# Investigation of *Giardia lamblia* and other parasites in tap water as a potential source of transmission in some regions of Baghdad

**Ihsan M. Al-Sagur[*1], Souhaila H. Mahmood[2], Heba M. A. Al-Obaidi[2]**
[1]Biological Researches Unit for Tropical Diseases, College of Science, University of Baghdad
[2]Biology department, College of Science, University of Baghdad

**Abstract**

Samples from tap water were chosen to be examined as one of the possible modes of *G. lamblia* and other parasites transmission. 144 domestic filter units were randomly distributed to houses in Baghdad in Al-Sadr City and surrounding regions then the suspended materials were examined microscopically by direct wet mount after centrifugal sedimentation method, and after staining with modified Ziehl-Neelsen (acid fast) stain. The results showed that 5/144 (3.47%) samples contained *G. lamblia* cysts, 3/144 (2.08%) contained *E. histolytica/E. dispar* cyst, while each of *Cryptosporidium* spp. and *Cyclospora cayetanensis* oocysts were observed in two samples (1.38%); also free living amoeba, ciliates and flagellates were detected in all samples. This result reveals a potential role of drinking water in the infection with these intestinal parasites in the study's area. Accordingly, the present study recommends the need to ensure the purification and sterilize drinking water and the safety of pipeline network. On the other hand, this new method, at the local level, by using domestic filter units for the detection of parasites and other microorganisms in tap water provide a simple, effective, and applicable research's tool beside saving time and effort in the samples' collection and filtration.

**Keywords**: *Giardia lamblia*, Giardiasis, Amoebiasis.

# التحرّي عن الجيارديا لامبليا والطفيليات الأخرى في ماء الحنفية بوصفه مصدرا محتملا للانتقال في بعض مناطق بغداد

**احسان مهدي الصقر*[1]، سهيلة حيّاوي محمود[2]، هبة محمد علي العبيدي[2]**
1وحدة بايولوجية المناطق الحارّة، كليّة العلوم، جامعة بغداد.
2قسم علوم الحياة، كليّة العلوم، جامعة بغداد.

**الخلاصة**:

تمّ اختيار عينات من ماء الحنفية للفحص بوصفها احد المصادر المحتملة لانتقال طفيلي الجيارديا وغيره من الطفيليات. وزّع 144 مرشحا للماء منزليّ الاستعمال بشكل عشوائي على منازل في مدينة الصّدر والمناطق المحيطة بها ثم فحصت المواد العالقة بالمرشّحات مجهريا باستعمال المسحة الرطبة المباشرة بعد عمليّة الترسيب بالطرد المركزي وفحصت ايضا بعد التصبيغ بصبغة زيل-نيلسون المحوّرة (الصّامدة للحَمض). اظهرت النتائج احتواء 5 عينات (3,47%) على اكياس الجيارديا *Giardia lamblia*، 3 عيّنات (2,08%) على اكياس *Entamoeba histolytica/Entamoeba dispar* وعينتين (1,38%) على البيوض المتكيسة

*Emails: drihsanalsagur@yahoo.com.

لكل من *Cyclospora cayetanensis* و*Cryptosporidium* spp. كذلك تمّ الكشف عن اميبات وهدبيّات وسوطيّات حرّة المعيشة في جميع العيّنات. تبيّن النتائج دورا محتملا لماء الشرب في الاصابة بهذه الطفيليات المعوية في منطقة الدّراسة وبناءا عليه توصي الدّراسة الحاليّة بضرورة التأكد من عمليات تصفية وتعقيم ماء الشرب وسلامة شبكة الانابيب. من جانب آخر، فإن هذه الطريقة الجديدة على المستوى المحلي في استعمال وحدات الفلترة منزلية الاستعمال للتحرّي عن الطفيليات وغيرها من الأحياء المجهريّة في ماء الحنفيّة توفر اداة بحثية بسيطة، فعالة وقابلة للتطبيق كما توفر الوقت و الجهد في جمع وتصفية العيّنات.

