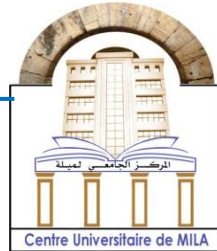


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وزارة التعليم العالي والبحث العلمي

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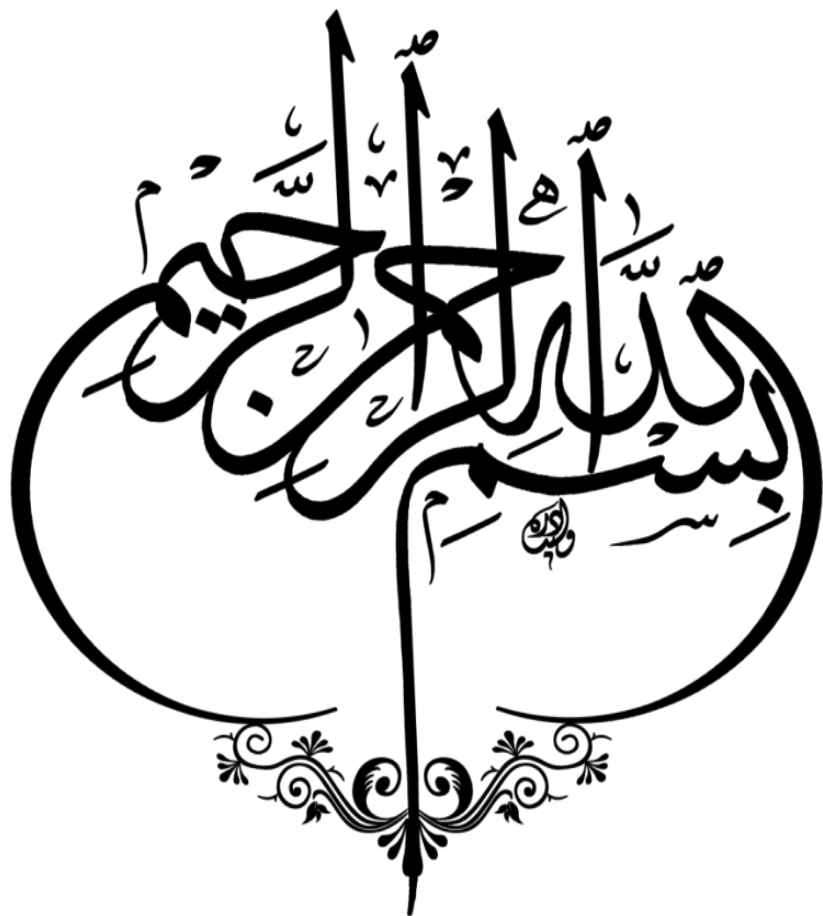
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Special appreciation

In the name of Allah, the most graceful, the most merciful
“and wherever you have of favor, it is from Allah”

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Amel & Ghadir

Dedication

Praise be to Allah, the Most Merciful,
the Most Compassionate, for His endless blessings and guidance.

To my beloved mother, **Zahrouna Bouhzam**, whom I love
beyond words and expressions. My favorite person in the whole
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I am always blown away by your brilliant mind, and how
amazing you are inside and out

Thank you all for your love, support, and unwavering belief in
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♥ A m a l ♥





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To our God almighty who been guiding me and gave me strength in my everyday life and for always looking out for me and being their for me. Thank you for making all of these happend and ended it with a good outcome.

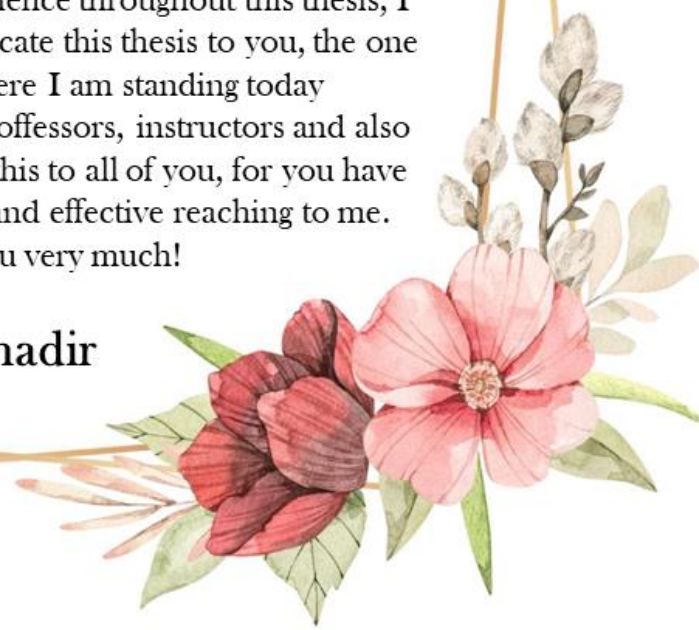
To my dear parents "**MAAMAR**" and "**AIB WARD A**". Thank you fir giving me the support to reach my dreams. Accomplishing this would hopefully make you proud of me as much as I am proud of having you as my parents. I love you,Dad and Mom.

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ABSTRACT

This study aimed to investigate the correlation between the epidemiology of giardiasis and weather parameters in the Mila region over the past ten years. Data on giardiasis infection rates were collected from four hospitals across the region: "Maghlaoui Brothers – Mila," "Mohamed Meddahi - Ferdijoua," "Boukhachem Brothers – Oudi El Atmania," and "Houari Boumadiene – Chelghoum El Aid," involving a total of 11433 individuals screened, with 138 testing positive, resulting in an infection rate of (1,20%). Among the positive cases, (62,32%) were males, and the most affected age group was 20-44 years. Monthly infection rates peaked in May at (14,49%), with annual peaks observed in 2013 and 2014, showing rates of (1,62%) and (2,00%), respectively. Seasonal patterns revealed higher infection rates during spring (35,50%) and autumn (28,98%).

Regionally, the highest number of cases was recorded in Mila (53,62%), while Ferdjioua had the highest infestation rate at (2,95%). Detailed analysis showed significant monthly and seasonal variations, with specific peaks in May, March, and November, and notable increases during autumn and spring. Annual trends indicated fluctuating infection rates across the regions over the decade.

A supplementary three-month study in early 2024 at Maghlaoui Brothers hospital in Mila confirmed these retrospective trends. Meteorological data sourced from the Ain Tin station formed the basis of our analysis, statistical analysis using SPSS and R software revealed significant correlations between giardiasis distribution and weather parameters, specifically, a positive correlation was identified between giardiasis prevalence and temperature and sunduration, while a negative correlation was found with precipitation, humidity, and wind speed.

An investigation was conducted to identify potential sources of contamination through water sampling at three distinct sites within the Beni Haroun Dam area: The Dike, Médious, and Boudmaghe, respectively. The parasite was detected specifically in the Boudmaghe locality, indicating a presence in the region.

Geospatial analysis and visualization using QGIS facilitated the creation of detailed maps showing sampling points. This approach enabled the generation of graphical representations illustrating statistical analyses, infestation levels, and prevalence rates across distinct regions within the state. These efforts provided a comprehensive overview of spatial variation, offering valuable insights into the geographic distribution and intensity of the parasitic disease.

The statistical analysis of prevalence rates provides a quantitative foundation for assessing disease burden and informing proactive public health strategies, reinforcing the importance of ongoing surveillance and intervention efforts to ensure safe water sources and minimize health risks in the population. Specifically, the detection of *Giardia* parasites in the Boudmaghe locality underscores the critical role of environmental surveillance in understanding and mitigating waterborne diseases.

These insights emphasize the urgent need for robust water quality monitoring programs and targeted public health interventions to safeguard community health against the transmission of giardiasis and related infections.

Keywords: Giardiasis, epidemiology, infection, prevalence, correlation, meteorological parameters, Mila.

RESUME :

Cette étude visait à investiguer la corrélation entre l'épidémiologie de la giardiase et les paramètres météorologiques dans la région de Mila au cours des dix dernières années. Les données sur les taux d'infection par la giardiase ont été collectées dans quatre hôpitaux de la région : "Frères Maghlaoui – Mila", "Mohamed Meddahi - Ferdjioua", "Frères Boukhachem – Oued El Atmania", et "Houari Boumadiene – Chelghoum El Aid", impliquant un total de 11,433 individus examinés, parmi lesquels 138 ont été testés positifs, résultant en un taux d'infection de (1,20%). Parmi les cas positifs, (62,32%) étaient des hommes, et le groupe d'âge le plus touché était celui des 20-44 ans. Les taux d'infection mensuels ont atteint leur pic en mai à (14,49%), avec des pics annuels observés en 2013 et 2014, montrant des taux de (1,62%) et (2,00%), respectivement. Les motifs saisonniers ont révélé des taux d'infection plus élevés au printemps (35,50%) et en automne (28,98%).

Au niveau régional, le plus grand nombre de cas a été enregistré à Mila (53,62%), tandis que Ferdjioua a présenté le taux d'infestation le plus élevé à (2,95%). Une analyse détaillée a montré des variations mensuelles et saisonnières significatives, avec des pics spécifiques en mai, mars et novembre, ainsi que des augmentations notables pendant l'automne et le printemps. Les tendances annuelles ont indiqué des taux d'infection fluctuants à travers les régions au cours de la décennie.

Une étude supplémentaire de trois mois au début de 2024 à l'hôpital "Frères Maghlaoui" à Mila a confirmé ces tendances rétrospectives. Les données météorologiques provenant de la station d'Ain Tin ont servi de base à notre analyse. L'analyse statistique à l'aide des logiciels SPSS et R a révélé des corrélations significatives entre la distribution de la giardiase et les paramètres météorologiques, en particulier une corrélation positive a été identifiée entre la prévalence de la giardiase et la température et la durée d'ensoleillement, tandis qu'une corrélation négative a été trouvée avec les précipitations, l'humidité et la vitesse du vent.

Une enquête a été menée pour identifier les sources potentielles de contamination à travers des échantillons d'eau prélevés dans trois sites distincts dans la zone du barrage de Beni Haroun : La Digue, Médious, et Boudmaghe. Le parasite a été détecté spécifiquement dans la localité de Boudmaghe, indiquant sa présence dans la région.

L'analyse géospatiale et la visualisation à l'aide de QGIS ont facilité la création de cartes détaillées montrant les points d'échantillonnage. Cette approche a permis la génération de représentations graphiques illustrant les analyses statistiques, les niveaux d'infestation et les taux de prévalence à travers les différentes régions de l'état. Ces efforts ont fourni un aperçu complet des variations spatiales, offrant des informations précieuses sur la distribution géographique et l'intensité de la maladie parasitaire.

L'analyse statistique des taux de prévalence fournit une base quantitative pour évaluer la charge de la maladie et informer les stratégies de santé publique proactives, renforçant l'importance des efforts de surveillance continue et d'intervention pour garantir des sources d'eau sûres et minimiser les risques

sanitaires dans la population. En particulier, la détection de parasites Giardia dans la localité de Boudmaghe souligne le rôle critique de la surveillance environnementale dans la compréhension et l'atténuation des maladies d'origine hydrique.

Ces Perspectives soulignent la nécessité urgente de programmes robustes de surveillance de la qualité de l'eau et d'interventions ciblées en santé publique pour protéger la santé communautaire contre la transmission de la giardiase et des infections connexes.

Mots-clés : Giardiase, épidémiologie, infection, prévalence, corrélation, paramètres météorologiques, Mila.

الملخص:

هدفت هذه الدراسة إلى التحقيق في العلاقة بين وبائيات الجيارديا وعوامل الطقس في منطقة ميلة بالجزائر على مدار السنوات العشر الماضية (2013-2023). تم جمع بيانات معدلات الإصابة بالجيارديا من أربعة مستشفيات في جميع أنحاء المنطقة: "مستشفى الأخوة مغلاوي بميلة"، "مستشفى محمد مداحي بفرجوية"، "مستشفى الأخوة بوخشم بوادي العثمانية"، و"هوارى بومدين بشلغوم العيد"، حيث تم فحص ما مجموعه 11,433 فردًا، تبين أن 138 منهم مصابون، مما أدى إلى معدل إصابة قدره 1.20%. من بين الحالات الإيجابية، كانت نسبة 62.32% من الذكور، والفئة العمرية الأكثر تضررًا كانت من 20 إلى 44 عامًا. بلغت معدلات الإصابة الشهرية ذروتها في شهر مايو بنسبة 14.49%، مع ذروات سنوية لوحظت في عامي 2013 و2014، بمعدلات 1.62% و2.00% على التوالي. أظهرت الأنماط الموسمية معدلات إصابة أعلى خلال فصل الربيع (35.50%) وفصل الخريف (28.98%).

إقليميًا، تم تسجيل أكبر عدد من الحالات في ميلة (53.62%)، في حين سجلت فرجوية أعلى معدل إصابة بنسبة (2.95%). أظهرت التحليلات التفصيلية تباينات شهرية وموسمية كبيرة، مع ذروات محددة في مايو ومارس ونوفمبر، وزيادات ملحوظة خلال فصلي الخريف والربيع. أشارت الاتجاهات السنوية إلى تذبذب معدلات الإصابة عبر المناطق خلال العقد.

أكدت دراسة تكميلية لمدة ثلاثة أشهر في أوائل عام 2024 بمستشفى إخوان مغلاوي في ميلة هذه الاتجاهات السابقة. تم أخذ معطيات معلمات الطفق للمنطقة عبر المحطة الجوية لعين التين. كشفت التحليلات الإحصائية باستخدام برمجيات برنامج الحزم الإحصائية للعلوم الاجتماعية SPSS وبرنامج الـ R عن ارتباطات كبيرة بين توزيع الجيارديا ومعلمات الطقس. بشكل خاص، تم تحديد ارتباط إيجابي بين انتشار الجيارديا ودرجة الحرارة، ومدة سطوع الشمس بينما تم العثور على ارتباط سلبي مع هطول الأمطار، والرطوبة، وسرعة الرياح.

تم إجراء تحقيق لتحديد المصادر المحتملة للتلوث من خلال أخذ عينات المياه في ثلاثة مواقع مختلفة داخل منطقة سد بني هارون: الحاجز، ميديوس، و بودماغ. تم اكتشاف الطفيلي تحديدًا في منطقة بودماغ، مما يشير إلى وجوده في المنطقة. سهلت التحليلات والتصويرات الجغرافية باستخدام QGIS إنشاء خرائط مفصلة توضح نقاط أخذ العينات.

أتاحت هذه المقاربة توليد تمثيلات بيانية توضح التحليلات الإحصائية ومستويات الإصابة ومعدلات الانتشار عبر المناطق المختلفة داخل الولاية. وفرت هذه الجهود نظرة شاملة على التباين المكاني، مما أتاح معلومات قيمة حول التوزيع الجغرافي وشدة المرض الطفيلي. توفر التحليلات الإحصائية لمعدلات الانتشار أساسًا كمياً لتقييم عبء المرض وإعلام استراتيجيات الصحة العامة الاستباقية، مما يعزز أهمية المراقبة المستمرة وجهود التدخل لضمان مصادر مياه آمنة وتقليل المخاطر الصحية على السكان.

بشكل خاص، تؤكد اكتشافات طفيليات الجيارديا في منطقة بودماغ على الدور الحاسم للمراقبة البيئية في فهم وتخفيف الأمراض المنقولة عن طريق المياه. تؤكد هذه الرؤى على الحاجة الملحة لبرامج قوية لمراقبة جودة المياه والتدخلات الصحية العامة المستهدفة لحماية صحة المجتمع من انتقال الجيارديا والعدوى المرتبطة بها.

الكلمات المفتاحية: الجيارديا، علم الأوبئة، العدوى، الانتشار، الارتباط، العوامل الجوية، ميلة.

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ABRIVIATION TABLE

%	Percentage
°C	Degree Celsius
AII	Sub-assemblage AII (a specific genetic group of <i>Giardia</i>)
ANOVA	Analysis of variance
BIII	Sub-assemblage BIII (a specific genetic group of <i>Giardia</i>)
BIV	Sub-assemblage BIV (a specific genetic group of <i>Giardia</i>)
DAS	Department of Agricultural Services
DNA	Deoxyribonucleic Acid
DPSB	The Directorate of Budget Programming and Monitoring
EIA	Enzyme immunoassay
ELISA	Enzyme-Linked Immunosorbent Assay
EM	Electronic microscopy
FIG	Figure
G	<i>Giardia</i>
g	Gramme
GDH	Glutamate Dehydrogenase
ITIS	Intergrated Taxonomic Information System
M	Meters
mm	Millimetres
NAID	The National Agency for Investment Development
NALIRA	The National Agency for Land Intermediation and Regulation of Algeria
PCR	Polymerase Chain reaction
PSE	Parasitological stools examination
pH	Hydrogen potential
R	Correlation Coefficient
SPSS	Statistical Package for the Social Sciences
SSU	Small Subunit
TAA	total agricultural area
TPI	Triosephosphate Isomerase
UAL	Useful agricultural land
EU	European Union
EEA	European Economic Area
ECDPC	European Center for Disease Prevention and Control

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INTRODUCTION



INTRODUCTION

The human environment hosts a diverse array of microorganisms, ubiquitous in the atmosphere, adorning the skin, entwined within mucous membranes, and colonizing the digestive tract; Digestive parasites constitute a serious public health problem worldwide **(Keiser and Ulziner, 2010)**. The parasite is defined as a microorganism that lives at the expense of another living being called a host **(Nicolas et al., 2001)**. The human intestine can be colonized by various parasitic species, causing benign parasitic diseases that can take on a serious aspect, potentially leading to the death of certain patients **(Nicolas, 2002)**. Several digestive parasites can infest humans on all continents and in all climates **(Desoubeaux and Duong, 2011)**, notably *protozoa* (*Giardia*, *Chilomastix*, *Entamoeba*, *Endolimax*, *Cryptosporidium*, *Balantidium*) **(Melhorne, 2008)**, *metazoans* (*nematodes*) (*Ascaris*, *Strongyloides*, *Ancylostoma*, *Trichuris*) **(Nicolas et al., 2001)**, *cestodes* (*Taenia*, *Hymenolepis*, *Diphyllobothrium*), and *trematodes* (*Fasciola*, and *Schistosoma*) **(Melhorne, 2008)**.

Giardia is a leading but neglected cause of infectious gastroenteritis worldwide **(Savioli et al., 2006)**. It is considered the most common human intestinal parasitosis in the world **(Savioli et al., 2006)**. The flagellated protozoan, *Giardia lamblia* (syn. *G. duodenalis* and *G. intestinalis*) comprises eight genetic “assemblages” (A-H) with only A and B affecting humans **(Alison Waldram et al., 2017)**. Assemblages A and B can also infect pets, livestock and wild animals showing the potential for zoonotic transmission **(Ryan and Cacciò, 2013)**.

The reported prevalence of *Giardia* in human populations is 4–43% and 1–7% in low and high income countries respectively **(Rogawski et al., 2017)**. It is believed that giardiasis is responsible for 2.5 million of diarrhea and nutritional deficiencies in children in developing countries **(WHO, 1988)**. The World Health Organization has also reported that about 200 million people have symptomatic giardiasis every year in Asia, Africa and Latin America **(WHO, 1996)**. This protozoan has been estimated to cause annually 184 million clinical cases **(Pires et al., 2015)**.

Recently, *Giardia lamblia* has become a significant public health concern due to its association with multiple waterborne outbreaks **(Karanis et al., 2007)**. It primarily affects children and the immunosuppressed and exists in two interconvertible forms: cyst and trophozoite. Infection is acquired by ingestion of food or water contaminated with cysts, which shed in human or animal faeces and become trophozoites that colonize the small intestine by attachment to the epithelial microvillus **(Zakai, 2004)**, **(Al-Braiken, 2008)**. The parasite is known to induce pathophysiological changes in the small intestines and trigger an

immune response that result in parasite clearance. The cause of disease is multifactorial. Host immune responses toward *Giardia lamblia*, however, are not completely understood (**Showgy and Staffan, 2024**). Some infected people present gastrointestinal symptomatology; however, in many cases the disease is completely asymptomatic. In such cases, patients act as carriers, releasing large numbers of cysts into the environment and promoting the transmission of infection (**Sullivan et al., 1988**). The clinical disease ‘giardiasis’ typically includes diarrhea, flatulence, abdominal pain and bloating (**Ali and Hill, 2003**). And weight loss due to malabsorption (**Nygård et al., 2006**). In addition to causing diarrhea, abdominal pain, flatulence, nausea, vomiting and other classical gastrointestinal manifestations, in some people, this flagellated protozoan has been linked with a broad range of clinical features (also extra-intestinal), including post-infectious- irritable bowel syndrome, chronic fatigue and, in young children, failure to thrive (**Escobedo et al., 2010**) (**Escobedo et al., 2016**) (**Litleskare et al., 2018**). All these features suggest a need for increased control efforts.

Correct diagnosis of giardiasis is important for treatment and prevention of diseases. The laboratory diagnosis of *Giardia lamblia* is mainly based on finding and demonstration of microscopic cyst in stool samples, but immunological-based assay and molecular methods also are available and are used for diagnostic or research proposes in developed countries. All diagnostic methods provide different sensitivity and specificity. This condition depends on some factors such as the method of test, the skill of operations and the stage that the test has been performed (**Elmi et al., 2017**). The most common way to detect *Giardia lamblia* being is the microscopic examination of stool specimens. However, this technique can give a false negative result. Detection of the parasite antigen in stool specimens through ELISA (enzyme-linked immunosorbent assay) using monoclonal antibodies is a more sensitive and accurate method (**Uchoa et al., 2018**). The disease is usually treated with Metronidazole or Tinidazole, but the numbers of treatment failures are steadily increasing (**Showgy and Staffan, 2024**).

Prevalence rate is high in areas with poor sanitation and varies from 2% to 5% in developed to 20% to 30% in developing countries. The variation in prevalence might be attributed to factors such as the geographical area, the urban or rural setting of the society, the age group composition, and the socio economic conditions of the study subjects (**Yibeltal and Simenew, 2015**).

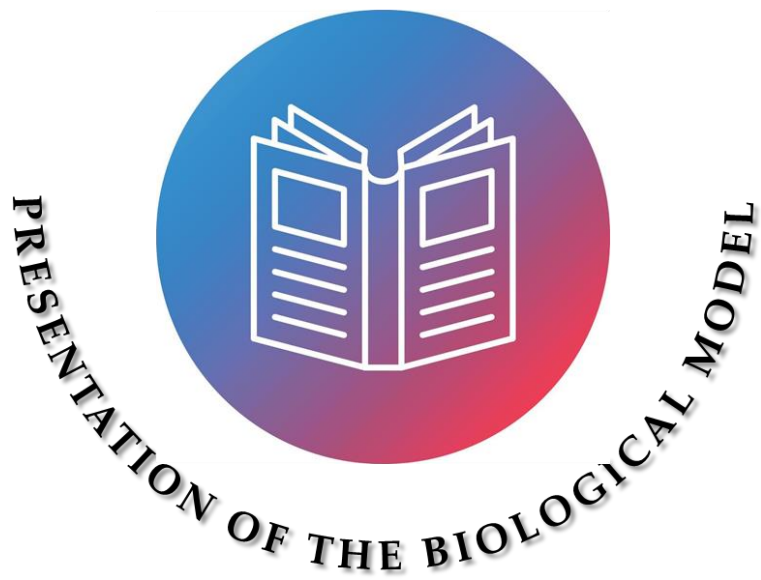
Recent studies have extensively investigated the identification and prevalence of *Giardia lamblia* in both human and animal populations across various regions worldwide (**adam, 2021**). The prevalence of *Giardia lamblia* exhibits intricate geographical disparities,

influenced by a multitude of environmental factors such as climate, terrain, and water sources. Additionally, public health infrastructure, including access to clean water and sanitation facilities, plays a pivotal role in shaping the prevalence patterns. Cultural practices further contribute to this variation, affecting hygiene standards and dietary habits, which in turn impact transmission rates. Moreover, demographic attributes such as gender, age, residential settings (urban vs. rural), educational attainment, and occupational exposure, intricately intertwine with epidemiological factors to modulate the severity and distribution of *Giardia lamblia* infection. These factors collectively delineate the complex landscape of *Giardia lamblia* prevalence, underscoring the necessity for targeted interventions and comprehensive public health strategies (Smith *et al.*, 2020; Jones *et al.*, 2019; Patel *et al.*, 2021; Brown *et al.*, 2018).

Giardia lamblia is heavily influenced by environmental conditions, which regulate its distribution, transmission, and developmental success (Xiao and Fayer, 2008). Meteorological parameters, such as temperature, humidity, and precipitation, significantly affect the prevalence and intensity of *Giardia* infections among host species (Karanis *et al.*, 2007). Elevated temperatures are known to enhance the survival and infectivity of *Giardia* cysts, thereby increasing transmission rates (Smith and Nichols, 2003). Precipitation plays a crucial role in waterborne transmission by promoting the spread of cysts in both surface and groundwater (Fayer *et al.*, 2000). Consequently, changes in climate patterns are expected to modify the geographical distribution and prevalence of *Giardia* infections, as these environmental factors directly impact host-parasite interactions (Harvell *et al.*, 2002).

In this study, we aim to conduct a retrospective epidemiological investigation into cases of human giardiasis. We have collected data from four distinct public hospital establishments situated across four regions within the Mila province. These hospitals include Brothers Maghlaoui located in Mila, Mohamed Meddahi - Ferdjioua, Brothers Boukhachem - Oued el atmania, and Houari Boumadiene - Chalhoun El-Aid, over the past decade (2013 to 2023). Our objectives are to establish the frequency of *Giardia lamblia* in the region of Mila and to investigate the distribution of this parasite according to gender, age groups, years, seasons, and months. Additionally, we aim to assess the prevalence of human giardiasis in the Mila province and explore any potential correlations with meteorological factors. Our work is divided into three chapters. The first section of our study is dedicated to a comprehensive bibliographic review focusing on general information pertaining to *Giardia lamblia* and giardiasis, synthesizing current scientific knowledge and key findings in the field. In the

second section, we detail the methodology employed at the Mila Parasitology Mycology Laboratory. This includes the application of various parasitological techniques for the detection of *Giardia lamblia*, as well as the methods utilized for analyzing and interpreting the obtained results. Additionally, the methodology encompasses a water sampling investigation into potential sources of contamination within our Beni Haroun Dam (including the dike, Medious, and Boudmaghe). The third section presents the results obtained from our study and provides a detailed interpretation of these findings, considering factors such as prevalence rates, demographic patterns, and any notable variations observed. Moving on to the fourth section, we engage in a comprehensive discussion of our results in light of existing scientific literature. This involves comparing our findings to previous studies, identifying similarities, differences, and potential implications for future research and public health interventions. Finally, the conclusion section synthesizes our study's key findings, highlights any significant implications or limitations, and offers recommendations for further investigation or practical application in the field of parasitology and public health.



2. PRESENTATION OF THE BIOLOGICAL MODEL

2.1. Historical background

Giardia (usually pronounced ‘jiardia’) has been considered one of the most ancient and primitive eukaryotic organisms on the planet (**Adam, 2001**). Recent studies have called into question this assertion (**Ankarklev et al., 2010**).

This parasite has existed for a very long period, and most of it without our awareness. Up until the second half of the 19th century, when a variety of bacteria and other microbiological diseases were identified and connected to certain infections, our understanding of microorganisms was very limited. However, as early as in 1681 the Dutch pioneer microscopist Antony van Leeuwenhoek “the father of microbiology” observed a small animalcule in great numbers in his own diarrheal stools. Based on his notes and drawings it has been concluded that this was *Giardia lamblia* (**Dobell, 1920**).

Vilém D. Lambl, a Czech physician, gave it a more thorough description in 1859 and named it *Cercomonas intestinalis*. Vilém D. Lambl, a Czech physician, gave it a more thorough description in 1859 and named it *Cercomonas intestinalis* (**Lambl, 1859**).

In 1888, Blanchard named it *Lamblia intestinalis* in his honor, and several creatures that were subsequently determined to be *Giardia* species were described during the same period (**Adam, 2001**).

The name *Giardia* was given in honor of the French zoologist Alfred M. Giard (**Adam, 2001**), which was later on used for the first time in 1882 by Kunstler for an organism he found in tadpoles.

In 1915 Kofoed and Christensen proposed *Giardia* for the genus and *lamblia* for the species (**Adam, 2001**). To this day, this is still the official name according to the Integrated Taxonomic Information System (**ITIS report**).

Giardia lamblia is the most common name in English literature, but *Giardia intestinalis* and *Giardia duodenalis* are also used and some argue that the latter is the most correct form (**Monis, et al**).

So far there is no agreement to choose one before the others. In medical literature the term “*Giardia*” is often used synonymously with the species *Giardia lamblia*.

2.2. Definition

Giardia is a genus of intestinal flagellates that infects a wide range of vertebrate hosts. The genus currently comprises six species, namely *Giardia agilis* in amphibians, *Giardia microti* and *Giardia muris* in rodents, *Giardia ardeae* and *Giardia psittaci* in birds, and *Giardia lamblia* (synonymous. *G. intestinalis*, *G. duodenalis*) in humans, although it is also found in other mammals, including pets and livestock (**Thompson, 2004**).

2.3. Presentation of the parasite (*Giardia lamblia*)

Giardia is a ubiquitous intestinal parasite. It is a unicellular, binucleate, flagellate protozoan found infecting all classes of vertebrate examined to date. *Giardia lamblia* is an obligate parasite with two life stages, the active trophozoite stage that attaches to the upper intestines with a suction disc and the environmental cyst that is passed between hosts by the fecal-oral route (**Adam, 2021**).

Transmission to humans occurs either through direct person-to-person contact in environments with compromised hygiene levels (**Colli et al., 2015**), or by the contamination of water or food by the cysts (**Silvestri et al., 2013**).

2.3.1. Taxonomy and classification

In the widely used 1980 classification, Protozoa was considered a subkingdom with seven phyla: Sarcomastigophora (containing Mastigophora and Sarcodina), Apicomplexa, Microspora, Myxozoa, and Ciliophora (**Cavalier-Smith, 2003**).

The most recent classification, however, establishes Protozoa as a diverse group within the larger domain Eukaryota, recognizing numerous phyla. The flagellates, previously grouped under Mastigophora, are now distributed among four distinct phyla: Metamonada, Parabasalia, Percolozoa, and Euglenozoa. These reclassifications have resulted from integrating molecular sequence studies with other lines of evidence, including genetic, structural, and biochemical data (**Cavalier-Smith, 2003; Cox, 2002**).

Therefore, based on modern taxonomy (**Table 1**), *Giardia* belongs to the Phylum Metamonada, Class Trepomonadea, Order Diplomonadida, and Family Hexamitidae (**Morrison et al., 2007**).

Table 01: Taxonomy and classification of giardia lamblia (Laura et al., 2022)

KINGDOM	<i>Eukaryotic Protista</i>
PHYLUM	<i>Metamonada</i>
CLASS	<i>Trepomonadea</i>
ORDER	<i>Diplomonadida</i>
FAMILY	<i>Hexamitidae</i>
GENUS	<i>Giardia</i>
SPECIES	<i>Giardia lamblia</i> (syn. <i>Giardia intestinalis</i> , <i>Giardia duodenalis</i>)

2.3.2. Classification of giardia species and Genotypes

The organism he called *Giardia lamblia* on the basis of light microscopy is found in humans and in a broad range of other mammals. However, subsequent investigation using techniques with greater discriminatory power, primarily electron microscopy and molecular characterization, have defined differences within *G. lamblia* (*duodenalis*) that are frequently associated with host specificity. The first round of subdividing *G. lamblia* into subgroups consisted of the formal designation of new species on the basis of morphologic differences seen by electronic microscopy (EM) and included *Giardia psittaci*, *Giardia microti*, and *Giardia ardeae* (Table 2). The distinctness of these new species has subsequently been supported by DNA sequence comparisons (Rodney, 2021).

Even after excluding the organisms with morphological differences at an EM level, there is substantial variability at a DNA sequence level, and for many of them, there is a difference in host specificity (Table 2) (Rodney, 2021).

The name “*Giardia lamblia*” has typically been used in medical writing, while “*Giardia intestinalis*” and, later, “*G. duodenalis*” have been commonly used in the scientific literature (Rodney, 2021).

Table 02: *Giardia lamblia (duodenalis)* species and genotypes (Rodney, 2021)

Genotype	Hosts	Proposed species name	Reference (s)
A1	Primarily animals, but also in humans	<i>Giardia duodenalis</i>	(Morrison <i>et al.</i> , 2007) (Nash <i>et al.</i> , 1985)
All	Humans, numerous other mammals	<i>Giardia duodenalis</i>	(Nash <i>et al.</i> , 1985) (Adam <i>et al</i> 2013)
B	Humans, numerous other mammals	<i>Giardia enterica</i>	(Nash <i>et al.</i> , 1985) (Franzen <i>et al.</i> , 2009) (Adam <i>et al.</i> , 2013)
C	Dogs	<i>Giardia canis</i>	(Monis and Andrews, 1998) (Monis <i>et al.</i> , 1999)
D	Dogs	<i>Giardia canis</i>	(Monis and Andrews ,1998) (Monis <i>et al</i> , 1999)
E	Cows , Sheep, alpacas, goats, pigs	<i>Giardia bovis</i>	(Jerlstrom <i>et al.</i> , 2010) (Ey <i>et al.</i> , 1997)
F	Cats	<i>Giardia cati</i>	(Monis <i>et al.</i> , 1999)
G	Rats and mice	<i>Giardia simondi</i>	(Monis <i>et al.</i> , 1999)
H	Seals (marine vertebrates)	<i>Giardia psittaci</i>	(Lasek-Nesselquist <i>et al.</i> , 2010)

2.3.3. Structure and morphology

2.3.3.1. General Structure

Giardia lamblia is a unicellular flagellated motile eukaryotic microorganism. Other *Giardia* species include *G. agilis* found in amphibians and *G. muris* found in rodents, but *G. lamblia* is the only one found in man. Within the species *G. lamblia* there are several genotypes dividing it into different “assemblages”, each with preference to different hosts and with possible variations in the clinical manifestations of infection. (Monis *et al.*, 2009).

