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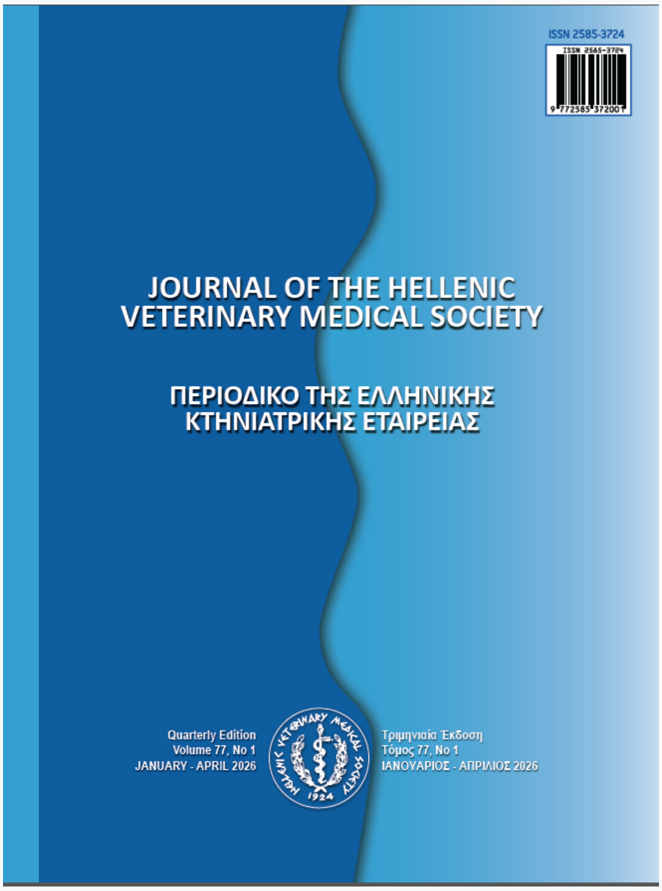

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Isolation and Identification of *Candida albicans*, *Macrorhabdus ornithogaster*, and *Giardia* spp. in Captive Pet Birds Referred to Aban Veterinary Clinic

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ABSTRACT: Fungal and parasitic infections pose significant health risks to avian populations, particularly under conditions of immunosuppression or poor husbandry. This study investigated the prevalence of *Candida albicans*, *Macrorhabdus ornithogaster*, and *Giardia* spp. in 756 birds referred to the Aban Veterinary Clinic over a two-year period (2023–2025). Fecal samples were analyzed via wet-mount microscopy, Gram staining, and clinical assessment to classify infections as mild, moderate, or severe. Results indicated *C. albicans* as the most prevalent pathogen (20.3% positivity), with severe cases concentrated in cockatiels and mynahs. *M. ornithogaster* infections were rarer (5% positivity), though an unusual predominance of intermediate cases in canaries suggested potential host-specific susceptibility. Giardiasis was detected in 40.6% of samples, primarily as mild infections (23.1%), with severe manifestations being rare (1.1%). Species-specific disparities were evident, with African grey parrots, pigeons, and finches showing no infections, while cockatiels and mynahs exhibited heightened vulnerability. These findings underscore the role of species-specific factors, immune status, and husbandry practices in disease manifestation. The study highlights the need for targeted surveillance in high-risk species and further investigation into atypical presentations, such as *M. ornithogaster* in canaries. Improved diagnostic protocols and management strategies are essential to mitigate these infections in avian populations.

