

(RESEARCH ARTICLE)



## Epidemiological profile of digestive protozooses at the parasitology-mycology laboratory of Chu Hassan II

Meryem Kadiri <sup>1,2,\*</sup>, Raounak El Yaacoubi <sup>1,2</sup> and Zineb Tlamçani <sup>1,2</sup>

<sup>1</sup> Department of Parasitology and Mycology, Central Laboratory for Medical Biological Analysis, CHU Hassan II, Fez, Morocco.

<sup>2</sup> Faculty of Medicine and Pharmacy Sidi Mohammed Benabdellah, Fez, Morocco.

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### Abstract

Digestive protozooses are caused by parasites of the protozoan group.

Some species are commensal and recognized as little or non-pathogenic for humans, while others are pathogenic and can induce malabsorption syndromes, diarrhea, altered general condition and statural-retardation in children.

Their prevalence reflects the hygiene status of the population and, given their frequency, represents a public health problem in developing countries. Our study will focus on the group of pathogenic digestive protozooses, notably Amoebiasis, Giardiasis and Intestinal Coccidiosis.

This is a descriptive retrospective study carried out in a population of 4400 patients, conducted in the parasitology-mycology department of the Hassan II University Hospital in Fez over a period spread between 2016 and 2021.

Each patient received at least one parasitological stool examination. Each stool was processed in two stages:

- Direct examination in the fresh state with and without staining (Lugol 2%, MIF...)
- Direct examination after concentration.

Followed by staining with Ziel Neelsen modified for immunocompromised patients.

**Keywords:** Protozooses; Entamoeba; Giardia; Coccidiosis; Immuno depression

### 1 Introduction

Digestive protozooses are caused by parasites of the protozoan group. Some species are commensal and recognized as little or non-pathogenic for humans, while others are pathogenic and can induce a syndrome of malabsorption, diarrhea, altered general condition and status-weightal retardation in children [1].

In many countries, they represent a real public health problem, especially in third world countries where promiscuity, lack of drinking water and sanitary facilities are a major scourge. In fact, their prevalence reflects the population's state of hygiene. Demographic growth, immigration and climatic conditions also favor the spread of parasitism in a population [2].

\* Corresponding author: Kadiri Meryem

Their distribution is ubiquitous, with a tropism for tropical and poor regions [2].

Protozooses are often intestinal in origin, but can also spread to extra-intestinal sites, notably the liver and lungs. In certain situations, particularly immunodepression, extra-digestive localizations are possible.

The protozoan group comprises:

- Rhizopods: *Entamoeba histolytica*, *coli*, *butschlii*, *gingivalis*, *hartmani*, *polecki*, *dientamoeba fragilis*, *endolimax nana*.
- Flagellates: *Giardia intestinalis*, *Trichomonas intestinalis*, *Chilomastix mesnili*, *Embadamonas intestinalis* and *Enteromonas intestinalis*.
- Ciliates: *Balantidium coli*.
- Coccidia: *Cryptosporidium sp*, *Cyclospora sp*, *Isospora sp*.
- Microsporidia: *Enterocytozoon sp*, *Encéphalisozoon sp*.
- *Blastocystis hominis*.

Worldwide, they are the third most common cause of death after malaria and bilharziasis, and the leading cause of morbidity in children [2].

Known protozoan pathogens include: *Entamoeba histolytica histolytica*, *Giardia intestinalis*, *Cryptosporidium sp*, *Cyclospora sp*, *Isospora belli* and *Sarcocystis hominis*.

Parasitological diagnosis of protozooses is not always straight forward, and their detection in stools in different forms: eggs, larvae and adult forms for helminths, cysts and vegetative forms for protozoa, calls for specific techniques and may require several samples.

The aim of our study, carried out at the parasitology-mycology laboratory of the Hassan II University Hospital, is to determine the epidemiological profile of digestive protozooses belonging to the group of pathogens, notably *Entamoeba histolytica*, *Giardia intestinalis*, *Cryptosporidium sp*, *Cyclospora sp*.

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## 2 Materials and methods

This is a descriptive retrospective study carried out in a population of 4400 patients conducted within the parasitology-mycology department of Hassan II University Hospital in Fez over a period spread between 2015 and 2021.

Each patient underwent at least one parasitological stool examination. Each stool was processed in two stages:

- Direct examination in the fresh state, without staining with physiological water. It highlights vegetative forms, their size, cytoplasmic content, mobility and also indicates the presence of leukocytes and yeasts.
- Direct examination with staining :
  - Double Lugol's stain
  - Merthiolate Iodine Formol (MIF) staining
  - Specific stains :

Modified Ziehl Neelsen: coccidial oocysts.

