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In Sector: Biological Sciences
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Protection of ecosystems

Theme

Prevalence of human blastocystosis in the region of Mila, correlation with abiotic factors

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Dedication

I dedicate this memory to the most loving mother in the world **Zohra** , little darling mom, every day you prove your love to me. My turn to give you the proof that I love you with all my heart. I promise to try to be more successful. I know that the greatest gift I can give you is to make an effort and to love you very much, may Allah keep you for me.

My first and my forever hero, my precious father **Abderrahmane** who leads me through the valley of darkness with light of hope and support who stand by me when things look bleak. He who always says : “I am here for you , I am living for you ”Almighty God preserves you and gives you health, happinessand long life.

to all my sisters Thank you, lovely sisters, **Meriem ,Widad , Sonia , Nour Elhouda, Aya** , for showing so much kindness and helpfulness towards me. May Allah, the Highest, grant you a happy life and a prosperous future.

To all my family: uncles, aunts, cousins and cousins , To all the members of my family, young and old Please find in this modest work the expression of my Affection.

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Dedication

In the name of Allah, the Merciful, the merciful

Thank God enough and pray for the beloved Mustafa and his family and those who are still faithful . To whom she spared no breath in my upbringing - affectionate mom To whom I reached out my hands in order to take care of me-my patient father

And to the kind family that has supported me and continues to be brothers and sisters...

And to the Companions of the journey who shared his moments with me, may Allah bless them and grant them: **Rania, Moufida , amina And to my mrs , Tayaa Hakima**

I hope that our research will be sincere to the face of Allah and that it will be useful, and that he will forgive us our slips in it and prove us for what we have agreed to and teach us and write us with the students of science following the Sunnah of his noble Prophet, May the best Prayer and peace be upon him.

Hadjer

ABSTRACT

In order to determine the epidemiological and clinical characteristics of *Blastocystis hominis* in the region of Mila, we developed a retrospective study during a period extending from January (2012 to December 2022), and the other prospective during three-months (January-March 2023). We collected the data at the level of parasitology-mycology laboratory of the General Hospital Maglaoui Brothers Mila, for the retrospective descriptive analytical study we treated 12941 examinations, 2356 were positive, an infestation rate of 18%.

-Of the positive cases, 57.51% were male and 42.48% were female.

- Patients aged (20-44 years) are the most exposed to this type of parasitism .

-The years 2019, 2021 and 2022 had the highest infection rates, 22.62 %, 20.92% and 19.31% respectively.

-The highest number of parasitized cases noted during the Autumn season followed by winter.

-A high rate of this *Blastocystis hominis* was noted during the months of October, September.

-The highest number of parasitized cases noted during the Autumn season followed by winter.

-The climatic conditions of Mila region, like the ambient temperature increase the dissemination of the bacteria. Sunshine duration, average humidity, precipitation, and average monthly wind speed cause a decrease in the bacterial index of *Blastocystis hominis*.

The results obtained during the three months of our prospective study (January- March 2023), confirmed what we deduced from the results above (The retrospective study from 2012 to 2022).

Keywords: *Blastocystis hominis*, parasite, prevalence, epidemiology, prevalence, correlation, meteorological parameters, Mila.

RESUME

Afin de déterminer les caractéristiques épidémiologiques et cliniques de *Blastocystis hominis* dans le district de Mila, nous avons élaboré une étude rétrospective durant une période s'étendant de Janvier 2012 à Décembre 2022, et l'autre prospective de trois mois (Janvier-Mars 2023). Nous avons collecté les données au niveau de laboratoire de parasitologie-mycologie de l'Hôpital Général Frères Maglaoui Mila, pour l'étude analytique descriptive rétrospective nous avons traité 12941 examens, 2356 étaient positifs, un taux d'infestation de 18 %.

-Parmi les cas positifs, 57,51 % étaient des hommes et 42,48 % étaient des femmes.

- Les patients âgés (20-44 ans) sont les plus exposés à ce type de parasitisme.

-Les années 2019, 2021 et 2022 ont enregistré les taux d'infection les plus élevés, 22,62 %, 20,92 % et 19,31 % respectivement.

-Le plus grand nombre de cas parasités noté pendant la saison d'automne suivie de l'hiver.

- Un taux élevé de ce Blastocystis hominis a été noté durant les mois d'octobre, septembre.

-Le plus grand nombre de cas parasités a été noté pendant la saison d'automne suivie de l'hiver.

Les conditions climatiques de la région de Mila, comme la température ambiante augmentent la dissémination de la bactérie. La durée d'insolation, l'humidité moyenne, les précipitations et la vitesse mensuelle moyenne du vent provoquent une diminution de l'indice bactérien de *Blastocystis hominis*.

Les résultats obtenus au cours des trois mois de notre étude prospective (janvier-mars 2023), ont confirmé ce que nous avions déduit des résultats ci-dessus (l'étude rétrospective de 2012 à 2022).

Mots clés : *Blastocystis hominis*, parasite, prévalence, épidémiologie, prévalence, corrélation, paramètres météorologiques, Mila.

الملخص

من أجل تحديد الخصائص الوبائية والسريرية للمتبرعمة الكيسية البشرية في منطقة ميلة ، قمنا بتطوير دراسة بأثر رجعي خلال فترة تمتد من يناير (2012 إلى ديسمبر 2022) ، والأخرى المرتقبة خلال ثلاثة أشهر أشهر (يناير - مارس 2023) .جمعنا البيانات على مستوى معمل علم الطفيليات وعلم الفطريات بـالمستشفى العام الإخوة مغلاوة ميلة ، من أجل الدراسة التحليلية الوصفية بأثر رجعي ، عالجنا 12941 فحصًا ، 2356 منها كانت إيجابية ، ومعدل الإصابة 18٪.

من بين الحالات الإيجابية 57.51٪ ذكور و 42.48٪ إناث.

–المرضى الذين تتراوح أعمارهم (20-44 سنة) هم الأكثر تعرضا لهذا النوع من التطفل.

– .سجلت الأعوام 2019 و 2021 و 2022 أعلى معدلات الإصابة بنسبة 22.62٪ و 20.92٪ و 19.31٪ على التوالي

- .أكبر عدد من حالات الطفيليات لوحظ خلال فصل الخريف يليه فصل الشتاء

- .لوحظت نسبة عالية من المتبرعمة الكيسية البشرية خلال شهري أكتوبر و سبتمبر.

- .تم تسجيل أكبر عدد من الحالات المصابة خلال فصل الخريف يليه فصل الشتاء.

الظروف المناخية لمنطقة ميلة ، مثل درجة الحرارة المحيطة تزيد من انتشار البكتيريا .تتسبب أشعة الشمس ، ومتوسط الرطوبة ، والتساقط ، ومتوسط سرعة الرياح الشهرية في انخفاض مؤشر البكتيريا المتبرعمة الكيسية البشرية . أكدت النتائج التي تم الحصول عليها خلال الأشهر الثلاثة من دراستنا المرتقبة (يناير - مارس 2023) ما استخلصناه من النتائج أعلاه (الدراسة بأثر رجعي من 2012 إلى 2022).

.**الكلمات المفتاحية:** المتبرعمة الكيسية البشرية ، الطفيلي ، الانتشار ، علم الأوبئة ، الانتشار ، الارتباط ، العوامل المناخية ، ميلة.

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Abbreviations list

AIDS / HIV	Human immunodeficiency virus
ANOVA	Analysis of Variance
B. hominis	Blastocystis hominis
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
GI	Gastrointestinal
GLMMs	Generalized linear mixed models
IBS	Irritable bowel syndrome
Ig	Immunoglobulin
NaCl	Sodium chloride
PSE	Parasitological stool examinations
R	Software for statistical computing and graphics.
RNA	Ribonucleic acid
sp	Species
spp	Species plurimae
SPI	Simple parasite index
SPSS	Statistical Package for the Social Sciences
SSU rRNA	Small Subunit ribosomal RNA
ST	Subtypes
WHO	World health organization
fig	Figure


Introduction

INTRODUCTION

A parasite is an organism that develops at the expense of another living being called (host). The degree of parasitism reflects the degree of damage brought to this host ranging from symbiosis to the death of the host (**Nicolas *et al*., 2001**).

The human digestive tract can be colonized by various parasitic species, whether protozoa or helminths. This strategic location within the host provides the parasite with a regular nutritional substrate and ensures the sustainability of its transmission cycle, which is mainly linked to faecal peril (**Ouraiba and Seghir, 2014**).

In the world, intestinal parasitoses constitue a serious problem of public health, especially in developing countries **(Sana *et al* ., 2015**). The intestinal parasites exists in all countries of the world with a predominance in tropical areas (**Adjeteyat *et al* ., 1997**). They can be observed all over the world. However, they are more frequent in tropical and intertropical zones than in temperate zones. Examples: amoebiasis, giardiasis, trichomonas, ascariasis, trichocephalosis, taeniasis, blastocystosis **(Bouree, 1983).**

Blastocystis spp. is a polymorphic organism; the vacuolar, granular, amoeboid, and cystic forms are the most frequent. Avacuolar, multivacuolar, and with filamentous inclusions are also recognized forms **(Khalifa, 1999 ; Devera, 2015)**. *Blastocystis hominis* is one of the most common parasites colonizing the intestines of humans and numerous animals (**Cian *et al*., 2017).** It can be part of the gut microbiota generally resulting harmless to the subject (**Scanlan *et al.*, 2014; Audebert *et al*., 2016**). Blastocystis is a frequent intestinal protozoa that causes *Blastocystosis* disease (**Tuti *et al.*, 2015**). The infection being associated with some gastrointestinal (GI) disorders (abdominal pain, diarrhea, flatulence, constipation, anorexia, nausea, vomiting, and fatigue), irritable bowel syndrome (IBS) and cutaneous urticaria lesions (**Wawrzyniak *et al.*, 2013**).

The oral-fecal route is the principal transmission pathway for infection, which occurs through human-to-human, animal-to-human, and human-to-animal (**Stensvold and Clark, 2016 ; Angelici *et al*., 2018**). The parasite infects one billion people around the world and is more commonly found in developing countries than industrialized nations (**El Safadi *et al*., 2014 ; Cifre *et al*., 2018**). The high prevalence of *B. hominis* in developing countries is usually related to consumption of contaminated water or food and poor hygiene practices (**Belleza *et al*., 2015**).

The prevalence of *B. hominis* is not the same in different regions. This may be due to the weather conditions, public health and food and varies cultural habits. Demographic factors such as gender, age, place of residence, level of education, type of occupation and epidemiological factors such as geographical conditions, also have a clear and obvious influence on the severity of *B. hominis* infection (**Horiki *et al*., 1997; Abe *et al*., 2002; Yan *et al*., 2006; Mokhtari, 2010; Anvari *et al*., 2018, 2019**).

Environmental conditions play an important regulating role in the distribution, transmission and developmental success of parasites and pathogens. Meteorological parameters can influence both the parasite species richness as well as the intensity of infection in the host species (**Mas-Coma *et al*., 2008**). Given the importance of environmental factors in host-parasite interactions and parasite life-history it is anticipated that the geographical range of most parasites is likely to shift in response to host movement and a change in climate (**Sutherst, 2001**).

In recent years, researches on the identification and prevalence of *Blastocystis* sp. both in humans and animals have been reported throughout the world (**Lee *et al*., 2012**).

in this context we have elaborated this work which aims to make a retrospective epidemiological study on cases of human blastocystosis collected at the level of public hospital Brothers Maghlaoui - Mila during the last ten years (2012 to 2022), which aims to establish the frequency of *B. hominis* in the region of Mila, and investigate the distribution of this parasite according to gender, age groups, years, seasons and months. This work aims to assess the prevalence of human blastocystosis in the Mila region as well as the correlation with meteorologecal factors.

Our work is divided into three chapters:

- ✓ The first devoted to a bibliographic study devoted to general information on *B. hominis* and blastocystosis.
- ✓ The second encompasses all the methodology followed in the Mila parasitology mycology laboratory, which consists of the application of different parasitological techniques in the search for *B. hominis* as well as the methods of exploiting the results obtained.
- ✓ The third axis: Results obtained and their interpretation.
- ✓ The fourth axis: Discussion of the obtained results in relation to the data of the scientific literature.

Finally, a conclusion closing the work.

Presentation of the biological model

1. PRESENTATION OF BIOLOGICAL MODEL

1.1. Historical

Blastocystis is the most common intestinal parasite found in humans. Although it was described for the first time at the beginning of the 20th century more than 100 years ago more exactly in 1911 by Alexeieff, and called *Blastocystis hominis* a year later by Brumpt (**Poirier *et al*., 2014**). It was described as a yeast-like fungus, while (**Zierdt *et al*., 1967**) reclassified it as a protozoa. So in 1912 Émile Brumpt considered it a new genus of fungus from the Ascomycetes group. Indeed, its morphological aspect (great variation in size, absence of locomotor organs, no cell division) made it classify as saprophytic intestinal yeasts. But for the past fifteen years, more and more publications have associated this organism with intestinal symptoms, particularly in immunodeficient subjects (**Bourée, 2007**).

In 1991, Zierdt by studying in depth the ultra-structural, biochemical and cultural aspects, concluded that it is a parasite of the protozoan and anaerobic type (Zierdt, 1991). In culture, first successful in 1921 in human serum, Blastocystis emits pseudopodia and reproduces by binary division (**Boreham and Stenzel, 1993**).

In 1996, molecular biology studies made it possible to classify this organism in a new species of Stramenophile heterogeneous group of unicellular eukaryotic microorganisms including diatoms, brown algae and oomycetes (**Arisue *et al*., 2002; Wawrzyniak *et al* ., 2008**). It presents in several aspects: most often vacuolar form in the stools and granular or amoeboid form in culture and sometimes also in the saddles (**Tan and Suresh ,2006**).

1.2. Presentation of the parasite

Blastocystis is an anaerobic eukaryotic parasite of the digestive tract of humans and many animals (**Silberman JD *et al* ., 1996**). This parasitic belonging to the stramenopila group which includes algae, diatoms and oomycetes. Which is responsible for frequent cosmopolitan infection. The Blastocystis group includes many species, living in the gastrointestinal tract of species as diverse as humans, farm animals, birds, rodents, reptiles, amphibians, fish and cockroaches. The waterborne disease they can cause is called blastocystosis (**Coyle *et al* ., 2012**)

Blastocystis hominis was long considered a saprophytic yeast. Indeed, its great variation in size, the absence of cell division and locomotory organs are the morphological aspects which in 1912 made it possible to classify this microorganism among the yeasts in the genus of Blastomycetes (**Bouree, 2007**). Seven decades later, Zierdt questions this classification and then classifies it among the protozoa. It has the following structural and morphological characteristics which allow it to be linked to protozoa (**Garavelli, 1992**)

1.2.1. Taxonomy and classification

Table 1. Classification of *Blastocystis hominis* **(Guéchi, 2014)**

Domain	**Eukaryota**
Phylum	Bigyra
Class	Blastocystea
Order	Blastocystida
Family	Blastocystidae
Genus	*Blastocystis*
Species	*hominis*

High genetic diversity of *Blastocystis* has been identified in isolates obtained from birds and mammals based on single nucleotide polymorphism at the small subunit ribosomal RNA (rRNA SSU) gene that allowed l establishment of different subtypes (ST) (**Stensvold *and al*., 2020; Maloney *et al* ., 2021a**). *Blastocystis* subtypes exhibit varying degrees of host specificity (**Jiménez *et al* ., 2019**). On time Currently, there are 31 proposed subtypes, although four of these subtypes are not currently validated (**Stensvold *et al* ., 2020; Maloney *et al* ., 2021a; Maloney *et al* ., 2021b**). Among these subtypes, ST1 to ST9 and ST12 have been found in humans (**Ramírez *et al* ., 2016**). ST1 to ST4 are the most common subtypes reported in humans (**Stensvold *et al* ., 2009**), and ST9 has only been reported in humans (**Andersen *et al* ., 2016**). Subtypes identified in humans have also been reported in domestic and wild animals (**Hublin *et al* ., 2020**). He There are thus several species of *Blastocystis* but the authors agree that only one is commonly found in humans, it is *Blastocystis hominis*.(**Fig. 01**)

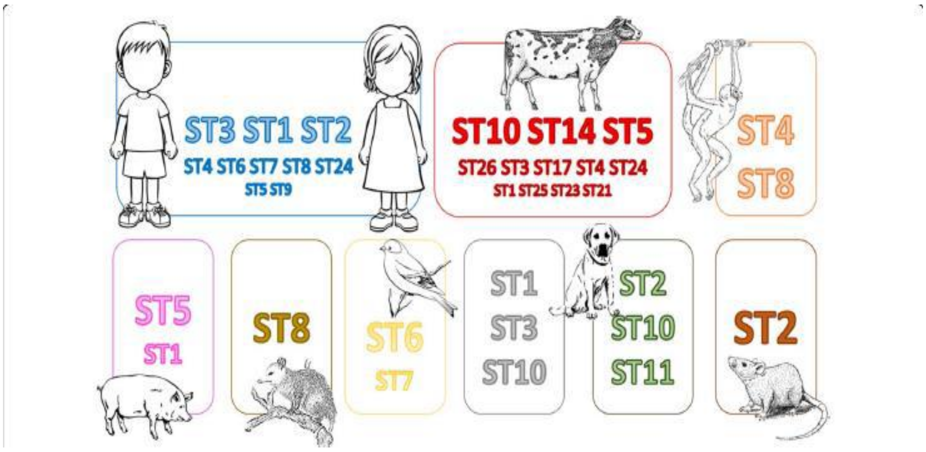


Fig. 01: *Blastocystis* subtypes found in humans and animals (**Jiménez *et al*., 2019**)

1.2.2. Structure and morphology

The blastocyst is a polymorphic unicellular eukaryotic microorganism inhabits the small bowel and colon of the human body and the animals. It can be identified in at least four forms: vacuolar, granular, amoeboid and cystic **(Trakia, 2008).** *B. hominis* has got several different morphological forms. Vacuolar, granular, ameboid, and cystic forms are the ones best described so far. Other morphological forms have also been found on electron microscopy (avacuolar and multivacuolar, of small dimensions and rarely present). In fresh stool samples and culture sam-ples, vacuolar and granular forms are the ones most commonly encountered; they can be visualized using phase-contrast microscopy, with light microscopy of native and stained sample preparations and with electron microscopy (**Zierdt, 1991**).**(Fig. 02).**

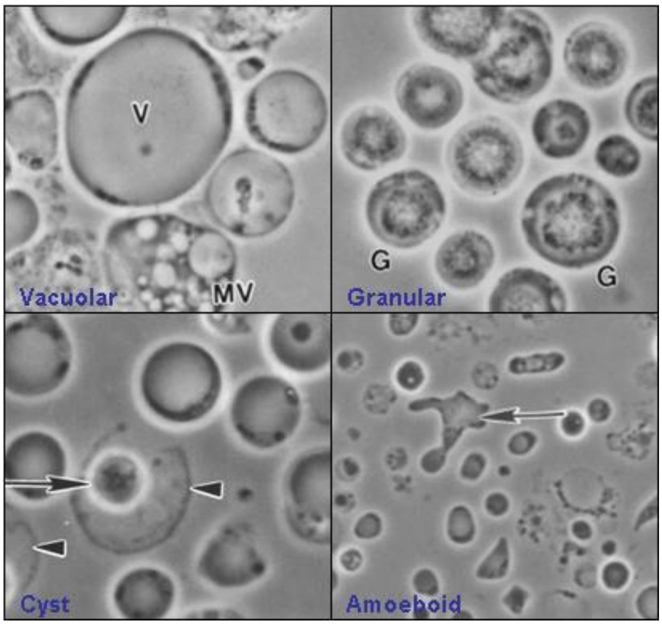


Fig. 02 : Various morphological forms of *Blastocystis hominis* (**Valentia, 2006**).

