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Microscopic, serologic and molecular diagnosis of *Giardia lamblia* in cattle of Al-Suwaira district, Wasit province (Iraq)

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ABSTRACT

This study was conducted from January to December 2023 for 200 samples from different places in Al-Suwayra District in Wasit Governorate, where the microscopical, ELISA test, and PCR and analysis of restriction segment length variation (PCR). The results of the current study used microscopically indicated that 75 cattle were infected with the *G. lamblia* parasite at a rate of 37.5%. The results showed that in the areas where cattle are located, the highest rate of infection with the *Giardia lamblia* parasite was recorded in the city (19.5%) and in the Rural (22.5%). concerning the rate of infection with the *G. Lamblia* parasite, based on the results of the ELISA test, was 20%. The test showed a sensitivity and specificity estimated at 22.6%, and 93.3%. The results of the PCR reaction for the three genes showed total infection rates with the *Giardia lamblia* parasite amounting to 10.5%. While recorded the infection rate with the *Giardia lamblia* parasite was based on the results of genes amounting to 4% for the *ssu rRNA* gene. This study concluded the high prevalence of *G. lamblia* parasite among the study region suggesting the role of increasing of furthermore studies to investigate the prevalence of parasite in other Iraqi areas.

1. Introduction

Giardia lamblia parasite is one of the most common intestinal parasites that infect both animals and humans causing diarrhea (Vandenberg *et al.,* 2006; Shati and Abdolgafor, 2022). Scientific statistics indicate that the infection rate with the *G. lamblia* parasite in cattle reached 60-80% in developing countries (Chang and Zhang, 2023). Previously, the traditional microscopic test was used to diagnose intestinal parasites and is considered a primary diagnosis (Al-Kaeebi and Ressan, 2019). While the specificity and sensitivity of the infection cannot be measured by microscopic examination, which is cumbersome and inaccurate, impossible to distinguish between types. Later, other, more advanced methods were used in terms of sensitivity to detect parasites, the most common of which are ELISA and PCR (Fink et al., 2020). The ELISA test depends on detecting the parasite antigen in the feces, although unable to distinguish whether the infection was recent or old (Lushbaugh and Pittman, 1979; Al-Kaeebi and Ressan, 2019), while PCR provides fast and accurate results and can detect pathogenic factors and genetic profiling, distinguishes between the main types of microorganisms, which allows conducting appropriate epidemiological studies to help evaluate the precise interactions between species and within a single species. This will also provide clear information regarding the distribution and variation of major species between animals and the environment (Acquah, 2010). There are more than 51 species of Giardia. Still, only six species identified can be distinguished using a microscope based on their external appearance, and they can also be distinguished by infecting the host (Hugo and Staffan, 2011). The study of Molecular epidemiologists in endemic areas has provided evidence supporting the role of dogs in the transmission cycles of giardiasis between humans, dogs, and domestic animals in local communities in some regions of the world, Assam in India, the inhabitants of the temples in Bangkok, and the Indigenous people in Thailand and northern Canada (Traub et al., 2004; Salb et al., 2008; Saleem et al., 2022). The technique analysis DNA has been applied to the *Giardia* parasite for two main purposes: detecting genetic differences between types of the parasite and distinguishing live and dead cysts of the parasite in terms of their effect on infection (Wielinga and Thompson, 2017). The Traditional conventional PCR reaction with direct testing or variation sequence in section lengths is the most widely used tool among different populations using specific primers for those parasitic populations (Geurden et al., 2008; Levecke et al., 2009) or use real-time PCR with special probes (Almeida et al., 2010; Madjeed et al., 2022).

2. Methodology

Ethical Approval
This study licensed by the Scientific Committee of the Kut University College (Wasit, Iraq).

Samples
Collecting 200 samples of feces cattle from various areas of Al-Suwaira District in Wasit Governorate from January to December 2023. The samples were collected in sterile plastic containers caps numbered.