2.3.3.2. Morphology

Giardia exists in two distinct life forms (**Fig 1**) Cyst, the resistant form, responsible for the transmission of the parasite between vertebrate hosts (**Fig 1/B**), and trophozoite (**Fig 1/A**), the vegetative form that replicates within the intestinal tract of the hosts and leads to clinical manifestation. *Giardia* differentiation occurs through the processes of excystation, in which the parasite changes from cyst to trophozoite, and encystment, when it returns to its resistant form (Lauwaet *et al.*, 2007).

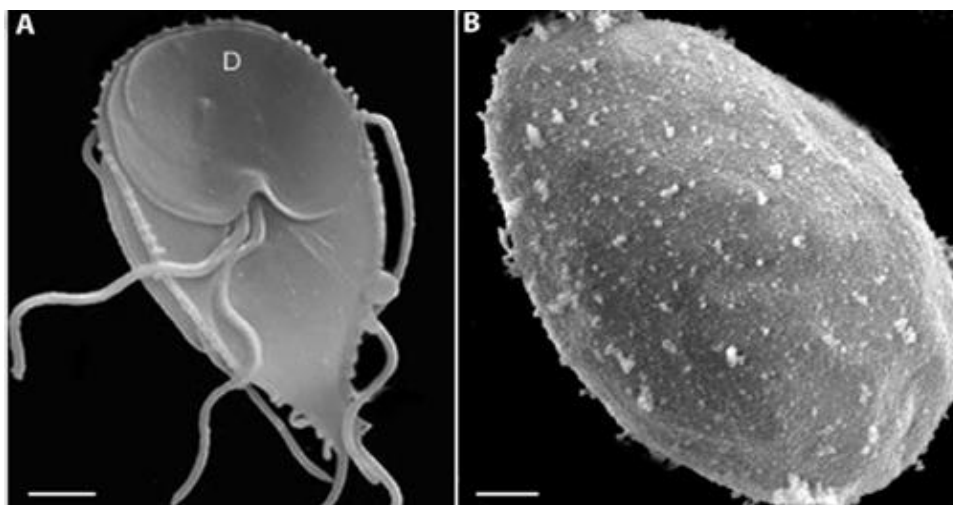


Figure 01: Scanning electron microscopy of *Giardia lamblia* in a trophozoite form (A) and as a cyst (B) (Marlene, 2007).

A. Cystic form-*

Cysts (**Fig 2/A**) are oval with a tough hyaline cyst wall and measure 8 to 12, μm long by 7 to 10, μm wide. The mature cyst contains four nuclei, usually situated at one end; curved median bodies, and the linear axonemes (**Fig 2/B**). The cysts can survive if kept cool and

damp and have been shown to survive in water for up to 3 months. They can also survive standard concentrations of chlorine used in water purification systems (Jones, 1988).

The cyst is resistant to environmental factors and stomach acid. After ingestion, it transforms into trophozoites in the small intestine, causing giardiasis symptoms. Some trophozoites encyst, and the cycle concludes when cysts are passed in feces, potentially infecting another host.

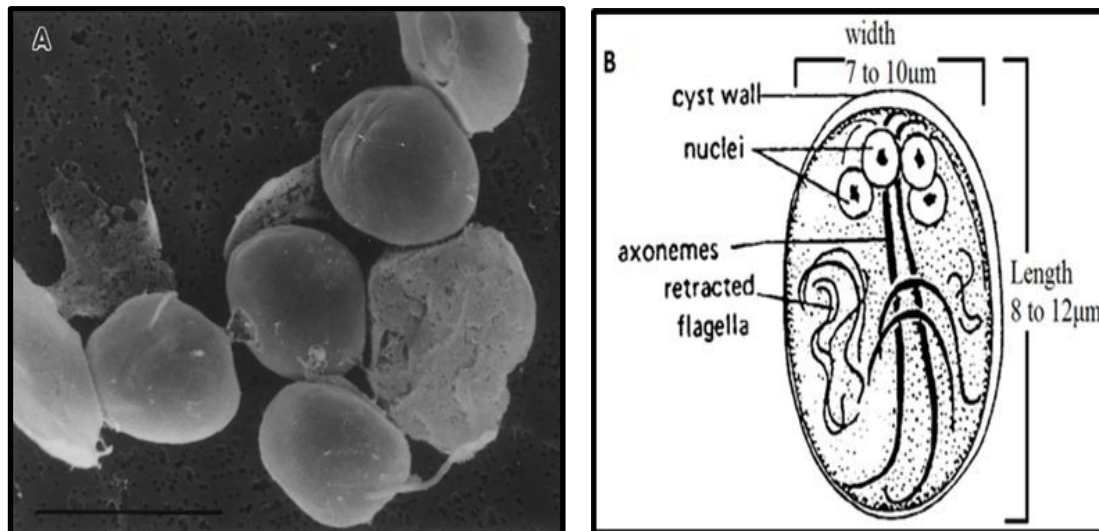


Figure 02: *Giardia lamblia* cystic form

A: Electron photomicrograph of *Giardia lamblia* form as a Cyst, Bar = 10mm (Ortega and adam, 1997).

B: *Giardia lamblia* morphology of cyste stage (Guillaume, 2007).

B. The Trophozoite form (vegetative)

The *Giardia lamblia* trophozoites are pear-shaped and are approximately 12 to 15 μ m long and 5 to 9 μ m wide (Fig 3). The cytoskeleton includes a median body, four pairs of flagella (anterior, posterior, caudal, and ventral), and a ventral disk (Fig 3/B).

Trophozoites have two nuclei without nucleoli that are located anteriorly and are symmetric with respect to the long axis. Lysosomal vacuoles, as well as ribosomal and glycogen granules, are found in the cytoplasm. Golgi complexes become visible in encysting trophozoites but have not been confirmed to be present in vegetative trophozoites (Gillin et al., 1996). However, stacked membranes suggestive of Golgi complexes have been demonstrated (Lanfredi et al., 1999) (Mayrhofer et al., 1995).

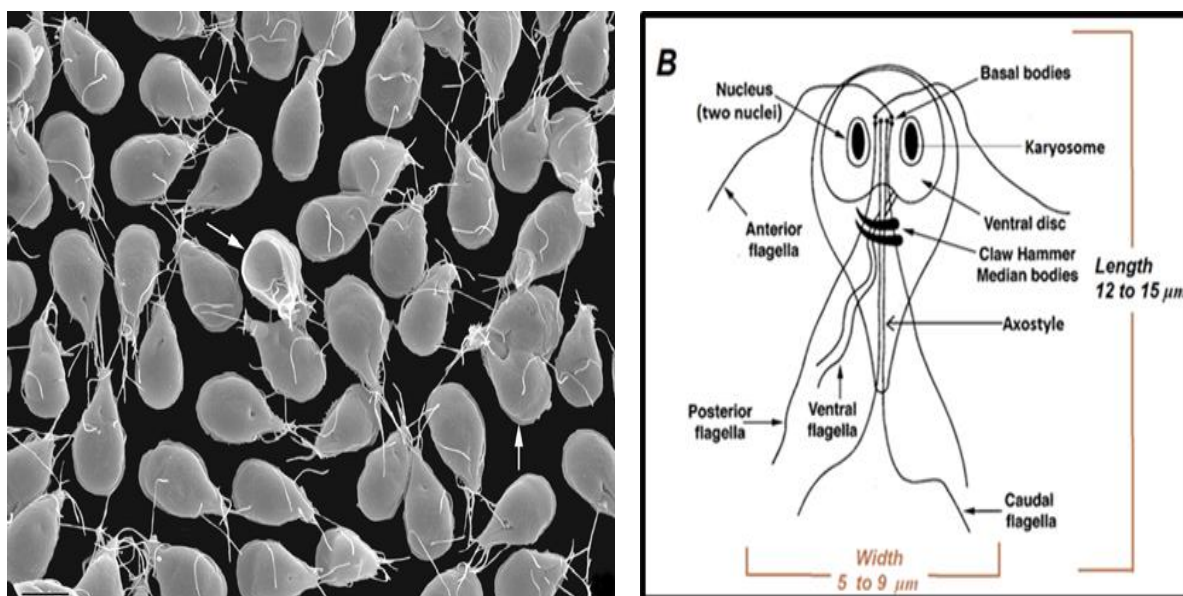


Figure 03: *Giardia lamblia* vegetative form

A: Low magnification of several *Giardia lamblia* adhered by the ventral region as seen in scanning electron microscopy. Arrows point to dividing cells. Bar = 5 µm (**Marlene, 2007**).

B: Morphology of *Giardia lamblia* on the trophozoite stage (**Guillaume, 2007**).

2.3.3.3. *Organelles of Giardia*

They have four pairs of flagella and a concave ventral side that is surrounded by the lateral crest and a flange (**Fig 4**) that allows the organism to attach to the intestinal epithelium. The dorsal surface is convex, and two symmetrically placed nuclei are in the anterior half of the organism (**Fig 5**), (**Table 3**). The organism is remarkably adapted for survival within the small intestine, even in the absence of tissue invasion, and the cytoskeleton plays a central role in this adaptation (**Rodney, 2021**).

The cytoskeletal components include the ventral disk, the median body, and the eight flagella with their basal bodies. The core composition of the cytoskeleton consists of microtubules that are formed from a family of alpha- and beta-tubulins (**Elmendorf et al., 2003**); (**Gadelha et al., 2020**). The ventral disk and the median body are unique to *Giardia* species. The ventral disk is composed of a spiral of microtubules and associated sheets that are called microribbons (**Fig 4**). There are also hundreds of disk-associated proteins, including ankyrins (initially called alpha-giardins) (**Peattie et al., 1989**), all of which add rigid structure to the concavity of the disk (**Brown et al., 2016**). The disk itself has the ability to contract during attachment and utilizes the lateral crest (**Fig 4**) to help generate the initial attachment

(Feely *et al.*, 1982); (House *et al.*, 2011), as well as to remain attached when confronted with shear forces (Woessner and Dawson , 2012).

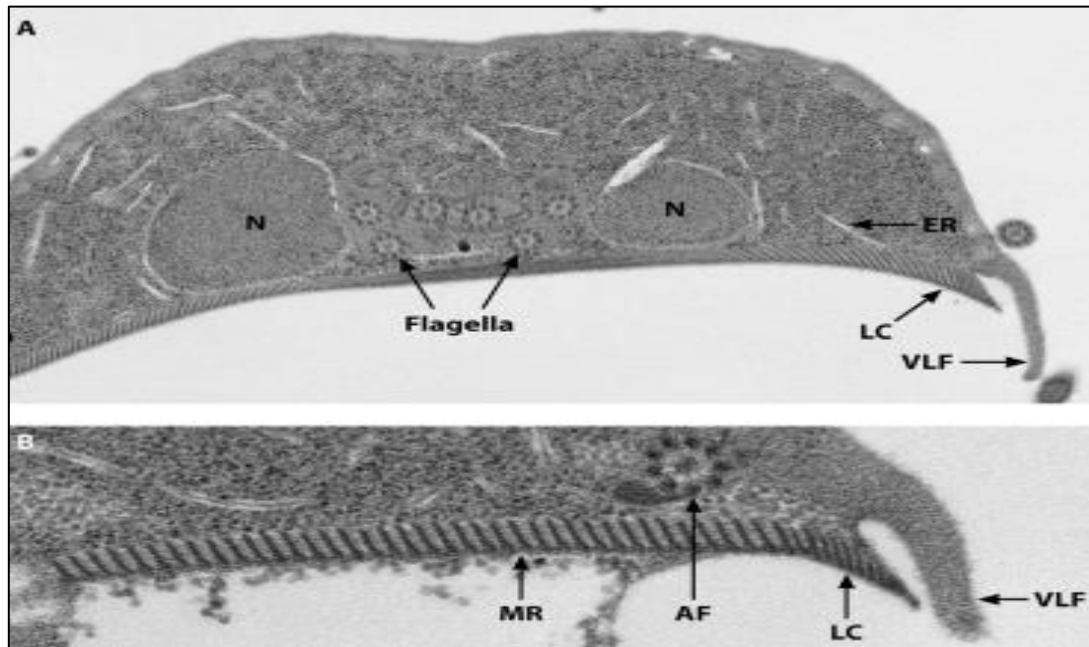


Figure 04: A transverse electron micrograph of a trophozoite through the two nuclei shows the nuclei (N), six flagellar axonemes between the nuclei with the canonical 912 arrangements, the ventral disk with the microribbons (MR), and the lateral crest (LC) and ventrolateral flange (VLF). (B) A higher magnification, including the anterior flagellum (AF) (Rodney, 2021).

The flagella have the conventional eukaryotic microtubule organization and are called the anterior, posterolateral, ventral, and caudal flagella according to the direction of their emergence from the basal bodies located between the nuclei (Brown *et al.*, 2016) (Fig 4 to 5). The flagella provide motility for the trophozoites, but their role in attachment continues to be debated. The beating of the ventral flagella occurs in conjunction with attachment to the intestinal epithelium and supports a model in which the ventral flagella would generate a hydrodynamic force resulting in suction by the ventral disk (Holberton, 1974). However, more recent data have suggested that the ventral flagella help generate suction through a force that pushes the ventral disk against the intestinal epithelium and helps remove fluid under the disk to allow the initial attachment to occur (Lenaghan *et al.*, 2011); Lenaghan *et al.*, 2013).



Figure 05: A coronal view through the nuclei (N) also demonstrate the multiple flagellar axonemes (F) traversing through posteriorly. The endoplasmic reticulum (ER) and peripheral vesicles (vacuoles) can also be seen (**Rodney, 2021**).

Table 04: Organelles of *Giardia lamblia* (Rodney, 2021)

conventional eukaryotic organelle	Equivalent Giardia organelle	Reference (s)
Nucleus / Nucleolus	Two nearly identical nuclei rDNA not organized in nucleolar pattern, but genes for nucleolus-localized proteins in genome and candidate nucleolus demonstrated by EM and confocal microscopy	(Tian <i>et al.</i> , 2010) (Gabaldon <i>et al.</i> , 2016) (Xin <i>et al.</i> , 2005)
Mitochondrion	Mitosome	(Tovar <i>et al.</i> , 2003)
Golgi	Numerous features of Golgi transport done by The endoplasmic reticulum	(Zamponi <i>et al.</i> , 2017)
Endosome/lysosome	Peripheral vacuoles or vesicles	(Rivero <i>et al.</i> , 2013) (Feely and Dyer, 1987) (Thirion <i>et al.</i> , 2003)
Peroxisome	Peroxisome-like proteins in cytoplasmic vesicles	(Acosta-Virgen <i>et al.</i> , 2018)
Exosome	Exosome-like vesicles with minimal ESCRT	(Moyano <i>et al.</i> , 2019)

The median body is not only unique to *Giardia* species but is one of the identifying features for the various species. The median body of *G. lamblia* has been called a “crooked smile” (Dawson, 2010); (Ankarklev *et al.*, 2010). Its function remains unknown, but hypotheses include a role as a reservoir of tubulin subunits during cytokinesis (Hardin *et al* 2017), or that it may play a role in detachment (Piva and Benchimol, 2004). A protein called the median body protein (MBP) was initially found in the median body, but a subsequent study found that it is also in the ventral disk and is required for a proper dome shape of the ventral disk and thus for attachment (Smith *et al.*, 1982). A single highly divergent actin has been identified in the *Giardia* genome (Morrison *et al.*, 2007), but canonical eukaryotic actin-binding proteins have not been identified.

However, recent studies have identified an actin cytoskeleton (Paredes *et al.*, 2011); (Paredes *et al.*, 2014). In addition, the eukaryotic actin associated protein phosphothreonine

has been shown to interact with actin in *G. duodenalis* (Krtkova *et al.*, 2017). However, the role of the actin cytoskeleton has not yet been determined.

2.3.4. Biology and metabolism of the parasite

2.3.4.1. The *Giardia* life cycle

The life cycle of *Giardia lamblia* (also known as *Giardia intestinalis*) is consisted of two stages: cyst and trophozoite. The cysts are about 7-10 µm in length and oval in shape. The mature cyst contains four nuclei. They are resistant through environmentally and responsible for transmission (Wolfe, 1992). Cysts may stay viable for several months in moist, cool conditions, and have been detected in natural surface waters (Farthing, 1996). They are also capable to survive standard concentrations of chlorine used in water purification systems (Jones, 1988).

Infection happens after cysts are ingested (Fig 6). After ingestion, mature cysts in the small intestine release trophozoites throughout a process called excystation (Jones, 1988). Cysts are able to endure exposure to gastric acid; gastric acid may actually trigger excystation (Farthing, 1996). The trophozoite stage is accountable for producing clinical disease in humans. Trophozoites have two different nuclei and four pairs of flagellae. They are 12-15 µm in length. Trophozoites colonize the small intestine, attaching to the mucosa of the bowel using ventral sucking disks. The trophozoites multiply through binary fission (Hill, 1993).

As the *Giardia* trophozoites travel toward the colon, they retreat into the cyst stage (known as encystation) and the fresh cysts are excreted in the feces. (Adrabbo, 2002).

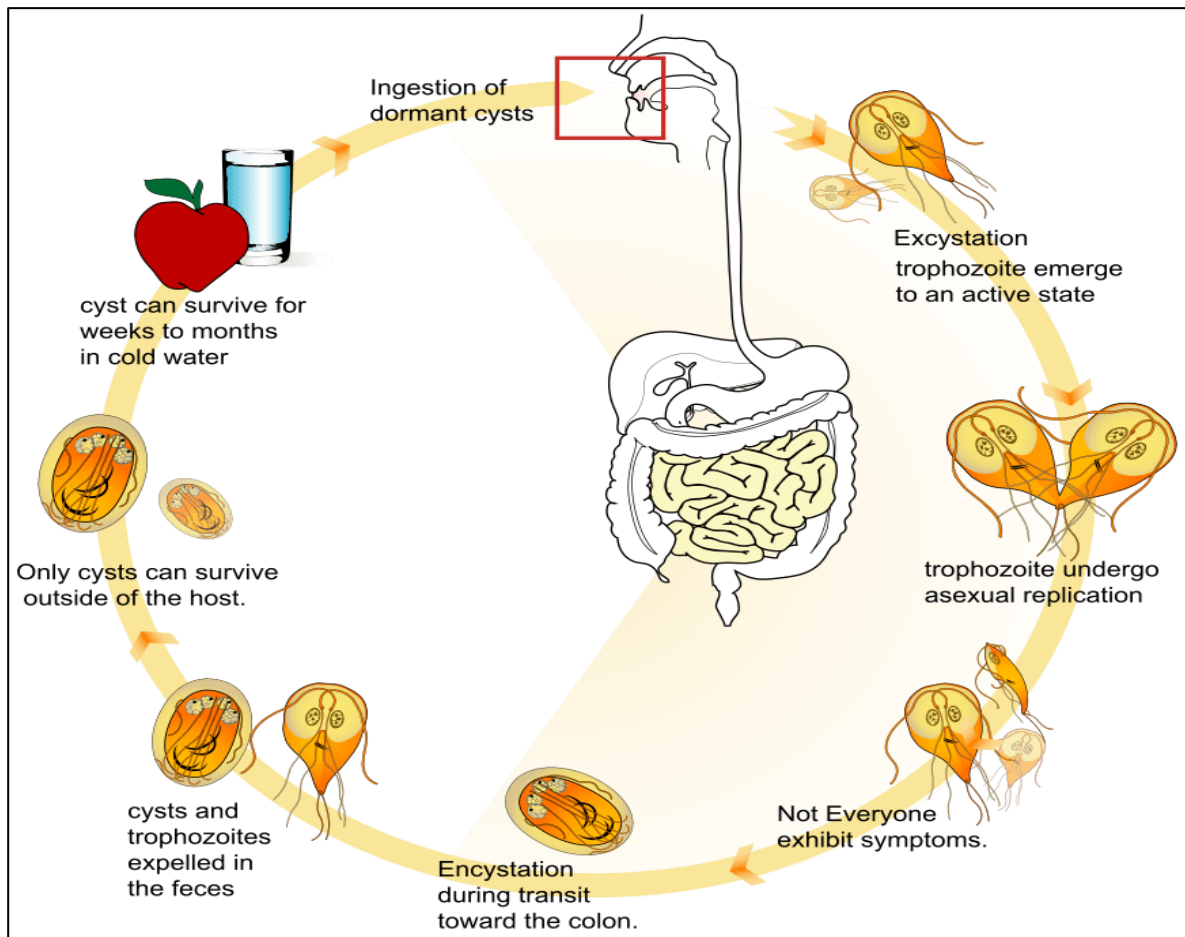


Figure 06: *Giardia* life cycle (ingestion- Excystation and Encystation) (Katelaris *et al.*, 1995).

A. The Cyst

Giardia cyst is the environmentally stable stage of the parasite life cycle that facilitates the transmission of cysts passed in the feces of one host into the environment to be ingested by the subsequent host. The cyst is 5 mm by 7 to 10mm in size with a two-layered cyst wall. The outer filamentous layer is covered by filaments 7 to 20 nm in length (Erlandsen *et al.*, 1989) and with N-acetylgalactosamine as the major sugar (Jarroll *et al.*, 1989). The cyst has four nuclei, rather than the two found in trophozoites. The metabolic rate is only 10% to 20% that of the trophozoite (Paget *et al.*, 1989), allowing prolonged survival in the environment, especially in cool, moist settings, perhaps explaining the higher frequency

of giardiasis in the northern part of the United States compared to that in the southern areas (**Coffey et al., 2021**).

B. Ingestion and Excystation

Infection of the host is initiated when cysts are ingested and pass through the acidic stomach into the duodenum where excystation occurs upon exposure to bile and a more alkaline pH. In vitro application of that sequence of pH change and exposure to bile results in a high percentage of successful excystation (**Bingham et al., 1979; Rice et al., 1981**). However, it is also possible to excyst *Giardia* at a neutral pH (**Feely et al., 1991**), and humans whose gastric pH is raised naturally or by medical intervention are susceptible to *Giardia* infection (**Cook et al., 1985**). In fact, they may be more susceptible to chronic giardiasis, as suggested in a report of two cases of gastric giardiasis in patients with hypochlorhydria as a result of treatment with a proton pump inhibitor, one of which resolved after discontinuing the proton pump inhibitor and without specific antimicrobial treatment (**Reynaert et al., 1995**).

C. Encystation

The trophozoites replicate in the small intestine, where some of the organisms will differentiate into cysts. Recent animal model data suggest that the trophozoites cluster into foci throughout the small intestine and even into the cecum and that encystation begins shortly after infection and peaks in a week. The encystation occurs in these clusters of increased organism density (**Barash et al., 2017**). The development of in vitro encystation was accomplished by three different laboratories (**Schupp et al., 1988; Gillin et al., 1987**). The initial report of in vitro encystation used primary bile salts to induce encystation (**Gillin et al., 1987**), an approach modified to a two-step process in which the second step utilized an alkaline pH of 7.8 and porcine bile (**Boucher and Gillin, 1990**). Alternative approaches have included high-bile medium (**Kane et al., 1991**) or cholesterol starvation (**Lujan et al., 1997**). One of early events in encystation is the development of encystation-specific vesicles (**Reiner et al., 1990**). These encystation-specific vesicles have a number of properties of the Golgi apparatus, including their involvement in protein trafficking that includes cell wall proteins 1 to 3, sensitivity to brefeldin A, and their emergence from active ER sites (**Faso et al., 2013**). During encystation, the trophozoites become rounded, and some of the key trophozoite cytoskeletal components are disassembled (**Palm et al., 2005**).

There are two cycles of chromosome replication and one cycle of nuclear division, resulting in a mature cyst that has four nuclei, each of which is 4n. Then, when viable cysts

are exposed to appropriate conditions, an opening at one pole of the cyst allows the emergence of the flagella and the cell body of the excyzoite (**Bernarder et al., 2001**), which undergoes two rounds of division, resulting in four trophozoites that are each 4n (two diploid nuclei).

The Giardia genome has 27 cysteine proteases that are part of the CA clan of cysteine proteases (**DuBois et al., 2008**). Twenty-five of those cysteine proteases are expressed, with GlCP2 (CP2) being the most abundantly expressed. Cysteine protease 2 is a cathepsin B-like protease that is involved in excystation (**Ward et al., 1997**) and encystation (**DuBois et al., 2008**); (**Touz et al., 2002**). It is found in the encystation specific vesicles, and purified cysteine protease is able to cleave recombinant cell wall protein 1 into a 26-kDa fragment, which is the size found in encysting organisms (**Dubois et al., 2008**). The roles of the other cysteine proteases remain to be confirmed. The transcriptomics of encystation using serial analysis of gene expression (**Birkeland et al., 2010**) and microarray (**Morf et al., 2010**) identified that cell wall protein 1 to cell wall protein 3, a high-cysteine nonvariant cyst protein, and the transcription factor Myb are highly upregulated in the first 3 h. high cysteine nonvariant cyst protein was the first characterized protein from a family of high-cysteine membrane proteins (**Davids et al., 2006**). And additional genes are upregulated at 7 h, including genes with Myb-binding sequences (**Morf et al., 2010**). A subsequent proteomic evaluation of encystation using tandem mass spectrometry found that the variety of variant-specific proteins had decreased 4 h after initiation of encystation in comparison to the baseline level (**Faso et al., 2013**).

In addition, there were multiple changes in metabolic and cytoskeletal proteins. The mechanisms of initiating encystation are not well understood, but there are data suggesting the inhibition of encystation by nitric oxide (**Eckmann et al., 2000**), histone deacetylase inhibitors (**Carranza et al., 2016**), or by the presence of lactoferrin (**Frontera et al., 2018**). In addition, a study of the one Rho family GTPase found in Giardia (Rac) showed its localization to the active sites and encystation specific vesicles and demonstrated that its expression increased cell wall protein 1 expression. Cell wall protein 1 then accumulated in the extracellular environment and was used by surrounding trophozoites to support encystation. The authors proposed that this mechanism may explain why encystation is frequently found in clusters of organisms (**Krtkova et al., 2016**).

2.3.4.2. *Giardia* cell cycle:

Giardia is unusual in that it contains two apparently identical, synchronously replicating nuclei in the vegetative trophozoite stage (**Wiesehahn et al., 1984**); (**Adam, 2000**).

Based on the pulse field analysis of the WB isolate it was showed that it contains five different chromosomes resulting in a haploid genome size of 12 Mb (**Adam, 2000**). Recently it determined the nuclear and cellular genome ploidy of *G. lamblia* cells during all stages of the life cycle (**Bernarder et al., 2001**). Results show that, during vegetative growth (**Fig 7**), each nucleus cycle between a diploid (2N) and tetraploid (4N) genome (**Bernarder et al., 2001**), giving a cellular ploidy of 4N and 8N (**Fig 7**). The fundamental task of the cell cycle is to make sure that the genome is replicated once in every cell cycle (phase 1) and that the chromosomes are distributed equally to the daughter cells (phase 2) (**Staffan et al., 2003**). The onset of differentiation in many cell types is associated with arrest of the cell cycle, suggesting that cell cycle proteins influence the transition into the differentiated state. The normal function of these proteins has to be impaired when the cell exits the cell cycle and differentiates (**Staffan et al., 2003**). A study of the *Giardia* ploidy during the complete life cycle (**Bernarder et al., 2001**) showed an importance of DNA replication in the encystation process and cytokinesis in the excystation process.

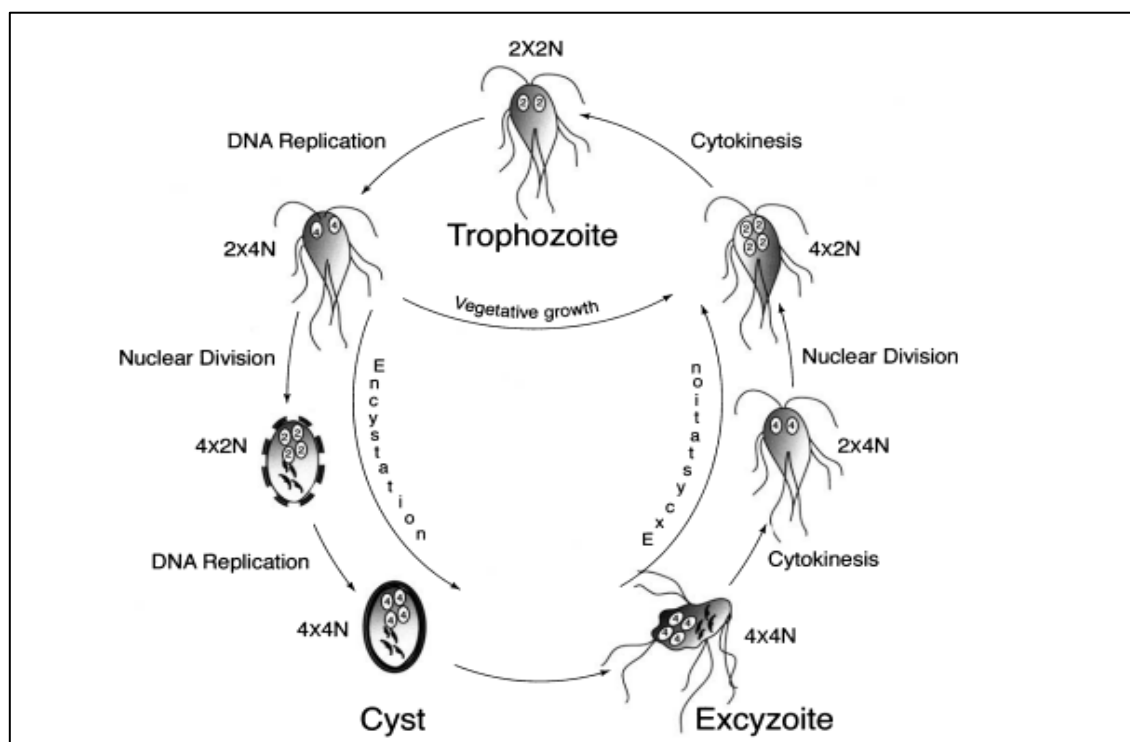


Figure 07: *Giardia lamblia* cell cycle (**Staffan et al., 2003**).

Giardia has a vegetative cycle where trophozoites cycle between cellular ploidies of 4N and 8N. Upon induction of encystation *Giardia* differentiates out from the phases. Late in encystation the two nuclei divide and the DNA is replicated generating cysts with a ploidy of 16N. Cysts excyst, releasing an excyzoite with four nuclei and a ploidy of 16N, the excyzoite divides twice without DNA replication and four trophozoites are formed from one excyzoite (Staffan *et al.*, 2003).

2.4. Study of disease (Giardiasis)

2.4.1. Epidemiology

Giardiasis occurs worldwide, with higher prevalence where sanitation is poor. Persons of all ages are affected, though in endemic areas infection is more frequent in infants. Specific areas of recognized increased risk for travellers include the Soviet Union, Southeast and South Asia, tropical Africa, Mexico, and western South America (Gradus, 1989). *Giardia* is mainly spread through contaminated drinking water, but other pathways of transmission are also recognised (Osterholm *et al.*, 1981). Giardiasis has also been linked to interactive water fountains, the first report came from Florida in 2006 (Eisenstein *et al.*, 2006). In 2003, 30 primary cases of giardiasis during a large outbreak in Boston, Massachusetts, were linked to exposure to a children's pool, but as many as 105 secondary cases probably resulted from person-to-person spread (Katz *et al.*, 2006). *Giardia* may also spread through sexual activity, and increased prevalence among homosexual men has been reported (Meyers *et al.*, 1977); (Hakansson *et al.*, 1984). It is also widespread throughout the world. It is endemic in tropical and subtropical areas where hygienic conditions are poor. The prevalence of infection or symptomatic disease is not well established. The World Health Organization (WHO) has given the highest estimate to date in a 1996 report, stating that 200 million people in Asia, Africa and Latin America had symptoms of giardiasis (WHO, 1996). Numbers frequently quoted in review articles are prevalence rates of 2-5% in the industrialised world and 20-30% in low-income countries (Farthing, 1996); (Marshall *et al.*, 1997).

Infection is spread directly from person to person by fecal-oral contamination with cysts or indirectly by transmission in water (Barbour *et al.*, 1976); (Craun, 1986) and occasionally food (Petersen *et al.*, 1988). Under favourable conditions of temperature and humidity, such as water at 4-10 C, the cysts may remain viable for several months (Wolfe, 1992). The cysts are relatively resistant to chlorination and to disinfection by ultraviolet light.