Keyword: *Candida albicans*; *Macrorhabdus ornithogaster*; Giardiasis; avian infections; fungal pathogens; parasitic disease

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INTRODUCTION

Fungal infections occur less frequently than viral or bacterial diseases, primarily due to differences in how these pathogens interact with the host. A robust, functioning immune system can typically prevent fungal overgrowth and infection (Romani, 2011). However, certain conditions—such as underlying health disorders, immune suppression, or physical barriers breaking down—can create opportunities for severe fungal diseases to develop in both animals and humans (Seyedmousavi, Bosco et al., 2018). Candidiasis, known clinically as thrush or sour crop, is a common fungal infection in birds caused by the opportunistic overgrowth of *Candida albicans*, a yeast naturally present in the avian digestive tract (Ibrahim, 2020). The condition primarily affects the upper gastrointestinal system, including the mouth, esophagus, and crop, though it may occasionally spread further down the digestive tract (Garces, 2023). Several factors contribute to disease development, particularly in young birds with immature immune systems or those suffering from immunosuppressive conditions like malnutrition or Psittacine Beak and Feather Disease (Talazadeh, Ghorbanpoor et al., 2022). Poor hygiene practices, improper cleaning of feeding equipment, and diets high in sugars create favorable conditions for yeast proliferation (Doneley, 2018). Additionally, crop stasis leading to alkaline pH changes further promotes fungal overgrowth (Talazadeh, Ghorbanpoor et al., 2023). The disease has been reported across various avian species, Psittacines like cockatoos, budgerigars, and cockatiels, as well as in pigeons and poultry raised under suboptimal conditions. Effective management requires addressing both the fungal infection and predisposing husbandry factors (Samanta and Bandyopadhyay, 2017).

Macrorhabdus ornithogaster is an elongated, yeast-like fungus measuring approximately 2–3 µm in width and 20–80 µm in length. This pathogen primarily colonizes the stomachs of birds, affecting both companion species (such as budgerigars and canaries) and poultry (including chickens and ostriches) (Rinder, Schmitz et al., 2017, Kojima, Osawa et al., 2022). It typically resides at the junction between the proventriculus and ventriculus, attaching to the luminal surface and potentially invading the koilin layer (Hanafusa, Costa et al., 2013). While many infections remain subclinical, *M. ornithogaster* can cause severe chronic wasting disease in birds. Clinical signs may persist even after treatment. In some cases, chronic infection has been linked to gastric cancer (Speer, Phalen et al., 2004, Powers, Mitchell et al., 2019, Kojima, Osawa et al., 2022). Due to its potential severity, early detection and intervention are critical for effective management.

For *Candida albicans* diagnosis typically begins with clinical examination and microscopic evaluation (Abou-Zahr, 2023). Gram staining demonstrates gram-positive, oval yeast cells that may show pseudohyphal growth. Fungal culture on Sabouraud dextrose agar remains a gold standard (Banik, 2024).

For *Macrorhabdus ornithogaster* wet-mount examinations with saline or Gram staining are commonly employed, though the organism's uneven shedding may lead to false negatives (Baron, Stevenson et al., 2021). PCR testing has shown higher sensitivity compared to microscopy in research settings, particularly for subclinical infections, but remains underutilized in practice due to cost, laboratory requirements, and turnaround time (Murray, 2014).

Giardiasis is a parasitic infection in birds caused by *Giardia* protozoa, typically contracted through contaminated water or food sources (Egan, Barbosa et al., 2024). Though many birds may carry the parasite without visible symptoms, young or stressed individuals often develop signs such as diarrhea, weight loss, and lackluster plumage. Overcrowded living spaces and poor hygiene accelerate the spread of the disease. Diagnosis is usually confirmed through fecal testing, and treatment involves administering antiprotozoal medications along with thorough cleaning of the environment. Preventing giardiasis relies on maintaining sanitary conditions, minimizing stress, and isolating new or sick birds. Good husbandry practices are essential to protect both captive and wild avian populations (Malik, Arun Prince Milton et al., 2021).

Compared to bacterial or viral diseases, fungal infections in birds are less frequent but more deadly, especially in birds kept in captivity or with compromised immune systems (Dykstra, Charlton et al., 2013). Fungal diseases are still underdiagnosed and challenging to treat, whereas bacterial and viral infections are extensively researched because of their zoonotic and economic effects (Schmitz, Rinder et al., 2018). Lack of vaccines, few approved antifungals, and limited diagnostics are important research gaps. Although there are few studies on wild birds, environmental factors like climate change may increase the risks of fungi (Melo, Stevens et al., 2020). Closing these gaps is essential for zoonotic prevention and bird conservation, necessitating improved ecological research, detection techniques, and treatments to reduce neglected fungal threats. In this study we evaluated the presence of *Giardia* spp. alongside these fungi in birds referred to the Aban Veterinary Clinic.