**Introduction:**

*Giardia lamblia* is one of the most prevalent intestinal protozoan parasites of humans in both developing and developed countries. In Asia, Africa, and Latin America, about 200 million people have symptomatic giardiasis with about 500,000 new cases reported each year [1]. Despite being known since the 17th century by the work of Antony van Leeuwenhoek, *G. lamblia* has only become recognized as a major public health concern in the last three decades after its identification in community outbreaks, travelers to endemic areas and immune-compromised individuals [2]. *Giardia lamblia* infection is transmitted by the fecal-oral route and results from the ingestion of *G. lamblia* cysts through the consumption of fecally contaminated food or water or through person-to-person (or, to a lesser extent, animal-to-person) transmission. The cysts are infectious immediately upon being excreted in feces. The infectious dose is low; ingestion of 10 cysts has been reported to cause infection [3]. The cyst can survive for weeks to months in cold water; they may also occur in the city water-reservoirs and resist the conventional water treatment methods, such as chlorination and ozonolysis [4]. According to data, the waterborne route is particularly important in comparison with foodborne transmission [5]. Since 1954, there were at least 132 reported waterborne outbreaks of giardiasis world wide [6].

In Iraq, little is known about the transmission routes of giardiasis and some other intestinal parasitic disease which are endemic in country according to surveillance studies [7-9]. Therefore, the present study was conducted to exam tap water, the main source of drinking water to check their role in the transmission of parasitic pathogens.

**Materials and methods:**

Direct filtration process was performed by using commercial filters for domestic use which were distributed in randomly-chosen houses in Baghdad in Al-Sadr City and surrounding regions like Al-Habibiya, Al-Baladiyat, Al-Shammai'ya. Examination was performed through 2011 in two seasons: summer season (April, May and June), and winter season (October, November and December). Twenty four filters were examined in each month by examining 12 filters every two weeks. The final yield was thus 144 examined filters in 6 months (diagram-1). Processing was carried out according to the following steps:

1- Each filter was fixed to a faucet (Fig. 1) and left for 2 weeks or until filter's pours were closed by accumulative materials.
2- Filter was transferred into a 60 ml plastic cup which contained 30 ml of 50 mM Tris dispersing solution or d. w.
3- Cup was shaken vigorously for few seconds then put on a shaker for 30 min.
4- The suspension was transferred into centrifugal tubes and centrifuged for 3 min at 2500 rpm.
5- Supernatant was discarded and sediment was resuspended with 1-2 ml of normal saline and left in room temperature until microscopic examination.
6- Microscopic examination was performed directly with normal saline, Lugol's iodine solution, and vital stain (0.1% eosin or 0.4% trypan blue) by using 40x, 100x objectives. In addition, modified Ziehl-Neelsen (acid fast) staining technique was performed according to [10]. All observations were recorded.

Each participant was asked a few questions about: using water tank or water pump, water supplies in the area, and whether or not he/she had any observations about water's color, taste, and odor.

