

Epidemiology of Amoebas in Marrakech (Morocco): Experience of Parasitology Service from the Military Hospital Avicenne

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Abstract: Amoebiasis is the third leading cause of parasite mortality and morbidity worldwide and still remains a serious public health problem today. In order to determine the epidemiological profile of amoebae and intestinal amoebiasis in the population of Marrakech. This is a prospective study on the results of parasitological examinations of stool (EPS) performed in adults and children in the department of Parasitology Mycology at the Military Hospital Avicenne (HMA) of Marrakech over a period of two months from 1 May 2018 to 30 June 2018. 70 parasitological examinations of stool (EPS) were performed. Of the 38 positive samples 71.05% were male with a sex M / F ratio of 2.08. Each patient received at least one EPS, including fresh reading, after Lugol staining, and finally after concentration by the Willis and Ritchie technique. Among the parasites encountered, after identification of the species, the percentage of *Blastocystis hominis* is 45.76% followed by *Entamoeba coli* 35.59%, followed by *Chilomastix mesnili* 10.17% then *Entamoeba histolytica histolytica* and *Entamoeba histolytica minuta*, the latter having the same proportion 3.39% and in late *Giardia intestinalis* with a low proportion of 1.69%. Amoeba remains, even today, a real public health problem. For example, broadening the scope of these studies by similar surveys focusing on the rural environment would be desirable.

Keywords: Amoebae, Entamoeba Histolytica, Entamoeba Dispar, Intestinal Amebiasis, Hygiene, Morocco

1. Introduction

The intestinal amoebae are protozoans constituting the subphylum rhizopods and characterized by cell motility through pseudopodia and asexual reproduction. They are classified according to the morphology of the nucleus in two groups: the nucleus type "*Entamibe*" present in amoebae of the genus *Entamoeba* and type "*Limax*" in amoebae of the genus *Pseudolimax* and *Endolimax*. The genus *Entamoeba* gathers several species of which only one is pathogenic certain, because of its necrotic action on the cells, responsible for amebiasis (current name amoebiose). According to WHO, this predominantly intestinal parasitosis is the state in which the human organism hosts, with or without clinical manifestations, a protozoan of the rhizopod class *Entamoeba histolytica*. The latter has long been considered as an infectious agent of variable virulence because of the

important difference between the number of healthy carriers and the number of cases of amoebiasis [1].

Amoebiasis is a cosmopolitan parasitosis. About 10% of the world's population is infected with parasitic amoebae. 10% of the parasitized subjects have an intestinal or extra-intestinal invasive form and of which 90% are asymptomatic. Amoebiasis is the third leading cause of death by parasitic diseases in the world after malaria and schistosomiasis. It affects approximately 50 million people, of whom 40,000 to 110,000 die each year [2]. It constitutes a real public health problem mainly because of the favorable climatic conditions, the absence or insufficiency of hygiene and sanitation measures most often linked to poverty. These factors which contribute to the perpetuation of the transmission of intestinal parasitoses remain very diverse and complex. Although not pathogenic, all other species of amoeba have an undeniable epidemiological interest, because they are considered

indicators of orofecal contamination and low levels of hygiene of the carrier subject.

Optical microscopy, during a stool parasitology examination, makes it possible to differentiate specifically the different species of amoebae as well as several other parasitic species. However, mere morphological study may be insufficient; in particular, the differentiation, in non-hematophagous cystic and trophozoite forms, of the pathogenic species: *E. histolytica* and non-pathogenic: *E. dispar*, which were long confused [3].

The differentiation between *E. histolytica* and *dispar*, recommended by WHO, has considerable epidemiological, diagnostic and therapeutic implications. Indeed, *E. dispar* would be 10 times more common than invasive *E. histolytica* that requires specific treatment [4].

In recent years, several enzymatic, molecular and genetic techniques have been developed to complement the parasitological examination of feces and improve the detection of *E. histolytica* [5].

The objective of our study is to estimate the prevalence of pathogenic and non-pathogenic intestinal amoebae at the service of mycology parasitology of the Avicenne military hospital of Marrakech (HMA) to assess the degree of orofecal contamination, to study poly-parasitism general and that of amoebas specifically.

2. Materials and Methods

2.1. Type, Place and Duration of Study

This is a prospective study on the results of stool parasitological examinations (EPS) performed in adults in the department of Parasitology Mycology at the Avicenne Military Hospital (HMA) of Marrakech over a period of two months ranging from 1 May 2018 to 30 June 2018.

2.2. Patients

The patients recruited in our study associate adults (military and their families) hospitalized or consultants at the Avicenne Military Hospital (HMA) of Marrakech and the civilian patients addressed hospitals Mohamed VI or the private sector.

