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Microscopical and phylogenetic identification of *Giardia lamblia* from fecal samples of chickens in Baghdad governorate, Iraq

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Abstract

Giardia lamblia is a zoonotic protozoan that causes diarrhea in chickens. This feature makes this microorganism a potential research material. The presented study was conducted to identify *Giardia lamblias* presence in fecal samples of chickens in Baghdad Province, Iraq. The investigation involved 60 fecal samples collected over different months of the year. The samples were examined using microscopic and PCR-based methods and partial gene sequencing that targeted the glutamate dehydrogenase (GDH) gene. The microscopic observations revealed the presence of the cysts at their highest rate of 4/5 (80%) during January. The PCR demonstrated that 13/60 (21.66%) of the chickens were infected. The phylogenetic tree reported that the current strains were closely related to global isolates from Poland and Australia. The results of the present study determined a significant distribution of *Giardia lamblia* in the fecal content of chickens, which reflects human infection.

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Introduction

Giardia lamblia is a microscopic parasite that belongs to the phylum Sarcomastigophora, class Zoomastigophorea. This single-celled organism is commonly found in freshwater environments but also infects humans and causes a gastrointestinal illness known as giardiasis. In this essay, we will explore the descriptive details of *Giardia lamblia*, including morphology, life cycle, pathogenesis, and clinical manifestations. Various studies have provided comprehensive information about the parasite (1-5). *Giardia lamblia* exists in two distinct forms: the trophozoite and the cyst. The trophozoite form is the active motile stage of the parasite, while the cyst form is modified for survival outside the host and transmission to new hosts. The trophozoite measures approximately 10-20 micrometers long and possesses a characteristic pear shape. It has two nuclei and a ventral adhesive disc that aids attachment to the host's intestine. The anterior end of the trophozoite features flagella, which enables its locomotion. In contrast, the cyst is oval-shaped, measuring around 8-12 micrometers. It contains four nuclei and a protective outer layer, allowing it to withstand harsh environmental conditions (6-11). The life cycle of *Giardia lamblia* involves two stages: the trophozoite and the cyst. The trophozoites reside in the hosts small intestine, where they attach to the intestinal wall using their adhesive disc. They reproduce asexually through binary fission, which leads to an exponential increase in the number of colonies. Some trophozoites transform into cysts, excreted in the host's feces, and can survive in the environment for extended periods. When a new host ingests contaminated water or food, the cysts pass through the stomach and reach the small intestine. Once in the small intestine, the cysts become active trophozoites, restarting the life cycle (12-18). *Giardia lamblia* causes pathogenesis by attaching to the epithelial cells of the small intestine, leading to malabsorption and tissue damage. The adhesive disc of the trophozoites disrupts the microvilli, reducing the absorptive surface area and impairing nutrient absorption. Additionally, the trophozoites release toxins that further contribute to

tissue damage and inflammation. The host's immune response plays a crucial role in the pathogenesis of giardiasis as both humoral and cellular immune mechanisms are activated to eliminate the parasite. However, *Giardia lamblia* has developed mechanisms to evade the host's immune system, allowing it to persist and cause chronic infections (19-25). Giardiasis is characterized by gastrointestinal symptoms such as diarrhea, abdominal pain, bloating, and flatulence. These symptoms arise due to the impaired absorption of nutrients and increased fluid secretion in the intestine. In some cases, giardiasis can lead to weight loss and malnutrition, especially in vulnerable populations such as children and immunocompromised individuals. The severity and duration of symptoms can vary, ranging from acute self-limiting infections to chronic infections that persist for several weeks or months (26-30).

Giardia lamblia is a zoonotic protozoan that causes diarrhea in chickens. This importance makes this microorganism a potential research material. The presented study was conducted to identify *Giardia lamblias* presence in fecal samples of chickens in Baghdad governorate, Iraq.

Materials and methods

Ethical approve

All the authors of the present work ensure that all procedures of our experiment were performed under the Ethical Norms approved by the scientific board of the College of Veterinary Medicine, University of Al-Qadisiyah (committee approval number 1314 on 18/10/2022).

Sample collection

The investigation involved the collection of 60 fecal samples distributed over different months of the year.

