






DETECTION AND PREVALENCE OF *Leucocytozoon* spp. IN LOCAL CHICKEN BREEDS IN AL MUTHANNA PROVINCE OF IRAQ

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↳Supporting Information

ABSTRACT: *Leucocytozoon* spp., a hemiparasite commonly found in birds with economic impacts in poultry production. The present study investigates the presence of *Leucocytozoon* in chickens in Al Muthanna province of Iraq. Eighty-one blood samples were collected from chickens in areas like Samawah, Rumaitha, Warkaa, and Kidhre to examine the prevalence of *Leucocytozoon*. The investigations found a 6.1% infection rate among chicken breeds. The study highlighted the main symptoms which were decreased egg production, anemia, and loss of appetite as indicators of infection. Notably, infections were most prevalent in the Rumaitha, Khidr and Samawah regions while no cases were reported in Warka. Treatment methods included Primaquine and Pyrimethamine alongside care to manage the condition effectively. It is important to mention that the observed prevalence rate in chickens was lower compared to studies on birds in Iraq where an overall blood parasite prevalence of 15% was documented. This difference could be attributed to factors like habitat variations, vector presence, or differing susceptibility, among bird species. Our recommendation for future work can be applied to new programs for diagnosing and controlling parasites in chickens.

Keywords: Al-Muthanna Province, Avian health, Flocks, Hemoparasite, *Leucocytozoon* spp.

INTRODUCTION

Leucocytozoon belongs to a group of alveolates, in the Apicomplexa phylum, which is also home to malaria parasites. These parasites are recognized for their life cycle involving blackflies (*Simulium* species) or biting midges as hosts and birds as intermediate hosts. There have been more than 100 *Leucocytozoon* species identified worldwide infecting avian hosts (Adler, 2019). In the life cycle of *Leucocytozoon* gametocytes are present in the blood of hosts. Are acquired by female blackflies. The parasite undergoes a process of malaria. It does not produce hemozoin deposits as *Plasmodium* does. Instead, merogony takes place in organs such as the liver, heart, and kidneys (Adler, 2019).

Pathogenic avian blood parasites can cause harm to poultry farming (Zhou et al., 2020). Infections by these blood-dwelling parasites can result in issues like anemia, weight loss, stunted growth decreased egg production, and higher mortality rates in poultry flocks (Adamu, 2017). This widespread presence of haemoparasites poses a risk to poultry due to exposure to insect vectors and environmental contamination (Wamboi et al., 2020).

Infections by haemoparasites can lead to changes in blood parameters in chickens which could affect their productivity (Wamboi et al., 2020). For example, *Haemoproteus* infections have been found to lower blood glucose levels in chickens significantly likely because the parasites consume glucose, for their metabolic needs (Wamboi et al., 2020). Moreover, these parasitic infections are often asymptomatic, which makes them challenging to identify and manage without monitoring. Helminth infections are widespread, in free-range chickens, at levels leading to hidden illnesses that impact health and productivity (Sharma et al., 2018). Hence it is vital to establish control measures and regularly monitor these parasites to uphold poultry well-being and enhance production outcomes in both backyard environments (Wamboi et al., 2020). Major avian haemosporidian genera include the potentially dangerous *Plasmodium* spp., *Haemoproteus* spp., and *Leucocytozoon* spp. (Bennett et al., 1993). Birds can acquire leucocytozoonosis from numerous species of the genus *Leucocytozoon* that are spread via vectors. While *Leucocytozoon* is abundant, only a small subset of species is known to cause disease in birds (Forrester and Greiner, 2008). Waterfowl, pigeons, galliforms, raptors, and ostriches are all vulnerable to the phylum Apicomplexa, order Haemosporina, family Plasmodiidae, genus *Leucocytozoon* (Bennett et al., 1993). There are at least 67 identified species, with 66 infecting birds (Hsu et al. 1973). *Leucocytozoon* is birds' biggest and most prevalent haemoparasite (Ahmadov et al., 2019). *Leucocytozoon* has two subgenera: *Akiba* and

Leucocytozoon (Ahmadov et al., 2019). In Al-Muthanna, different epidemiological studies used physiological parameters to show more information on microbiological infections in animals (Hameed et al., 2022; Al-Yasari et al., 2024).

