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MOLECULAR IDENTIFICATION OF ENTAMOEBA BOVIS IN ASYMPTOMATIC SMALL RUMINANT

Zuhair Mohammed Abed

Salah Al-Din Education Directorate, Ministry of Education, Tikrit, Iraq
*Corresponding author, email: zuhairmohammedabed@gmail.com, zuhairaljanabi0@gmail.com

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Abstract

The genus Entamoeba is a parasite infects many species of ruminant such as sheep and goats, A collection of 192 freshly passed fecal specimens was obtained from asymptomatic ruminants (sheep and goats). the sample were collected from the outskirts of Tikrit, during the period from January-May 2025, A quantity of 200 mg of stool sample was used for the purpose of extracting genomic DNA, a fragment of approximately 900bp from the 18s rRNA gene was amplified employing specialized primers for identify of Entamoeba spp. the sample that scored positive was subsequently sequenced to determine the species. The result demonstrated that the total prevalence of Entamoeba spp. was 11.97% (23/192). the prevalence rate in sheep was 15.23% (16/105) in goats 8.04% (7/87), a higher prevalence rate was observed in sheep lees than 12 months old (16.45%) compared to over 12 months old (11.53%), but without was not statistical significance, note the higher prevalence rate in spring compared winter 9.57% (9/94),7.14% (7/98) respectively, the difference did not reach statistical significance, based on sequence analysis, Entamoeba bovis has been identified in asymptomatic ruminants.

1. Introduction

The genus *Entamoeba* is a parasite that live in many hosts, such as human and animals (Servian et al., 2024; Wilson et al., 2019), most of cases are asymptomatic, but in other cases it develops into extensive intestinal disease and leads to weight loss, stomach pain, cramps and diarrhea that lasts for weeks. Extra intestinal infection can lead to liver abscess, purulent pericarditis, brain amoebiasis and pneumonia (Kantor et al., 2018).

Molecular methods have been used to identify *Entamoeba* species because it is difficult to distinguishing between morphologically similar species under the light microscope. this has led to a significant understanding of their classification, phylogenetic relationships and epidemiology (Calegar et al., 2021; Huaman et al., 2022). The extensive genetic variation within morphologically similar *Entamoeba* parasites, combined with their ability to infect a wide range of hosts, there is a continuing need to characterize the genetic variant and parasite hosts, especially for pathogenic and zoonotic species (Huaman et al., 2022), the term ribosomal lineage (RLs) have been recently used to classify lately identified *Entammoeba* 18s rRNA sequences that differ by more than 5% from the previously identified species of *Entamoeba* and to date 11RLs have been detected (Ren et al., 2021). Until now, several species or genotypes of *Entamoeba* have been documented in ruminants hosts, including *E. bovis* and *Entamoeba* RL1, *Entamoeba* RL2, and *Entamoba* RL4 (Stensvold et al., 2011). The presence of *E. bovis* and *Entamoeba* RLs was also documented in goats (Ai et al., 2021).

The *E. bovis* infects several ruminant animals and infection can occur as a result of consuming food or water contaminated with parasite cysts through the oral (Stensvold et al., 2010), *E. bovis* and closely related species live in the rumen of mammalian ruminants and are characterized by their morphological similarity to *Entamoeba histolytica* (Stensvold et al., 2010), the rate of *Entamoeba* infection in sheep reached 49.1%, slightly exceeding the rate recorded in cattle. As observed in cattle, *E. bovis* was the most common parasite, while remaining positive sample was limited to one case of RL2 and three cases in which *E. bovis* was detected in mixed infection. The inclusion of RL2 in this category represents an expansion of the host range for this lineage, previous reports of it were limited to cattle only (Stensvold et al., 2011). Only limited studies have been performed on the genetic characteristics of *Entamoeba* species that infect ruminants. The aim of this research is to identify some common species of *Entamoeba* in the study area.