Microscopic examination

The smears were Lougal Iodine-stained according to a method described by Newman *et al.* (31).

Molecular methods

To perform PCR on Giardia DNA, the parasite's DNA must be extracted. The Geneaid (Korea) extraction kit was used with its protocol.

PCR Steps targeting GDH

GDH is an enzyme that converts glutamate to alpha-ketoglutarate, which plays a crucial role in energy metabolism. The PCR steps included the use of F: ATCTTCGAGAGGATGCTTGAG and R: AGTACGCGACGCTGGGATACT designed by Feng and Xiao (32).

PCR Amplification

Once the DNA has been extracted, the primers designed for PCR amplification can be performed. The PCR reaction mixture included the extracted DNA template forward and reverse primers deoxynucleotide triphosphates (DNTPs), DNA polymerase, and buffer solution. The reaction mixture was subjected to a series of temperature cycles, typically 95ºC - 3minute (95ºC -35 seconds, 54ºC - 35 seconds, and 72ºC -35 seconds), and 95ºC -3minute for initial denaturation, 39-cycle (denaturation, annealing, and extension), and final extension, respectively.

Analysis of amplified products

After PCR amplification, the products were analyzed to confirm the presence and specificity of the target sequence. Agarose gel (1.5%) electrophoresis was used for this purpose. The PCR products were loaded onto an agarose gel and subjected to 100 volts and 80 AM for one hour. The resulting bands were visualized using ethidium bromide. A UV-imager was used to visualize the products.

DNA sequencing

Ten PCR - positive products were sent for sequencing (Macrogen, South Korea). The phylogenetic tree was built using NCBI-websites and MEGA11.

Results

The microscopic findings revealed the presence of cysts at the highest rate, 4/5 (80%), during January (Figure 1 and Table 1). The PCR demonstrated that 13⁄60 (21.66%) of the chickens were infected (Figure 2). The phylogenetic tree reported that the current strains were closely related to global isolates from Poland and Australia (Figure 3).

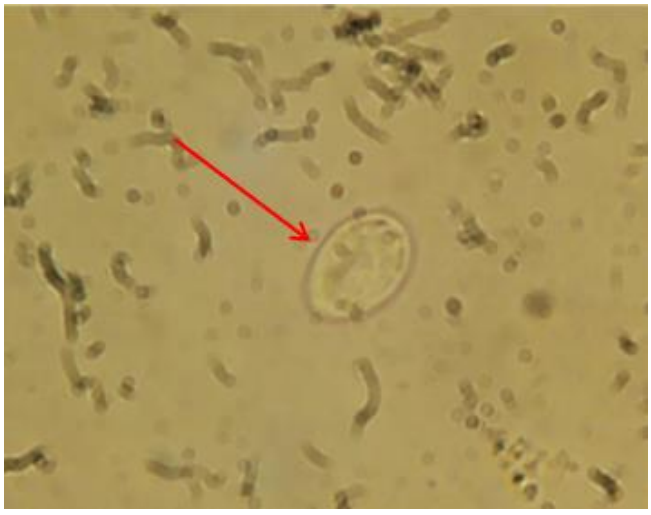

Figure 1: *Giardia lamblia* cysts from chicken fecal samples. (x100) were obtained by employing a Zinc Sulfate-based floatation method.

Table 1: Incidence of Giardia spp. in chickens

Month	Samples (n)	Infected (n)	%
October	6	0	0
November	5	0	0
December	12	0	0
January	5	4	80 [a]
February	0	0	0
March	17	6	35.3 [b]
April	15	3	20 [b]
Count	60	13	21.66%

Similar letters = $P>0.05$, Different letters = $P<0.05$.

