



# Detection of *Cryptosporidium* Infection in Domestic Pigeons in Baghdad City Using Conventional PCR Technique

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## Abstract

*Cryptosporidium* infection poses a significant threat to domestic pigeon populations worldwide, Pigeons serve as vectors and may transmit the parasite to other avian species, therefore playing a significant role in zoonotic transmission. This study aimed to investigate the prevalence of *Cryptosporidium* spp. in domestic pigeons in Baghdad, Iraq, using conventional PCR and to assess the influence of age, sex, and seasonal variation on infection rates. During the study period of six months, from 1<sup>st</sup> October to end of March, 120 domestic pigeons were sampled, and fecal samples were collected for genetic analysis by using a conventional PCR. The overall infection rate was found to be 70.83%, with no significant difference between male and female pigeons, and infection rate of females was highest 82.98%. However, younger pigeons below 6 months of age exhibited a higher infection rate 78.26% compared to adults with significant differences. Seasonal variation was observed, with the highest infection rates recorded in February 95% and January 85%. According to molecular analysis the results confirmed the high presence of *Cryptosporidium* spp. between domestic pigeons in Baghdad city. In conclusion the study reported high prevalence of *Cryptosporidium* infection in domestic pigeons in Baghdad, Iraq, with significant age-related variations and seasonal fluctuations.

## 1. Introduction

*Cryptosporidium* species, a detrimental parasite, is often found in the gastrointestinal tracts of several families. Tyzzer first characterized it in 1907, drawing on findings made in mice [1]. Birds may be affected by cryptosporidiosis, which is caused by three species of *Cryptosporidium*: *Cryptosporidium meleagridis*, *Cryptosporidium baileyi*, and *Cryptosporidium galli* [2]. Out of them, *C. meleagridis* is of zoonotic significance and is often identified as the primary cause of human cryptosporidiosis [2]. Pigeons serve as vectors and may transmit the parasite to other avian species, therefore playing a significant role in zoonotic transmission. Birds infected with *Cryptosporidium* may have respiratory, renal, and digestive problems, such as diarrhoea [3]. Direct, concentration, staining, and Polymerase Chain Reaction (PCR) as molecular procedures were among the diagnostic techniques for *Cryptosporidium* spp [4]. The molecular epidemiology of *Cryptosporidium* in pigeons has been the subject of several investigations, which have shown the parasite's evolutionary history and genetic variety. Worldwide, pigeons have been shown to be infected with *Cryptosporidium*, and Iraq is no exception [5]

[6]. Pigeons were a potential vector for human cryptosporidiosis, thus it's important to keep an eye on them at all times because of the human-pigeon interaction [7]. Therefore, the objective of the research was to identify *Cryptosporidium* spp. in domestic pigeons by conventional PCR, and investigate the influence of age, sex, and months on infection rates.

## 2. Experimental Part

### 2.1. Ethics Statement

This study was conducted in accordance with ethical guidelines for the use of animals in research. Sample collection and handling procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the College of Veterinary Medicine, University of Baghdad. Efforts were made to minimize stress and discomfort to the pigeons during sample collection. No invasive or harmful procedures were performed.

### 2.2. Pigeons and Samples Collection

From October 2022 through March 2023, a total of 120 domestic pigeons of varying ages and sexes were studied in Baghdad city. The birds were studied by collecting fresh dropping samples. Each sample was promptly placed in a sterile, labelled container and kept in a refrigerator [8]. Separate portions of the samples were used for molecular methods maintained at -20°C tests in the internal and preventive medicine department of the veterinary college [9].

### 2.3. Genetic Analysis and Primers

Genomic DNA was extracted from faeces samples using DNA extraction kit supplied by Genaid / Korea, Polymerase Chain Reaction (PCR): designed primers according to NCBI-Genbank and an ACCESSION number (AF442484.1) with using primer 3 plus. Primers sequences have been to amplify the (*18SrRNA* gene) (1320 bp). Forward: 5' GGG TAT TGG CCT ACC GTGG -3', and Reverse: 5'- ACC GGA TCA TTC AAT CGG TAGG -3' The parameters for cycling were start at 95°C for 5 min, 35 cycles with denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, annealing at 72°C for 2 min, and final extension at 72°C for 5 minutes, with final keeping at 4°C forever [10]. Amplification was confirmed through electrophoresis on a 1.5% agarose gel that had been pre-stained with 3 µl of ethidium bromide in a 1× TBE buffer. After sending their data to NCBI through Macro Gen Company (Korea).

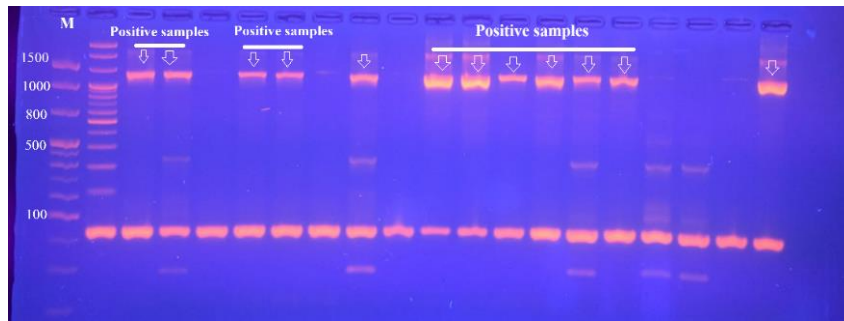
### 2.4. Statistical Analysis

Data analysis utilized SPSS Version 23.0 (SPSS Inc., USA) for Windows, employing Chi-square tests to assess the relationship between the parasite's presence and various studied variables. The data were organized in a Microsoft Office Excel 356 database. Significant was established at  $P \leq 0.05$ .