MATERIALS AND METHODS

Sampling

This study was conducted from March 2023 to March 2025 to evaluate the prevalence of *C. albicans*, *M. ornithogaster*, and giardiasis in birds referred to Aban Veterinary Clinic. An aggregate number of 1123 birds were referred in this period which 756 cases were sampled and tested for our study. About 0.3 gram of feces was collected from the suspected cases. Fresh fecal samples were collected and immediately prepared as wet smears for microscopic examination. The samples were transported to the clinical pathology laboratory under appropriate conditions to ensure specimen integrity. Upon receipt, the laboratory performed standardized diagnostic protocols, which included direct microscopic evaluation for characteristic microbial morphology and staining procedures when indicated. This approach allowed for timely identification of potential pathogens while maintaining diagnostic accuracy.

DIAGNOSIS

Fresh fecal samples were processed as wet smears in the laboratory and examined under a light microscope (JENUS, China) at three objective magnifications: 40×, 100×, and 400×. For enhanced visualization and diagnostic confirmation, Gram staining was additionally performed on selected cases. This staining procedure facilitated both morphological verification and photo documentation of the microbial findings. The multi-magnification approach allowed for comprehensive evaluation of sample characteristics, from initial low-power screening to detailed high-resolution analysis.

Microscopic evaluation of stool samples from clinically suspect birds revealed varying degrees of microbial contamination, which were categorized into three distinct severity levels:

1. **Mild Contamination** - Characterized by occasional fungal elements observed sporadically throughout the smear preparation
2. **Moderate Contamination** - Demonstrated consistent presence of fungal organisms across multiple microscopic fields
3. **Severe Contamination** - Exhibited dense aggregation of fungal structures with extensive pseudohyphal networks

This tiered classification system enabled standardized reporting of mycological burden, facilitating correlation between laboratory findings and clinical manifestations. The grading scale proved particularly valuable for tracking disease progression and evaluating therapeutic efficacy in follow-up examinations.

Ethics Statement

This study was conducted in accordance with the ethical standards of the institutional review board (IRB) and the 1964 Declaration of Helsinki and its later amendments.

RESULTS

During past 8 seasons, among the 1,123 birds referred, 756 were tested, while 367 remained untested. *Candida albicans* infections were more prevalent overall, with 20.3% of tested birds showing positive results (mild, intermediate, or severe). Adult cockatiels and Mynahs exhibited the highest number of severe *Candida* cases (4 cases each), while mynahs had the highest proportion of infections, with 45 out of 75 tested birds showing *Candida* presence. In contrast, *Macrorhabdus* infections were less common, affecting only 5% of tested birds. Adult cockatiels again showed the highest number of cases, including 12 mild, 10 intermediate, and 4 severe infections. Notably, canaries displayed an unusual pattern for *Macrorhabdus*, with 5 out of 10 tested birds showing intermediate infections—a finding that warrants further investigation as this severity level is uncommon for this pathogen.

The data reveals significant species-specific variations in infection patterns. While some species like African grey parrots, pigeons, and finches showed no positive cases for either pathogen, others such as adult cockatiels and mynahs demonstrated higher susceptibility. The presence of *Macrorhabdus* infections in species like lovebirds and budgerigars (typically less affected) suggests potential husbandry or environmental factors influencing disease spread. These findings highlight the importance of targeted surveillance for high-risk species and further research into the unusual *Macrorhabdus* presentation in canaries. The overall low incidence of severe cases (<1% for both pathogens) indicates that while infections are common, they rarely reach critical levels in the tested populations.