Boiling is very effective for inactivating *Giardia* cysts, but some cysts may survive after freezing for a few days. Most human infections result from ingestion of contaminated water or by direct fecal oral transmission such as the occurring in child care centres, food contamination, and male homosexual sexual contact (**Hill, 1993**).

2.4.2. Source de parasite

Infection is spread directly from person to person by fecal-oral contamination with cysts or indirectly by transmission in water (**Barbour et al., 1976**); (**Craun, 1986**) and occasionally food (**Petersen et al., 1988**). It can also be animal to animal, zoonotic (animal to human, human to animal), waterborne from humans or animals through drinking water or recreational contact such as in swimming and foodborne from contamination of water used in food preparation and manufacture or from food handlers (**Karanis et al., 2007**); (**Porter et al., 1990**); (**Shields et al., 2008**); (**Takizawa et al., 2009**). Travellers often become infected when they ingest contaminated water that usually originates from groundwater (wells) or surface water (lakes or streams). In the United States, most infections are sporadic, especially in campers and hikers who drink untreated stream water (**Barbour et al., 1979**). Numerous community-wide outbreaks have resulted from fecally contaminated central water supplies (**Craun, 1986**). *Giardia lamblia* is frequently identified as the etiologic agent in waterborne diarrheal outbreaks from contaminated surface water that has been ineffectively filtered or pretreated (**Gradus, 1989**).

Thus, contaminated water sources include unfiltered surface waters, shallow wells, and house hold water from either of these sources (**Chute et al., 1987**); (**Gradus, 1989**). Infections occur in outbreak and endemic forms within nursery schools and other institutional settings and among family members of infected children (**Pickering et al., 1984**). Transmission also occurs among male homosexuals engaging in sexual practices (**Schmerin, 1978**). Ingestion of 100 or more cysts is required to ensure infection in humans, but ingestion of as few as 10 cysts has resulted in infection in volunteers (**Rendtorff, 1954**). The cysts survive in the environment longer in cool, moist areas. Thus, in temperate climates, which tend to be low-prevalence areas, giardiasis is often seen in the form of symptomatic waterborne outbreaks (**Coffey et al., 2021**); (**Benedict et al., 2019**).

2.4.3. Reservoir of the parasite

Humans are the main reservoir of the parasite, but a variety of animals carry *Giardia*, similar to those infecting humans. At first, the genus was thought to contain numerous host

specific species. However, it is now believed that perhaps only two morphologically distinct species infect animals. One of these, *Giardia* (includes *G. lamblia*) naturally infects humans, beavers, coyotes, cattle, cats, and dogs and can experimentally infect certain other mammals. Whereas studies analyzing isolates of *Giardia* from different hosts suggest that intraspecific variation occurs within the group, host specificity is still considered unreliable as a means of classifying *Giardia*. The other morphologically distinct species, *Giardia muris*, infects primarily mice and rats **(Smith and Wolfe, 1980)**. Community water supplies can be contaminated by *Giardia* cysts from beavers, which have been infective for humans experimentally **(Davies and Hibler, 1979)**. Experimental infection of dogs has been induced by cysts from humans, but so far there is no documented evidence of transmission from dogs to humans **(Healy, 1990)**.

2.4.3.1. Contaminated water

Regions of the world where giardiasis thrives tend to be in those with poor living conditions, often the population lives within systems of malnourishment, squalor and unsanitary conditions which promote spread of the disease **(Sylvia and Ryan, 2017)**. Children are particularly at risk of acquiring the disease within these locations and are often impacted heavily due to commonly being already unhealthy and potentially infected with other pathogenic organisms such as bacteria or other protozoan species **(Daniels et al., 2018)**. A study of US outbreaks found that drinking water was the source in 75% of cases, with recreational and environmental waters, such as swimming baths and lakes, respectively; resulting in accidental ingestion of contaminated waters **(Porter et al., 1988); (Slifko et al., 2000); (Katz et al., 2006); (Adam et al., 2016)**. Detection methods for standard water monitoring typically utilize microscopy, though molecular-based studies would be needed, to identify the presence of different *Giardia lamblia* assemblages in water to determine public health risk posed **(Horton et al., 2018)**.

2.4.3.2. Contaminated food

It is well known that *Giardia lamblia* infection within humans can occur due to the consumption of contaminated food items, with historical data of outbreaks highlighting this **(Osterholm et al., 1981); (Petersen et al., 1988); (Porter et al., 1990); (Mintz et al., 1993); (Smith et al., 2007)**. Food-related outbreaks of giardiasis are however less commonly reported than those originating from water supplies, as shown in the USA between 1971 and 2011, whereby 74.8 and 15.7% of outbreaks were reported as waterborne and foodborne,

respectively (**Adam et al., 2016**). The difference however may also be due to the difficulty in the reporting/detection of foodborne outbreaks in the population, compared with water source outbreaks, which tend to have a more identifiable point of origin. Waterborne outbreaks tend to be confined regionally with increased cases detected within a single health authority, whilst foodborne outbreak cases tend to be far greater dispersed and thus increasingly difficult to notice (**Horton et al., 2018**). Commercially prepared contaminated food, such as vegetables or salad items, can often travel a great distance before being consumed by the individual who will potentially become infected, masking point of contamination of food. Salad items and vegetables have been often highlighted as a foodborne risk for giardiasis. Salad and vegetable items which are (a) improperly washed to remove cysts or (b) washed, but in contaminated water, and (c) poor food handler hygiene can lead to human giardiasis (**Horton et al., 2018**). Additional methods of foodborne giardiasis include infection via cold drinks (contaminated water frozen to make ice) (**de Lalla et al., 1992**), shellfish which are contaminated with cysts (filter feeding shellfish can accumulate cysts within them) (**Giangaspero et al., 2014**), mechanical transmission to food items via insects such as filth flies (**Graczyk et al., 2005**).

2.4.3.3. Infected persons

Due to the cysts being infectious when excreted in feces, humans have the potential to pass on the pathogen to other humans either from direct contact or via transmission from contaminated sources, e.g. foods, most likely due to poor hygiene. Cases may be mild, short-lived or even asymptomatic and therefore individuals may be highly infectious without knowing, and will therefore not seek medical attention. In addition, long time delays between infection and consultation of a general practitioner (GP) can lead to delayed diagnosis which increases the chances of spreading the infectious cysts (**Cacciò and Sprong, 2011**). To improve detection and obtain more accurate information regarding the extent of *Giardia* infection in humans, there are significant improvements required to laboratory testing algorithms and procedures to minimize delays and under-reporting of this pathogen (**Horton et al., 2018**). Diagnostic microbiology laboratories mostly use microscopy for the detection of cysts and trophozoites (**Alexander et al., 2017**).

2.4.3.4. Livestock

Farm animals and their environments are often suggested as potential reservoirs for human infections (**O’Handley et al., 1999**); (**Appelbee et al., 2003**); (**Ralston et al., 2003**); (**Trout et al., 2007**); (**Hoar et al., 2009**); (**Santin et al., 2009**); (**Sprong et al., 2009**);

(Miguella *et al.*, 2012); (Ryan and Cacciò, 2013). This is due to the large numbers of livestock animals present globally in combination with the potential of these animals to create widespread contamination due to infected waste (Ryan and Cacciò, 2013). Their potential to contaminate the environment is however thought to be very dependent on the practices of the farm and region of the world. It has been previously highlighted that livestock pastures are significant sources of water contamination worldwide (Plutzer *et al.*, 2010). Farm practices that allow cattle direct access to rivers or streams permit defecation directly into water sources, potentially spreading infective cysts downstream to other environments (Budu-Amoako *et al.*, 2011). A direction to reduce land contamination could be to improve the education of farmers on the subject, however is difficult to enforce in practice (Horton *et al.* 2018).

2.4.3.5. Companion animals

Companion animals have been thought to be potential sources of infection for human infective of *Giardia*, not ably cats and dogs in which there are 77 and 93 million in the USA alone (Esch and Petersen, 2013). However, studies into giardiasis in dogs and cats are far and few between and often differ in results, similar to other animals (Horton *et al.*, 2018). It was claimed the presence of *Giardia* in dogs will increase the levels of human infection in the population as a consequence (Ballweber *et al.*, 2010).

In addition to potential animal–human transmission, it was also noted that there is another infection cycle from dog to dog, passing assemblages C and D to each other, resulting in two potential separate cycles within the canine population (Feng and Xiao, 2011). It was also noted that there is another infection cycle from dog to dog, passing assemblages to each other, resulting in two potential separate cycles within the canine population (Feng and Xiao, 2011). Dogs and cats generally come into contact much more frequently with humans due to behavioural habits, such as licking humans after cleaning themselves, which could enable transmission of the parasite from dog to human to be much more frequent (however rare access for dogs to human feces will limit the infection being passed back to the dogs, especially in high-income countries) (Horton *et al.*, 2018).

2.4.4. Infection modes

Giardia cysts are increase by the fecal-oral route. Cysts may be ingested with contaminated water or food, or acquired from unwashed hands (Fig 8). *Giardia lamblia* has been to be spread either from person to person, animal to person or from the environment to person and via fecal-oral contamination with cysts or occasionally through food. (Craun,

1986); (Petersen, 1988). *Giardia lamblia* infect infants in both industrialized and developing countries, making it one of the most common causes of day care-center outbreaks of diarrhea. U.S. day care-centers have reported the prevalence of *Giardia* to range from 17% - 90%. Infection often extends among the family members of infected children (Wolfe, 1992). Male homosexual contact has also been associated as an additional transmission factor with oral-anal sexual practices serving as a route of transmission (Peters et al. 1986).

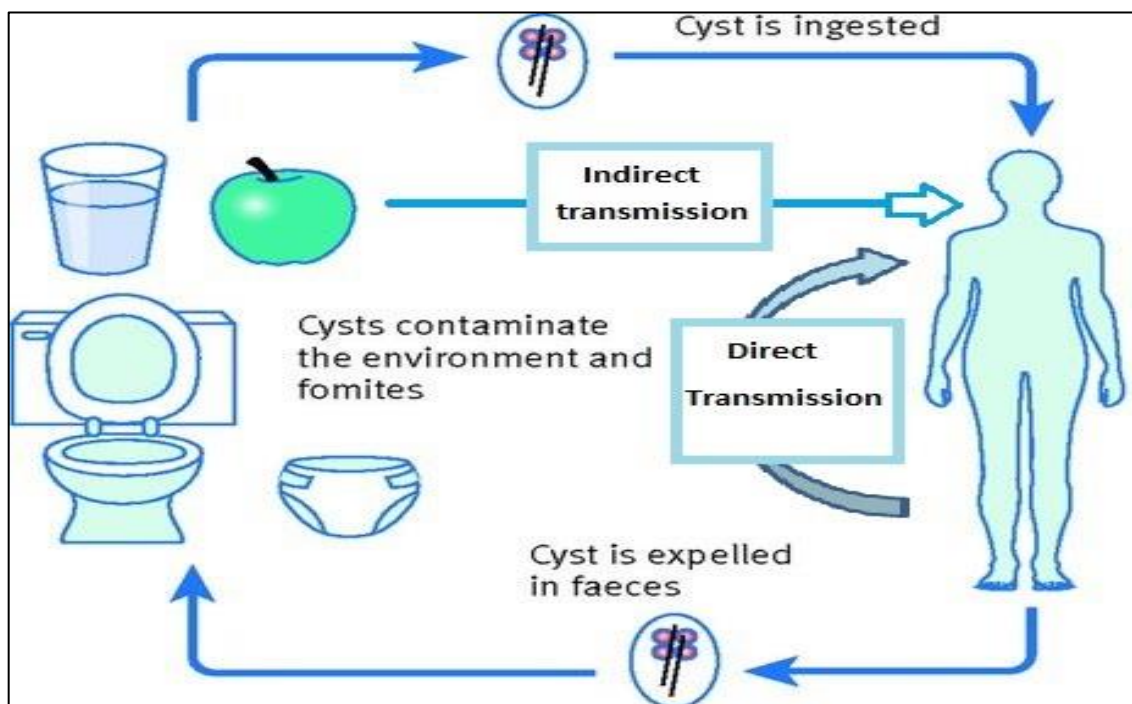


Figure 08: *Giardia* transmission modes (Direct transmission – indirect transmission) (Bridle, 2014).

2.4.5. Factors related to the parasite

The transmission modes are well favored by high temperatures and moist climatic conditions, poor personal hygiene and unsanitary habits of individuals (Noor et al., 2007); (Ayeh-Kumi et al., 2009); (Wongjindanon et al., 2005); (Kaur et al., 2002).

2.4.5.1. Climate related factors

Environmental factors (including climate, soil, and water) can influence the survival/infectiveness of *Giardia* cysts or the incidence of giardiasis. Various studies have come to different conclusions. Whether environmental factors become risk or protective factors depends on multiple factors and the mechanisms are complex. Although some

previous studies have investigated the influencing factors of cryptosporidiosis/giardiasis, the attention paid to the environmental perspective remains insufficient. Climatic characteristics: Factors such as temperature, rainfall, humidity, wind speed, and solar radiation play important roles, and they can be considered as risk or protective factors for *Giardia* (**Xihan Wang et al., 2023**).

1) Temperature

High temperatures can improve the survival of *Giardia* cysts and promote disease progression to some extent, although conversely, high temperatures increase the inactivation of cysts (**Wang et al., 2020**). While temperature inactivation is a key abiotic factor affecting both: cyst survival and infectivity in the environment (**King et al., 2005**). Temperature might increase the risk of infection by promoting pathogen infectivity, shedding in animal hosts, and increasing the interactions between pathogen sand hosts (**Semenza et al., 2012**).

2) Rain fall

Rainfall and extreme weather events are associated with the cyst concentration, which depends on the situation. The correlation is positive in some cases because excessive rainfall and runoff might mobilize cysts (**Chhetri et al., 2017**); (**Cizek et al., 2008**); (**Lal et al., 2012**), which then leads to an increase in microbial infections (**Rechenburg et al., 2006**). More intense precipitation events cause more sediment disturbance and resuspension of infectious cysts. Meanwhile, this might result in cyst sedimentation due to increased turbulence and binding of organic matter in water (**King and Monis, 2007**). Drought caused an increase in concentration of pathogens in water sources, which could be washed out by subsequent rainfall (**Lal et al., 2013**).

3) Humidity

Studies have shown positive and negative associations between relative humidity and *Giardia*, while wind speed and solar radiation were mainly negatively associated with both (**Xihan Wang et al., 2023**).

4) Soil characteristics

Relevant factors include the slope, vegetation cover, soil moisture, porosity, pH value, soil type, and texture. Soil moisture can increase the survival of cysts and affect disease transmission, while dry soil partially inhibits the survival of cysts (**Barwick et al., 2003**). Inactivation of cysts in the soil depends to a large extent on soil temperature, but to a lesser

extent on soil texture, and precipitation can mobilize cysts in the soil. Studies of other land features have not provided definitive conclusions, which also vary among studies (**Xihan Wang et al., 2023**).

5) Water characteristics

The main factors involved are runoff, dissolved oxygen, water pH, the water level, and turbidity. Among them, runoff has been studied more extensively. When water flow conditions are insufficient to dilute or flush pathogens from waterways, it can be assumed that pathogens will concentrate. Conversely, high flow rates might dilute or flush pathogens from waterways. When runoff was extremely low and cyst concentrations could not be diluted, the risk of disease increased (**Wilkes et al., 2011**). Other water features are less studied and further investigation of their mechanisms of action is required (**Xihan Wang et al., 2023**).

2.4.5.2. Socio-environmental factors

The social and environmental factors underlying the transmission of intestinal pathogens are varied and different in nature. Enteroparasites infections are commonly associated with the age (**Freeman et al., 2015**); (**Njenga et al., 2011**), hygiene habits and nutritional or immunological conditions (**Humphrey, 2009**); (**Zonta et al., 2014**) of the affected children, indicating the importance of the characteristics at the individual level. Socio-environmental factors at the family or community levels also have been demonstrated as critical because of the relationship between intestinal parasites and WASH inequalities (water, sanitation & hygiene) (**Benjamin et al., 2015**); (**Prüss-Ustün et al., 2014**), parents' education level (**Buor, 2003**); (**Chen and Li, 2009**), and sanitization and health of pets (**Dantas, 2014**); (**Zanzani et al., 2014**) among others. Given the characteristics of the life cycle of intestinal parasites, the environment is also a key player in the maintenance of these infections either as sites of maturation to their infective forms as well as dispersion vehicle (**Brooker et al., 2006**); (**Eisenberg et al., 2007**).

2.4.6. Pathogenesis

Giardia lamblia is a non-invasive pathogen of the small intestine and produces a wide range of clinical presentations, including chronic diarrhea with weight loss, post infectious complications of irritable bowel and chronic fatigue, growth stunting, and asymptomatic infections. These varied manifestations may result from host, parasite, or microbiota differences and make it particularly difficult to elucidate the mechanisms resulting

in this range of illness. Nevertheless, there has been substantial progress over the past 2 decades in elucidating some of the mechanisms. Recent in-depth reviews of these advances are also available (**Certad *et al.*, 2017**); (**Fink and Singer, 2017**). The trophozoites adhere tightly to the small intestinal mucosa, leaving a detectable imprint when they detach from the intestinal epithelium (**Erlandsen and Chase, 1974**), so the possibility of direct pathogenesis from the mechanical attachment has been raised. However, there is currently no evidence to support this possibility. Rather, current data suggest that a combination of secreted proteases and other *Giardia* factors, the host immune response, and the interaction of these factors with the intestinal microbiota contribute to the various manifestations (**Fink and Singer, 2017**). Through the entire immune response, it is actually remarkable that inpatients who have biopsies for symptomatic giardiasis, the findings consist of flattening of the villi but no obvious inflammatory changes. However, an inflammatory picture can be seen and may actually be separate from the location of the trophozoites. A series of cases was reported in which trophozoites were seen in ileal biopsy specimens from ileocolonoscopy of symptomatic patients but inflammatory changes were found in the duodenal biopsy specimens of these patients (**Oberhuber, 2016**).

All patients had ileal blunting or atrophy, 6 of 11 had neutrophilic infiltration, and one had findings consistent with celiac disease. The finding of trophozoites in the ileum is consistent with the animal model in which trophozoites were concentrated in the proximal small intestine but were sometimes found in the ileum or even the cecum (**Barash *et al.*, 2017**).

2.4.7. Immunity response

It is well accepted that both humoral and cellular immune responses to giardial infection are generated by the host. Secreted immunoglobulin A and immunoglobulin M antibodies seem to play a role in eradicating parasites. Immunoglobulin A and G antibodies coat *Giardia muris* trophozoites in the bowel lumen of rats and mice and may reduce parasite motility and prevent adhesion to the epithelium (**Farthing and Goka, 1987**). *Giardia* specific immunoglobulin A and G antibodies have also been demonstrated in serum and may be helpful in differentiating patients with acute or recent infection from those with past or previously treated infections (**Jones, 1988**). Studies also indicate that, during waterborne outbreaks of diarrheal illness, the presence of serum antibody of *G. lamblia*, particularly immunoglobulin A, may be helpful in detecting exposure to *G. lamblia* contaminated water and in diagnosing giardial disease (**Birkhead *et al.*, 1989**). Experiments with a mouse model

have demonstrated that cell function is necessary for development of resistance to infection **(Roberts et al., 1976); (Stevens et al., 1978)**. It has also been shown that, after epidemic outbreaks, patients develop some resistance to subsequent infection **(Istre et al., 1984); (Wright and Vernon, 1976)**. However, even after repeated exposure to the organism, symptomatic infections do recur in the same individuals. Current evidence suggests that, by adulthood, a degree of resistance to this organism is present. One study found that 14% of a group of asymptomatic adults had serum antibodies to *G. lamblia* **(Smith et al., 1981)**.

2.4.8. Symptomatology clinic

Giardiasis was at the start observed as non-pathogenic and frequently found in asymptomatic patients. Though, there is now abundant proof for the pathogenic nature of *Giardia lamblia*. The main symptom of acute giardiasis as indicated in **(Fig 9)** and mostly seen in travelers, is prolonged diarrhea. The incubation period for infection is usually 9-15 days. The acute stage frequently begins with a feeling of intestinal uneasiness followed by anorexia and nausea **(Wolfe, 1992)**. Low grade fever and chills may found followed by watery, foul-smelling, explosive diarrhea, abdominal pain, passage of foul gas and belching. This stage lasts for 3- 4 days, frequently resembling other causes of traveler's diarrhea. If missing untreated, symptoms may persevere for months. Malabsorption due to chronic *Giardia* infection has also been reported. Other frequent symptoms of giardiasis consist of abdominal pain, flatulence, vomiting, bloating and weight loss **(Flanagan, 1992)**.

Symptoms differ from person to person, frequently depending on the inoculum size, period of infection, and individual host and parasite factors. The diarrhea can be gentle and create semi- solid stools, or it can be intense and debilitating. Keeping in mind, numerous individuals infected with giardiasis exhibit no apparent signs or symptoms of illness. **(Mank, 2001)**.

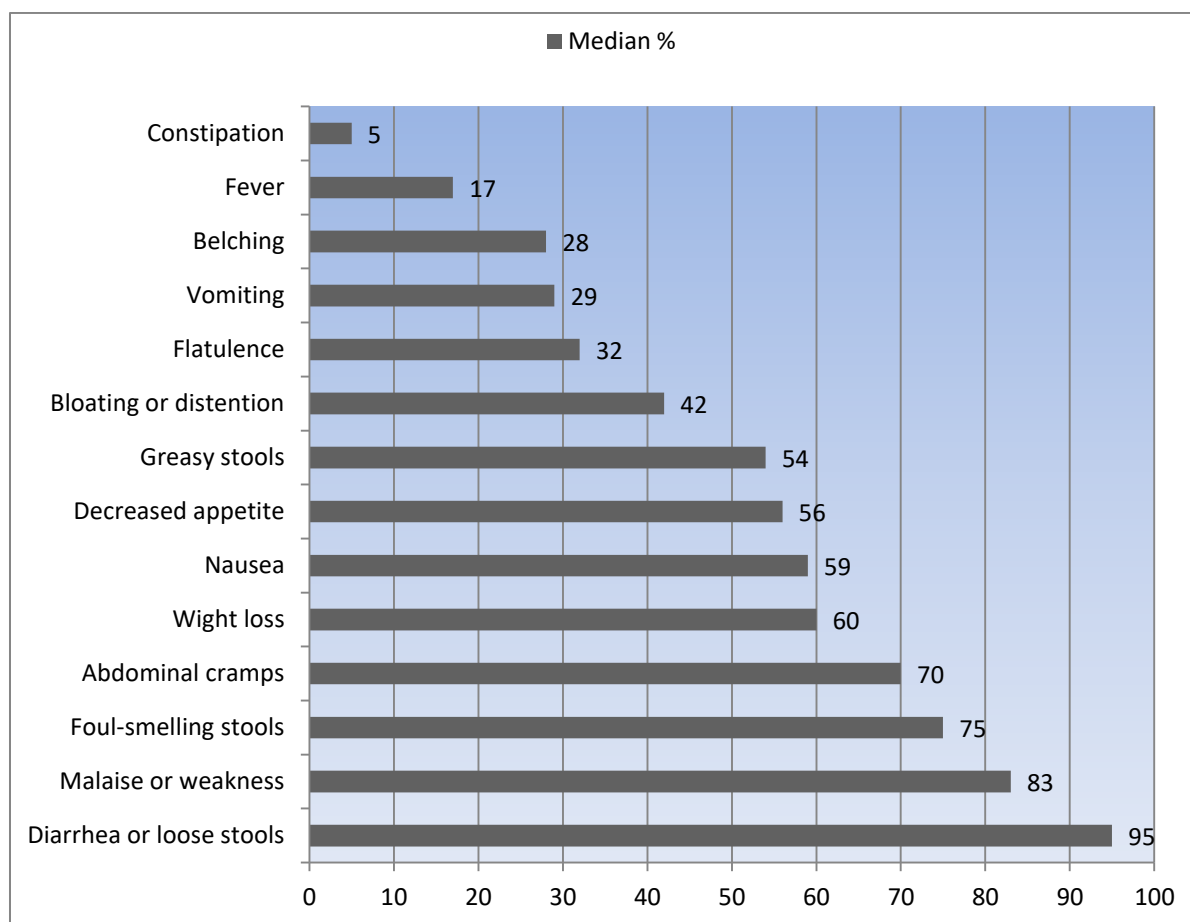


Figure 09: Median Percentage of Signs and Symptoms of Giardiasis that been noticed on patients (Rodney, 2021).

2.4.9. Diagnostic of the illness

The diagnosis of giardiasis is most commonly established by identification of cysts or, less frequently, trophozoites in fecal specimens that are stained with trichrome, or iron hematoxylin. Stool samples can be concentrated by formalin-ethyl acetate or zinc sulfate concentration methods. The passage of cysts is somewhat sporadic, and if the first specimen from a patient with suspected giardiasis is negative, the sensitivity can be improved by repeating the examination once or twice (Adam, 1991). Direct examination of wet mounts of fresh stool specimens (Fig 10) may also be useful because the detection of the motile trophozoites correlates with symptomatic giardiasis.

Giardia antigens in feces can be detected by EIAs, indirect and direct immunofluorescent assays using monoclonal antibodies (Meridian Diagnostics, Cincinnati), and direct fluorescent assays. All of these procedures are highly sensitive and specific for assaying environmental and stool samples (Marshall *et al.*, 1997). For some patients with

chronic diarrhea and malabsorption, the results of stool examinations are repeatedly negative despite on-going suspicion of giardiasis. For such patients, direct examination of small-intestinal contents may be useful in establishing the diagnosis. This can be done with the string test (**Beal et al., 1970**), for which a patient swallows a capsule on the end of a string. The string moves to the jejunum, where the trophozoites attach. After 4 hours to overnight, the string is withdrawn and inspected microscopically for trophozoites. Esophagogastroduodenoscopy, with duodenal aspiration or biopsy can also be performed. In addition, biopsy material can be obtained without endoscopy by using Rubin's tube, a Crosby capsule, or Carey's capsule (**Adam and Ortega, 1997**). Although biopsy is more invasive, use of this procedure allows detection of other diseases, including Whipple's disease, other protozoan forms of diarrhea (e.g., cryptosporidiosis, isosporiasis, or cyclosporiasis), Crohn's disease, or lymphoma that may also present as diarrhea and malabsorption. Abnormalities will also be seen in patients with tropical or nontropical sprue, but such abnormalities are not diagnostic of these entities (**Adam and Ortega, 1997**). Conversely, completely normal biopsy results for a patient with diarrhea and weight loss would suggest diagnoses other than giardiasis. Despite the value of duodenal biopsy or aspiration for the diagnosis of giardiasis, it should be emphasized that biopsy supplements stool examination: biopsy is less sensitive than stool examination but will identify patients for whom the diagnosis cannot be ascertained by stool examination alone (**Kamath and Murugasu, 1974**); (**Rosenthal and Liebman, 1980**).

Sero-diagnosis cannot be used to differentiate between present and prior infection and is therefore not useful for the diagnosis of giardiasis. The sensitivity of PCR for diagnosing giardiasis is relatively low because inhibitors of PCR are present in fecal specimens (**Adam and Ortega, 1997**).

2.4.9.1. Examine diagnostic

It is well known that no traditional or new methods can detect all cases of *Giardia* infection. Several immunodiagnostic tests of rapid diagnosis of giardiasis have been developed particularly in the last three decades, mainly based on the detection of *Giardia* antigens in faecal specimens. While to the high sensitivity of these methods (**Table 04**), microscopy stool examination especially using concentration methods, most frequently has performed laboratory procedure worldwide as a good performance diagnostic strategy and should still be held as the golden standard (**Hossein Hooshyar et al., 2019**). A non-morphological diagnostic method particularly immunoassay is recommended to detect

coproantigen is recommended as a complementary test to the traditional technique and has been applied in larger laboratories that process a large number of stool samples daily. The stool concentration techniques such formalin-ether method can be used as a routine and economical method in medical diagnostic laboratories in developing countries (**Hossein Hooshyar et al., 2019**).

1) Fecal microscopy examination

The microscopic identification of *Giardia lamblia* in fecal samples is considered as the gold standard method for the diagnosis of giardiasis. This method is performed to detecting cysts and trophozoites. The sensitivity of microscopy techniques depends on using direct or concentration methods, the number of examined fecal samples and employment of professionally trained persons (**Gutiérrez et al., 2011**); (**Soares and Tasca, 2016**).

- *Direct examination methods*

The diagnosis of giardiasis in most cases is mainly confirmed by stool examination. Fecal suspension in physiological salt solution (0.85 NaCl) or fixation in sodium acetate–acetic acid–formalin is used to prepare wet mounts in order to the observation of *Giardia* trophozoite in diarrhea or loose samples. Wet mounts smear (**Fig 9**) can be examined either unstained or iodine stained (2-5% lugol's solution) (**Hossein Hooshyar et al., 2019**).

Examination of direct wet saline preparation of a fresh stool specimen allows motile trophozoites to be seen, but in stained preparation smears the trophozoites will be non-motile. If diarrhea stool sample containing trophozoite left too long without fixations or preservatives solution, the organisms tend to degeneration, thus preventing has been recommended for sample transfer and protection of the typical trophozoite morphology. A number of commercial kits with preservative solutions are available or can be made manually. The most commonly used preservation kit contains of 10% buffered formalin, polyvinyl alcohol, merthiolate-iodine-formalin, and acetic acid-formalin solution (**Wolfe, 1992**); (**John et al., 2006**). Polyvinyl alcohol is suitable for preparation of smear in order to permanent staining in the asymptomatic individuals and healthy carrier who do not have diarrhea, the cyst stage is more likely to be seen in a fecal sample examination. Fecal suspension in saline or lugol's solution or in a fixative solution may be used for cyst identification The number of cysts may be low in the fecal specimens, thus the wet mount examination of stool samples may not detect the parasite. It has been recommended for preparation and examination of two or more, even to six wet mounts smear for increasing the chance of finding parasite agents. Also in

some cases, the examination of more than three stool samples is necessary due to intermittent or low levels of cyst shedding (15), Direct microscopy method has been considered economical and rapid for the diagnosis in the medical diagnostic laboratory (**Hossein Hooshyar et al., 2019**).

- *Concentration methods*

Fecal concentration is a recommended and routine procedure that allows the detection of a small number of Giardia cysts may be missed by using wet mounts direct smear. Concentration methods have been designed to separate protozoan cysts and helminthes eggs from excess fecal debris. Flotation and sedimentation are two types of concentration procedure that have been used in the parasitological laboratory. Flotation methods permit the separation of some protozoan cysts and helminthes eggs through the use of a liquid with high specific gravity such as NaCl, NaNO₃, ZnSO₄ (final specific gravity of about 1.20). Zinc sulfate has been recommended as the best saturated solution to detection of Giardia cyst (**Smith and Mank, 2011**). Giardia cysts and other parasitic elements are floated and visible on the surface and the debris aggregate at the bottom of the tube. A modified technique has been made by adding a centrifugation step after the samples emulsified in flotation methods for increasing the efficiency of cyst recovery. Yields of this technique are cleaner than sedimentation methods, but in flotation techniques the walls of cysts will often be collapsing. The sedimentation procedures are the recommended methods as being the easiest to perform and less prone to technical errors (**Smith and Mank, 2011**). In this method, using centrifugation has been led to the recovery of Giardia cyst and other intestinal parasite in fecal sediment. These methods are the easiest but the preparation contains more debris. Many sedimentation methods have been employed for detection of *Giardia lamblia* and another intestinal protozoan cyst. Among them, the formalin-ether/ formalin-ethyl acetate, sedimentation technique is best to employ and generally applicable.

In these methods, 10% formalin has been fixed and preserved cyst stage and also provides user protection due to microbicidal activity of formalin. The ether or ethyl acetate has been used to remove the fat drop and oils. In this method, less distortion of Giardia cysts occurs in comparison with zinc sulfate flotation. Comparison of wet mounts smear and formalin-ether concentration techniques in the diagnosis of intestinal parasite has showed that formalin-ether concentration technique detected 65.26% of positive specimens for one or more intestinal parasites while the direct wet mount smear was only 34.74% sensitivity

(Oguoma, 2007). The formalin-ether concentration technique can be adopted and used as a routine method in medical diagnostic laboratories (Hossein Hooshyar *et al.*, 2019).

