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Prevalence of *Blastocystis* sp. among cooks in the region of Fez-Meknes (Morocco)

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Summary

Cooks have an important role in the dissemination and transmission of enteropathogenic microorganisms, including intestinal parasites such as *Blastocystis* sp. responsible for blastocystosis.
Blastocystis is a unicellular, anaerobic, eukaryotic protist that colonizes the intestinal tract of many hosts. It is the most common parasite found in human stool. It can be the cause of acute digestive disorders which could lead to chronic syndromes such as irritable bowel syndrome. The aim of the present study is to determine the prevalence of this protozoan in cooks in the Fez-Meknes region. This is a retrospective descriptive analytical study carried out in the Parasitology-Mycology laboratory of the Moulay Ismail Military Hospital in Meknes.
Out of a total of 200 parasitological examinations of cooks' stools, 88 (44 %) cases were positive for intestinal parasites. *Blastocystis* sp. is the most frequently encountered protozoan in our study. It represents 39.78 % of those infected. It is associated in 48.57 % of cases with another intestinal parasite. *Dientamoeba fragilis* (*D. fragilis*) is the most found in 31.43 % of co-infections, respectively followed by *Entamoeba coli* (*E. coli*) (8.57 %), *Endolimax nana* (*E. nana*) (5.71 %) and *Pseudolimax boutschlii* (*P. boutschlii*) (2.86 %).
Our results showed a high prevalence of *Blastocystis* sp. in the kitchen staff population. These workers must be considered as reservoirs of this microorganism, and therefore a source of infection in those around them. Consequently, preventive measures are necessary, including raising awareness among this population in order to effectively fight against the infestation by this protozoan in particular and other parasites in general.
Keywords: *Blastocystis* sp.; Prevalence; Cooks; Co-infections; Protozoa

Introduction

Parasitic infestations, especially those caused by protozoa, have long been a public health problem. According to the World Health Organization (WHO), nearly a third of people in developed countries are affected by parasitic intestinal infections, while the rate is five times higher among people in developing countries (Gizaw *et al.,* 2014; Tulu *et al.,* 2014). *Blastocystis* sp. is one of these protozoa, the most identified in symptomatic and asymptomatic patients during parasitological stool examinations (PES) (Thathaisong *et al.,* 2003; Wong *et al.,* 2008). It is a unicellular anaerobic eukaryotic parasite, frequently reported in humans and animals worldwide (Wongthamarin *et al.,* 2018). Its prevalence varies from 55 to 100 % in developing countries and from 7 to 50 % in devel-

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Table 1. Prevalence of intestinal parasitic infections, as determined by analysis of stool specimens, according to age group and sex of 200 food handlers in the Parasitology-Mycology laboratory of the Moulay Ismail Military Hospital in Meknes.

Variable	Total n (%)	Parasitic infection n (%)	*p* – value
Sex			
Male	192 (96)	83 (94.32)	0.284
Female	8 (4)	5 (5.68)	
Age group			
30 – 34	27 (13.5)	11 (12.5)	0.792
35 – 39	137 (68.5)	61 (69.32)	
40 – 45	36 (18)	16 (18.18)	

oped countries (Lhotská *et al*., 2020; Wakid *et al*., 2022)
Currently, 28 subtypes (ST) have been described based on sequence comparison of the 18S rRNA gene denoted ST1 to ST28, while only 24 subtypes meet the currently recommended criteria for subtype identifications, their numbers are: ST1-ST17, ST21, ST23-ST28. Subtypes between them (for example ST18-20, ST22) are chimeras (Stensvold & Clark, 2020). The contribution of this microorganism to pathogenicity is increasingly proven, although long considered non-pathogenic, recent studies have suggested a strong correlation between infection with *Blastocystis* sp. and digestive disorders (Yakoob *et al.,* 2010; Hayashi *et al.,* 2006). In particular, food handlers constitute a population at high risk of contamination and spread of this parasite (Idowu & Rowland, 2006; Khurana *et al.,* 2010). The risk in this population lies in the absence of digestive symptoms in some cases, making carriers asymptomatic (Aoun *et al.,* 1999; Takizawa *et al.,* 2009). The importance of this group of workers in the transmission of parasitic diseases has been emphasized by several authors (Tsega & Nadew, 1972; Sahlemariam & Mekete, 2001; Dagnew *et al.,* 2012). This study aims to assess the prevalence of *Blastocystis* sp. in cooks in the Fez-Meknes region, who go to the Parasitology-Mycology laboratory of the Moulay Ismail military hospital in Meknes, for medical examinations in routine.

