Scientific Research Publishing

Advances in Bioscience and Biotechnology, 2017, 8, 399-410
http://www.scirp.org/journal/abb
ISSN Online: 2156-8502
ISSN Print: 2156-8456

Prevalence of Two Gastrointestinal Parasites *Entamoeba histolytica* and *Giardia lamblia* within Samarra City, Iraq

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How to cite this paper: Bazzaz, A.A., Shakir, O.M. and Alabbasy, R.H. (2017) Prevalence of Two Gastrointestinal Parasites *Entamoeba histolytica* and *Giardia lamblia* within Samarra City, Iraq. *Advances in Bioscience and Biotechnology*, **8**, 399-410.
https://doi.org/10.4236/abb.2017.811029

Received: September 28, 2017
Accepted: November 7, 2017
Published: November 10, 2017

Abstract

The prevalence of two gastrointestinal parasites the *Entamoeba histolytica* and *Giardia lamblia* parasites and their impact on some blood parameters, *i.e.* packed cell volume (PCV), hemoglobin (Hb%) and total protein (TP) of a total 780 patients (children and adults) admitted to Samarra General Hospital were assessed. Samples of fresh feces were collected in normal physiological saline and examined using Olympic microscopes. The frequency of the parasite *E. histolytica* was 12.8% (46.3% male and 53.6% female). The highest frequency of infection of *E. histolytica* (13.8%) was found at age group (1 - 5 years old) followed by <1 year old children while the lowest (7.4%) was at ages (>41 years old). The highest rate of infection (33.9%) was found in September and the lowest (2.2%) in January. Similarly, the general infection frequency of the parasite *G. lamblia* was 3.9% with the highest rate at ages 1 - 20 years old and the lowest rate was 7.3% for >50 years old. The monthly, highest rate of infection (5.2%) was in August and least (2.2%) in January (2.2%). The frequency of total protein (TPD) in the blood relevant to the presence of parasite *E. histolytica* and *G. lamblia* was 4.6% and 1%, respectively. It is concluded that the above two parasites are the most common gastrointestinal parasite in Iraq whose pathogenesis to be which is likely to escalate during the summer seasons and at low hygienic services environment. There has been an irrelevance neither to anemia nor total protein deficiency. It is recommended that Ministry of Health in Iraq should not share the global idea of defining the giardiasis as a neglected disease.

Keywords

Entamoeba histolytica, *Giardia lamblia*, Samarra, Iraq, Total Protein Deficiency, Blood Parameters

1. Introduction

Infection with parasites may take place by several routes e.g. direct contact with an infected person, a vector animal or indirectly via human carriers of the microbes while parasites mainly enter the body through the skin or mouth. Infection could also be achieved via a close contact with pets particularly dogs and cats, which are host to many parasites. However, food contamination allows spread of the germs to many people through a single source [1]. Diseases caused by parasites are numerous but most common are amoebiasis and giardiasis which spread worldwide including Iraq, and lead to severe diarrhea. Although the diarrhea refers to a pathogenic status of excreted stool it may sometimes mean otherwise, *i.e.* a quick waste passage through the gut. The latter could be due to a few reasons, e.g. infection of the large intestine and rectum by intestinal parasites, food poisoning, contaminated dairy or may result in certain diseases *i.e.* tuberculosis, cholera, typhoid, or dysentery. Bacterial diarrhea is often accompanied by fever, intestinal colic, bleeding, abdominal pain, pale and underweight.

Gastrointestinal parasites are also considered as the main source of diarrhea, in the third causative of mortality in the world where number of death tolls could reach above 1.8 million people a year [2]. The World Health Organization (WHO) has estimated that currently around 3.5 billion people are infected with intestinal parasites and 450 million people suffer from the results of those injuries [3]. The *Entamoeba histolytica* is the most common protozoan intestinal parasite of humans which affects large intestinal areas leading to ulcerations and spread dysentery particularly in the third world nations, *i.e.* equatorial, Far Eastern and South American countries [4]. Approximately 500 million people are infected with the parasite each year leading to almost 100 thousand deaths a year and about 10% of cases are asymptomatic. Others may not be accompanied by severe injuries depending on species, nutritional status, immunity of the host, and the coexistence of other intestinal bacteria [5].

