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Comparative detection of *Giardia lamblia* by immunochromatographic assay from stool samples in Erbil Province

Hataw Fryad Saber
Medical Laboratory Science, Lebanese French University, Erbil, Kurdistan Region, Iraq.
Email: hataw.fryad@lfu.edu.krd

Heshu J. Ahmed
Biology Education Department, Tishk International University, Erbil, Kurdistan Region, Iraq.
Email: Heshu.jalal@tiu.edu.iq

Hawri M. Bakr
Department of physiology and microbiology, College of Medicine, Hawler Medical University, Erbil, Iraq.
Email: hawri.mustafa@hmu.edu.iq

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ABSTRACT

Background: A flagellated protozoan parasite *Giardia lamblia* is one of the most significant causes of parasitic gastrointestinal illnesses. The main method of transmission is through the fecal-oral route, and the life cycle is straightforward and direct, including the motile trophozoite and nonmotile cyst stages. The aim of the study was to compare microscopy and immunochromatographic tests for diagnosing *Giardia lamblia*. Methods: This cross-sectional study was done by selecting 100 microscopically negative samples from groups of people In Erbil city, which were collected and stored at -20 °C for further testing. The result of the study conducted showed that, from 100 frozen samples, 3% were positive by immunochromatographic rapid testing. Males were more likely to participate in this study because most of the participants were food handlers. There was no statistically significant association between the gastrointestinal symptoms of positive patients; 1 (2.8%) has symptoms, and 2 (3.1%) are asymptomatic. It is concluded that Immunochromatographic assays are easy

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to perform, rapid, sensitive, and specific. It showed that the test may be useful in the diagnosis of infection in large cases or in studying the epidemiology of *Giardia lamblia.*

1. Introduction

Giardia Lamblia (also known as *Giardia duodenalis*) is commonly reported throughout the world as the most important non-viral cause of human diarrhea. Annually, it affects an estimated 280 million people worldwide; however, incidence of the disease is highest in developing countries (Fink, Shapiro, & Singer, 2020). A protozoan intestinal parasite *Giardia lamblia* can infect any mammalian host, including people, livestock, wild animals, and pets. The prevalence of *Giardia* infection varies from approximately 2–5% in the industrialized world, with children typically being more frequently infected than adults (Belkessa et al., 2021). Owing to the elevated burden of *Giardia lamblia* – related illnesses in developing countries, its impact on developmental and socioeconomic improvements, and its close connection with poverty, this parasite has been included in the WHO's Neglected Diseases Initiative since 2004 (Al-Mekhlafi, 2017).

Rapid diagnostic testing is still popular, there may be waterborne epidemics, there aren't enough skilled technicians; and there's growing proof that *Giardia lamblia* and *Cryptosporidium parvum* can cause serious symptoms in people. Therefore, laboratories are looking into the test ordering options for immunoassay kits that they can use in their standard diagnostic procedures (Garcia et al., 2003). Transmission of *Giardia lamblia* protozoa occurs through direct or indirect ingestion of infectious cysts via the fecal-oral route (Krumrie et al., 2022). The period between the ingestion of infectious cysts and the appearance of the symptoms differs from 9 to 15 days. Clinical signs of *Giardia lamblia* infections differ between individuals, ranging from acute to chronic infections. While some hosts are asymptomatic carriers, individuals suffering from acute giardiasis may have abdominal pain, foul-smelling sensing explosive, watery diarrhoea, steatorrhoea, nausea, and vomiting. In chronic infections with *Giardia lamblia,* patients may present with diarrhoea, abdominal pain, malabsorption, and weight loss (Feng & Xiao, 2011; Ryan & Cacciò, 2013).

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Adaptive immunity plays a role, especially Immunoglobulin M (IgM), Immunoglobulin G (IgG), and Immunoglobulin A (IgA)-specific antibodies, which seem to have a major effect on the clearance of the parasite, but T-cell subsets, neutrophils, macrophages, and complement also contribute (Faubert, 2000). In the last few years, research utilizing gene-targeted mice has established the importance of Giardia-specific IgA in the clearance of infections. Numerous signs suggest that IgA antibodies contribute to protective immunity against giardiasis (Solaymani-Mohammadi & Singer, 2010(Singer, Fink, & Angelova, 2019)).