Materials and Methods

This is an analytical cross-sectional study carried out in the Parasitology-Mycology laboratory of the Moulay Ismail Military Hospital in Meknes, it was conducted on 200 asymptomatic persons (apparently healthy), spread over a period of 2 years from 03/01/2019 to 11/02/2021. In our study, we included all the stool samples emitted by the kitchen staff of the various military units in the Fez-Meknes region, as part of periodic systematic screening. Three fecal samples were taken from each patient in a clean plastic container. The samples were examined for the presence of intestinal parasites.
Each coprological sample was subjected to a macroscopic examination and microscopic in the fresh state (a pea of stools diluted in physiological water), after staining with Lugol's iodine and after concentration according to the Ritchie method modified and Bailenger. Occasionally, the rapid antigenic test for the diagnosis of *Giardia intestinalis* (*G. intestinalis*) and microsporidia has been performed. The x10 and x40 objectives were used for the detection of eggs and larvae of helminths, cysts and trophozoites of protozoan parasites.
Blastocystis sp. positivity has been accepted when the parasite count is greater than or equal to 5 elements per field when microscopically examined at 40x magnification (Nimri, 1993). The

Table 2. Prevalence of different parasites found.

	Parasite	Number of parasitized subjects	SPIp1 (%)	SPIp2 (%)	Parasitic index in relation to parasitized subjects (%)
Amoebas	*Entamoeba coli*	27	13.5	23.90	30.68
	Entamoeba histolytica/E.dispar	2	1	1.77	2.27
	Endolimax nana	16	8	14.16	18.18
	Pseudolimax boutschlii	3	1.5	2.65	3.41
	Total	**48**	**24**	**42.48**	**54.54**
Flagellates	*Giardia intestinalis*	2	1	1.77	2.27
	Chilomastix mesnilii	3	1.5	2.65	3.41
	Dientamoeba fragilis	25	12.5	22.12	28.41
	Total	**30**	**15**	**26.54**	**34.10**
Blastocystis	*Blastocystis sp.*	35	17.5	30.97	39.77

