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Traditional diagnosis of *Entamoeba histolytica* species at Babylon province, Iraq

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Abstract

In this study the (100) samples that was taken from different areas from Babylon province shows that the most infection rate in female (74%) male (26%) since most of sample collected were form female and most of cases found in barns were female, the infection with samples examined microscopically was higher in Al-Qasim- All-fayathea since most of area live with cattle and barns and the lower infection in the center of Babylon province which is the same result that's showed by flotation technique and in Almosyab 9 hor – hussain and highest infection rate showed by the flotation technique was in Al-Qasim in Al-Fayathea, most of cases was normal in shape but seen an infection with if with both examination methods which proves that diarrhea is not the only shape for eye examining, it was found that the group of age from (1-2 years) are most positive infection cases (24%), and because most of them females they most expose to poor hygiene and poor sanitation.

Keywords: Entamoeba histolytica, protozoa, diagnosis, species, amoebiasis

Introduction

Amoebiasis, an infection by intestinal protozoa belongs to the genus Entamoeba consist of several species (e.g. E. histolytica, E. dispar, E. moshkovskii, E. coli, E. poleki and E. hartmanni). E. histolytica considered the third most important parasitic cause of human death rate after malaria and schistosomiasis. The transmission of amoebiasis in developing countries is usually due to poor sanitation, poor hygiene, and crowded living conditions, whereas it is mostly transmitted in developed countries by people who travel from endemic countries (Fotedar et al., 2007)^[10]. Most cases arise from human carriers, or cyst passers, which pass in formed or semi formed stools. Monkeys, dogs, cattle, and probably pigs are naturally infected with E. histolytica, although these animals are only a small source for human exposure when compared to man himself (Watanabeand Petri, 2015), Entamoeba histolytica, a protozoan, is the source of the illness sometimes known as amoebic dysentery. The majority of cases are asymptomatic, but extensive intestinal illness can develop and cause weight loss, cramps, stomach discomfort, and diarrhea (could be bloody) that lasts for many weeks. Diffused extraintestinal illness is reported to cause purulent pericarditis, liver abscess, pneumonia, and even brain amoebiasis. E. histolytica is thought to affect up to 50 million people globally, mostly in underdeveloped nations, and it is the cause of over 100,000 fatalities each year. The most common methods of transmission are through the consumption of contaminated food or water brought on by the fecal expulsion of cysts, as well as fecal-oral transmission and due to homosextuality, a man having sex with another man. Amoebiasis is a disease that affects people everywhere, especially in countries with poor sanitary systems. Infection is rising in wealthier nations like those in North America as a result of an upsurge in emigration and travel from highly disease-prevalent countries. India, Africa, Central and South America, especially Mexico, have the greatest infection rates. Males and females almost equally contract amoebic colitis at the same rate. Males are ten times more likely to develop an amoebic liver abscess (ALA) than females, and those between the ages of 18 and 50 are most frequently affected E. histolytica is an invasive intestinal protozoan. When mature quadrinucleated cysts are ingested in food or drink contaminated with fecal materials, an infection usually starts. Motile trophozoites are released after excystation in the small intestine to the large intestine. Both trophozoites and cysts are produced by binary fission, and both are excreted in feces, but only

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cysts have the capacity to transmit infection because of their hard wall. While trophozoites are quickly killed when they leave the body or by stomach secretions if consumed, cysts can persist days to weeks in such environments. Trophozoites are able to attach to and destroy the colonic epithelium before blood-stream, spreading to far-off locations including the peritoneum, liver, lung, or brain via the portal vein system.

Leonard Rogers proposed the first effective treatment for amoebiasis in 1912. Treatment for intrusive disease contrasts from treatment for painless contamination. Harmless contaminations might be treated with paromomycin. The backbone of treatment for obtrusive amebia 2016). Nitroimidazoles, such as tinidazole, secnidazole, and ornidazole, are more easily tolerated and can be administered more rapidly. Roughly 90% of patients with gentle to direct amebic diarrhea responsed to nitroimidazole treatment. Different helpful methods are utilized for digestive and extra gastrointestinal amoebiasis. Collecting 100 samples from infected cattle diagnosed through clinical examination and directed fecal by microscopic. Collecting animal samples from Cow barns and recording infection rates according to their age, sex and regions.

