2024, Scienceline Publication
World's Veterinary Journal
World Vet J, 14(1): 53-57.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2024.wvj7
PII: S2322456824000007-14

Molecular Detection of *Entamoeba* spp. in Monkeys (*Macaca* spp.) in Babylon Province, Iraq

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ORIGINAL ARTICLE
Received: January 07, 2024
Revised: February 20, 2024
Accepted: February 25, 2024
Published: March 25, 2024

ABSTRACT

Amoebiasis is a widespread parasitic disease caused by *Entamoeba histolytic*a (*E. histolytic*a), affecting various hosts, such as humans, birds, and pigs. This study aimed to investigate *Entamoeba* spp. in monkeys (*Macaca* spp.) diagnose them using molecular methods. A total of 33 fecal samples were collected from monkeys (*Macaca* spp.) aged 3-5 years in Babylon province to investigate a common and zoonotic parasitic disease. Initially, microscopic examination was conducted on all samples, and those yielding positive results were preserved for molecular study. The DNA was extracted, and conventional PCR was carried out with a pair of primers to detect the 857 bp fragment of *E. histolytica* SSU rRNA gene. PCR results for 19 fecal samples, previously identified as positive by the direct smear method, from monkeys in the reserves of Babylon province indicated that the presence of the SSUrRNA gene with an 857 bp was 45% in only 15 samples. Sequencing of the SSUrRNA gene revealed 98-100% similarity with *E.histolytica* sequences deposited in International GenBank, which have the sequence numbers OP522013, OP522014, OP522015, OP522016, OP522017, Op626161, Op626162, Op626163, Op626164, and Op626165.

Keywords: *Entamoeba histolytica*, Gene, Monkey, SSU rRNA

INTRODUCTION

Nature reserves and public zoos play a crucial role in housing diverse animal species, ranging from pets to predators. These environments provide a secure habitat for animals, fostering their growth and reproduction. Additionally, these facilities serve as invaluable resources for researchers, facilitating easy access to specimens and species that are under study (Thawait et al., 2013).

Captive animals of various kinds are generally susceptible to infections by a multitude of parasites. This susceptibility is influenced by several factors, including nutrition, the management system in place for the animals, and environmental conditions, such as temperature, humidity, and the pollution levels in the surrounding environment (Kashid et al., 2003; Goossensa et al., 2005; Singh et al., 2006; Atanaskova et al., 2011). Some types of parasites may be harmless to some animals, but they threaten the lives of others. Contact between humans and captive animals increases the chance of the spread of zoonotic parasitic diseases, which pose a threat to the health of the animals as well as those working in those places (Panayotova-Pencheva, 2013).

Entamoeba histolytic causes an intestinal disease called Amebiasis. It attacks the intestinal wall of the host, causing intestinal symptoms, abdominal discomfort, and bloody or loose mucous stools (Guillén, 2023). The infection period lasts for approximately 1-3 weeks. In advanced cases of infection, the infection may spread to other organs of the body, causing liver abscesses, lung abscesses, or brain abscesses, which leads to severe symptoms and may be fatal (Shirley et al., 2020; lin et al., 2022; Guillén, 2023)

This study aimed to undertake a molecular investigation of a parasitic species, analogous to human parasites, with the objective of confirming the potential presence of shared parasite species, such as Entamoeba spp. The methodology involved the application of conventional polymerase chain reaction (PCR) and DNA sequencer technologies.

Material and methods

Ethical approval

All procedures conducted on animals were in accordance with the ethical standards of the institution. and the current study was approved by the Committee of the Department of Biology, Faculty of Education, University of AL-Qadisiyah, Al-Diwaniyah, Iraq (No.456).

To cite this paper: Abdul Abbas ZT and Aziz Anah S (2024). Molecular Detection of *Entamoeba* spp. in Monkeys (*Macaca* spp.) in Babylon Province, Iraq. *World Vet. J.*, 14 (1): 53-57. DOI: https://dx.doi.org/10.54203/scil.2024.wvj7

Sampling

A total of 33 fecal samples were collected from *Macaca* spp. monkeys situated in both governmental and private reserves in the central region of Babylon, including its districts. These monkeys, aged between 3 and 5 years, were imported from Japan and Afghanistan. The direct smear method was employed, utilizing iodine Lugol's dye, to examine the presence of Entamoeba spp. cysts. Positive smears were identified using a Compound light microscope (Olympus, Japan; 40x). Subsequently, the samples were preserved at -20°C for molecular confirmation.