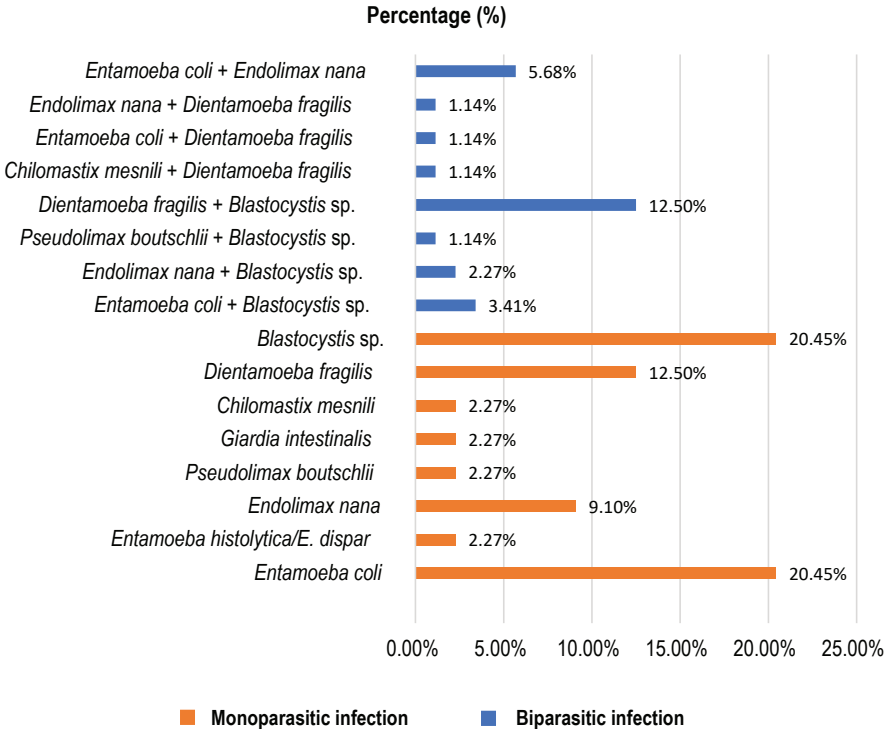

Fig. 1. Distribution of intestinal parasitic species in infected individuals.

results of the macroscopic and microscopic analyzes of the PES were collected from the registers of the service. All results were entered using Microsoft Office Excel 2010 software and processed by IBM SPSS Statistics 21.0 software.

Ethical Approval and/or Informed Consent

The study was retrospective. All data were collected from registers available at the Parasitology Mycology Laboratory of the Moulay Ismaïl Military Hospital in Meknes. The authors did not have any contact with the patients. The stool samples received previously were within the framework of routine care: screening for intestinal parasite carriage in cooks without any additional procedure. It has been taken care of to maintain patient anonymity. So the authors have an approbation of ethics committee.

Results

During the study period (01/03/2019 to 02/11/2021), we included 200 PES of asymptomatic cooks (192 Men and 8 Women). The mean age of the participants was 37.5 ± 3.245 years, with extremes of 30 and 45 years. The largest food handler age group was 35 – 39 years (68.5 %).

Fecal parasite testing was positive in 88 individuals, which corresponds to an overall prevalence rate of 44 %. The prevalence of intestinal parasitism in male cooks is higher compared to that observed in female cooks 94.32 % versus 5.68 %. No significant statistical differences regarding parasitic infections related to gender (*P*=0.284) or age groups (*P*=0.792) were observed. The results of the study are shown in Table 1.

The intestinal parasites identified in our study all belong to the protozoa, the latter are represented respectively by the Amoeba (42.48 %) followed by the Blastocystis (30.97 %) then the Flagellates (26.54 %) (Table 2).

Blastocystis sp., *Entamoeba coli* (*E. coli*), *Dientamoeba fragilis* (*D. fragilis*) and *Endolimax nanus* (*E. nanus*) were the most identified protozoan species in our study with respectively 30.97 %, 23.90 %, 22.12 % and 14.16 % (Table 2). Among the pathogenic or potentially pathogenic parasites, we note the presence of *D. fragilis* with a frequency of 22.12 %, followed by *G. intestinalis* and *Entamoeba hystolitica* (*E. hystolitica*)/ *Entamoeba dispar* (*E. dispar*) with the same prevalence rate of 1.77 %.

The parasitic association is recorded in 25 cases, or 12.5 % of the cooks tested, made only of a combination of protozoa. The species identified are found alone (monoparasitism) in 71.60 % or in double bond (biparasitism) in 28.41 % of infected persons. The bi-parasitic association between *D. fragilis* + *Blastocystis* sp. is the most important, it represents 12.50 %, followed by *E. coli* + *E. nana* with a rate of 5.68 %, then we find *E. coli* + *Blastocystis* sp. with a frequency of 3.41 %. *E. nana* + *Blastocystis* sp. occupy the fourth position with a percentage of 2.27 % and finally we find *E. nana* + *D. fragilis*, *E. coli* + *D. fragilis* and *C. mesnili* + *D. fragilis* with a rate of 1.14 % for each of them (Fig. 1).

The overall prevalence of *Blastocystis* sp. infection is 17.5 %

Table 3. Prevalence of *Blastocystis* sp. as determined by analysis of stool specimens, according to age group and sex of 200 food handlers in the Parasitology-Mycology laboratory of the Moulay Ismail Military Hospital in Meknes.