1.2.2.1. Vacuolar form

This form of *B. hominis* is spherical, containing a central body representing a large vacuole, occupying approximately 90% of the cell, and a thin layer of peripheral cytoplasm situated immediately beneath the cell membrane (**fig.03**) .The nuclei can be distributed peripherally throughout the cytoplasm. There can be seven nuclei at the most, but there are two nuclei on the average, situated at the opposite ends of the cell (**Bergamo and Carvalho, 2013**).

Mitochondrion-related organelles and Golgi apparatus are located peripher-ally in the cytoplasm. Mitochondria look like roses placed around the nucleus. These structures may protrude within the central body and can have a fiberlike appearance (**Sekar and Shanthi ,2015**). It has been discovered that the central body is a membrane-enclosed vacuole, containing carbohydrates, fats, and basic proteins. These substances are accumulated within the vacuole by way of the action of the Golgi apparatus and via clathrin-mediated endo-cytosis (**Yamada and Yoshikawa, 2012**). Vacuolar forms can be of different sizes (ranging from 3 µm to 120 µm), but meas-uring 5 µm to 15 µm on the average (**Sekar and Shanthi ,2015**). It is generally accepted that this form is most commonly seen in asymptomatic carriers of *B. hominis* (**Otašević *et al.*, 2011**).

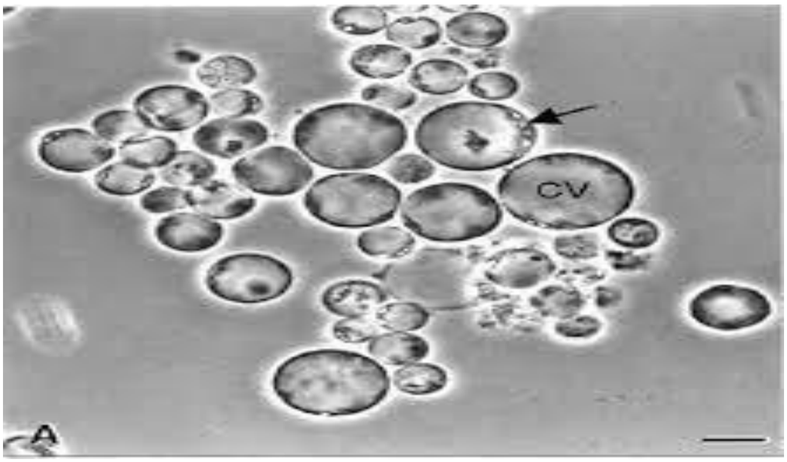


Fig. 03 : Vacuolar form of *Blastocystis hominis* (**Tan, 2004**).

1.2.2.2. Granular form

This form is very similar to vacuolar forms of *B. hominis*, but contains the granules within the cytoplasm which are often centrally situated (**fig. 04**) In 1989, **Dunn *et al.*** proposed that these structures were similar to myelin-like inclusions, small vesicles, crystal granules, and drops of fat. The granules can be metabolic, reproductive, and lipid ones (**Sekar and Shanthi ,2015**). On the average, there are two nuclei in the cytoplasm (four at the most).

They have a slightly smaller diameter compared to vacuolar forms, and measure 9,0 µm to 28,3 µm (**Bergamo and Carvalho, 2013**). They are more frequent in older cultures and the cultures treated with antibiotics, and there has also been the hypothesis that their existence is an indicant of cell death (**Otašević *et al*., 2011**).

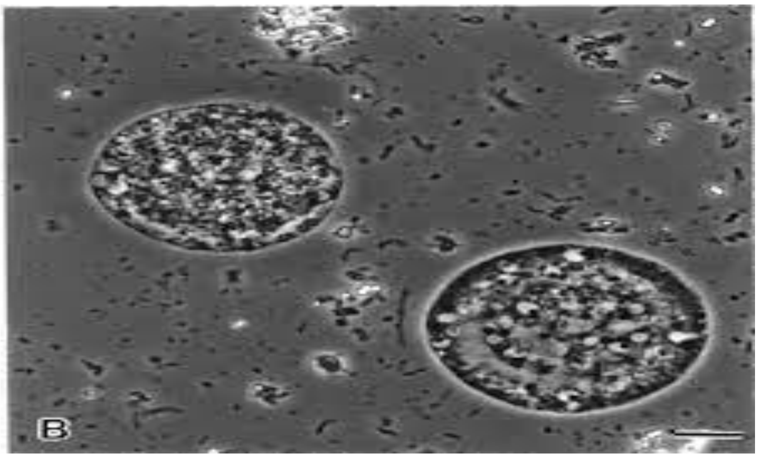


Fig. 04: Granular shape of the blastocyst (**Tan, 2004**)

1.2.2.3. Ameboid form.

This form of *B. hominis* is most rarely encountered. It is irregular in shape, with 1-2 pseudopodia (being stationary nevertheless), with con-siderable adhesion abilities, enabling its attachment to the bowel mucosa (**Otašević *et al*., 2011**). There is a large vacuole in its cytoplasm, and this form is in fact transformed into cystic form (**fig. 05**). It is more commonly present in individuals with symptoms of digestive tract infections and in cultures, indicating the pathogenic potential of this form of *B. hominis* (**Otašević *et al*., 2011** ; **Vassalos *et al*., 2010).**

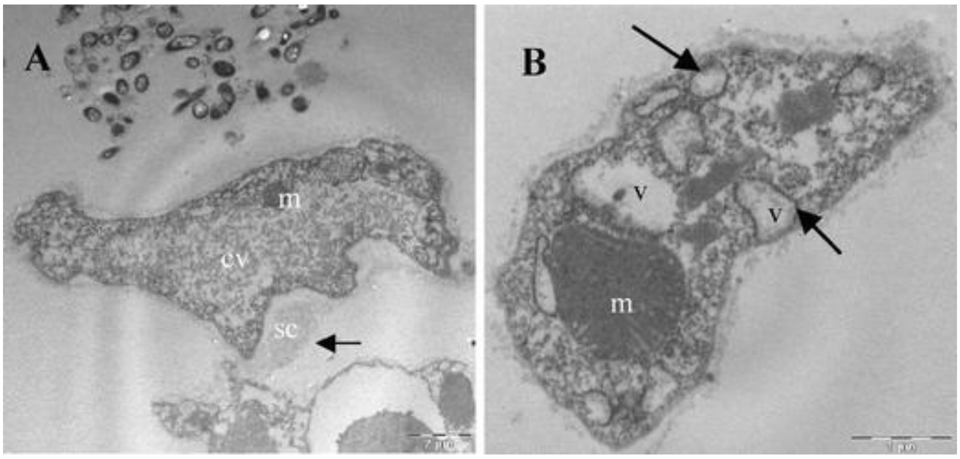


Fig. 05 : Amoeboid form (**Tan and Suresh, 2004**).

1.2.2.4. Cystic form.

These are round or oval, and with smaller dimensions (3-6 µm) (**Sekar and Shanthi ,2015**). These forms found in certain animals are larger (**Stenzel *et al*., 1997**). Cystic forms have a thin, multilayered wall with/without a surface envelope (**Bergamo and Carvalho, 2013**). Their condensed cytoplasm has got several mitochondria and storage vacuoles (**fig. 06**). The number of nuclei within the cysts varies from 1 to 4. A cyst may survive about a month exposed to air and the temperature of 25°C and enables further spread of the infection – it is a form infec-tious for humans (**Otašević *et al*., 2011 ; Tan ,2008**).

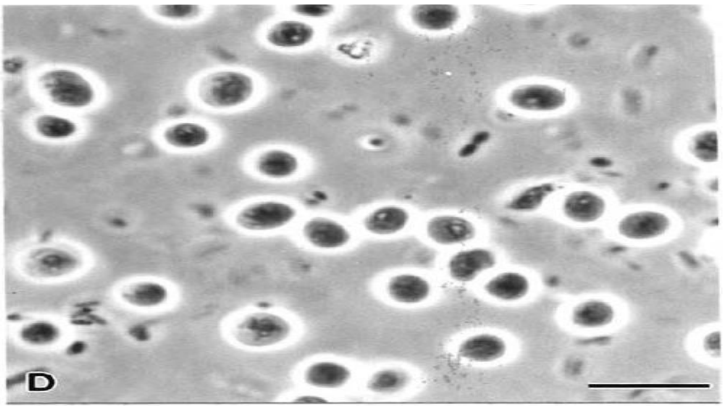


Fig. 06 : Cystic form of *Blastocystis hominis* (**Tan, 2004**)

1.2.2.5. Vegetative forms

Vegetative forms are transformed into other vegetative forms with different morphology and can thus escape identification in stool samples (**Parija and Jeremiah, 2013**). Avacuolar and multi-vacuolar forms are the most dominant forms *in vivo*, and these also most commonly remain unrecognized on microscopy (**Vdovenko, 2000**).

1.2.3. Life cycle of parasite

Infectious, cystic forms of *B. hominis* are transmitted by the fecal-oral route (**Yoshikawa *et al*., 2004**). The infection may occur after an intake of untreated water or uncooked water plants contaminated with cysts, and also via dirty hands (**Lee *et al*., 2012 ; Li *et al*., 2007**). In an adequate host, the cyst develops via the pro-cess of excitation into vegetative forms within the large bowel (**Tan, 2008**).

Further continuation of the life cycle depends on the subtype compatibility with the host (**Sekar and Shanthi ,2015**). Other forms can also develop from vacuolar ones. After a period of time after the infection, vacuolar forms form the cysts in the bowel lumen

(**Vdovenko, 2000**). The encystation occurs during the passage through the large bowel, and the cysts are then excreted via feces. Fecal cysts can be covered with a fiber-like layer which gradually disappears during the cyst development. A thin fibrillar surface layer detected in stool samples plays a significant role in the survival of this parasite *in vivo* (**Vdovenko, 2000**). It is thought that different modes of reproduction exist when this organism is concerned (binary fission, budding, plasmotomy, multiple fission, endodyogeny, schizogony). Binary fission is nevertheless the most common mode (**Zhang *et al*., 2007**).

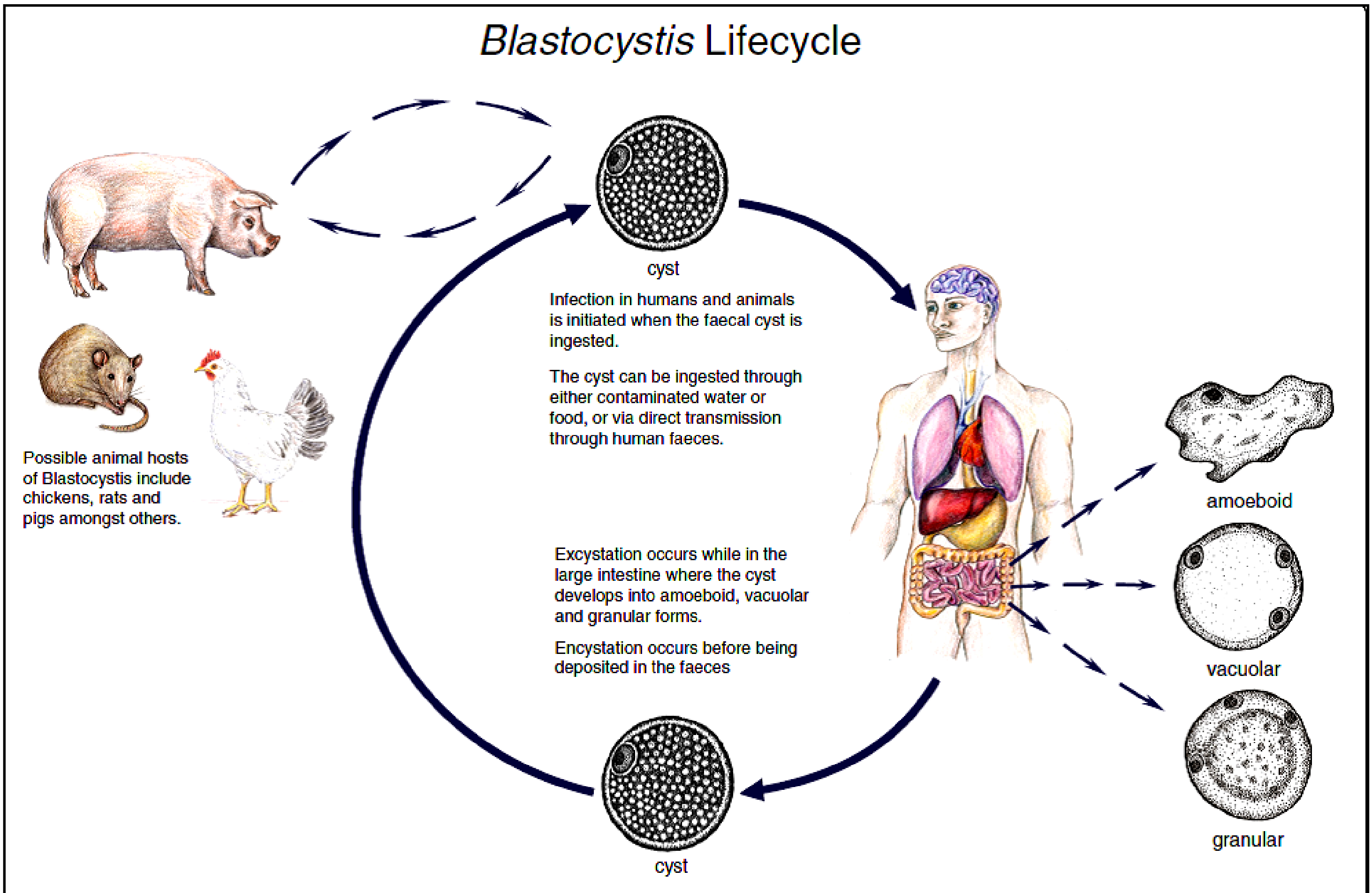


Figure 1 Lifecycle of *Blastocystis* sp.

Fig. 07 : Life cycle of *B. hominis*. (**Roberts *et al*., 2014**)

1.3. Study of the disease blastocystosis

1.3.1. Definition

Blastocystosis or **Zierdt and Garavelli** disease is a cosmopolitan parasitosis: *B. hominis* is widespread worldwide, living mainly in the colon and being eliminated in the stools of humans and certain animals (**Bandjee, 2017**). In some cases, it is a pathogen causing gastrointestinal symptoms such as diarrhoea, nausea, abdominal pain, vomiting and bloating (**Wawrzyniak *et al*., 2013**).

1.3.2. Epidemiology

1.3.2.1. Geographical distribution

Blastocystis sp infection is reported worldwide (**Stenzel *et al*., 1996**). Early reports from developed countries gave lower levels of prevalence of *Blastocystis* (approximately 1.5-10%), while it seemed more prevalent (approximately 30-50%) in developing countries (**Stenzel *et al*., 1996**). Overall, the prevalence of *Blastocystis hominis* would be low in developed countries due to the absence of faecal peril (**Abe *et al*., 2022; Abdel-Hafeez *et al*., 2016**) and particularly in some Asian countries ranging from 0.5% in Japan up to 60% in Malaysia (**Clin Microbiol Rev , 2008**). In general, this parasitosis is more frequent in developing countries, and to a lesser extent, in European countries such as Italy (7.1%) **(Bourée, 2007**) Spain (7%) (**Bourlioux, 2014**) On France, there is little epidemiological data (**Pinel *et al*., 1999**) Netherlands (24.2%) (**Abdulsalam *et al*., 2013**) and Denmark (23%) (**Badparva *et al*., 2014**). The average prevalence of the parasite would ther efore be around 20% of the European population.

In developing countries, the prevalence of *Blastocystis* is generally higher (**Abe *et al*., 2022 ; Abdel-Hafeez *et al*., 2016**) as a result of the consumption of more easily contaminated water or food, closer contact with animals that are potential sources of contamination, and more precarious hygiene conditions. Thus, the prevalence of Blastocystis can exceed 40% as in Brazil (40.9%), Nigeria (49%), Indonesia (60%) or Liberia (70%) (**Abe *et al*., 2022** ; **Calderaro *et al*., 2014**).

Considerable variations in the prevalence of Blastocystis have been observed between studies conducted in the same country, for example in Malaysia (3.9% to 52.3%), in China (1.9% to 32.6%) or Thailand (0.19% to 45.2%) (**Abe *et al*., 2022**). If the same identification method has been used, these fluctuations may then reflect real distinctions between geographical regions for which the climate, food habits or socio-economic or industrial fabric may be very different.

1.3.2.2. Transmission mode

Transmission occurs via the faecal-oral route by absorption of water or food contaminated with cystic forms of *B. hominis*. It is therefore an easy indirect transmission linked to faecal peril. There is also a direct transmission which is mainly encountered in homosexuals or during auto-infestation. Three types of transmission are known (**Capron *et***

***al*., 2003**); these are human-to-human transmission and the two human-animal and animal-human cross-transmissions.

A**. Fecal-oral transmission**

Currently, it is accepted that the mode of contamination by Blastocystis spp. occurs in a faecal-oral and zoonotic manner (**Yoshikawa *et al*., 2004c**). The presence of a cystic form resistant in water and stool in the parasite cycle, suggests that the contamination is linked to poor hygienic conditions, in particular through the consumption of dirty water and contact with animal reservoirs of the parasite (**Banaticla and Rivera, 2011**). Tests carried out on Thai military personnel showed the presence of Blastocystis spp. as being a predominant intestinal parasite, with a prevalence of 22 to 36%. This prevalence has been linked to the consumption of unfiltered or unboiled water. In the same study, 19% of schoolchildren were positive for Blastocystis spp. (**Yoshikawa *et al*., 2004c**).

B. Zoonotic transmission

Exposure to animals is another cause of contamination of populations due to the apparent low host specificity of the parasite. Thus, *Blastocystis spp*. is revealed as a very common parasite within the animal kingdom (**Yoshikawa *et al*., 2004c**). It has been reported in mammals, birds, reptiles, amphibians and arthropods. Some animals even have a very high prevalence, it can go up to 95% in pigs and 71% in sheep (**Nasirudeen *et al*., 2004; Osman *et al*., 2016**). Similarly for domestic animals where the observed prevalence varies between (17 and 67)% in cats, it reaches 70%. , in the dog. Zoo animals also show a high prevalence. Zoonotic contamination is due to the proximity of animals (**Nasirudeen *et al*., 2004**). The zoonotic transmission of the parasite would, according to the few figures cited above, have a significant impact on the prevalence of Blastocystis spp. in the human population (**Han *et al*., 2018**).

C. Human-to-human transmission

A direct mode of human-to-human contamination through contact with infected patients in a crowded situation remains very difficult to prove and therefore few studies have been published on this subject (**Banaticla and Rivera, 2011**). Other studies, however, have shown that patients and staff at two health facilities shared the same species of *Blastocystis spp.* (**Yoshikawa *et al*., 2004c**). Similarly, Thathaisong had shown that almost all young girls living in the same residence in Thailand were infected by the parasite and moreover by the same species (**Osman *et al*., 2016**). On the other hand, statistical analyzes carried out in the context of several epidemiological studies have shown that contact with members of the same family presenting with gastrointestinal disorders was a risk factor associated with infection by *Blastocystis spp*. (**Yoshikawa *et al*., 2004c; Osman *et al*., 2016**).

