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STUDY THE PREVALENCE OF ENTAMOEBA SPECIES IN CHILDREN AND FARM ANIMALS IN WAIST PROVINCE

1 Russul Wassit Kadhum,
2 Nawar Jaber Hussein Al-Asadi
3 Zainab Abbas Jasim Al-Maliki
1, 2, 3 Department of Biology, College of Sciences, University of Wasit, Iraq
Corresponding author: russul.wassit@uowasit.edu.iq

Abstract
The study's objectives are to determine the prevalence of Entamoeba species in Waist Province's farm animals and children. 80 Stool samples were collected from villager children (1-6 years) whom attended to (Al –kut Hospital for Gyneclogy and obstetrics and pediatrics) and 75 Stool samples from farm animals (25 Sheep, 25 cattle, and 25 goats) . In children the results were revealed that 45(56.2%) which infection with Entamoeba spp. The age group of 3–4 years had the highest infection rate (87.5%), whereas the age group of 1-3 years had the lowest infection rate (26.66%). A statistical examination of the data revealed five Entamoeba species and a significant variance ($P<0.05$) in the total prevalence of these species among child groups. E. disper, E. moshkovskii, E. polecki, E. hartmanni, and E. histolytica were found in samples of feces. Three farm animals in Waist Province had an overall prevalence of 84% (75/63) Entamoeba spp., with infection rates varying from 72% to 100% for various animals and Entamoeba species. Three species were identified: E. bovis, E. moshkovskii, and E. histolytica. The findings gave insight into the extent of animal infections with Entamoeba spp. in Waist Province and emphasized the pressing necessity to put preventative measures in place to shield humans from this zoonotic illness.

Keywords: Entamoeba spp, children, zoonotic disease, farm animals.

Introduction

Entamoeba species' protozoans are zoonotic parasites that are found around the world and infect both humans and a variety of animal hosts. (1). According to (2), the main cause of Entamoeba infection transmission in affluent countries is travel from endemic countries, while poor sanitation, poor hygiene, and crowded living circumstances are the main causes in underdeveloped countries (3). Human carriers, or cyst passers, who pass in formed or semi-formed feces, are the cause of the majority of instances (4). Goats, cattle, and sheep are all naturally infected with E. histolytica, but their numbers pale in comparison to the human population (5). Though more prevalent in the tropics and subtropics, Entamoeba histolytica infection is found globally. Worldwide, an estimated 500 million people are thought to have amoebiasis (6). According to earlier research, amoebiasis kills 100,000 people annually and

affects 50 million people annually. (7). Nevertheless, conventional diagnostic techniques fail to distinguish between the disease-causing species (8). Molecular methods have made it easier to identify E. histolytica at the genotype level because E. dispar, E. Bangladeshi, and E. moshkovskii share the same morphology as the species (9).

Materials and methods

Sample Collection:

80 Stool samples were collected from villager children (1-6 years) whom attended to (Al –kut Hospital for Gyneclogy and obstetrics and pediatrics) and 75 Stool samples from farm animals (25 Sheep, 25 cattle, and 25 goats) during the period from 3rd December 2022to 15 July 2023 The samples were collected from many villages in waist province per week, with one visit every region.

Direct wet smear Apin point fecal sample and put on a glass slide.

One drop of regular saline was added, then combined with a wood stick and covered with a coverslip. Light microscopy was used for the study, with magnification powers of 10X and 40X (10).

Wet with iodine

Diluted in 1:5 distilled water, Lugol's iodine and a small volume of feces sample were separately mixed to create an iodine wet mount, which was then put on a glass slide, covering the smear with a coverslip (11).

Flotation method

heather's solution was used to carry out the flotation process. The procedure involved using 10 milliliters of distilled water to examine a batch of 2-4 grams of excrement. Subsequently, a forty-angle filter was used to remove large fragments from the feces combination. The filtrates were put in sterile plastic tubes, and after three minutes of centrifugation at 1000 rpm, the supernatant was disposed of. Following a thorough mixing process and a 2-minute centrifugation at 1000 rpm, a 5 ml amount of the sugar solution was applied using wooden sticks for precipitation. To allow the pipette to drop solution vertically, all of the plastic test tubes were placed on hold while the tanks were filled. The glass cover slide was then installed on the tubes at the end for was carefully lifted and placedunder a microscope at magnification strength between 10x and 40x (12).