Prepared DNA and primers

A pair of primers for the gene SSU rRNA [F:5'-GTCAGAGACCACATGAAC-3, R: 5'-GTTGTCCCGACCTAATCC-3], based on Al-Abodi et al. (2014) was designed to confirm the diagnosis of *Entamoeba* using conventional PCR technique with a molecular weight of 857bp. DNA was extracted using Stool Genomic DNA Extraction Kit (Bioneer, Korea) following the manufacturer's protocol and the concentration and purity of the extracted DNA were measured by a Nano-drop spectrophotometer (Thermo, USA).

The PCR mix was prepared using AccuPower® Premix Kit (Bioneer, Korea) according to the manufacturer's instruction and the PCR reaction was performed with initial denaturation for 5 minutes at 95°C, followed by 35 cycles of 95°C for 30 seconds (denaturation), annealing at 58°C for 30 seconds, extension at 72°C for 40 seconds, with a final extension at 72°C for 5 minutes. The PCR products were analyzed by electrophoresis in a 1% agarose gel. Then, PCR product was sent to Bioneer Company in South Korea for the purpose (by DHL fast and saving DNA samples at -20°C) of knowing the sequence of DNA fragments using the DNA sequencing system to determine the type of *Entamoeba* spp. through phylogenetic tree analysis and the National Center for Biotechnology Information Genbank-Primer-Blast database program.

Statistical analysis

The data was analyzed using the statistical program (SPSS 24) where the Chi-Square test was used to determine the significant differences under the probability level ($p \leq 0.05$). T-test was also used to analyze the data.

RESULTS

The PCR examination of 33 stool samples collected from the monkeys (*Macaca* spp.) showed that only 15 samples produced positive results, accounting for a rate of 45%. The results of the statistical analysis indicated no significant differences in the presence of the SSU rRNA gene in collected samples using the direct swab method followed by PCR technique, as shown in Figure 1 ($p > 0.05$). The results revealed that only 10 samples were identical to the global isolates registered in NCBI representing *Entamoeba histolytica* (*E. histolytica*). Upon comparing the local parasite sequences bearing the serial numbers OP522013, OP522014, OP522015, OP522016, OP522017, Op626161, Op626162, Op626163, Op626164, Op626165, with the global isolates, the percentage of congruence ranged 99-100%. Utilizing the MEGA 6 program, a genetic tree was constructed to illustrate the genetic relationship between local isolates of *E. histolytica* bacteria and global isolates registered in NCBI, as depicted in Figure 3. The results of the genetic tree analysis of local isolates showed the presence of common ancestors, where the local isolates of *E. histolytica*, which carry the serial numbers OP522013, OP522014, OP522015, OP522016, OP522017, op626161, op626162, op626163, op626164, op626165 isolates, showed a genetically close relationship with isolates L00636.1, KP233840.1, AB002793.1, ON724174.1 and AB608092.1 globally registered in NCBI.