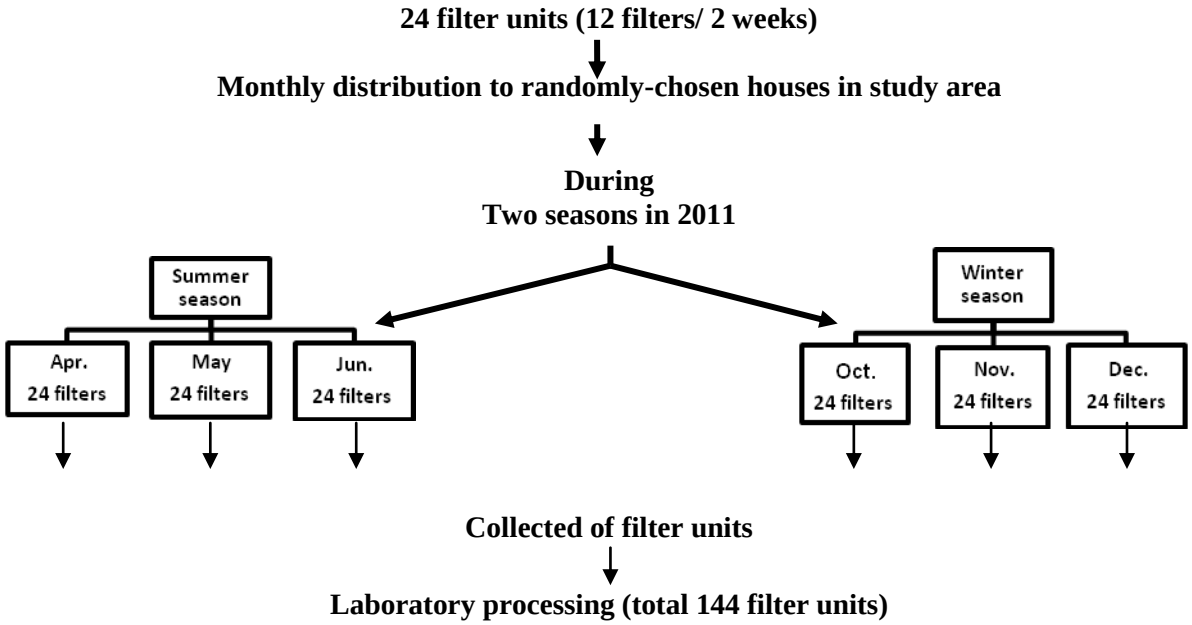


**Diagram 1-** Scheme for the distribution method of total 144 filter units in study's area during two seasons in 2011.

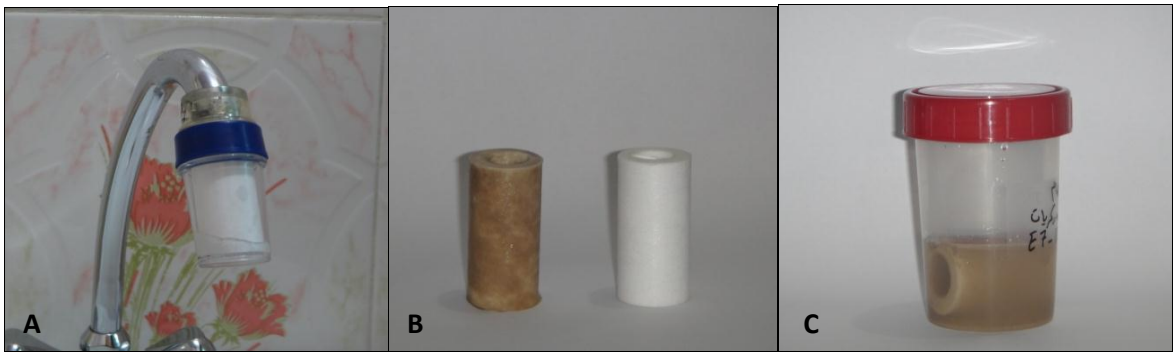


**Figure 1-** A: Domestic filter unit that used in the present study, fixed in a faucet. B: The cartridge filtes, before and after use. C: The cartridge filter immersed in dispersing solution.

**Results and discussion:**

Accessing to safe drinking water is essential to health a basic human right and a component of effective policy for health protection [11]. To the best of our knowledge, that is the first study which use a household cartridge filters for monitoring parasites in the tap water the main drinking water source in the study area. These filters provided an excellent way to collect sediments from large amounts of tap water as the typical sample volume for *Giardia* and *Cryptosporidium* monitoring for distribution systems water range from 10-1000 liters [12]. Moreover, direct filtration process have been done by these small devices in each participants' house so there was no need to collect water samples in large containers and transfer it to laboratory for filtration method by membranous filters or centrifugation. etc. which is time-consuming and cumbersome method.

Of 144 examined water samples, 5 (3.47%) samples contained *G. lamblia* cysts, 3 (2.08%) *E. histolytica*/*E. dispar* cysts, 2 (1.38%) *Cryptosporidium* spp. oocysts, and 2 (1.38%) *Cyclospora cayetanensis* oocysts (figure -2; table-1). Also free-living microorganisms were detected: 7 amoebae, 11 small flagellates and 4 ciliates (figure -3). All samples were turbid in different intensities, all samples contained fungi and algae especially diatoms (figure -4-5). Pathogenic, free-living protozoa, and other detected microorganisms were found in the samples from Al-Sadr City as well as the surrounding regions. In addition, almost all pathogenic parasites were detected in summer season

while free-living protozoa and other microorganisms were detected in both summer and winter seasons.