The life cycle of the parasite is summarized in a direct transition of cystic phase through contaminated food and water extremes to the gut cavity where the trophozoite phase is immerged to invade the mucosa layer of the intestine leading to various severe symptoms pending on the location and density of ulcers. The latter could cause intestinal colic and diarrhea and may occasionally be accompanied by bleeding of mucosa, loss of appetite, vomiting, loss of weight, fatigue, loses of electrolytes and minerals as a result of diarrhea [6]. Loss of sodium and potassium ions is considered the most important reason of protozoan intestinal infection leading to malnutrition and anemia [7]. Infection could be transmitted to the extra-intestines regions, *i.e.* through pyloric hepatic vein leading to liver and lung abscesses [8] [9].

The *Giardia lamblia*, another intestinal parasite found in large numbers in the gastrointestinal lumen could cause giardiasis represented in damage and changes in inflammatory in the mucosal cells lining lumen of gallbladder or/and bile duct leading to weight loss and mal-absorption [10]. These parasites will compete the

host in their food, particularly, irons, vitamins, carbohydrates, minerals, proteins and fats elements giving rise to malnutrition and anemia.

A few similar surveys have already been carried out in some parts of the world, *i.e.* Pakistan [11], Brazil [12], Iran [13], Portugal [14], Ethiopia [15], Ghana [16] Similar [17]-[26]. Most recently, a study was carried out covered years 2009-2014 involved six most common parasitic pathogens within Kerkuk province, while studies to cover the Samarra city is scanty [1]. The objective of the present research has been to assess the prevalence of these two common gastrointestinal parasites which covers 8 months to provide a bio-informative data associated with both anemia and total protein deficiency for medical purposes. The results could well be beneficial to the Health Ministry towards to prescribe a suitable cure to siege their prevalence.

2. Materials and Methods

2.1. Detection of Parasites

A total of 780 patients from both genders whose age ranged (4 months-70 years old) admitting *Samarra General Hospital* complaining diarrhea for check up and treatmentcovers8 months (August 2015-March 2016). Stool samples were collected inside clean plastic bottles coded with specific personal details, *i.e.* names, ages and dates. Some characters of the stools were also recorded as textures, e.g. mucous, serous, greasy bloody as well as color, e.g. yellow, brown, semi brown, greenish. Olympus light microscope following mixing a double blind check mixed with physiological saline (Normal saline solution) prepared from dissolving 8.5 gm of sodium chloride pure NaCl in one liter of distilled water were used to detect the two gastrointestinal parasites *Entamoeba histolytica* and *Giardia lamblia*. To assess the impact of them on blood parameters the blood samples collected from patients were analyzed using one of the following two methods.

2.2. Measurement of Blood Parameters

To determine the size and percentage of blood cells the packed cell volume (PCV) was measured using capillary test tubes, micro-centrifuge and haematocrite reader to determine the percentage of the size of blood cells [27] Hemoglobin concentration (Hb%) was also determined using cyanomethemoglobin method. Drabkin solution was used for dilution which contains cyanide ferric potassium [$K_3Fe(CN)_6$] and potassium cyanide (KCN), where the Ferro-cyanide oxidize the ferrous in the hemoglobin to ferric then to methemoglobin which is associated with potassium cyanide to form a hard compound of methemoglobin. The latter is brown in color as a result of the interaction, and match the intensity of the color formed with the amount of iron in hemoglobin and color intensity measured at 540 nm wavelength.

2.3. Method of Total Protein Assay Total Protein Test

Total protein involves albumin, Globulin, and fibrinogen while serum lacks fi-

brinogen where it enters the blood clotting process and it affects the nutritional status and functions of liver and kidney. Moreover, certain metabolic diseases and cases of abuse may result from infections with parasites and disease, mal-absorption and cases of fatigue or/and necrotic occur to hepatocytes. The latter has been scanned via biuret test to estimate the serum proteins and read the intensity of color at length [28]. It is a chemical test used for detecting the presence of peptide bonds where a copper (II) ion forms violet-colored coordination complexes in an alkaline solution (**Figure 1**). The intensity of the color, and hence the absorption at 540 nm, is directly proportional to the protein concentration, according to the Beer-Lambert law.

