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

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ABSTRACT: *Leucocytozoon* species are avian haemoparasites with economic impacts on poultry production. The present study investigates the presence of *Leucocytozoon* in chickens of Al Muthanna province, Iraq. Eighty one blood samples were collected from chickens in Samawah, Rumaitha, Warkaa, and Kidhre regions to examine the prevalence of *Leucocytozoon*. An infection rate of 6.1% was found among chicken breeds. The study highlighted that the main symptoms of infection were decreased egg production, anemia, and loss of appetite. Notably, infection was more 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 suggestion for future work can be the application of 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 phylum *Apicomplexa*, 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. They 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 high 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 hematologic parameters in chickens, might 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 controlling measures and regularly monitor these parasites to uphold poultry well-being and enhance production outcomes in backyard environments (Wamboi et al., 2020). Major avian haemosporidian genera include the potentially dangerous *Plasmodium spp.*, *Haemoproteus* spp., and *Leucoccytozoon* spp. (Bennett et al., 1993). Birds can acquire leucocytozoonosis from numerous species of the genus *Leucocytozoon* that spread via vectors. While *Leucocytozoon* is abundant, only a small subset of species is known to cause disease in birds (Forrester and Greiner, 2008). Waterfowls, pigeons, galliforms, raptors, and ostriches are all vulnerable to the phylum *Apicomplexa*, order *Haemospororina*, 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 the 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

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physiological parameters to show more information on microbiological infections in animals (Hameed et al., 2022; Al-Yasari et al., 2024).

The aim of the present study was to compare our results with the previous epidemiological studies, including those carried out in Iraq, about *Leucocytozoon* in birds.

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 chicken flocks in different regions of Al-Muthanna Province (Samawah, Rumaitha, Warkaa, and Kidhre), Iraq. Fresh samples were transferred in sterile containers to the Protozoology Laboratory at the College of Veterinary Medicine/Al-Muthanna University and aliquoted into 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 sample containers. Finally, the samples were evaluated by preparing thin and thick smears, stained with Giemsa, and examined under the light microscope.

Statistical analysis

After collecting the samples, the data, were recorded. These included clinical signs, sex, the main cities in Al-Muthanna Province, 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 ($P \leq 0.05$).

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

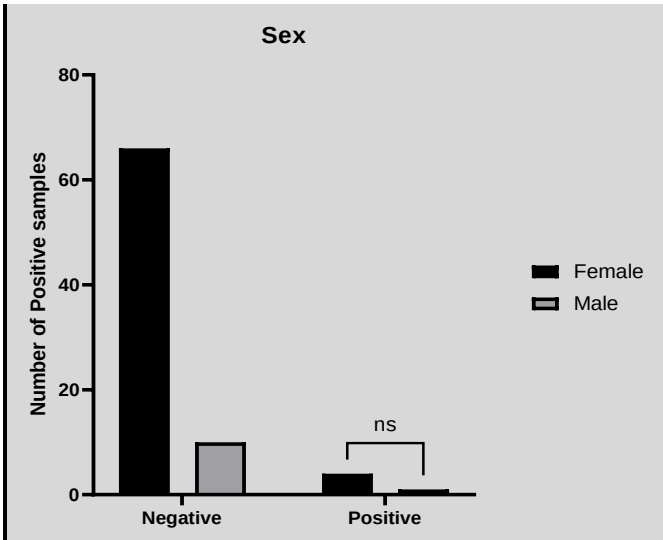
Epidemiology

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

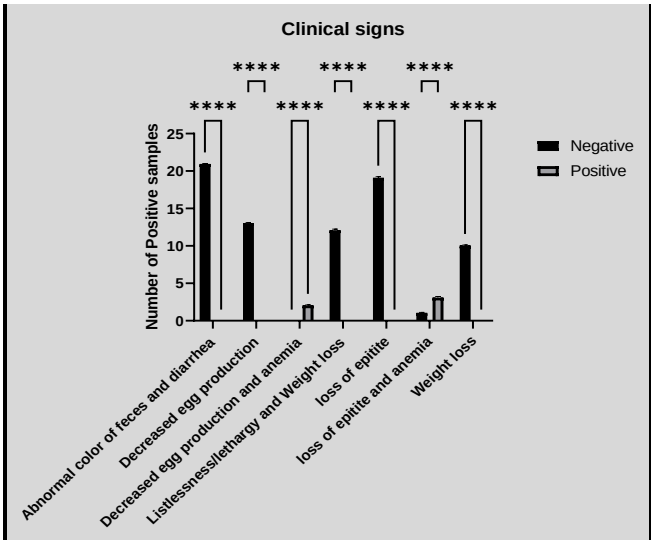
The life cycle of *Leucocytozoon* spp. involves two hosts. *Simuliid* black flies and *Culicoid* midges exhibit sporogony, after that sporozoites travel to the insect's salivary glands. Subsequently, the vertebrate host is infected, and internal organs such as the liver, brain, spleen, and lungs undergo schizogony. It is important to study intermediate hosts for both medicinal and veterinary purposes (Alkhuzaie 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 *Leucocytozoon* spp. infection rates of 10.52% in quail in Baghdad City. They found out that female quails had a greater infection rate than males.