For giardiasis, the majority of samples—449 (59.4%)—tested negative, while the remaining exhibited varying degrees of infection severity. Among positive cases, mild infections (G mild) were the most common, accounting for 23.1% (175 cases), followed by intermediate infections (G intermediate) at 16.4% (124 cases). Severe infections (G severe) were rare, representing only 1.1% (8 cases) of the total samples. This distribution suggests that while *Giardia* infections are relatively frequent, most cases are mild to moderate in severity, with severe clinical manifestations being uncommon. Results are summarized in Table 1.

DISCUSSION

Over eight seasons, 1,123 birds were referred, with 756 tested and 367 untested. *Candida albicans* was more prevalent (20.3% of tested birds), with severe cases highest in adult cockatiels and mynahs (4 each). Mynahs had the highest infection rate (45/75). Macrorhabdus was less common (5% of tested birds), with cockatiels having the most cases (12 mild, 10 intermediate, 4 severe). Notably, canaries showed an unusual pattern—5/10 had intermediate Macrorhabdus infections, which is atypical. In the case of Giardiasis, 40.6% of all referred cases were positive, making *Giardia* a common finding in clinical case, while it is rarely severe (1.1%). In the study of Talazadeh et al. (2022), 196 samples were collected from various types of birds (4 orders of Anseriformes, Columbiformes, Passeriformes, and Galliformes) showing clinical signs. They used PCR to detect *Candida albicans* and other non-*albicans*, which resulted in detecting 14.28% *C. albicans* and 10.72% non-*albicans* isolate, a total of 25% candida isolates (Talazadeh, Ghorbanpoor et al., 2022). Given that our diagnostic approach relied solely on light microscopy, the sensitivity and specificity of pathogen detection may have been limited, however, the detection rate of Candida is similar in both studies. Queiroz-Aaltonen et al. (2021) evaluated the droppings of three different orders (Columbiformes, Passeriformes, and Psittaciformes) by PCR in 3 residences and 4 districts of Brazil. They collected 71 samples (30 captive poultry, 41 pigeon) and all of them were candida positive (Queiroz-Aaltonen, Melo et al., 2021), which shows a much higher incidence compared to our study. Vieira and Coutinho (2009) studied an illegal trade flock, captured by forest police, comprised of 29 blue-fronted amazons and 11 orange-winged amazons using Gram-staining. Twenty-three Candida spp. were isolated from 40 parrots (57.5%) (Vieira and Coutinho, 2009). In the study of Cafarchia et al. (2006), occurrence of yeasts was evaluated in the migratory birds. A total of 421 wild birds was tested out of 1726 (24.39%) caught in Romania, Hungary and Bulgaria. The presence of yeasts was observed in 15.7% of birds and *C. albicans* was detected in 9.2% of total tested birds (Cafarchia, Camarda et al., 2006).

In our study, 4 out of 13 referred and tested budgerigars were positive (30.7%) for *Macrorhabdus ornithogaster*. Powers et al. (2019) conducted a retrospective review of avian cases submitted to a zoo and exotic pet pathology service from 1998 to 2013 in Washington, USA. Out of 28,128 avian submissions analyzed, 1,006 were budgerigars housed in zoos, aviaries, or private homes. Histopathological examination revealed that 177 (0.6%) of these budgerigars were infected with *Macrorhabdus ornithogaster* at the time of necropsy (Powers, Mitchell et al., 2019), which is not consistent with the results of our study. One important factor in this discrepancy can be the limited time of our study (2 years vs 15 years). However, Pustow et al. (2017) also conducted a 2.5 years study at the Clinic for Birds and Reptiles at the University of Leipzig (Leipzig, Germany) and detected *Macrorhabdus ornithogaster* in 66 out of 580 budgerigars (12%) by microscopic and radiographic examination (Püstow and Krautwald-Junghanns, 2017), which is almost one third the results of our study. Blagojevic et al. (2024) investigated the prevalence of *Macrorhabdus ornithogaster* in exotic birds between 2019 and 2022 using direct smear microscopy and Gram staining. The study found the highest detection rates in budgerigars (55.5%), followed by African grey parrots (33.3%) and cockatiels (34.3%), while canaries showed a lower prevalence (10%). Overall, 31% of the examined birds tested positive, indicating that *M. ornithogaster* is a common finding in exotic avian species. Notably, the majority of infected birds were asymptomatic, demonstrating that clinical signs are frequently absent despite infection (Blagojević, Davidov et al., 2024). Findings of this study does not entirely support the results of our study, which implies the *M. ornithogaster* infection is rare in pet birds, with the exception of budgerigar (30.7%) and canary (60%). Also, Piasecki et al. (2012) conducted a study to determine the prevalence of *Macrorhabdus ornithogaster* in both exotic and wild bird populations in Poland. The researchers examined 399 birds representing 45 species, utilizing fecal smears for live birds and proventricular cytology during