Materials and Methods

Study composed of two lines; the first one is examination all samples that has been collected based on traditional technique, and the second line include examination the same samples by flotation technique. Stool samples (<0.1) grams were collected 100 sample from cattle at different ages, and of both sexes (males and females) during the period from the last quarter of October (2023) to end of November (2023) The samples were collected from included (Al-fayathia, Al-deseem, 9-hor hussain, Al-mossayab and hilla noun) in sequence.

Stool samples were collected directly from the intestine directly and mostly fresh, in a clean plastic container and were tightly closed, given sequential numbers, with taking off protective measure such as wearing disposable gloves. All information included age, sex, and date of sampling. The samples were transported in refrigerated bags to a Parasitology laboratory in the College of Veterinary Medicine- Al-Qasim green University in Babylon province.

All (100) fecal sample has examined microscopically by take a drop of the sample after mixed with normal saline by wood stike and covered by a coverslip, the examination was done with light microscopy at 10X and 40X magnification powers.

Then all the samples were examined by flotation technique by using Sheather's solution which includes suger, distilled water, sieve, wooden stick, tubes and centrifuging process was applied on all samples after treated with sheather solution then microscopic examination.

Direct Wet Smear

One drop of fecal sample taken and put on a glass slide. A coverslip was applied after addition of one drop of normal saline and mixed with a wood stick. The examination was done with light microscopy at 10X and 40X magnification powers.

Flotation method

The flotation method was performed by use of Sheather's solution. The steps of the method were by examining a batch of 2-4 gm of feces with 10ml of distilled water. Then to extract big fragments, the mixture of feces was washed out with sieve sized of forty angles. The filtrates were placed in sterile plastic tubes and centrifuged at 1000 rpm for 3 min then the supernatant was discarded. 5ml volume of sugar solution was applied for precipitation using wooden sticks; then the latter mixed properly and centrifuged at 1000 rpm for 2 min. all plastic test tubes were put on hold to fill the tanks letting the Pipette to drop solution vertically. Finally, the glass cover slide was then put on the tubes for 10-15 min. to scan the Entamoeba spp stages cyst and trophozoite, the glass cover slide was carefully lifted and placed under a microscope at magnification strength between 10x and 40x (Charles and Robinson,2006; Al-Kaabi, 2009).

Results

According to the results below which have been divided in to sex, age, area and examination methods, the (100) feces samples were taken directly form cattle in cattle barns with the colleting measurements and examined directly by microscopic examination and then with floatation tcqunice and microscopic examination after treated with shatter's solution.

The reading shows that the rates of infections by area that samples were examined ith microscopic examination was Al-Qasim – alfayatha (35.29%), Al-Qasim – aldseem (30.77%), Al-mosyab - 9 hor hussain (11.54%), Al-latifiya (18.92%), Babil center (0.00%), the higest infection rate was in Al-Qasim – alfayatha (35.29%) and the lowerest infection rate was in babil center by (0.00%).

And the distribution of sample study according to Flotation techquine with difference Area that's are positive and shows that Al-Qasim – alfayatha (35.29%), Al-Qasim – aldseem (38.46%), Al-mosyab - 9 hor Hussain (0.00%), Al-latifiya (2.70%), Babil center (0.00%), accordind to the listed results shows the highest positive rates was in Al-Qasim – aldseem (38.46%) and with lowerest positive cases in Babil center (0.00%) and Al-mosyab - 9 hor hussain (0.00%) and the figure and table 2 shows that.

The samples take different shapes during collecting Normal, bloody, mucoid, semi-mucoid, watery and semi-watery.