2. Method

2.1. samples collections

A collection of 192 freshly passed fecal specimen was obtained from herds of sheep and goat, which were asymptomatic. the sample were collected from the outskirts of Tikrit, during the period from January-May 2025, all sample were kept in sterile container labeled with the date of collection ,age ,sex, and the type of animals and transported to the laboratory at Tikrit University and preserved at-20℃.

2.2. DNA extraction

Use 200 mg of stool specimens for genomic DNA extraction as stated in the manufacturer's instruction of stool DNA extraction Kit, in the PCR reaction, specific primers (5'-TAAGATGCACGAGAGCGAAA-3') (5'-TACAAAGGGCAGGGACGTA-3'), were employed to "amplify a fragment of the 18s rRNA gene" (about 900bp) aimed molecular identification of the genus *Entamoeba* (Khairnar & Parija, 2007), the reaction were carried out at volume of 25 µl. the reaction mixture include 1µl for both forward and reverse along with 3 µl of template DNA. The PCR program include: an preliminary denaturation step was performed at 95℃ for 5 min, 30 cycles composed of 94℃ for 30 s, 30 s at 58℃ , 72℃ for 30 s, with a final elongation of 72℃ for 5 min. the resulting PCR products were subjected to electrophoresis on 1.5% agarose gel for separation to detect positive samples for *Entamoeba* sp. Six PCR-positive sample were sequenced and the species or genotype was determined by aligning the 18s rRNA sequences with those available in the NCBI database, was carried out by BLAST for analysis and identification. The phylogenetic tree was by MEGA11 (Tamura et al., 2021).

3. Result and Discussion

3.1. Result

The overall prevalence of *Entamoeba* spp. was 11.97% (23/192) by PCR amplification of 18S rRNA gene, as shown in Figure 1. The prevalence rate in sheep was 15.23% (16/105) and in goats was 8.04% (7/87). The prevalence rate found in male sheep were almost identical to those of females, 15.78% (6/38) and 14.92% (10/67) respectively, no statistically significant difference was observed (p = 0.906). Similarly, prevalence of the disease in goats was similar between males 8.82% (3/34) and females 7.54% (4/53) (p = 0.833). However, the prevalence of *Entamoeba* was detected in sheep over 12 months of age 11.53% (3/26), which was less than in sheep under 12 months of age 16.45% (13/79), but without statistical significance (p = 0.514). While in goats, the prevalence rate in the group under 12 months of age was 9.09% (2/22), higher than in the group over 12 months 7.69% (5/65) (p = 0.841). Notably, the prevalence rate in spring was higher compared to winter, 9.57% (9/94) and 7.14% (7/98) respectively, however the difference did not reach statistical significance, as presented in Table 1.

Table1. *Entamoeba* spp. infection prevalence in sheep and goat.

Variable		No. sample	No. positive	Percentage	p.value
Host	Sheep	105	16	15.23%	
Sex	Mel	38	6	15.78%	0.906
	Female	67	10	14.92%	
Age	< 12	79	13	16.45%	0.514
	>12	26	3	11.53%	
Host	Goat	87	7	8.04%	
Sex	Mel	34	3	8.82%	0.833
	Female	53	4	7.54%	
Age	>12	65	5	7.69%	0.841
	<12	22	2	9.09%	
Season	Winter	98	7	7.14%	0.543
	Spring	94	9	9.57%	
Total		192	23	11.97%	

The species or genotype of *Entamoeba* was determined by carried out at sequence analysis of PCR product (Amplify about 900bp of 18s rRNA gene) , sequencing results indicated that infection in sheep and goats is limited to a single species, *E. bovis* .