The Primers

The primers were supplied in lyophilized form and were diluted in highly clean water to produce primer stocks with a final concentration of 10 Pico mole/µl. Until they were used in a concentration, these were stored at -20 oC. 10ul Korea/Macrogen (13) Table(1). The Genotyping isolation DNA were used according to manufacturer instructions.

Table (1): Primers of 18S rRNA gene of Entamoeba species

Prime name	Sequence (5'-3')	Product Size	Reference
Entamoeba 18SrRNA	F: ATTGGAGGGCAAGTCTGGTG R: CATACTCCCCCTGAAGTCCA	600bp	Albanse *et al*., 2019

Sequencing and phylogenetic analysis

The athletic PCR results. The Entamoeba species or genotype infected by the sample was determined by matching it to the first homologous sequence (E value = 0 and Ident >96%) found after BLAST analysis of all the available sequences. With accession numbers MN749972–MN750000, the representative nucleotide sequences that were obtained were uploaded to the GenBank database. Using a previously reported procedure, the MEGA6 program was used to create the phylogenetic tree. (14).

Statistical Analysis

the Chi-square test was used to analyze the current study's data using the SPSS program (version 18), software (2010), and P values of ($p \leq 0.05$) were deemed to indicate statistical significance (15).

Result and Discussion

Isolation of *Entamoeba* spp from children stool

80 Stool samples were collected from villager children (1-6 years) whom attended to (Al – kut Hospital for Gyneclogy and obstetrics and pediatrics. The children president in rural area and directly contact with farm animals. Out of 80 suspected cases were examined microscopically, the results were revealedthat 45(56.2%) which infection with *Entamoeba* spp were show in figure (1). The age group of 3–4 years had the highest infection rate (87.5%), whereas the age group of 1-3 years had the lowest infection rate (26.66%). Table (2) presents the statistical analysis of the data, which revealed a significant variation ($P<0.05$) in the total prevalence of Entamoeba species among the child groups.

Table (2) . prevalence of *Entamoeba* species among children

Age	The Positive sample	The negative samples
1-2	2 (4.4%)	6(17.1%)
3-4	30(66.6%)	19(54.2%)
5-6	13(28.8)	10(28.5%)
Total	45 (100%)	35(100%)
$\chi 2$	21.71	
P value	0*	