Microscopic testing
Direct Wet mount method, they were examined with a microscope under 40X and 100X under magnification power. They examined the fecal samples by preparing a direct swab to search for the feeding or molting stages of the parasites. placed a small drop of topical 1% iodine on the glass slide and mixed the feces with a small portion of the feces using small wooden chopsticks. The slide cover was placed well, and the samples were examined using a light microscope using the direct wet swab method to search for the feeding and encysted stages of the parasites as previously utilized (Alkefari et al., 2017; Gharban et al., 2022; 2023a).

ELISA
The parts of the samples were taken for examination with an ELISA test to detect ELISA the kit for this test, manufactured by Germany / DRG International with 98% sensitivity and 97% specificity for the detection of *G. lamblia* parasite-specific antibodies, the test was performed according to the manufacturer's instructions, using recombinant antigen at a dilution of 1:101, the combination of antigens with antibodies found in wells during the first incubation period, during the second incubation period, the anti-second antibody linked to the peroxidase enzyme is attached to the formed complex, where the anti-second antibody confines the antigen in sandwiches. Lastly, in the next incubation period, the chromogen dye-generating substance (substrate) is added, which shows a blue color in the presence of the binding complex. As for the stop solution, ends the reaction and converts blue to yellow color. while the repeated washing operations perform the task of removing unbound materials, lastly the rest was concentrated and then stored without adding any preservatives at a temperature of 20- until DNA was extracted from them (Al-Gharban and Dhahir, 2015; Al-Hassani et al., 2018; Al-Graibawi et al., 2021).

Molecular Examination
The detection parasite DNA is detected using PCR from stool samples of the study population, DNA was extracted according to the Fast CTAB DNA isolation method used by the researcher, modified from the method based on amplification of the diagnostic parasite gene according to the method described (Gharban et al., 2023b).
Preparation of PCR reaction for *Giardia lamblia* parasite primers for the according to the method El-Badry *et al*. 2010 This method is called Single Round-PCR to detect the presence of the gene specific to the parasite, using primers designed for this purpose, Giardia-80F, and Giardia-127R, which produce a band with a size of 292 bp. The work method was conducted using PCR Microtubes with a diameter of 0.2 m. The reaction components were mixed well using a microcentrifuge for 4 seconds to deposit the reaction solution's droplets on the tube's wall. Then the tubes were inserted into the Thermal Cycler with caution and care to complete the reaction using the following program start steps (Initial denaturation, Denaturation, Annealing, Extension and Final extension) with temperature and time (95°C/15 min, 95°C/15 sec, 60°C/30 sec, 72°C/30 sec and 72°C/7 min) respectively. After the end of the reaction time, the samples were removed from the device and electrophoresed for 75 minutes with 5 microliters of DNA samples duplicated in an agarose gel at a concentration of 1.5 (Gharban, 2022).

Statistical analysis
Results were conducted using the Chi-Square test at a significance level of 0.05 and 0.01 (Gharban et al., 2023b).

3. Results

The results of the detection of *Giardia lamblia* infection by microscopic examination in the current study showed a rate of 37.5% from January to December 2023 for 200 samples from different places in Al-Suwayra District in Wasit Governorate (Figure 1).

