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Research article

Molecular and microscopic study of *Entamoeba histolytica* in Camels in Al-Qadisiyah & Al-Najaf Al-Ashraf Provinces

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Abstract

Using a PCR technique, the detection of Entamoeba histolytica in camels was the main reason for performing of this study. Two hundred fecal samples of the camels from the slaughterhouses & nomadic areas of Al-Qadisiyah and Al-Najaf Al-Ashraf provinces. Some of camels suffered from diarrhea & other asymptomatic animals, their ages range from less than 1 year to more than 1 year. Whereby showed the prevalence of Entamoeba histolytica infection was (30.5%) by Lougal iodine stain method according to using microscopic examination for description of E. histolytica cysts. While the prevalence rate depending the PCR technique there was (61%).

Key words: *Entamoeba histolytica*, Camels, Lugol's iodine stain, PCR, Iraq.

Introduction

Camels are valued as one of the important sources that provide people with food from their meat and milk. This importance could reach to the use of their wool in industries such as wool-based clothing production. In addition and for thousands of years, camels have been used for transportation. For quality assurance and especially in the desert-based climate, the meat of camels is considered to be among the best according to its quality and nutritional components. Moreover, comparing the quality of camel milk to other animal types of milk, the first is estimated to be with high quality criteria (1, 2). (3) Point to that the numbers of parasites are responsible for enteric infections in ship of deserts (camel), parasitic infection causes great losses to camel. *Entamoeba* is a single-celled protozoan parasite belonging to the subphylum Sarcodina (4) .When an infection is induced by *E. histolytica*, colitis and liver abscess are the main conditions that these protozoa could cause. The protozoal activity is divided into two stages of movable-pervade trophozoite and the effective cyst. While the single-nuclear trophozoites are characterized by 10-50µm of diameter, the 4-nuclear cyst is characterized by 10-15µm of diameter. These cysts are resistant to chemical-based destruction and weeks of dampness-based environment. Contaminated food and water-based beverages are the main method of infection induction (5). The main pathogenesis steps for these moving stages are started when the trophozoites are settled on intestinal-mucosal to pass through them. A dissemination process is followed to invade body organs and cause spotty infections such as in colons and livers. After that, these trophozoites are involved in cyst formation that gets out with feces (6). In Iraq, *Entamoeba* spp. infection recorded in cattle and sheep by using PCR method by (7). Generating primers that target the 18S rRNA gene was followed to identify those protozoa via PCR in fecal samples from various sources (8,9). Moreover, *Entamoeba histolytica* was also diagnosed in fecal

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samples using some reliable tests such as ELISA, direct fluorescent-antibody assay, and PCR. The technique represents a full-specificity and -sensitivity method for identifying these protozoa in feces (10). To make sure the process of the protozoal identification go well, a successful and high quality DNA extraction method should be followed (11). Optimization of the PCR technique had been done before the current study was started (12).

Aim of study: Detection of *Entamoeba histolytica* cause diarrhea in camels by microscopic examination & by PCR.

Materials and Methods

Ethical approval

The Animal Ethical Committee of Veterinary Medicine College, University of Al-Qadisiyah, Iraq, has approved the present study under permission No: 439

Fecal samples collection

Total of 200 camel's fecal specimens were collected from abattoirs and nomadic areas in Al-Qadisiyah and Al-Najaf Al-Ashraf Provinces. Some of these camels was suffered from diarrhea and other asymptomatic camels in age ≤ 1 year to more than 1 year. These samples were collected in the sterile plastic containers and stored in the large containers containing ice bags, then transported to the parasitology laboratory in the College of Veterinary Medicine, University of Al-Qadisiyah to perform the examinations.

Microscopic examination:

Using a direct-wet smear technique involving the use of Lugol's iodine, the detection of protozoan stages was initiated (13).

Concentration method

For active diagnosis of small numbers of protozoan stages in fecal samples, a concentration method was followed to notice these parasitic forms via either flotation or sedimentation (14).

Polymerase chain reaction (PCR)

The PCR technique was used for determined *Entamoeba histolytica* according to subunit ribosomal rRNA gene from camel fecal samples. This test was carried out based on test described by (13) as following steps:

A-DNA Extraction genomic material: Fecal samples of camels were subjected to AccuPrep® stool DNA Extraction Kit® (Bioneer, South Korea) to extract DNA.

B-DNA evaluation: The DNA that was resulted from the extraction process was checked for its quality and quantity. Nanodrop spectrophotometer (THERMO, USA) was used to perform this step. The unit of ng/µL was followed to measure the concentration of the DNA.

C- Generation of the Mastermix: The manufacturer's protocol was used to prepare this Mastermix (AccuPower® PreMix Kit).

D-PCR Thermocycler Conditions: All PCR reactions were done at same thermocycler conditions by using convential PCR thermocycler system.

E-Analysis of the PCR-based products: Agarose-based gel electrophoresis was utilized to perform this process.

Statistics analysis

Multiple evaluations of effectors were carried out using Chi–square (x^2). Significant analyses were decided if $p \leq 0.05$ (15).