- Direct examination after concentration: These are two-phase methods using the properties of ether:
  - Bailanger
  - Modified Ritchie

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## 3 Results

### 3.1 Prevalence of protozooses

Over a six-year period from 2016 to 2021, 4,400 subjects had undergone a parasitological stool examination, 610 of whom were positive (14%), 530 of whom (86.88%) had digestive protozooses.

The prevalence of intestinal protozoan parasitism will be studied by calculating the Specific Parasite Index (SPI), which is the percentage of subjects parasitized by a parasite or group of parasites in relation to the total number of subjects examined (table 1):

$$\text{S.P.I.} = (\text{Number of positive cases} / \text{Total number of cases}) \times 100$$

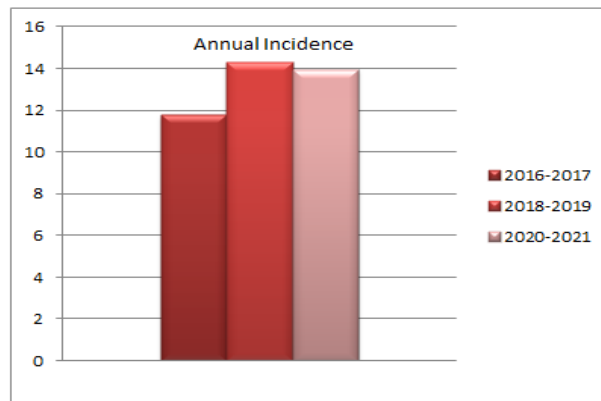
**Table 1** SPI of intestinal parasitism to protozoa

Number of cases in serie	4400
Number of positives	530
SPI of protozoa	12%

SPI is estimated at 12%.

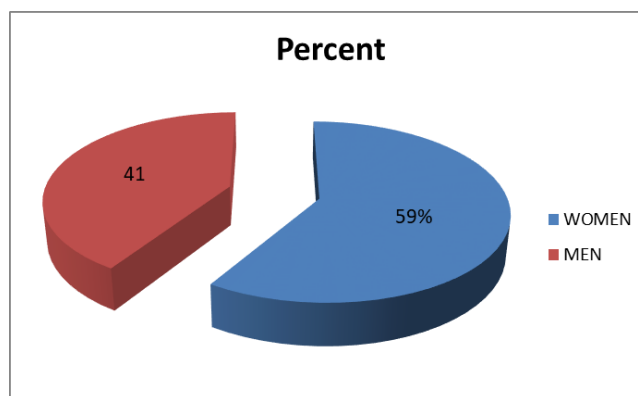
### 3.2 Annual incidence

With regard to annual incidence, we noted low figures between the years 2016-2017 then an increase between 2018 and 2019 with a decrease between 2020 and 2021. This decrease can be explained by the improvement in hygiene measures during this period, which coincided with the appearance of the Covid 19 virus (Figure 1).



**Figure 1** Annual incidence of digestive protozooses

### 3.3 Age and gender distribution



**Figure 2** Distribution of protozooses by gender

The age range affected varied from one month to 85 years, with an average age of 32 years and a percentage of children of 4%.

Based on our statistics, we can see that protozooses occur less frequently in children, although this finding remains insignificant given that we receive few parasitological stool examinations from the under-15 age group.

In our series, infection with digestive protozoa was more prevalent in women (59%) than in men (49%), with a gender ratio of 1.24 in favor of women (figure 2).

We can see that gender has no impact on digestive protozoan infection.

### 3.4 Observed parasites

In our series, the pathogenic parasites observed were: *Entamoeba histolytica/Dispar*, *Giardia intestinalis*, *Cryptosporidium sp* and *Cyclospora sp* (figures 3, 4, 5, 6 ).