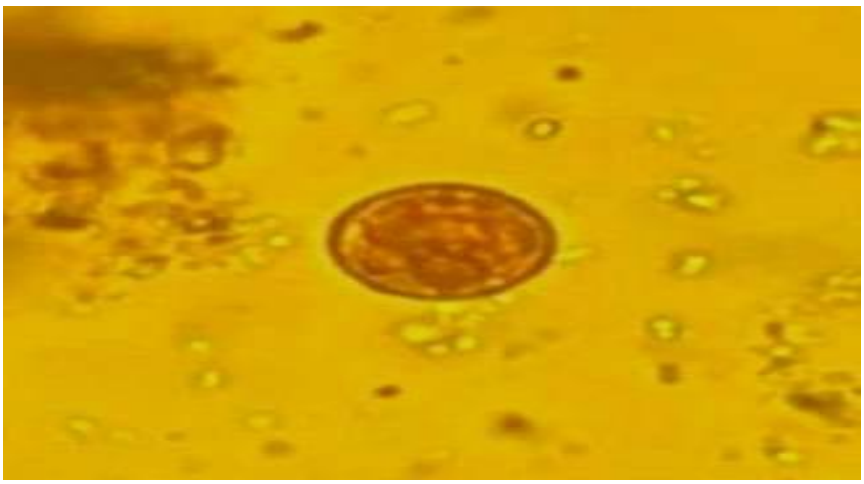

Figure 1. Cyst of *Entamoeba histolytica* (400x) isolated from the feces of monkeys (*Macaca* spp) in Babylon province

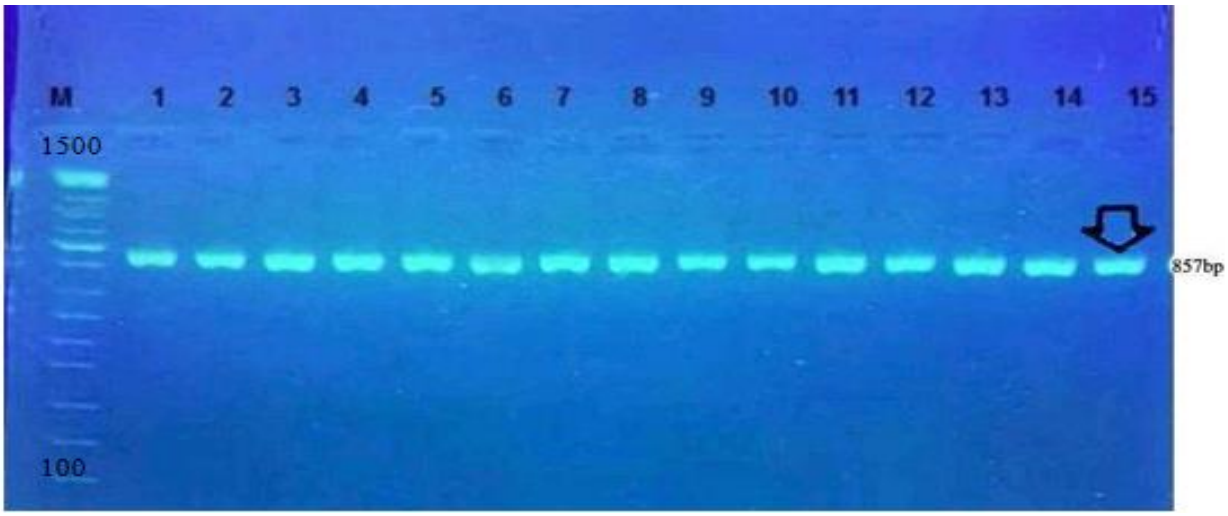

Figure 2. Amplified SSU rRNA gene electrophoresis in PCR. Columns 1-15 represent fecal samples (of monkeys at ages ranging between 3-5 years and both sexes) positive for polymerase chain reaction, showing the 857bp SSUr RNA gene of the *Entamoeba* spp. Column M represents a Ladder bearing molecular weight 100-1500 bp.