3. Results

In general the infection frequency with the gastrointestinal parasite *E. histolytica* was significantly higher (3 folds) than those of *G. lamblia* while the difference in their distribution between the gender appeared insignificant (**Figure 2**). Within 8 months the highest record of infection was in August and September, the two hottest months in Iraq but had declined proportionally with temperature dropped down during the autumn and winter while it began to gradually increase starting from March when the temperature begins to increase. The proportions of infection between the females was insignificantly higher than of males e.g. (53.7% to 46.3%); and (61.3% to 38.7%) for by *E. histolytica* and *G. lamblia*, respectively (**Table 1**).

Regarding the age related infection, the highest infection proportion was detected at age ranged (>1 - 5 year old) with almost 15% while the lowest was recorded at age ranged above 40 years old (7.4%). The differences between low infection in male and higher infection of female showed insignificance ($p > 0.05$) (**Table 2**). Again, the proportion of infection with *E. histolytica* was 2 - 4 folds

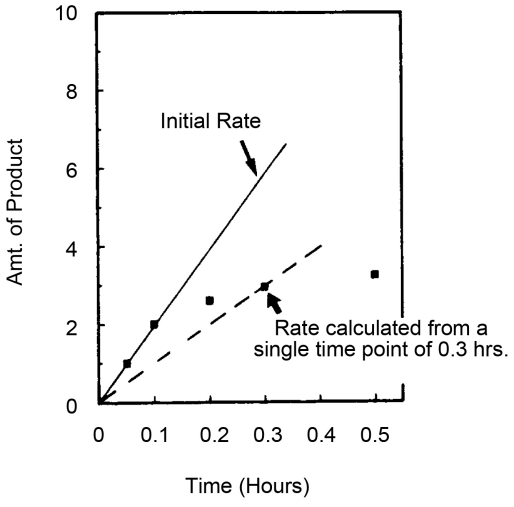
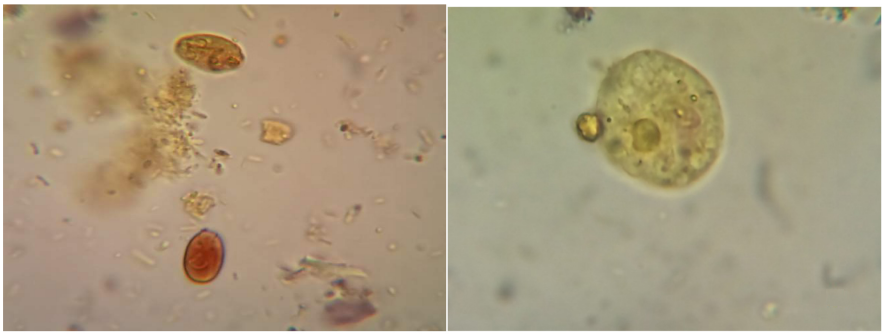


Figure 1. Dependence of measured rate on protein concentration (μg/mL) for a typical enzyme assay measured using a discontinuous system.



(a) (b)

Figure 2. (a) Trophozoite of the parasites *Giardia lamblia*; and (b) *Entymoeba histolytica* as seen from the stool of patients stained with Lugol's iodine (100×).

Table 1. Distribution of both *Entamoeba histolytica* and *Giardia lamblia* for the periods (August 2015-March 2016), within Samarra City. (n): number of infected patients and their percentages (%). An insignificant difference was seen between the genders. According to student T-test, there has been an insignificant difference ($p > 0.05$) between the genders.

	Entamoeba histolytica			*Giardia lamblia*		
Months & number of infected patients	Infection & (%)	Males & (%)	Female & (%)	Infection & (%)	Male & (%)	Female & (%)
August (n = 172)	23 (13.3%)	13 (56.5%)	10 (43.4%)	9 (5.2%)	4 (44.4%)	5 (55.5%)
September (n = 186)	27 (14.5%)	12 (44.4%)	15 (55.5%)	7 (3.7%)	3 (42.8%)	4 (57.1%)
October (n = 93)	12 (12.9%)	4 (33.3%)	8 (66.6%)	4 (4.3%)	2 (50%)	2 (50%)
November (n = 78)	7 (8.9%)	3 (42.8%)	4 (57.1%)	3 (3.8%)	1 (33.3%)	2 (66.6%)
December (n = 53)	4 (7.5%)	3 (75%)	1 (25%)	2 (3.7%)	1 (50%)	1 (50%)
January (n = 45)	5 (11.1%)	4 (80%)	1 (20%)	1 (2.2%)	0 (0%)	1 (100%)
February (n = 66)	7 (10.6%)	2 (28.5%)	5 (71.4%)	2 (3%)	0 (0%)	2 (100%)
March (n = 87)	10 (11.4%)	3 (30%)	7 (70%)	3 (3.4%)	1 (33.3%)	2 (66.6%)
Total n = 780	95	44	51	31	12	19