- *Staining techniques*

Identification of *Giardia* trophozoite and cyst are dependent on morphological criteria. Sometimes the correct identification of this morphological character may need to the examination of the permanent stained smear for revealing some details of the organism that cannot be seen in unstained or temporary stained smears. Several temporary and permanent stainings have been existed for identification and diagnosis of *Giardia* trophozoite and cyst. Although an experienced microscopic can identify this organism on a slide of concentrated and temporary stained sample, the permanent stain is not recommended for all stool samples that submitted for *Giardia* examination (Hossein Hooshyar *et al.*, 2019). A number of staining technique is available for the staining of *Giardia* trophozoite or cyst. Temporary stain such as methylene blue and iodine or lugol solution are primarily stains that have been used after preparation of a wet mount (Fig 10) or concentration smears for better detection (Hossein Hooshyar *et al.*, 2019). Some permanent stains have been used for *Giardia lamblia* diagnosis. Giemsa stain is an easy to use permanent stain for routine clinical laboratory use. In this staining, flagella and nuclei are reddish pink stain, and cytoplasm stains grey-blue. Iron hematoxylin is a useful staining procedure for demonstrating trophozoite and cyst of *Giardia*, additionally; automated staining machines can be used for this method (Mank *et al.*, 1995); (Palmer, 1991). Although Chlorazol Black is not widely used, it is another stain that has been used for permanent staining of trophozoite and cyst of *Giardia lamblia* and other intestinal protozoa. In this staining, the background of smear is light blue/grey, the cytoplasm of organism stains blur/grey and nuclei tend to dark (blue/black) (Garcia, 2007); (Bullock, 1980). Trichrome is a shorter permanent stain (Fig 11) that is simple and well- stained smears in about 45 min to 1 hour. This procedure is of value for staining fresh faecal specimens as well as stool fixed. In this staining, the background materials stain green or blue-green (Hossein Hooshyar *et al.*, 2019). So the cytoplasm of trophozoites and cyst stain green or greenish-blue, nuclei and nuclear chromatin stain red or red-purple (Garcia, 2007).



Figure 10: *Giardia lamblia* Cysts in bright-field microscopy using the wet mount staining technique (Smith *et al.*, 2024).

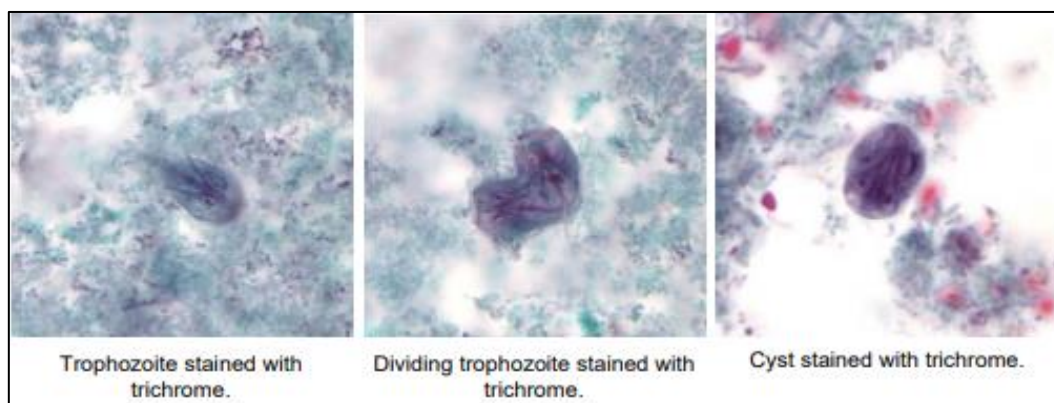


Figure 11: *Giardia lamblia* cyst and trophozoite under microscope using the trichrome stain technique (Smith *et al.*, 2024).

2) Culture methods

Although cultivation of human intestinal protozoa is a useful method for detection and diagnostic purpose, routine culture techniques were not established for *Giardia lamblia* in the clinical diagnostic laboratory. Cultivation of *Giardia spp* is applied in the research laboratory for many types of studies that require a large number of trophozoite (Hossein Hooshyar *et al.*, 2019). The *Giardia lamblia* is grown in the monoxenic and xenic type of culture system. In monoxenic system, the parasite has been grown in the presence of a single additional flora organism species and in axenic, parasite has been grown in the absence of any other accompanied alive cell (Graham and Diamond 2002). Monoxenic cultivation is an introduction to xenic growth. However, *Giardia lamblia* can be established directly into axenic media (Hossein Hooshyar *et al.*, 2019). The most common and suitable used medium for *Giardia* axenic culture is Diamond's medium which modified by Keister (Graham and Diamond 2002); (Keister, 1983).

- *String test (Entero-Test)*

In some cases of giardiasis that routine laboratory methods are unable to confirm infection, examination of fluids obtained from duodeno-jejunal by endoscopy or using string test (entro-test) may be useful for revealing the *Giardia* trophozoites **(Wolfe, 1992); (Beal et al., 1970)**. The Entero-Test consists of a lead-weighted gelatin capsule containing a length of nylon string 90 or 140 cm. After ingestion, the capsule dissolves and the nylon releases down into the duodenal area by peristaltic action. The string was left in this area for a recommended of 4 hours, the nylon string was withdrawn, the fluid from the bile-stained portion of the string was extracted and examined by direct microscopy or inoculated to the culture medium **(Beal et al., 1970); (Korman et al., 1990)**. Some studies have demonstrated that application of the string test resulted in an increase of the successful axenic cultivation of *Giardia lamblia* than other detection methods **(Korman et al., 1990); (Gordts et al., 1984)**. Also, a drop of mucus can be fixed directly on the slide and used for permanent staining **(Wolfe, 1992)**. The value of Entero-Test to fecal examination for *Giardia lamblia* diagnosis is little known and reported inconsistent. Some researchers have reported that Entero-Test is reliable and superior to stool examination for identification of *Giardia lamblia* in human and dog **(Rosenthal and Liebman, 1980); (Hall et al., 1988)** while others do not support it. Goka et al. showed that giardiasis was diagnosed in 73% of 229 patients with the first fecal specimen while it was found in only 44% of the patients via duodenal aspirates examination **(Wolfe, 1992); (Goka et al., 1990)** However, further studies need to investigate this difference.

- *Immunodiagnostic tests*

A variety of antibody and antigen detection methods have been developed and used for immunodiagnostic of giardiasis during the last three decades. Nevertheless, immunodiagnostic of giardiasis is still has a complementary role for microscopy stool test in the diagnosis of giardiasis. Immunodiagnostic test for *Giardia lamblia* diagnostic includes immunoassay techniques such as ELISA for antibody detection and methods dependent on detection of *Giardia intestinalis* antigens in human fecal specimens **(Heyworth, 2014)**.

- *Antibody detection*

Both cell-mediated and humeral immunorespons stimulated in human giardiasis **(Faubert, 2000); (Fink and Singer, 2017)**. The presence of immunoglobulin A and G and secretary humeral response to acute giardiasis has been noted previously **(Faubert, 2000); (Adam, 2001); (Heyworth, 1986)**.

In persons with acute giardiasis level of immunoglobulin M antibody falls to levels of healthy persons between two or three weeks after drug treatment. This indicates that detection of immunoglobulin M antibody may be a useful indicator for diagnosis of current infection. IgG antibody response may remain for up to 18 months after infection, so it has been applied in epidemiological studies **(Smith and Mank, 2011)**. Specific immunoglobulin G antibody response to trophozoite is detectable in 81% of infected asymptomatic *Giardia* and only in 12% of healthy control individuals **(Smith and Mank, 1984)**.

It is well known that *Giardia* spp. induces a strong production of immunoglobulin A antibody in human and animal infections. Secretory Immunoglobulin G as the predominant antibody has been detected in duodenal fluid and saliva samples of infected people. The production of secretory immunoglobulin A has been developed during active giardiasis, so detection and monitoring this antibody may be a useful tool for serodiagnosis **(Rodríguez et al., 2004)**; **(El-Gebaly et al., 2014)**. A study on *Giardia*-infected children in Egypt has showed that salivary and serum for A and G responses against *G. duodenalis* infection were significantly higher than non-*Giardia* infected children ($p < 0.001$) **(El-Gebaly et al., 2014)**.

A variety of assays such as Enzyme immunoassay, Immunofluorescent **(Fig 12)**, Western blot have been used for the serodiagnosis of giardiasis, but these methods may be problematic as the antibody may be detectable as long times after treatment of acute diseases. Commercially produced kits were not developed for detection of serum antibodies to *Giardia* infection **(Hossein Hooshyar et al., 2019)**.

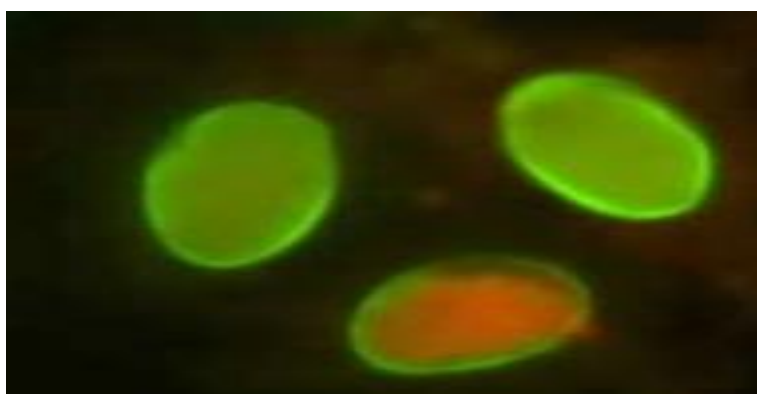


Figure 12: *Giardia intestinalis* labeled with fluorescent antibodies **(Smith et al., 2024)**.

- *Antigen detection*

Some methods include immunoassay techniques (including enzyme-linked immunosorbent assays [ELISAs], and rapid antigen detection tests [RDTs] such as non-

enzymatic immunochromatographic assays) have been used to detect fecal antigens in both preserved formalin- and fresh stool specimens **(Heyworth, 2014)**. Several commercially kits are available and ELISAs are the basis for detection of faecal *G. intestinalis* antigens. Using immunoassay kits have been described for simultaneous detection of Giardia and Cryptosporidium or Giardia spp., Cryptosporidium and Entamoeba species antigens in faecal specimens **(Heyworth, 2014); (Den Hartog et al., 2013); (Soares and Tasca., 2016)**.

There are many published articles about comparing the sensitivity of immunoassays methods and the faecal microscopy in diagnosing Giardia infection. The overall conclusion of them with some exceptions is that immunoassay is more sensitive than or as sensitive as, microscopy fecal examination **(Heyworth, 2014); (Soares and Tasca, 2016)**.

One of the best antigens that have ever been used is Giardia stool antigen with a relative molecular mass of 65 Kda (GSA65) which present in both trophozoites and cysts **(Smith and Mank, 2011)**. The reported sensitivity of Elisa-GSA65 for a single specimen varies between 95 and 100% with 100% specificity. Elisa-GSA65 can detect giardiasis in at least 30% more cases than microscopy examination **(Pestechian et al., 2014)**. There are some non-enzymatic immunochromatographic techniques for identification of *G. intestinalis* antigen in faecal specimens. In these methods the captured antigen is detectable with an antibody conjugated to a visible marker. The presence of *G. intestinalis* antigen indicated by a dark band and it is visible to the naked eye **(Smith and Mank, 2011); (Pestechian et al., 2014); (Gutiérrez et al., 2011); (Soares and Tasca, 2016); (Chan et al., 2000); (Garcia et al., 2003)**.

Results of immunochromatographic techniques are visible in 10–15 min, in contrast to the longer time that required enzyme-linked immunosorbent assays. Considering the cost of antigen detection tests, faecal microscopic which has been used in medical laboratories examination is cheaper and easier **(Hossein Hooshyar et al., 2019)**.

3) Molecular methods

Molecular diagnosis of giardiasis is not used in routine medical laboratories. PCR-based methods are often restricted to research laboratories and mostly used for sub-typing propose such as determination of assemblages or sub-assemblages of *Giardia duodenalis* **(Smith and Mank, 2011); (Hooshyar et al., 2017)**. The major target gene sequence which has been used in different molecular studies of Giardia species are genes encoding small subunit (SSU) ribosomal RNA, glutamate dehydrogenase (gdh), triosephosphate isomerase

(tpi) and β -giardin genes (a protein in the adhesive disk of *Giardia*) (**Hosseini Hooshyar et al., 2019**). Comparison and polymorphisms of glutamate dehydrogenase (gdh), the small-subunit of ribosomal RNA (SSU), and triosephosphate isomerase (tpi) genes, showed that *G. duodenalis* is classified to at least eight distinct genetic groups (A to H) or assemblages (**Feng and Xiao, 2011**); (**Hooshyar et al., 2017**). All these assemblages are indistinguishable by light microscopy. Two assemblages A and B are mainly isolated from human. Genotyping study of human isolates of *Giardia* in different regions of Iran and neighboring countries indicated that AII as the most common sub-assemblage is followed by BIII and BIV, respectively (**Hooshyar et al., 2017**); (**Babaei et al., 2011**). Using multiplex real-time PCR have been described for the simultaneous detection of *Giardia* spp., *Cryptosporidium*, *Dientamoeba* and *Entamoeba histolytica* with a high sensitivity and specificity (**Bruijnesteijn et al.; 2009**).

In recent years PCR-based methods have been used for detecting *G. intestinalis* and other human parasites in environmental sources such as water, and sewage (**Moreno et al., 2018**); (**Imre K et al., 2017**). There is an extensive literature that compares the molecular methods and other diagnostic technique in diagnosing *Giardia* infection (**Heyworth et al., 2014**); (**Bruijnesteijn et al., 2009**); (**Hijjawi et al., 2018**). Real-time PCR has been reported to be more sensitive and beneficial than Elisa and faecal microscopy for diagnosing *G. intestinalis* infection (**Beyhan and Tas., 2017**). Using a real-time PCR-based as routine parasitological examination for the identification of *G. intestinalis* displayed an average 92% sensitivity and 100% specificity (**Laude et al., 2016**).

Table 04: Comparison of sensitivity and Specificity of different method in Giardia diagnosis
(Hossein Hooshyar *et al.*, 2019)

Methods	Sensibility	Specificity	Refrence (s)
Direct stool examination	34.7 – 55	96 – 100	(Elmi <i>et al.</i> , 2017) (Oguoma <i>et al.</i> , 2007) (Elsafi <i>et al.</i> , 2013)
Stool concentration	65.2 – 83	58 – 97	(Elmi <i>et al.</i> , 2017) (Oguoma <i>et al.</i> , 2007) (Schuurman <i>et al.</i> , 2007) (Barbecho <i>et al.</i> , 2018)
Surcrose density gradient	42 – 94	97 – 100	(Elmi <i>et al.</i> , 2017) (Xiao et Herd, 1993)
String test (Entero-Test)	44 – 73	97 – 100	(Wolfe, 1993) (Goka <i>et al.</i> , 1990) (Hall <i>et al.</i> , 1988)
Antigen detection	44 – 100	68 – 100	(Faubert <i>et al.</i> , 2000) (Garcia <i>et al.</i> , 2003) (Aldeen <i>et al.</i> , 2003) (Barbecho <i>et al.</i> , 2018)
Molecular assay	58 – 92	56 – 100	(Beyhan <i>et al.</i> , 2017) (Uehlinger <i>et al.</i> , 2017)

2.5. Treatment

Number of effective agents, including quinacrine, the nitroimidazoles metronidazole and tinidazole, and furazolidone are available for treatment of patients with giardiasis (**Table 05**) (Davidson, 1984). Metronidazole has no effect on cyst viability, whereas quinacrine has some effect (Paget *et al.*, 1998). However, it is unknown whether cysts produced in the small intestine then have the ability to excyst in the same host and continues the infection, and whether the major therapeutic effort is therefore directed against the trophozoite (Adam, 1991).

Table 05: Commonly used treatment regimens for giardiasis (Adam, 1991)

Agent	Daily dose (frequency)	Duration	Efficacy	References
Quinacrine	6 mg/kg/day (three times daily), 300 mg/day maximum	5 days	>90%	(Davidson, 1984)
Metronidazole	15 mg/kg/day (three times daily), 750 mg/day maximum	5 days	>90%	(Davidson, 1984)
Tinidazole (b)	50 mg/kg, 2 g (maximum)	Single dose	>95%	(Jokipii and Jokipii, 1979) (Speelman, 1985)
Furazolidone	8 mg/kg/day (three or four times daily), 400 mg/day maximum	10 days	>80%	(Davidson, 1984)
Paromomycin (c)	30 mg/kg/day (three or four times daily)	10 days	Unknown	(Carter <i>et al.</i> , 1962)

B: Not available in the United States.

C: Its efficacy is probably lower than that of the other agents, but it is sometimes recommended for pregnant women because it is poorly absorbed.

2.5.1. Quinacrine

It is an antimalarial agent that is highly effective for treatment of giardiasis when given for a 5- to 10-day course (Davidson, 1984) and is considered by some to be the agent of choice. However, gastrointestinal side effects are common, sometimes causing difficulty with compliance (Murphy and Nelson, 1983). Rare side effects include toxic psychosis and hemolysis in glucose6 phosphate dehydrogenase-deficient patients. The mechanism of action of quinacrine is possibly due to its effect on the flavoprotein and quinone components of respiration (Paget *et al.*, 1998).

2.5.2. The nitroimidazoles metronidazole and tinidazole

These are also highly effective for the treatment of giardiasis. They have a broad spectrum of activity against anaerobic bacteria and protozoans as a result of their reduction to nitro anion radical metabolites through ferredoxin or ferredoxinlike molecules (Finegold,

1980); (Moreno *et al.*, 1983). The radicals or an intermediate, such as hydroxylamine, then bind to DNA or protein molecules (Ings *et al.*, 1974). Although the interaction with DNA has been proposed as an important component of the effect of metronidazole (Ings *et al.*, 1974), the rapid inhibition of respiration in *G. muris* trophozoites (Paget *et al.*, 1989) suggests that the radicals may have a toxic effect on the enzyme(s) of the respiratory pathway. Metronidazole is widely used in the United States for treatment of *giardiasis* (although not approved by the Food and Drug Administration for treatment of *giardiasis*) and is more than 90% effective when given for a 5-day course. Nausea and general malaise are common during therapy, and a disulfiramlike interaction with ethanol can be seen, but serious side effects are rare. Metronidazole is mutagenic in bacteria (Legator *et al.*, 1975); (Rosenkranz and Speak, 1975) and, in high doses for prolonged periods, is carcinogenic in mice (Rustia and Shubik, 1972), so concerns have been raised about its use in humans. However, two case-control studies have shown no increased frequency of cancer in people who have taken metronidazole (Beard *et al.*, 1988); (Beard *et al.*, 1979).

2.5.3. Tinidazole

It is effective when given as a single dose and is very well tolerated For these reasons, it is probably the treatment of choice in countries where it is available; however, at the time of writing, it has not been approved in the United States (Jokipii and Jokipii., 1979); (Speelman, 1985).

2.5.4. Furazolidone

It is frequently used in children because the bitter taste and gastrointestinal side effects of quinacrine make the latter difficult to use, but furazolidone may be somewhat less effective than quinacrine and metronidazole (Davidson, 1984). It most probably acts by inhibiting anaerobic respiration (Crouch *et al.*, 1986); (Edwards *et al.*, 1973), but it also binds to DNA. Like metronidazole, it is carcinogenic in animals (rats), but has not been shown to be carcinogenic in humans.

2.5.5. Paromomycin

It is a nonabsorbable aminoglycoside that has some in vitro activity against *G. lamblia* (Edlind, 1989). Aminoglycosides inhibit protein synthesis through binding the smallsubunit (16S-like) rRNA (Edlind, 1989). The sequence of the DNA encoding the small-subunit rRNA was used to predict susceptibility to paromomycin, but not to many of the other

available aminoglycosides, and the prediction was confirmed by in vitro testing. Clinical data regarding the efficacy of paromomycin are very limited (**Carter et al., 1962**); (**Kreutner et al., 1981**), but it is frequently recommended for treatment of symptomatic *giardiasis* during pregnancy because of concerns about possible teratogenic effects of the other available agents (**Rotblatt, 1983**).

2.5.6. Mebendazole

A benzimidazole, is a broad-spectrum antihelminthic agent that probably works through its interaction with P-tubulin. It demonstrates in vitro activity against *G. lamblia* (**Edlind et al., 1990**), and in one uncontrolled clinical trial with a dose of 200 mg/kg/day, 38 of 40 patients (95%) were cured (**Al-Waili et al., 1988**). The duration of therapy was not specified, but was apparently at least 5 days. In another study, the same dose given for 1 day was ineffective (**Gascon et al., 1989**). Therefore, mebendazole is probably effective for treatment of *giardiasis*, but at much higher doses than used for helminthic infections (**Keystone and Murdoch, 1979**). The benzimidazole albendazole also has in vitro activity against *G. lamblia* (**Meloni et al., 1990**) and was effective in five patients (**Ward et al., 1982**). Controlled trials are necessary to compare the safety and efficacy of the benzimidazoles with those of the standard agents.

2.5.7. Other agents with in vitro activity

They include chloroquine (**Gordts, 1985**), pyrimethamine (**Gordts, 1985**), mefloquine (**Crouch et al., 1986**), rifampin (**Crouch et al., 1986**), azithromycin (**Crouch et al., 1986**), and the lipophilic tetracyclines, such as doxycycline (**Crouch et al., 1986**); (**Edlind, 1989**), but clinical studies have not been performed. Patients who fail to respond to treatment usually respond to a second course of treatment with the original or another agent. Decreased in vitro susceptibility to metronidazole (**Boreham et al., 1988**) and furazolidone (**McIntyre et al., 1986**) has been documented, but has not been clearly correlated with treatment failure, and high grade in vitro resistance to these agents has not been reported. However, it is difficult to culture *Giardia spp.* In samples from patients, and in vitro testing for susceptibility is not standardized. Therefore, in vitro susceptibility is difficult to evaluate in an individual patient, and so true drug resistance is difficult to document. Combined treatment with quinacrine and metronidazole has been used successfully in infections that were refractory to treatment with a single agent (**Borehm et al., 1988**); (**Smith et al., 1982**); (**Taylor et al., 1986**).

2.6. Prevention and control

Conventional water treatment plants that use coagulation sedimentation-filtration methods are needed to prevent waterborne giardiasis outbreaks (**Wolfe, 1984**). According to centers for Disease Control surveillance report on waterborne disease in the United States between 1986 and 1988, *Giardia lamblia* were identified as the causative agent in 9 of 50 outbreaks, the largest of which affected more than 500 people. Eight outbreaks were associated with deficiencies in community water systems, and six were associated with unfiltered surface water systems in which chlorination was the only treatment (**Levine et al., 1990**). While chlorination alone is often effective in killing most enteric organisms, *Giardia* cysts may require higher concentrations of chlorine and longer contact times to be inactivated, particularly in cold water (**Jarroll et al., 1981**). For individual protection, bringing water to a rolling boil for 1 min destroys *Giardia* cysts. If boiling is not possible, 2 to 4 drops of household bleach or 0.5 ml of 2% tincture of iodine can be added to each liter of water and the water can be held for 60 min before drinking. A longer treatment time (overnight) is recommended if the water is cold. Eating hot, cooked foods helps to prevent ingestion of viable cysts from foods contaminated by infected water or fingers (**Jarroll et al., 1981**).

No drugs are available to use for *Giardia* prophylaxis. Considering the many sources from which giardiasis may be acquired, prophylactic therapy may not be of much value except for travel in highly endemic areas (**Wolfe, 1984**). Continued research on the epidemiology, ecology, sociology, anthropology, pathogenesis, diagnosis, treatment and prevention of giardiasis can most optimally be pursued in the endemic regions which, unfortunately, also suffer from a lack of research capacity, funding support, and institutional infrastructure. Much needs to be done to promote and strengthen giardiasis research in these regions if true progress is to be made (**Escobedo et al., 2020**). It is necessary that scientific communities from both, the industrialized and developing world, share knowledge and experiences, and jointly face this parasitic disease (**Escobedo et al., 2020**).

Giardia is mainly harbored among people having limited financial resources and access to care. This infection may, in some cases, persist for years or people become chronically re-infected, and those affected have no recourse but to live with their symptoms. It is likely that many of these people have had persistent infection for so long that they do not report their clinical manifestations because most of their signs and/or symptoms have become the norm. It might be assumed that a high proportion of those infected received either no treatment, inappropriate treatment or incomplete treatment. The result is that this treatable IPI

continues its spread within the community even though its transmission could be prevented or reduced with attention to water quality, food hygiene, and a few cents worth of generic drugs previously mentioned, when these measures fail (**Escobedo and Cimerman, 2007**).

2.7. Global spread of the illness

Human giardiasis has been reported from most countries in the world. Prevalence rates of infection differ between < 1 and 50 % depending on the population sampled, being higher in under-developed than developed countries and in urban rather than rural areas (**Janoff *et al.*, 1990**); (**Miotti *et al.*, 1986**). In developed countries the group with the highest rates of infection appears to be children attending day-care and they might be a source of infection to their families and the different community. Just about one-third of the population in Riyadh has been reported to be infected through Giardia was the most common species, indicating the significance of parasitic infections in public health in Saudi Arabia (**Al-Shammari *et al.*, 2001**).

For 2019, 18 004 confirmed giardiasis cases were reported by 25 countries in the EU/EEA, with an overall rate of 5.2 cases per 100 000 populations. The highest number of confirmed cases (**Fig 13**) was reported by the United Kingdom, followed by Germany and Belgium. These three countries combined accounted for 58% of all confirmed giardiasis cases in the EU/EEA. Belgium had the highest notification rate of 18.0 cases per 100 000 populations, followed by Bulgaria at 16.3 cases per 100 000 populations. More than two-thirds (68.7%) of giardiasis cases with reported information were domestically acquired. The proportion of travel-associated cases exceeded 50% in three countries reporting more than 50 cases (Germany, Norway and Sweden). Sweden, in particular, reported that 81.1% of its giardiasis cases were travel-associated. Among cases with complete travel information, India accounted for the highest proportion of cases (19.3%) with known travel destination (**ECDPC, 2022**).

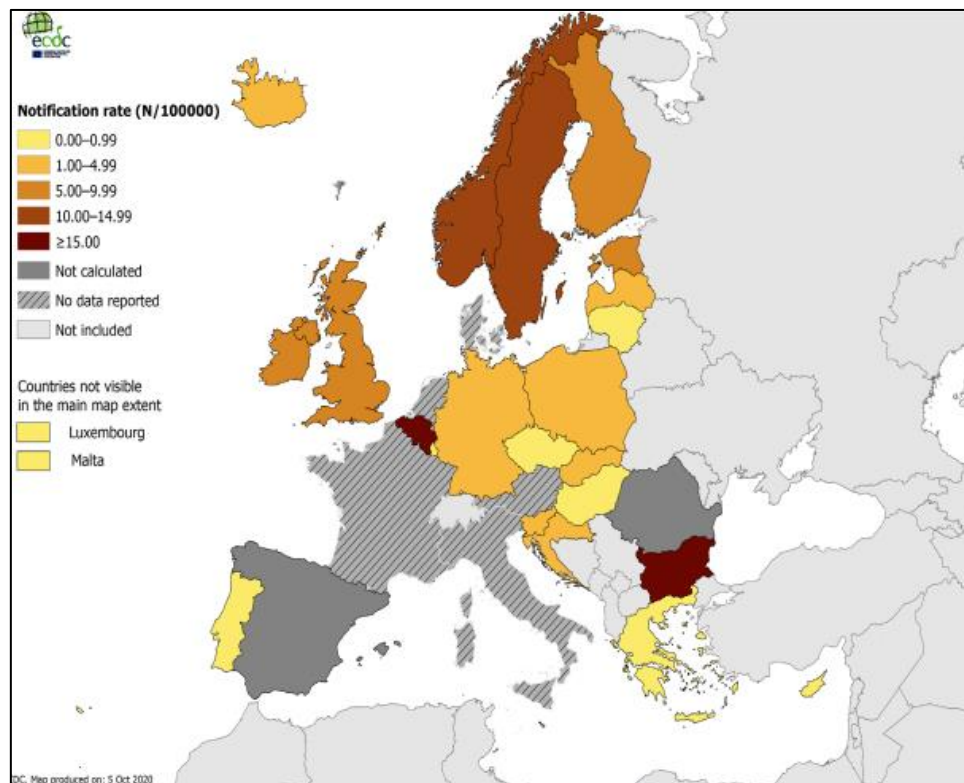
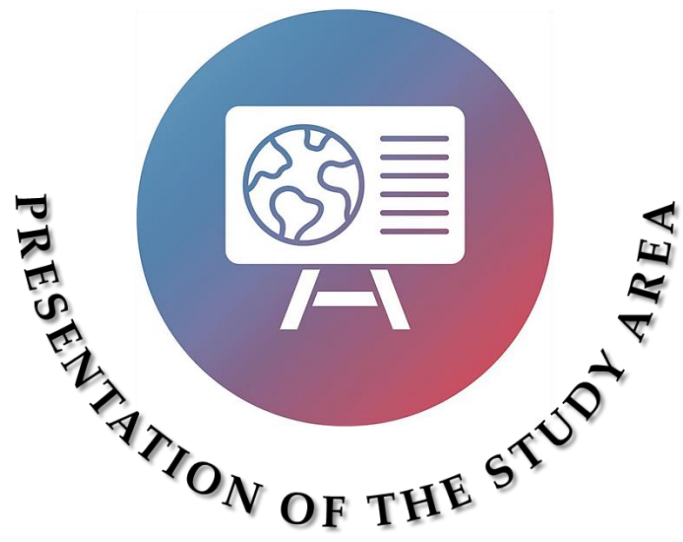


Figure 13: Distribution of confirmed giardiasis cases per 100 000 populations by country, EU/EEA, 2019 (ECDPC, 2022).



3. Presentation of the study area

3.1. Geographical Description

Mila region is located in the northeast of Algeria (**Fig 14**), about 391 km from Algiers and 50 km northwest of the city of Constantine. It belongs to the Mila basin, which covers an area of 3500 km² often known as the Mila-Constantine basin. Located between latitude 244,591.01 and 263,859.46 m N and longitude 4,027,529.17, 4,043,981.06 m E (WGS 84 UTM 32), where the area of this municipality is 129.95 km², it represents 0.13% of the country's surface area (**Bounemoeur et al., 2022**).

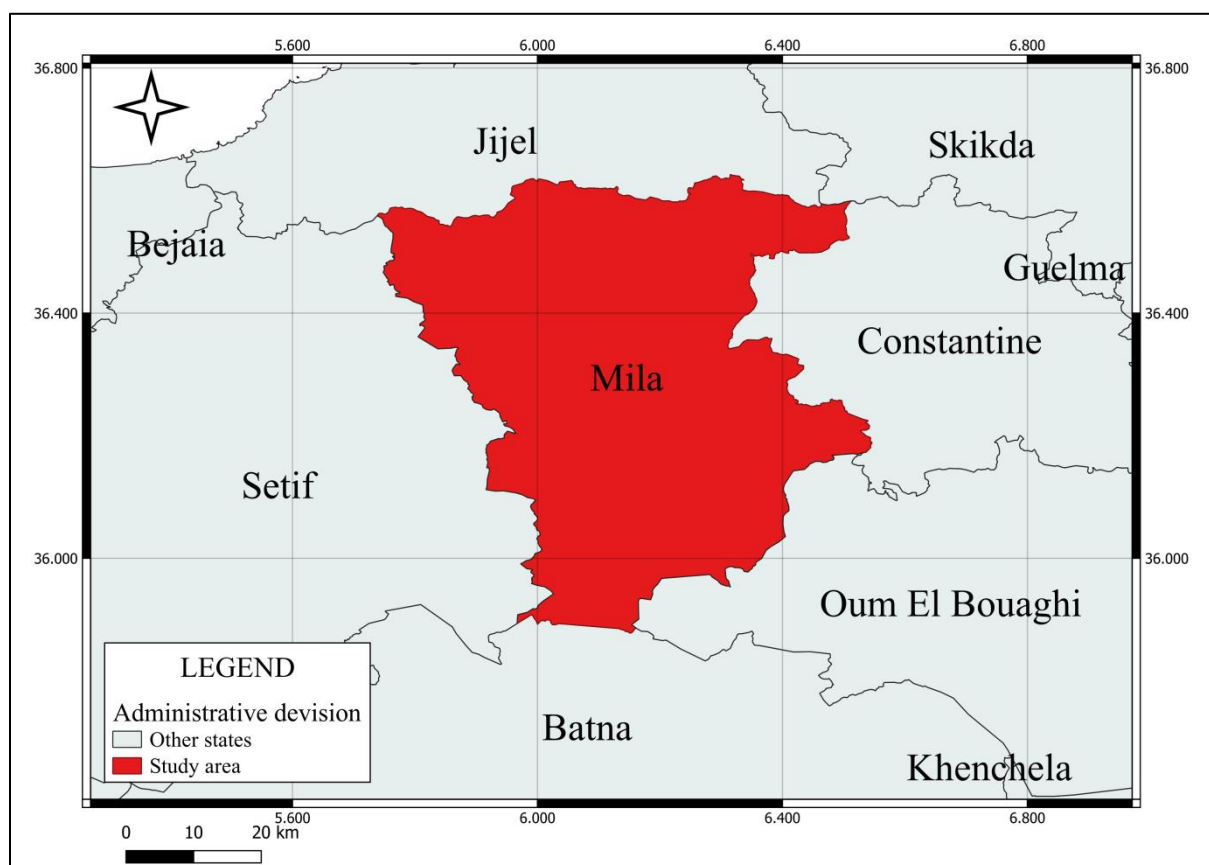


Figure 14: situation of the Mila province, north- east Algeria (**personal photo, 2024**).