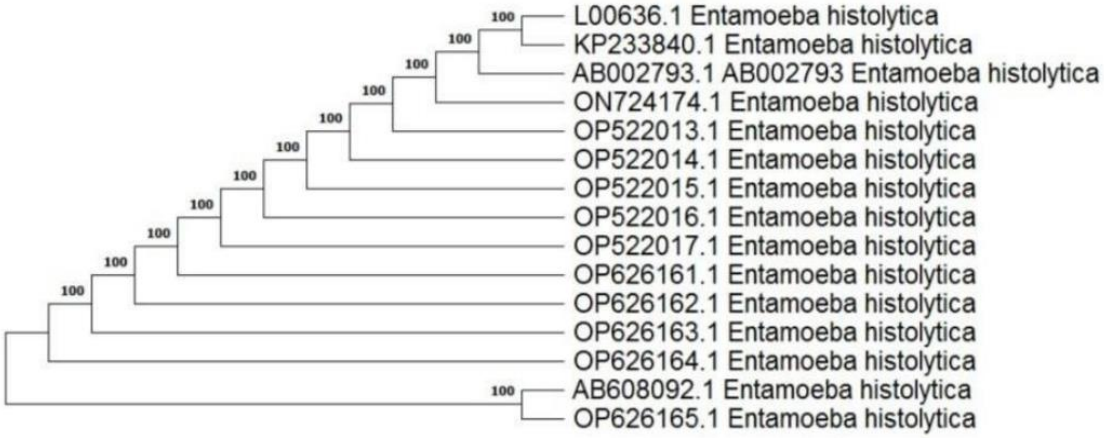

Figure 3. Genetic tree analysis using MEGA 6 program. The results show a common genetic relationship in local parasite samples taken from the feces of monkeys with *E. histolytica* parasite samples registered in NCBI-Genbank.

DISCUSSION

The results of the current study, utilizing PCR to detect the SSU rRNA gene of the *Entamoeba* species with a molecular weight of 857bp in 15 fecal samples from monkeys that tested positive by the direct smear method, revealed an overall presence rate of 45% in only 15 samples. The absence of a positive result for the PCR reaction in 4 samples may be attributed to potential uncontrolled laboratory conditions. The detection of *E. histolytica* in monkeys using molecular methods is consistent with some studies that indicated the presence of this parasite in different species of captive nonhuman primates. A study by Levecke et al. (2010) indicated that the tantalus monkey, greater spot-nosed monkey, Sunda pig-tailed macaque, olive baboon, and Bornean orangutan were infected with *E. histolytica* at a various rate. Amoebiasis has also been reported in other species of monkeys as mentioned in studies by Márquez-Monter et al. (1991) and Takano et al. (2005). Moreover, *E. histolytica* was not found in the patas monkey (Beaver et al.,1988), mandrill (Verweij et al., 2003; Mätz-Rensing, 2004), mantled guereza (Loomis et al., 1983; Suzuki et al., 2008), and Western gorilla (Sleeman et al., 2000).

The identification of the *E. histolytica* parasite in Iraqi monkeys, whether through the direct smear method or molecular methods, represents one of the first reports in Iraq concerning a parasite shared between monkeys and humans. In line with the current study, some international studies indicated the possibility of infecting species in different types of monkeys with *E. histolytica* (Márquez-Monter et al., 1991; Takano et al., 2005; Levecke et al., 2010).

It is noteworthy that 10 samples from the current study were identical to globally recorded *E. histolytica* species, demonstrating a genetic relationship with isolates registered in the National Center for Biotechnology Information. These include the German isolate recorded by Tannich et al. (1991), the Japanese isolate recorded by Tanaka et al. (1997), and Thailand isolates recorded by Koi et al. (2012), the Iraqi isolate recorded by AL-Abodi et al. (2014), and the Iraqi isolate

taken from livestock and recorded by ALseady et al. (2022), with a match rate of 100%. A recent study by Liu et al. (2022) in non-human primates in a Zoological Garden in Nanjing, China, further supports the findings of the current study, particularly in the Macaca species.

CONCLUSION

This study confirmed the presence of *Entamoeba histolytic* in monkeys of Iraq which is one of the first isolation reports in the Middle East. This study recommends conducting further studies on other zoonotic parasites in monkeys in the study area.

DECLARATIONS

Funding

Funding was provided by Dr.Sadiya Aziz Anah and Zaman Turkey Abdul Abbas with the fund number 103.

Authors' contributions

Zaman Turkey Abdul Abbas contributed to collecting samples and statistically analyzing data. Sadiya Aziz Anah contributed to the implementation of PCR. All authors discussed the results, commented on the manuscript, and gave final approval of the final version of the manuscript.

Competing interests

The authors report no conflicts of interest.

Ethical considerations

Ethical issues, such as data fabrication, double publication and submission, redundancy, plagiarism, consent to publish, and misconduct, have been checked by all the authors before publication in this journal

Availability of data and material

All data of the current study are available in the present article.

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