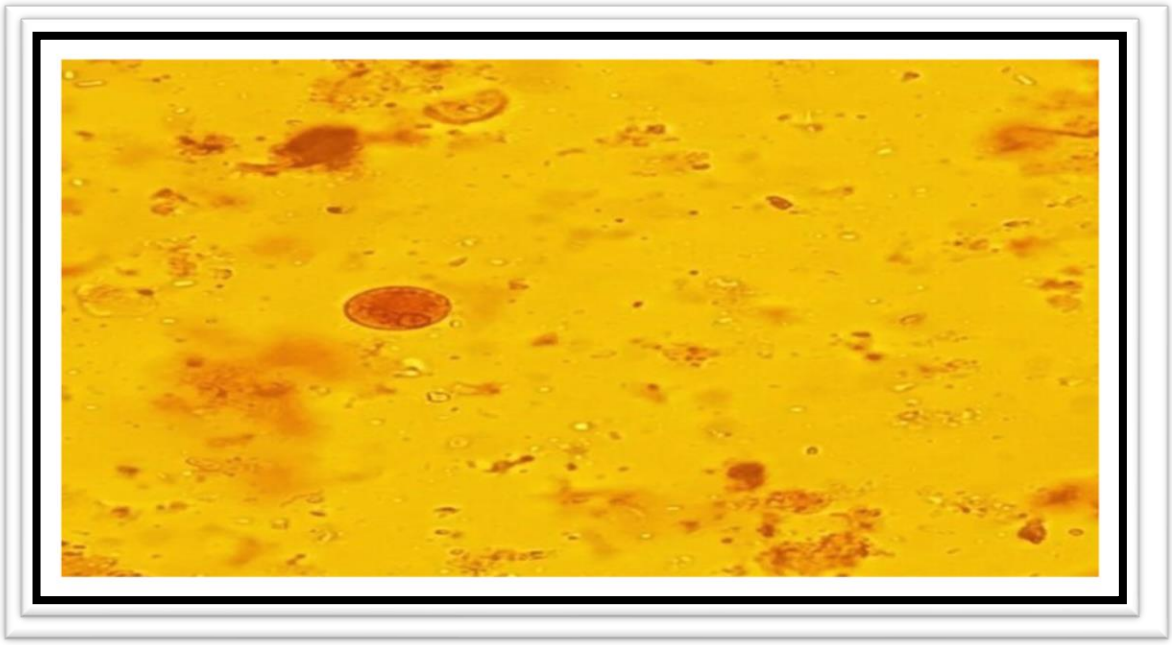

Fig. 1. The cysts of *Entamoeba* spp at (40X)

The result of the present study agreed with results recorded by (16) 66 % of 200 samples from the province of Al-Wasit were found to have Entamoeba species.Infection rates in rural regions were found at 68.6% in Thi-Qar Province (17).The study's infection rate was found to be lower than that of Baghdad, where results from (18) recorded 33.33%. Additionally, the study's findings did not align with those of (19). who found the prevalence of *Entamoeba* 32% in Basrah province, in Baghdad, (20) recorded infection rate was 42.2%, in Basrah , (21)) found the rate of infection was 32.5%. (22) in Baqubah province recorded the rate of infection 30% , (23)in Al-Diwaniya province who recorded infection rate 44%, In Egypt (24) found rate of *Entamoeba* was(32%), in United Arab Emirates, Ali (25) recorded infection rate(19%).In other studies, Zahida *et al*.(26) in Pakistan found overall prevalence of *Entamoeba* was 21.69.Study in Americans by Andrea (27) recorded the infection rate was(30%).The high prevalence of *Entamoeba* species among children potentially be linked to a number of risk factors, including lack of knowledge, crowded living conditions, and tainted water sources. inadequate urban services, age, place of residence, inadequate sanitation, and consumption of raw veggies (28) demonstrated the high prevalence of intestinal parasite infections in poorer nations as a result of inadequate personal hygiene and poor sanitation. The occurrence is strongly correlated with environmental factors, including climate. workers in homes and sanitation (29). Furthermore, families who share a plate, people who eat with their hands, people who eat out, and sanitary professionals are among the groups with higher incidence of Entamoeba species (29).

Genetic characterization of *Entamoeba species*/genotypes in children

Twenty five samples examined, five Entamoeba species, . *E.histolytica* , *E. disper* ,*E. moshkovskii* , *E. polecki*,*E. hartmann*i , were identified . The high prevalence was *E.histolytica* infection was detected in 11(44%) fecal samples, while the low prevalence was E. *hartmann*i 1 (4%) as shown in Table (3). Fig. (2). The result of study agreed with results of Alazawi *et al*.(2009) in Baghdad who recorded infection rate 77% with *Entamoeba* spp in children The nucleotide sequencing of the *18SrRNA gene* local *Entamoeba* spp was checked and confirmed by the NCBI. Based on the Clustal W alignment tool in (MEGA X version), sequence alignment of the local isolated with reference strain of Entamoeba spp. previously recorded in GenBank (30) was utilized to apply for and obtain accession numbers for the current study's sequence, which was filed to the NCBI-Genbank. Because of this, the sequencing data produced by this work will help to comprehend the genetic diversity and geographic distribution of Entamoeba species that infect humans worldwide. Additionally, the DNA sequences followed by phylogenetic analysis represent a useful tool to learn about the evolutionary relationships between organisms.