The Mila province is bordered:

- ✚ To the north, by the Jijel province
- ✚ To the east, by the Constantine province
- ✚ To the northeast, by the Skikda province

- ✚ To the west, by the Sétif province
- ✚ To the south, by the Batna province
- ✚ To the southeast, by the Oum El Bouaghi province

3.1.1. Administrative aspect

The province of Mila was created during the last Algerian administrative division of 1984, with the city of Mila as the capital of wilaya 43 (NAID, 2013). The province has 13 daïras comprising 32 municipalities (Table 06, Fig 15) represents the administrative division of the Mila region.

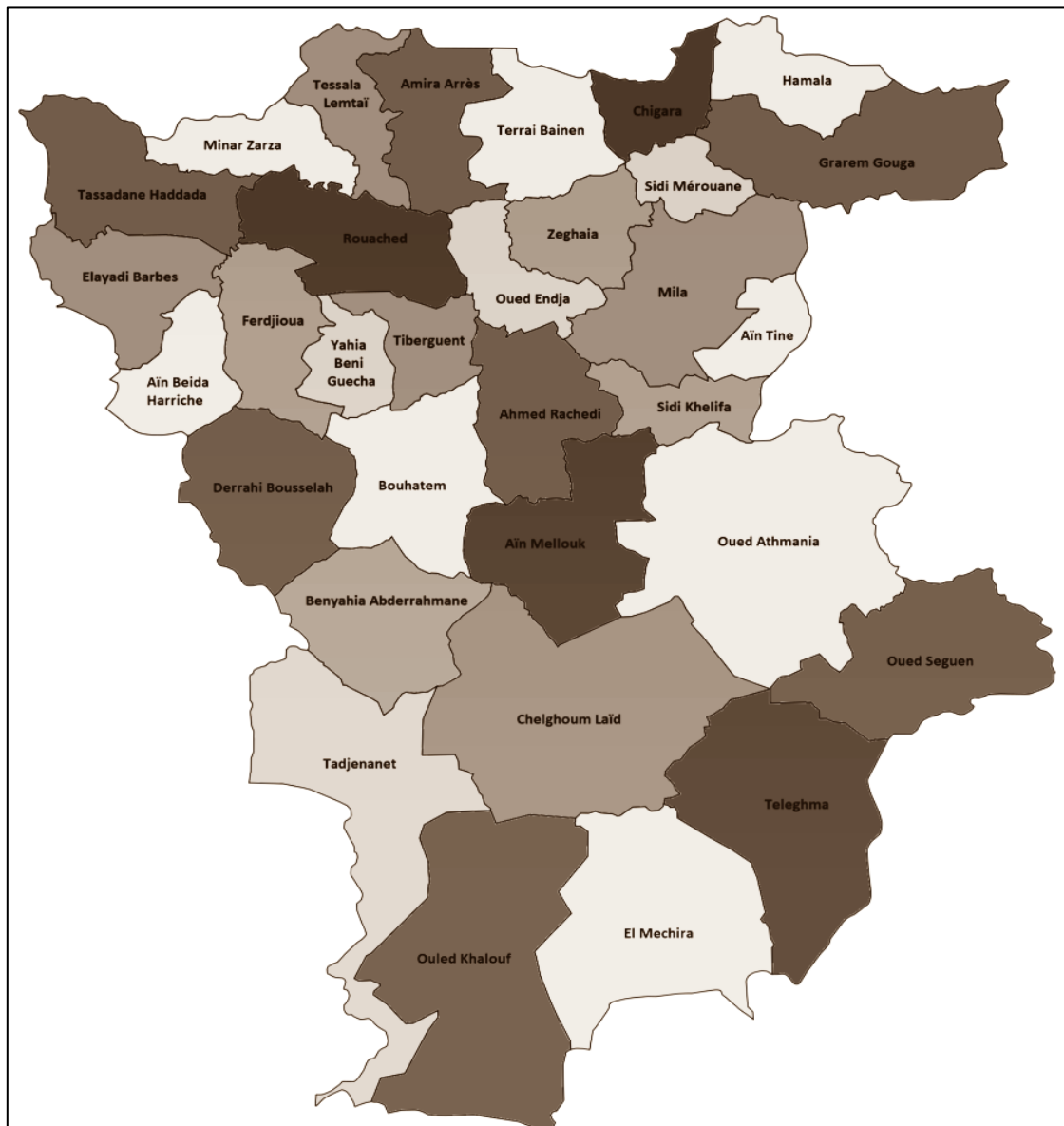


Figure 15: Administrative division of the Mila province (Personal photo, 2024)

Table 06: Administration division of the state (Mila) (Boularas and Kadjoudj, 2016).

Daïra	District	Surface (Km²)
Mila	Mila / Ain Tinn / Sidi Khelifa	212,56
Grarem Gouga	Grarem Gouga / Hamala	199,13
Sidi Merouane	Sidi Merouane / Chigara	86,52
Oued Endja	Oued Endja / Ahmed Rachedi / Zeghaia	186,97
Rouached	Rouached / Tiberquent	145,82
Terral bainen	Terral bainen / Amira arres / Tassala Lamtai	220,25
Ferdjioua	Ferdjioua / Yahia ben Ghecha	109,07
Tassadane Haddada	Tassadane Haddad / Minar Zarza	162,9
Bouhatem	Bouhatem / Derradji Bousselah	233,34
Ain Beida Harriche	Ain Beida Harriche / Elayadi Barbes	153,53
Teleghma	Teleghma / Oued seguin / M'chira	484,85
Chelghoum Laid	Chelghoum Laid / Oued Atmania / Ain Mellouk	634,54
Tadjenanet	Tadjenanet / Ouled Khlouf / Benyahia Abderrahmane	577,52
TOTAL		3407,00

3.2.Demographic informations

3.2.1. Population distribution by districts

The population of the Mila province was estimated by 931 363 inhabitants (**Fig 16**) as of the end of 2023 (**DPSP, 2023**).

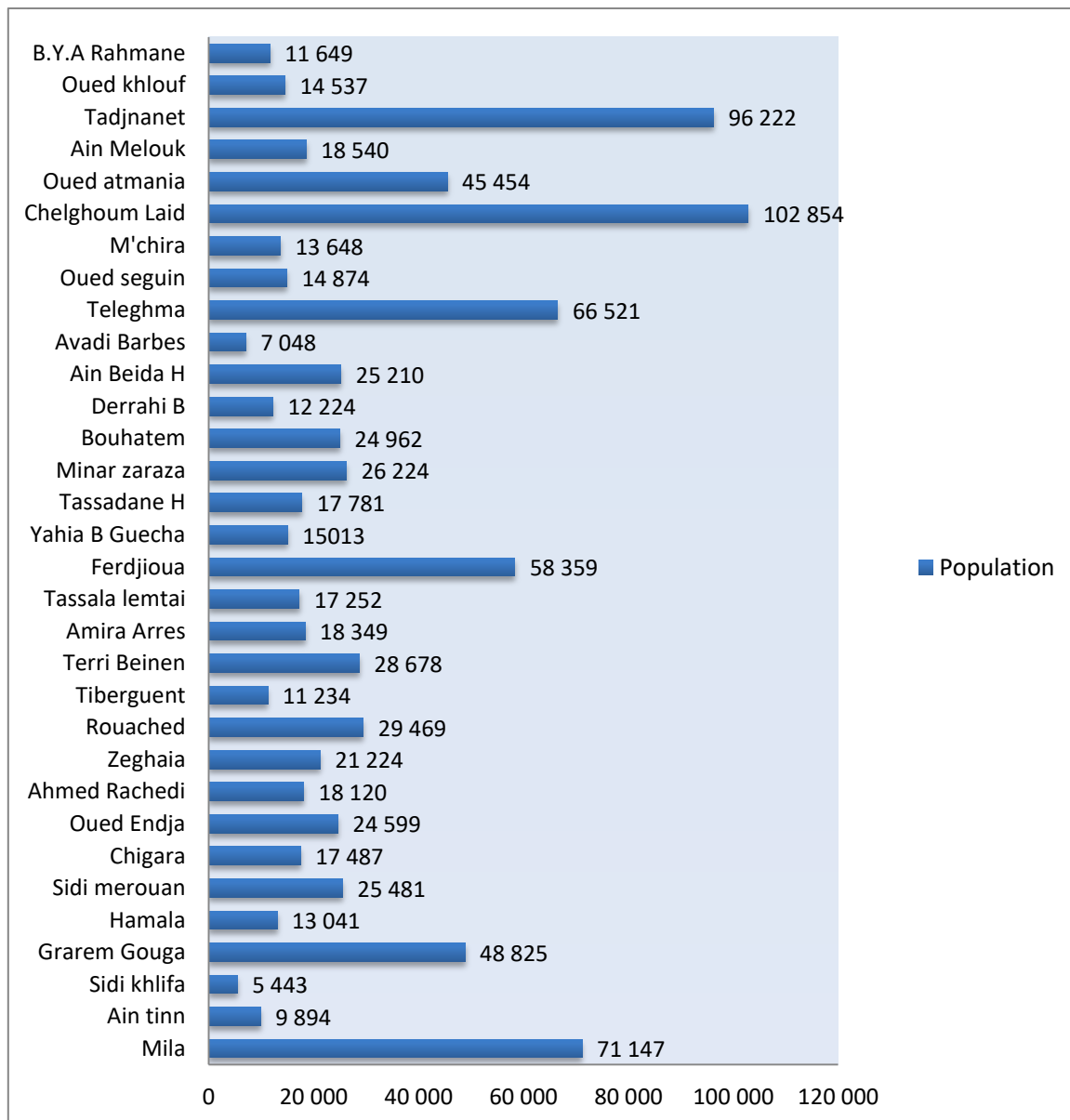


Figure 16: Chart representing the population distribution by district, totaling 1,006,199 individuals (DPSP-Mila, 2023).

3.2.2. Population distribution by gender groups

The population of the Mila province was estimated by gender categories, men represented 468444 inhabitants, while females represented 462920 inhabitants (**Fig 17**) (DPSP-Mila 2023).

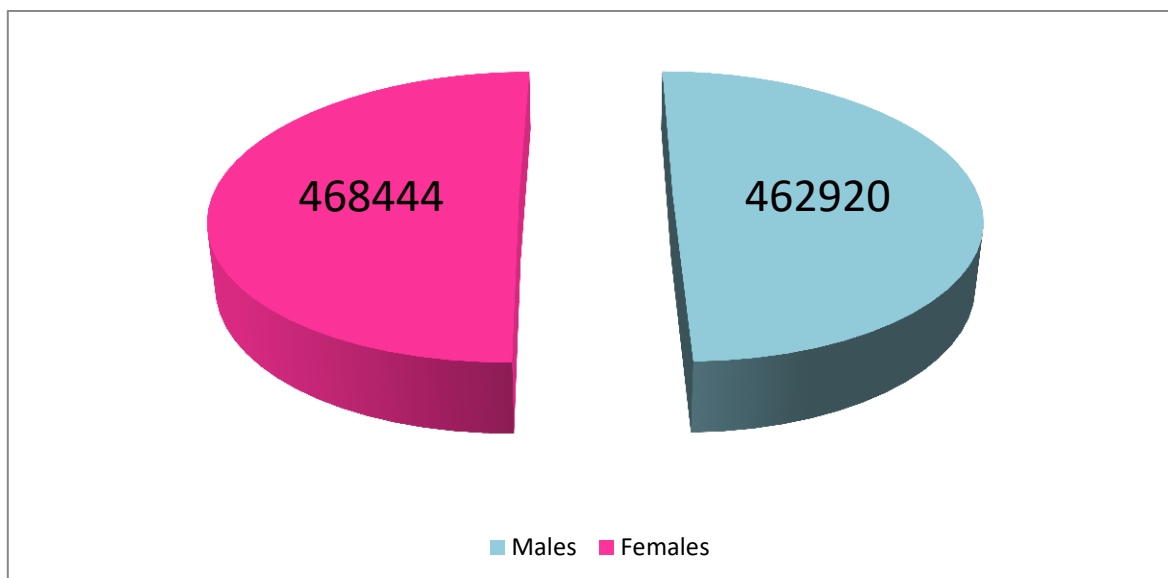


Figure 17: Chart depicting the population distribution in Mila Province by gender categories (DPSP-Mila, 2023).

3.2.3. Population distribution by age groups

The population distribution based on age categorization showed that the highest numbers of individuals within the state are around 22 to 24 and 15 to 19 (**Fig 18**) (DPSP, 2023).

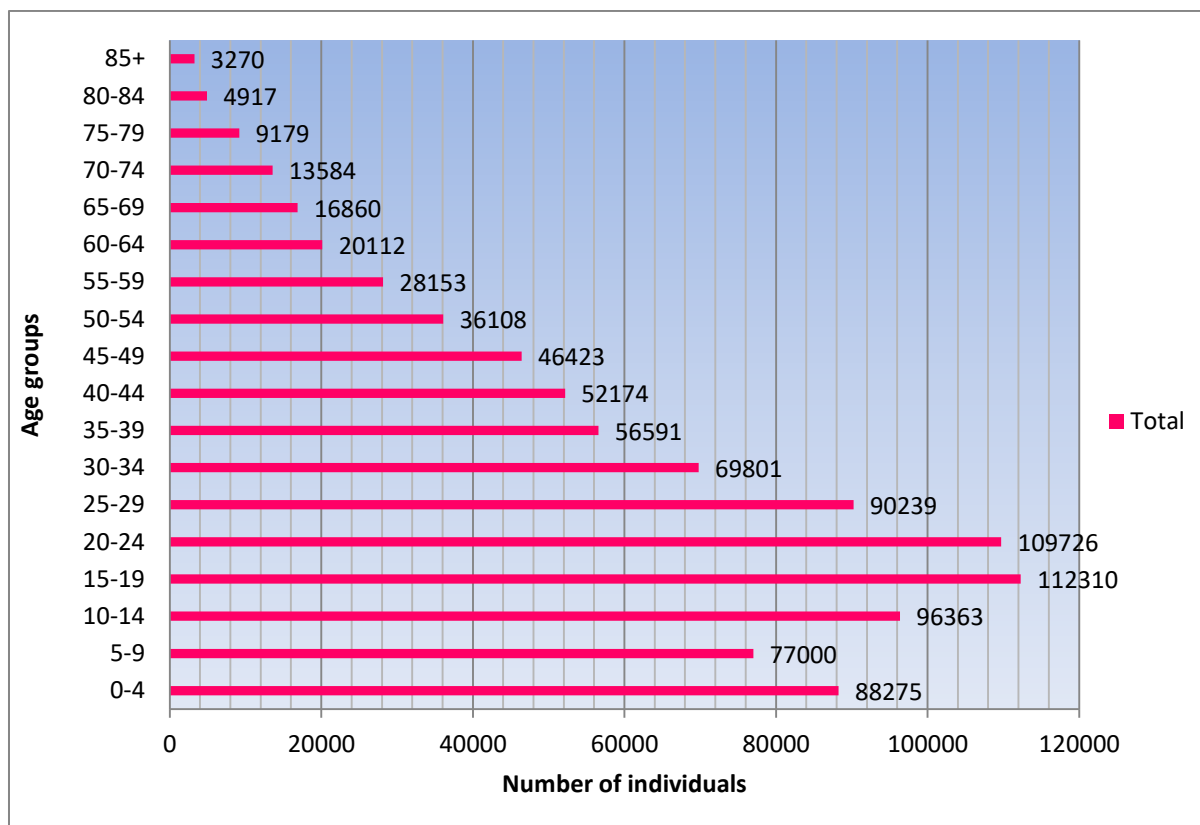


Figure 18: Chart representative of the population distribution by age groups from 2010-2023 (DPSP-Mila, 2023).

3.2.4. Population growth rate

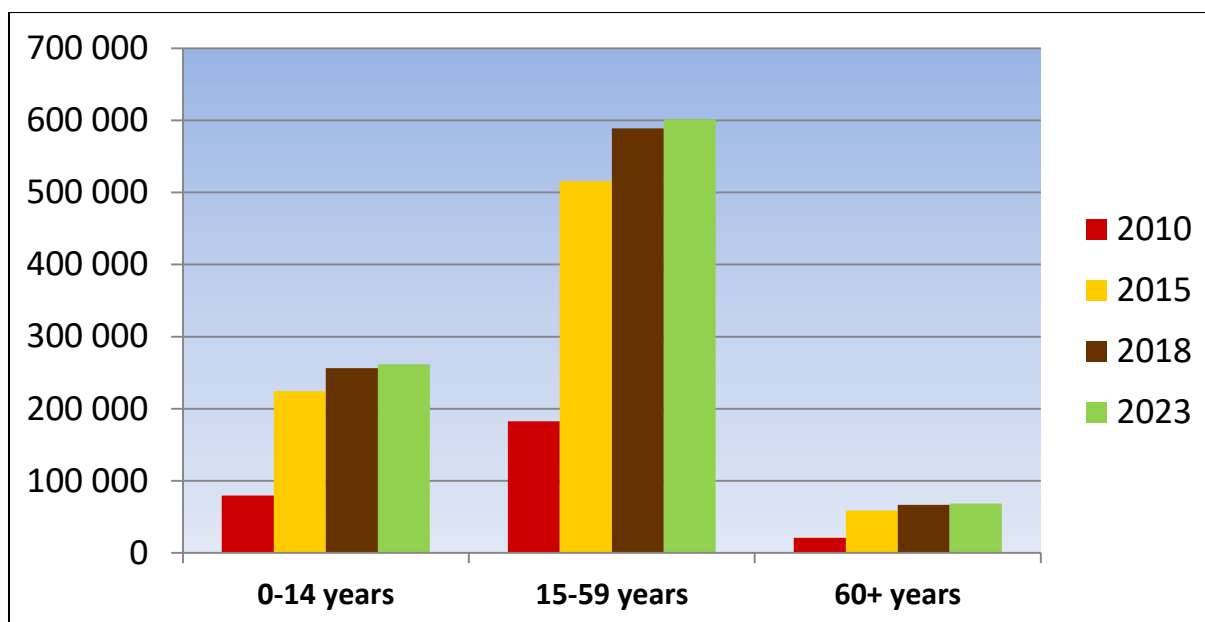


Figure 19: Chart illustrating the growth rate of the population distribution by age groups from 2010 to 2023 (DPSP-Mila, 2023).

3.2.5. Population distribution by the economic Activity

The total of active population estimated to be 671,543 inhabitants, representing 66,74 % of the total population, in which employed population in the state was determined to be 603,247. The activity rate determined to be 66,74%, noting public works also includes construction and hydraulics (**Fig18**).

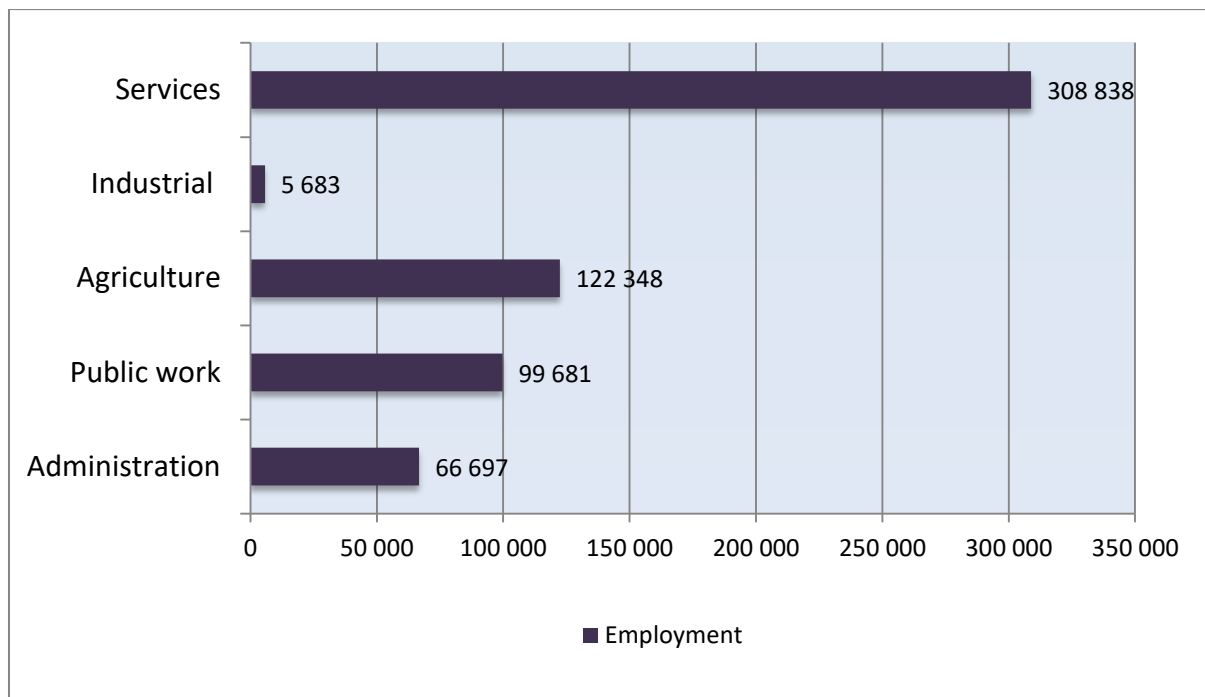


Figure 20: Chart representative of the population distribution by activity sector (DPSP-Mila, 2023).

Population distribution by areas (country side/ urban)

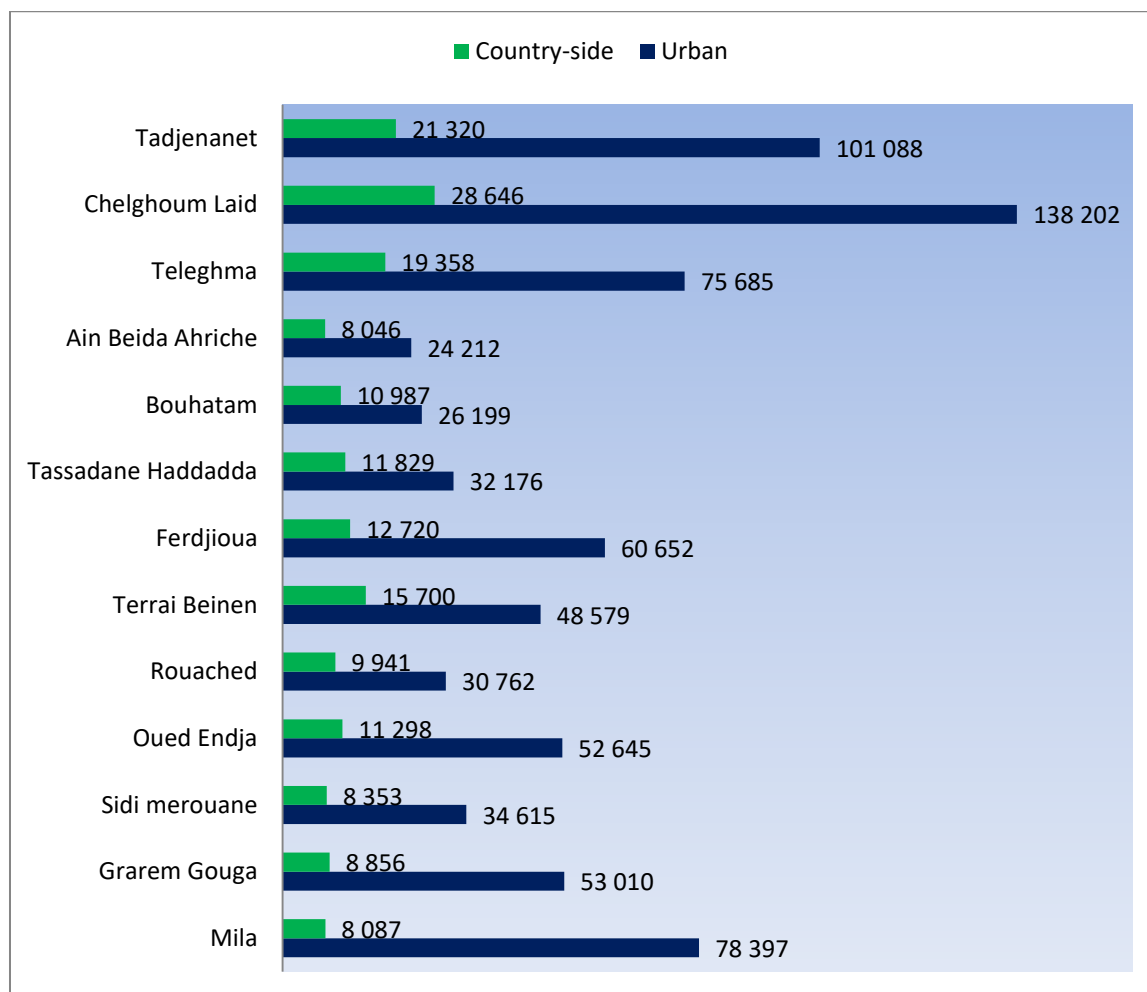


Figure 21: Chart representative of the population distribution based on the occupied area (Urban/country-side) (DPSP-Mila 2023).

3.3.Environmental Characteristics

3.3.1. Climate

The Mila region is characterized by a Mediterranean climate that is cold and rainy in winter, hot and dry in summer, and receives a height of precipitation varying between 500 and 600 mm/year (**Bounemoeur et al., 2022**). So it governed by three microclimates, which correspond to the arrangement of the three major morphological units: Humid For the mountainous reliefs of the North and the middle part, this extends from Bouhatem to Ain Tine. Semi-arid to sub-humid for the middle part of the province (its slopes). And semi-arid for the ‘high plains’ (**NAID, 2013**).

3.3.2. Natural potentials

3.3.2.1. Forest cover

The Mila province, covering an area of 340,684 hectares, is endowed with a forest area of 33,670 hectares, representing a rate of 9.80%. These are distributed according to the following domains:

- ✓ Natural forests: Covering 6,762 hectares, representing 20.08% while the dominant species being cork oak (*Quercus suber* L.).
- ✓ Reforestation: With an area of 18,493 hectares and representing 54.92%, the main species being Aleppo pine (*Pinus halepensis* Mill.) and cypress.
- ✓ Maquis: Covering an area of 8,415 hectares and representing 25%: (maquis of holm oak (*Quercus ilex* and juniper) (**DSA, 2000**).

3.3.2.2. Agriculture potentials

Out of its total geographical area, which exceeds 3.48 thousand km², the total agricultural area is estimated at 315745 hectares, of which 237557 hectares are arable, or 75.23 percent. Of the total agricultural area, this area represents 90% of the area of the State, which is classified as a peasant state par excellence.

Agricultural land is distributed according to the type and nature of exploitation for each municipality, as the municipalities located in the southern region of the upper plains contain the most significant percentage of agricultural land, in particular (Shalghoum Al-Eid, Oued El-Othmania, Tagnant, Awlad Khalouf, Al-Mouchira, Al-Talaghma, and Oued Saqqan) with a rate of more than 48%. Among the arable lands are the same municipalities that exploit the most irrigated areas (**Bendjouad, 2023**).

At the same time, the pastoral lands are concentrated in the municipalities of the north and the basins by way of allocation (Minar Zarza, Hamala, Al-Ayadi Barbas, Tassala Lamta'i, Al-Shigara, Tassadan Hadada, Terai Bayan) where The pastoral lands in it constitute between 20 to 46% of the total agricultural lands for each municipality. However, they do not exceed 9% of the total arable lands. (**DPSB/DAS, 2020**).

3.3.2.3. Relief

The relief of Mila province is characterized by great geographical diversity

a. In the north

there is a mountainous area formed by a succession of mountain ranges (the Tellian massifs) stretching across the territories of the municipalities of Hamala, Chigara, Terrai Beinen, Amira Arres, Tassala Lemtai, Minar Zarza, and Tassadane Haddada. The highest points in this area are (Djebel Tamezguida: 1600 m / Djebel M'cid Aicha: 1400 m / Djebel Zouagha: 1300 m / Djebel Boufroun: 1300 m).

b. In the centre part

There is a zone of foothills and hills constituting the central region of the southern Tellian foothills, which covers almost all of the districts of Ferdjioua, Oued Endja, and the municipality of Grarem Gouga. It comprises Intra-mountain plains in the Ferdjioua and Oued Endja regions with an average altitude of 400 m. Hills and foothills located in the eastern part of the province; they are bordered to the north by the mountainous region and form the southern boundary of the high plains. The region of high foothills, which extends north westward as an extension of the Tellian reliefs, it encompasses the Ferdjioua and Oued Endja depression and extends from the municipality of Derrahi Bouslah to the reliefs of Sidi Khelifa and Ain Tine. The lowland of Mila formed by a set of low hills ranging from 500 to 600 m in altitude and isolated massifs such as Djebels Akhel, Boucharef, Ouakissen, and the Ahmed Rachedi massif.

c. In the south

There is a high plateau zone characterized by gentle slopes of less than 12.5%. It covers almost the entire district of Chelghoum Laid and the vast cereal plains of Tadjenanet and Teleghma. In this southern region of the province, with an average altitude generally ranging between 800 and 900 m, isolated mountainous massifs emerge, such as Kef Lebiod (1,408 m), Djebel Tarioulet (1,276 m), Kef Lsserane (1,276 m), Djebel Gherour (1,271 m).

3.3.2.4. Water resources

The province has a total of 330 identified water sources, with a combined mobilized flow of 7,804,812.16 m³/year. Among these sources (**Table 7**), there are 40 identified wells with a total mobilized flow of 1,814,942.16 m³/year, and 104 boreholes with a combined mobilized flow of 9,713,065.71 m³/year. Additionally, the province is home to 425 reservoirs

with a total capacity of 157,010 m³, 67 water towers with a combined capacity of 21,700 m³, and 4 hillside reservoirs with a total capacity of 1,573,000 m³

The irrigated area at the state level is estimated at 16030 hectares, or 6.7 percent of the arable area, which is a very weak percentage compared to the area irrigated by precipitation, despite the availability of a large dam that holds more than one billion cubic meters. The precipitation rate is estimated at 700 mm annually in the northern mountainous regions and 350 mm in the southern regions. In comparison, in the central regions, it ranges between 400 and 600 mm annually, as the level of precipitation varies according to each season and from month to month (**Bendjouad, 2023**).

Concerning groundwater, it is estimated at 56 million cubic meters per year. Wells exploits it, and explorations, 36 by 46, are exploited, with a combined flow of 922301 liters/second. As for the unexploited explorations, their number is 15 explorations, of which eight (08) are in the municipality of Shalghoum Al-Eid. The statistics of the agricultural season for the year 2020/2021 indicate that the annual irrigation yield is estimated at 16030 hectares, of which 2952 hectares are by flow, 12892 hectares are by sprinkler, and 174 hectares are by local irrigation. The irrigated areas are 330 hectares of dams, 75 hectares of water barriers, 507 hectares of water sources, 8052 hectares of excavations, 1779 hectares of wells, and 2590 hectares of waterways. Three central valleys in the State flow continuously through the four seasons. Constantine, while the second is Wadi Al-Naga, whose length is 110 km, on a line parallel to the northern mountain range. Its most important tributaries are Wadi Jamila, Wadi Bou Salah, Wadi Ragas, and Wadi Al-Malah, which are 45 km long. On the other hand, there are three dams in the State, Bani Haroun al-Kabir Dam (960 million cubic meters), Wadi al-Uthmaniyah Dam (35 million cubic meters), and Karouz Dam (41 million cubic meters). All three dams are used as drinking water, in addition to partial exploitation for irrigation, especially for the southern extension line. Dam Bani Haroun towards Batna is used for irrigation at the municipality level of El Talagama (**Bendjouad, 2023**).

Table 07: Hydrography sources in the Mila province (flow and capacity) (DPSB/DAS, 2020).

Water supply	Total	Flow/Capacity
Recoded sources	330	7,804,812.16 m ³ /year.
Recorded well	40	1,814,942.16 m ³ /year.
Boreholes	104	9,713,065.71 m ³ /year.
Reservoirs	425	157,010 m ³
Water tower	67	21,700 m ³
Hill reservoirs	4	1,573,000 m ³

3.4. Sanitaire structure

According to the National Agency for Land Intermediation and Regulation of Algeria, the province has the following facilities/service represented in (Table 8/9), and qualified human resources consist of the total represented in (Fig 22).

Table 8: Total facilities and services of the province and their capacity (NALIRA, 2022)

Facility / Service	Total	Capacity
Hospitals	5	Total of 835 beds
Polyclinics	40	5 of them provide maternity services
Health centers	167	/
Maternity hospitals	9	/
Hygiene Laboratoried	3	/
Privat clinics	2	/

Table 9: Public Sector Healthcare Establishments within the State of Mila (NALIRA, 2022).