Table 1. Results

	Total	Not tested	Tested	Ca* negative	Ca Mild	Ca Intermediate	Ca Severe	M# negative	M mild	M intermediate	M severe	G negative	G mild	G intermediate	G Severe
Cockatiel chick	21	7	14	5	4	5	0	14	0	0	0	3	6	5	0
Adult cockatiel	535	142	393	327	33	29	4	367	12	10	4	134	141	110	8
Mynah	114	39	75	30	23	18	4	75	0	0	0	72	2	1	0
Lovebird	93	33	60	52	3	5	0	58	2	0	0	50	8	2	0
Green-cheeked parakeet	156	56	100	85	10	4	1	98	0	2	0	90	6	4	0
African grey parrot	64	30	34	34	0	0	0	34	0	0	0	32	2	0	0
Pigeon	22	13	9	9	0	0	0	9	0	0	0	6	1	2	0
Rose-ringed parakeet	39	21	18	15	0	3	0	18	0	0	0	17	1	0	0
Budgerigar	28	15	13	12	0	1	0	9	3	1	0	10	3	0	0
Alexandrine parakeet	13	4	9	8	1	0	0	9	0	0	0	7	2	0	0
Finch	7	0	7	7	0	0	0	7	0	0	0	5	2	0	0
Canary	16	6	10	8	1	1	0	4	1	5	0	10	0	0	0
Cockatoo	6	0	6	4	1	1	0	6	0	0	0	6	0	0	0
Peafowl	5	1	4	4	0	0	0	4	0	0	0	3	1	0	0
Macaw	4	0	4	2	0	2	0	4	0	0	0	4	0	0	0
Total	1123	367	756	602	76	69	9	716	18	18	4	449	175	124	8

*Candida albicans
#Macrorhabdus ornithogaster

necropsy for deceased specimens. The overall detection rate was 28.7% in exotic birds and 26.1% in wild birds. Among the affected species, budgerigars showed the highest infection rate (65.0%), followed by macaws (41.6%), African grey parrots (33.3%), cockatiels (26.9%), and lovebirds (16.7%). In contrast, canaries exhibited a lower prevalence (9.3%), while finches had minimal infection rates (1.2%) (Piasecki, Prochowska et al., 2012). This study also does not align with our results.

Based on our results, Giardiasis was detected in almost all species (except canary, cockatoo, and macaw) and was prevalent in cockatiel chick (11/14; 78.5%), cockatiel (259/393, 65.9%), pigeon (3/9; 33.3%), finch (2/7; 28.5%), and peafowl (1/4; 25%). Kazemi Lifshagerd et al. (2023) evaluated the occurrence of Giardiasis during 2022 in Mashhad, Iran. Among the tested birds, 60 Cockatiels (47.6%), 10 cockatiel chicks (43.4%), 4 lovebirds (14.2%), 2 Green-cheeked parakeets (8.6%), and a single African grey parrot (6.6%) were positive (KazemiLifshagerd, BehrouziNasab et al., 2023), which aligns with our study findings. Chamanara et al. (2024) ran a molecular survey of *Giardia* in pet avian species from April to July 2020 in Tehran, Iran. In total, they evaluated 150 cases of 4 orders using microscopy and PCR, yet did not detect *Giardia* (Chamanara, Arabkhazaeli et al., 2024). Their study can suggest a very low incidence of *Giardia* in Tehran compared to Mashhad. In the study of Dong et al. (2021), PCR was used to identify Giardiasis in captive pet birds from July 2018 to May 2019 in Henan province, Central China. In a total of 1005 birds, *Giardia duodenalis* was detected in 33 cases (3.3%) (Dong, Cheng et al., 2021), which is much lower compared to our study.

