, and stomach issues. Severe cases may cause intestinal blockage or anemia, two potentially fatal outcomes. Respiratory problems might also arise in severe situations. To reduce these risks, it is imperative to implement effective management methods, such as routine screening, hygienic practices, and perhaps preventive therapies depending on data on area prevalence (Greiner and Ritchie, 1994).

#### Limitations

Acknowledging the limitations of our study is crucial, particularly the dependence on qualitative techniques for parasite identification.

#### Conclusions and Recommendations

The study conducted at RASSA bird aviary in Gujranwala, Punjab, Pakistan, investigated *Ascaridia* spp. infections in 110 Psittaciformes across nine species. Using direct microscopic examination and flotation techniques, *Ascaridia galli* and *Ascaridia platyceri* were identified as predominant parasites. Overall, 40.9% of birds tested positive, with varying prevalence among species. This study underscores the significant presence of *Ascaridia* spp. in captive parrots and highlights the importance of effective management strategies to safeguard their health and welfare.

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the owner of RASSA Birds Farm and Aviary for their cooperation and assistance in making the birds and samples accessible, which was essential for the success of this research.

### Novelty Statement

In a pioneer revelation, this study documents the first-ever presence of *Ascaridia platyceri* in Psittaciformes (parrots) in Pakistan, uncovering a striking 40.9% prevalence of *Ascaridia* spp. such as *Ascaridia galli* and *Ascaridia Platyceri*, highlighting *Ascaridia platyceri* and *Ascaridia galli* as a major threat to captive parrot populations. This critical discovery not only fills a vital knowledge gap but also urges aviculturists and veterinarians to rethink preventive measures and protect these vibrant birds from the growing menace of *ascaridiosis* essential reading for those dedicated to parrot health and conservation!

### Author's Contribution

**Shahzadi Sarrah Atique:** Designed the study, performed the research, and prepared the manuscript.

**Ishrat Aziz:** Supervisor, provided guidance throughout the research process.

### Conflict of interest

The authors have declared no conflict of interest.

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