PHELH	Total	Region	Capacity
Psychiatric hospital	1	Oued atmania	270 beds
Public hospital	5	Meghlaoui Mila	166 beds
		Tobal Mila	88 beds
		Ferdjioua	240 beds
		Chelghoum Laid	220 beds
		Oued Atmania	110 beds

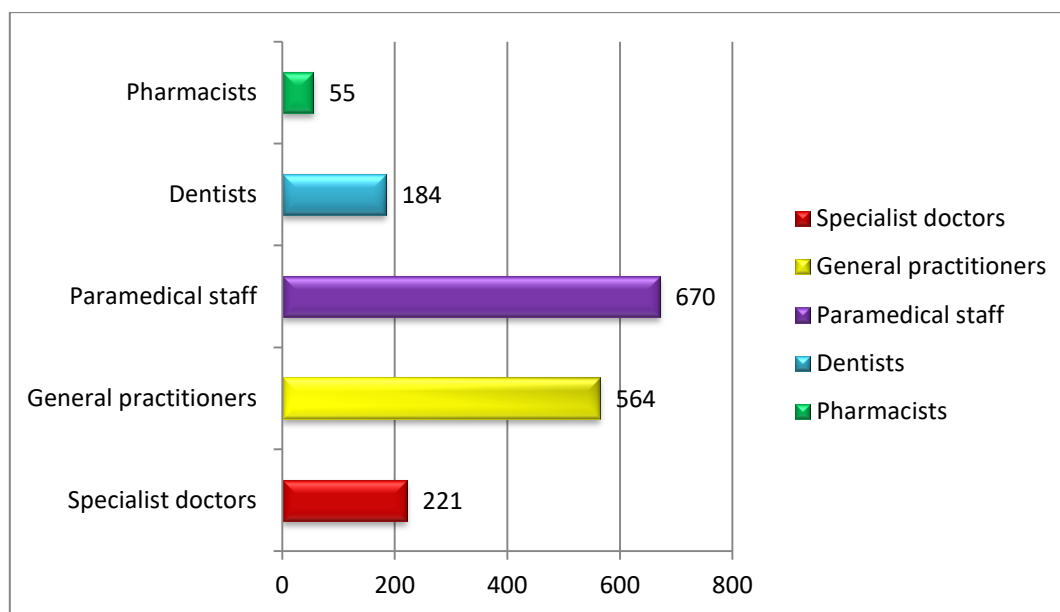


Figure 22: Total qualified human resources of the province and their capacity (NALIRA, 2022)



4. Material and methods

4.1. Epidemiological study

4.1.1. Location, Type, and duration of the descriptive study

The descriptive epidemiological study of *Giardia lamblia* (syn. *G. duodenalis* and *G. intestinalis*), was done on four different hospital establishments within the state of mila (**Fig 23**):

- The central laboratory service, medical parasitology and mycology unit of the “Maghlaoui brothers “public hospital establishment of Mila region
- The central laboratory service, medical parasitology and mycology unit of “Mohamed Meddahi” public hospital establishment of Ferdjioua region
- The central laboratory service, medical parasitology and mycology unit of the “Boukhachem brothers” public hospital establishment of Oued el atmania region
- The central laboratory service, medical parasitology and mycology unit of “Houari Boumadiene” public hospital establishment of Chelghoum El-Aid region.

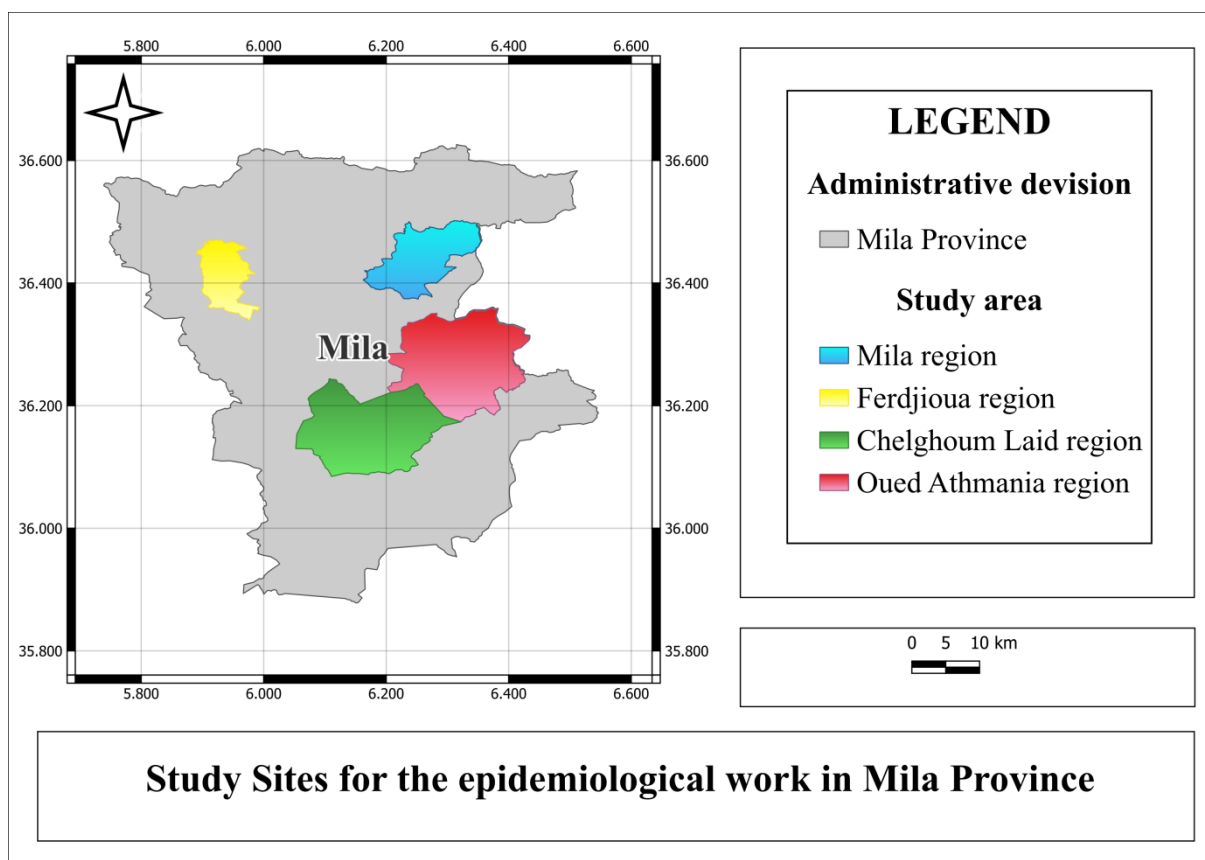


Figure 23: Mapping of study sites for the epidemiological work in the province of Mila
(Personal photo, 2024)

4.1.2. Location, Type, and duration of the prospective study

The prospective epidemiological study of *Giardia lamblia* (*syn. G. duodenalis* and *G. intestinalis*) was done on:

- The central laboratory service, medical parasitology and mycology unit of the “Maghlaoui brothers “public hospital establishment of Mila.

4.1.2.1. Patients status

This epidemiological study focused on general stool examinations (Both physical and microscopic examination) from patients assigned to the parasitology laboratory. The study included patients (adults and children) that were either hospitalized or consulted in various departments of the hospital, coming from varied geographical areas (different municipalities in Mila region) and different social backgrounds (patients from both the public and private sector).

Our prospective study focused on 293 patients referred to the parasitology laboratory during the first three months (January/February/March) of 2024.

4.1.2.2. Parasitological analysis (January - March 2024)

❖ Samples collection

Sampling is a fundamental aspect for guaranteeing result quality. Stool collection is typically conducted either in the morning at the laboratory or at home using a clean, dry plastic pot with a wide opening (**Fig 24**). Occasionally, specific precautions are recommended prior to performing parasitological stool examinations (PSE).



Figure 24: Plastic pots of stool collection given by patients to the parasitological examination
(Personal photo, 2024).

❖ **Material and equipment**

It has been used as in (Fig 25):

- ✓ Pots;
- ✓ Microscope slides and cover glass;
- ✓ Light optical microscope;
- ✓ Benzene burner;
- ✓ Inoculating loop;
- ✓ Pasteur pipette.



Figure 25: Laboratory equipment used for the diagnosis of *Giardia lamblia* (Personal photos, 2024).

❖ **Reagents**

It has been used as in **(Fig 26)**:

- ✓ Lugol's iodine (iodine-potassium iodide) solution ;
- ✓ Physiological saline solution.

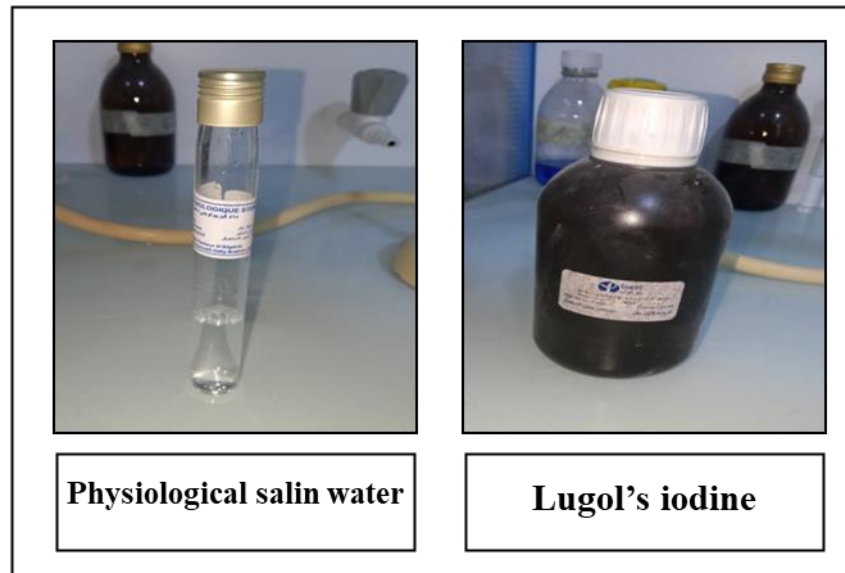
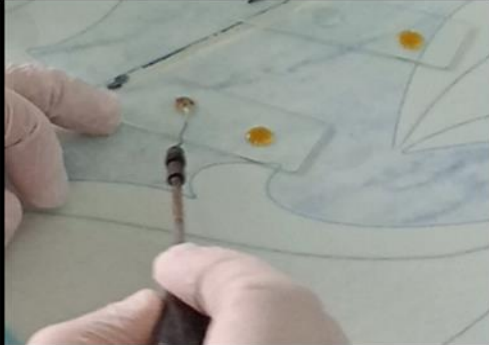


Figure 26: The reagents used in the laboratory for (PSE) of stools **(Personal photos, 2024)**.

4.1.2.3. Stages in the Parasitological Analysis of Stool Samples

The definitive diagnosis of *Giardia lamblia* primarily relies on parasitological examination of stools (PES), which aims to detect parasites in their various forms: cysts and vegetative forms. This typically involves both macroscopic and microscopic examinations as standard procedure **(Table 10)**.

Table 10: the parasitological examination and analysis of stools steps taken in the labotrary work (Personal photos, 2024).

1	
	Placing a small drop of 0.85% saline solution on the left side of the slide
2	
	Placing a drop or two of lugol's iodine solution on the slide
3	
	Sterilizing the application loop by holding it over the Bunsen burner flame until it turn red-hot
4	
	Adding a small portion of the specimen (1-2 mg) and mix well to obtain a uniform suspension
5	
	Placing a 22 x 22 mm coverslip (no. 1) over the preparation and examine as soon as possible
6	
	Placing the slide into the microscope stage, ensuring it is cantered , select the objective 40x - 100x

4.1.3. Collecting data

In the initial phase, data collection involves accessing the records of the parasitology department, where the samples were obtained, as well as gathering clinical information and farm sheets. These farm sheets typically include details such as patient identity (surname, first name, sex, and age), sampling date, services involved, and results of both macroscopic and microscopic examinations of the PES. The data collected span a period from January 2013 to December 2023 and were recorded in a Windows Excel file.

4.2. Meteorological data

The essential data for conducting this study were sourced from the Ain Tin meteorological station. These data pertain to the Mila province and encompass five key climatic parameters:

-  The average temperature;
-  The average humidity;
-  The average sunshine duration;
-  The average monthly wind speed;
-  The average monthly precipitation.

4.3. Investigation into any source of contamination

4.3.1. Location, type and duration of the study

Considered the largest dam in Algeria with a capacity of 1 billion m³, the dam of Beni Haroun is of great economic importance for the entire Eastern Region. Located 500 km east of Algiers, the Beni Haroun dam was put into operation in 2003 (**Fig 27**).



Figure 27: Beni Haroun Dam within the state of Mila (Remini, june 2014).

3.4.2. Investigation

Water samples were collected from three distinct regions: The Dike, Médious, and Boudmaghe. Then laboratory analysis aimed to detect *Giardia lamblia* (syn. *G. duodenalis* and *G. intestinalis*) in water samples through microscopic examination (Fig 28 and 29).

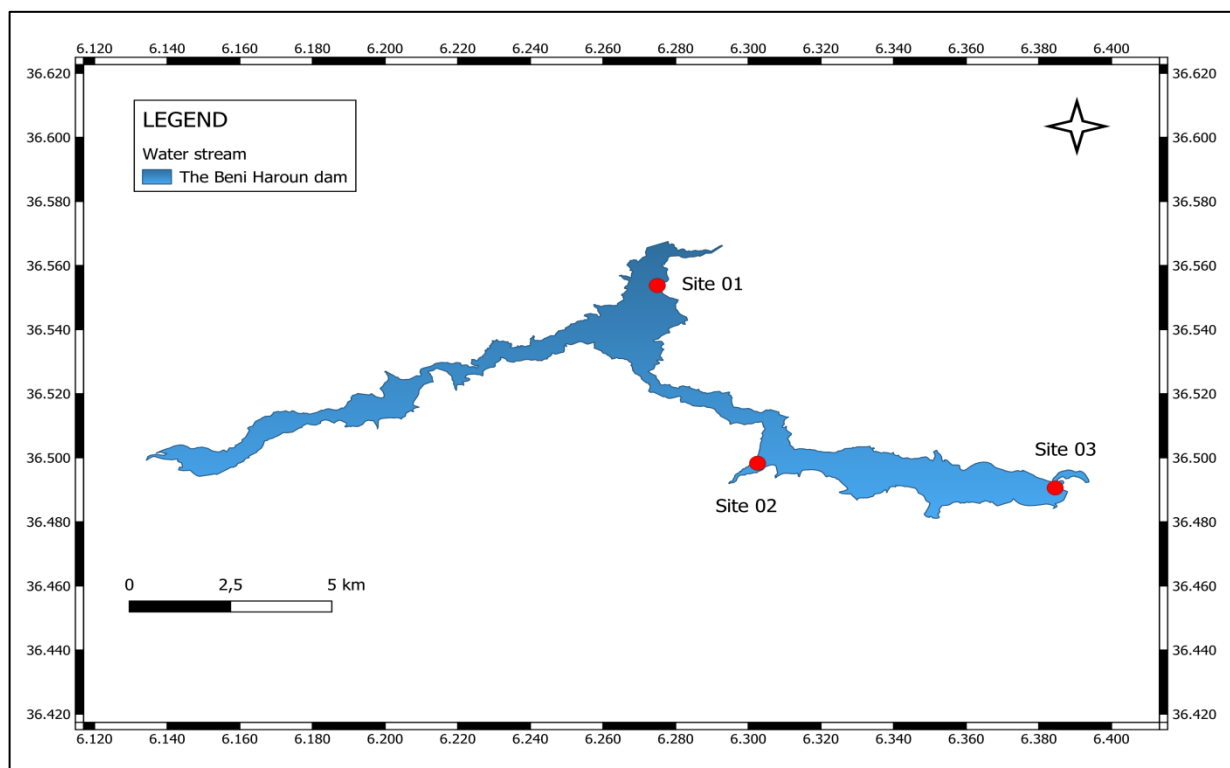


Figure 28: Sampling Points for *Giardia lamblia* water detection search in the Beni haroun dam – Mila (Personal photo, 2024).

4.3.1.1. Area 01 (The Dike)

The Dike is a significant region in Mila Province, known for its vital water resources and strategic location. It is situated on the northern side of the Beni Haroun Dam (Fig 29/ Area 01), one of Algeria's most important reservoirs. The approximate coordinates for La Digue are 36.4610° N latitude and 6.2390° E longitude. This region is a crucial sampling site for our water analysis due to its proximity to the dam and the variety of water bodies it encompasses. The findings from La Digue will be instrumental in assessing the water quality and detecting the presence of *Giardia lamblia* in the area.

4.3.1.2. Area 02 (Médious)

Médious is a notable region located in the Mila Province, known for its strategic position and significant water sources. It is situated on the eastern side of the Beni Haroun Dam (Fig 29/ Area 02), one of the largest dams in Algeria. The precise coordinates for Médious are approximately 36.4470° N latitude and 6.2640° E longitude. This location is crucial for our water sampling and analysis due to its varied aquatic environments influenced by both the dam and local water usage patterns.

4.3.1.3. Area 03 (Boudmaghe)

Boudmaghe is an important region within the Mila Province, recognized for its distinct geographical features and vital water sources. It is located on the southern side of the Beni Haroun Dam (Fig 29/ Area 03), a major water reservoir in Algeria. The approximate coordinates for Boudmaghe are 36.4310° N latitude and 6.2710° E longitude. This region serves as a key sampling site for our water analysis due to its proximity to the dam and the diverse aquatic environments present. The data collected from Boudmaghe will provide valuable insights into the water quality and the presence of *Giardia lamblia* in the area.

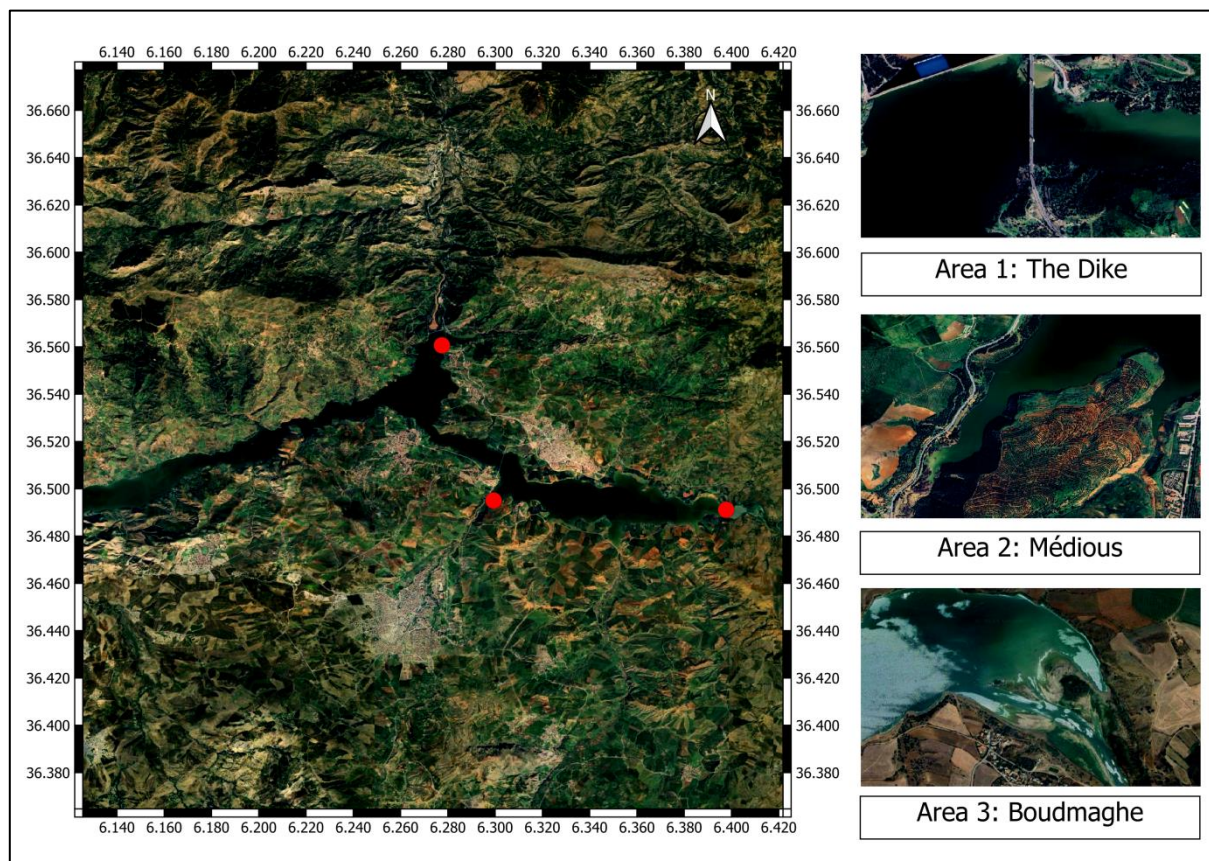


Figure 29: Google satellite location of each sampling point with close ups (Google Satellite, 2024).

4.3.2. Sampling

On the designated day, we undertook a scientific search aimed at detecting *Giardia lamblia*, a protozoan parasite commonly found in water sources. Our initial step involved the systematic collection of plankton's samples using the plankton net. Utilizing a filtration apparatus, we deployed a net with a conical mouth designed to ensnare planktons (Fig 30). These organisms serve as reservoirs for various pathogens, including *Giardia lamblia*. With meticulous precision, we submerged the net into the water, allowing it to immerse for a standardized duration of 15 to 30 seconds. Subsequently, we executed a controlled retrieval, ensuring minimal disturbance to the captured specimens. Following retrieval, we carefully transferred the collected planktons into designated sample bottles, repeating this process six times at distinct locations within the water body.

Through strict adherence to meticulous sampling protocols and conducting the procedure within the same day, we guarantee methodological consistency and data reliability. These collected samples will enable thorough analysis, revealing the presence of *Giardia*

lamblia and aiding in the assessment of water quality and safety. Our scientific approach underscores our dedication to advancing knowledge and safeguarding public health from waterborne pathogens.



Figure 30: Sampling in water utilizing a Plankton Net (**Personal photo, 2024**).

4.3.3. Preservation and storage

Samples were fixed on-site using Lugol's solution, with approximately 8 drops added to 250 ml of sample.

4.3.4. Transport and packaging

The samples intended for analysis were transported in a portable refrigeration unit maintained at 4 to 10°C. Subsequently, the water bottles were stored in a laboratory refrigerator for 24 to 48 hours post-sampling (**Fig 31**).

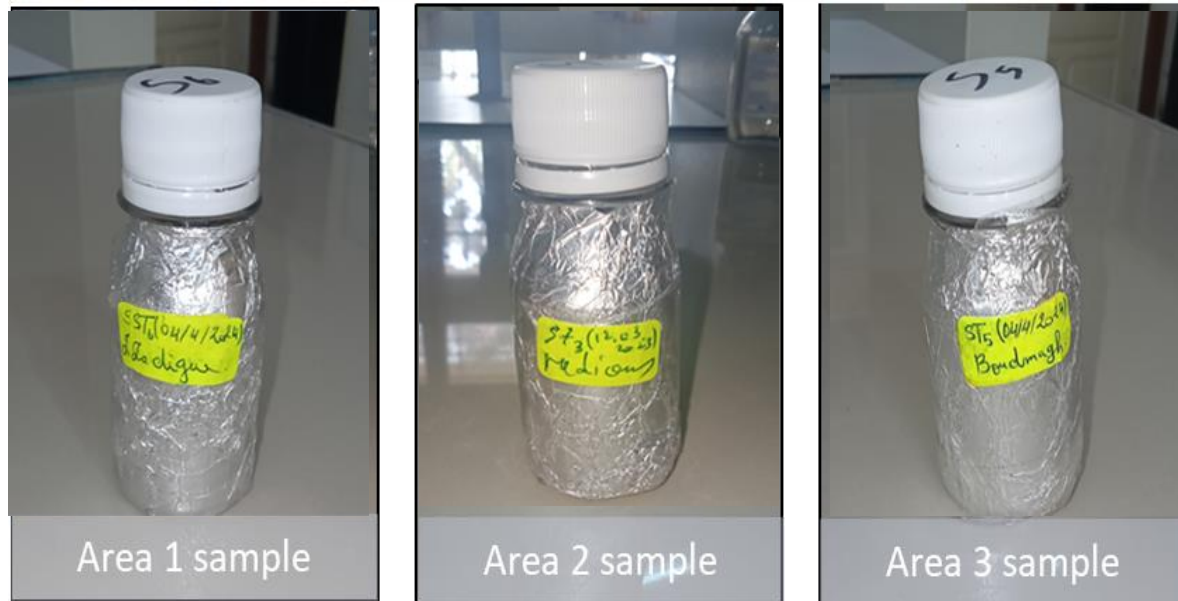


Figure 31: collection of water samples (Personal photo, 2024).

4.3.5. Analysis and examination

4.3.5.1. Material and equipment

It has been used through out this testing for *Giardia lamblia* (Fig 32):

- ✓ Microscope ;
- ✓ Microscope slides and covers ;
- ✓ Pasteur pipette ;
- ✓ Water samples ;
- ✓ Lugol solution.



Figure 32: Material and equipment used in the experimental procedure (Personal photo, 2024).

4.3.5.2. Steps to Experimental procedure

Upon arrival at the laboratory, the samples underwent microscopic observation following these steps:

- ✓ Sample Collection: Using a Pasteur pipette, a small drop or two of the sampled water was put.
- ✓ Slide Preparation: The collected sample was placed between a microscope slide and cover slip.
- ✓ Microscopic Observation: The sample was observed under a microscope using a 40x objective lens, suitable for identifying waterborne parasites.
- ✓ Identification: For the purpose of identification, images were acquired using mobile phones and a camera of "Optica" type to document and discern the morphological features of the parasites. I
- ✓ Identification was conducted based on morphological criteria including size, shape, and distinctive characteristics by the parasitologic doctors at the hospital establishment brother's meghlouai Mila.

4.4. Data statistical analysis

The data underwent input and processing using Excel software, alongside SPSS (Statistical Package for the Social Sciences) Version 26 and R software. Descriptive statistics concerning sex, age groups, years, seasons, and months were depicted using boxplots generated through the {ggplot2} package within R, which was utilized for all statistical analyses and graphical representations. Variations in each parameter across sex, age groups, months, seasons, years, and their interactions (sex $\times$ age groups) were assessed through one-way and two-way analysis of variance (ANOVA).

QGIS was employed to create maps and diagrams for each region of the state, providing a detailed visual analysis of the prevalence and epidemiology variations. Subsequently,

Student's t-tests were performed to discern variability within each parameter group. Pearson correlation tests were then employed to explore the relationships among *Giardia lamblia* dissemination parameters (including age, sex, months, seasons, years, and meteorological parameters), aiming to elucidate their behaviour and interconnections under the meteorological conditions prevailing in the state of Mila.

The resultant correlation matrix was visualized through an interactive correlation diagram, employing the {corrplot} package within R. Subsequently, utilizing the {nlme} package in R, we implemented generalized linear mixed models (GLMMs) to examine the

relationships between *Giardia lamblia* dissemination variation and the effects of temperature (T), sunshine duration (Sun), precipitation (P), wind speed (WS), and humidity (H).

Statistical significance for all tests was established at $p < 0.05$, with a confidence interval of 95%.



Results

5. Results

This survey reveals cases of giardiasis diagnosed at the parasitological analysis laboratories of the state of Mila during the period from 2013 to 2023. According to the prescriptions of attending physicians, patients presenting with digestive disorders, diarrhea, and abdominal pain were referred for a parasitological stool examination (PSE). During this period, 11433 patients were tested. In witch 138 tested positive for giardiasis. The epidemiological study covers four areas within the wilaya: Mila, Ferdjioua, Oued Athmania, and Chelghoum Laïd.

✚ The Simple Parasite Index (SPI)

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

$$\text{SPI} = (\text{the number of positive cases} \div \text{Total number of subjects examined}) \times 100$$

5.1. Overall prevalence of giardiasis during the descriptive study period (2013-2023)

5.1.1. Comprehensive state-wide retrospective analysis of the study population

5.1.1.1. *Distribution of patients according to infestation rate during the descriptive study period (2013-2023)*

(Table 11) and (Fig 33) shown that from 11433 subjects screened for giardiasis infection, 138 are infected; the infection rate of (1, 20%) during the descriptive study period (2013-2023).

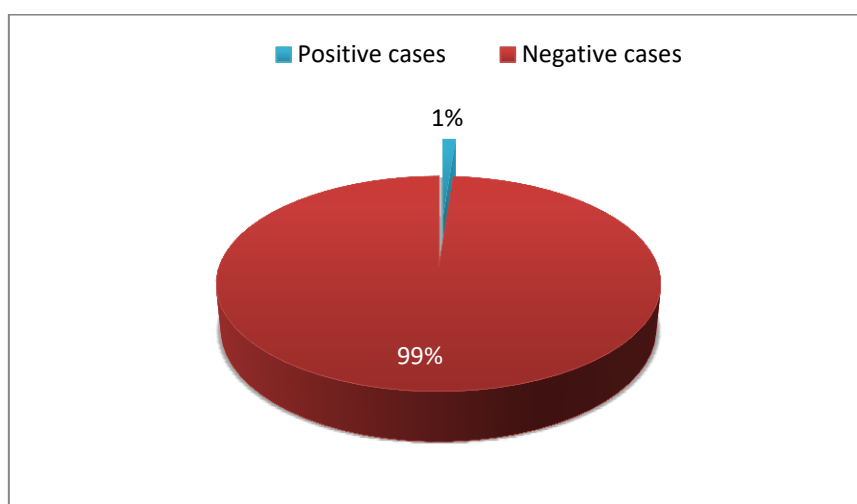


Figure 33: Distribution of patients according to infestation rate during the descriptive study period (2013-2023)

5.1.1.2. Distribution of infected patients according to sex ratio during the study period (2013-2023)

Our study revealed that the males are more exposed to giardiasis by (62,32%) in the state of Mila (**Table 12, Fig 34**), the graphs

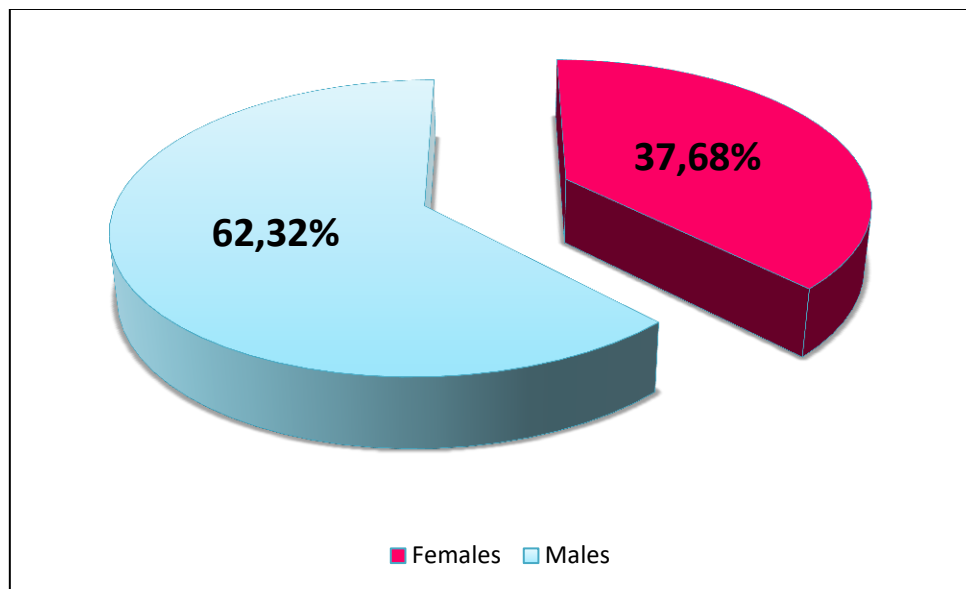


Figure 34: Distribution of infected patients according to sex ratio during the study period (2013-2023)

5.1.1.3. Distribution of infected patients according to age slices during the study period (2013-2023)

According to the data presented in (**Fig 35**) and (**Table 13**), the most affected age group is [20-44] years, with 78 cases and a rate of (56.52%). Followed by two categories, [5-9] age group and [15-19] age group which both show 16 cases and a rate of by 16 cases and a rate of (11,59%), The category [0-1] years and the over 65 is the less representative group by zero cases and a rate of zero percentage. Our data analysis revealed there is a significant difference between groups for Age ($p = ,035$).

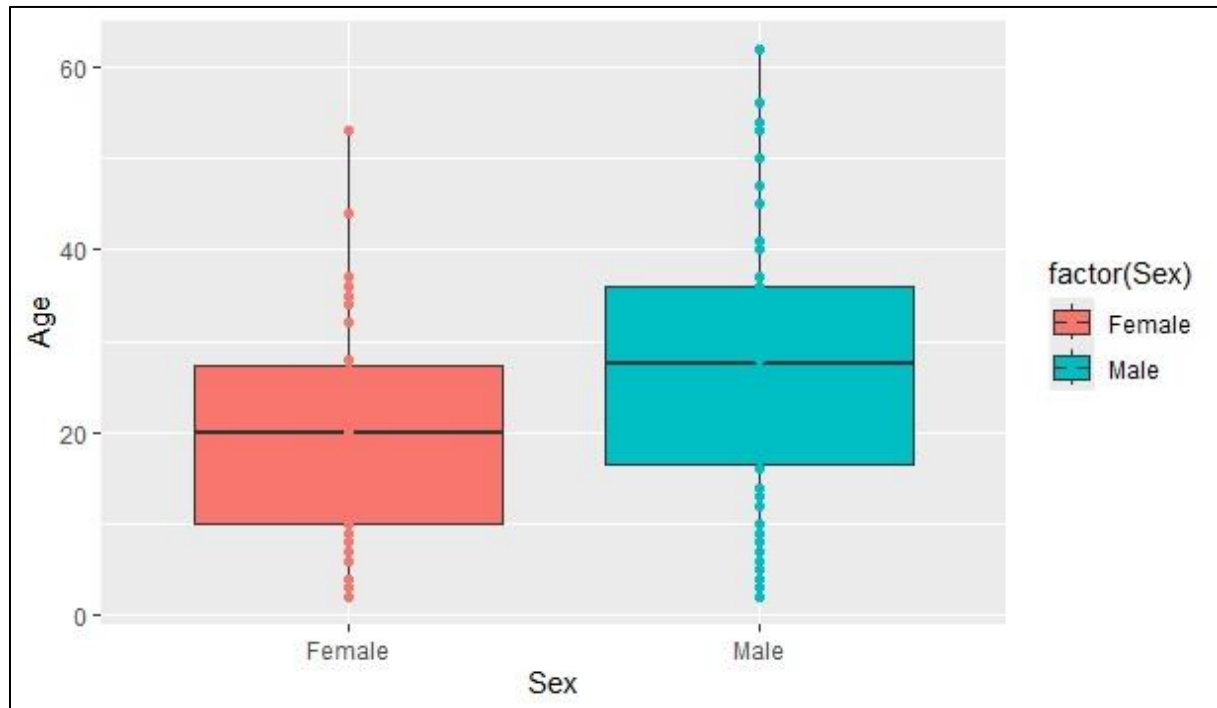


Figure 35: Boxplots displaying the distribution of infected patients according to sex ratio during the study period (2013-2023).