This study aimed to compare our results with the previous epidemiological studies of *Leucocytozoon* in birds including that carried out in Iraq.

MATERIALS AND METHODS

Study area

The samples were collected from the Veterinary Teaching Hospital in four selected regions of Al-Muthanna Province/Iraq (Samawah, Rumaitha, Warkaa, and Kidhre).

Samples collection

Eighty-one (5-13/month) blood samples were collected from local chickens farming in different regions in AlMuthanna province of Iraq. These regions are Samawah, Rumaitha, Warkaa, and Kidhre. Fresh samples were transferred in sterile containers to the Protozoology Laboratory at the College of Veterinary Medicine/Al-Muthanna University and then divided into two tubes with EDTA and without EDTA. The study period was nine months from October 2022 to June 2023. All the information about chickens including sex, region, date of collection, clinical signs, and treatment were recorded on the containers of the samples. Finally, the samples were evaluated by making thin and thick smears and staining with Giemsa then examined under the light microscope.

Statistical analysis

After the collection of the samples, the data were recorded including clinical signs, sex, the main cities in Al-Muthanna Province and months of study, and treatment measurements (Graphs 1-4). These data were analyzed after the examination of samples. The analysis was done using GraphPad Prism 9, Chi-Square program under $P \leq 0.05$.

Ethical approval

This study was part of a bigger project that was technically approved by the Scientific Committee at the College of Veterinary Medicine at Al-Muthanna University (Registered code: REF-3-Iman K Alabadi).

RESULTS AND DISCUSSION

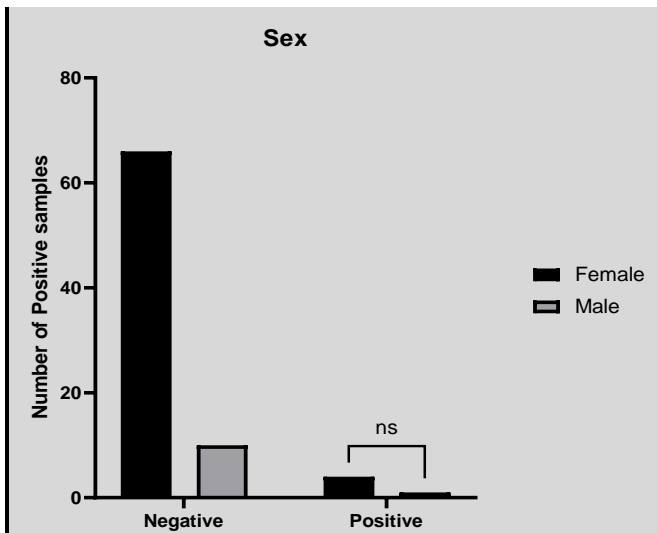
Epidemiology

Present results revealed that the total infection rate of *Leucocytozoonosis* was 6.1% in local Iraqi breeds of chickens. Many bird host families have been infected with several *Leucocytozoon species*. *Leucocytozoon's* gametogony takes place in leukocytes or erythrocytes, whereas its schizogony takes place in a wide variety of parenchymal and endothelial cells. *Leucocytozoon* gametocytes are pleomorphic, with certain species showing fusiform and exclusively spherical forms.