higher than *G. lamblia* respectively. However, the frequency of infection with *G. lamblia* at age ranged between (11 - 20 years old) was higher than other age ranges.

The criteria adopted to measure the anemia incidence was based on both hemoglobin percentages (Hb%) in the red blood corpuscles (RBC) and protein deficiency. The study implied checking the parasitic infection of both parasites in

Table 2. Prevalence of the gastrointestinal parasites *Entamoeba histolytica* and *Giardia lamblia* between male and female patients according to the ages. Differences are insignificant ($p > 0.05$).

	Entamoeba histolytica			*Giardia lamblia*		
Ages (years)	General & %	Male & %	Female & %	General & %	Male & %	Female & %
<1 year (n = 123)	17 (13.8%)	9 (52.9%)	8 (47%)	3 (2.4%)	1 (33.3%)	2 (66.6%)
1 - 5 (n = 128)	19 (14.8%)	10 (52.6%)	9 (47.3%)	5 (3.9%)	2 (40%)	3 (60%)
6 - 10 (n = 94)	12 (12.7%)	5 (41.6%)	7 (58.3%)	4 (4.2%)	1 (25%)	3 (75%)
11 - 20 (n = 82)	10 (12.1%)	5 (50%)	5 (50%)	6 (7.3%)	3 (50%)	3 (50%)
21 - 30 (n = 105)	15 (14.2%)	6 (40%)	9 (60%)	7 (6.6%)	3 (42.9%)	4 (57.1%)
31 - 40 (n = 87)	10 (11.4%)	4 (40%)	6 (60%)	4 (4.5%)	2 (50%)	2 (50%)
41 - 50 (n = 94)	7 (7.4%)	3 (42.8%)	4 (57.1%)	2 (2.1%)	0 (0%)	2 (100%)
>50 (n = 67)	5 (7.4%)	2 (40%)	3 (60%)	0 (0%)	0 (0%)	0 (0%)
Total (n = 780)	95 (12.1%)	44 (46.3%)	51 (53.6%)	31 (3.9%)	12 (38.7%)	19 (61.2%)

the anemia patients and protein deficiency patients. Generally only 16% of the 780 patients checked had confirmed to be anemia, divided as 12% infected with *E. histolytica* and only 4% with *G. lamblia*. However, only 36% of the anemia patients were infected with *E. histolytica* while 25% were infected *G. lamblia*. In both cases the proportion of infected female patients was almost double of the male (Table 3).

Similarly, out of 780 patients only 42 patients (5.4%) showed protein deficiency. However, only 36 patients out of 42 (4.6%) showed parasitic infection. Again female patients showed more susceptibility for infection with gastrointestinal parasites than male (Table 4).

4. Discussion

Generally, a population of 780 patients involved in this study is considerably high enough to eliminate any bias conclusion may be driven otherwise in comparison with lower numbers. The most common gastrointestinal parasites *histolytica* followed by *G. lambelia* are transmitted via the ingested unhealthy foods [1]. The 3 fold higher infection of *E. histolytica* than *G. lambelia* was similar to most recent study done in other parts of Iraq [17]-[26] and could be interpreted that amoebiosis is the most prevailed parasitic diseases in comparison with other diseases [1] or the people are more susceptible to *E. histolytica* than *G. lambelia*.

Table 3. Prevalence of anemia between male and female patients according to the ages *Entamoeba histolytica* and *Giardia lamblia*. Differences are insignificant ($p > 0.05$).