5.1.1.4. *Distribution of infected patients according to the months during the study period (2013-2023)*

(Table 14) and (Fig 36) show that the highest number of parasitical cases was recorded during the month of May at (14,49%), followed by March and February at (10,86%), (10,86%), respectively. The lowest percentages were detected in the months of December and July at a rate of (2,89%) and (2,17%), respectively over the study period. The results obtained

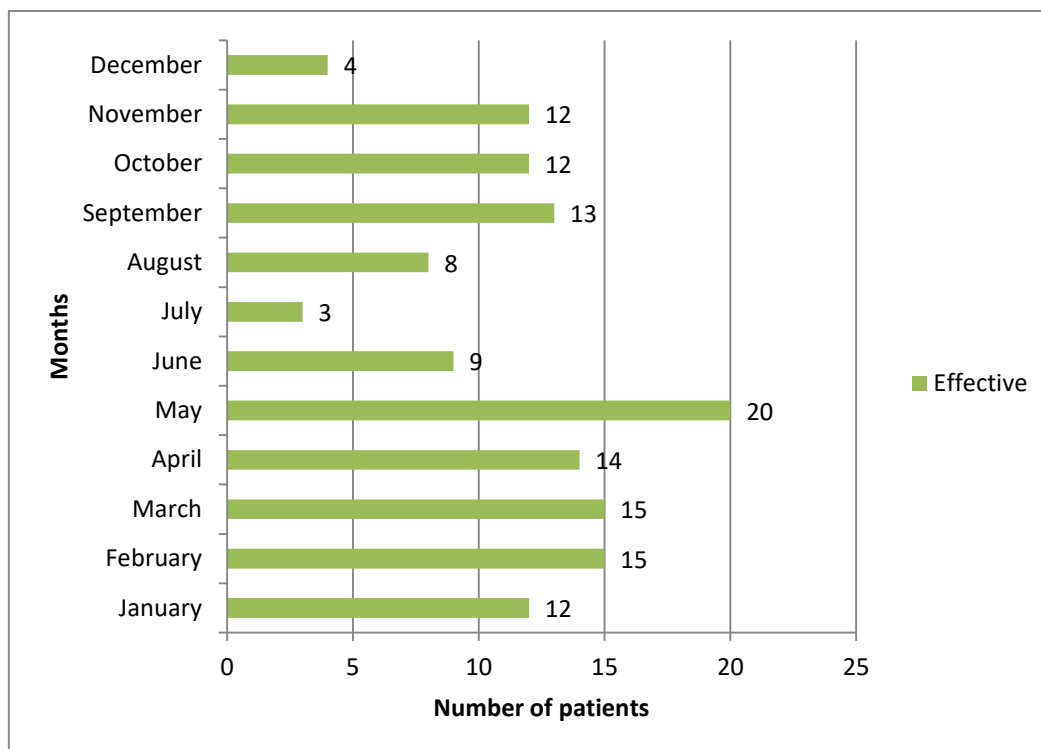


Figure 36: Distribution of infected patients according to the month during the study period (2013-2023)

5.1.1.5. *Distribution of infected patients according to the seasons during the study period (2013-2023)*

(Fig 37) and (Table 15) shows that the highest number of infected cases was observed during the Spring season, followed by the autumn season, with a rate of (35,50%) and (28,98%), respectively. While the lowest number of cases was recorded during the summer season, with a rate of (15,94%), respectively. The results obtained indicate statistically significant differences in giardiasis infection rates between seasons.

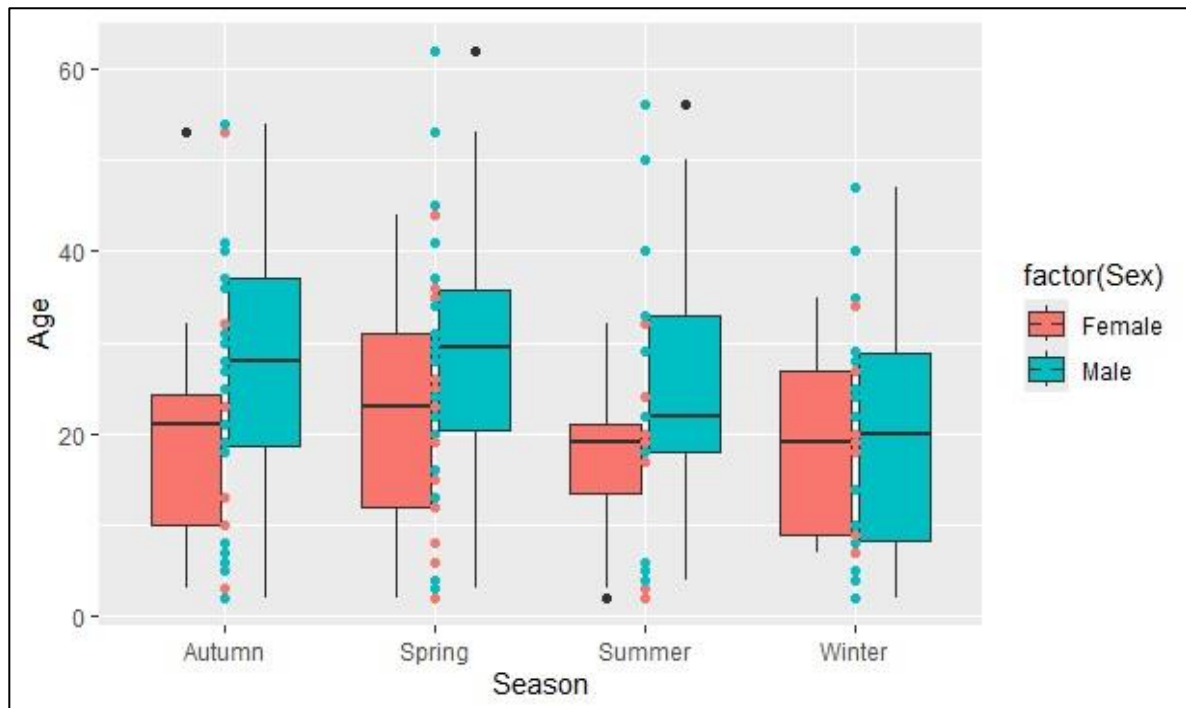


Figure 37: Boxplots displaying the distribution of age slices and genders of infected patients according to seasons during the study period (2013-2023).

5.1.1.6. Distribution of the infected patients according to the years during the study period (2013-2023)

The years 2014 and 2013 exhibited the highest rates of human giardiasis, registering a rate of (2%) and (1,62%), respectively, compared to other years (**Table 16**), (**Fig 38**). Conversely, the rates for human giardiasis ranged from (0,60%) to (0,19%) during the years 2020 and 2018, respectively. The analysis of data revealed a highly significant interaction between year and region ($p < 0,0001$) (**Table in annex**).

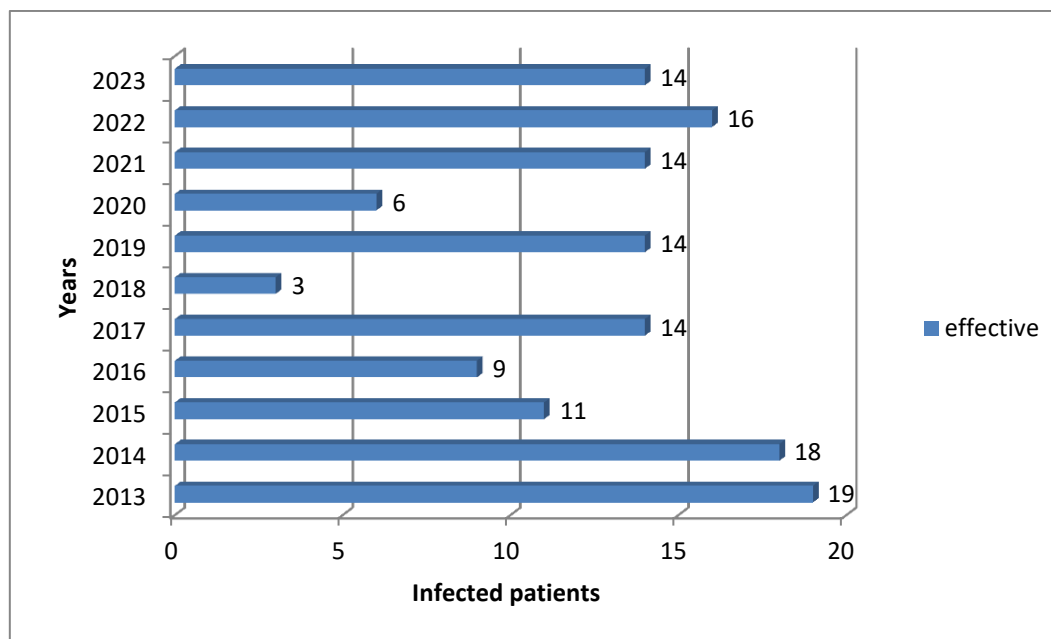


Figure 38: Distribution of infected patients according to the years during the study period (2013-2023).

5.1.2. Specific retrospective analysis of each region of the study area

5.1.2.1. Distribution of patients according to the region during the descriptive study period (2013-2023)

(Fig 39) and (Table 17) illustrates that during the study period (2013-2023), the Mila region recorded the highest number of patients, accounting for (53,62%) of the total. Following Mila, the Chelghoum Laid region and the Ferdjioua region recorded patient rates of (18,84%) and (18,11%), respectively. Then at last, the Oued Athmania region with a rate of (9,42%). The analysis of the data showed statistically a very highly significant difference between groups for Region ($p < ,001$) (Table in annex).

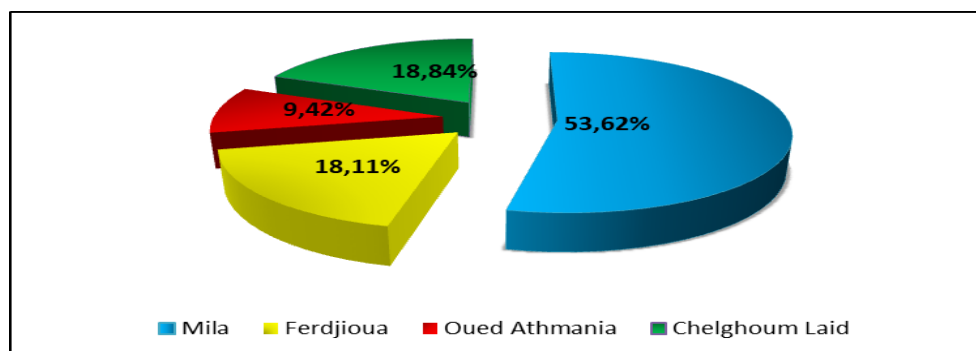


Figure 39: Distribution of patients according to the region during the descriptive study period (2013-2023)

5.1.2.2. Distribution of patients according to infestation rate during the descriptive study period (2013-2023)

The data presented in (Fig 40), (Table 18) indicate that the infestation rates of giardiasis in the regions of Mila, Ferdjioua, Oued Athmania, and Chelghoul Laid are (0,87%), (2,95%), (1,76%), and (1,86%), respectively. Notably, the highest infestation rate is observed in Ferdjioua.

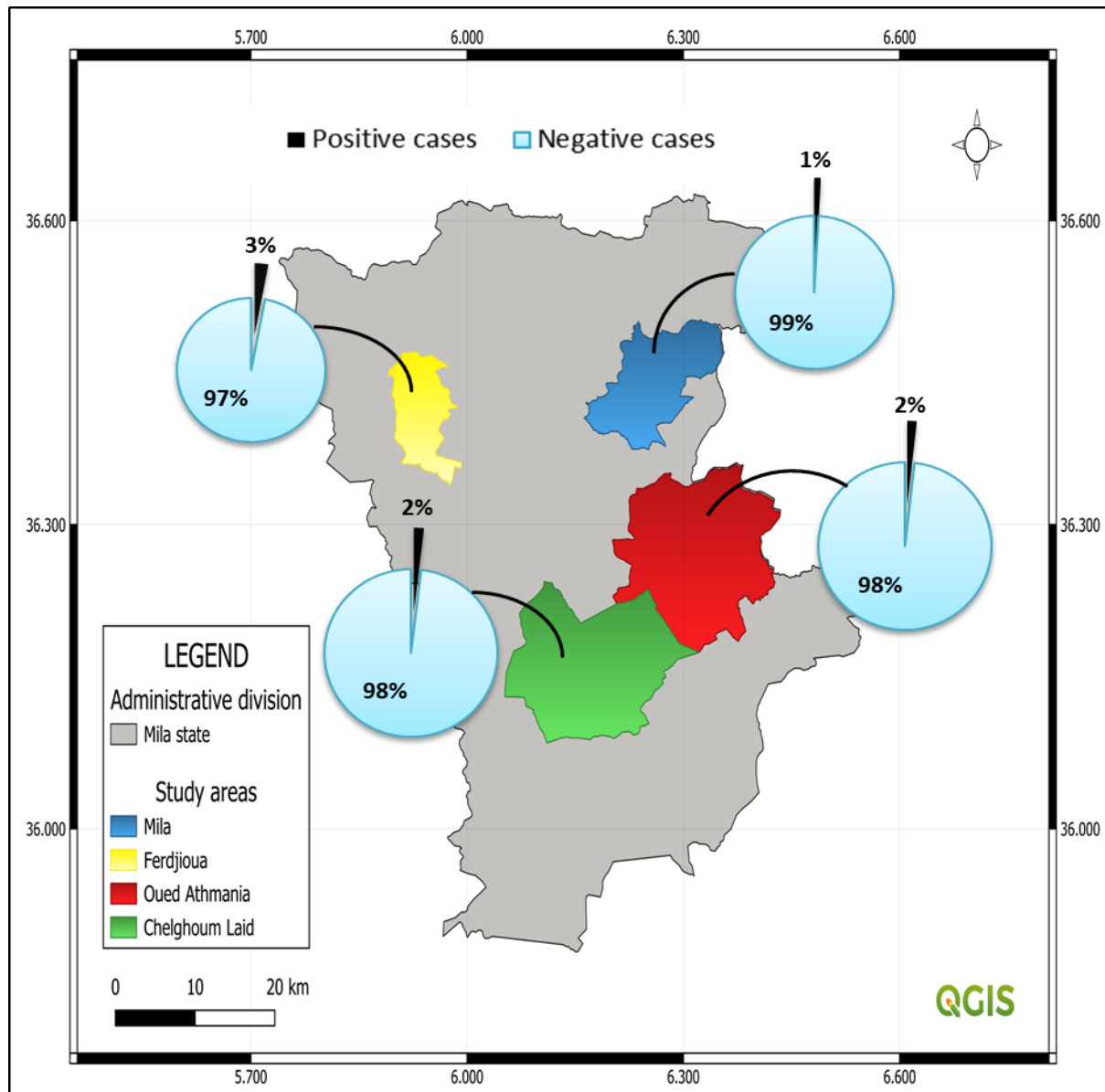


Figure 40: Regional distribution of patients according to infection rate during the study period (2013-2023). Detailed: (A) In the region of Mila (74 positive cases out of 8526). (B) In the region of Ferdjioua (25 positive cases out of 820). (C) In the region of Oued Athmania (13 positive cases out of 737). (D) In the region of Chelghoul Laid (26 positive cases out of 1396).

5.1.2.3. Distribution of infected patients according to sex ratio during the study period (2013-2023)

Our study revealed that in **(Fig 41), (Table 19)** the highest prevalence was along men in the regions of Chelghoum Laid and Oued Athmania and Mila, at (76,92%), (76,00%), and (56%), respectively. Conversely, the Ferdjioua region showed significant high infection rates among females at (56,00%).

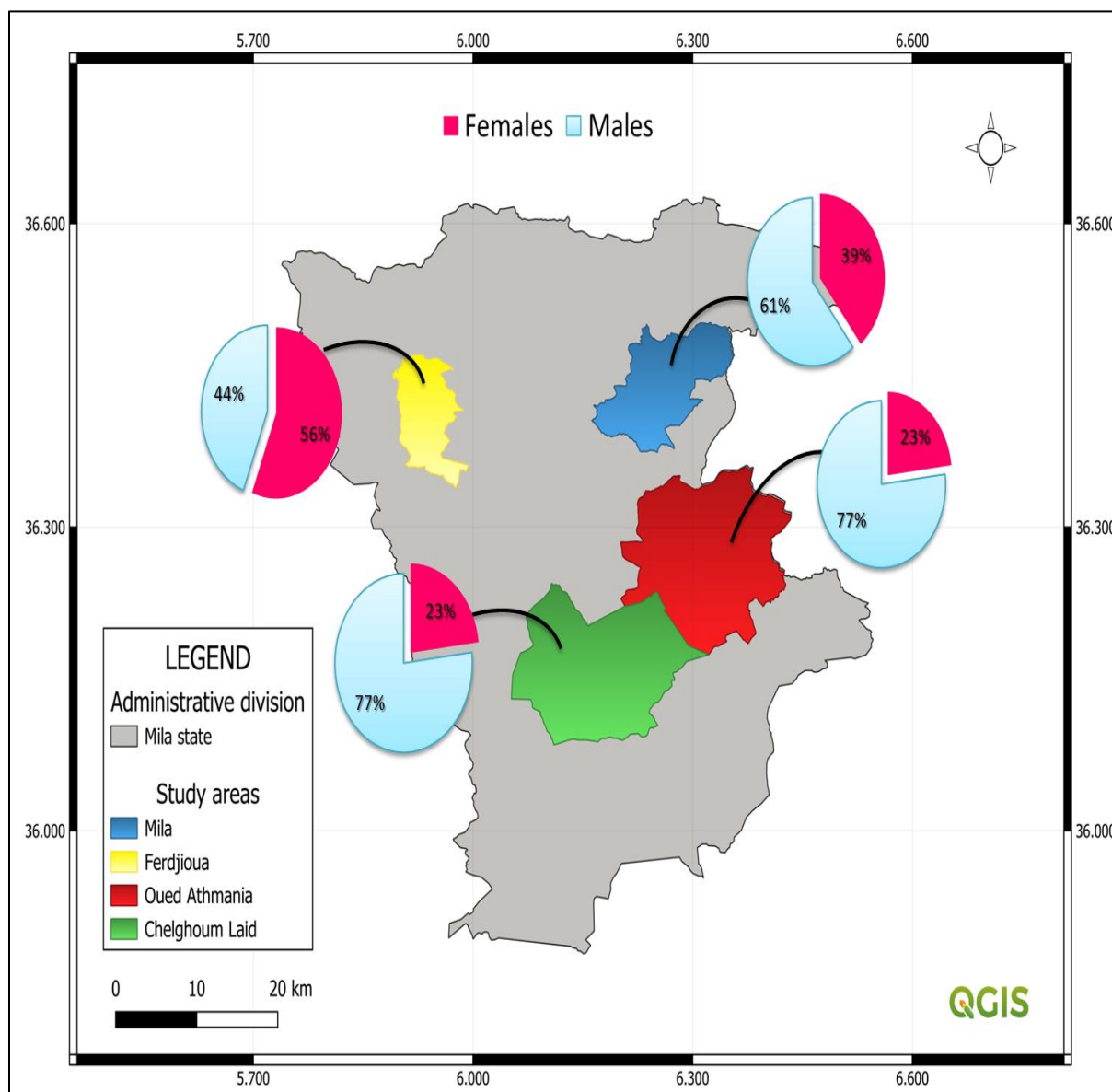


Figure 41: Regional distribution of infected patients according to the sex ratio during the period (2013-2023). Detailed: Mila (45 males/29 females), Ferdjioua (11 males/14 females), Oued Athmania (10 males/3 females), Chelghoum Laid (20 males/6 females).

5.1.2.4. Distribution of infected patients according to age slices during the study period (2013-2023)

Based on the data represented in the (Fig 42), (Table 20) we noted that the most affected age group within the four regions of the study is [20-44] years with 40 cases in Mila, 16 cases in Chelghoum laid, 12 cases in Ferdjioua, and 11 cases in Oued Athmania, and infection rate of (54,05 %), (61,53 %), (50 %), (84,61 %), correspondingly.

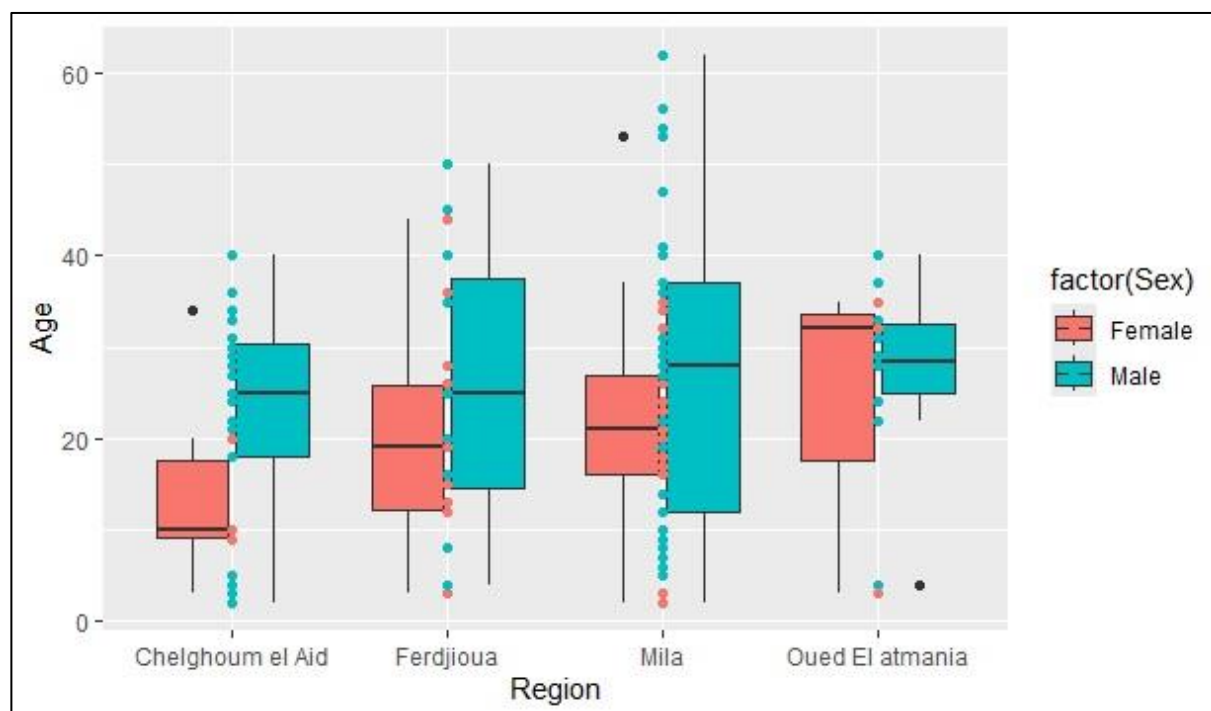


Figure 42: Bloxplots displaying the distribution of age slices and gender of infected patients by region during the study period (2013-2023).

5.1.2.5. Distribution of infected patients according to the months during the period (2013-2023)

The data presented in (Fig 43), (Table 21) indicate that both the region of Mila and Ferdjioua shared higher prevalence in May with a rate of (16,21%) and (20,00%), respectively. While regions like Ferdjioua and Chelghoum Laid shared a high prevalence rate in of March with a rate of (20,00%) and (15,38%), respectively. At last a pattern was also noticed between the regions of Mila and Chelghoum Laid indicating a common high prevalence rate in November with a rate of (10,81%) and (11,53%), respectively.

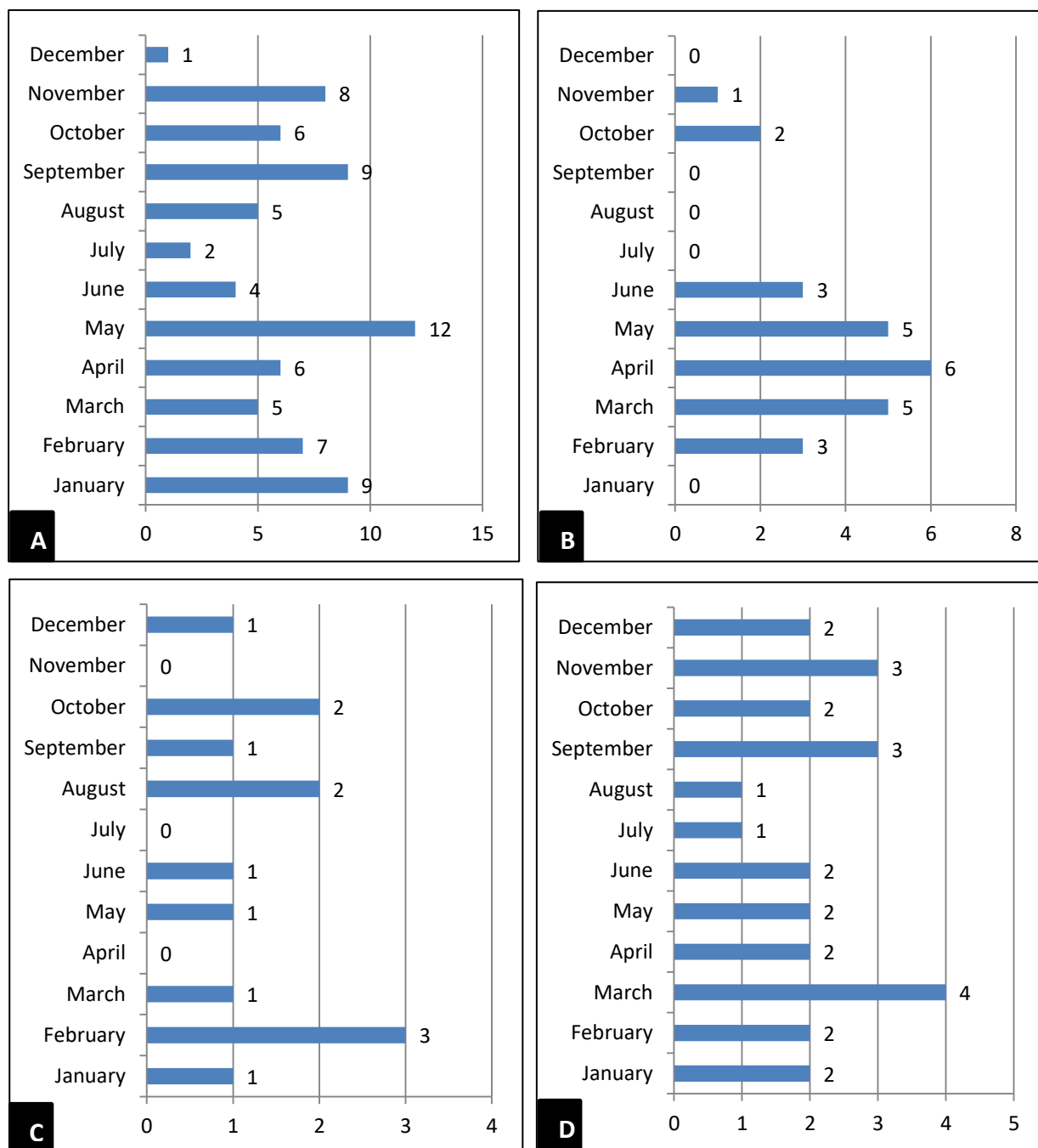


Figure 43: Regional distribution of infected patients by month during the period (2013-2023).
Detailed: **(A)** In the region of Mila. **(B)** In the region of Ferdjioua. **(C)** In the region of Oued
Athmania. **(D)** In the region of Chelghoum Laid.

5.2.1.6. Distribution of infected patients according to the seasons during the period (2013-2023)

(Fig 44), (Table 22) demonstrates that the number of infected cases in regions such as Mila, Oued Athmania and Chelghoum Laid followed a pattern, with the highest infection rates occurring during the autumn months, with a rate of (32,43%) and (38,46%), (30,76%) respectively. On the other hand a pattern also been followed by the regions of Mila, Ferdjioua and Chelghoum Laid with an increased rate of infections during the spring months, with rates of (31,08%), (64,00%) and (30,76%), respectively. It is noteworthy that the winter season showed an increase of cases in both the region of Mila and Oued athmania with a rate of (21,62%) and (30,76%).

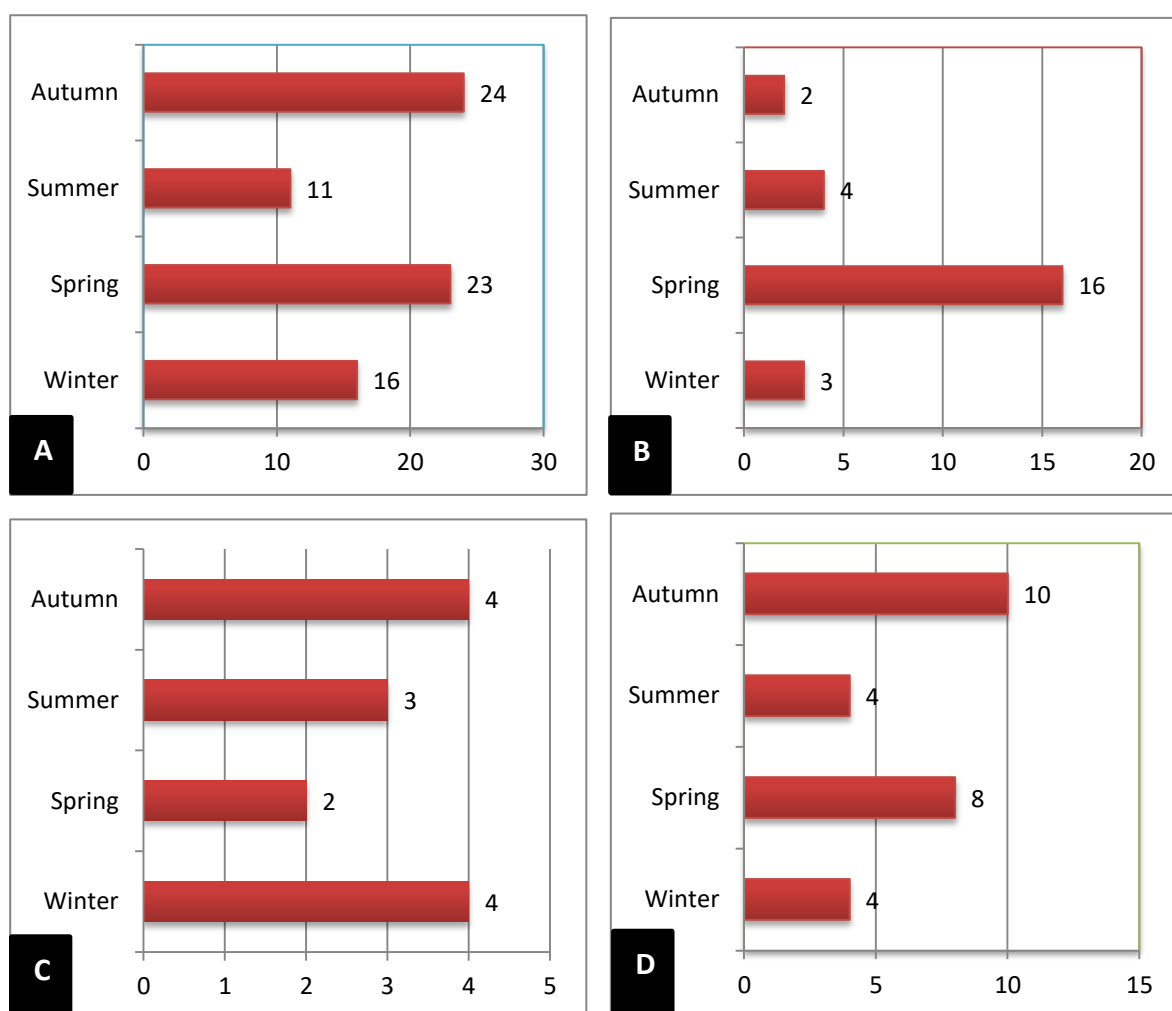


Figure 44: Regional distribution of infected patients by season during the period (2013-2023). Detailed: **(A)** In the region of Mila. **(B)** In the region of Ferdjioua. **(C)** In the region of Oued Athmania. **(D)** In the region of Chelghoum Laid.