Pathogenesis and clinical signs

From present recordings, decreased egg production, anemia, and loss of appetite were the only significant signs (Graph 2). Different clinical signs were recorded such as anorexia, weight loss, feed conversion drawbacks, anemia, green feces, and frequent mortality. These can result from parasitic 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 prevalent in flocks of ducks, especially those 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).



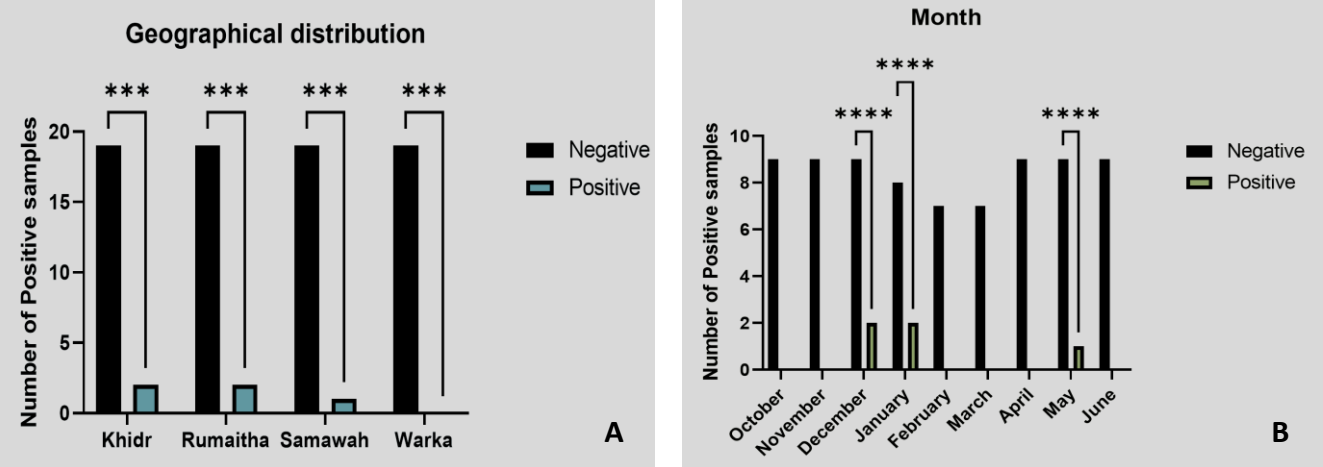
Graph 1 - Number of infected samples according to sex.



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

Geographical distribution

The collected data showed that the infection was centered mainly in Rumaitha, then Khidr and Samawah. It is 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 be related to the nature of environmental conditions in Al-Muthanna, which could increase the growth rates of the transmission vector (Graph 3 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. were the least common haemoparasite according to a study by Abdullah (2013), and their prevalence was low (13.5%), with no signs of sickness present among the chickens in the Qaradagh district of the Kurdistan region of Iraq, around 45 kilometers from Sulaimani Province. 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). However, Hasson (2015) did not find any Leucocytozoon spp. record in adult chickens in Diyala. While mixed infection with the triple hemiparasites (*Plasmodium* spp., *Heamoproteus* spp., and *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% (Ibrahim and Al-Rubaie, 2020). Additionally, mature chickens have a higher infection rate than young chickens, just as females compared to males. In Nineveh Villages, where geese were examined, Shamaun et al. (2007) found that the prevalence of *Leucytozoon simondi* was 33.33%. Al-Shuaibi (2008) reported a 10.7% infection rate of *Leucocytozoon* spp. in geese in different areas of Al Ramadi. This rate was reported as 5.37% and a mixed infection with *Plasmodium* spp. + *Leucocytozoon* spp., and 14.1% in geese at Sulaimani Province (Mohammed, 2014a). 14.2% was the rate reported 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 *Diploposthe*

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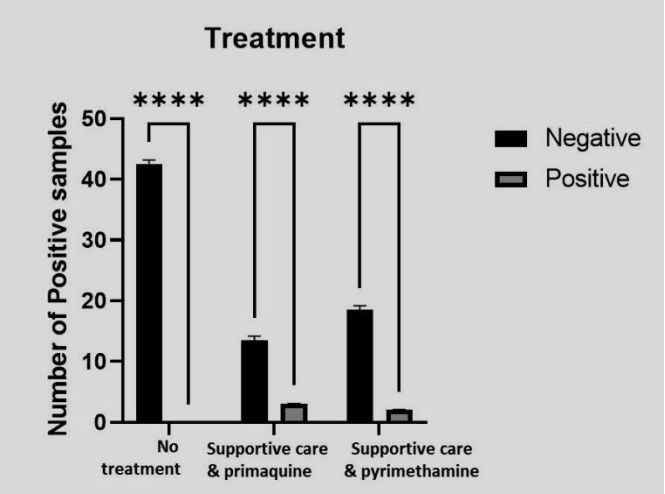
laevis with total infection rates of 3% and *Leucocytozoon simondi* was the rarest among parasites. According to Mohammad (2015), ferruginous ducks were infected with *Leucocytozoon* spp. at the Dalmaj Lake, Al-Diwaniya Province, where the infection rate was 9.09%. At a few neighborhood markets in Baghdad, 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%. 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 suggested 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). These measures 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*. 6.1% was the infection rate among the chicken breeds. 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 similar 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, etc. it is suggested to conduct studies mapping out the distribution of *Leucocytozoon* comprehensively in Iraq, as well as identifying haemosporidian parasites which can vary significantly across different regions and bird species.

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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