Ages (years) & No. of patients	General & %	*Entamoeba histolytica* and anemia			General & %	*Giardia lamblia* and anemia		
	Anemia	Anemia & *E. histolytica*	Male & %	Female & %	Anemia	Anemia & *G. lamblia*	Male & %	Female & %
<1 year (n = 123)	17 (5.8%) 1	1	1 (100%)	0 (0%)	3 (2.4%) 1	0	0 (0%)	0 (0%)
1 - 5 (n = 128)	19 (57%) 11	11	5 (45.4%)	6 (54.5%)	5 (3.9%) 11	0	0 (0%)	0 (0%)
6 - 10 (n = 94)	12 (75%) 9	8	2 (25%)	6 (75%)	4 (4.2%) 9	1	1 (100%)	0 (0%)
11 - 20 (n = 82)	10 (80%) 8	6	2 (33.3%)	4 (66.6%)	6 (7.3%) 8	2	1 (50%)	1 (50%)
21 - 30 (n = 105)	15 (60%) 9	5	1 (20%)	4 (80%)	7 (6.6%) 9	4	1 (25%)	3 (57.1%)
31 - 40 (n = 87)	10 (80%) 8	5	2 (40%)	3 (60%)	4 (4.5%) 8	3	1 (33%)	2 (50%)
41 - 50 (n = 94)	7 (100%) 7	5	2 (40%)	3 (60%)	2 (2.1%) 7	2	0 (0%)	2 (100%)
>50 (n = 67)	5 (80%) 4	4	3 (75%)	1 (25%)	0 (0%) 4	0	0 (0%)	0 (0%)
Total (n = 780)	95 (60%) 57	45	18 (40%)	27 (60%)	31 (3.9%) 57	12	4 (33.3%)	8 (61.2%)

Table 4. Prevalence of protein deficiency between male and female patients according to the ages *Entamoeba histolytica* and *Giardia lamblia*. Differences are insignificant ($p > 0.05$).

Ages (years)	General &	*Entamoeba histolytica*				*Giardia lamblia*			
No. of patients	No. of PD	PD & *E. histolytica*	Male & %	Female & %	*Gl* & %	PD only	Both PD & G.l	Male & %	Female & %
<1 (n = 123)	13 (10.6%) 6	4 (11%)	2 (50%)	4 (50%)	3 (2.4%)	6 (35%)	2 (28.5%)	0 (0%)	2 (28.5%)
1 - 5 (n = 128)	14 (42.1%) 8	5 (13.8%)	3 (60%)	2 (40%)	5 (3.9%)	8 (42.1%)	3 (42.8%)	0 (0%)	3 (42.8%)
6 - 10 (n = 94)	6 (58.3%) 6	6 (16.6%)	2 (33.3%)	4 (66.6%)	4 (4.2%)	7 (58.3%)	2 (28.5%)	0 (0%)	2 (28.5%)
11 - 20 (n = 82)	5 (50%) 5	5 (13.8%)	2 (40%)	3 (60%)	6 (7.3%)	5 (50%)	0 (0%)	0 (0%)	0 (0%)
21 - 30 (n = 105)	11 (26.6%) 5	4 (11.1%)	1 (25%)	3 (75%)	7 (6.6%)	4 (26.6%)	0 (0%)	0 (0%)	0 (0%)
31 - 40 (n = 87)	3 (30%) 3	3 (8.3%)	1 (33.3%)	2 (66.6%)	4 (4.5%)	3 (30%)	0 (0%)	0 (0%)	0 (0%)
41 - 50 (n = 94)	5 (71.4%) 5	5 (13.8%)	1 (20%)	4 (80%)	2 (2.1%)	5 (71.4%)	0 (0%)	0 (0%)	0 (0%)
>50 (n = 67)	4 (80%) 4	4 (11.1%)	3 (75%)	1 (25%)	0 (0%)	4 (80%)	0 (0%)	0 (0%)	0 (0%)
Total (n = 780)	61 (44.2%) 42	36 (37.8%)	15 (41.6%)	21 (58.3%)	31 (3.9%)	42 (44.2%)	7 (7.3%)	0 (0%)	7 (100%)

Occasional discrepancy in the results amongst these researches was noticeable which could be attributed to the methodology used in these studies. Recently, a similar work [29] preceded ours carried out within Samarra but reveled different outcomes than ours The discrepancy found is attributable to a few reasons involved in his work, *i.e.* shorter duration (5 months), less samples (40%) in comparison with ours, rural patients only, different season chosen for his research which all could accumulatively, and inevitably have produced such a variation. In general, such infection could well be attributed to the decline in general hygienic condition in the city due to eruption of civil wars within Iraq and the consequent immigration and displacement of the victims who might have exposed to various unhealthy circumstances in comparison with previous years. Similar researches involved the same two gastrointestinal parasites or/with other relevant ones carried out in other parts of the Mediterranean or and developing world refers, which are classified as over populated, to their prevalence in these countries, *i.e.* Pakistan [11], Brazil [12], Iran [13], Portugal [14], Ethiopia [15], and Ghana [16].