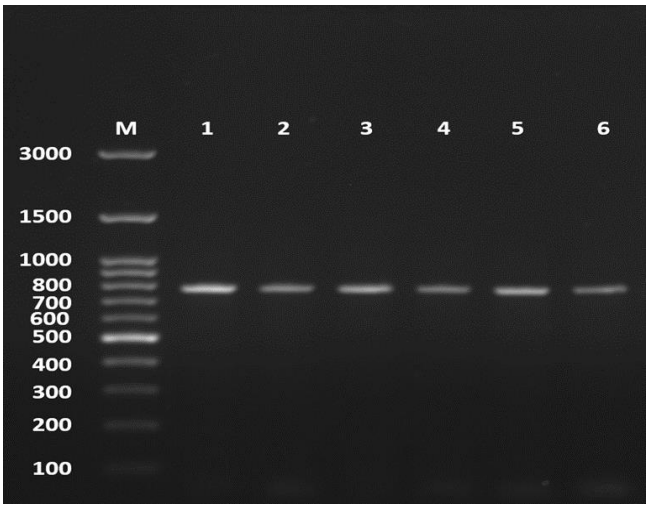

Figure 2: Image of agarose gel electrophoresis (1.5 % agarose). 1-6: Giardia sp. Positive PCR *GDH* gene (size= 778 bp) in chicken fecal samples. M: Ladder (100- 3000bp).

Discussion

Giardia lamblia is a flagellated protozoan parasite that causes gastrointestinal infection, and giardiasis, in humans. Microscopic examination plays a crucial role in diagnosing and characterizing this parasite. The microscopic examination of *G. lamblia* involves identifying and characterizing unique morphological features. The most common method for visualizing this parasite is the direct microscopic examination of stool samples using wet mounts or staining techniques. Microscopically observing the trophozoite and cyst forms of *G. lamblia*, various vital findings can be identified (33-36). According to Behr *et al*. (37), the microscopic findings of Giardia include the presence of pear-shaped trophozoites measuring approximately 10-20 micrometers in length, two visible nuclei, and four pairs of flagella emerging from the anterior end. Furthermore, the authors reported that the trophozoites exhibit a characteristic falling leaf motility pattern due to the movement of their flagella.

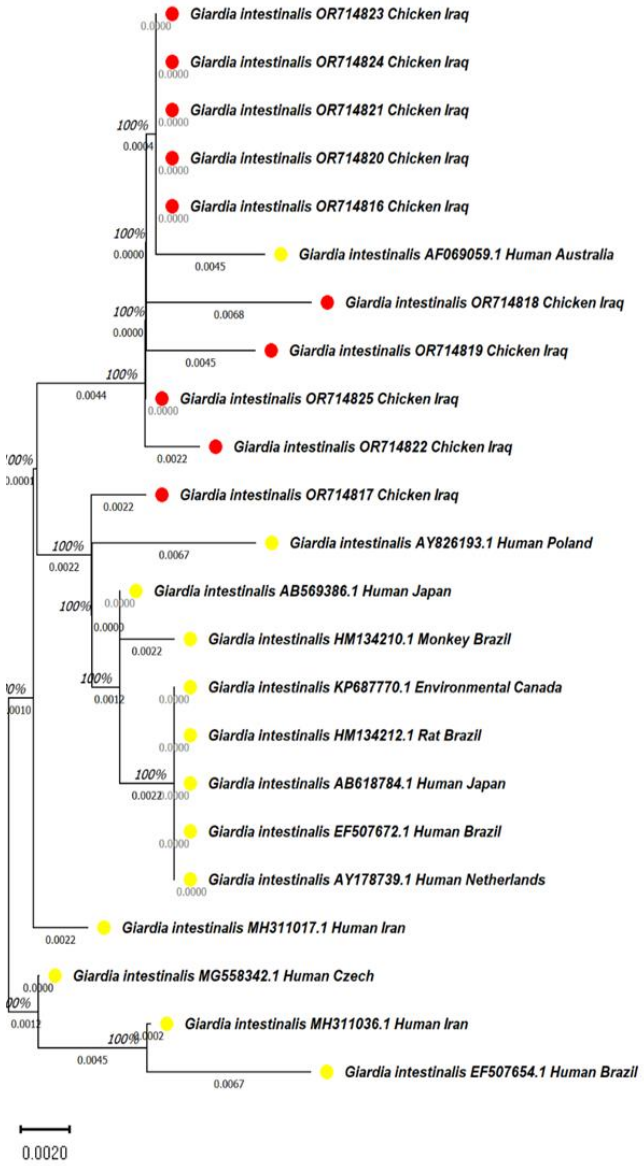

Figure 3: Phylogenetic tree of *Giardia intestinalis* in Chicken (red dots: Current isolates, yellow dots: global isolates).