Table 1: Distribution of sample study according to Microscopic ex with difference Area

Area	Quantity of complex	Micros	D volue					
Alea	Quantity of samples	Positive No. (%)	Negative No. (%)	r-value				
Al-Qasim – alfayatha	17	6 (35.29%)	11 (64.71%)	0.295 NS				
Al-Qasim – aldseem	13	4 (30.77%)	9 (69.23%)	0.307 NS				
Al-mosyab - 9 hor hussain	26	3 (11.54%)	23 (88.46%)	0.0016 **				
Al-latifiya	37	7 (18.92%)	30 (81.08%)	0.0009 **				
Babil center	7	0 (0.00%)	7 (100%)	0.0086 **				
Total	100	20 (20.00%)	80 (80.00%)	0.0001 **				
P-value		0.0085 **	0.0001 **					
** (<i>p</i> ≤0.01).								



Fig 1: Distribution of sample study according to microscopic ex with difference Area

Area	Quantity of complex	Flotation	D voluo				
Alea	Quantity of samples	Positive No. (%)	Negative No. (%)	r -value			
Al-Qasim – alfayatha	17	6 (35.29%)	11 (64.71%)	0.295 NS			
Al-Qasim – aldseem	13	5 (38.46%)	8 (61.54%)	0.406 NS			
Al-mosyab - 9 hor hussain	26	0 (0.00%)	26 (100%)	0.0001 **			
Al-latifiya	37	1 (2.70%)	36 (97.30%)	0.0001 **			
Babil center	7	0 (0.00%)	7 (100%)	0.0086 **			
Total	100	12 (12.00%)	88 (88.00%)	0.0001 **			
P-value		0.0219 *	0.0001 **				
* (<i>p</i> ≤0.05), ** (<i>p</i> ≤0.01).							

Table 2: Distribution of sample study according to Flotation technique with difference Area



Significant ($p \le 0.05$). Highly Significant ($p \le 0.01$). NS. Non-Significant.

Fig 2: Distribution of sample study according to Flotation technique with difference Area

Table 3: Shows the groups of ages and infection by sex, examination methods together

Age		Sex		Sample state			Examination methods				
More than two years	More than year to two tears	3 months to 1 year	Male	Female	Normal	Bloody	Watery	Mucoid	Microscopic	Flotation	
2	24	1	10	19	18	1	7	3	22	18	Positive
11	51	11	16	55	63	0	6	2	78	88	Negative



Pictures of cyst and trophozoite under microscope 40x

The picture above shows clearly the cysts and the multinucleated under microscope with 40x cyst considered the infective form of *Entamoeba histolytica*.

And in the second tow pictures shows the trophozoite and with red blood cells that ingested by the trophozoite since the last is the nutritional shape of *Entamoeba histolytica*.

Discussion

In the present study, investigation about *Entamoeba* species in 100 e cattle samples revealed high infection rate (32%) with

20% microscopic examination and 12% with flotation technique. The high prevalence of *Entamoeba* species of cattle in Babylon province could be attributed to weakness and emaciation in animals as a result of lack of food, which leads to immunodeficiency, climate change, severe drought, hunger and nutritional deficiency, especially in minerals, mixing of animals and contact with infected animals or importing animals from different countries helps in the spread of the parasite.

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According to the sex, the present study recorded high infection rate in females 74% than males 26%.

According to the age, the higher infection rate was recorded in age> 1 years (), while the lower infection rate () was recorded in age () years, statistical analysis of the data showed significant variation () on the overall prevalence of *Entamoeba* species between both age groups. Both of these factors lead to immunosuppression, making cattle that are malnourished or constantly exposed to a stressful environment.

Conclusion

The study revealed that the total infection rate with *Entamoeba* spp was high in cattle in Babylon province according to study samples isolates could be related to many risk factors such as poor sanitation, contaminated water supplies age, The study recorded significant difference of infection rate between male and females in cattle higher infection rate was in females than males with significant difference which was 74% with female 26% males since most of examined samples was female and most of the cases founds in barns was females.

The group of age also showed significant difference form the other groups 24% in the range from (1-2years) since most of these are female and pregnant and expose to not fully sanitation food and other environmental agents.

Conflict of Interest

Not available

Financial Support

Not available

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