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Sequencing and phylogenetic analysis of *Entamoeba histolytica* in cattle in Al-Diwaniyah governorate, Iraq

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Abstract

This study is carried out to evaluate the occurrence of *Entamoeba histolytica* in cattle in Al-Diwaniyah Governorate, Iraq. Fifty fecal samples were collected from cattle in different regions of the province. Microscopy and polymerase chain reaction (PCR) were used to examine the samples. Four-PCR-product-dependent gene-sequencing was conducted, besides the microscopy, targeting the small subunit *rRNA* (*SSU rRNA*) gene of the parasite. The outcomes of the microscopy demonstrated the presence of the parasitic cyst and trophozoite in 66%, (33/50) of the fecal samples of the examined cattle. The results of the PCR showed that the genus level of *Entamoeba* was positive in 72%, (36/50) of the fecal samples. The sequencing reported the existence of four closely similar isolates to isolates registered in fecal samples of cattle in Baghdad City, Iraq. The study concludes that *Entamoeba histolytica* is currently present in the cattle tested in the current governorate, and the zoonotic disease could strongly be induced in people with contact with the animals testing positive.

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Introduction

Entamoeba histolytica, a protozoan, is the source of the illness known as amoebiasis, sometimes known as amoebic dysentery. The majority of cases are asymptomatic, but extensive intestinal illness can develop and cause weight loss, cramps, stomach discomfort, and diarrhea (could be bloody) that lasts for many weeks. Diffused extraintestinal illness is reported to cause purulent pericarditis, liver abscess, pneumonia, and even brain amoebiasis (1). *E. histolytica* is thought to affect up to 50 million people globally, mostly in underdeveloped nations, and it is the cause of over 100,000 fatalities each year. The most common methods of transmission are through the consumption of contaminated food or water brought on by the fecal expulsion of cysts, as well as fecal-oral transmission and due to homosexuality, a man having sex with another man (2-11). Amoebiasis is a disease that affects people everywhere, especially in countries with poor sanitary systems. Infection is rising in wealthier nations like those in North America as a result of an upsurge in emigration and travel from highly disease-prevalent countries (1,12). India, Africa, Central and South America, especially Mexico, have the greatest infection rates. Males and females almost equally contract amoebic colitis at the same rate. Males are ten times more likely to develop an amoebic liver abscess (ALA) than females, and those between the ages of 18 and 50 are most frequently affected (13). *E. histolytica* is an invasive intestinal protozoan. When mature quadrinucleated cysts are ingested in food or drink contaminated with fecal materials, an infection usually starts. Motile trophozoites are released after excystation in the small intestine to the large intestine (14). Both trophozoites and cysts are produced by binary fission, and both are excreted in feces, but only cysts have the capacity to transmit infection because of their hard wall. While trophozoites are quickly killed when they leave the

body or by stomach secretions if consumed, cysts can persist days to weeks in such environments. Trophozoites are able to attach to and destroy the colonic epithelium before blood-stream, spreading to far-off locations including the peritoneum, liver, lung, or brain via the portal vein system (15-17).

This study is carried out to evaluate the occurrence of *Entamoeba histolytica* in cattle in Al-Diwaniyah Governorate, Iraq.

Materials and methods

Samples

Fifty fecal samples were collected from cattle in different areas of Al-Diwaniyah Governorate, Iraq. The samples, which were collected in May-October, 2022, were capped in containers that were cold-transported to the Parasitology Laboratory, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Diwaniyah Governorate, Iraq.

PCR and DNA Sequencing

Each fecal sample (200 mg) was washed in 1ml-sterile Phosphate Buffer saline pH 7.2, 5mins-14,000 × g-centrifuged, according to the instructions of QIAGEN kit (QIAGEN, Hilden, Germany), then, DNA was extracted. The primers employed in the current study were F: TGGACTTCAGGGGGAGTATG and R: TCAATCTCGGTACACCACTCA for a product of 545bp (18). DNA polymerase at 0.2µl 5U/µl, DNA at 2µl 100ng, each primer at 0.6µl 40µM, and 16.5µl PCR-water were used for the PCR reaction component. At the conditions, 94°C-Denaturation, 40 cycles of (92°C-60s denaturation, 47°C-60s annealing, and 72°C-90s-extension), and 72°C-7mins final extension were employed for the thermocycler. The PCR products were purified and sent to Macrogen (Korea) to perform partial gene sequencing. The phylogenetic tree was generated using MEGA X, and similar world sequences were detected using NCBI websites.

Results

The outcomes of the microscopy demonstrated the presence of the parasitic cyst and trophozoite in 66% (33/50) of the fecal samples from the tested cattle (Figure 1). The results of the PCR showed that the genus level of *Entamoeba* was positive in 72%, (36/50) of fecal samples (Figure 2). The sequencing reported the existence of four closely similar isolates to isolates registered in Baghdad City, Iraq, (MW426065 and MW426066) (Figure 3).

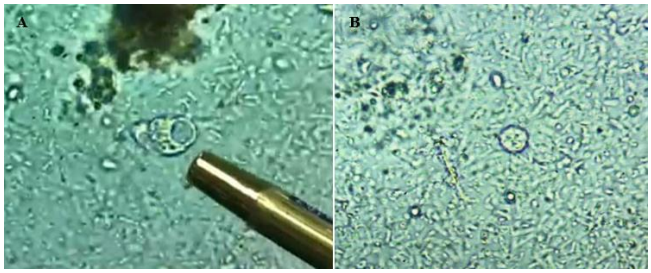

Figure 1: (A) Trophozoite and (B) cyst of *Entamoeba histolytica* identified from fecal samples of cattle. (X100)

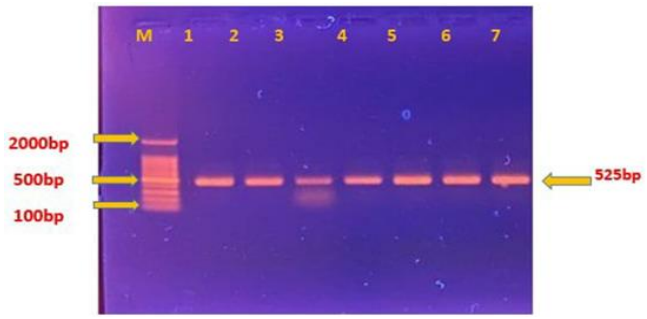

Figure 2: Image of SSU rRNA gene-based agarose gel electrophoresis of *Entamoeba histolytica* identified from fecal samples of cattle. M: Ladder. Lanes 1-7: bands of positive detection.