D. Foodborne transmission

Besides this direct transmission, it is logical to think that indirect faecal-oral transmission is more common from media such as contaminated water or food by human or animal stool cysts (**Yoshikawa *et al*., 2004c; Banaticla and Rivera, 2011; Osman *et al*., 2016).** Also, the impact of this transmission in a region is directly linked to the sanitary conditions encountered and the hygiene rules followed by the populations (**Osman *et al*., 2016**). The other possible source of indirect transmission remains of course the consumption of contaminated food. However, no data has yet come to confirm this transmission (**Ribas *et al*., 2017; Angelici *et al*., 2018**). On the other hand, *Blastocystis spp*. was recently identified in salad bags sold in supermarkets in Italy (**Imre *et al*.,. 2017**). On the other hand, several studies carried out in Malaysia, Iran, Haiti, Gambia, Egypt, Brazil or Mexico have all shown that the professional category of meat sellers or handlers is significantly more infected by the parasite than the general population

E. Waterborne transmission

Even if water seems to be the major vector of transmission, practically no data is available concerning the identification of a possible epidemic of blastocystosis. waterborne (**Banaticla and Rivera, 2011**). *Blastocystis spp*. was placed in 2006 by the WHO in the list of waterborne parasites but it is not yet sought within the framework epidemic episodes. On the other hand, the prevalence of the parasite in certain geographical regions is such that any epidemic of limited scale is difficult to detect. A case of an epidemic of *blastocystosis* affecting more than a thousand people has however been suggested in a Chinese province but without molecular confirmation of the agent involved. Similarly, two cases of family epidemics of gastroenteritis in Morocco (**Heyland *et al*., 2012**) and Italy report probable contamination of drinking water by this parasite. More recently, and still in Italy, a case of blastocystosis with diarrhea was reported with molecular evidence of parasite transmission through the consumption of undrinkable water on a farm (**Angelici *et al*., 2018**).

At the same time, many studies conducted in several mainly Asian and African countries have identified Blastocystis spp. with variable frequencies but can be significant in different sources of drinking water, whether rivers, reservoirs or directly tap water, in water used for irrigation (**Moreno and Para, 2018**), in wastewater or in recreational water (**Ribas *et al*., 2017; Pan *et al*., 2018**).

In some of these investigations, the isolates identified may have been subtypes and these subtypes are found to be identical to those found in the human or even animal population that consumes this water or lives near these different water sources (**Moreno *et al*., 2018**). This circulation of the parasite is probably favored by the fact that conventional wastewater treatments are not effective against cystic forms of the parasite (**Haresh *et al*., 1999; Moreno *et al*., 2018**). This was demonstrated by Suresh in 2005 who observed only a very small decrease in the number of cysts before and after wastewater treatment in sewage treatment plants in Scotland and Malaysia, which may have the effect of facilitating the spread of Blastocystis spp. in the environment (**Fréalle *et al*., 2015**). On the other hand, it should be noted that the statistical analyzes carried out within the framework of several epidemiological studies have shown that drinking untreated or unboiled water was a risk factor associated with infection by Blastocystis spp. (**Osman *et al*., 2016**).

F.Air transmission

Finally, a last possible mode of transmission of the parasite which has hardly ever been evaluated is the possible dissemination of cysts by the wind. Interestingly, Blastocystis spp. was detected in an air collector in the city of Seoul in South Korea (**Han *et al*., 2018**).

Blastosystis hominis can be spread by:

- Accidentally swallowing *B. hominis* picked up from surfaces (such as bathroom fixtures, changing tables, diaper pails, or toys) contaminated with feces from an infected person or animal.
- Drinking water or using ice made from contaminated sources (eg, lakes, streams, shallow [less than 50 feet] or poorly monitored or poorly maintained wells).
- Drinking recreational water contaminated with *B.hominis*. Recreational water includes water from swimming pools, water parks, hot tubs or spas, fountains, lakes, rivers, springs, ponds or streams that may be contaminated with feces or water waste from humans or animals.
- Consuming raw foods contaminated with *B.hominis*.

- Having contact with a person with blastocystosis.
- Traveling to countries where blastocystosis is common and being exposed to the parasite (**Yoshikawa *et al*., 2004c**).

1.3.2.3. Population at risk

Travellers to developing tropical countries were thought to be more prone to diarrhoea associated with Blastocystis (**Sohail *et al*., 2005**). A hig percentage of Blastocystis infection was found in soldiers transferred to endemic countries or regions, such as Egypt, the Middle

East and Central America (**Sohail *et al*., 2005**). Blastocystis was also highly prevalent among refugees and immigrants (**Stenze *et al*., 1996**).

An extremely high Blastocystis prevalence was noted in pre-school children and school children in developing countries (**Stenze *et al*., 1996**). Food and animal handlers were found to be at risk of being infected by *Blastocystis* (**Smithand *et al*., 2005 , Tan *et al*., 2002**). In addition, individuals who come into contact with animals, mainly pets, could be found to give positive results for Blastocystis (**Doyle *et al*., 1990** the culture conditions used for culturing human Blastocystis might not have been appropriate for growth of feline Blastocystis or improved sanitation could contribute to the absence of the parasite (**Duda *et al*., 1998 ; Horiki *et al*., 1997**).

Thus, further molecular studies will be required to clarify this point. On the other hand, faecal specimens from primate pets that came into close contact with humans were positive for Blastocystis in Indonesia and in the Rift Valley in Ethiopia (**Jones *et al*., 2004 ; Legesse *et al*., 2004**). In addition, Blastocystis sp. isolates were detected in rural regions of developing countries and in lowincome community groups (**Kobayashi *et al*., 1995 ; Tan *et al*., 2002**). Poor hygiene and sanitation facilities were regarded as major contributing factors (**Tan *et al*., 2002**)

1.3.2.4. Risk factors

A. Socio-cultural conditions

A study conducted by (**Apezteguia *et al*., 2004**) correlated the socio-cultural and environmental conditions and the presence of *B. hominis*. They demonstrated that precarious sanitary conditions such as malnutrition, poor hygiene defective food, the presence of latrines, dirtiness and frequent flooding in the dwellings were conducive to infections by *B. hominis*. In addition, these same authors observed that population density was related to the incidence of blastocystosis; the denser the population, the higher the prevalence rate of *B. hominis* and vice versa. (**Apezteguia *et al*., 2004**)

B. Immunological conditions

A Turkish study (**Koltas *et al*., 2000**) revealed that the occurrence of *B. hominis* infection depended on the immunological status of individuals. The subject of the study was to compare the prevalence of blastocystosis in people with hematological malignancies and in healthy people. They concluded that the prevalence of the parasite is much higher in immunocompromised subjects than in healthy subjects. Other studies carried out on AIDS patients or kidney transplant recipients under immunotherapy come to the same conclusion. (**Koltas *et al*., 2000).**

1.3.3. Clinical study

1.3.3.1. Symptomatology

The time to onset of symptoms in humans remains unknown. However, it is known that in mice, the symptoms appear 2 days after inoculation of *B. hominis* cysts and last two to three weeks (**Chen *et al*., 1997**). Human disease manifests itself in very variable ways. There are asymptomatic carriers, but it is undeniable that some subjects present digestive symptoms without any other organism, apart from *B. hominis*, being identifiable. Symptoms commonly associated with blastocystosis are no specific. The most frequently encountered clinical signs in the presence of *B. hominis* are digestive signs such as diarrhea, nausea, vomiting, abdominal pain and flatulence. Diarrhea is the predominant sign and is defined as frequent passing of loose or semi-liquid to liquid stools. The character of the stools is variable: glairo-bloody, glairo-aqueous, rice-like or molded with traces of blood. Abdominal pain is frequent and relatively moderate. They are most often diffuse reaching the abdomen in its entirety or localized in the iliac fossa. They can be spontaneous or triggered by palpation or passing stools.Flatulence is a common symptom; it is an emission by the anus of intestinal gas.
Colitis and ileitis associated with *B. hominis* infection were demonstrated through biopsies.
An alteration of the general state is often encountered. It is manifested by mild asthenia, malaise, anorexia and weight loss ranging from two to ten kilos related to water loss secondary to diarrhea.

Other symptoms may occur during blastocystosis such as headache, dizziness, heartburn, profuse sweating, fever, chest tightness, sleep disturbances, constipation alternating with diarrheal attacks, rectal bleeding, arthritis, tenesmus, intestinal meteorism , itching, hives and even hair loss.

Blastocystis hominis thus causes digestive symptoms but it is not limited to the intestinal sphere. Indeed, **Greco *et al*., in 2003**, found that blastocystosis was sometimes associated with cutaneous manifestations such as rashes (**fig. 08**), urticaria or pruritus. In the majority of cases, it is a palmoplantar pruritus, associated or not with edema and swelling. The researchers associated *B. hominis* with these cutaneous manifestations since the latter resolve when the blastocystosis is eradicated. The parasite would induce inflammation by recruitment of inflammation cells and the accumulation of neutrophils, eosinophils and lymphocytes. The toxins of *B. hominis* would activate to them the complement system generating the anaphylotoxins C3a and C5a which interact with the specific receptors of mast cells and basophils leading to the release of histamine at the origin of the cutaneous symptoms.

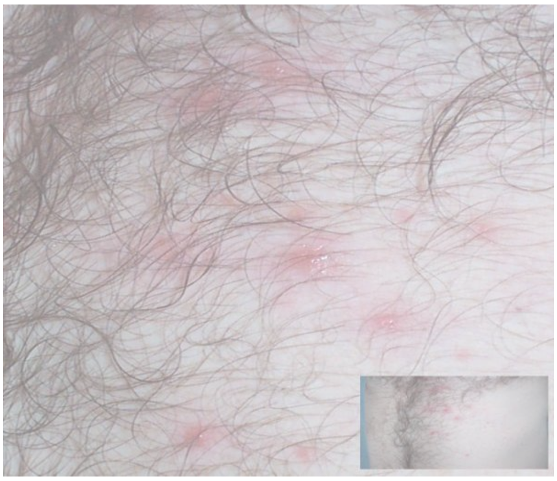


Fig. 08: Recurrent and very itchy rash in a 39-year-old man with chronic blastocystosis, according to **Boorom *et al*., 2008**

Two teams of researchers (**Abrar *et al*., 1997 ; Cirioni *et al*., 1999**) demonstrated the involvement of *B. hominis* in irritable bowel syndrome. It is a very common gastrointestinal disorder characterized by abdominal pain accompanied by diarrhea and/or constipation. Its etiology is not yet fully established. After initially thinking that this disorder was of psychosomatic origin, it has been shown that this syndrome results from a chronic activation of the immune system (**Adam *et al*., 2007**).

This syndrome is the only functional intestinal disorder for which a parasitic infection is found concomitantly in half of the cases (**fig. 09**). According to the work of **Abrar *et al*., and Cirioni *et al*., 1999**, *B. hominis* would be one of the agents responsible for this syndrome since they observed that significant blastocystosis were accompanied by a significant prevalence of irritable bowel syndrome. However, this finding can be reversed: irritable bowel syndrome – caused by an imbalance of the intestinal flora, for example – can constitute an environment conducive to the proliferation of *B. hominis*. In the latter case, *B. hominis* would play the role of indicator of intestinal dysfunction or disruption of the intestinal ecosystem rather than that of the causative agent of a gastrointestinal disorder (**Chan *et al*., 2003**). It can thus be seen that opinions differ when it comes to considering *B. hominis* as a simple indicator of intestinal disorder or else its cause.

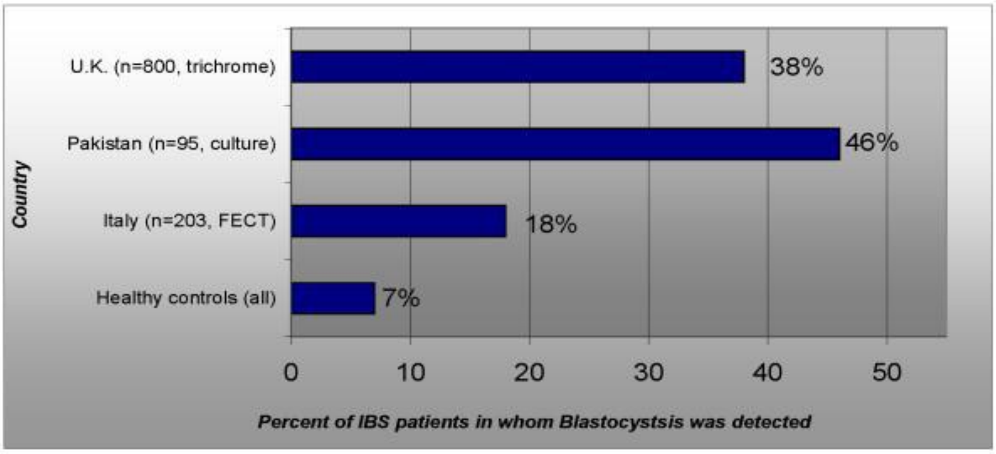


Fig. 09: Percentage of patients with irritable bowel syndrome in which *Blastocystis hominis* was detected (**Boorom *et al*., 2008**).

1.3.3.2. Diagnostic

Detection of *Blastocystis hominis* in biological samples The diagnosis of blastocystosis is essentially based on direct biological diagnosis. Its polymorphism makes its diagnosis difficult in the laboratory and requires an experienced technician to be able to detect it by direct observation in stool samples.

A. Clinical diagnosis

Digestive symptoms such as nausea, abdominal pain, bloating, excessive gas, loss of appetite, weight loss, anal itching, diarrhea or alternating constipation, pathogenicity has not been detected should raise suspicion of blastocystosis. It is up to biologists to confirm whether or not *B. hominis* is carried.

B. Biological diagnosis

The parasite is mainly found in freshly passed stools

*** Direct diagnosis**

The detection of *B. hominis* can be carried out by different methods, namely: A coprological examination in the fresh state by (electron microscope), staining, concentration, culture, extraction and amplification of DNA.

a/ Fresh stool examination

Electron microscopic examination is the basic technique for detecting *Blastocystis hominis.* The procedure for this examination is very simple: several fresh stool samples are taken from different places and placed on a slide, then diluted in a drop of 9% NaCl solution per 1000 mL, then observed immediately. The electron microscope detects the existence of two amoebic forms, the first containing a large central vacuole full of micro-granules, while the second containing several small vacuoles in the center (**Bourée, 2007**).

b/coloring

Several commonly used dyes can be used to aid in the detection of *B.hominis* namely

b.1. Slide staining

*** Staining on slide (with Lugol)**

Lugol staining is used to identify cystic forms of protozoa (especially amoeba) in stool. It allows to better visualize certain identification elements: vacuole, nucleus, karyosome. It gives the blue black color.

- **Staining on slide (Gram)**

Since the amoeboid form of Blastocystis is less frequently encountered. As its name suggests, it is irregular in shape with one or two pseudopodia but are not mobile hence its name. The cytoplasm contains a single large vacuole and this form develops into a cyst, because it resembles neutrophils and macrophages, it can easily go unnoticed during conventional stool examination. So to identify it, Zierdt suggested simultaneous Gram staining of an unfixed smear where this forms lyses upon exposure to air, while leukocytes remain intact (**Zierdt, 1991; Tan, 2008**).

- **Staining on slide (with Trichrome)**

Trichromic staining highlights vacuolar and amoeboid forms. Polyvinyl alcohol is used as a fixative. If the staining is successful, the images obtained are pure

- **Staining on slide (with Hematoxylin)**

Hematoxylin is a naturally occurring chemical used as a dye base in laboratories to stain nuclei. It requires two preliminary steps: spreading the stools on a slide and fixing with the Duboscq-Brasil fixer. Hematoxylin remains the most common nuclear stain in histology.

b.2. Coloring in tube

Tube staining (with MIF)

Merthiolate-Iodine-Formaldehyde staining is the main method of tube staining. It makes it possible to better visualize certain identification elements: the cytoplasm is colored in red and the nuclear structures in dark red or black (**Miné and Rosa, 2008**) .

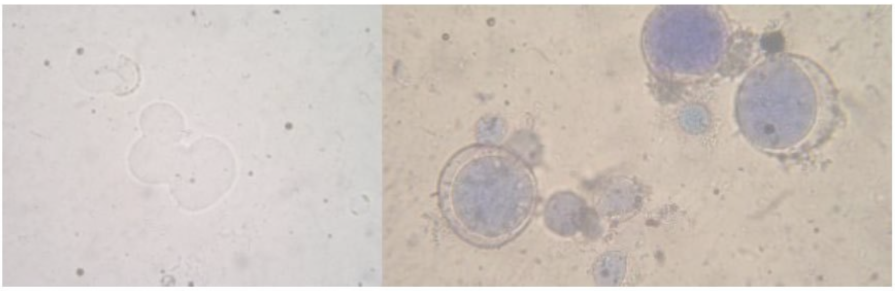


Fig. 10: Photograph of *Blastocystis hominis* with and without staining (**Miné and Rosa ,2008**)

c/ Concentration

By concentrations we mean the techniques by which we try, from a large quantity of collected faecal matter, to obtain in a small volume the different forms of the parasite, by eliminating the residues of digestion. Two techniques are used to highlight *B.hominis*:

- the RITCHIE technique which is a solution composed of physiological saline at 9 per 1000 and formalin at 10%.
- the TELEMAN technique which uses a 5% acetic acid solution. Concentration techniques are rarely used because they destroy *B. hominis*. However, they are necessary to search for other parasites. Indeed, the symptoms presented by the patient can only be attributed to in the absence other pathogenic agents (bacteria, virus, fungi, parasites) or functional or organic alteration of the intestine.

d/ Culture

Culture techniques have proved more sensitive than coproparasitoscopic routine techniques in identifying *Blastocystis* (**Roberts *et al.*, 2011**). But, their use in routine diagnosis is not viable. However, they are the tool of choice for the recovery of viable cysts from drinking water, surface water, and wastewater samples (**Suresh *et al.*, 2005 ; Flores *et al.*, 2011**).

*__Indirect diagnosis__

Is a recent technique because it was long believed that there was no humoral response to *B. hominis* infestation. A study by **Rivera and Santos, in 2009**, confirms this. Performed on mice infested with *B. hominis*, it demonstrated the production and activity of three isotypes of anti-blastocyst antibodies: immunoglobulins A (IgA), B (IgB) and M (IgM) present in serum or intestinal secretions. This is the first study to report the kinetic analysis as well as the antigenic targets of the different antibodies by flow cryometry. IgM are only found in the serum and correspond to the early immune response.

These are therefore indicators of a recent *B. hominis* infection. IgA are found in intestinal secretions and show strong activity against the blastocyst, suggesting a vital role in the immune response against *B. hominis*. IgG can be serum and/or intestinal and appear after IgG; they are the sign of an older *B. hominis* infection. It is more than likely that B. hominis causes the same humoral immune response in humans. Indirect diagnosis is carried out using an immunological method: the ELISA test (Enzyme-linked immunosorbent assay) which makes it possible to highlight specific antibodies directed against *B. hominis* antigens. It was **Nagy *et al*., in 1995**, who first performed this method on the blastocyst.