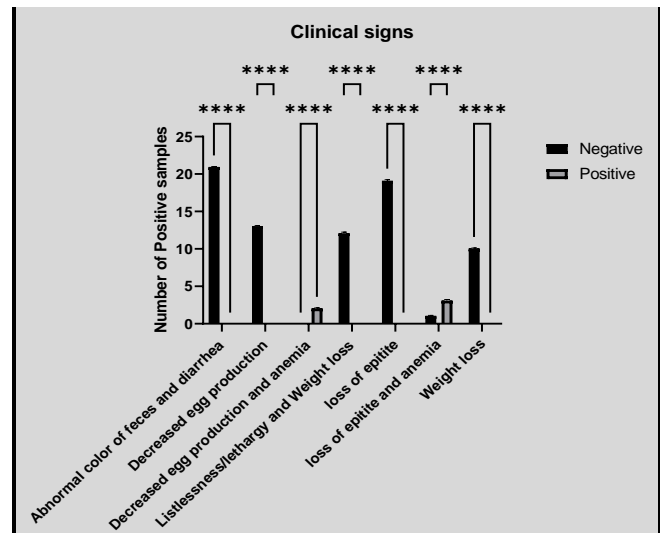
The life cycle of *Leucocytozoon spp.* involves two hosts. *Simuliid* black flies and *Culicoid* midges exhibit sporogony, after that sporozoites travel to the insect's salivary glands. Subsequently, the vertebrate host is infected, and internal organs such as the liver, brain, spleen, and lungs undergo schizogony. It is important to study intermediate hosts for both medicinal and veterinary purposes (Alkhuzai et al., 2019 and Shaker et al., 2024) as they can infect many organs such as the nasal cavities and sinuses (Alhayali et al., 2022). Our results revealed no significant differences between males and females infected with *Leucocytozoon* (Graph 1). Al-Biatee (2014) recorded that *Leucocytozoon spp.* infection rate was 10.52% in quail in Baghdad city also female quails have a greater infection rate than males.

Pathogenesis and clinical signs

From present recordings, decreased egg production and anemia and loss of appetite and anemia were the only signs that were significantly found (Graph 2). Different clinical signs were recorded such as anorexia, decreasing weight, drawback of the conversion feed, anemia, green feces, and frequent mortality can result from parasite infections with *Plasmodium* and *Leucocytozoon spp.* Infections with *Leucocytozoon spp.* cause severe anemia. Pneumonia, lung congestion, and the resulting occlusion of alveolar capillaries are also all potential outcomes in Turkey. Moreover, liver necrosis, enlarged spleen, lymphocytic infiltration of the liver and heart, and hemosiderosis may be present (Atkinson and Van Riper, 1991). Illness and mortality in young ducks, both domestic and wild, can be caused by *Leucocytozoon simondi*. Infection can be more spread in flocks of ducks especially close to lakes. Recovering ducklings may be permanently dwarfed. Adult birds are sometimes severely impacted to the point of death. Most of the time, they can make a full recovery, however, they continue to carry the parasite in their blood and spread it to other birds, especially young ones (Wehr and Farr, 1956).



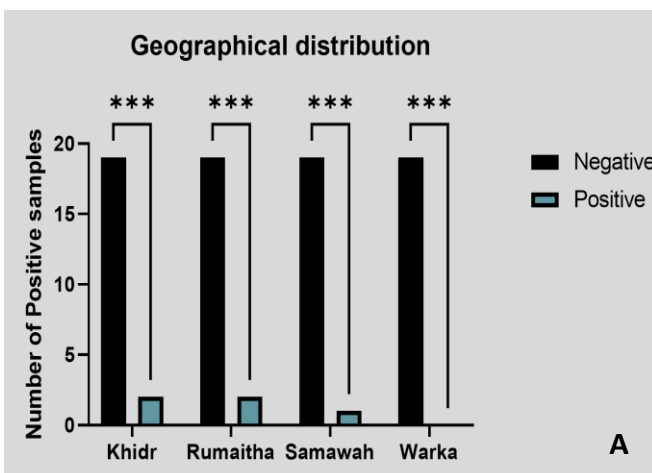
Graph 1 - Number of infected samples according to sex.