Frequently, diagnosis of *Giardia lamblia* infection is performed through the microscopic identification of trophozoites and cysts in fecal samples (Cama & Mathison, 2015; Guimarães & Sogayar, 2002). In the microscopic examination, at least three fecal samples have been recommended to be taken and examined over 3 days to achieve 94% accuracy in diagnosing positive *Giardia lamblia* cases because examination of a single stool sample has a low sensitivity (46% sensitivity) due to irregular shedding of the cysts (Hanson & Cartwright, 2001; Weitzel et al., 2006).

In addition to the microscopic identification, a variety of different diagnostic tests have been available: immunoassays such as enzyme-linked immunosorbent assay (ELISA) that measure antibodies or antigens (Alharbi et al., 2020); rapid tests (immunochromatographic tests) that are easy and sensitive to perform (Heyworth & Hygiene, 2014); and the detection of *Giardia*-specific genes by conventional (polymerase chain reaction) (PCR) and other types of PCR (e.g., nested PCR). PCR is a more significant technique not only for epidemiological studies but also as a diagnostic tool for low-density *Giardia* infections (Jahan, Khatoon, Ahmad, & JCDR, 2014; Platts-Mills, Operario, & Houpt, 2012).

Immunological analysis based on the detection of anti-*Giardia* antibodies and *Giardia* antigens in stool samples. Recently, direct fluorescent antibody (DFA) tests and enzyme immunoassays (EIAs) have become the most commonly used antigen detection immunoassays for *Giardia lamblia*. Both enzyme immunoassays (EIAs) and direct fluorescent antibody (DFA) tests may identify soluble antigen in stool samples. EIA has two advantages over DFA: first, several samples can be screened at one time, and secondly, the tests can be read accurately on a spectrophotometer instead of individually on a fluorescence

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microscope (Duque-Beltrán et al., 2002; Jahan et al., 2014)(Hooshyar, Rostamkhani, Arbabi, Delavari, & Bench, 2019). The aim of the study was to compare the prevalence of *Giardia lamblia* protozoan parasite by using a microscope and immunochromatographic tests.(Alharbi et al., 2020).

2. Method

2.1. Study design and Setting

This is a cross-sectional study. The study was carried out in laboratories and different schools at different locations in Erbil city.

2.2. Sample collection and population

After macroscopic and microscopic examination of 576 samples, 100 negative samples for *Giardia lamblia* were randomly selected and stored without adding any preservatives or chemicals at -20 °C for immunochromatographic assay (Ghieth, Kotb, Abu-Sarea, & El-Badry, 2016).

2.3. Macroscopic examination (physical analysis) and Wet mount examination procedure

For macroscopic examination was performed as previously described A small amount of stool was placed on the slide and mixed with a drop of normal saline; after that, the slide was covered with the coverslip. (Organization, 2019)
Examined for the presence of a trophozoite or cyst of *Giardia lamblia under* 10X and 40X objectives.

2.4. *Giardia lamblia* Immunochromatographic rapid test

Giardia lamblia rapid test is an immunochromatographic lateral-flow test for qualitative detection of Giardia lamblia antigen in stool samples. In the present study RIDAQUICK Giardia (cassettes) kit (R-Biopharm) was used.

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2.5. Procedure of assay

Followed the directions of the manufacturer as follows**:**

Collection of samples and storage.

According RIDAQUICK Giardia (cassettes) kit that used in this study, it can be used for both fresh and frozen stool samples, the stool sample collected in sterile cup without adding any type of preservatives and for frozen samples must store at –20 °C.

Preparing the solution

1 ml (1000 µl) of extraction Buffer (Diluent) placed in test tubes, with solid stool samples, 50 mg of stool sample weighed and suspended in the buffer, in the case of liquid samples, 100µl of stool sample pipetted. After that the sample well homogenized. This can be completed either by repeated suction and ejection of the suspension using the disposable pipette or, by mixing on a vortex mixer. Later, permit the homogeneous suspension to settle for 3 minutes until a clear supernatant is formed.

Running the test

Disposable pipette used for pipet 4 drops of clear supernatant of the stool suspension into the round opening of the test cassette, 5 minutes after could read the result

Result interpretation

In all cases the C (control) line appears blue, if the sample positive for *Giardia lamblia* the Control and Test band appears, C (blue) and T (red) would be visible. In cases of the blue band, not appears, the test becomes invalid and if the color of the band changed after 10 minutes the test not be evaluated figure 1.