In practice, indirect serological diagnosis is little used. It is of little interest since antibodies can also be detected in asymptomatic but chronically infected subjects. However, immunological investigation may be necessary to confirm a diagnosis of blastocystosis when other methods of detecting *B. hominis* have not proved conclusive. This method also makes it possible to assess the evolution of blastocystosis under treatment (**Kaneda *et al*., 2000).**

1.3.3.3. Treatment

The need for systematic treatment in the event of discovery of the parasite is discussed since the pathogenic role of *B. hominis* is controversial. In practice, drug treatment is only justified if there is clinical symptomatology. Witness of a dirty diet and which can hide another associated parasitosis or enteroviruses and pathogenic bacteria, the presence of the blastocyst should encourage the clinician to prescribe digestive antiseptics associated with a specific anti-B treatment. hominis.A large number of drugs can be active against *B. hominis* (**Albrecht *et al*.,1995)**

Metronidazole (Flagyl®) is the most widely prescribed and most appropriate drug: it is effective, inexpensive, can be used orally or parenterally. The recommended dosage for adults is 750 mg to 1 g, 3 times a day for 5 to 10 days. In children, the dose is 50mg/kg/day in three doses. Metronidazole is a 5-nitroimidazole derivative which acts by modifying the DNA structure of parasites. It allows a rapid disappearance of cases of diarrheal disease and a gradual elimination of symptoms in patients suffering from chronic disease. This drug is also very effective against anaerobic bacteria which are often associated with the presence of *B. hominis*. Its digestive resorption is rapid and its levels are effective in less than an hour. It is concentrated in the liver where it is oxidized, glycuro-conjugated before being eliminated mainly via the kidneys **(Coyle *et al* .,2012).**

1.3.3.4. Prevention

Prophylactic measures are essentially based on life style and dietary measures, the three pillars of which are health education, environmental sanitation and food hygiene (**Coudert and Dreyfuss, 2010**). These measures mainly concern developing countries since in Europe - and in other industrialized countries - the hygiene rules for water and food are strict and governed by legislation guaranteeing maximum safety for populations.

A. Health education

Health education consists of informing people about the dangers associated with faecal peril and instilling in them the basic rules of hygiene. This education must be done from an early age. Workers in contact with animals should be advised to wear a mask and gloves to avoid possible infestation by *B. hominis* (**Mak *et al*., 1999**).

B. **Environmental sanitation**

Sanitation of the environment is essential to avoid the dissemination of blastocysts and other parasites, this implies:

- the regulation of the use of human or animal manure.
- construction of latrines.
- the treatment of waste water in order to protect crops against the dispersion of blastocyst cysts.
- garbage collection and destruction.

C. Food hygiene

- Good food hygiene is essential to avoid infestation by B. hominis.
- It requires systematic washing of hands before meals and any handling of food as well as following contact with an animal and after each bowel movement.
-Vegetables and fruits eaten raw should always be washed thoroughly with clean water. When this is not possible, they must be peeled and then systematically cooked.
- As far as drinking water is concerned, it must be filtered or boiled for at least one minute or disinfected with bleach (dilute 1 to 2 drops in a liter and wait ½ hour before consumption) or chloramine T Hydrochlorazone® (dissolve 1 tablet per liter of water to be treated and you must wait 1 hour before using the water).
- Populations in developing countries as well as travelers to tropical and subtropical regions should be advised to preferably drink bottled water uncapped in front of them and to avoid the consumption of ice cubes and ice cream as much as possible (**Fischer and Sohail, 2005**).

Finally, screening and systematic treatment of healthy carriers could make it possible to control blastocystosis but this is not done in practice since it is only treated when there is clinical symptomatology since the pathogenic role of this parasite is still discussed

Presentation of the study site

2. PRESENTATION OF THE STUDY SITE

2.1. Geographical situation of Mila's wilaya

The wilaya of Mila is located in the North-East of Algeria at an altitude of 464 m, and 70 km from the Mediterranean Sea. It occupies a total area of 3481 km² or 0.14% of the total area of the country for a population which amounted in December 2011 to 810,370 inhabitants, i.e. a density of 90,75 inhabitants per km². It was in the administrative division of 1984 that Mila was broken down into 32 municipalities (**Chaalal, 2012**).

The Wilaya of Mila is limited by 6 wilayas (**Fig.11**):

- To the North-West by the Wilaya of Jijel.
- To the North-East by the wilaya of Constantine and Skikda.
- To the west by the wilaya of Sétif.
- To the South-East by the wilaya of Oum-El Bouaghi and Batna

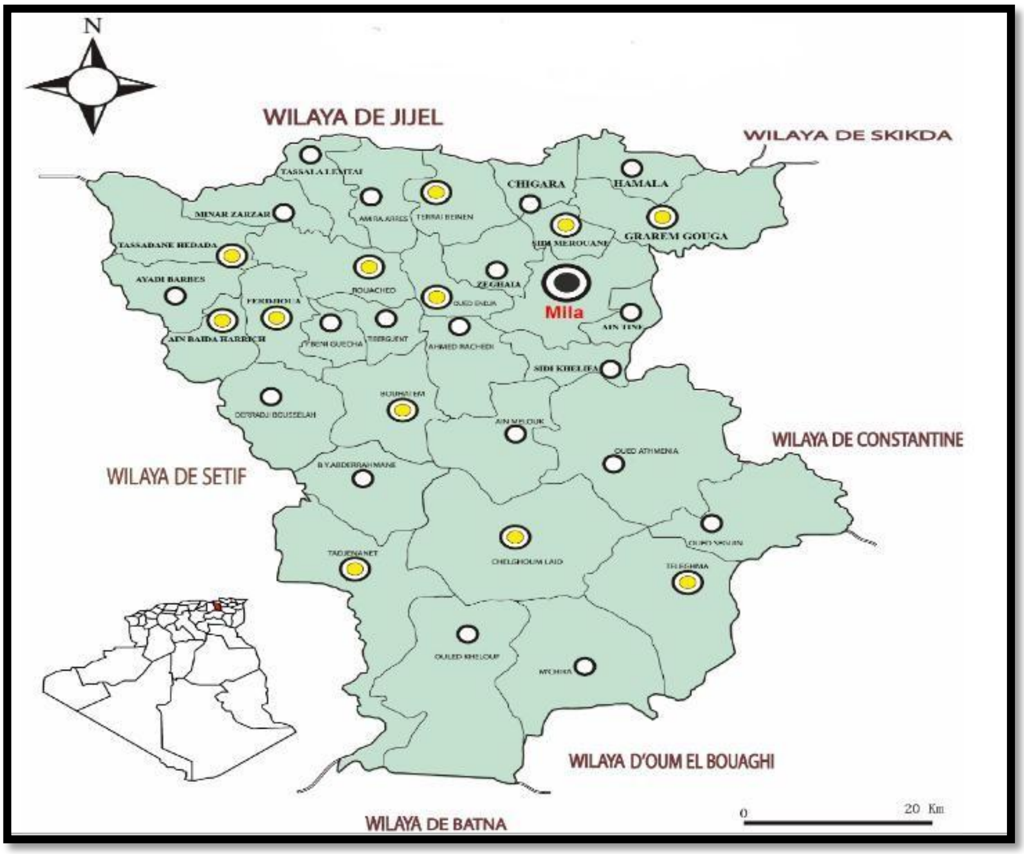


Fig. 11: Geographical location of the wilaya of Mila (**Cetic, 2009**).

2.2. Administrative aspect

The wilaya of Mila was created during the last Algerian administrative division of 1984, with the city of Mila as the capital of wilaya 43 (**ANDI, 2013**). The wilaya of Mila has 13 daïras comprising 32 municipalities. **Table 02** represents The administrative division of the Mila region.

Table 2: Administrative division of the Mila region (**Boularas and Kadjoudj, 2016**).

Dairas	Municipalities
Mila	Mila- Ain Tine- Sidi Kkhlifa
Grarem gouga	Grarem Gouga- Hamala
Sidi merouan	Sidi. Mérouane- Chigara
Oued Endja	Oued Endja- Zeghaia- A. Rachdi
Rouached	Rouached- Tiberguent
Terrai Beinen	Terrai Beinen- AmiraArres- TassalaLamtai
Ferdjioua	Ferdjioua- Y. B. Guecha
Tassadane.H	ZarzaTassadane Hadda- Minar
Bouhatem	Bouhatem- D. Bousselah
Ain Baidah H	Ain B.Ahrich- AyadiBerbes
Teleghma	Telaghma- OuedSeguen - El M'chira
Chelghoum Laid	Chelgoum El Aid- O. Atmania- AinMelouk
Tadjenanet	Tadjnanet- Ben Yahia A- OuledKhlouf

2.3. Demographics

The wilaya of Mila is divided into 13 daïras. It covers an area of 3,480 km² for a population of 865,370 inhabitants, giving a density of 248.7 inhabitants/Km^2. This density varies from one municipality to another due to multiple regional specificities of an economic order (agriculture, industry and trade), geomorphological (nature and reliefs of the land) and administrative (area allocated to each municipality during the administrative division) . (**Abid , 2014**)

2.4. Geology

The study region in the Alpine chain of North Africa whose complex geological framework is characterized by the presence of thrust sheets. These aquifers constitute vast sets of terrains from the Antecambrian to Lower Miocene age which moved (in the form of "thick" scales) horizontally over distances of several kilometers and were deposited according to varied and complex methods (**Belahlou, 2016**)

2.5. Relief and hydrography

The orography of Tamentout is expressed through two parameters namely: the slope and the hydrography. The non-existence of the slope class (0-3%) again confirms the rugged relief in places, thus giving a hilly landscape. In addition, the classes of slopes (greater than 45%). This demonstrates that the area studied is located on moderately steep terrain (**Bneder, 1992**).

The relief of the wilaya of Mila is structured in three morphological sets:

-To the north: a set of high mountains, characterized by very high altitudes and excessively marked slopes.

- In the centre: an ensemble combining valleys – hills and foothills, and even some high slopes.

- In the south: a set of high plains (plains and hills) (**ANDI, 2013**).

The wilaya is home to an important hydrographic network made up of rivers and dams: the largest water dam at the national level, Béni-Haroun dam, Oued Athmania dam, and Oued Seguène dam. Oueds Rhumel and Oued Endja (Oued El Kebir) are the main supply sources of the Béni Haroun dam (**Abid, 2014**). There are 415 water sources in the wilaya; 57 wells and 87 boreholes located in the southern part of the wilaya (**Soukehal and Cherrad, 2011**).

The Beni Haroun dam located in the heart of a huge hydraulic complex, with a storage capacity of 960 million cubic meters, and a height of 120 meters (**Seddiki *et al*., 2013**). It is the largest artificial reservoir in Algeria and the second on the African continent (after the Al Sad El Alli dam in Egypt) with a reserve of 1 billion m^3 of water reached in February 2012 (i.e. 40 million m^3 above beyond its target capacity), spread over 3,900 hectares. Located on the Oued el Kébir, it is fed by two main arms, with the Rhumel and Endja wadis (**Seddiki *et al*., 2013**)

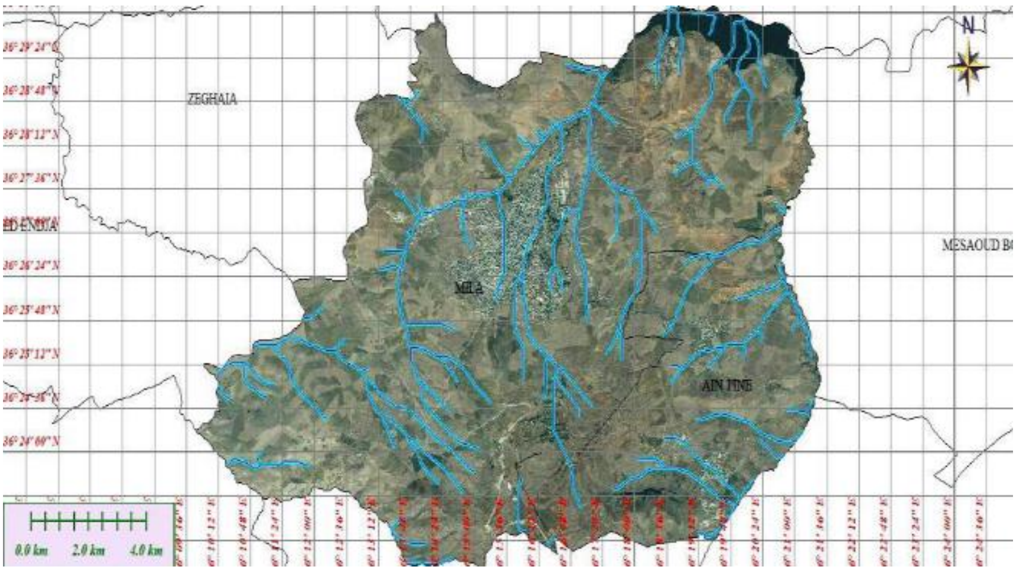


Fig. 12. Hydrographic network of the Mila region

2.6. Forest cover

The forest area of the wilaya covers 348,054 ha, divided into 13,158.20 ha of natural forests, 2,266.80 ha of maquis, and 18,022 ha of reforestation, i.e. a rate of forest cover of 9.61% (**Seddiki *et al.*, 2013**), which are divided into the following areas: Natural forests representing 6,762 ha or 20,08%; including the dominant species (**Zouaidia, 2006**). Reforestation with an area of 18,493 ha or 54,92%; the main species are Aleppo pine and cypress. The maquis represent an area of 8,415 ha or 25% (**Zouaidia, 2006).**

2.7. Climatology

Climatology is the set of meteorological characteristics of a given region. However that, climate is the set of meteorological phenomena that characterize the average state of the atmosphere at a point on the earth's surface (**Soukehal, 2009**). The most important environmental factor is certainly the climate. It has a direct influence on the fauna and flora. It demonstrates an impact on migratory birds: shifting of migration periods, modification in the reproduction and survival of species, displacement of breeding and wintering areas.

The climate of the wilaya of Mila is a typical Mediterranean climate. It is characterized by :

- A wet and rainy season (winter) extending from November to April.
- And a long hot and dry summer period from May to October **(Zouaidia, 2006)**.

➢ **Temperature**

Temperature is an essential and fundamental ecological climatic factor for the life of living beings. Temperature can affect organisms directly or indirectly because thermal conditions affect other organisms to which an individual is ecologically related, although these relationships could be complex. It acts directly on the reaction rate of individuals, on their abundance and their growth **(Faurie *et al.*, 1980)** and it explains that living beings can only carry out their activities in a range of temperatures ranging from 0 at 35°C. A moderate mediterranean temperature during the months of Autumn, Winter and Spring. During the summer, the temperature increases rapidly, especially inside the wilaya. In any case, the temperature is favorable for crops both in summer and winter **(Soukehal, 2011)**.

Table 3: Average monthly temperature of the Mila region. **(Mila meteorological station, 2012 to 2022).**

Months	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
T °C	9,36	9,81	11,9	16,18	20,72	26,9	30,36	29,54	25,09	20,36	14,54	11,18

..

According to **table 03**, which gives the average monthly temperature changes for our region, we note that the maximum temperature is recorded during the month of July when it reaches 30.36 degrees Celsius, while the month of January is characterized by cold degrees, with a temperature not less than 9.36 Celsius.

- **Precipitation**

Precipitation refers to any type of water that falls from the sky, in liquid or solid form **(Dajoz, 2000)**. It represents an essential climatic factor with regard to the ecological cycle, the hydrographic regime and agricultural activity. The variation of annual precipitation is the striking fact in this wilaya. Rainfall in Mila is unevenly distributed across the months of the year and precipitation is, naturally, confined to the cool semester which begins in November and ends in March. The lack or abundance of precipitation has a significant effect on water reserves; quantities mobilized and quantities exploited. The drought acts directly on the behavior of the population in this area **(Soukehal, 2011)**.

Table 4: Average monthly precipitation in the Mila region. **(Mila meteorological station, 2012 to 2022).**

Months	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
P(mm)	77,81	75,18	104,36	56,72	58,9	22,18	10,36	28,36	47,18	57,18	67,9	44,9

The study area is considered one of the wettest areas. From **the table 4** above, we see that March is the month with the most rain, as it experienced an excess of 104.36 mm, and on the contrary, July experienced a deficit of 10.36 mm, which is the driest month and the annual average of precipitation.

- **Humidity**

It is the ratio between the quantity of water vapor in a given volume of air and the quantity possible in the same volume at the same temperature **(Villemeuve, 1974)**. It depends on several climatic factors such as rainfall, temperature and wind **(Faurie *et al.*, 1980)**.

Table 5: Average monthly humidity variations in the Mila region. **(Mila meteorological station, 2012 to 2022).**

Months	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Hum%	84,81	84,54	85,45	81,36	72,36	57,63	50,45	56,09	69,18	74	76,9	88,66

According to **table 05**, the month with the highest humidity is December with 88.66% and the month with the lowest value is July with 50.45%.

- **Wind speed**

The wind is one of the most characteristic elements of the climate. It acts by activating the precipitation which can induce a drought **(Seltzer, 1946).**

Table 6: Average monthly wind speed variations in the Mila region. **(Mila meteorological station, 2012 to 2022).**

Months	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Wind speed (knots)	11,54	12,54	13,72	12,9	13,36	14,45	15,18	15	12,54	11,54	12,63	10,9

Table 6 shows that the maximum wind speed is recorded in the month of July with a maximum value of 15.18 knots, and the minimum speed is in the month of December with a value of 10.9 knots.

2.8. Sanitary infrastructure

On the epidemiological level, the wilaya registers each year several hundred cases of notifiable diseases, tuberculosis occupying the 1st place followed by meningitis and a few dozen cases of zoonoses (leishmaniasis and brucellosis) as well as a few cases of viral hepatitis B and C. (**Larbi, 2014**)

The public health sector has (**Larbi, 2014**):

▪ 05 general hospitals (EPH Maghlaoui Mila, EPH Tobal Mila, EPH Ferdjioua, EPH

▪ Chelghoum-Laid, EPH Oued-Athmania) with 749 beds.

▪ 01 hospital specialized in psychiatry in Oued Athmania with 340 beds

▪ 01 general hospital with 60 beds in Grarem Gouga, under construction.

▪ 38 polyclinics with 25 medical analysis laboratories.

▪ 05 rural maternities.

▪ 149 treatment rooms.

Table 07: Public Hospital Establishments (EPH) **(DSPM, 2014)**.

Denomination	Number of services
Public Hospital Establishments Brothers Maghlaoui Mila.	7
Public Establishments Hospitaliers Brothers Tobal Mila.	9
Public Hospital Establishments Brothers Boukchem Oued El Athmania.	8
Public Hospital Establishments Ferdjioua.	11
Public Hospital Establishments Chelghoum Laid	12

Table 08: Local public health establishments **(DSPM, 2014)**.

Denomination	Number of polyclinics	Number of treatment rooms	Communes covered
Mila	14	34	08
Ferdjioua	10	46	09
Chelghoum laid	09	29	06
Ain Beida ahrich	06	29	06
Tadjnanet	02	19	03
Total	40	157	32

With a specialized hospital establishment, the EHS in psychiatry of Oued Athmania, 38 polyclinics, 145 treatment rooms and 02 private clinics **(DSPM, 2014)**.