This study presents significant new information on the prevalence of *Candida albicans*, *Macrorhabdus ornithogaster*, and *Giardia* spp. in captive pet birds, while also identifying key diagnostic limitations and knowledge gaps. The most striking finding is the prevalence of intermediate Macrorhabdus infections in canaries (50% positivity) when literature has previously reported <10% prevalence in canaries, additionally, cockatiels and mynahs in this study were highly susceptible to Candida, while African greys, pigeons and finches were apparently not susceptible, suggesting geographic based and perhaps husbandry related resistance. The reported 40.6% prevalence of *Giardia* also exceeds rates reported in China (3.3%) and Tehran (0%), indicating considerable geographical variation in risk of infection.

Many gaps compared to the literature emerge in the paper. First, each reported prevalence is likely underestimated due to the use of microscopy - especially for Macrorhabdus (intermittent shedding creates false negatives) and for *Giardia* where genus rather than genotype was reported. Second, the unexpected presentation of Macrorhabdus in canaries is in conflict with multiple previous studies - suggesting either regional strain differences between studies or an artifact of diagnosis. Third, the absence of any infection in particular species (African greys, pigeons) raises the prospect of potential unexplained resistance mechanisms that should be examined.

In future studies, PCR offers substantial benefits over traditional diagnostic techniques for these pathogens: For *Candida albicans*, PCR (ITS/18S rRNA), has better sensitivity to detect fungi at low levels in feces and will be able to differentiate species, while culture still has a role for antifungal susceptibility testing. For *Macrorhabdus ornithogaster*, PCR (ITS region) is essential because it cannot be cultured and is intermittently shed, therefore the limitations of microscopy that has considerable false negatives can be avoided. For *Giardia* spp., PCR has better sensitivity and specificity than microscopy or ELISA, while also providing improved detection despite variability in cyst shedding, while also allowing for strain discrimination which is imperative for understanding zoonotic potential. Collectively, PCR provides improved sensitivity and specificity for each rate pathogen and offers the best approach for describing these pathogens while reducing the shortcomings of other methods.

CONCLUSION

This research found important epidemiological profiles of *Candida albicans*, *Macrorhabdus ornithogaster*, and *Giardia* spp. in captive pet birds and highlighted that the host species demonstrated extremely variable susceptibilities to the pathogens. The exceptionally high prevalence of intermediate infections of Macrorhabdus in canaries was contrary to the previous literature base. This may suggest that there could be regionally distinct strains of Macrorhabdus, or the reliance on a microscopy diagnosis was limited. Both Candida and Macrorhabdus were most prevalent in cockatiels and mynahs, while African greys and pigeons had no infections—implying unexplained mechanisms of resistance to the pathogens. The prevalence of 40.6% *Giardia* was also much greater than rates reported in other places, highlighting that prevalence can be geographically dependent. However, the reliance on a microscopy diagnosis limited widespread prevalence estimates, especially for intermittently shed Macrorhabdus and *Giardia* genotypes. Overall, the study highlights the need for future studies to use PCR-based diagnostics, which will increase the sensitivity and ability to differentiate between strains, and clarify host-pathogen interactions, and can eliminate misdiagnosis. Additionally, higher levels of surveillance of at-risk species and assessments to evaluate the features of birds that may confer resistance to the pathogens.

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