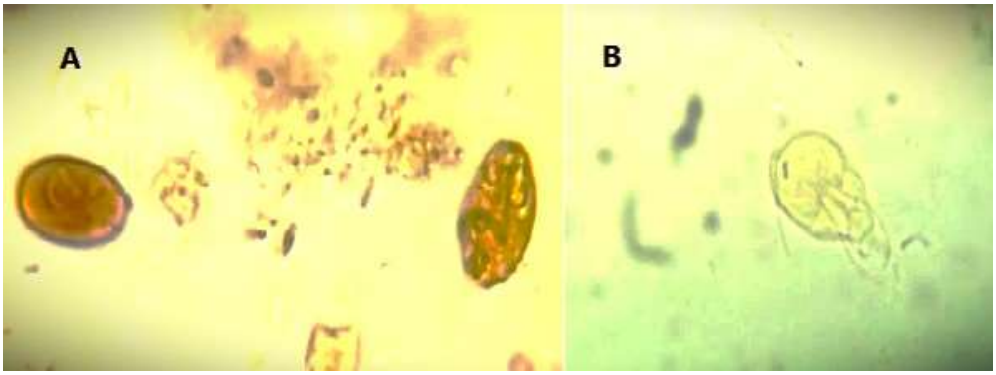

Figure 1. Giardia lamblia was examined under a microscope and at 100X magnification with stained Lugol's iodine, and appeared A: (cyst), B: (Trophozoite).

Regarding sex, there are significant differences in infection rates between females and males as shown in Table 1

Table 1. Rates of infection with the *Giardia lamblia* parasite according to sex.

Sex	No. of sample infection	%	No. of sample not infection	%
Female	63	63.6	38	38.3
Male	50	49.5	49	48.5
Total	113	-	87	-

As for residential areas, infection rates with the *Giardia lamblia* parasite in the countryside were recorded at 64.4% from 62 samples. The analysis showed that the rate of infection recorded in the city is higher than that of 48% of 50 samples. Statistically, there are highly significant differences in infection rates between rural and urban residents (Table 2).

Table 2. Rates of infection with the *Giardia lamblia* parasite according to area.

Area	No. of sample infection	%	No. of sample not infection	%
Rural	62	64.4	42	43.6
City	50	48	46	44.1
Total	92	-	108	-

Regarding ELISA test results the infection rate with the *G. lamblia* parasite about the ELISA test in the current study was 20% from of a total of 90 samples (Table 3). The test showed a degree of sensitivity compared to the microscopic test, estimated at 22.6%, while the test showed a high degree of specificity of 93.3%. as shown in Table 4.

Table 3. Rates of infection with *Giardia lamblia* parasites, according ELISA test.

ELISA	No. of sample infection	%
Positive	18	20
Negative	72	80
Total	90	100

Table 4. Comparison of the sensitivity and specificity of the ELISA and microscopy test.

Microscopy	Total	ELISA test		Specificity	Sensitivity
		Positive	Negative		
Negative	15	1	14	-	14/15(93.3 %)
Positive	75	17	58	17/75 (22.6%)	-

The Results of replication of the ssu rRNA gene in the current study showed that the rate of infection with the parasite reached 4%, as a show in Table 5.

Table 5. Infection Rates with the *G. lamblia* parasite according to PCR test

Result	Total of sample	%
Negative	8	4
Positive	192	96
Total	200	100

Based on the results of the PCR test for the ssu rRNA gene, the total number of samples showed the required band with a size of 760 bp (Figure 2).

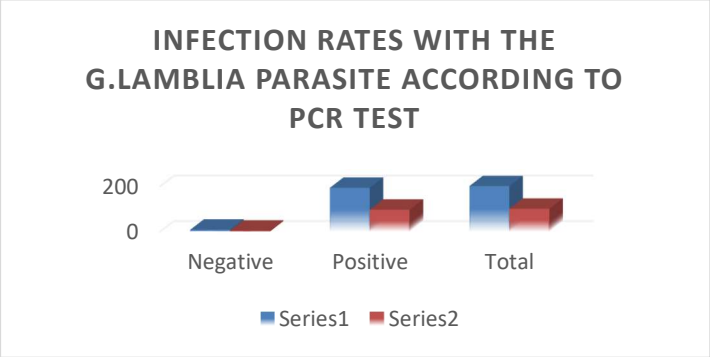

Figure 2. infection Rates with the *Giardia Lamblia* parasite according to PCR test.