2.3. Methodology

2.3.1. Data Collection

The collection of information is made from the binders and registers of the mycology parasitology department of the Avicenne Military Hospital of Marrakech (HMA). The records of exploitation included: the identity of the patients (name, first name, sex and age), the date of the analysis, the service and the macroscopic and microscopic results of the

parasitological examinations of the saddles (EPS).

2.3.2. Parasitological Examination of Stool

Each patient received a sterile jar to pick up his morning saddle from the day of the exam. Patients referred to emergency departments defecated in mycology parasitology department.

The stool collected is examined as soon as possible after its emission, first macroscopically to note the appearance, consistency, color and possible presence of blood, mucus or adult parasitic forms.

Stool is also carefully examined microscopically in the fresh state (0.9% saline solution) after staining with 2% Lugol or Merthiolate Iodine Formol (MIF) and after concentration by different techniques, two of which are commonly prepared and used in the HMA parasitology department in Marrakech: the simplified RITCHIE method and WILLIS.

3. Results

3.1. The Characteristics of the Population

During two months, 70 parasitological examinations of the stool (EPS) were received.

The sex ratio is 2.08 (Figure 1).

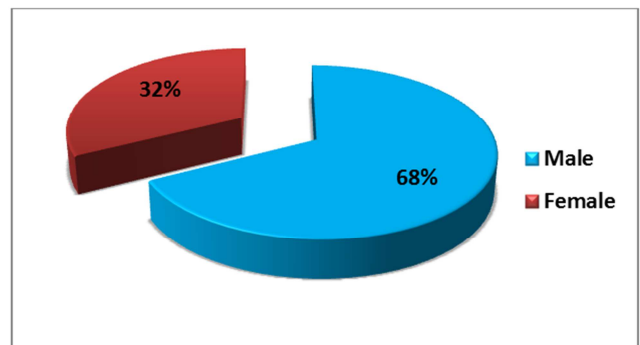


Figure 1. Distribution of the study population by sex.

3.2. Distribution of Parasitic Species According to the Sex of the Patients

Le pourcentage des espèces parasitaires isolées enregistré chez les hommes est presque le double que celui chez les femmes pour le cas d'*Entamoeba coli* et de *Blastocystis hominis*. Ce pourcentage est plus de 5 fois chez les hommes que chez les femmes pour *Chilomastix mesnili*. De même aucune femme n'a été parasité ni par *Entamoeba histolytica* ni par *Giardia intestinalis*.

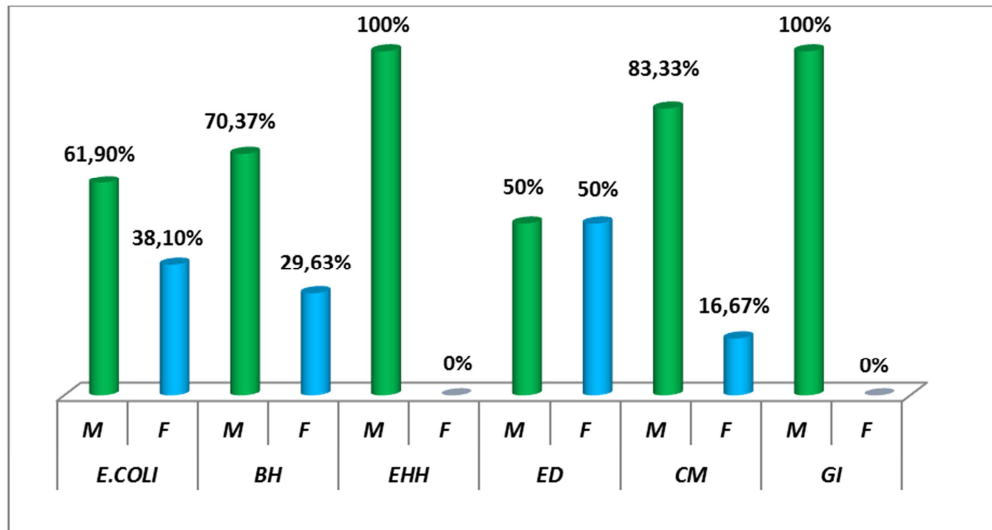


Figure 2. Distribution of the isolated parasite species by sex.

M: Male F: Female E. coli: *Entamoeba coli*
 BH: *Blastocystis hominis* EHH: *Entamoeba histolytica histolytica*
 ED: *Entamoeba dispar* CM: *Chilomastix mesnili* GI: *Giardia intestinalis*

3.3. Identification of the Isolated Parasite Species

Parasitological examination of stool was used to isolate 5 parasite species, of which *Blastocystis hominis* is the most common (45.76%), followed by *Entamoeba coli* (35.59%)

and *Chilomastix mesnili* (10.17%). *Entamoeba histolytica histolytica* and *Entamoeba histolytica minuta* and *Giardia intestinalis* were only weakly identified with proportions of 3.39% and 3.39% and 1.69% respectively (Figure 3).