Variable	Total number of infected subjects n (%)	Infection with *Blastocystis* sp. n (%)	*p*-value
Sex			
Male	192 (96)	34 (97.14)	0.706
Female	8 (4)	1 (2.86)	
Age group			
30 – 34	27 (13.5)	5 (14.29)	0.888
35 – 39	137 (68.5)	23 (65.71)	
40 – 45	36 (18)	7 (20)	

(35/200). The rate of infection with this parasite is higher in men (97.14 %) than in women (2.86 %). However, no significant differences between infection with Blastocystis related to sex (*P*=0.706) or age groups (*P*=0.888) were observed (Table 3).

Blastocystis sp. is the most frequently encountered protozoan in our study. It represents 39.78 % of those infected. It is associated in 48.57 % of cases with another intestinal parasite. *D. fragilis* found in 64.71 % of co-infections followed by *E. coli*, *E. nana* and *P. boutschlii* with 17.65 %, 11.76 % and 5.88 % respectively (Fig. 2).

Discussion

Our study series is composed of 192 men and 8 women, this very low female representation is due to the fact that most military structures employed men. In fact, several studies have found no significant difference in parasite infection rates between the two sexes. In our series, the average age of the participants was 37.5 ± 3.245 SD. Data on education level, place of residence, and economic status were not collected.

In the present study, the PES was positive in 88 patients, for an overall parasitism prevalence rate of 44 %. This rate of parasitism is almost identical to that recorded Addis Abeba in Ethiopia (45.3 %) (Aklilu *et al.,* 2015). However, it is higher than the results of other studies which have reported: 29.3 % in India (Ghosh *et al.,* 2014), 23.7 % in Kenya (Damen *et al.,* 2015), 20.11 % in Saudi Arabia (Haouas *et al.,* 2021), 14.3 % in Ghana (Abaka-Yawson *et al.,* 2020), 21.5 % in Tunisia (Masmoudi *et al.,* 2012), 28 % in Algeria (Belkessa *et al.,* 2021), 17.9 % in France (Rousset *et al.,* 1993), 19.3 % in Iran (Teimouri *et al.,* 2021) and 14.5 % Northern Ethiopia (Gezehegn *et al.,* 2017).

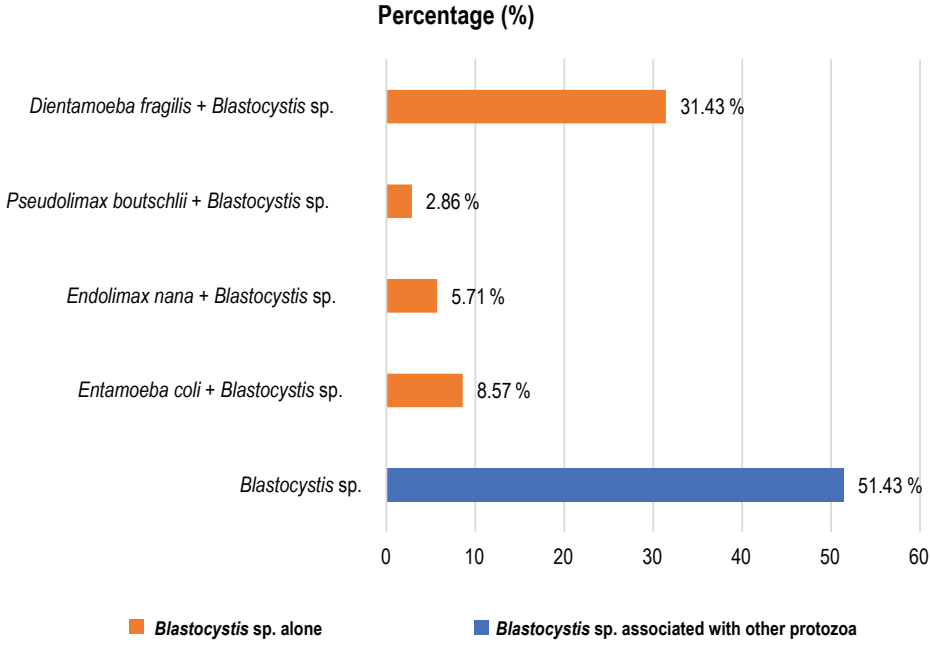

Fig. 2. Prevalence of infection with *Blastocystis* sp. alone or associated with other protozoa.