Table (3). The genotype and species distribution of Entamoeba spp. in children

Entamoeba species	*E. histolytica*	*E. disper*	*E. moshkovskii*	*E. polecki*	*E. hartmann*i
25	11(44%)	6(24%)	4(16%)	3(12%)	1(4%)

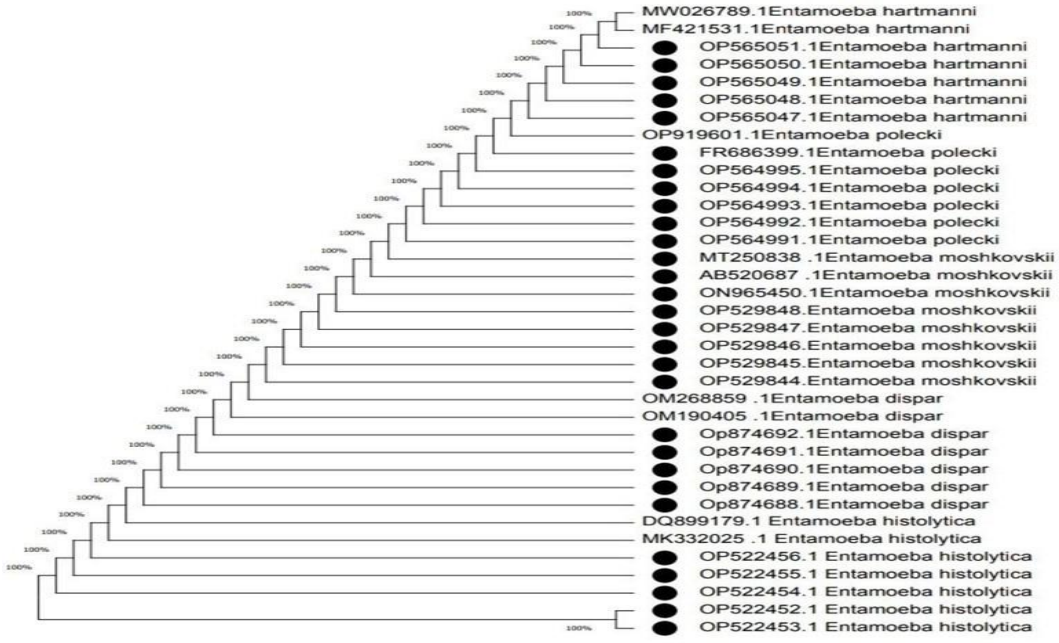

Figure (2). Comparing the multiple sequence alignment of the detected juvenile *Entamoeba* species to global homologous strains

Isolation of *Entamoeba* spp from farm animals

Three farm animals in Waist Province had an overall prevalence of 84% (75/63) Entamoeba spp. infection rates varied from 72% to 100% depending on the animal. In this study, 100% of the cattle tested positive for Entamoeba spp. infection, compared to 80% of the sheep and 72% of the goats.(Table 4).

Table (4). The prevalence of *Entamoeba spp*. in farm animals

farm animals	Positive	Negative
Cattle	25(100%)	0 (0%)
goat	20(80%)	5 (20%)
Sheep	18 (72%)	7 (28%)
$\chi 2$	18.07	
P value	0*	

Entamoeba parasites are easily transmitted to humans and non-human primates due to their simple transmission pattern and zoonotic nature (32). The results of the study agreed with result (33) in that the recorded prevalence of infection in Basrah city was 72.7%. However, the results of the current study disagreed with result (34) in that the observed rate of infection was 23.57.(35). In Baghdad, the infection rate was found to be 15.55%. Additionally, result (36) recorded the prevalence of infection as 25.18%.

Farm animal Entamoeba species and genotypes characterized genetically

Out of thirty samples, three kinds of Entamoeba were analyzed. Three species were identified: E. bovis, E. moshkovskii, and E. histolytica. It was shown that *E. bovis* infection had a high prevalence in 15(50%) fecal samples, while the low prevalence was *moshkovskii* 6 (20%) as shown in Table (5). Fig. (3). The prevalence of *Entamoeba species* according to the species of farm animals were *E. bovis* in Cattle 7(46.6%),in goat 5(33.3%) and in Sheep3(20%).while the *E. histolytica* in Cattle 4(44.4%), goat2(22.2) and Sheep 3(33.3%). and the *E. moshkovskii* were both in Cattle and goat 3(50%), while in Sheep 0(0%). as shown in Table (5).