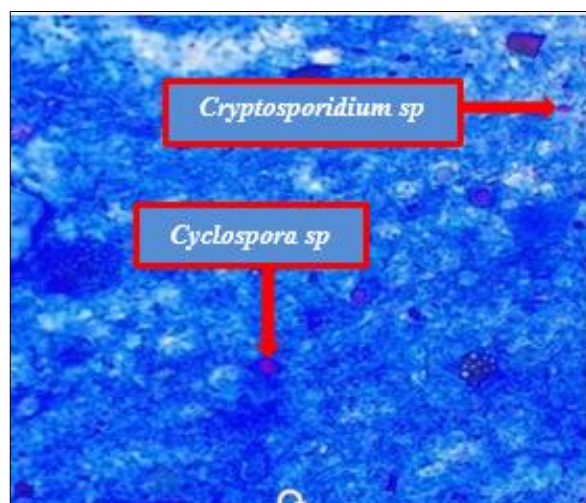
**Figure 3** Appearance of the vegetative form of *E.histolytica histolytica* fresh in physiological water (x40)



**Figure 4** Appearance of the cystic form of *E.histolytica/Dispar* in the fresh state (x40)



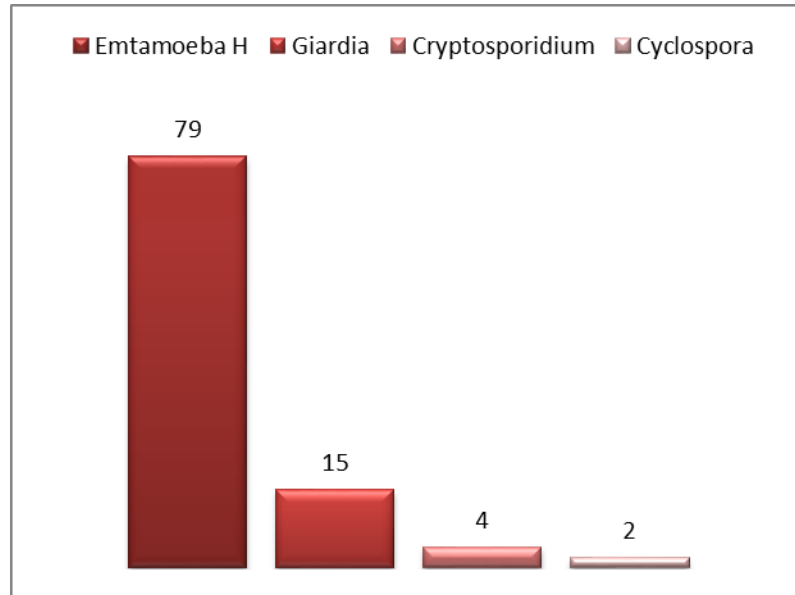
**Figure 5** Aspect of the cystic form of *Giardia intestinalis* in the fresh state (x40)



**Figure 6** Appearance of oocysts of: *Cryptosporidium sp* and *Cyclospora sp* with modified Ziehl Neelsen stain (x100)

### 3.5 Species prevalence

79% of protozooses were caused by *Entamoeba histolytica*, and 15% by *Giardia intestinalis*. Cryptosporidiosis and Cyclosporiasis were less frequent, accounting for 4% and 2% respectively (figure 7).



**Figure 7** Prevalence of pathogenic protozooses (%)

### 3.6 Parasitic associations

Only 1.5% of our patients (8 out of 530) carried more than one parasite. The various associations described in our series were as follows:

- *Entamoeba h/Dispar/Giardia* (3 of 8 patients)
- *Entamoeba h/Dispar/Cryptosporidium sp* (3 of 8 patients)
- *Entamoeba h/Dispar/Cryptosporidium sp/Cyclospora sp* (2 of 8 patients)
- *Cryptosporidium sp /Cyclospora sp* (2 of 8 patients)

We noted that *Entamoeba histolytica/Dispar* was associated with all species, but not significantly. We also noted that *Cryptosporidium sp Cyclospora sp* species were frequently associated with each other, but always without any significant link. On the other hand, these two species are often associated with immunocompromised subjects.

### 3.7 Parasites

Unlike *Giardia intestinalis* infection, which was more frequent in children (62%), 73% of *Entamoeba histolytica* parasites were in adults.

Infections with *Cryptosporidium sp* and *Cyclospora sp* were more common in immunocompromised patients (54%).

The majority of our immunocompromised patients were undergoing chemotherapy and followed up in the oncology department for cancer (acute leukemia, lymphoma, Hodgkin's disease, pulmonary carcinoma) and patients on immunosuppressants followed up in the gastroenterology department for chronic inflammatory bowel disease (IBD). The remaining patients were nephrology patients with chronic end-stage renal failure, with only one kidney transplant patient. Only one patient was diabetic (Table 2).