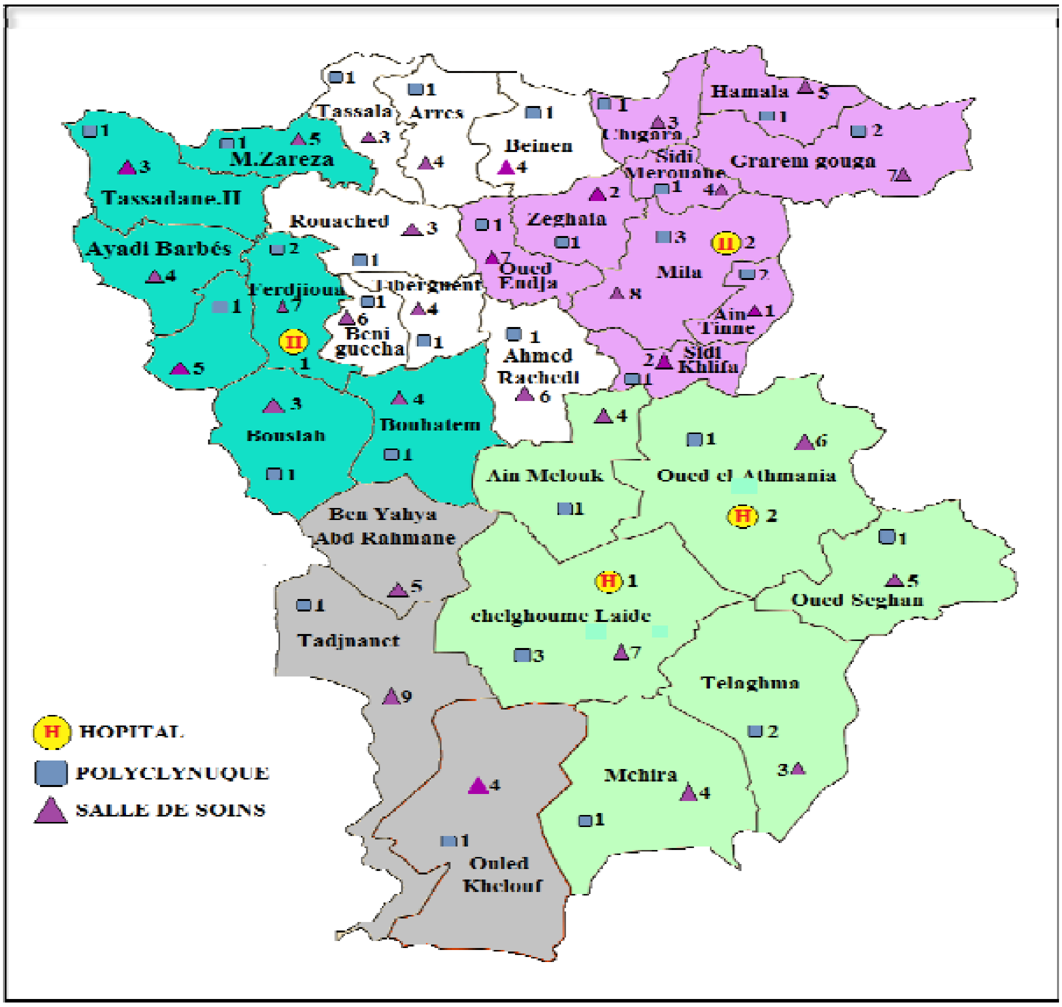


Fig. 13: Health structure in the wilaya of Mila (**DSPM, 2014**).

Material and methods

3. MATERIAL AND METHODS

3.1. Epidemiological investigation

3.1.1. Location, type and duration of study

This epidemiological study of human blastocystosis takes place at the central laboratory service level, medical parasitology-mycology unit of the Maghlaoui Brothers – Mila public hospital establishment. The present study was conducted following the retrospective analytical descriptive method based on the documentary analysis of registers. During the study period which was carried out from January 2012 to December 2022 over a period of 11 years. We have associated this part with a prospective study of human intestinal amoebiasis during the period (January-March 2023) at the same service level.

3.1.2. The patients

This epidemiological study focused on all parasitological stool examinations (PSE) of patients referred to the parasitology laboratory. The patients in our study bring together adults and children hospitalized or consulted in the various departments of the hospital and employees of restaurants, from very diverse origins both geographically (different municipalities in the region of Mila), and socially (patients from public and private sector). Our prospective study focused on 304 patients referred to the parasitology laboratory during the first three months of 2023.

3.2. Parasitological analysis (January-March 2023)

3.2.1. Material

The material used: (**fig. 14**)

- Blade and lamella.
- Optical microscope.
- Bunsen burner.
- The application handle.

Fig. 14: Laboratory equipment used for the diagnosis of *blastocystis hominins* (**Personal photo, 2023).**

3.2.2. Reagents

- Lugol.
- Physiological water

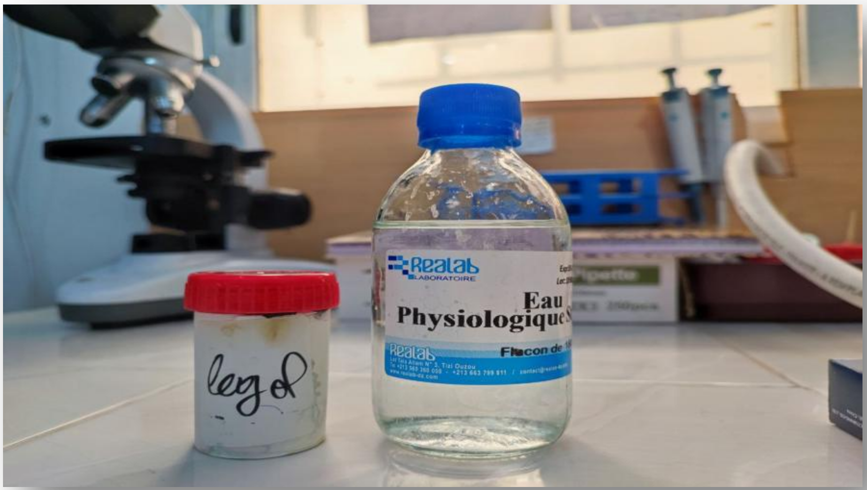


Fig. 15: The reagents used in the laboratory for the (PES) of stools (**Personal photo, 2023**).

3.2.3. Sampling conditions

Sampling is an essential step for the quality of the results. Stool collection is done in the morning in the laboratory or at home in a clean, dry plastic pot with a wide opening. Sometimes, certain precautions are advised before the parasitological stools examination.

3.2.4. The stages of parasitological examination of stool

A. Procedure

- Place a drop of physiological water on the left half of the slide:

-Using the applicator take the stool sample and mix evenly with the drop then cover the preparation with a covrslip.(**Table 09**)

- Place a drop of parasitological lugol on the right half of the slide:

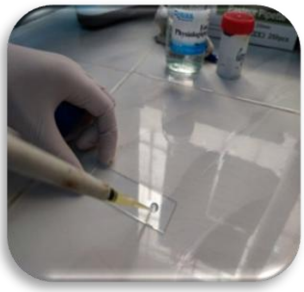
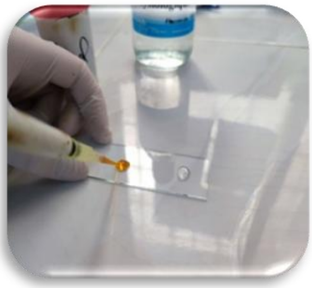
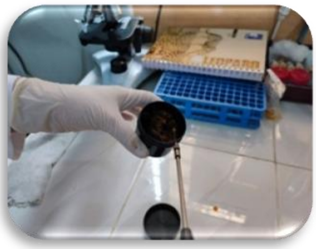
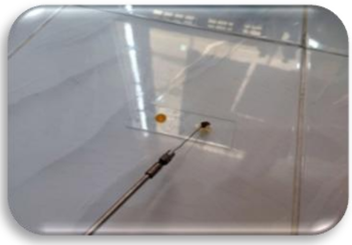
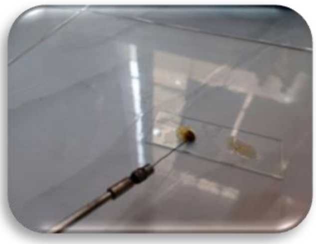
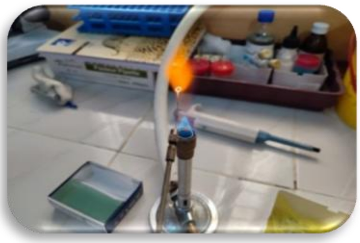
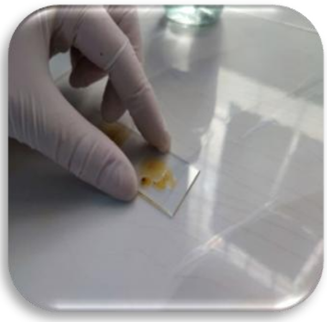
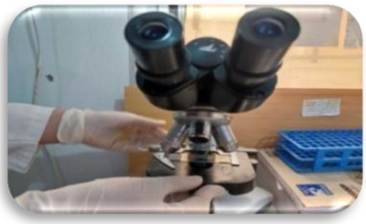
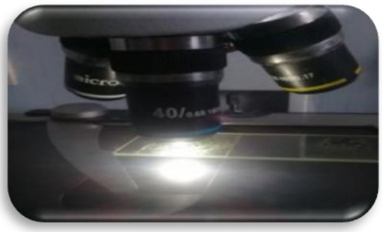
-Then take the stool sample and mix evenly with the drop of lugol and cover the preparation with a cover glass.

B. observation

-For preparation with physiological saline, use the 4X and 10X objective

-For the preparation with the parasitological lugol use the 10X and 40X objective (It allows to highlight the cysts)

Table 09: The stages of PSE in the fresh state and lugol staining. (**Personal photos, 2023**)

 Drop of physiological water	 Drop of lugol
 Stool sampling in different locations	 Spreading of stool on physiological water
 Spreading of stool on lugol	 Loop sterilization application
 Lamella deposit	  Observation at Gx10 and Gx40

3.2.5. Collection of data

The collection of information in the first part is made from the registers of the parasitology department, where the samples were taken, as well as the collection of clinical information, farm sheets including: the identity of the patients (surname, first name, sex and age), the date of sampling, the services and the results of the macroscopic and microscopic examinations of the PES . The data collected over a period from January 2012 to December 2022 were recorded on a Windows Excel file.

3.3. Meteorological data

The data necessary for the realization of this study were provided from the meteorological station of Ain Tin, It is the meteorological data relating to the wilaya of Mila concerning five climatic parameters which are:

- ➢ The average temperature;
- ➢ Average humidity;
- ➢ Average sunshine duration;
- ➢ average monthly wind speed
- ➢average monthly precepitation

3.4. Statistical analysis of data

Data were entered into Excel software and processed using SPSS [(Statistical Package for the Social Sciences) V 26] and the software R. Descriptive statistics of these variables for sex and age slices, years, seasons, months were represented in boxplots using the package {ggplot2} of the software R that was employed in all statistical tests and graphics. The variation each parameter following sex , age slices,months seasens years and the interaction (sex × ages slices) was tested usingone way and two-way analysis of variance (ANOVA). Then student’s t test were conducted to distinguish the variability in each group of parameters. Pearson correlation tests were applied between all *Blastocystis hominis* dissemination parameters measured (age, sex, months, seasons, years and meterologecal parameters) in order to understand the behavior and relationships between *Blastocystis hominis* dissemination combined under the meterologecal parameters conditions in the Mila state.

The resulting correlation matrix was plotted in an interactive correlation diagram using the package {corrplot} in R. Using the package {nlme} in R, we implanted generalized linear mixed models (GLMMs) with to test the following relationships: effects of T, Sun, P, WS and H on *Blastocystis hominis* dissemination variation.The statistical significance of all tests was set at $p < 0.05$ and 95% of the confidence interval.

Results

4. RESULTS

4.1. Retrospective analysis of the study population

This survey reveals cases diagnosed at the parasitological analysis laboratories of the wilaya of Mila during the period (2012-2022), according to the prescription of the attending physician, patients with digestive disorders, diarrhea and abdominal pain are referred to a parasitological stool examination (PSE). During this period 12941patients are testing.

***Simple parasite index (SPI)**

The Simple Parasitic Index is the percentage of people infected with parasite out of the total number of subjects examined.

$$SPI = \frac{\text{Number of positive cases}}{\text{Total number of subjects examined}} \times 100$$

4.1.1. Distribution of patients according to infestation rate during the descriptive study

Fig.16 and **table 10** have show that from 12941 subjects screened for the *Blastocystis hominis* infection, 2356 are infected; the infection rate is 18% during the descriptive study period.

Table 10: Distribution of patients according to infestation rate during the period (2012-2022).

	Effective	Frequency
Positive cases	2356	18%
Negative cases	10585	82%

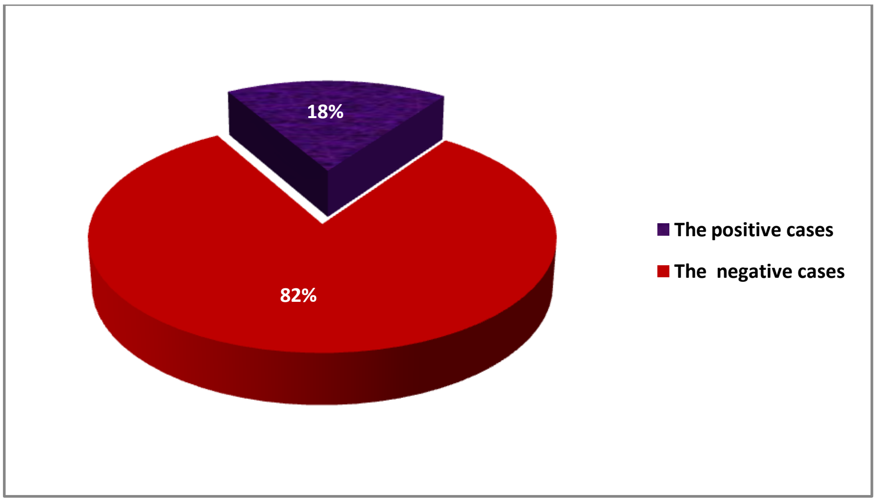


Fig. 16: The distribution of patients according to the infestation rate during the study period.

4.1.2. Distribution of infected patients according to sex ratio during the study period

Our study showed that men are more exposed to *Blastocystis hominis* by 57,51% in the state of Mila (**table 11, figure 17**), the graphs represented in **fig. 18, 19,** and **20** show that men and women were more infected during the years (2019,2021). The highest rates of infection were recorded in the autumn and winter seasons for both sexes. Also, the months of October, September, and November have seen an increase in the number of cases for females and for male's sex the highest rates of infection have been noted in the October, September, and January months. The results obtained showed statistically significant differences ($p < 0.001$) between sex ratio for *Blastocystis hominis* infection**.** One-way ANOVA reveals a not significant sex effect ($p > 0.05$).

Table 11: Distribution of infected patients according to the sex ratio during the study period.

Sex	Effective	SPI%
Woman	1001	42,48%
Male	1355	57,51%

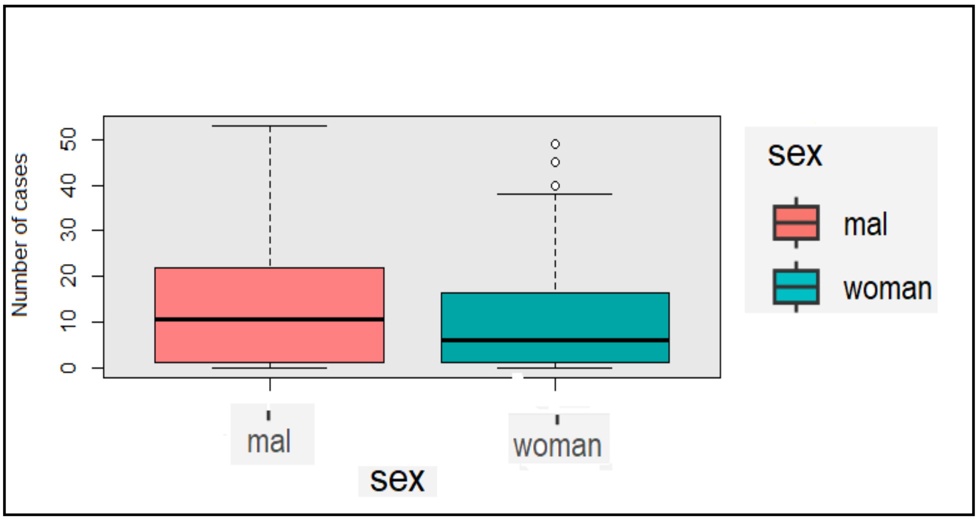


Fig. 17. Boxplots displaying the distribution of infected patients according to sex ratio during the period (2012-2022).

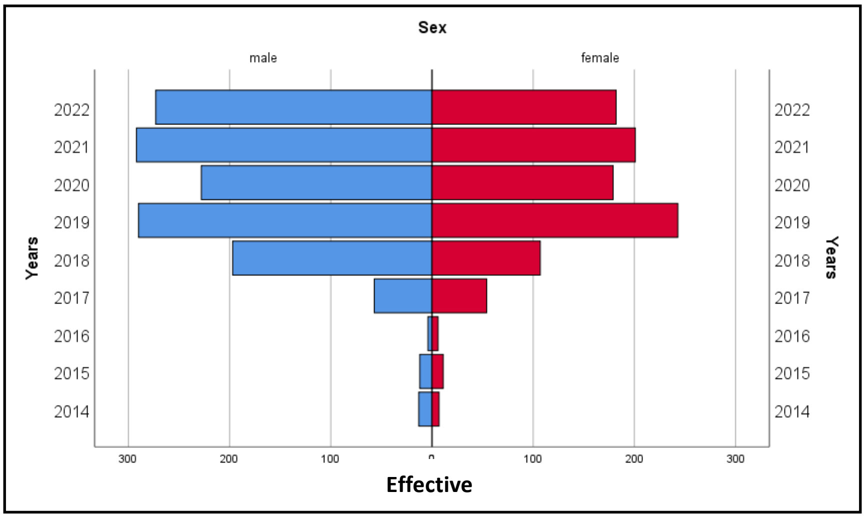


Fig. 18: Distribution of patients according to the sex ratio during the study period according to years.

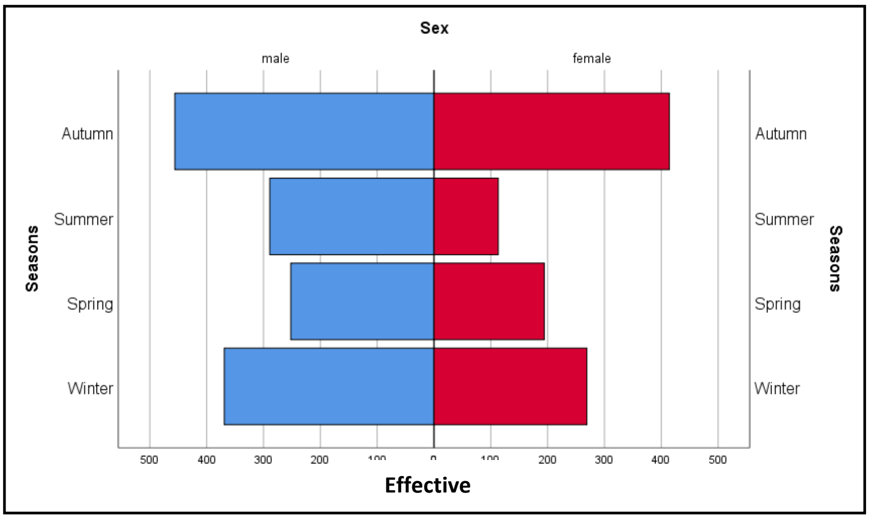


Fig. 19: Distribution of patients according to the sex during the retrospective study period according to seasons.