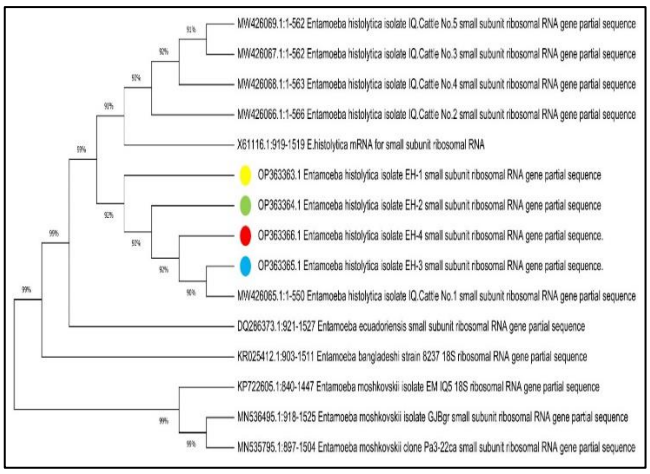

Figure 3: Phylogenetic tree of SSU rRNA gene of *Entamoeba histolytica* identified from fecal samples of cattle. Colored labels: Isolates of the current study.

Discussion

Entamoeba histolytica is commonly known as highly infectious parasite that causes a severe diarrheal disease in humans; however, some reports noted that this protozoan could cause a disease in animals (19). The current study

demonstrated, via the use of microscopy, the species identification of the parasite; however, it is said that microscopy can often be employed to diagnose protozoa in stool samples, but to the genus level. In disagreement with the current result, researchers suggested that this approach is unable to distinguish *E. histolytica* from other non-pathogenic species like *E. dispar*, which has physical similarities with it (20). As a result, the WHO urged the creation and use of novel techniques for a precise diagnosis of *E. histolytica* infections (20), including the PCR, which can be a better choice for the significant detection of the protozoan.

The results of the current study ensured that the PCR is highly effective in the detection of *E. histolytica*. Hence, utilizing PCR is another option for separating the species. Compared to microscopy, this method has high sensitivity and specificity. A study by López-López *et al.* (20) used a piece of the adh112 gene to identify and to differentiate between *E. histolytica* and *E. dispar*. They found that this gene showed five variations in single nucleotide between the two species. *E. histolytica* and *E. dispar* were successfully detected using a variety of techniques, and real-time PCR. Unfortunately, the expensive price restricts their application in developing countries, and some of them also, provides falsely negative findings (21-37). Moreover, the *SSu rRNA* gene based PCR was highly effective in the current detection, and this agrees with López-López *et al.* (20).

According to the results of the microscopic study and PCR tests by Aryal *et al.* (38), it was revealed that 80% of the wild water buffaloes had *E. bovis* infection. Also, a research conducted in China showed that *E. bovis* infection affected 100% of cattle and more than 90% of yak and some small ruminants (39). In a Ugandan investigation, *E. bovis* infection was detected in 60% of goats and 80% of cattle (40).

Conclusion

The study suggested that *Entamoeba histolytica* is currently present in the cattle tested in Al- Diwaniyah Governorate, and that zoonotic infection could strongly be induced in people in contact with animals testing positive.

Acknowledgments

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Conflict of interests

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عملية السلسلة والتحليل الوراثي للأميبا المعوية المحللة للأغشية الخلوية (إنتاميبا هستوليتيكا) في الماشية في محافظة الديوانية، العراق

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الخلاصة

أجريت هذه الدراسة لتقييم تواجد طفيلي الأميبا الحالة للنسيج في الماشية في محافظة الديوانية، العراق. جمعت خمسون عينة برازية من الماشية في مناطق مختلفة من المحافظة. أُجري الفحص المختبري المجهري وكذلك تفاعل إنزيم البلمرة المتسلسل لفحص هذه العينات. استهدف كلا الاختبارين، تسلسل القواعد النتروجيني الجينية، الذي تتيحه تقنية التفاعل لأنزيم البلمرة المتسلسل والفحص المختبري المجهري الوحدة الثانوية الصغرى الجينية للطفيلي. أظهرت نتائج الفحص المجهري وجود كيس الطفيلي وطوره النتغذي في ٦٦٪، (٥٠/٣٣) من عينات البراز. كما وأظهرت نتائج التفاعل لأنزيم البلمرة المتسلسل وجود طفيلي من جنس الإنتاميبا في ٧٢٪ (٥٠/٣٦) من عينات البراز. وكذلك، أظهر فحص تسلسل القواعد وجود أربع عزلات متطابقة إلى حد كبير مع عزلات مسجلة في مدينة بغداد، العراق. تستنتج الدراسة الحالية أن طفيلي الإنتاميبا هستوليتيكا موجود في الماشية التي تم فحصها في محافظة الديوانية، وأن العدوى الحيوانية-المصدر يمكن أن تحدث بشدة للأشخاص الذين لهم اتصال مع الحيوانات المصابة.