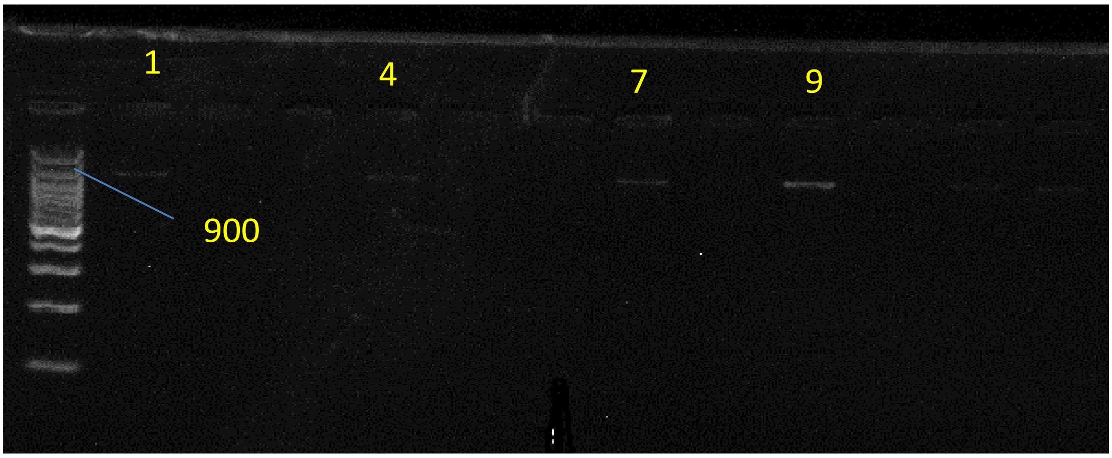

Figure1. Lane 1 ,4,7 and 9 are positive for *Entamoeba* spp.

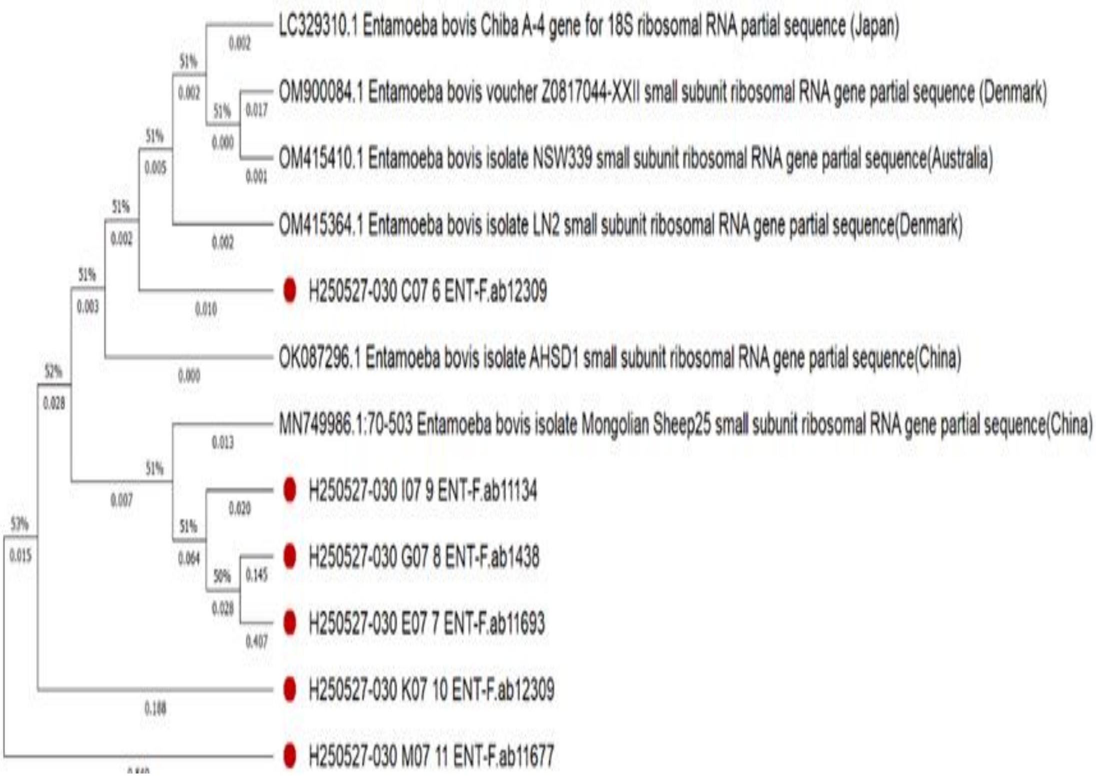

Figure 2. Phylogenetic tree of *Entamoeba* spp. isolated from asymptomatic ruminants and reference sequence from GenBank.