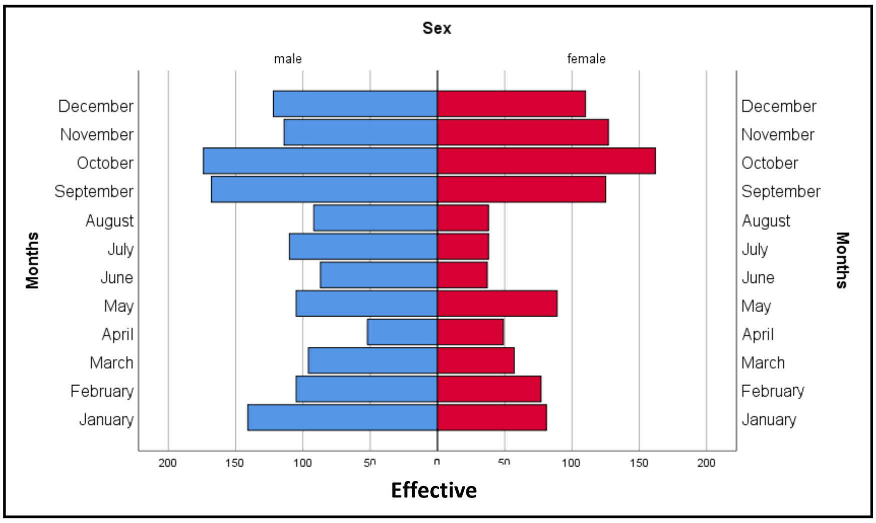


Fig. 20: Distribution of patients according to the sex ratio during the study period according to months.

4.1.3. Distribution of infected patients according to age slices during the period (2012-2022)

According to the data presented in the **table 12** and **figures 21,22** we note that the most affected age group is [20-44] years with 1767 cases and a rate of 75%, followed by the category [45-65] years by 347 cases and a rate of 14, 72%. The category [0-1] years is the less representative group by 4 cases and a rate of 0.16% over the study period. The **figure 23** shows that the age category most threatened with this parasite is wider in male, especially during the autum season**.** The analysis of the data showed a statistically significant differences (p< 0.001) between ages slices for *Blastocystis hominis* infection**.** One-way ANOVA reveals a not significant age effect (p > 0.05).ANOVA bivariate analysis show a not significant sex-age interaction (p > 0.05). (**table in annex**).

Table 12: Distribution of infected patients according to age during the period (2012-2022)

Age slices	Effective	SPI%
[0-1]	4	0,16%
[2-4]	52	2,20%
[5-9]	89	3,77%
[10-14]	31	1,31%
[15-19]	51	2,16%
[20-44]	1767	75%
[45-65]	347	14,72%
≥65	15	0,63%

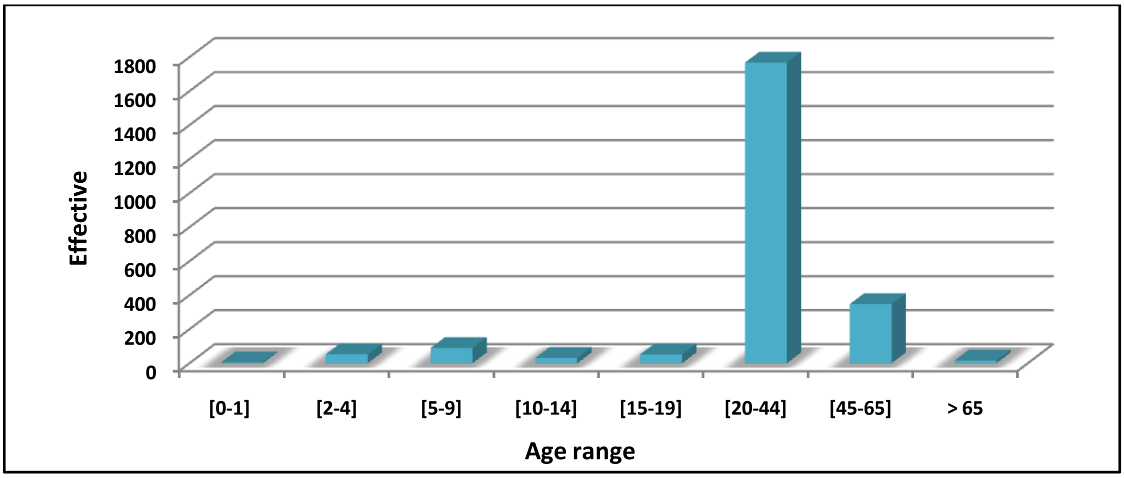


Fig. 21: Distribution of infected patients according to age slices during the period (2012-2022).

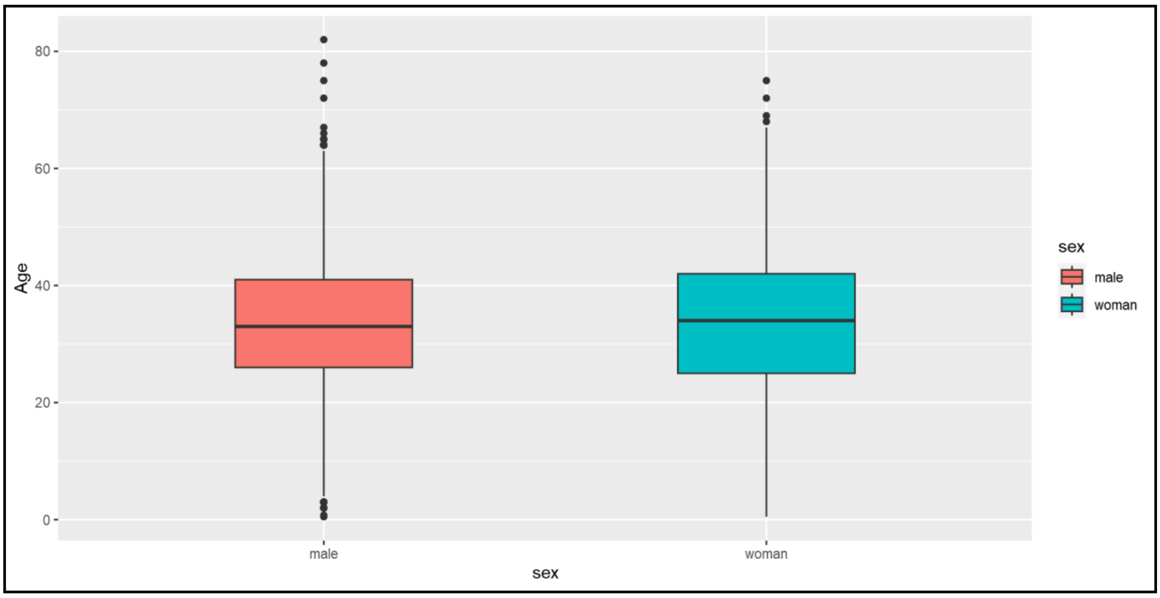


Fig. 22: Boxplots displaying the distribution of age slices of infected patients according to sex ratio during the period (2012-2022).

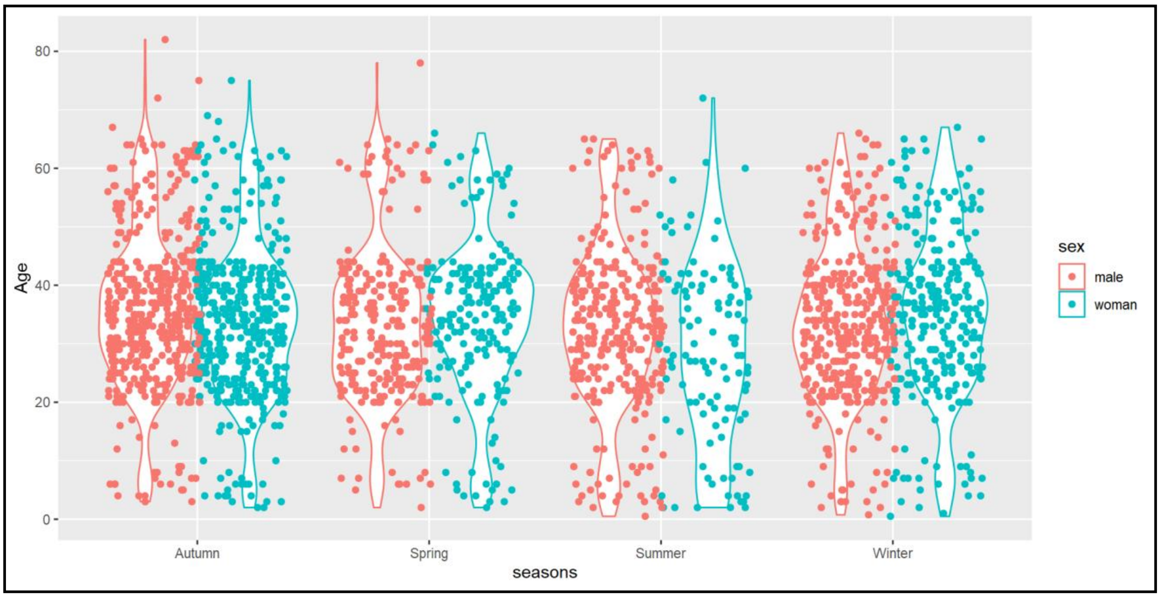


Fig. 23: violin plots displaying the distribution of age slices of infected patients according to seasons during the period (2012-2022).

4.1.4. Distribution of infected patients according to the months during the period (2012-2022)

The **table 13** and **fig. 24** show that the highest number of parasitical cases was recorded during the months of October, September recorded at 14.21%, 12.52%, the lowest percentage was detected in the months of April and June at a rate of 4.28%, 5.26% over the study period. The results obtained showed statistically significant differences ($p < 0.001$) between months for *Blastocystis hominis* infection**.** One-way ANOVA reveals a very highly significant month's effect ($p > 0.001$).

Table 13: Distribution of infected patients by months during the period (2012-2022).

Months	Effective	SPI%
January	222	9.42 %
February	184	7.80 %
March	152	6.45 %
April	101	4.28 %
May	195	8.27 %
June	124	5.26 %
July	149	6.32 %
August	127	5.39 %
September	295	12.52%
October	335	14.21%
November	239	10.14%
December	233	9.88%

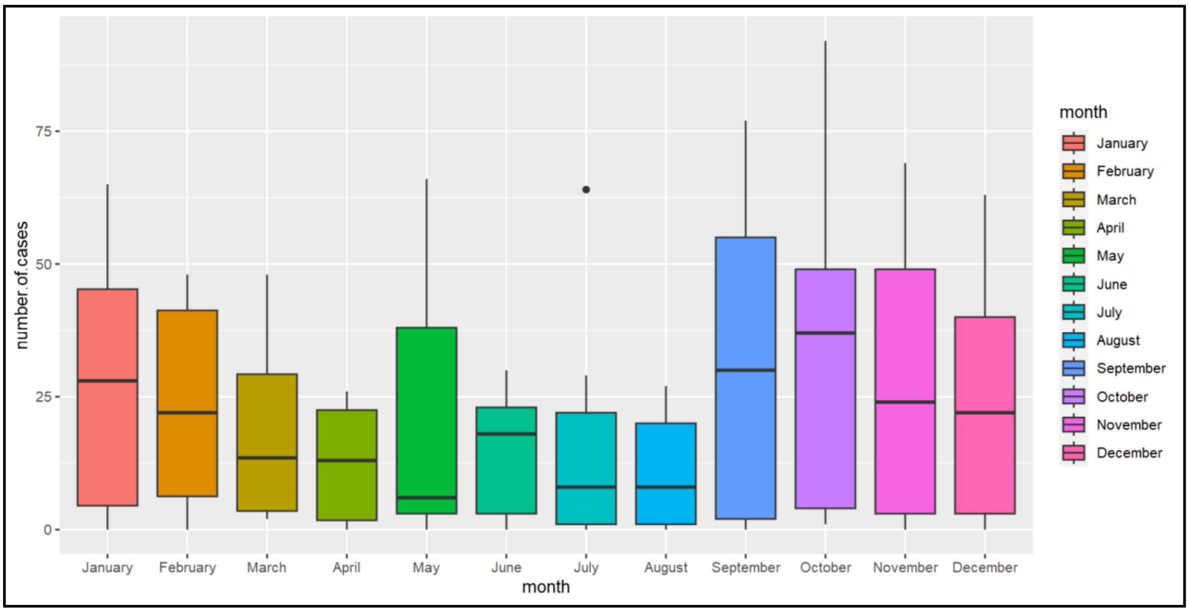


Fig. 24: Boxplots displaying the distribution of infected patients by months during the period (2012-2022).

4.1.5. Distribution of infected patients according to the seasons during the period (2012-2022)

The **table 14, fig. 25** have shown that the highest number of infected cases was observed during the autumn season, followed by the winter season, while the lowest cases were recorded during the summer season. The results obtained showed statistically significant differences ($p < 0.001$) between seasons for *Blastocystis hominis* infection**.** One-way ANOVA reveals a very highly significant season's effect ($p > 0.001$).

Table 14: Distribution of infected patients according to the seasons during the period (2012-2022).

Seasons	Effective	SBI%
Winter	639	27,12%
Spring	448	19,01%
Summer	400	16,97%
Autumn	869	36,88%

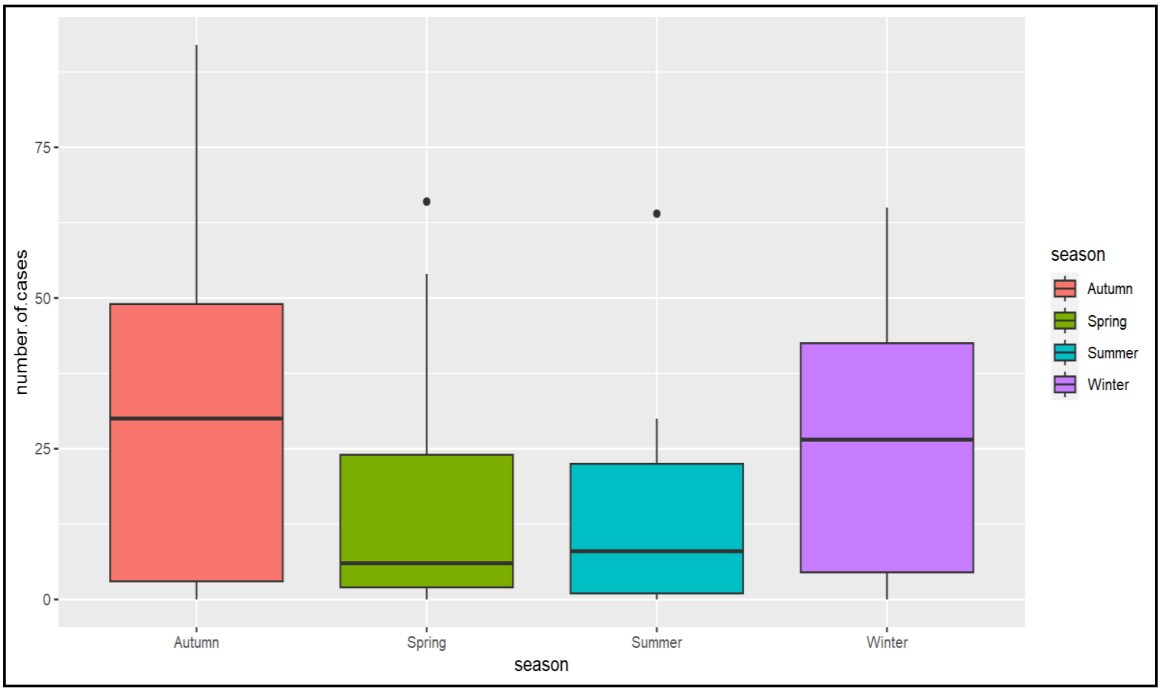


Fig. 25: Boxplots displaying the distribution of infected patients according to the seasons during the period (2012-2022).

4.1.6. Distribution of infected patients according to the years during the period (2012-2022)

The years 2019, 2020, 2021 and 2022 recorded the highest rates of *Blastocystis* infection, 22.62%, 17.27%, 20.92%, 19.31% respectively, compared to other years (**table 15, fig. 26**), the rate of *Blastocystis hominis* ranged from 042% to 12.90%.over the period (2012-2022). The results obtained showed statistically significant differences ($p < 0.001$) between years for *Blastocystis hominis* infection**.** One-way ANOVA reveals a very highly significant year's effect ($p > 0.001$).

Table 15: Distribution of infected patients according to the years during the period (2012-2022).

Years	Number total	Positive cases(+)	SPI%
2012	677	0	0%
2013	786	0	0%
2014	685	20	0,84%
2015	1126	23	0,97%
2016	1106	10	0,42%
2017	1294	111	4,71%
2018	1638	304	12,90%
2019	1825	533	22,62%
2020	1107	407	17,27%
2021	1406	493	20,92%
2022	1291	455	19,31%

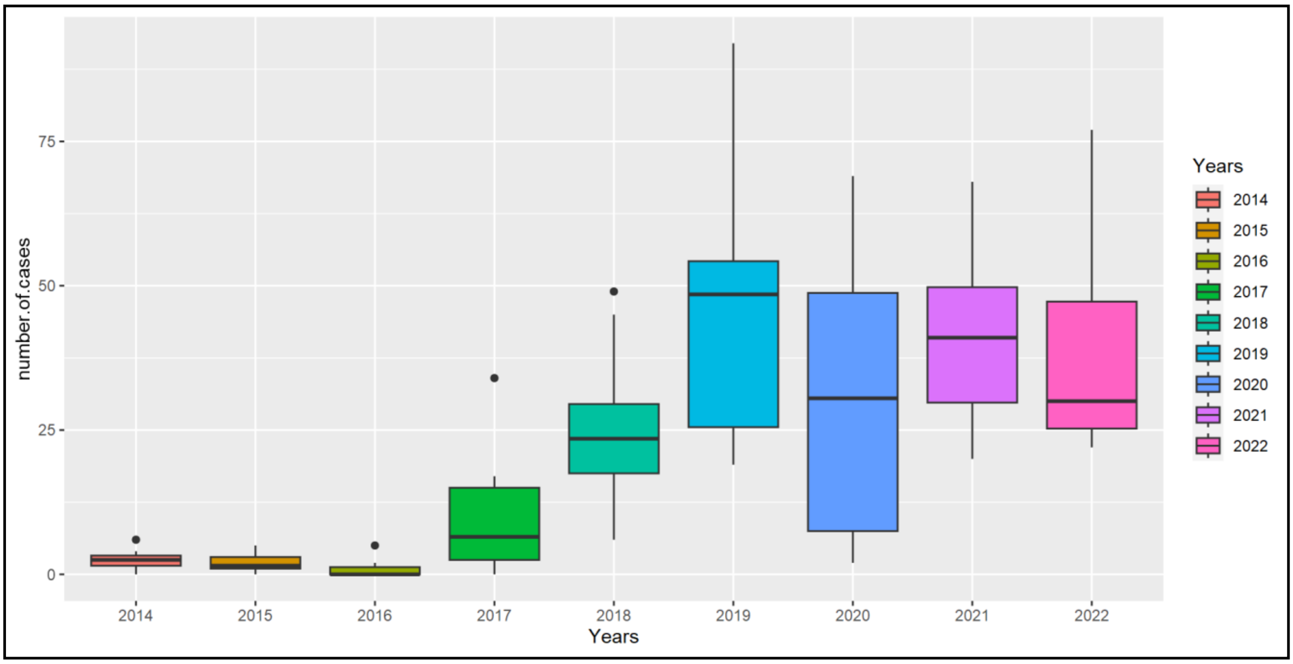


Fig. 26: Boxplots displaying the distribution of infected patients according to the years during the period (2012-2022).