The differences in the prevalence reported in various studies may be related to several factors such as socioeconomic status, climatic conditions, poverty, personal and community hygiene, the population studied, and the year in which these surveys have been carried out.

The prevalence of parasitic infection shows a clear male predominance, 94.32 % versus 5.68 %, but without statistical significance (P = 0.284). This difference can be explained by the fact that the majority of military structures employed men. Therefore, we cannot draw conclusions based on gender. Moreover, several studies have not found a significant difference in the rates of parasitic infection between the two sexes (Incani *et al.,* 2017)

Age groups analysis (Table 1) revealed that the prevalence of intestinal parasitic infections was 12.5 % among subjects aged 30 – 34 years (11/88), 69.32 % in the group aged 35 – 39 years (61/88), and 18.18 % in the group aged 40 – 45 years (16/88). These results no showed any significant association (P = 0.792) between age and intestinal parasitic infection.

Protozoa are the only parasitic group identified in our series, and they are represented by 54.54 % of Amoebas, 39.77 % of Blastocystis, and 34.10 % of flagellates of all parasitized subjects.

Blastocystis sp. is the most widely detected parasite in our study population, with a prevalence of 39.77 %, followed by *E. coli* (30.68 %). These results corroborate those from the literature since it is accepted that *Blastocystis* sp. is often recorded as being the parasite most frequently encountered in the stool, followed by non-pathogenic protozoa such as *E. coli* or *E. nana* (Sebaa *et al.,* 2021; Heydari-Hengami *et al.,* 2018; Tan, 2008) (Table 2). The prevalence of *Blastocystis* sp. recorded in our study series (39.77 %) is very close to that recorded in Egypt (35.5 %) (Fathy, 2011), but it is lower than the results of other studies carried out in a population of food handlers in Tunisia (13 %) (Abda *et al.,* 2017), Iran (24.3 %) (Heydari-Hengami *et al.,* 2018). In contrast, surveys of school children in Senegal, Morocco, and Egypt reported prevalence rates of *Blastocystis* sp. of 100 %, 33.4 %, and 33.3 %, respectively (El Safadi *et al*., 2014; Rahmouni, 2010; Rayan *et al*., 2007).

Concerning co-parasitism, *Blastocystis* sp. is associated with another parasite in 48.57 % of cases. The most frequent association combines *Blastocystis* sp. and *D. fragilis* with a frequency of 64.71 %. Our results are correlated with those from the literature which have shown that the most common co-infections are those between protozoa and that biparasitic infection between *Blastocystis* sp. and *D. fragilis* is the most mentioned (Rahmouni, 2010; Piubelli *et al.,* 2019). This co-infection can be explained by the fact that these two parasites have the same mode of transmission. Recent studies have reported that there is a significant association between co-infection with *Blastocystis* sp. and *D. fragilis* and the gastrointestinal symptoms (Maas *et al.,* 2014; David *et al.,* 2015; Piubelli *et al.,* 2019). *D. fragilis* is the most detected pathogenic parasite in humans (Kallel *et al.,* 1999; Dickinson *et al.,* 2002; Piubelli *et al.,* 2019) with a prevalence varying between 0.9 % and 82.9 % depending on the country, the diagnostic method and the population studied (Preiss *et al.,* 1990; Ögren *et al.,* 2016). It should be noted that the presence of parasitic associations shows a low level of sanitary, food and fecal hygiene as well as unfavorable living conditions of these subjects (El Moutaallik Billah, 2019). The predominance of co-infection with protozoa is explained by the similarity of risk factors and transmission modalities of these parasites (Devera *et al.,* 1998; Dupouy-Camet, 2000).