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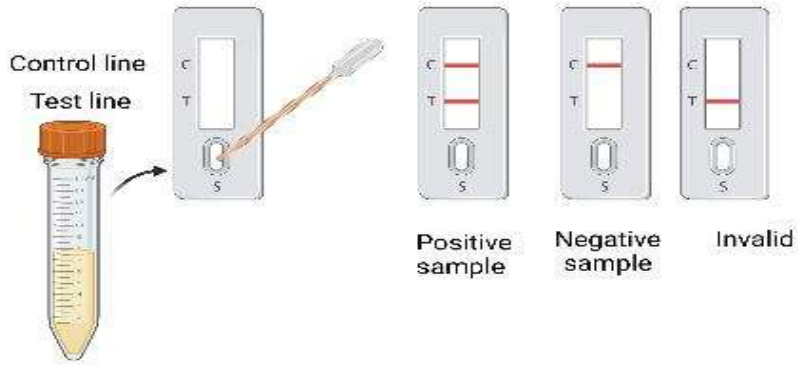

Figure 2.1. Evaluation of *Giardia* rapid test

2.6. Statistical analysis and Ethical approval:

The research ethics committee of the College of Medicine at Hawler Medical University gave its approval to the study project, and participants gave verbal consent after being told of its purpose and promised that any information they provided would be kept confidential. The statistical program for social sciences (SPSS) version 25 was used to examine the data. A p-value of 0.05 or lower was regarded as statistically significant for determining the significance of the responses using the chi-square and Fischer's exact tests.

3. Results

3. 1. Comparison of direct microscopy and immunochromatographic tests for detecting *Giardia lamblia:*

From 100 microscopically negative samples for *Giardia lamblia* that randomly selected from three groups (food handlers, primary school students, others participants), only 3(3.0%) of negative samples were positive by immunochromatographic test and 97(97.0%) of samples were negative by both methods.

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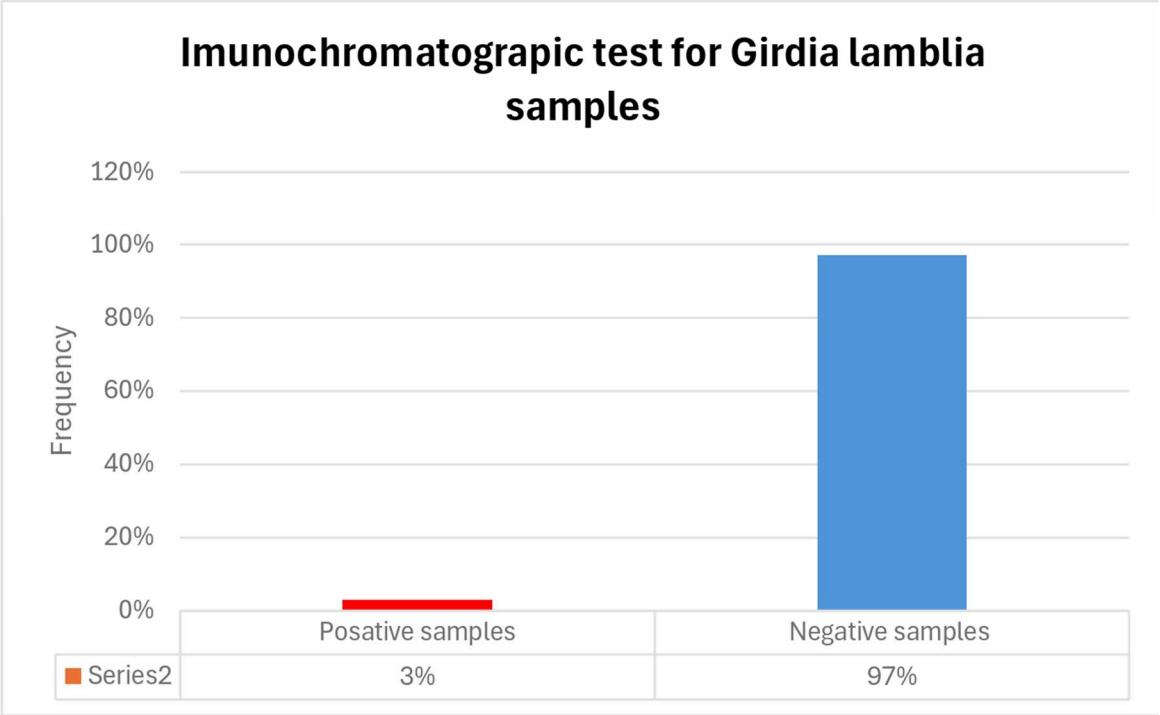

Figure 3.1: One hundred negative microscopic samples were selected and compared with immunochromatographic assay for the detection of *Giardia lamblia* infection.