The increase in infection frequency of both parasites within August and September in comparison with November-February could be attributed to higher temperature in summer months when these gastrointestinal parasites become more epidemic leads to decrease in food hygiene than in winter months. These results are in concomitant with most recent work [30] as well as in other parts of Iraq, *i.e.* Tikrit [26] [31] [32] Karbala [23]. However, the insignificant differences between them might be attributable to the methodology and some difference in personal and public health service as well as environmental factors, *i.e.* demographical, climate and age differences for the samples involved in these works. The insignificant higher differences in proportions of infection between the females than of males are similar to those obtained elsewhere in Baghdad [33] but were higher in Mosul city [25]. These gender variations might be due to physiological, behavioral as well as immune differences between genders, e.g. endocrine activity as male bodies are more tolerant than those of females [34].

The higher records of infection of these two gastrointestinal parasites during the months August and September in comparison with December could be attributed to the environmental differences, *i.e.* higher temperature during summer in comparison with winter (December) were cyst phases are more active during summer months than in winter [1] [6].

The highest infection proportion was detected at lower ages (>1 - 5 year old) while the lowest was at age ranged >40 years old. This is a clear indication of hygiene related cause where children are more susceptible to be exposed to unhealthy food than adults or less developed immune system [35].

The epidemic of *E. histolytica* appeared to be stronger than *G. lamblia.* Such a result might refer to people's extra susceptibility to the *E. histolytica* than *G. lamblia* at early ages [1]. However, the *G. lamblia* showed insignificant difference at ages (11 - 20 years old) as higher than other age ranges. The latter could be attributed to the differences in the quality of outdoor food by readymade takeaways

or restaurants being consumed by these ages. Recently, the Centers for Disease Control and Prevention (CDC) in USA government public health agency alerted towards the possibility of great risk to generate from travelers to countries where giardiasis is common, people in child care settings, carriers of disease, contaminated or untreated drinking water, backpackers, *i.e*. campers, people in contact with infected animals [36] [37]. It occurs more often during the summer in the USA which is believed to be due to a greater amount of time spent on outdoor activities. Wilderness travel within the USA is believed to be a risk factor with poorly treated or untreated water playing a role [38].

The study also implied checking the parasitic infection of both parasites in both anemia and protein deficiency patients. With anemia associated parasitic diseases, *i.e*. amoebiasis or giardiasis, people affected might have developed anemia due to loss of blood [39]. Generally only 16% of the 780 patients checked had confirmed to be anemia (12% with *E. histolytica* and 4% with *G. lamblia*). Such a percentage is not unfeasible proportion of anemia at developing countries in comparison with higher health service in developed countries according to annual WHO reports [3] [8] [9] Similar percentages of anemia, particularly in girls, was found amongst students with no parasites in an Egyptian study infected with *Giardia lamblia*, as the parasitic infections were insignificantly associated with anemia (El-Sahn, *et al*., 2003) [40].

Protein deficiency was assessed in all 780 patients involved in this study showed generally low records *i.e*. (5.4%) and only (4.6%) with parasitic infection. All vital organs *i.e*. muscles, tissues, some hormones of the body are made from proteins, which also create hemoglobin, important antibodies, control blood sugar levels, to healing wounds and fighting off bacteria [41]. Although such a low percentage of protein deficiency was not possible to link with the parasitic infection it may still internationally be considered as a feasible record in developing countries.

More studies are necessary to hold in other parts of the country towards linking the gastrointestinal parasites with other disorders for national medical health strategy and international data base purposes.

5. Conclusion

It is concluded that both gastrointestinal parasites, *E. histolytica* and *G. lamblia*, are epidemic particularly during the summer seasons due to increase of temperature and decline in hygienic services. It is recommended that Ministry of Health in Iraq should take extra actions toward surveillance of the complications caused by these two active parasites.

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