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Molecular Diagnostic Study of Intestinal Protozoa in Wild Pigeons in Diwaniyah Province

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Abstract Parasitic infestations in wildlife are significant biomarker of the present state of ecosystems, and studying these infections is essential for better understanding natural epidemiological hotspots. Animals infected with parasites are therefore susceptible to pathogens of medical and veterinary importance, wild birds have an important role in transmitting and spreading many zoonotic diseases, which has a great impact on the health of humans and birds. In this study, samples were collected from the intestinal contents of (50) wild pigeons from different areas in the city of Diwaniyah during the period extending from the beginning of September 2024 to the end of March 2025. The birds were dissected and samples were taken from the intestinal contents to investigate intestinal protozoa. The results of the microscopic examination showed the infection of 14 infected pigeons were frozen and preserved for polymerase chain reaction (PCR) testing. The results revealed two genera of intestinal protozoa, Cryptosporidium sp. and Isospora sp., with infection rates of 85.71% and 14.28%, respectively.

Keywords: *Cryptosporidium spp*., *Isospora spp*., intestinal protozoa

Introduction

Birds are among the most important animals in the environment as they are essential for maintaining ecological balance and also serve as seed dispersers, pollinators, and prey regulators. They are considered vital indicators for environmental conservation. They are animals known for their high sensitivity to habitat changes. The extinction of wild species leads to the deterioration of natural environments, affecting not only animals but also plants and microbes, and indirectly contributes to the emergence and spread of parasitic diseases Parasites may be a major player in maintaining this biodiversity by maintaining diversity in ecological communities [1] .Wild and domestic birds also play an important role in transmitting and spreading many zoonotic diseases (parasitic, bacterial, viral and fungal), which has a significant impact on the health of humans and birds [2] . Wild birds are often responsible for contaminating water sources with zoonotic diseases due to their presence near water sources and in urban areas and their ability to transmit intestinal pathogens over long distances [3] . Parasitic infection in wildlife is an important biomarker of the current state of ecosystems and the study of these infections is essential for a better understanding of natural foci by epidemiology. Animals infected with parasites are therefore susceptible to pathogens of medical and veterinary significance [4]. The digestive system is more susceptible to infection by various types of parasites than other organs of the body due to the entry of water or food containing the infective stage of the parasite through the digestive system [5]. The species of Cryptosporidium sp. and Coccidia parasites that cause Coccidiosis, including species of the genus Isospora sp., are considered common intestinal protozoa in birds [6]. The species of Cryptosporidium sp. cause Cryptosporidiosis. It is considered a parasitic disease of animal origin that infects multiple hosts, including birds [7]. The oocyst is considered the infectious stage, as it is infectious when it is excreted from the host's body. It is characterized by its tolerance to environmental conditions and standard water treatments [8]. Wild birds infected with the parasite also pose a threat to public health due to the nature of their life in the wild, which leads to the difficulty of controlling their infection with Cryptosporidium, which increases the possibility of this disease being transmitted to humans and pets. It is also necessary to monitor infection in wild birds, its methods of transmission, and its prevention [9].The genus Isospora sp. is considered one of the causes of coccidiosis. The oocysts contain two sporocysts, and each sporocyst contains four spores [10]. Many studies have shown that birds are infected in different organs of the bird's body. In wild birds, it was found in the intestines, liver, and spleen. The stages of Isospora sp. in the tissues located

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outside the intestines are often accompanied by lymphocytic inflammation of varying severity [11].

Materials and methods

Ethical Approval

The procedures for the current study were approved by the Department of Life Sciences, College of Education. In this study, samples of the intestinal contents of (50) wild pigeons were collected from different areas in the city of Diwaniyah during the period extending from the beginning of September 2024 to the end of March 2025. The results of microscopic examination using traditional methods showed that 14 wild pigeons were infected. These were examined using the conventional polymerase chain reaction technique according to the method of [12]. To detect Cryptosporidium sp. and Isospora sp. parasites, DNA was extracted using the Presto™ Stool DNA Extraction Kit from Geneaid/Taiwan, which was provided by Scientific Researcher. Co. Ltd. The extraction was carried out according to the steps in the company's instruction manual. Nano-drop spectrophotometer was used to measure the concentration and purity of DNA, and primers specific to the Small-subunit 18 Sr RNA gene were used to detect Cryptosporidium sp. and Isospora sp. using PCR technology, as shown in Table (1). These primers were prepared by Scientific Researcher. Co. Ltd, Iraq, where it was designed using the NCBI-Genbank gene bank and the Primer 3 Plus design program. A Nano-drop spectrophotometer was then used to measure the concentration and purity of the extracted DNA. The polymerase chain reaction mixture was prepared in tubes and then transferred to a BIO RAD T 100 Thermocycler for thermal cycling. The PCR product was analyzed by Agarose gel electrophoresis.

Table (1) shows the primers used in this study.

