Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

Sheep-human genetic similarities of *Entamoeba histolytica* isolates recovered from farms and human hospitals in Al-Diwaniyah Province, Iraq

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Received:10/3/2023. Accepted:16/4/2023. Published:1/6/2023

Abstract

The current comparative investigation was conducted to identify sheep-human genetic similarities of *Entamoeba histolytica* isolates recovered from animal farms and human hospitals in Al-Diwaniyah Province, Iraq. The study involved the use of 50 (25 sheep and 25 human) fecal samples that were subjected to microscopic examination and a polymerase chain reaction (PCR) method and a partial gene sequencing, of which both targeted the *18S rRNA* gene as a molecular marker. The findings of the microscopy documented that 21 (84%) and 20 (80%) of the sheep and human fecal samples, respectively, contained the cyst of the *E. histolytica*. The PCR reported the identity of the parasites in the tested samples from both sheep and human at 17 (68%) and 15 (60%), respectively. The phylogenetic analysis revealed close-alignment between sheep and human isolates due to high similarities between the sequences of nucleotides between both host isolates. The finding of the present work reveals high presence of *Entamoeba histolytica* in fecal samples of sheep and humans with high similarity between the isolates of both hosts, which may indicate a highly transmissible pathogen between sheep and human beings.
Keywords: *Entamoeba histolytica*, parasitic evolution, phylogeny.

Introduction

Amoebiasis, often known as amoebic dysentery, is an illness induced by the protozoan *E. histolytica*. Normally, the illnesses have no symptoms, but invasive intestinal illnesses may cause painful abdominal cramping, watery or bloody diarrhea, and loss of weight. Liver abscess, purulent pericarditis, pneumonia, and even cerebral amoebiasis are all examples of extraintestinal manifestations of the illness (1–3). *E. histolytica* is responsible for over 100,000 fatalities annually, and it is believed that up to 50 million individuals worldwide are infected, mostly in impoverished nations. Cysts excreted in feces may contaminate water or food, and fecal-oral transmission can develop between members of the same household (4).*E. histolytica* is known to cause severe illness and even death in humans. Developed, quadrinucleated cysts detected in fecal-contaminated food or water are the primary cause of infection. Motile trophozoites are released after excystation in the small intestine and go to the large intestine (5). The trophozoites generate the cysts that they produce during binary fission are both excreted in the feces, but only the cysts may cause disease transmission because of the protective barrier provided by their cell wall. Whereas trophozoites are quickly killed once outside the body or by stomach secretions if consumed; however, cysts may persist for days to weeks in the external environment (6). Although many other types of harmless amoeba live in similar environments, *E. histolytica* is uniquely able to use gene regulation and activation

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pathways to respond to changes in the intestinal ecology caused by the parasite (7). The cyst, the infectious form, promotes the continuation of the species since it is resistant to environmental alterations and can be spread with relative ease. Cysts are so resistant that they may survive even after the host immune system or antibiotics have eliminated the underlying disease. Adequate hand cleanliness adherence in endemic places substantially corresponded with a lower risk of *E. histolytica* and other intestinal parasite illnesses, and this result was mostly validated during the COVID-19 pandemic (8).

The current comparative investigation was conducted to identify sheep-human genetic similarities of *Entamoeba histolytica* isolates recovered from animal farms and human hospitals in Al-Diwaniyah Province, Iraq.

Materials and methods

Samples

The study involved the use of 50 (25 sheep (0.5-5 years old) and 25 human (15-60 years old)) fecal (diarrheic or normal) samples collected from animal farms and human hospitals in Al-Diwaniyah Province, Iraq, during July, 2022 to February, 2023. The samples were plastic-cooled-container-transported to a parasitology laboratory to perform the required tests.

Microscopic examination

These samples were subjected to microscopic examination using a light microscope.

Molecular techniques

Extraction of protozoal nucleic acid

The stool DNA extraction Kit (Bioneer. Korea) with the utilization of its protocol procedures was employed to extract the DNA from the parasite located in the fecal samples from both sheep and humans. A NanoDrop was recruited to understand the quality and quantity of the extracted DNA.

18S rRNA gene dependent PCR

The primers designed in the present study, F: TGAGTTAGGATGCCACGACA and R: CGAGCGTTTTAATCACAGCA, were used in the current study that targeted the *18S rRNA* gene. The master mix for the PCR reaction solution (20µl) contained 5µl DNA, 2µl (10pmol) of each primer direction, 10µl water (For molecular purposes). The conditions for the thermocycler procedures were one-cycle for 95ºC-3mins, 35 cycles for (95ºC-30s, 58ºC-30s, 72ºC-1min), and one cycle for 72ºC-5mins of initial denaturation, (denaturation, annealing, and extension), and final extension, respectively.

18S rRNA gene dependent sequencing

A partial gene sequencing, of which both targeted the *18S rRNA*-gene PCR-products purified utilizing the EZ EZ-10 Spin Columni DNA Gel Extraction Kit (Biobase, Canada), was performed. The extracted products were sent for the sequencing at Bioneer Company (Korea). The sequences were processed and analyzed by employing different tools, including NCBI-based websites and MEGA X software, by which a phylogenetic tree was generated.