Table (5). Genotype and species distribution of *Entamoeba spp*. in agricultural animals

farm animals	*E. bovis*	*E. histolytica*	*E. moshkovskii*
Cattle	7(46.6%)	4(44.4%)	3(50%)
goat	5(33.3%)	2(22.2)	3(50%)
Sheep	3(20%)	3(33.3%)	0(0%)
Total	15(50%)	9(30%)	6(20%)
$\chi 2$	23.24		
P value	0*		

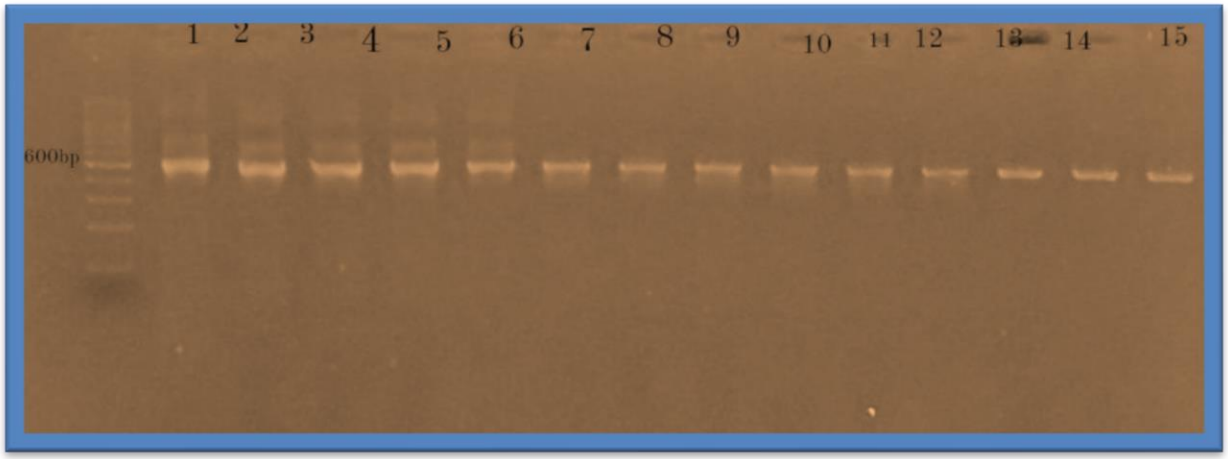

Figure (3): PCR product analysis of the 18S rRNA gene in Entamoeba species of DNA extracted from fecal samples, where ladder (3000-100bp), lanes (1-25) showed positive Entamoeba species at 600bp Pcr product size. Agarose gel electrophoresis image (1.5% agarose).

The result of the current study agreed with result of (37) Alidressi (2019) in Baghdad who recorded infection rate 58.3% with *Entamoeba* spp in the domestic dogs . (38) Sabreen *et al.(*2019) in Baghdad who recorded infection rate 51% in domestic Cattle .The result of the study was incompatible with (39) Baghdad who recorded infection rate 15% in domestic animals A study of (40) 30% of domestic animals in Baghdad had a prevalent infection rate of Entamoeba spp.The National Center for Biotechnology Information (NCBI) examined and validated the nucleotide sequencing of the 18S rRNA gene of the local Entamoeba spp. of dogs used in this investigation. The sequences from this work were registered and assigned accession numbers when they were submitted to the NCBI – Genbank. Sequence alignment of the local isolates with reference strains of Entamoeba spp. already registered in GenBank was carried out using the clustal W alignment tool in MEGA6.0 (41).The possibility that the similarity between country isolates and local isolates resulted from ongoing animal imports into Iraq while bringing in new strains is suggested by the similarities between the two types of isolates. The fact that this parasite can infect a broad variety of hosts may contribute to genetic diversity is true. The parasite's invasion technique may be encouraged by this genetic diversity (42).

Conclusion

The host ranges of Entamoeba species/genotypes were enlarged in this study. The findings contributed to a better knowledge of theWasit province has seen illnesses with Entamoeba spp. in both farm and humane animals, highlighting the critical need to take action to stop this zoonotic disease from spreading to people.

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