**Table 2** Types of immunosuppression seen in patients with *Cryptosporidium sp/Cyclospora sp*

Immunodepression type	percentage	Department
Patients undergoing chemotherapy/Cancer	51%	Onco-hematology
Patients on immunosuppressive therapy (IBD/renal transplant patients)	34%	Gastroenterology and nephrology
Chronic end-stage renal disease	12%	Nephrology
Diabetes	3%	Endocrinology

#### 4 Discussion

With regard to studies carried out in Africa, our results are lower than those found in the Central African Republic, where the percentage of sufferers was 34.1% [1], and those found in Oran: 19.96% [3] and in the Senegal River basin: 30.6% [2], but still higher than the statistics found in the Tunis region: 12.55% [4]. At national level, when we compared our results with those of a study carried out on the population of the city of Agadir, we found that our percentages were close: 15.01% of the population of Agadir compared with 14% of the population of Fez [5].

Protozoa are the most dominant parasites in our series (86.88% of digestive parasitoses), and our results concur with those of the Tunis region (94.53%) [4], Oran (95.7%) [3], and Agadir region (92.54%) [5]. On the other hand, compared with those recorded at the Guadeloupe university hospital, our results are discordant, with 27.7% of Protozoa. This can be explained by the different climatic conditions in each region.

In our series, the prevalence of positivity according to gender showed that 58% of our patients were females, compared with 42% male. Despite the predominance of females, the rate of parasitism by gender remains insignificant. This is in line with research carried out in the Central African Republic [1], the Oran region [3] and Agadir [5]. We can explain this finding by the fact that both genders are subject to the same hygienic conditions and the same risks of infestation.

The age groups involved in our study ranged from one month to 85 years, with an average age of 32. Adults (96%) were more affected than children (4%), and our results concur with those obtained in the Oran region (adults 71.15% and children 28.8%) [3]. In the Agadir region, the most-affected population is that of adults aged between 21 and 30 [4]. According to a study carried out in Kénitra [6], children were more affected than adults (adults 19.97%, children 80.03%).

The most frequently isolated pathogenic species in our series was *Entamoeba histolytica*, with a percentage of 79% (based on a sample of pathogenic protozoa only). These results are similar to those found in the Agadir and Kenitra regions, where *Entamoeba histolytica* is the most dominant pathogenic protozoan specie, with percentages of 19.28% and 24.51% respectively (the sample studied included all protozoan species) [5] [6]. The same observation was made in relation to studies carried out in the Central African Republic, where *Entamoeba histolytica* reached a percentage of 30.37% [1]. In other Maghreb countries, and more specifically in the Tunis region, the most frequent pathogenic protozoan is *Giardia intestinalis*: 1.48% [4]. The same specie was isolated in Oran with a percentage of 15.32% [3].

Intestinal coccidiosis is known to occur in immunocompromised conditions. The major determinant of the severity of these infections is immune status. Their incidence has risen rapidly in recent years with the multiplication of transplants and the spread of AIDS, then fallen back sharply with the progress of triple therapy. These parasites represent an important and not inconsiderable role in opportunistic diseases.

In our series, as in the literature, coccidiosis occurs mainly in immunocompromised subjects. The most prevalent types of immunosuppression we encountered in our subjects parasitized by one or both of the previously described coccidial species (*Cryptosporidium sp* and/or *Cyclospora sp*) were chemotherapy, cancer, particularly hematological malignancies, and immunosuppressive therapy in IBD patients. We also described cases of renal failure, one renal transplant patient and one diabetic. These results are not similar to those reported in the literature, given that intestinal coccidiosis primarily affects HIV-infected patients, followed by haematological malignancies and immunosuppressive therapy [7] [8].

Cryptosporidiosis, for example, has a prevalence ranging from 2% in industrialized countries to 6% in developing countries, with 4% in our study, a figure close to that in the literature. Cryptosporidiosis in HIV-positive patients can reach 14% in industrialized countries and 24% in developing regions [9].

#### 5 Conclusion

Digestive protozooses are one of the leading causes of morbidity worldwide. Their epidemiology is linked to faecal peril, which explains their frequency in developing countries, and is therefore an indicator of a population's level of hygiene.

*Entamoeba histolytica/Dispar* infection remains the most common in adults and Giardiasis in children.

Coccidiosis mainly affects the immunocompromised, and persists in regions of the world where AIDS is rife.

Parasitological examination of stools plays a vital role in the detection and diagnosis of intestinal parasitosis in general, and protozoosis in particular.

The prevention of intestinal protozoan infections is linked to raising public awareness of faecal hygiene, good toilet maintenance, particularly for children, and the proper treatment of water and food intended for consumption.

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### **Compliance with ethical standards**

#### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

#### *Statement of informed consent*

Informed consent was obtained from all individual participants included in the study.

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