4.2. Overall prevalence of *Blastocystis hominis* during the prospective study

Our prospective study was interested in 317 patients who were referred to a parasitology laboratory during the first three months of 2023 and allows us to perform microscopic examination of a sample in its fresh condition and can detect pathogenic *Blastocystis hominis*.

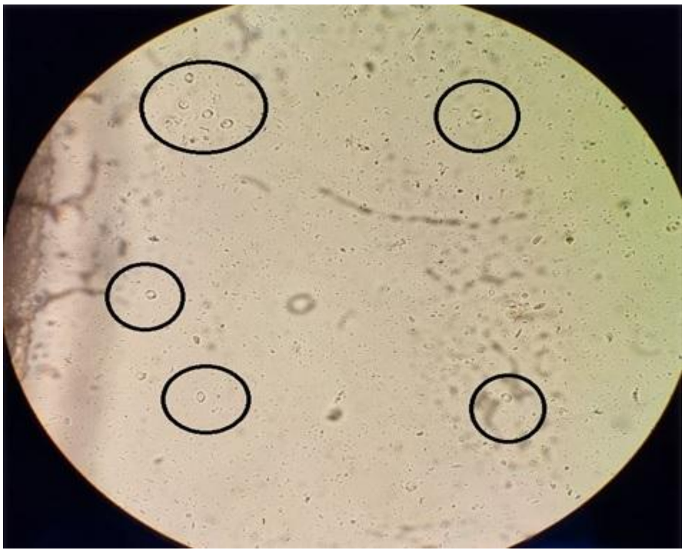


Fig. 27: *Blastocystis hominis* staining with physiological water. (Objective X 40) (**Personal photo, 2023**).

Fig. 28: *Blastocystis hominis* staining with lugole . (Objective X 40) (**Personal photo, 2023).**

Fig. 29: *Blastocystis hominis* staining with lugole . (Objective X 40) (Personal photo, 2023).

4.2.1. Distribution of patients according to infestation rate during the period (January-March 2023)

Table 16 and **figure 30** have shown that from 317 subjects examined for human *Blastocystis hominis*, 132 were found infected, from January-March 2023 with an infestation rate of (1.69%).

Table 16: Distribution of patients according to infestation rate during the period (January-March 2023)

	Effective	Frequency
positive cases	132	41,64%
negative cases	185	58,35%

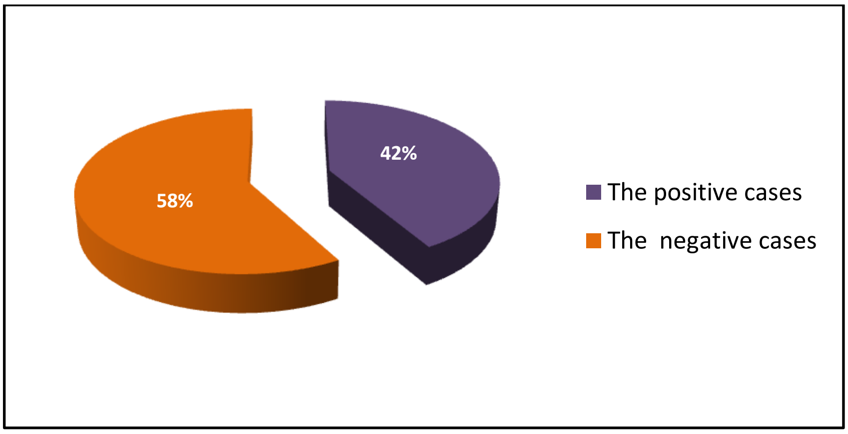


Fig. 30: Distribution of patients by infestation rate over the period (January-March 2023).

4.2.2. Distribution of infected patients by sex ratio during the period (January-March 2023)

Table 17 and **figure 31** reveal that the most of infected cases were man (69.69%).

Table 17: Distribution of infected patients according to the sex ratio (January-March 2023):

Sex	Effective	SPI%
Male	92	69.69%
Woman	40	30.30%

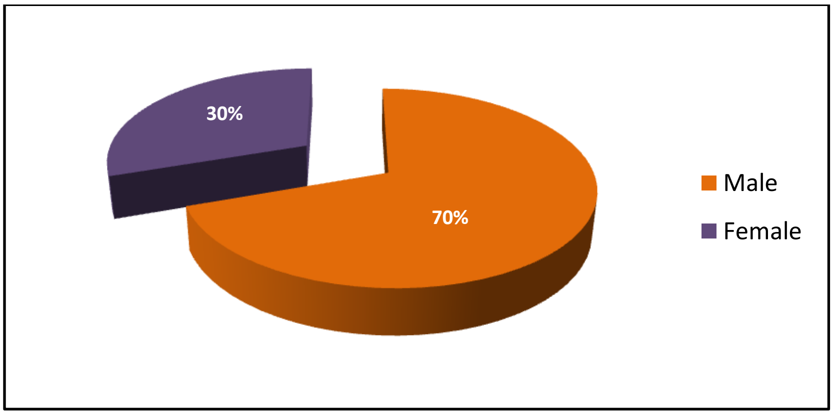


Fig. 31: The distribution of infected patients according to the sex ratio during the period (January-March 2023)

4.2.3. Distribution of patients by age group during the period (January - March 2023)

We notice that the age group [20-44] years is the most affected by *Blastocystis hominis* with 97.72% ,followed by the age groups included between [45-65] years with a percentage of 0.75% (**table 18, figure 32)**.

Table 18: Distribution of infected patients according to age (January-March 2023):

Age slices	Effective	SBI%
[0-1]	0	0%
[2-4]	0	0%
[5-9]	0	0%
[10-14]	1	0,75%
[15-19]	0	0%
[20-44]	129	97,72%
[45-65]	1	0,75%
≥65	0	0%

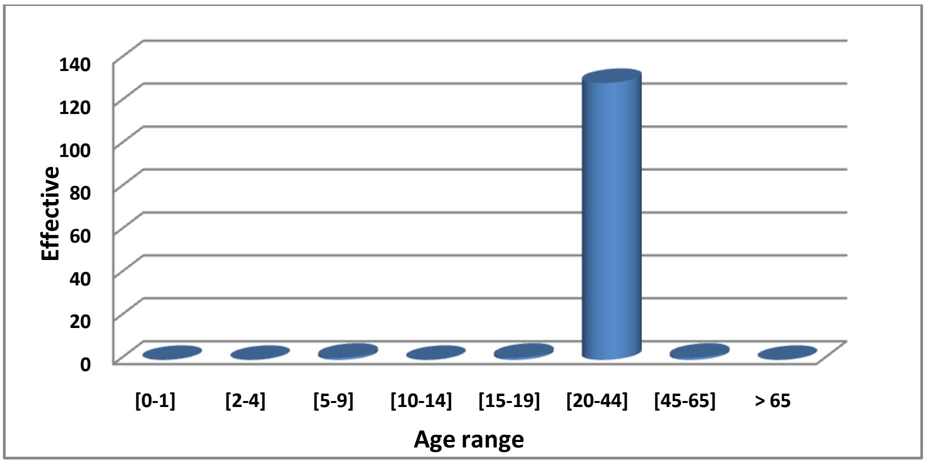


Fig. 32: Distribution of patients according to age groups during the prospective study period.

4.2.4. Distribution of infected patients by months during the period (January-March 2023)

Table 19 and **figure 33** shown that the highest number of *Blastocystis hominis* cases was noted during the month of March 46.21%, followed by the months of January and February with 28,03% and 25,75 % during the period (January-March 2023).

Table 19: Distribution of infected patients by months during the period (January-March 2023).

Months	Effective	SPI%
January	37	28,03%
February	34	25,75%
March	61	46,21%

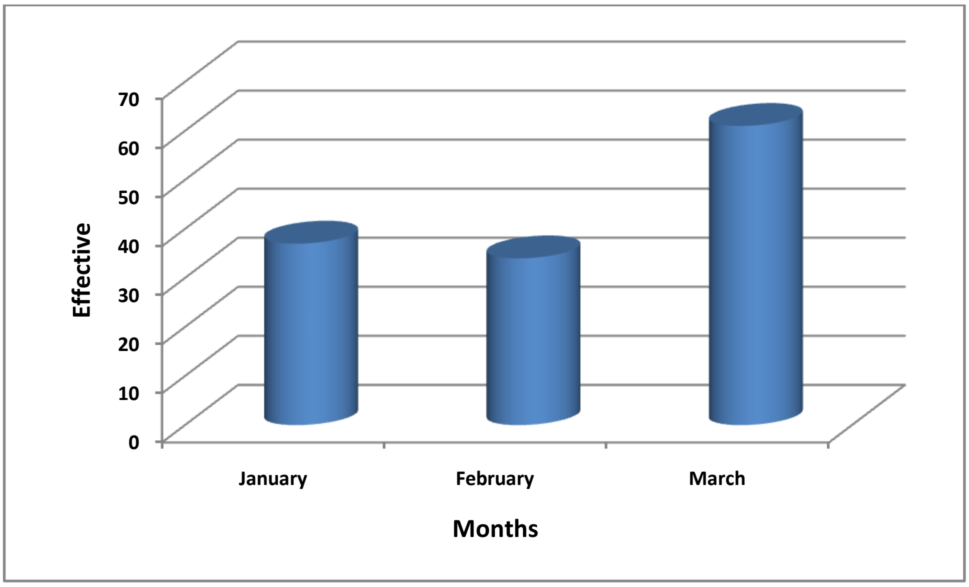


Fig. 33: Distribution of infected patients by months during the period (January-March 2023).

4.3. Correlation between the variation of meteorological parameters and the propagation of *Blastocystis hominis* during the period (2012-2022)

To identify the relationship between meteorological parameters and the propagation of human *Blastocystis hominis* in the Mila region, we have used the model of diagram using the package {corrplot} in R. Using the package {nlme} in R, we implanted generalized linear mixed models (GLMMs) to following relationships: effects of T, Sun, P, WS and H on *Blastocystis hominis* dissemination variation. Pearson correlation will be used to clarify the correlation between the dissemination of *Blastocystis hominis* and the variation of different meteorological parameters.

4.3.1. The relationship between the variation of the average temperature and the number of infected cases during the period (2012-2022)

Linear regression and Pearson correlation (**figure 34,39**) showed that the number of infected cases increases progressively with the increase in the average temperature so there is a very strong positive correlation between the variation of the average temperature (°C) and the number of infected cases during the period (2012-2022).

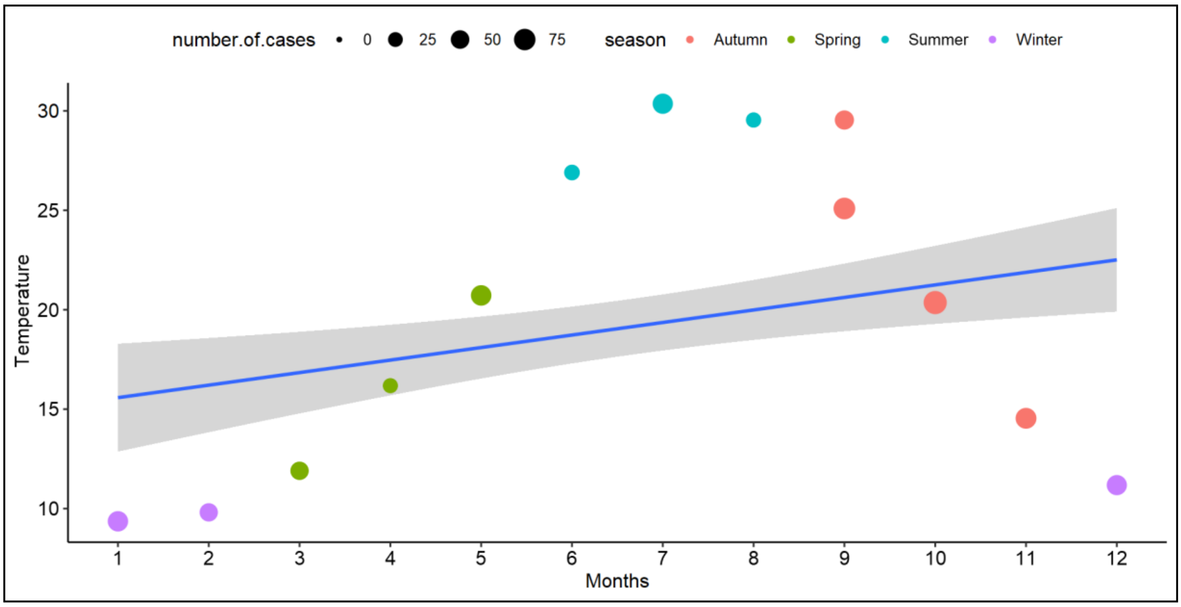


Fig. 34: The correlation between the average temperature (°C) and the number of infected cases during the period (2012-2022).

4.3.2. The relationship between the variation of the average precipitation and the number of infected cases during the period (2012-2022)

Linear regression and Pearson correlation (**figure 35,39**) showed that the number of infected cases decreases progressively with the increase in mean precipitation so there is a strong negative correlation between the variation in mean precipitation (mm) and the number of infected cases during the period (2012-2022).

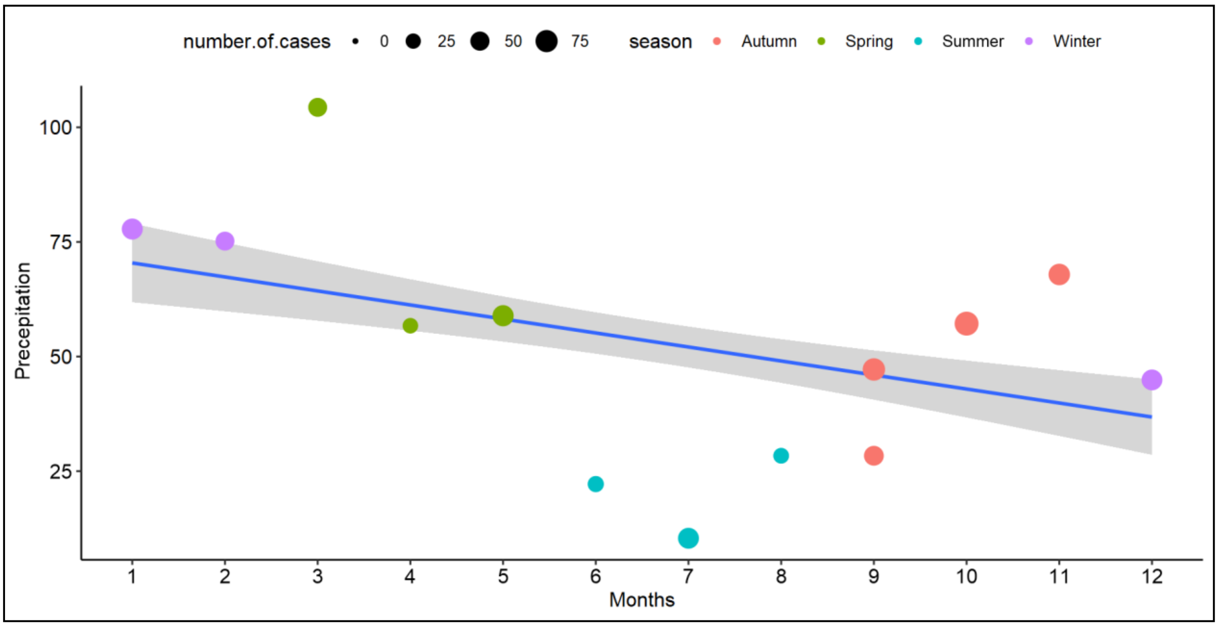


Fig. 35: The correlation between the average wind speed (knots) and the number of infected cases during the period (2012-2022).

4.3.3. The relationship between the variation of the average wind speed and the number of infected cases during the period (2012-2022)

Linear regression and Pearson correlation (**figure 36,39**) showed that the number of infected cases decreases progressively with the increase in mean wind speed so there is a medium negative correlation between the variation in mean wind speed (knots) and the number of infected cases during the period (2012-2022).

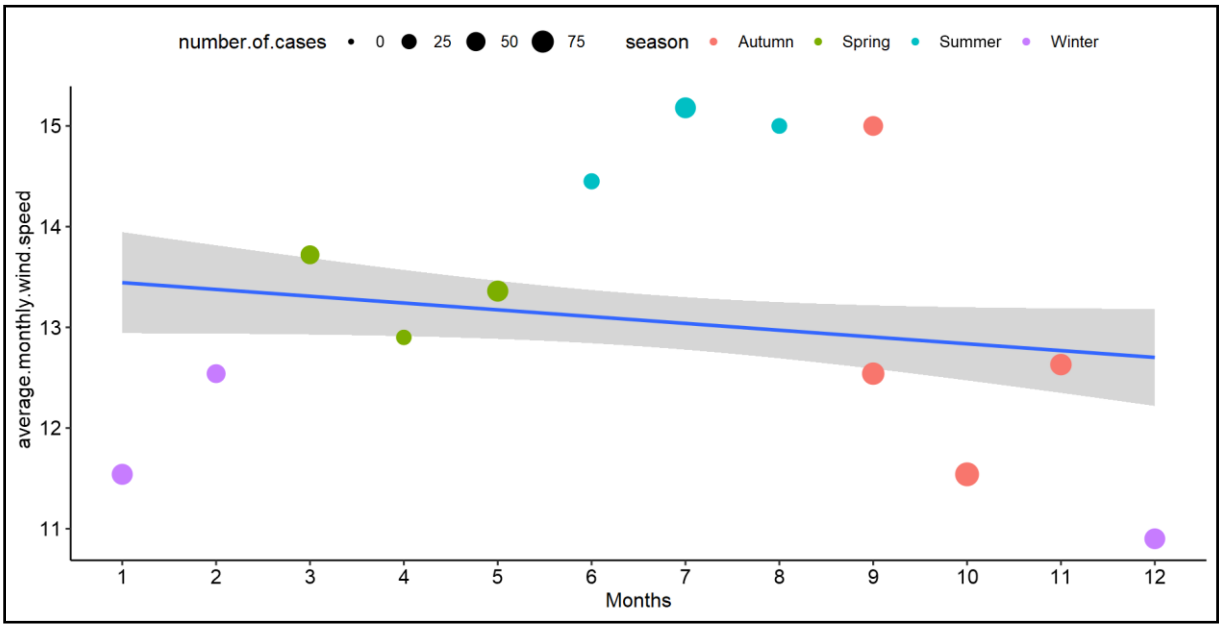


Fig. 36: The correlation between the average wind speed (knots) and the number of infected cases during the period (2012-2022).

4.3.4. The relationship between the variation of the average humidity and the number of infected cases during the period (2012-2022)

Linear regression and Pearson correlation (**figure 37,39**) revealed that the number of infected cases decreases progressively with increasing humidity, so there is a very strong negative correlation between the average humidity (g/m^3) and the number of cases infected during the period (2012-2022).