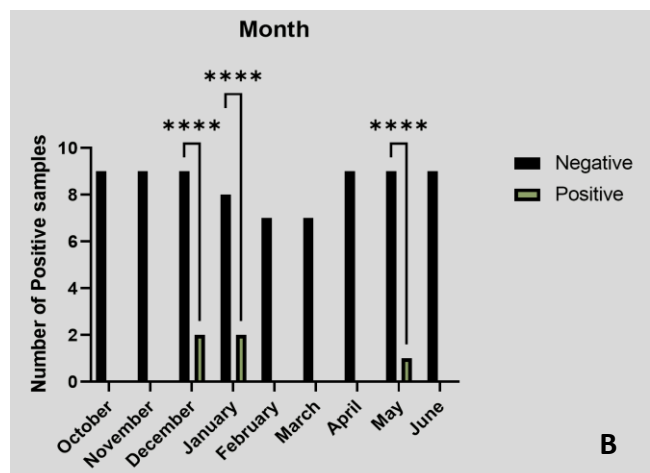
Graph 2 - Number of infected samples according to clinical signs.

Geographical distribution

The collected data showed that the infection was centered mainly in Rumaiitha then Khidr and Samawah, while there are no recorded positive results in Warka (Graph 3 A). These cities represent the main places in Al-Muthanna province. In addition, the researchers recorded positive results only in December, January, and May. This may relate to the nature of environmental conditions in Al-Muthanna which could increase the growth rates of the transmission vector (Graph 3 B).



A



B

Graph 3 - Number of infected samples according to geographical distribution (A) and months (B).

Al-Shuaibi (2008) did not find the *Leucocytozoon* infection in chickens in Al Ramadi about 110 kilometers west of Baghdad. *Leucocytozoon spp.* was the least common haemoparasite according to a study by Abdullah (2013), and its prevalence was low (13.5%) with no signs of sickness present among the chickens in the Qaradagh district of the Kurdistan region of Iraq, which is around 45 kilometers from Sulaimani province. While the prevalence of mixed hemoparasite infections in local chickens was 7.5% for *Plasmodium spp.* and *Leucocytozoon spp.* and 1.5% for *Leucocytozoon spp.* and *Haemoproteus spp.* (Abdullah, 2013). Hasson (2015) did not find any *Leucocytozoon spp.* record in adult chickens in Diyala. While mixed infection with the triple hemiparasites (*Plasmodium spp.*, *Haemoproteus spp.*, *Leucocytozoon spp.*) was found in adult chickens at a 36.8% rate. *Leucocytozoon species* are widely dispersed in farmed chickens (*Gallus gallus domesticus*) in Baghdad city, with a higher infection rate of 30%, according to Ibrahim and Al-Rubaie (2020). Additionally, mature chickens have a higher infection rate than young chickens, as well as female more so than male. In Nineveh villages, where geese were examined, Shamaun et al. (2007) found that the prevalence of *Leucocytozoon simondi* was 33.33%. Al-Shuaibi (2008) reported a 10.7% infection rate of *Leucocytozoon spp.* on geese in different areas of Al Ramadi, while it was reported as 5.37% and a mixed infection with *Plasmodium spp.* + *Leucocytozoon spp.* was 14.1% in geese at Sulaimani province (Mohammed, 2014a) and 14.2% in different areas of Mosul governorate in northern Iraq (Mohammed, 2020).