Ethical approval:

The researchers obtained ethical approval from the research Ethical Approval Committee of the College of biotechnology/ University of Al-qadisiyah

Results

Microscopic examination

The findings of the microscopy documented that 21 (84%) and 20 (80%) of the sheep and human fecal samples, respectively, contained the cyst of the *E. histolytica* (Figure 1).

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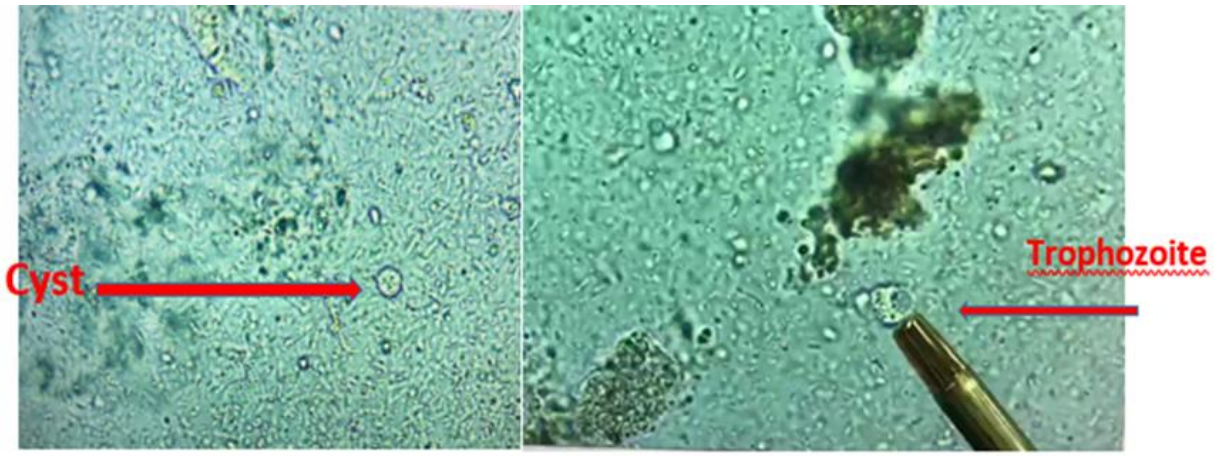

Figure 1: Cyst and trophozoite of *Entamoeba histolytica* from fecal samples of sheep and humans.

***18S rRNA* gene dependent PCR**

The PCR reported the identity of the parasites in the tested samples from both sheep and human at 17 (68%) and 15 (60%), respectively, (Figure 2).

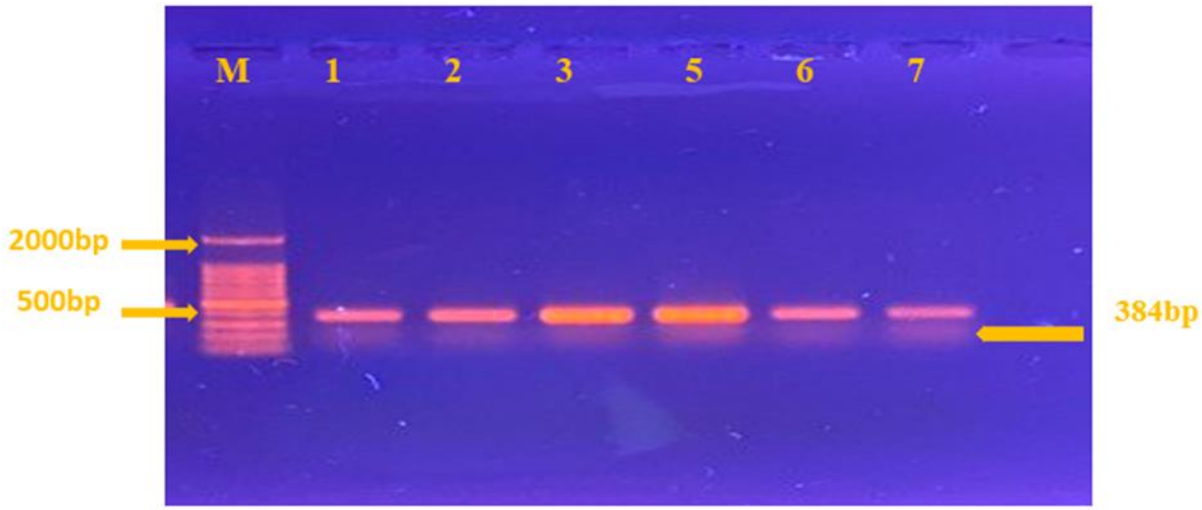

Figure 2: Image of agarose gel electrophoresis. It reports the *18S rRNA* gene dependent PCR of *Entamoeba* spp. Where M: Ladder, 1-7: Positive PCR products.

***18S rRNA* gene dependent sequencing**

The phylogenetic analysis revealed close-alignment between sheep and human isolates due to high similarities between the sequences of nucleotides between both host isolates (Figure 3).