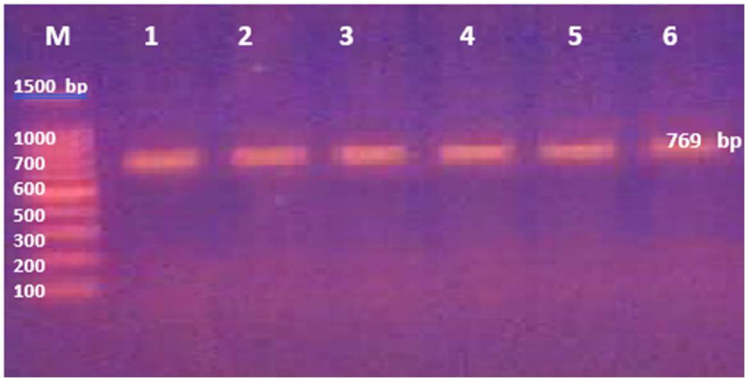

Figure 3. Electrophoresis of PCR using agarose gel (1.5%) for ssu rRNA gene M represents the size index of the PCR product (769 pb) DNA ladder.

In the current study, 97.3% (73 samples) were negative by PCR test and were positive by microscopic examination and 4.8% (6 samples) were positive by PCR test and were negative by microscopic examination.

Table 6. Comparison of the rate of infection with the G. lamblia parasite by PCR test and microscopic test

Microscopy	PCR		
	Positive	Negative	Total
Positive	2	73	75
Negative	6	119	125
Total	8	192	200

Table 6. Host-Parasite Interaction Parameters for *T. vaginalis* Strains T1 and T2

Interaction Parameters	*T. vaginalis* T1	*T. vaginalis* T2
Adherence (%)	78 ± 5.2	85 ± 4.8
Invasion (%)	65 ± 6.0	72 ± 5.5
Induced cellular changes (%)	58 ± 6.8	67 ± 6.4

3. Discussion

The results of the current research agree with the recorded results of *Giardia lamblia* parasitic infection in a study conducted in Dohuk Governorate in northern Iraq, where the percentage reached 38.5% (AL-Khayat and Ahmed, 2015), and disagree with

Al-Sharqat (20%), and Ryan et al. (2009) reported in Tikrit, 12.42%, as well as Al Mosawy and Al-Kinany (2020) in AL-Kut (4%).

The difference in the infection rate recorded in the current study compared to the studies mentioned above may be due to the difference in the level of health and hygiene services, population density, geographical location, climatic conditions, the total number of samples examined, examination methods and examination techniques during the study period, and also the failure to include some unsatisfactory beginnings. Such as colon amoeba in calculating proportions, all of these are factors that explain the reasons for the discrepancy in the findings of various studies, while the infection rates recorded are close to the current study, perhaps the reason is due to the similarity in the environment, conditions, and levels of coexistence in these areas (Al-Bayati et al., 2021).

This result agreed with a number of researchers; Saleem et al. (2021) in Baghdad, Al Mosawy and Al-Kinany (2020) in Karbala, and Stark et al. (2007) in Baghdad; while, these results did not agree with Al-Saad and Al-Emarah (2014) in Kufa, Hussein (2010) in Tikrit as well as Al-Mohammed (2011) in Al-Douz. The compatibility of the results of this study with other studies may be explained based on the existence of the same hypothesis that both sexes become infected with intestinal parasites through food and water contamination. However, the lack of agreement may be due to an unknown cause yet, and it may be related to genetic or physiological factors linked to sex as indicated by Turki and Kremsh (2015).

These results agreed Al Mosawy and Al-Kinany (2020) in Karbala, and Stark et al. (2007) in Baghdad, while these results did not agree with, Hussein (2010) in Tikrit, and Al-Mohammed (2011) in Al-Douz. This similarity in the results between the current study and other studies may be due to the similarity of the nature of life in the countryside, which tends to a low level of health and culture of the population and the use of animal and sometimes human waste as organic fertilizer. The high infection rates in cities that were recorded in this study may be explained as a result of many unnatural conditions. The country is currently experiencing, and its negative impact and bad environmental conditions have worsened, which has led to the migration of residents from grazing areas. The usual agriculture is to live in cities that mainly suffer from a severe lack of sanitation services, broken networks, and the accumulation of waste in the streets, as these factors are a suitable environment for insects that transmit many of these intestinal parasites.