3.1. Prevalence of *Giardia lamblia* according to socio- demographic characteristics:-
Table 3.1 showed that, according to gender, males are more infected than females. Those in the age group (6–12) years got *Giardia lamblia* infection 1 (4.3%). The lower infection rate was detected in the age group (more than 18 years). In contrast, in the age group (13–18 years), no positive samples were detected.

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Table 3.1. Prevalence of *Giardia lamblia* according to socio- demographic characteristics.

Variable	Category	*Giardia lamblia* infection rate	
		Positive No. (%)	**Negative No. (%)**
Gender	Male	2(2.6)	74(97.4)
	Female	1(4.2)	23(95.8)
Age in groups	6-12 years	1(4.3)	22(95.7)
	More than 18 years	2(2.8)	70(97.2)
	Total	**3(3.0)**	**97(97.0)**

3.2. Prevalence of *Giardia lamblia* infection according to occupation:

One (4.0%) of 100 microscopically negative samples from primary school pupils were positive by immunochromatographic test; all samples from food handles were negative by immunochromatographic test, as were other positive samples. 2 (8.7) were from various jobs; p = 0.06; there was no statistically significant relationship between the participants' occupations.

Table 3.2. Prevalence of *Giardia lamblia* infection according to occupation.

Occupation	*Giardia lamblia* infection rate by immunochromatographic test		P.value	Total
	Positive samples No. (%)	**Negative samples No. (%)**		
Student	1 (4.0)	24 (96.3)		25
Food handlers	0 (0.0)	52 (100.0)		52
Another career	2 (8.7)	21 (91.3)	*0.064	23
Total	3 (3.0)	97 (97.0)		100 (100.0)

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* Fisher's Exact Test

4.3. Prevalence of *Giardia lamblia* infection according to gastrointestinal symptoms:

From positive samples, a lower infection rate was detected in 1 (2.8%) asymptomatic sample, while 2 (3.1%) infections were detected from asymptomatic samples.

Table 3.3. Prevalence of Giardia lamblia infection according to Gastrointestinal symptoms.

Gastrointestinal symptoms	*Giardia lamblia* infection		
	Positive No. (%)	**Negative No. (%)**	**P.value**
Yes	1(2.8)	35(97.2)	***1.000**
No	2(3.1)	62(96.9)	
Total	**3(3.0)**	**97(97.0)**	

* Fisher's Exact Test

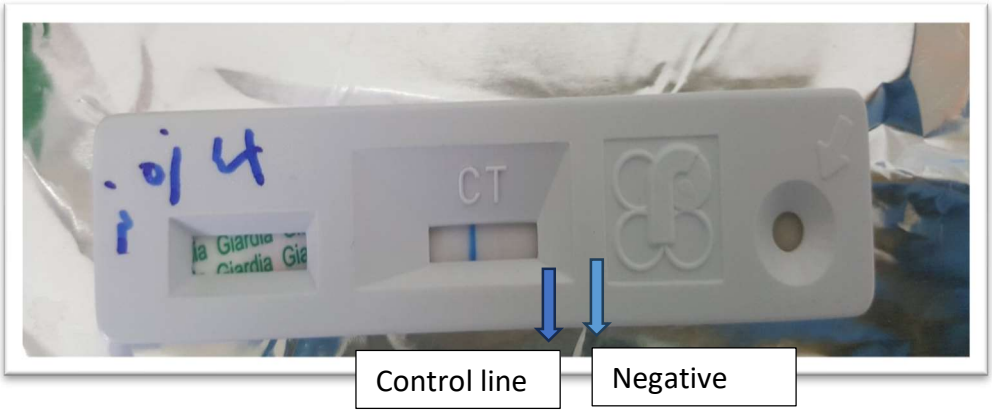

Figure 4.2: Negative immunochromatographic test for detection of *Giardia lamblia* infection.

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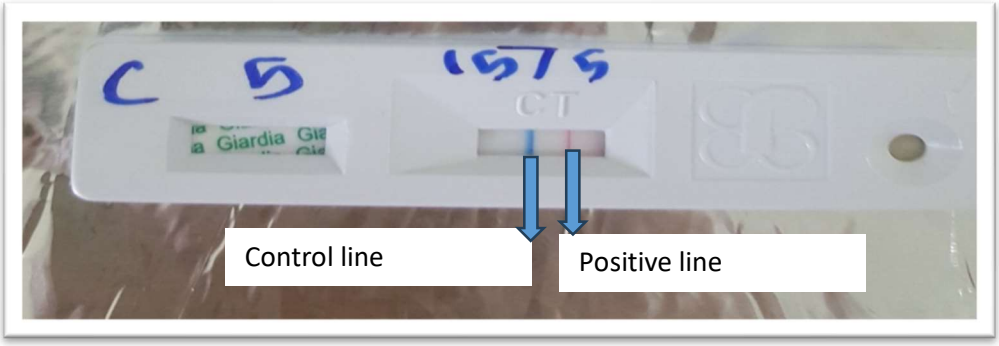

Figure 3.2. Positive immunochromatographic test that used for detection of Giardia lamblia infection.