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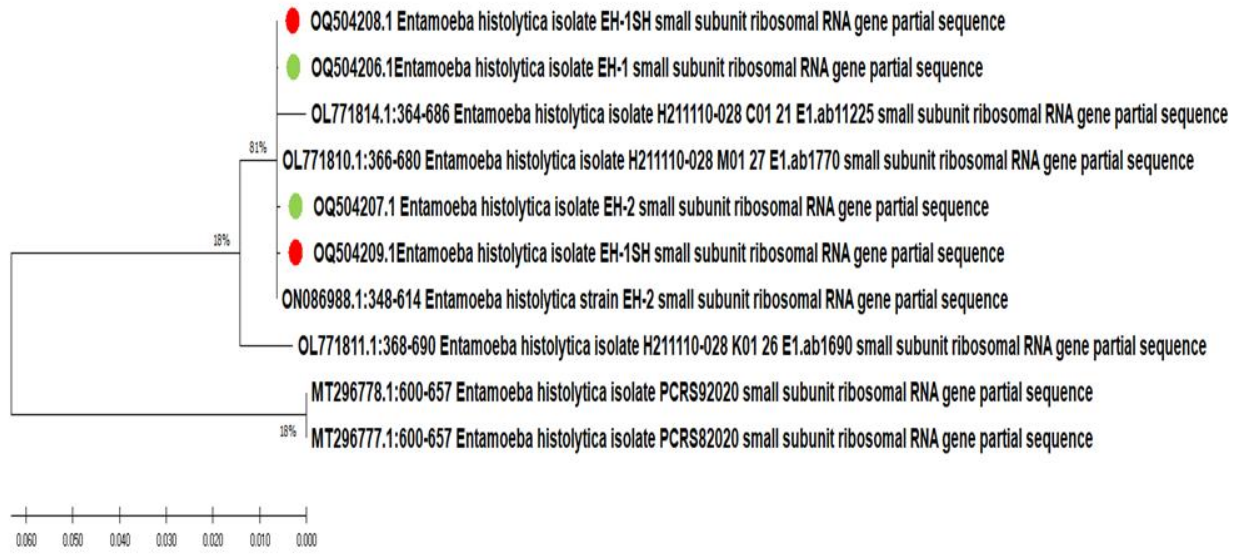

Figure 3: *18S rRNA* gene dependent phylogenetic tree of *Entamoeba histolytica* in sheep (red dots) and human (green dots) fecal samples.

Discussion

Most tropical and subtropical parts of the world's poorest nations are affected by parasitic illnesses. The protozoan parasite *E. histolytica* lives in the human gastrointestinal system, where it produces asymptomatic infections in around 90% of infected persons. Amebae have the potential to infect the liver, leading to amoebic liver abscesses if they make their way there through the digestive system and produce ulcerations and abscesses. While visually similar to *E. histolytica*, the nonpathogenic *E. dispar* and *E. moshkovskii* are distinct on the molecular and cellular levels (9).Mahmood et al (10) reported that samples from the feces in Northern, Iraq, were inspected under the microscope, and in the cases of a positive result, a nested PCR was used to specifically target the 18S-rRNA gene. Their first morphological data indicated a 7.4% prevalence rate for Entamoeba. Females and those with lower incomes were more likely to get infected than men and those with middle- to upper-level incomes, respectively. Their molecular characterization found the following occurrence rates: 6%, 4.3%, and 0.3% of *E. histolytica*, *E. dispar*, *and E. moshkovskii*, respectively. Out of all the specimens that tested positive for Entamoeba, 41.4% were found to have a single infection with *E. histolytica* (10).Recent research has shown that the infection rate of *E. dispar* is much greater than that of E. histolytica in industrialized nations. There have been reports of very high rates of infection with *E. moshkovskii* in the Indian region. Nevertheless, there hasn't been a lot of research done on the frequency of this species. A number of countries, including South Africa, the United States, Italy, and Bangladesh, have recorded human isolates (9,11,12).Fecal specimens may include polymorphic nuclear leukocytes and macrophages with a comparable appearance to the commensal *E. dispar*/*moshkovskii*, leading to false positives and an increased infection rate. Separating pathogenic E. histolytica from nonpathogenic organisms in a fecal sample is

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now easier because to newly developed procedures. The specificity, or true positive rate, of tests for the targeted *E. histolytica* DNA has been enhanced by the development of modern molecular-based methods, such as PCR. Hence, molecular approaches are used in the most up-to-date epidemiological investigations of *E. histolytica* (13–15). The finding of the present work reveals high similarity between the isolates of both hosts, which may indicate a highly transmissible pathogen between sheep and human beings. These similarities could be developed due to genetic evolution that may have occurred on the genetic materials of the parasite of either hosts (16).

Conclusion

The finding of the present work reveals high presence of *Entamoeba histolytica* in fecal samples of sheep and humans with high similarity between the isolates of both hosts, which may indicate a highly transmissible pathogen between sheep and human beings.

Conflict of Interest: there is no conflict of interest.

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Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
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