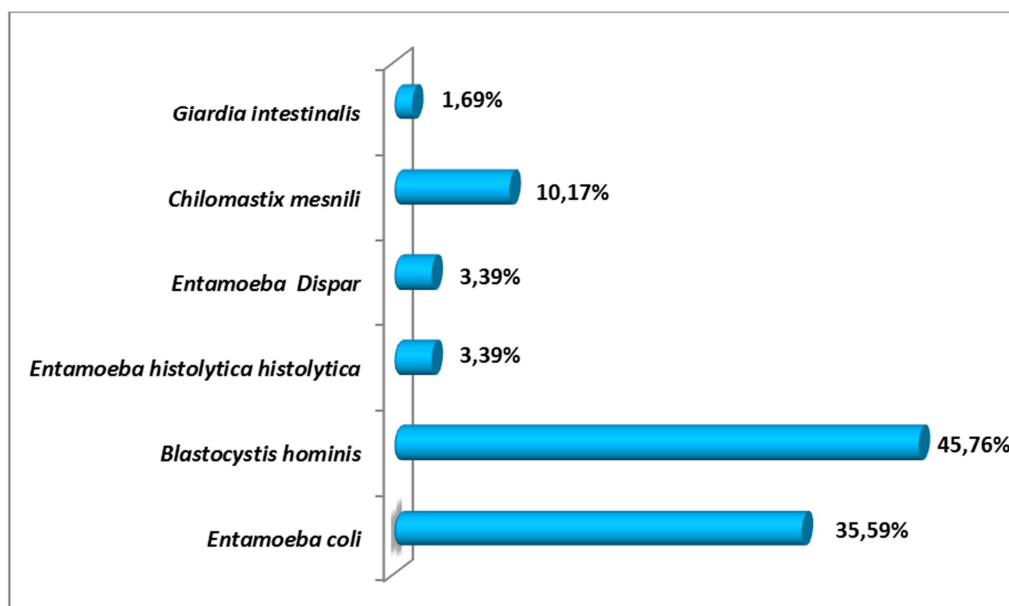


Figure 3. Distribution of isolated parasitic species.

3.4. Distribution of Species According to the Type of Parasitism

The analysis of the results according to the parasitism association mode shows that 47.36% of the cases are in mono-parasitism mode, while 52.64% of the subjects presents more than one parasite (Table 3).

In the case of poly-parasitism, there are two types of parasite association:

Table 1. Distribution of species by parasitism.

Mono-parasitism	Poly-parasitism
47.36%	52.64%
18	20
	2 parasites
	3 parasites
	19
	1
	95%
	5%

Figure 4 show the different percentages of the most dominant association (95%) that of the two parasites. The *Blastocystis hominis* and *Entamoeba coli* association is most dominant with a percentage of more than 85.71%.

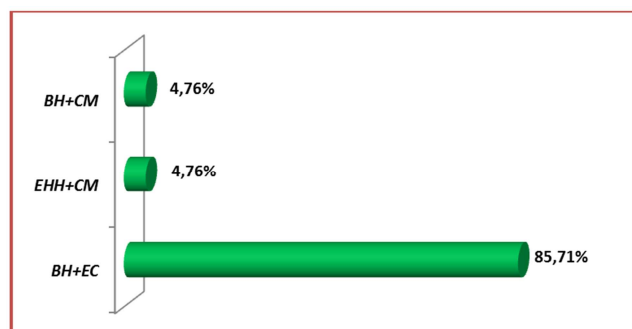


Figure 4. Type of associations observed.

BH: *Blastocystis hominis* EHH: *Entamoeba histolytica histolytica*
ED: *Entamoeba dispar* CM: *Chilomastix mesnili* EC: *Entamoeba coli*

4. Discussion

Our study was based on a sample of 70 samples, consisting of 50 men and 24 women.

The results showed that 51.35% of the samples analyzed at least one intestinal parasite, this percentage being higher than that found in Morocco, and in Algeria with prevalences of 14.15% and 35.06% respectively [6, 7] (Table 2). This difference can be explained by the fact that the other studies were carried out over a wider period (between 01 and 10 years) and a larger sample (between 358 and 4285) [7,6].

However, the prevalence found in this study is lower than that reported in Burkina Faso with prevalences of 54.7% and 71.05% respectively [8,9]. This is probably due to geographical distribution where it is noted that countries of southern Africa are the most affected because of several factors including hygienic factors adverse effects and socio-economic factors. Likewise, the numbers on which the studies are based and also the methodology used to collect the data was different.