3.2. Discussion

Information is currently available on the Pathogenic effects of *Entamoeba* species in ruminants, which have been documented worldwide, however, previous studies have, shown that the species of *Entamoeba* were detected frequently in cattle do not show clinical sign such as diarrhea, so consider the species of *Entamoeba*, including *E. bovis* and *Entamoeba* RLs are non-pathogenic or have low effect in ruminant (Huaman et al., 2022; Matsubayashi et al., 2018). According to the results of this study, the overall prevalence of *Entamoeba* spp. in asymptomatic ruminants was 11.97%, as shown in Table 1. In a previous study, a higher prevalence of *Entamoeba* was found in animals without disease compared to those with diarrhea (Fu et al., 2023), this result is lower consistent with the result Jasim & Alardi (2015), who recorded 65.9% infection rate in human and animals. In Diwaniah reached of rate infection in cattle 66% by microscope and 72% by PCR (Jawad et al., 2023), Baghdad province 58% (Al-Dabbagh et al., 2024) in china 51.5% and 93.67% (Wu et al., 2025a; Wu et al., 2025b) Greenland 14% (Stensvold et al., 2023) the prevalence rats vary depending on several factors, climatic, geographical location, the nature of the environment, in addition to the level contamination with the causative agent, as well as the irregular distribution of parasite in different countries (Roy et al., 2005). The study result indicate the prevalence did not significantly differ between the sexes of both sheep and goats, with respect to age group, the highest increase in the prevalence of *Entamoeba* was observed in sheep less than 12 months old but without statistical significance, this may indicate that age may contribute to the spread of *Entamoeba* in sheep and may be attributed to several factors including development of host immunity (Liu et al., 2022) in addition, the study reported an elevated incidence of *Entamoeba* during the spring compared to winter, the difference did not reach statistical significance, this increase may be attributed to moderate temperature and abundant pasture, which increases the possibility of ingesting parasite cysts.

In present study, based on sequence analysis, *E. bovis* has been identified in asymptomatic ruminants. According to SSU rRNA gene analysis, the *Entamoeba* commonly found in ruminants has been classified into four species or genotype, *E. bovis*, *Entamoeba* RL1, *Entamoeba* RL2 and *Entamoeba* RL4 (Matsubayashi et al., 2018) in previous studies based on phylogenetic tree analyses, most of the *Entamoeba* spp. isolates from goats exhibited closed related with *E. bovis* sequences deposited in the NCBI-BLAST database, while others were related to *E. histolytica* (Mohammed, 2017). According to one study, *E. bovis* has only been recorded in sika deer (Liu et al., 2022), in other studies, several species of *Entamoeba* (*E. bovis*, RL2, RL4 and RL8) were identified based on SSU rRNA sequences in ruminant (Wu et al., 2025a), in study, four species of *Entamoeba* were found *E. bovis*, *E. histolytica*, *E. moshkovskii*, *E. ecuadoriensis*, and most animals infected with the genus *Entamoeba* (94.2%) were caused by *E. bovis*, six genotype of *Entamoeba* have also been identified (RL1, RL2, RL3, RL4, RL8 and RL9) by sequence analysis (Ai et al., 2021), phylogenetic analyses have indicated a close relationship between *E. bovis* and both *Entamoeba* RL3 and RL8 (Stensvold et al., 2011), which is consistent with the phylogenetic tree constructed in the present study, as shown in Figure 2.

4. Conclusion

In this study, the species of Entamoeba in small ruminants and their prevalence were investigated. The overall prevalence rate was 11.97%, the species or genotype of Entamoeba was determined by carried out at sequence analysis of PCR product (Amplify about 900bp of 18s rRNA gene), Entamoeba bovis has only been identified in asymptomatic ruminants.

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