Their results agree with the rates of infection with the *G. lamblia* parasite in Tikrit by AL-Khayat et al. (2014) (12.42%), while they do not agree with the result by Míriam et al. (1999) in Brazil, where he recorded an infection rate of (36.6%) and as well Al-saeed and Issa (2010) in Dohuk (65.5%).

As for the sensitivity of the tests, the results recorded for ELISA Kits For *G. lamblia* antigen did not agree with those recorded by researcher Míriam et al. (1999) 100% and 95% for soft samples preserved in 10% formalin, respectively, as appeared by Al-Saeed and Issa (2010) in Dohuk (76.4%). When comparing the specificity of the test recorded in this study with other studies, the recorded percentages agreed with what was recorded by Hassan et al. (1995) in Egypt (91%) and Míriam et al. (1999) (90%) and (98.3%) for soft samples preserved in 10% formalin, respectively, and Al-Saeed and Issa (2010) in Dohuk (100%), and they did not agree with the results are consistent with what was recorded by Ozekinci et al. (2005) in Turkey (80.8%).

The current study showed that the extraction method using Proteinase K and CTAB was the best method used in this study to extract DNA. The extraction results using Proteinase K enzyme and chemicals agreed with what was reported by Hooshyar et al. (2012), Khairnar and Parija (2007), Pietsch (2010) and Shati et al. (2022).

El-Badry et al. (2010) reported in KSA that the percentage of infection with the parasite using a PCR test reached 11%, a rate similar to what was recorded in the current study, while the percentage recorded disagreed with Nantavisai (2016) in Thailand (51.4%), Haque et al. (2017) (38.8%), Samuel (2020) in Ghana (33.97%) and Tungtrongchitr et al. (2020) in Thailand 23%.

These results have been recorded in many other studies. Haque et al. (2017) showed that there were two samples (2.7%) were positive by the PCR test despite being negative before the test, and that 6 samples (11.1%) became negative by the PCR test and had been positive before as El Badry et al. (2010). There were 8 samples (7.6%) positive in the PCR tests that were negative upon microscopic examination out of 105. A negative sample and this percentage are close to what was recorded in the current study. Samuel (2020) recorded a rate of 22.8% (233 samples) that were negative in microscopic examination became positive in the PCR test, and 7.22% (14 samples) were recorded as positive by microscopic examination and no PCR in the presence of the infection.

Several reasons emerge to explain the similarities and differences in the results between the current study and previous studies, including those that gave similar results in the conventional PCR test, which explains its use in our current study, but the contradictory studies used the real-time test, which recorded Sensitivity and specificity in diagnosis were estimated at 100%, and considered more capable of controlling and identifying inhibitory substances in stool (Kadhim et al., 2020; Hassan et al., 2023). The presence of inhibitory substances in some fecal samples that may bind to DNA polymerases, inhibiting its work and preventing the DNA amplification process from occurring is another reason for giving different results between studies (Kadhim

et al., 2020). Also, possible that the presence of small numbers of parasites or the presence of only the vegetative phase, which is destroyed over time, is one of the reasons that leads to failure of the DNA replication process, many studies have confirmed that the presence of cysts in large numbers compared to the vegetative stages increases to some extent the chances of a successful PCR test (Saleem and Al-Samarai, 2018; Alhachami et al., 2023).

4. Conclusion

This study concluded the high prevalence of *G. lamblia* parasite among the study region suggesting the role of increasing of furthermore studies to investigate the prevalence of parasite in other Iraqi areas.

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