These results match with the finding of [13] concerning the presence of *E. histolytica* in tap water, also the result in agreement with the finding of [14] who reported the presence of *Cryptospordium* in tank water samples. On the other hand, none of the two studies mentioned about *G. lamblia* cysts detection. This may be due to the differences in detection methods. Somehow, the present study confirm the importance of filtration process to ensure the safety of drinking water , as cysts are relatively resistant to chlorine and ozone [15]. Filtration tap water by the filter unit was effective in capturing *G. lamblia* cysts as well as *Cryptosporidium* spp. oocysts and other microorganisms and sandy particles. In addition, the presence of these parasites in drinking water may be represented a possible source for the occurrence of co-infections in the study's area which were reported frequently in hospitals.

**Table 1-** Detected microorganisms during both summer and winter seasons in 144 tap water samples by microscopic exam.

| **Microorganisms** | **No.** | **(%)** |
|---|---|---|
| 1- *Giardia lamblia* cysts | 5 | 3.47% |
| 2-*Entamoeba histolytica/E. dispar* cysts | 3 | 2.08% |
| 3-*Cryptosporidium spp.* | 2 | 1.38% |
| 4- *Cyclospora cayetanensis* | 2 | 1.38% |
| 3-Free living amoebae | 7 | 4.86% |
| 4-Free living flagellates | 11 | 7.63% |
| 5-Free living ciliates | 4 | 2.77% |
| 6-Algae | 144 | 100% |
| 7-Fungi spores | 144 | 100% |

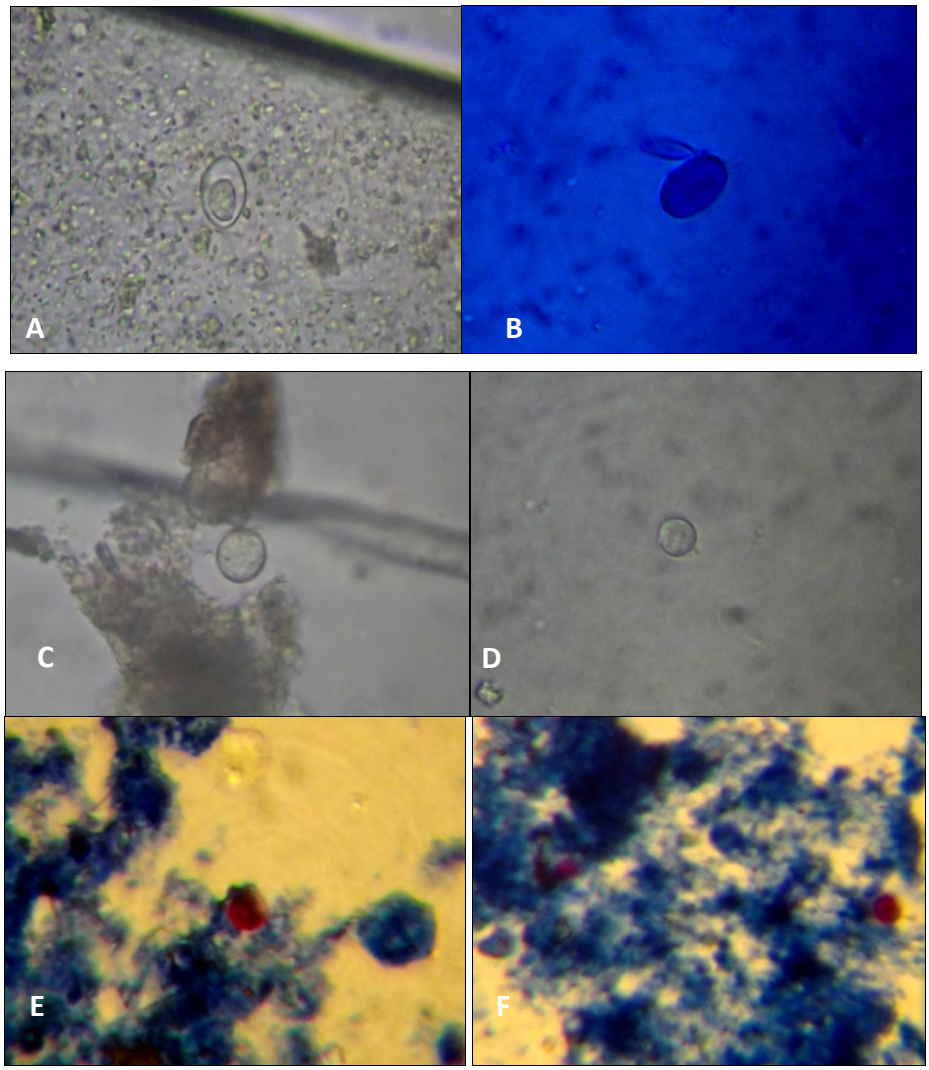


**Figure 2- A:** *G. lamblia* cyst in wet mount under 40x, B: *G. lamblia* cyst in methylen blue wet mount under 40x, C & D: *E. histolytica/E. dispar* cysts in wet mount under 40x, E: *Cyclospora caytanensis* oocyst by acid fast staining under 100x, F: *Cryptosporidium* spp. oocyst by acid fast staining under 100x.