Results

1-The prevalence according to microscopic examination:

Out of 200 fecal samples, 61(30.5%) were positive to the infection according to microscopic examination in which trophozoite & cysts were observed Figure (1, 2), Table (1)

Table (1): The infection with *Entamoeba histolytica*

N0. of tested samples	No. of positive samples	Percentage (%)
200	61	30.5%

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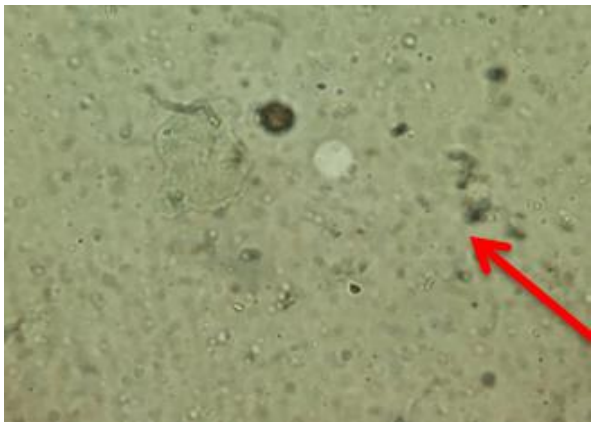

Figure (1) *Entamoeba histolytica* trophozoite (x100) by using lougal iodine stain.

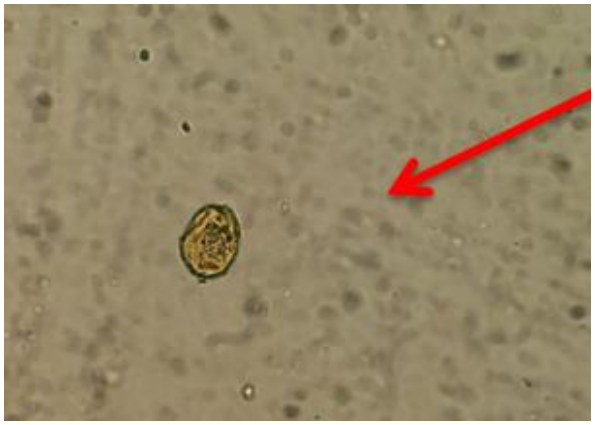

Figure (2) *Entamoeba histolytica* cyst (x100) by using lougal iodine stain.

2-The prevalence according to animal age:

Out of 61 infected camels, (8) were recorded in age less than 1 year and (53) more than 1 year, Table (2)

Table (2): The prevalence of infected camels with *Entamoeba histolytica* according to the animal age

Age	Infected	Percentage (%)
Less than 1 year	8	13.11% [a]
More than 1 year	53	86.89% [b]
Total	61	100%

Different letters = significant differences (p<0.05)

3-The prevalence according to polymerase chain reaction (PCR) technique:

Out of 100 fecal samples were examined by PCR technique there were 61% were positive according to PCR technique Table (3), Figure (3).

Table (3): Total prevalence of *E. histolytica* by using PCR.

No. of tested samples	No. of positive samples	Percentage (%)
100	61	61%

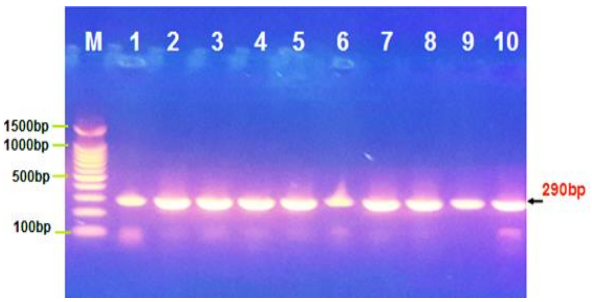

Figure (3): *E. histolytica*-18S rRNA gene based PCR product analysis via gel electrophoresis. M is the ladder; 1500-100bp. Columns 1 to 10 are the gene-positive products of 290bp.

Discussion

The prevalence reported by microscopic diagnosis according to Lugol's iodine stain under light microscopy in camel (30.5 %). These results were agreed with (16) when he recorder infection rate of *Entamoeba* spp. in the animals that belonged to some animal zoos when study the intestinal parasites which spread at the zoo. These results are in agreement with (17), who recorded an infection rate of *Entamoeba histolytica* reached 39.44% in sheep in Wasit Governorate/ Iraq. (18) Identified the infection rate was 24.6% in some animals of public parks in Britain. The results were in opposition to those from a study that was initiated on camels in Najaf, Iraq) 13), who found that the rate was 20%. In addition (19) showed *Entamoeba* sp. infection were (17.85%) in camels in Nagpur region. (7) Recorded the presence *E. histolytica* by microscopic examination were (58.3%) in cows and sheep. Also, disagree with the results (20) which showed that the infection rate of *Entamoeba* spp. in dogs of Ibadan were 58.2 %. In Al-Qadisiyah-Iraq, (21) reported that the infection rate was 57.17% for some animals such as donkeys and horses.While the prevalence rate of *Entamoeba* spp. infection depending on the PCR technique in our study (61%). The

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results are agree with results, which recorded by (7) that infection of *E. histolytica* was 78.5% in cows and sheep. Moreover, conformed to (22) were found prevalence rate of *Entamoeba* spp. 79% in rhesus macaques (*Macaca mulatta*) in India by using a genus-specific PCR.

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