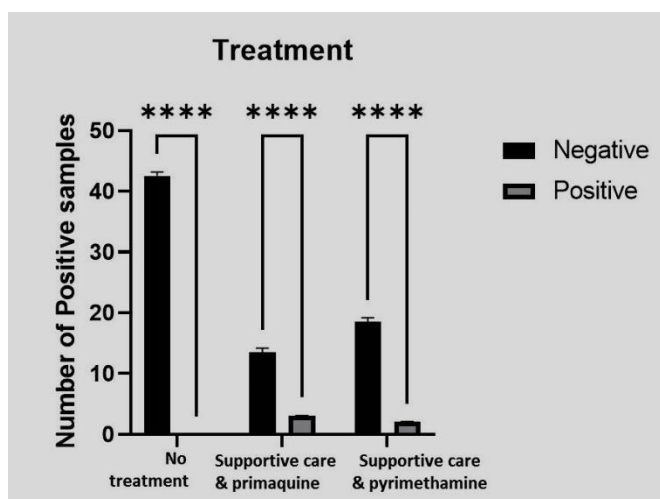
In the marbled teal from Al-Tharthar Lake, Salahuddin province, in the northern part of the middle region, Mohammad (2014b) observed triple infection with *Leucocytozoon simondi*, *Epomidiostomum uncinatum*, and *Diplopsthe*

laevis with total infection rates of 3% and *Leucocytozoon simondi* was the lowest among parasites. According to Mohammad (2015), ferruginous ducks were infected with *Leucocytozoon* spp. at the Dalmaj Lake, Al-Diwaniya province where the infection rate was 9.09%, and at a few neighborhood markets in Baghdad where the infection rate was 10%, whereas it was 5.56% in Ferruginous ducks at the center of Iraqi territory (Mohammad, 2016). AL-Zurfi and AL-Rubaie (2016) discovered that *Leucocytozoon simondi* in mallards was prevalent in local markets of Baghdad City at 16.66% and it was reported as 5.06% in the middle of Iraq by Mohammad (2016). Phasianid birds from various locations in the north, middle, and south of Iraq recorded free of *Leucocytozoon* infection (Mohammad et al., 2001).

Treatment and control

Our data revealed that the main therapies used to treat *Leucocytozoon* in chickens were Primaquine and Pyrimethamine. In addition, the supportive care plays a significant role in treatment measurements (Graph 4).

Primaquine was found to be effective against *Leucocytozoon* spp. gametocytes, however treatment with pyrimethamine mixed with sulfadimethoxine was reported to be partially successful in treating avian leucocytozoonosis (Zhao et al., 2016). According to Chiang et al. (2022); daily treatment of 0.5 g of *Artemisia annua* powder in chickens boosted body weight gain and decreased *Leucocytozoon caulleryi* parasite concentration, which in turn decreased mortality, pale comb, and the production of green feces. Based on present work, it's recommended to use both laboratory and field efforts for the control of *L. caulleryi* by immunization with an oil-adjuvanted rR7 vaccine (Recombinant R7 protein from second-generation *L. caulleryi* schizonts) have yielded encouraging results (Ito and Gotanda, 2004; Saeed et al., 2022).



Graph 4 - Number of infected samples according to treatment measurements.

CONCLUSION

In conclusion, a total of 81 blood samples were taken from chickens in different areas of Iraq, like Samawah, Rumaitha, Warkaa and Kidhre to investigate the presence of *Leucocytozoon*, and 6.1% infection rate among the chicken breeds was discovered. The study highlighted signs of infection such as decreased egg production, anemia and loss of appetite. Infections were more prevalent in regions like Rumaitha, Khidr and Samawah compared to Warka region, where no cases were found. Notably the observed prevalence of *Leucocytozoon* in chickens was lower than studies on birds in Iraq which reported an overall blood parasite prevalence of 15%. This difference could be attributed to factors like habitat variations, availability of vectors or varying susceptibility, among bird species. Conducting studies to map out the distribution of *Leucocytozoon* comprehensively in Iraq as well as recognizing that haemosporidian parasites which can vary significantly across different regions and bird species, is suggested.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Author contributions

The designing of the study and writing the manuscript were done by Iman Aabadi. Sura Alkhuzei and Zahraa Abbas rewrote the article and revised the whole manuscript. HK revised the final version of the article.

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Competing Interests

The authors state that there is no conflict of interest regarding the publication of this article.

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