Al-Zurgani and Al-Khanaq (38) explored the microscopic features of *G. lamblia* in Wasit province, Iraq. They reported similar findings with pear-shaped trophozoites, two visible nuclei, and the characteristic flagella arrangement. However, they also noted that the size of the trophozoites may vary slightly, ranging from 8 to 15 micrometers. This discrepancy in size could be attributed to the regional variations or different physiological factors, strains of the parasite, and staining techniques used.

Kaya *et al*. (39) investigated the microscopic findings of *G. lamblia* in immunocompromised individuals. They

observed that the trophozoites tended to be larger, measuring around 15-25 micrometers. Additionally, they highlighted the importance of conducting multiple stool examinations at different times to increase diagnostic accuracy, as the shedding of *G. lamblia* cysts may not occur consistently. Furthermore, a comprehensive review by Lujan (40) analyzed multiple studies on G. lamblia microscopy findings. He emphasized distinguishing between trophozoites and cysts, as the latter form is crucial for transmission and infectivity. The author mentioned that cysts are typically round or oval-shaped, measuring around 8-15 micrometers, and contain four nuclei. He also highlighted that staining techniques such as iodine or modified acid-fast stains can enhance the visibility of both trophozoites and cysts.

PCR targeting the GDH gene has proven valuable for detecting Giardia infections in chickens. Several studies have reported successful amplification and detection of Giardia DNA in chicken fecal samples using this technique. Villalba-Vizcaíno *et al*. (41) investigated the prevalence of Giardia in chickens raised for meat production in the Colombian Caribbean Coast. The researchers utilized GDH gene PCR and found that 48.1% of the tested chickens were positive for Giardia infection. This study highlighted the applicability of GDH gene PCR in diagnosing Giardia in chickens and emphasized the importance of monitoring this parasite in poultry production (42-45). Similarly, Cao *et al*. (46) in China determined the prevalence of Giardia in commercial broiler chickens using GDH gene PCR. The researchers reported a relatively low prevalence rate of 8.25%, indicating a lower incidence of Giardia infection in Chinese broiler chickens than in previous studies. These findings highlighted the importance of geographical variations and management practices in influencing the prevalence of Giardia in chicken populations.

Conclusions

The current study provides significant information on the occurrence of *Giardia lamblia* in chickens in the Baghdad governorate.

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Conflict of interests

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التعرف المجهري وتحديد النشوء والتطور لطفيلي الجياردیا المعوية في عينات براز الدجاج في محافظة بغداد، العراق

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الخلاصة

الـجياردیا المعوية هو حيوان أولي يسبب الإسهال في الدجاج ويسبب الإصابات الخمجية في الأنسان. هذه الصفة تجعل من هذه الكائنات الحية الدقيقة مادة مهمة للبحث. أجريت الدراسة للتعرف على وجود طفيلي الجياردیا لامبليا في عينات براز الدجاج في محافظة بغداد، العراق. وشمل التحري ٦٠ عينة براز موزعة على أشهر مختلفة من السنة. تم فحص هذه العينات باستخدام الطرق المجهرية والقائمة على تفاعل البلمرة المتسلسل ودراسة تعاقب تسلسل القواعد النتروجينية الجيني الجزئي الذي استهدف جين الـكلوتامين ديهايدروجينيز. أظهرت نتائج الطريقة المجهرية وجود الأكياس بأعلى نسبة ٥/٤ (٨٠٪). خلال شهر يناير. وأظهر فحص البلمرة المتسلسل إصابة ٦٢/٨٣ (٢١,٦٦٪) من الدجاج بالعدوى. ذكرت شجرة النشوء والتطور أن السلالات الحالية كانت مرتبطة ارتباطا وثيقا بالعزلات العالمية من بولندا وأستراليا. حددت نتائج الدراسة الحالية توزيعا نوعيا لطفيلي الجياردیا لامبليا في محتوى براز الدجاج، مما ينعكس على إصابة الإنسان.