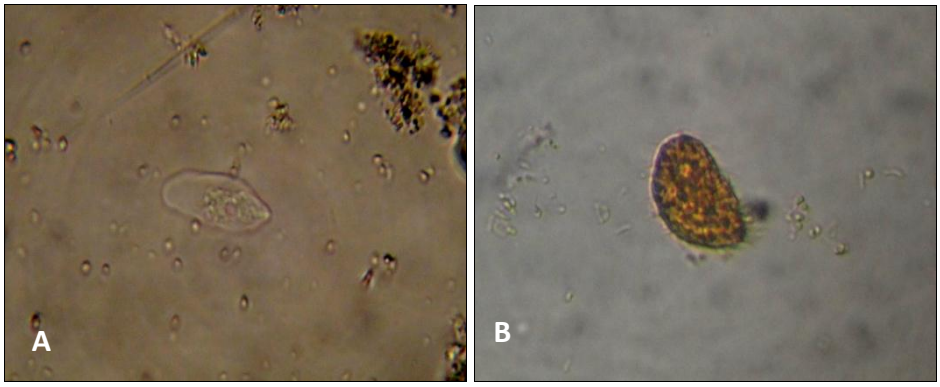


**Figure 3-** A- Free-living amoeba trophozoite in wet mount under 40x, B: Free-living ciliate trophozoite (*Colpoda* spp.) in iodine wet mount under 40x.