Primer	Sequence 5'- 3'		Product size
Cryptosporidium sp	F	AGACGGTAGGGTATTGGCCT	784bp
	R	TACGAATGCCCCCAACTGTC	
Isospora sp	F	ATTTCTGTCGGTGCGATCGG	423bp

Results

PCR results of the intestinal contents of 14 wild pigeons tested showed infection with Cryptosporidium sp. (12/14) birds, representing 85.71%, and Isospora sp. (2/14) birds, representing 14.28%. Agarose gel electrophoresis of the DNA amplification results from the tested samples for Cryptosporidium sp. PCR using the small subunit ribosomal RNA gene primer revealed DNA bands of approximately 784 bp, as shown in Figure (1).Electrophoresis on agarose gel of the DNA amplification product of the samples examined to diagnose the Isospora sp parasite using the primer specific to the Small subunit ribosomal RNA gene showed DNA bands of approximately (423 bp) as in Figure (2).

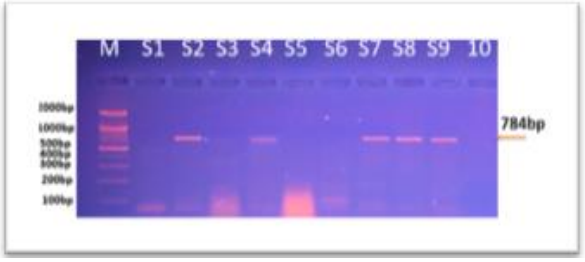

Figure (1): Acarose electrophoresis image showing the analysis of the polymerase chain reaction product of the small subunit ribosomal RNA in Cryptosporidium from bird samples. Where: M represents the index for the PCR product (2000-100bp) and the lines (S2, S4, S7, S8 and S9) are some positive samples of Cryptosporidium sp at (784 bp).

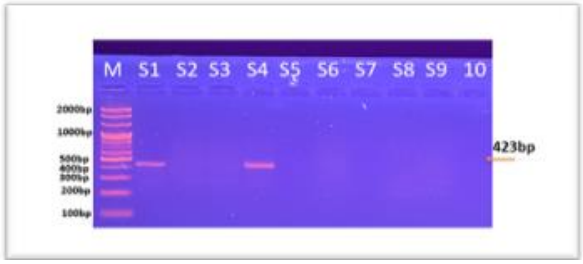

Figure (2): Acarose electrophoresis image showing the analysis of the polymerase chain reaction product of the small subunit ribosomal RNA in Isospora sp. from bird samples. Where: M represents the index for the PCR product (2000-100bp) and the line (S1,S4) for the positive Isospora sp samples when the product appears around (423bp).

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Discussion

Wild pigeons are found in large numbers around us and may be infected with or harbor numerous types of parasites that can be transmitted to humans or other animals. [13] The use of PCR-based diagnostic methods has increased significantly in the field of parasitology in recent decades. [14] This technique has had a significant impact on the progress achieved in the field of diagnosing, characterizing, tracking, and investigating the evolution, epidemiology, and biology of protozoan parasites. [15] The conventional PCR technique was used in this study, and the infection rate with Cryptpsoridium spp. in wild pigeons was recorded at 85.71%, which is consistent with what was recorded by [16]. A 90% infection rate of pigeons with the parasite was recorded in a study of primary infection in clinically ill pigeons in Assiut, Egypt. The rate in our current study was close to the rate recorded by [17], who recorded 62% infection in pigeons in his study of Cryptosporidiosis in domestic pigeons in Iran. The infection rate in the current study was higher than that recorded by [18], as the infection rate of wild birds in their study was 25.71% using Nested PCR technology. This may be due to the fact that the samples examined in their study were larger in number, and also the samples in our current study were previously diagnosed positive upon microscopic examination to diagnose the parasite Cryptpsoridium spp., so it is considered a selective sample and not a random one, several studies have confirmed the prevalence of the Cryptosporidium sp. parasite in pigeons worldwide, and molecular methods have yielded higher rates of infection compared to traditional methods. The highest infection rates were observed in Iraq [19]. The results of the current study also recorded that wild pigeons were infected with the Isospora sp. parasite, which was recorded at 14.28%, which is close to what was recorded by [20], who recorded a 19.4% infection rate in pigeons when studying intestinal parasites in pigeons in Venezuela. Several studies have confirmed bird infections with the Isospora sp. [21] recorded a 2% infection rate in ostriches when studying the isolation of intestinal parasites from ostriches from central Iraq. [22] also recorded a 2.91% infection rate in domestic birds with the Isospora sp. parasite in Bangladesh. [23] He also recorded that wild birds in Europe were infected with 5.8% of the parasite. [24] He also mentioned that wild birds in Britain and Ireland were infected with 32% of the parasite when he studied the prevalence of parasites in 13 families of wild passerine birds. He mentioned that the severity of infection was higher in birds that used a wider range of habitats and increased in areas that contained feeders. The infection of birds in these studies was either higher or lower than what was recorded in our current study. The reason may be due to the difference in the types and numbers of birds studied and the climatic conditions.

Conflict of interest

No conflict of interest is found for the present study.

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