4.Discussion

Giardia lamblia has been identified as one of the most common causes of gastrointestinal illness in both human and animals (Ayeh-Kumi et al., 2009).

The present study focused on the prevalence of *Giardia lamblia* by using an immunochromatographic rapid test taken from 100 microscopically negative samples to compare the techniques used for detecting infection caused by *Giardia lamblia* in groups of participants that have an important role in the Erbil population. The main finding in the present study was to determine immunochromatographic test sensitivity and compare it to microscopy technique.

Conventional microscopy wet mount or concentration techniques are still recommended for diagnosing intestinal parasites, especially *Giardia lamblia*, but their sensitivity is found to be low in comparison to other techniques (50–70%), even after multiple examinations. Furthermore, it needs more time and special technicians, and sensitivity can be lower in chronic giardiasis (Barazesh et al., 2010). Antigen detection assays for the detection of *Giardia lamblia* have been established to be very valuable, with the advantage of requiring less time and labor in the diagnosis of infections (Jelinek & Neifer, 2013).

In the current study, 3% of negative samples identified by wet mount identification were positive through the immunochromatographic method. This might be due to a low intensity of infection that could not be diagnosed by microscopic wet mount

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examination or a false positive that occurred, and sometimes miss diagnoses occur that need professional technicians to diagnose them by microscope (Selim, Taha, Abd El-Aal, Farag, & Yousef, 2015). Other research done showed that *the Giardia lamblia* copro-antigen test was more effective and easier to perform (Sorell et al., 2004; Weitzel et al., 2006). This result agreed with a study done by (Garcia et al., 2003), and sample result was showed with a study done by Abbas et al.,, 2011 same equation that more positive samples were detected by immunoassay.

According to present study males were more infected that the females which may refer to that more participants were male than females, the result was similar to results of study that done in Iraq by (khudair, 2010). Age affects the frequency and prevalence of Giardia infection. Giardiasis affects people of all ages (Samie et al., 2020), in present study showed the higher rate of infection was detected in age group more than 18 years. While in other study showed that highest prevalence was detected among children ages less than 12 years old (Choy et al., 2014). The study done by (Viesyet al., 2020) showed the highest infection rate was detected in adult or more than 18 years, which depends on different factors, one of them people live in village People have direct contact with one another, the employment of villagers in agriculture and animal husbandry, as well as their continued contact with agricultural soils and contaminated soils, the use of non-drinkable water, and the use of human fertilizers to strengthen agricultural land, seem to be the main causes, especially among those living in villages (Viesy et al., 2020).

The symptoms of giardiasis are extremely variable. Some persons have the capacity to eliminate infectious cysts in their feces even in the absence of any overt symptoms. It's unclear why some patients exhibit clinical symptoms while others don't. But it appears that host factors, such immune system health and parasite strain diversity, are at play in these variations (Pestechian et al., 2014). In a study based in sub-Saharan Africa and South Asia, (Kotloff et al., 2013) *Giardia* lamblia was not significantly associated with moderate-to-severe diarrhea; a similar observation was made in another report of endemic pediatric giardiasis concluding that there was an ostensibly paradoxical association with protection against acute diarrhea from other specific entero-pathogens, yet an enhanced risk of persistent diarrhea in *Giardia* carriers (Muhsen & Levine, 2012). In contrast, asymptomatic infections

patients were carrying the parasite without any clinical symptoms in present study the higher rate were asymptomatic.

5. Conclusion

Giardia lamblia is a common protozoan parasite that causes intestinal infection in humans in Erbil city. False-negative results sometimes occur by microscopical examination, which may also occur if the patient does not have symptoms or is asymptomatic. The Immunochromatographic assay is an alternative test that is easy to perform, rapid, sensitive, and specific. The test may be useful in the diagnosis of infection in patients that carry the parasite or, in large cases, in studying the epidemiology of *Giardia lamblia*.