Table 2. The prevalence of intestinal parasitism.

Our study	Maroc 2009 EL Guamri	Algérie 2016 EL Kassmi	Burkina Faso 2011 Cissé et al	Burkina Faso 2014 Zida et al
51.35%	14.15%	35.06%	54.70%	71.05%

Regarding the prevalence of amoebae, the study estimated 33.78% of the subjects infected by amoebae, this frequency is higher than that in Burkina Faso and Algeria with prevalences of 29.8% and 29.5% respectively [8,7].

On the other hand, it is lower than that reported in Burkina Faso and Morocco with proportions of 66.71% and 47.04% respectively [6, 9, 10] (Table 3).

The inequality of the prevalence of amoebae in these studies is always related to the geographical difference, moreover the case of Burkina Faso in the year of the study, this can be explained by the rise in temperature during this year. study, knowing that amoebae resist much better in the cold climate than in the hot climate which is an unfavorable condition for the maintenance of their life cycle.

Table 3. The prevalence of amoebae.

Our study	Maroc 2009 EL Guamri	Algérie 2016 EL Kassmi	Burkina Faso 2011 Cissé et al	Burkina Faso 2014 Zida et al
33.78%	47.04%	29.5%	29.8%	66.71%

After khi-two test, the present study showed that the difference is not significant, so patients are at the same risk of infestation regardless of gender. This can be explained by the nature of the population studied which is mainly military, and also by the fact that men are in contact with agricultural fields more than women and less hygiene compared to them.

After identification of the species, the percentage of *Blastocystis hominis* is 45.76% followed by *Entamoeba coli*

35.59% followed by *Chilomastix mesnili* 10.17% then *Entamoeba histolytica histolytica* and *Entamoeba histolytica minuta*, the latter having the same proportion 3.39% and in late *Giardia intestinalis* with a low proportion of 1.69%.

The low rate of the pathogenic species *Entamoeba histolytica histolytica* could be explained by the improvement of the standard of living and hygiene of our population (Table 4).

Table 4. The prevalence of parasitic species.

	Our study	Algérie 2016 EL Kassmi	Burkina Faso 2011 Cissé et al
<i>Entamoeba Coli</i>	35.59%	7.70%	—
<i>Entamoeba Histolytica Histolytica</i>	3.39%	5.60%	—
<i>Entamoeba Histolytica Minuta</i>	3.39%	—	10.40%

In this study, 47.36 % of the subjects presented a mono-parasitism and 52.64 % a poly-parasitism. These results differ with those in Algeria where mono-parasitism represents a percentage of 85% and 15% of subjects who have poly-parasitism [11] (Table 5).

Table 5. The prevalence of intestinal parasites according to the mode of association.

	Our study	Algérie 2016 EL Kassmi
Mono-parasitism	47.36%	85%
Poly-parasitism	52.64%	15%

This difference between the results can be explained by the duration of the study, which is wide for Algeria and also the studied population which is larger. In the case of poly-parasitism, the association of pathogenic and non-pathogenic parasites such as case of *Blastocystis hominis* which is, according to the studies, probably not pathogenic, but if it exists with a high quantity, gives a strong indication on the existence of pathogenic parasites as the case of association between *Blastocystis hominis* and *Entamoeba histolytica histolytica* and *Giardia intestinalis* [12, 13].

The percentages of positivity of the three techniques used (Ritchie and Willis and MIF) are very close and very compatible. All three techniques identified all parasites. But the quality of vision of the parasite differs from one technique to another. In our study, the Willis technique is the best technique to isolate parasites, which corrected the percentage of positivity of 9.25% followed by Ritchie (8.65%) and MIF (6.98%), last is the only technique that allows better vision for amoebae.

Many studies showed that Ritchie's technique is a good technique for concentrating the majority of parasitic elements. This method corrected the negativity of direct examination for 12 cases outside of *Blastocystis hominis* [14].

5. Conclusion

Intestinal amoebiasis, this cosmopolitan parasitosis caused by *Entamoeba histolytica histolytica*, affects according to the who 10% of the world population of which 10% of them could be symptomatic.

The present work is a modest contribution to the study of the prevalence of amoeba in the regional military context for a period of time two months. *Entamoeba histolytica histolytica* and *Entamoeba Dispar* were only weakly identified with proportions of 3.39% for each.

This should be interpreted by improving the standard of living and hygiene of the population studied. Population that is predominantly military and urban with a medium socio-economic level. Similarly, the study period is short enough to have a large staff to confirm the results found. These results do not reflect, the state of resident patients in rural or precarious environments. For example, broadening the scope of these studies by similar surveys focusing on the rural environment would be desirable.

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