## 3. Results and Discussion

### 3.1. Result

According to study of 120 pigeon's sample, founded that Infection rate was 70.83% over period of study for six months. *Cryptosporidium* infected both sexes, according to the study. The infection rate in pigeons was 46 (63.01%) out of 73 males and 39 (82.98%) out of 47 females, with no significant difference between gender ( $P < 0.05$ ) Table (1). The risk of infection in pigeons with *Cryptosporidium* was high at younger age below 6 months 18 (78.26%) out of 23 examined and adults above 6 months were 67 (69.07%) out of 97 examined pigeon with significant differences ( $P \leq 0.05$ ) between age groups Table (2). While the prevalence of *Cryptosporidium* was recorded all over the months of the study in both pigeon and handlers. The highest infection rate in pigeons was recorded in February 95% (19/20), January 85% (17/20) while the lowest rate was recorded in December 35% (7/20) with significant differences ( $P \leq 0.05$ ) between rate of infection Table (3). The unique 1320 bp on agarose gel (1.5%) for *Cryptosporidium* spp. was found 120 Baghdad domestic pigeons as shown in Figure (1).



**Figure (1):** Agarose gel (1.5%) electrophoreses of *Cryptosporidium* spp. M: marker (1500-100bp); lanes showed positive *Cryptosporidium* spp. at (1320 bp).

**Table (1):** Infection rate of *Cryptosporidium* spp. in pigeon according to sex.

Sex	Examined Samples	Positive Samples	Percentage
Males	73	46	63.01
Females	47	39	82.98
Total	120	85	70.83
<i>P</i> -value			0.1

Without significant difference ( $P < 0.05$ )

**Table (2):** Infection rate of *Cryptosporidium* spp. In Domestic pigeons according to age groups.

Age group	Examined Samples	Positive Samples	Percentage
Adult	97	67	69.07
Young	23	18	78.26
Total	120	85	70.83
<i>P</i> -value			0.002

With significant differences ( $P < 0.05$ )

**Table (3):** Infection rate of *Cryptosporidium* spp. In domestic pigeon according to months.

Month	Examined pigeon	Positive pigeon	Percentage
2022 October	20	16	80.00
November	20	10	50.00
December	20	7	35.00
2023 January	20	17	85.00
February	20	19	95.00
March	20	16	80.00
Total	120	85	70.83
<i>P</i> -value			0.0001

With significant differences ( $P < 0.05$ )

### 3.2. Discussion

The study infection rate was 70.83%, more than studies conducted in Baghdad, Iraq, by [6], [11], and [12], which reported lower rates 40%, 41%, and 58.1%, respectively. These consistent findings support the observed infection rate, emphasizing the significance of *Cryptosporidium* in the pigeon population.

A higher infection rate reported by the study [13] reported an even 62%, indicating regional variations in prevalence. Conversely, the study by [14] observed a lower rate of 35% (70 out of 200) in chickens, hinting at potential species-specific susceptibility differences. Additionally, study conducted by [15] identified 37% infection rate, while a study in Bangladesh by [16] reported a prevalence of 39%. These varying rates across different regions and species underline the dynamic nature of *Cryptosporidium* infections and emphasize the importance of understanding local ecological factors in disease transmission dynamics. These findings collectively highlight the need for comprehensive surveillance and control measures to mitigate the impact of *Cryptosporidium* in domestic pigeon populations.

This study aligns with studies that have similarly found no significant variations in infection rates based on pigeon sex. Notably, research conducted by [17] in Iraq, [6] in Iraq, and [18] in Brazil all supports this finding. This consensus among studies conducted in different regions underscores the robustness of this observation and suggests that the susceptibility to *Cryptosporidium* infection in pigeons may not be strongly influenced by sex. This finding contributes valuable insights to the broader understanding of *Cryptosporidium* dynamics within avian populations.

The investigation revealed younger pigeons exhibited a higher incidence of *Cryptosporidium* infection compared to their adult counterparts. This aligns with prior research conducted by [19] in Egypt, where 40% of young pigeons were found to have cryptosporidiosis, potentially attributed to their less developed immune systems. However, this observation diverges from the findings of [20], who reported no significant difference in infection rates between young pigeons (12.26%) and adults (9.57%). This discrepancy underscores the complexity of *Cryptosporidium* dynamics and highlights the need for further investigation into age-related susceptibility factors [19], [20].

These findings are consistent with studies conducted by [21], who reported an 11.4% infection rate in February, and [6], who observed a 76% infection rate in pigeons during the winter [21], [6]. This consistency across studies suggests a plausible association between environmental variables, such as temperature and the prevalence of *Cryptosporidium* infections in pigeons. In contrast, results differ from those of study by [13], who reported a high infection rate of 94% in April (spring). Additionally, Iraqi study of [14] found 46% infection in April, while study of [20], who found a significant rate of 50% in March [13], [14], and [20]. These disparities may be attributed to differences in study populations, geographic locations, or variations in levels of exposure to *Cryptosporidium*.

#### 4. Conclusions

The study findings regarding the high prevalence of *Cryptosporidium* infection in domestic pigeons in Baghdad, Iraq, along with the observed age-related variations and seasonal fluctuations, indeed provide valuable insights. Such data can serve as a foundation for further industrial research aimed at developing vaccines or other interventions to mitigate the impact of *Cryptosporidium* infection in pigeons.

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**Conflict of Interest:** The authors declare that there are no conflicts of interest associated with this research project. We have no financial or personal relationships that could potentially bias our work or influence the interpretation of the results.

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