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دۆزینەوەی بەراوردکاری جیردیە لاملیا بە پشکنینی ئیمۆنۆکرۆماتۆگرافی لە نموونەی پیسایی لە پارێزگای هەولێر

پـوختـه:

پاشبنەما: مشەخۆری پرۆتۆزۆن جیاردیا لامبیلیا یەکێکە لە هۆکارە سەرەکییەکانی نەخۆشی گەدە و ڕیخۆڵەی مشەخۆر. ڕێگای سەرەکی گواستنەوە لە ڕێگای پیسایی-زارەکییە، وە سووڕی ژیان ڕاستەوخۆ و بەرەوپێشچووە، لەوانە قۆناغەکانی ترۆفۆزۆیتی جوڵاو و کیسی نەجوڵاو. ئامانجی لێکۆڵینەوەکە بەراوردکردنی تاقیکردنەوەکانی مایکرۆسکۆپی و بەرگرییە بۆ دەستنیشانکردنی جیاردیا لامبیلا. ڕێگاکانی ئەم لێکۆڵینەوەیە بە هەڵبژاردنی ١٠٠ نموونەی نەرێنی مایکرۆسکۆپی لە کۆمەڵێك خەڵکی شاری هەولێر ئەنجامدرا، کە کۆکراونەتەوە و لە -٢٠ پلەی سیلیزی هەڵگیراون بۆ تاقیکردنەوەی زیاتر. ئەنجامی لێکۆڵینەوەکە دەریخستووە کە لە ١٠٠ نموونەی بەستوو، ٣٪ پۆزەتیڤ بوون بە تاقیکردنەوەی خێرای بەرگری. پیاوان زیاتر بەشداری ئەم توێژینەوەیە دەکەن، چونکە زۆربەی بەشداربووان بەکارهێنەری خۆراک بوون. هیچ پەیوەندییەکی ئاماری گرنگ لە نێوان نیشانەکانی گەدە و ڕیخۆڵە پۆزەتیڤ نەبوو؛ ١ (٢.٨٪) نیشانەکانی هەیە و ٢ (٣.١٪) نیشانەیان نییە. بەمەش گەیشتینە ئەو ئەنجامەی کە هێرشەکانی ئیمۆنۆکرۆماتۆگرافی ئاسانن بۆ ئەنجامدانی، خێرایی، هەستیار و دیاریکراو. ئەوە نیشان دەدات کە تاقیکردنەوەکە لەوانەیە بەسوود بێت بۆ دەستنیشانکردنی نەخۆشییەکە لە حاڵەتی گەورەدا یان لە لێکۆڵینەوە لە پەتاناسی (نەخۆشیناسی) جیاردیا لامبیلادا.

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الكشف المقارن عن الجياردیا اللمبلية عن طريق مقايسة الكروماتوجرافي المناعي من عينات البراز في محافظة أربيل

الملخص:

خلفية: طفيلي أولي الجياردیا اللمبلية هو أحد أهم أسباب أمراض الجهاز الهضمي الطفيلية. الطريقة الرئيسية للانتقال هي من خلال طريق البراز عن طريق الفم ، ودورة الحياة مباشرة ومباشرة ، بما في ذلك مراحل التروفوزويت المتحركة والكيس غير المتحرك. كان الهدف من الدراسة هو مقارنة الفحص المجهري والاختبارات الكروماتوغرافية المناعية لتشخيص الجياردیا اللمبلية. الطريقة: أجريت هذه الدراسة المستعرضة عن طريق اختيار 100 عينة سلبية مجهريا من مجموعات من الناس في مدينة أربيل، والتي تم جمعها وتخزينها في -٢٠ درجة مئوية لمزيد من الاختبار. أظهرت نتيجة الدراسة التي أجريت أنه من بين 100 عينة مجمدة، كانت 3٪ إيجابية عن طريق الاختبار السريع للكروماتوغرافيا المناعية. كان الذكور أكثر عرضة للمشاركة في هذه الدراسة لأن معظم المشاركين كانوا من متداولي الطعام. لم يكن هناك ارتباط ذو دلالة إحصائية بين أعراض الجهاز الهضمي للمرضى الإيجابيين. 1 (2.8٪) لديه أعراض، و2 (3.1٪) بدون أعراض. استنتج أن المقايسات الكروماتوغرافية المناعية سهلة الأداء وسريعة وحساسة ومحددة. وأظهرت أن الاختبار قد يكون مفيدا في تشخيص العدوى في الحالات الكبيرة أو في دراسة وبائيات الجياردیا اللمبليا.