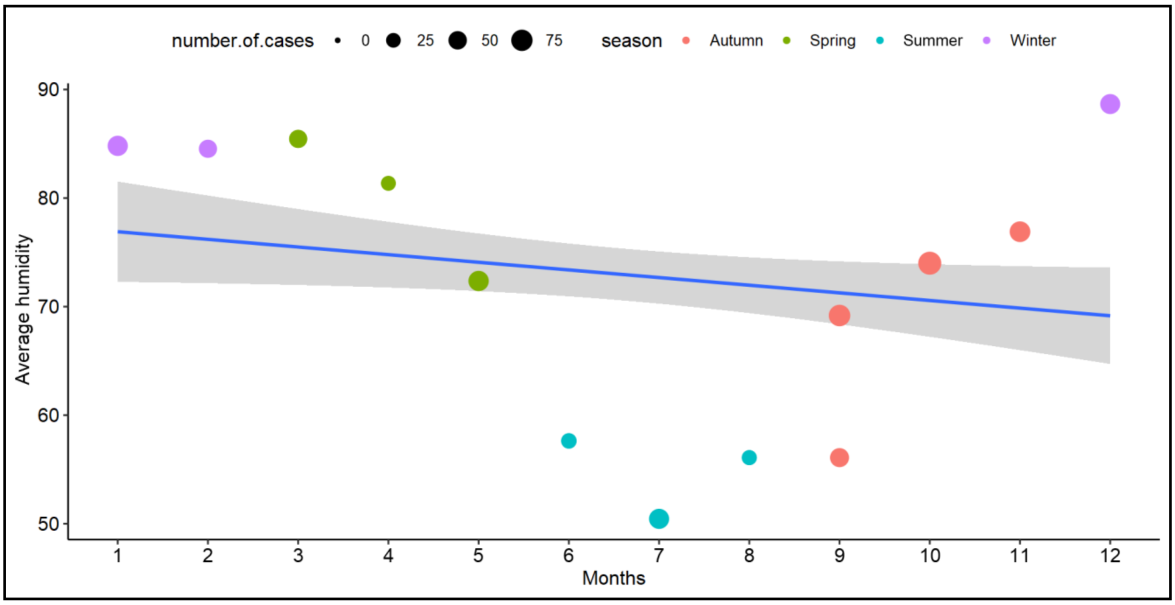


Fig. 37: The correlation between the average humidity (g/m^3) and the number of infected cases during the period (2012-2022).

4.3.5. The relationship between the variation of the average sunshine duration and the number of infected cases during the period (2012-2022)

Linear regression (**figure 38,39**) showed that the number of infected cases decreases progressively with the increase in the average duration of sunshine (hours) so there is a very strong negative correlation between the average duration of sunshine (hours) and the number of infected cases at during the descriptive study period.

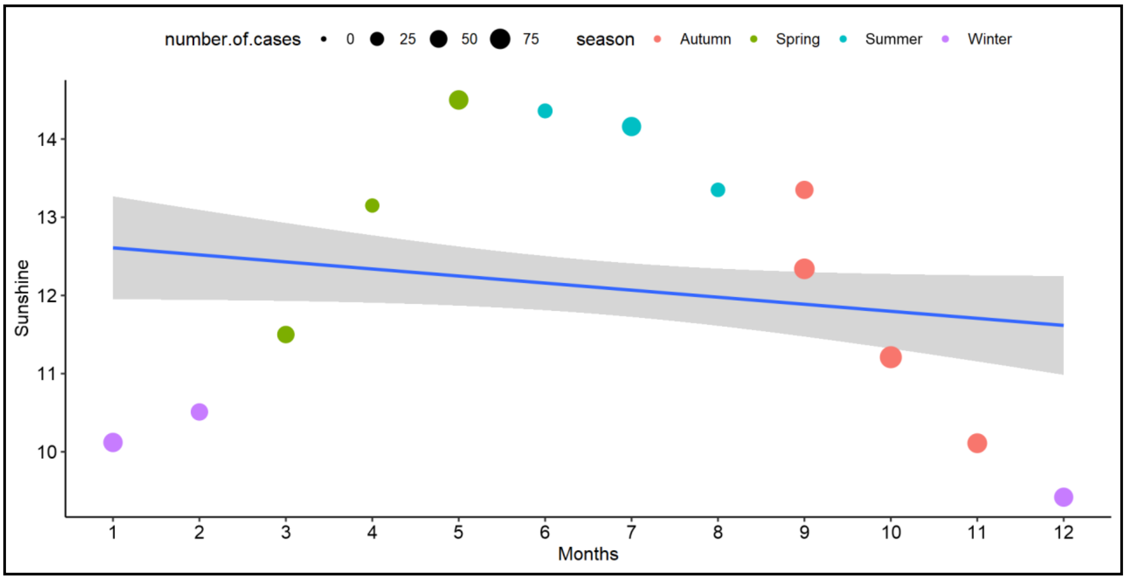


Fig. 38: The correlation between the average sunshine duration (hours) and the number of infected cases during the period (2012-2022).

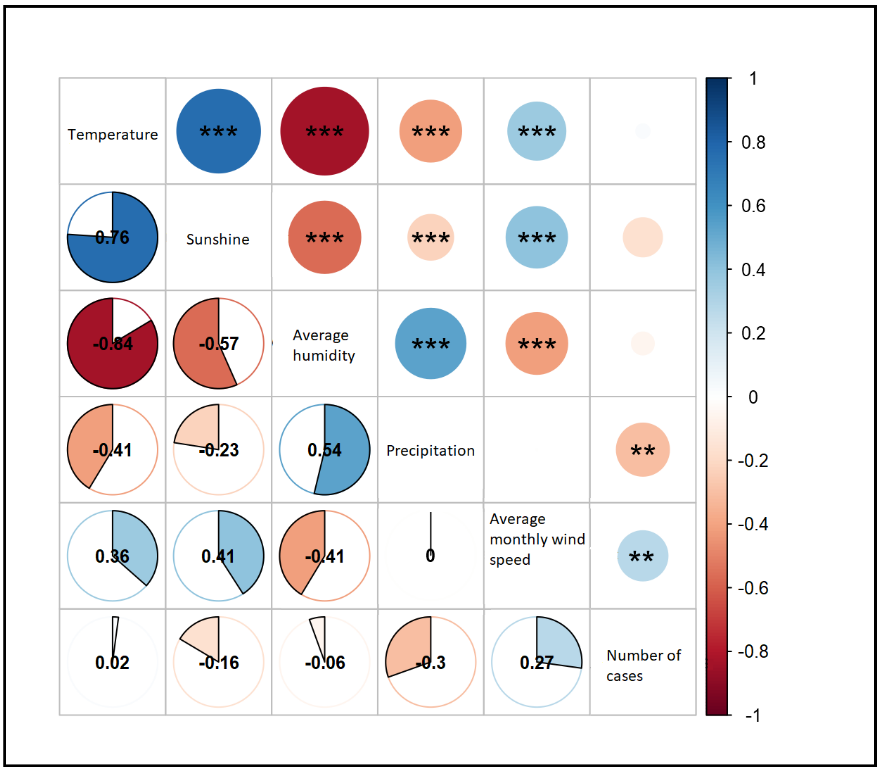


Fig. 39. Correlation matrix applied between meteorological parameters and number of cases of human blastocystosis. Pearson correlation tests are given as correlation coefficients (values below the diagonal, color shading, pie chart and square sizes) and the *p*-value (values above the diagonal). Significant correlations ($p \leq 0.05$)

Discussion

5. DISCUSSION

Blastocystis is the most prevalent parasite found in human digestive tract. *Blastocystis* was described for the first time one century ago, but knowledge about this parasite remains limited. The clinical prelevance of *Blastocystis* is disputable because the parasite colonizes both symptomatic and asymptomatic carriers **(Philippe ,2014).**

This job aims at determining the rate of prevalence of human blastocystose diagnosed at the level of service of parasitology-mycology of brother's hospital establishment Maghlaoui-Mila in the course of a period from (January 2012-December 2022) an analytical descriptive study was led and concerned with the results of exams parasitological of the stool. And a retrospective study during 3 months from January to March 2023. Also, this research aims to indicate the correlation between the prevalence of human blastocystosis in the region of Mila and abiotic factors. The comparison of our results with those of scientific literature allowed the identification of *blastocystis hominis* prevalence and put in a prominent place of relations between parasitical spread and various parameters such as the age and the sex of the patients, years, months, seasons, and meteorological parameters.

We treated 12941 cases among which 2356 cases were positiveduring the period (January 2012 to December 2022). The results of this study show that 18 % *B. hominis* was detected in of stool specimens examined. Very similar prevalence have been described in other parts of the world: 18% in Bethesda, 25% in Jordan 22.9% in Argentina, and 26.58 %in lybia **(Al-Fellani *et al* ., 2007)** We have recorded a higher infection rate than that seen in our Tunisian neighbors, who observed a rate of 7.27% (**Trabelsi *et al*., 2010**) , and a rate of 5.09% in Morocco in Marrakech (**Abdellah, 2019**) and 13.39% in Rabat **(Chabaa *et al*. ,2000).** If the same identification method has been used, these fluctuations may then reflect real distinctions between geographical regions for which the climate, food habits or socio-economic or industrial fabric may be very different.The prevalence of *Blastocystis* is generally higher (**abe *et al*., 2022**; **Abdel-Hafeez *et al*., 2016**) as a result of the consumption of more easily contaminated water or food, closer contact with animals that are potential sources of contamination, and more precarious hygiene conditions and faecal-oral hygiene. In developing countries Thus, the prevalence of *Blastocystis* can exceed 40% as in Brazil (40.9%), Nigeria (49%), Indonesia (60%) or Liberia (70%) (**abe *et al*., 2022** ; **Calderaro *et al*., 2014**).

Our study showed that all individuals are susceptible to *Blastocystis hominis*, males or females , but males are more susceptible with an infestation rate of (57.50%) than females. In contrast to the study conducted in Lebanon, in this study 163 patients were included, of whom 77 were men (47%) and 86 were women (53%)**(El safadi, 2014).** As the infection rate in women is greater than in men ,and Other studies have also reported higher infection rates in men compared to women (**Khoshnood *et al*.,2015 ; Beyhan *et al*.,2015 ; Badparva *et al*..2012**) but no significant relationship was found between gender and infection with this parasite **(Khalili, 2012)** And from this we conclude that There is no evidence to suggest that men are more susceptible to blastocyst disease than women. The susceptibility to the disease depends on various factors such as the immune system, hygiene practices, and exposure to contaminated food or water sources.

All age groups without exception are at risk of *Blastocystis*, In our study we found patients aged 6 months to 82 years. while the studies conducted in Turkey and Libya showed a significant relationship between age group and infection **(Beyhan *et al*., 2015, Abdulsalam *et al*., 2013).** Our study shows that blastocystosis is prevalent in a large percentage (75%) in patients whose age is [20 - 44] .By comparing our study to other studying in Spain in the study made by Salvador (**Fernando Salvador *et al*., 2016**), the average age of the cases was 36 years, the same thing in the study of Losada in Spain (**Ocana-Losada *et al*., 2018**), with an average age of 31 years, whereas in the In the study carried out by El Safadi in France (**El Safadi *et al*., 2016**) the mean age was greater at 45.7 years..This results could be explained by the fact that this age group has a more active community life also this age group is found among the able-bodied or hard workers who are exposed to the risk of contamination with *Blastocystis*.

The results obtained show that the rate of infestation during the period (2012-2022) was very high in 2019 (533 cases) compared to other years where we note that the prevalence of the parasite fluctuated from one year to the next. We have observed an increase in positive cases during the year 2019, this can be explained by a better mastery of clinical and biological diagnosis by health personnel, which would lead to the detection of a greater number of cases. This remarkable growth in 2019 shows that collective and individual prevention measures as well as hygiene rules applicable to risks related to water and food must always be maintained to fight against this parasite

The analysis of the distribution according to the seasons revealed that blastocystosis notes a higher predominance in autumn (36.88%) Then it begins to decline in winter (27.12%) And it is less prevalent in spring (19%) and summer (17%). so we can say that autumn is the most contagious season. In 2 studies done in Thailand and Malaysia showed a clear increase in the prevalence of Blastocystis h between July and October **(Suresh *et al*., 2005, Rhongbutsri *et al*., 2005).** and Compared with the study conducted in Iran (**Salehi *et al*., 2021**) the prevalence of *Blastocystis spp* was higher in the summer and spring compared to other seasons and a significant relationship was also observed between the prevalence of this parasite and the season **(Al-Fellani *et al*., 2007 , El Safadi *et al*., 2016, Khoshnood *et al*., 2015).** Changes and variability in the climate, in addition to current factors favorable to communicable diseases, such as seasonal weather variations, the socioeconomic factors, environmental changes and drug resistance are likely to influence the epidemiology of these diseases. The effects of this situation are likely to express themselves in a variety of ways, ranging from brief outbreaks to long-term gradual changes in disease trends **(Andrew *et al*., 2001).**

Environmental conditions play an important regulating role in the distribution, transmission and developmental success of parasites and pathogens. Meteorological parameters can influence both the parasite species richness as well as the intensity of infection in the host species **(Mas-Coma *et al*., 2008).** Climatic conditions can influence the spread of blastocystosis in several ways; the correlation between meteorological parameters (temperature, precipitation, wind speed, duration of sunshine, humidity) and the number of infected cases can shed light on the influence of climate change on the spread of this parasite.

Temperature is known to be able to increase parasite development rates **(Kutz *et al*., 2005 ; Hudson *et al*., 2006)**.Warmer temperatures favor the survival and spread of *Blastocystis spp.* in the environment. These pests can survive for months in water and moist soil at room temperature. Warmer temperatures also accelerate the life cycle of the parasite and sporulation in the environment. All of this contributes to increasing the risk of infection and transmission.Wetter conditions with heavy rainfall can lead to increased contamination of water sources and soils with *Blastocystis spp*. This increases the risk of transmission through contaminated water and food. High humidity also promotes prolonged survival of cysts in the environment. Strong winds can promote the dispersal of *Blastocystis spp.* in air, water and soil. This increases the likelihood of these cysts reaching human and animal hosts and contaminating water and food. Wind can carry cysts longer distances, allowing for greater geographic spread of blastocystosis **(Tan *et al.*, 2019 ; Eroglu *et al.*, 2010 ; Sarimento *et al.*, 2021 ; Abu-Madi *et al.*, 2016 ; Wu *et al.*, 2018).**

The prospective study that we carried out for 3 months (January-March 2023) at the level of the parasitology-mycology department of the hospital establishment of Brothers Maghlaoui-Mila affirms the retrospective study concerning sex and age. Thus we notice the dominance in March 61 cases (46.21%) this can be explained by it is its period of multiplication in favorable weather conditions.

Conclusion

CONCLUSION

Blastocystis remains a significant global health problem, mainly linked to faecal peril. These pathologies when they do not kill, they generate a harmful health and social impact, they constitute a powerful indicator of the level of hygiene of a population.

This study is the first survey of this kind at the level of the wilaya of Mila, their goal is to carry out a prospective study for three months (from January to March 2023) and another retrospective study in the field of *Blastocystis hominis* diagnosis in the wilaya of Mila, it consists in evaluating the prevalence of *Blastocystis hominis*, diagnosed in the parasitology-mycology laboratory of the Public Hospital Brothers Maghlaoui Mila establishment, over the period (2012-2022).

The results obtained show that 18% of the subjects examined are carriers of blastocystosis, among the positive cases the male sex is the most threatened. There may be biological or behavioral factors that make men more susceptible to *Blastocystis hominis* infection than women, including differences in sex hormones or social and environmental factors related to lifestyle.

The effect of patient age on the prevalence of human blastocystis and its dissemination differs from one study to another. To this end, during our study the results obtained according to age show that the age group from [20 to 44] years is the most exposed to parasites.This may be due to lifestyle and behavioral factors in this age group including diet, habits, and exposure to potential pathogens in the work or living environment.

According to our results, autumn and winter are the seasons that favor the spread of parasitosis because of the climatic conditions that favor the development of parasites.

The climatic conditions of the region of Mila, namely the temperature increase in the index of blastocystosis, the humidity, sunshine duration, and the high wind speed would be at the origin of the decrise of the parasite dissemination. This factors may have an effect on the spread and growth of *Blastocystis hominis*.

The prospective study confirms our retrospective analytical study because we obtained the same results concerning the effect of sex, age on the distribution of *Blastocystis*.Our results obtained regardless of the analytical retrospective study or the prospective study will be more conclusive and give a better appreciation of the epidemiology of *Blastocystis hominis*.

Therefore, the fight against Blastocystis hominis is essential. We advocate prophylactic measures that require a multidisciplinary intervention to bring together healthy living conditions with accountability and the active and serious participation of the community, this requires awareness of collective and individual hygiene.

In the end, we hope that our data will lead to better consideration of local epidemiology and that other studies will be carried out on a larger sample and over a longer period by targeting several regions. The results will be more conclusive and give a better appreciation of this problem.

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Annex

ANNEX

One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Sex	139,630	2355	,000	1,420	1,40	1,44

ANOVA one way

Sex

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3,501	8	,438	1,800	,072
Within Groups	570,497	2347	,243		
Total	573,998	2355			

One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Age	287,423	2355	,000	5,899	5,86	5,94

ANOVA one way

Age

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8,564	8	1,070	1,079	,375
Within Groups	2328,191	2347	,992		
Total	2336,755	2355			

ANOVA bivariate Table

			Sum of Squares	df	Mean Square	F	Sig.
Sex * Age	Between Groups	(Combined)	3,024	7	,432	1,777	,088
	Within Groups		570,974	2348	,243		
	Total		573,998	2355			

One-Sample Test

Test Value = 0

	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Months	95,022	2355	,000	7,076	6,93	7,22

ANOVA one way

Months

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1224,749	8	153,094	12,162	,000
Within Groups	29542,652	2347	12,587		
Total	30767,400	2355			

One-Sample Test

	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Seasons	104,160	2355	,000	2,638	2,59	2,69

ANOVA one way

Seasons

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	45,712	8	5,714	3,816	,000
Within Groups	3514,179	2347	1,497		
Total	3559,891	2355			

One-Sample Test

	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Years	200,762	2355	,000	6,853	6,79	6,92

ANOVA one way

Years

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	180,064	11	16,369	6,105	,000
Within Groups	6285,122	2344	2,681		
Total	6465,187	2355			

Field: Nature and Life Sciences	**Sector:** Ecology and environment	**Specialty:** Protection of Ecosystems

ABSTRACT

In order to determine the epidemiological and clinical characteristics of *Blastocystis hominis* in the region of Mila, we developed a retrospective study during a period extending from January (2012 to December 2022), and the other prospective during three-months (January-March 2023). We collected the data at the level of parasitology-mycology laboratory of the General Hospital Maglaoui Brothers Mila, for the retrospective descriptive analytical study we treated 12941 examinations, 2356 were positive, an infestation rate of 18%.

-Of the positive cases, 57.51% were male and 42.48% were female.

- Patients aged (20-44 years) are the most exposed to this type of parasitism.

-The years 2019, 2021 and 2022 had the highest infection rates, 22.62 %, 20.92% and 19.31% respectively.

-The highest number of parasitized cases noted during the Autumn season followed by winter.

-A high rate of this *Blastocystis hominis* was noted during the months of October, September.

-The highest number of parasitized cases noted during the Autumn season followed by winter.

-The climatic conditions of Mila region, like the ambient temperature increase the dissemination of the bacteria. Sunshine duration, average humidity, precipitation, and average monthly wind speed cause a decrease in the bacterial index of *Blastocystis hominis*.

The results obtained during the three months of our prospective study (January- March 2023), confirmed what we deduced from the results above (The retrospective study from 2012 to 2022).

Keywords: *Blastocystis hominis*, parasite, prevalence, epidemiology, prevalence, correlation, meteorological parameters, Mila.

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