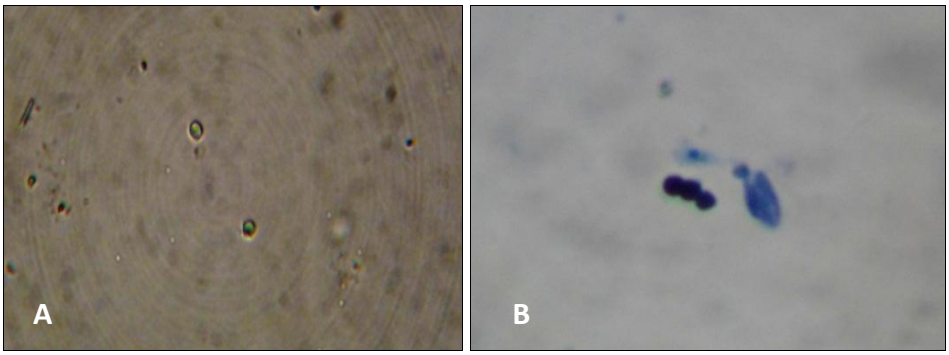


**Figure 4-** A- Fungi in wet mount under 40x, B: Budding yeast cells by acid fast staining under 100x.

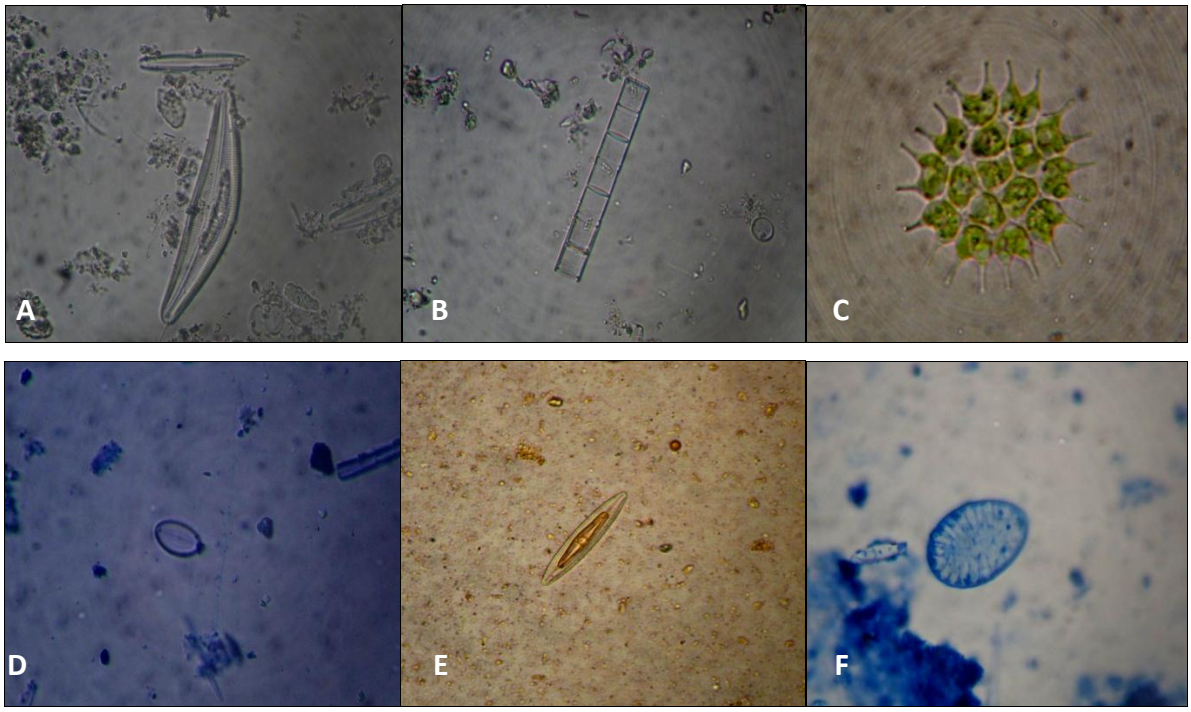


**Figure 5-** A & B- Diatoms in wet mount under 40x, C: green algae in wet mount under 40x, D: diatom in methylen blue mount under 40x, E: diatom in iodine mount under 40x, F: Diatom by acid fast stain under 100x.

The presence of pathogenic parasites even in low number in water may be one of the main sources to infections particularly with *G. lamblia* which can cause disease as 3-5 cysts/100 liters in treated drinking water [16]. One of the reported causes of giardiasis outbreak was the cross-connection between sewage effluent pipe and tap water pipes [17]. In Iraq, the received amount of tap water from the municipal supplies is inadequate. Consequently, people are used to delivering more of water amounts to their homes by the water pumps; this process may be a major cause of contamination of drinking water with various types of pathogen as the withdrawn water may interfere with pollutants and sewage materials that permeate by pumping power to old corroded water pipes. The clearly visible turbid water especially of the first flush after pumping water, which observed in the present study, may confirms this interpretation, beside the complaints of participants from the foul odor and unpleasant taste that they noticed sometimes. This problem may lead to significant increase in gastrointestinal illness especially by water that reached to the hospitals, schools, child - care centers and health institutions. Therefore, serious measures must be taken to prevent contamination of water supplies. Also it had been observed that samples of heavy sediment was rich of different microorganisms including the detected pathogenic ones, this is in agreement with the systematic review of [18] who

showed that an association could be found between turbidity and gastrointestinal illness exists in some setting or over a certain range of turbidity.

It is noteworthy that the presence of fungi in all water samples may be one of the main sources of fungal infections which were prevalent according to the hospitals' archives in the study's area. This is in consistent with the findings of several studies which detected fungi in tap water and some of them were human pathogenic [19-22]. Water contaminated with fungi is of important to hospitals where immunocompromised patients undergo treatment [23]. Different species of algae and diatoms were observed in all water samples, these organisms may affect the water quality besides some of them may cause a misdiagnosis for another organisms especially parasites, therefore, it must not depend on morphology but also diameter in diagnosis.

As the results demonstrated that the main source of drinking water, tap water, contained *G. lamblia* and intestinal parasite infective stages, the present study recommends to take reliable steps to ensure safety and quality of drinking water by effective methods of filtration, sterilization, and maintenance of the pipeline network. Also people can make water safer to drink by the boiling or the use of these types of domestic filter units which are cheap and easy to use. On the other hand, application of these filter units is recommended in research for monitoring microorganisms including pathogens in tap water, and takes advantage of the more sensitive and specific methods for microorganisms' detection in filtrates than microscopic exam.

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