Blastocystis sp. is the most common protozoan found in PES in both symptomatic and asymptomatic patients (Mohamed *et al.,* 2017; Kataki *et al.,* 2019), with prevalence ranging from 55 to 100 % in developing countries and 7 to 50 % in developed countries (Lhotská *et al*., 2020; Wakid *et al*., 2022). The presence of this protozoan in PES could be an indicator of poor personal hygiene and the existence of other parasites (Yaicharoen *et al.,* 2006; Kataki *et al.,* 2019). The identification of this parasite is done by several methods, namely the optical microscope, culture or molecular approaches such as PCR (Polymerase Chain Reaction). The comparisons made between these detection methods globally confirm the greater sensitivity of molecular methods compared to microscopic and cultural methods (Devera *et al.,* 1998; Dogruman-Al *et al.,* 2010). The transmission of this parasite takes place mainly by the fecal-oral route after direct or indirect contact with objects, food products (fruits, vegetables, salads) raw badly washed, or drinking water polluted by human or animal excreta. In addition to these risk factors for transmission of blastocystosis, there are food handlers and especially cooks with poor personal hygiene who can be potential sources of propagation of this protozoan (Idowu & Rowland, 2006; Khurana *et al.,* 2010). The risk in this population lies in the absence of digestive symptoms, in some cases, causing carriers to be asymptomatic (Takizawa *et al.,* 2009). The importance of this group of workers in the transmission of parasitic diseases has been emphasized by several authors (Sahlemariam & Mekete, 2001; Dagnew *et al.,* 2012). The absence of gastrointestinal symptoms in some cases can be explained by the presence of virulent and other non-virulent species of this protozoan (Fouad *et al.,* 2011). The pathogenicity of *Blastocystis* sp. remains controversial (Tan *et al.,* 2010; Osman *et al.,* 2016), although numerous clinical and epidemiological studies have examined this pathogenic potential in symptomatic and asymptomatic patients (Patino *et al.,* 2008; Tan, 2008). In this context, some authors have shown the association of this microorganism with certain gastrointestinal symptoms, particularly in immunocompromised people, in particular, patients with AIDS and cancer because it is considered to be an opportunistic pathogen (Patino *et al.,* 2008) and they have suggested a positive correlation between the density of the parasite and the symptoms (Kaya *et al.,* 2007), on the other hand, other authors have denied this pathogenic significance and confirmed that *Blastocystis* sp. is a commensal organism (Sun *et al.,* 1989; Kataki *et al.,* 2019). Other recent genetic analyzes have shown the existence of 10 different subtypes (STs) of Blastocystis in humans noted ST1 to ST9 and ST12 (Maloney *et al.,* 2021), this genetic

diversity, supports the hypothesis of virulent species and non-virulent of this protozoan (Wakid *et al*., 2022).

Conclusion

Our results showed a high prevalence of protozoan parasites in the kitchen staff population, *Blastocystis* sp. being the most predominant protozoan. These asymptomatic cooks will be considered as reservoirs of parasites, in particular of *Blastocystis* sp. and therefore a source of infection in those around them. Consequently, preventive measures are essential, in particular by sensitizing the populations by insisting on fecal hygiene, the good maintenance of toilets, the proper treatment of water and food intended for consumption, and the treatment of infected people in order to fight effectively. against infestation by this protozoan in particular and other parasites in general.

Recommendation

- Increase the study sample by recruiting food handlers from the private and public sectors.
- Obtain information on the epidemiological, clinical and immunological situation of each participant to study the risk factors for infection with *Blastocystis* sp.
- Use other diagnostic methods, namely culture and PCR technique.
- Perform sequencing to understand the correlation between pathogenicity and the subtypes of this parasite.

Conflicts of Interests

The authors declare that they have no conflicts of interest in relation to this article.

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