5.2.1.7. Distribution of patients according to the years during the study period (2013- 2023)

The years 2023, 2022, 2021, and 2019 recorded the highest rates of giardiasis infection in regions such as Ferdjioua, Chelghoum Laid, and Oued Athmania compared to other years, with rates of (20%), (32%), (28%), and (16%) for Ferdjioua; (11,53%), (23,07%), (11,53%), and (18,75%) for Chelghoum Laid; and (30,76%), (7,69%), (23,07%), and (23,07%) for Oued Athmania, respectively. Meanwhile, in the Mila region, the highest infection rates compared to other years were observed in 2013, 2014, 2015, and 2017, with rates of (20,27%), (20,27%), (14,86%), and (17,56%), respectively (**Fig 45**), (**Table 23**).

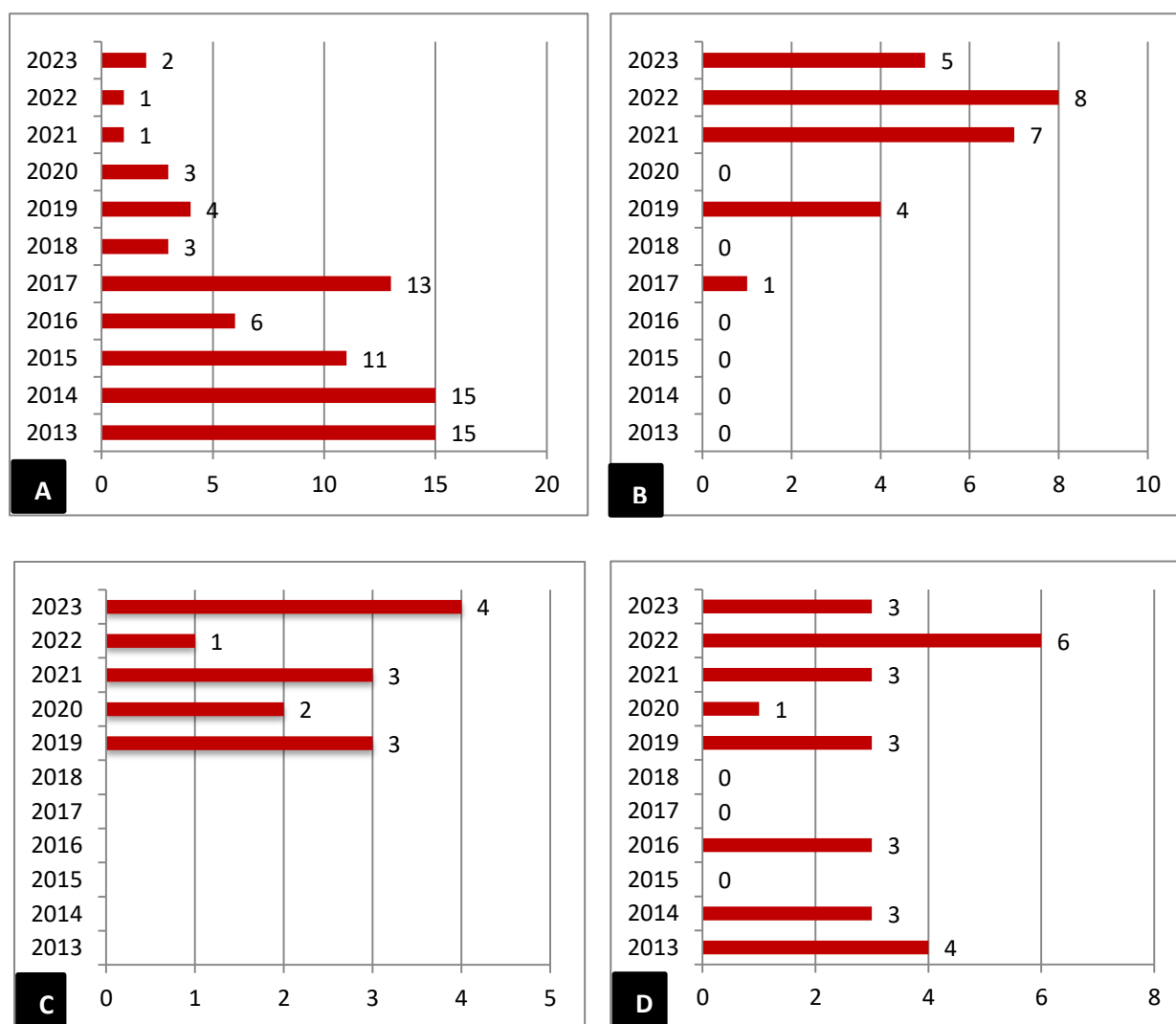


Figure 45: Regional distribution of patients according to the years during the period (2013-2023). Detailed: **(A)** In the region of Mila. **(B)** In the region of Ferdjioua. **(C)** In the region of Pued Athmania. **(D)** In the region of Chelghoum Laid.

5.2. Overall prevalence of giardiasis during the prospective study period

5.2.2. Distribution of patients according to infestation rate during the period (January – March 2024)

(Fig 46), (Table 24) have shown that from 293 subjects examined for human giardiasis, 5 were found infected, from January-March 2024 with an infestation rate of (1,70%).

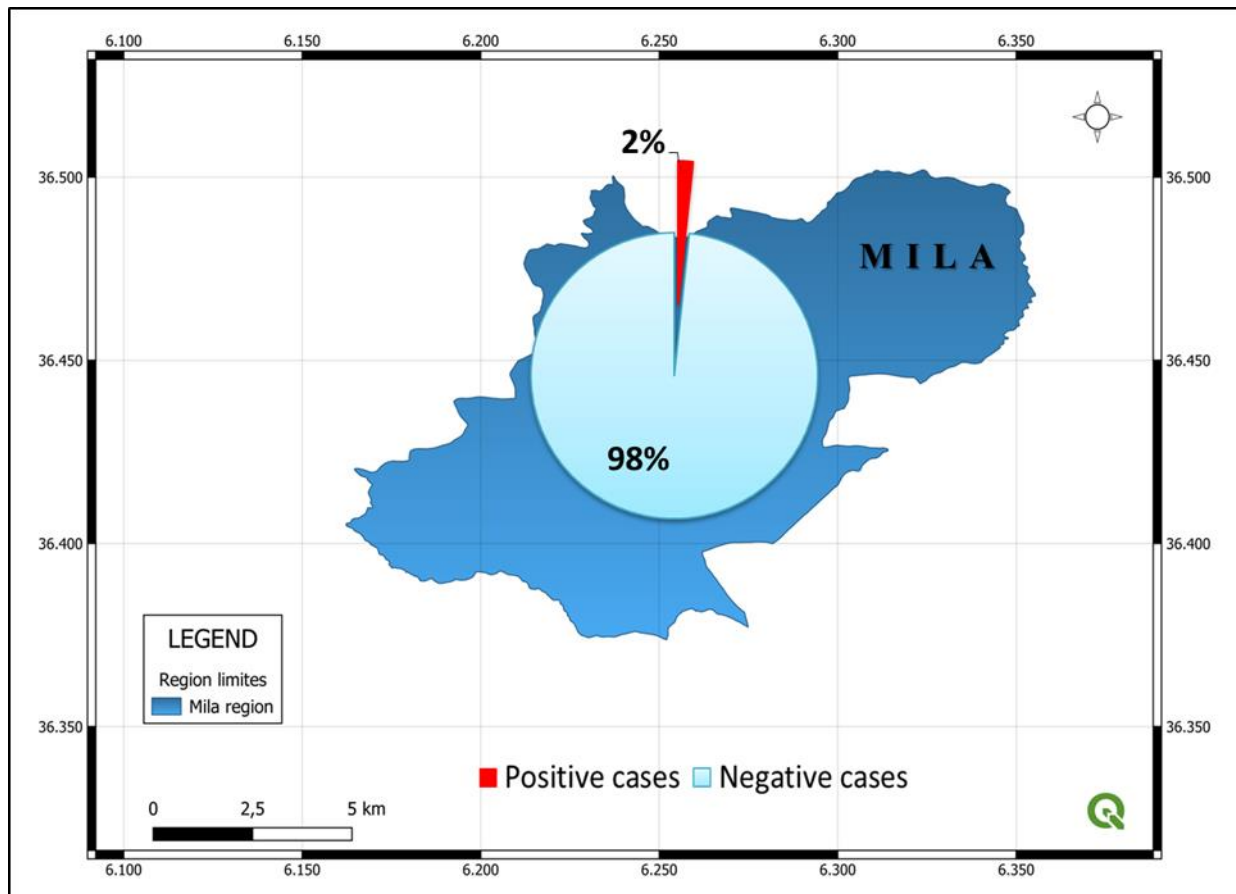


Figure 46: Distribution of patients by infestation rate in the region of Mila over the study period (January-March 2024).

5.2.3. Distribution of infected patients by sex ratio during the period (January-March 2024)

(Table 25), (Fig 47) revealed that the most of infected cases were man with a prevalence rate of (60%).

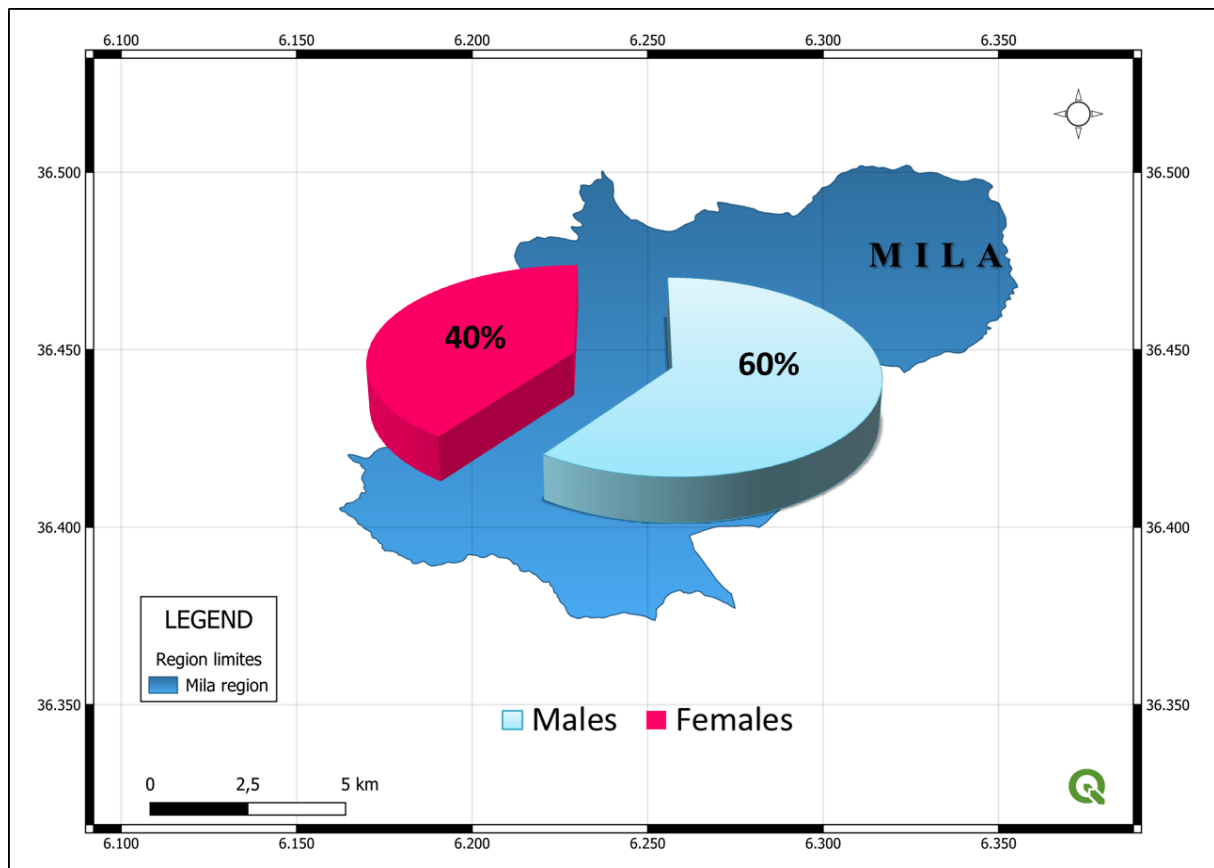


Figure 47: The distribution of infected patients in the region of Mila according to the sex ratio during the study period (January-March 2024).

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

In (Fig 48) and (Table 26) it was notice that the age group [20-44] years is the most affected by *Giardiasis* with (80%) followed by the age groups included between [10-14] years with a percentage of (20%)

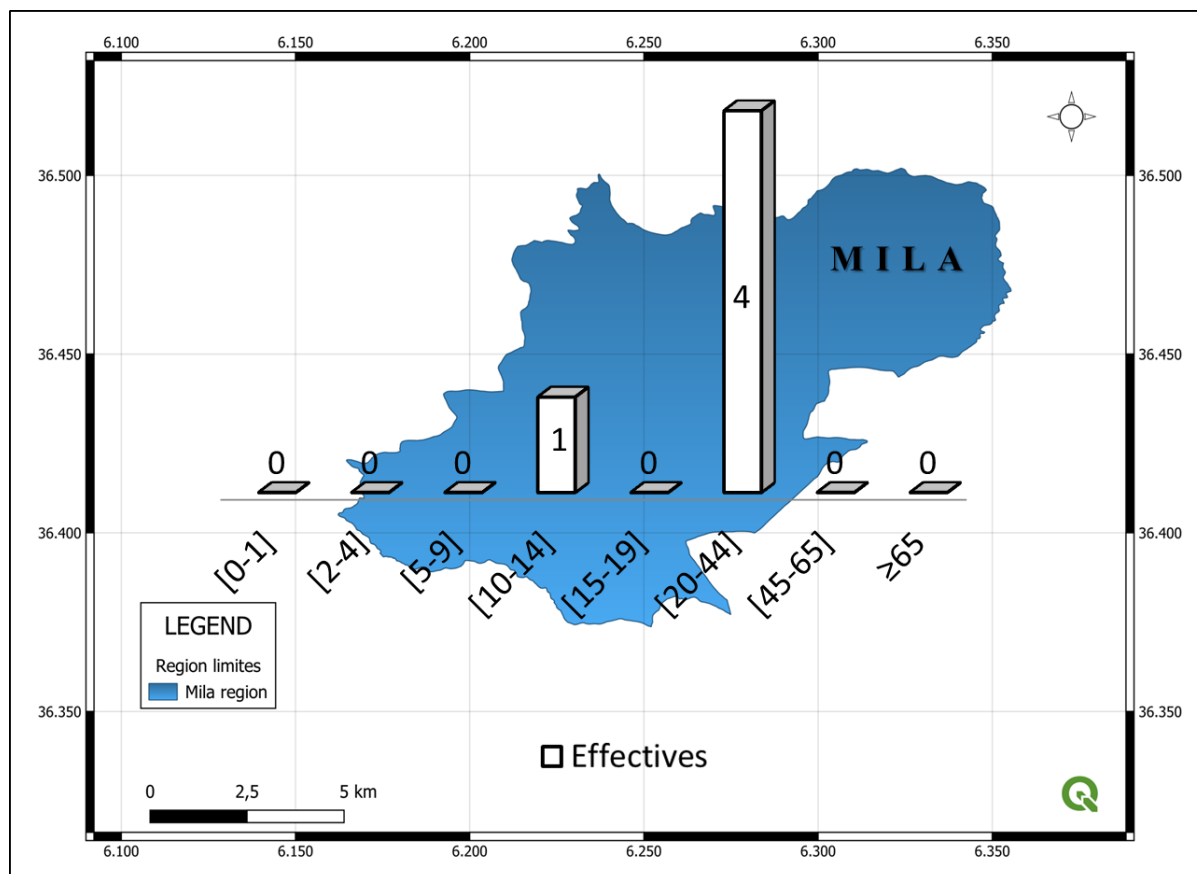


Figure 48: Distribution of patients in the region of Mila according to age groups during the prospective study period (January – March 2024).

5.2.5. Distribution of infected patients by months during the period (January-March 2024)

(Table 27), (Fig 49) shown that the highest number of Giardiasis cases was noted during the month of March (60%), followed by the months of January and February with (20%) and (20 %) during the period (January-March 2024)

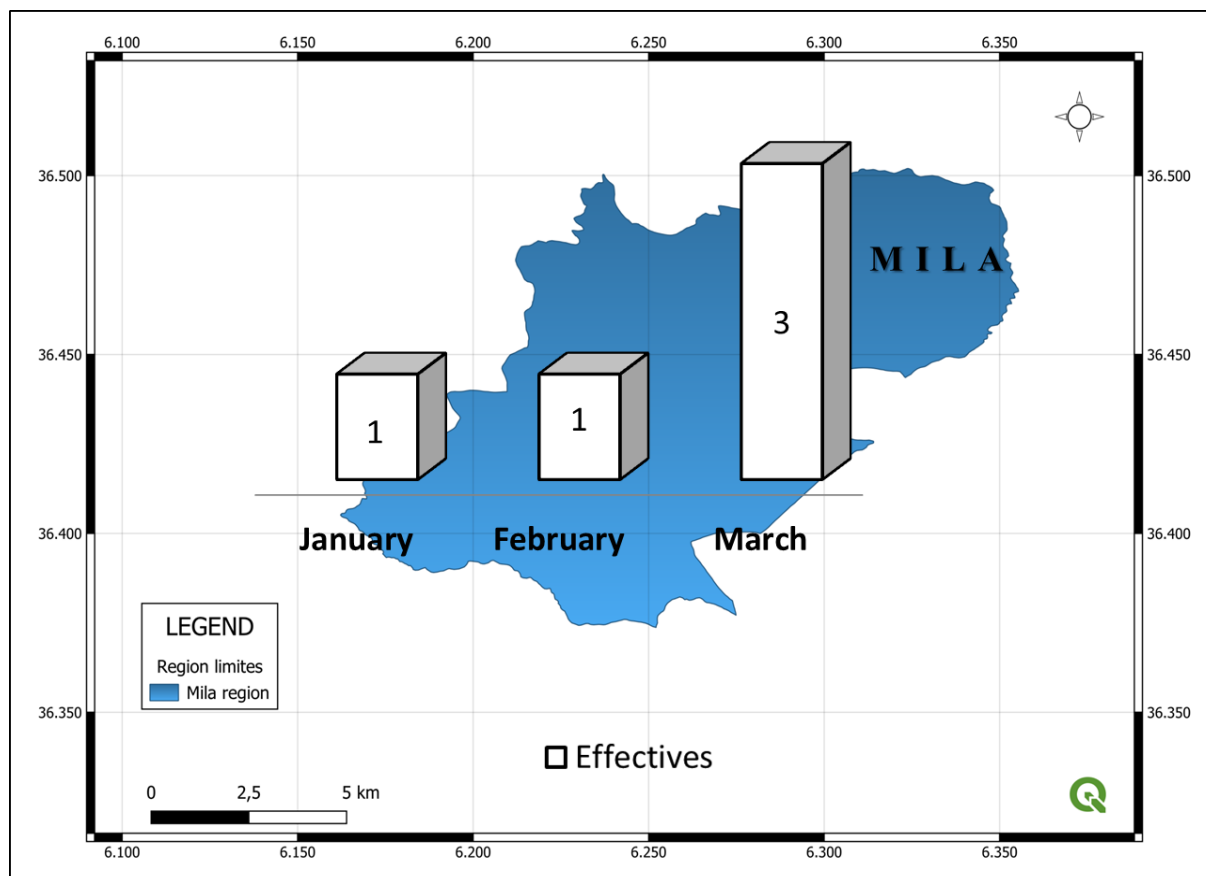


Figure 49: Distribution of infected patients in the region of Mila by months during the study period (January-March 2024).

5.2.6. Stool Examination for *Giardia lamblia* Detection in Patients: Prospective Study (January-March 2024)

Our prospective study involved 293 patients referred to a parasitology laboratory during the first three months of 2024 in which 5 were diagnosed with giardiasis. This approach facilitated the microscopic examination of fresh stool samples, allowing for the detection of the pathogenic parasite *Giardia lamblia* (Fig 47/48).



Figure 50: The detection of *Giardia lamblia* under microscope stained with physiological water (objective X 40) (Personal photo, 2024).



Figure 51: Close up into *Giardia lamblia* detected in fresh stool under microscope (objective X40) (Personal photo, 2024).

5.3. Correlation between the variation of metrological parameters and the propagation of Giardiasis during the period (2013-2023)

To identify the relationship between meteorological parameters and the propagation of human giardiasis 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 giardiasis dissemination variation. Pearson correlation will be used to clarify the correlation between the dissemination of giardiasis and the variation of different meteorological parameters.

5.3.1. The relationship between the variation of the average temperature and the number of infected cases during the period (2013-2023)

Linear regression and Pearson correlation analysis (**Fig 52**) demonstrated that the number of infected patients increase progressively with the increase in the average temperature. This indicates a very strong positive correlation between the variation in average temperature (°C) and the number of infected patients over the period from 2013 to 2023.

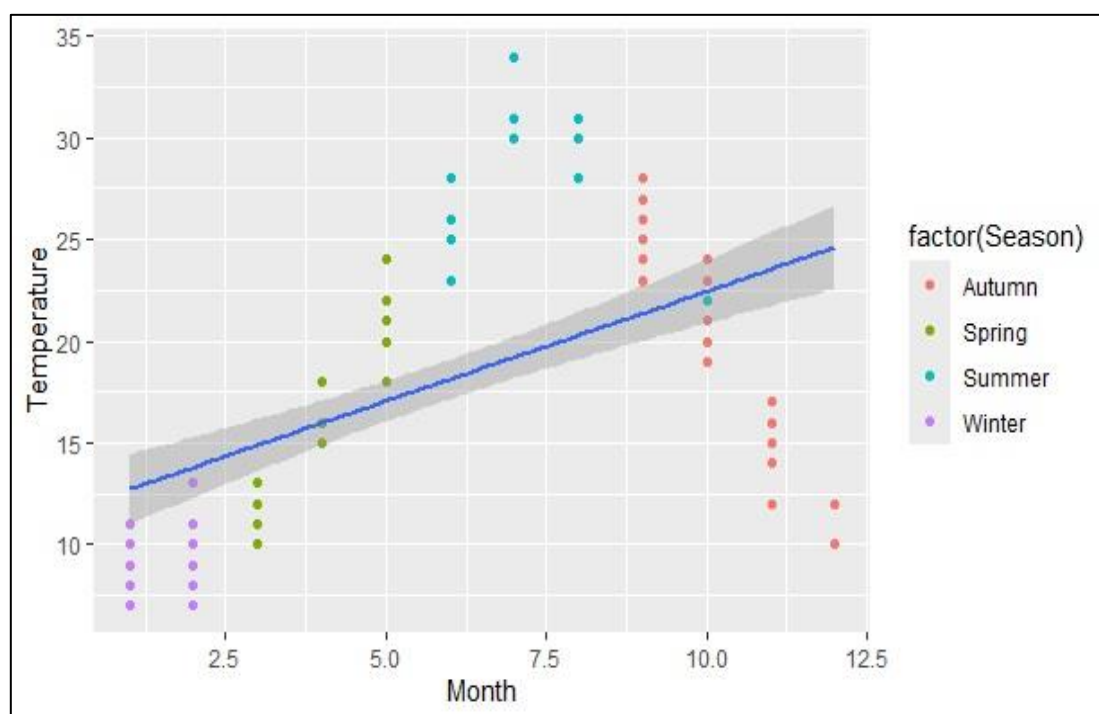


Figure 52: The correlation between the average temperature (°C) and the number of infected patients according to the months and the season during the study period (2013-2023).

5.3.2. The relationship between the variation of the average precipitation and the number of infected cases during the period (2013-2023)

Linear regression and Pearson correlation (**Fig 53**) showed that the number of infected patients decreases progressively with the increase in mean precipitation, so there is a very strong negative correlation between the variation in mean precipitation (mm) and the number of infected patients during the study period (2013-2023).

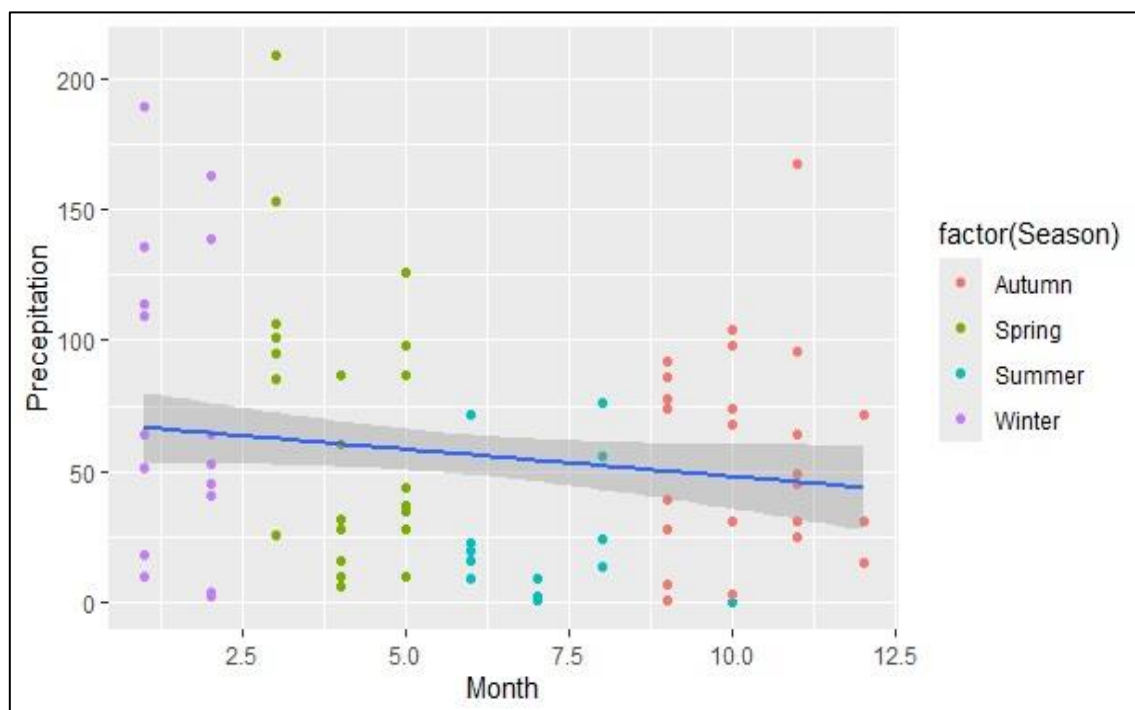


Figure 53: The coorelation between the average precipitation (mm) and the number of infected patients according to the months and the season during the study period (2013-2023).

5.3.3. The relationship between the variation of the average wind speed and the number of infected cases during the period (2013-2023)

Linear regression and Pearson correlation (**Fig 54**) 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 patients during the period (2013-2023).

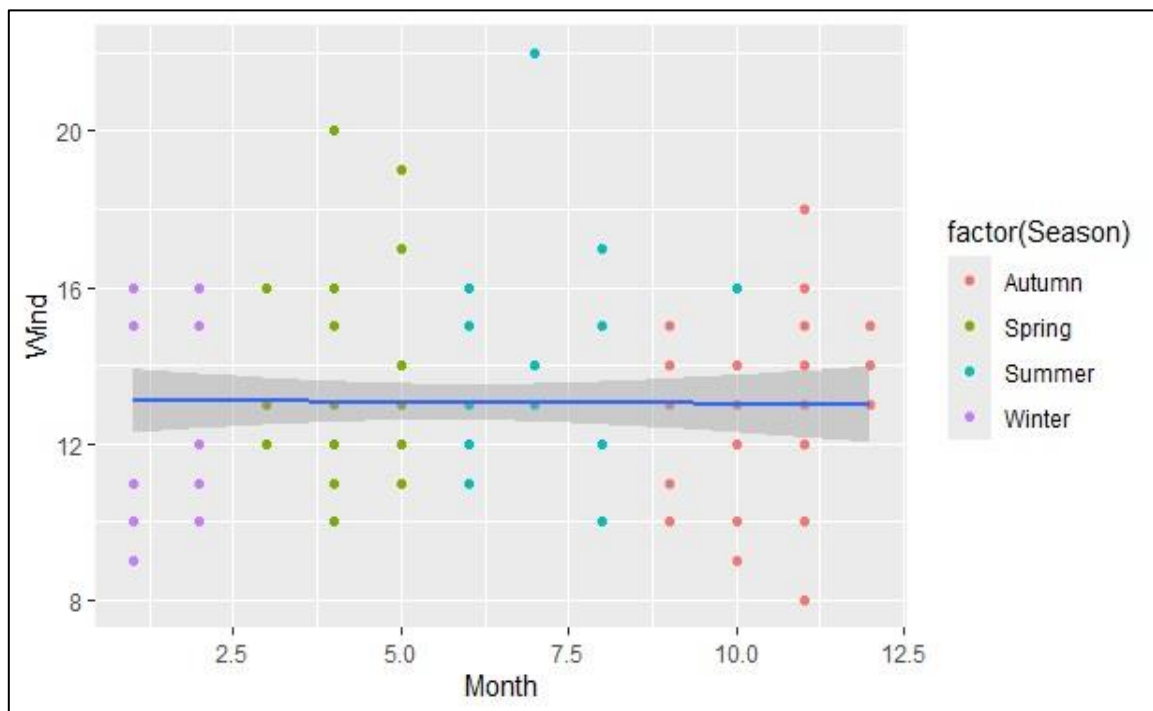


Figure 54: The correlation between the average wind speed (Knots) and the number of infected patients according to the months and the season during the study period (2013-2023).

5.3.4. The relationship between the variation of the average humidity and the number of infected cases during the period (2013-2023)

Linear regression and Pearson correlation (**Fig 55**) revealed that the number of infected patients decreases progressively with increasing humidity, so there is a very strong negative correlation between the average humidity (g/m³) and the number of infected patients during the study period (2013-2023).

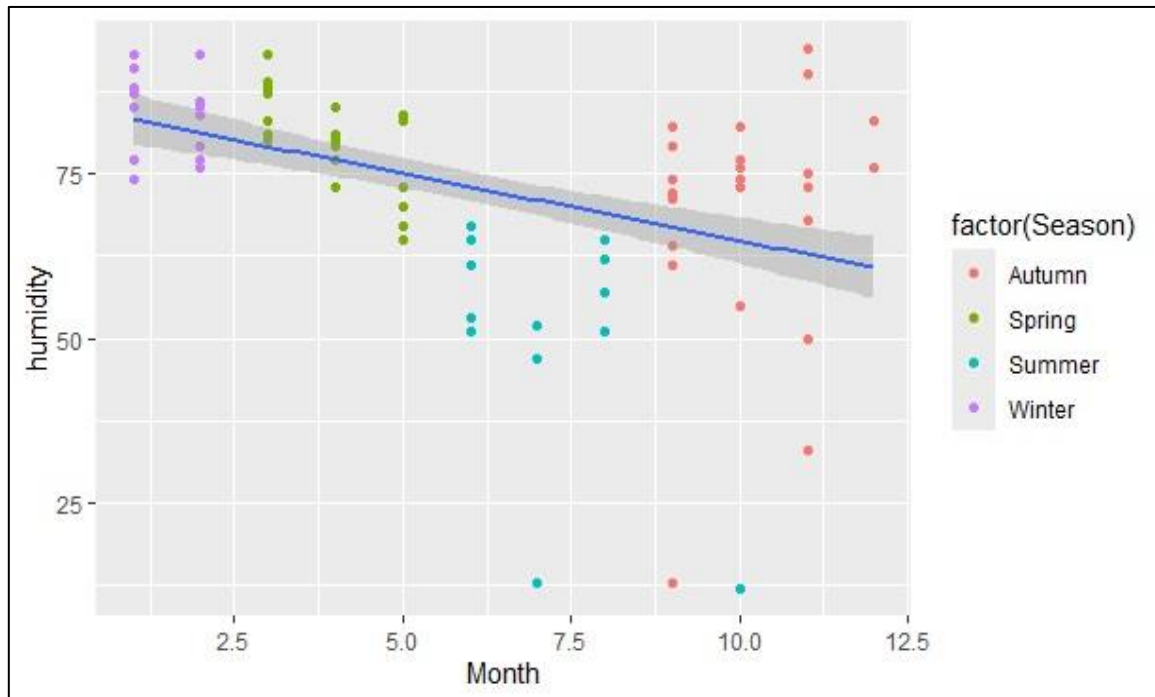


Figure 55: The correlation between the average humidity (g/m3) and the number of infected patients according to the months and the season during the study period (2013-2023).

5.3.5. The relationship between the variation of the average sunshine duration and the number of infected cases during the period (2013-2023)

Linear regression (**Fig 56**) showed that the number of infected patients decreases progressively with the increase in the average duration of sunshine (hours) so there is a medium negative correlation between the average duration of sunshine (hours) and the number of infected patients during the study period (2013-2023).

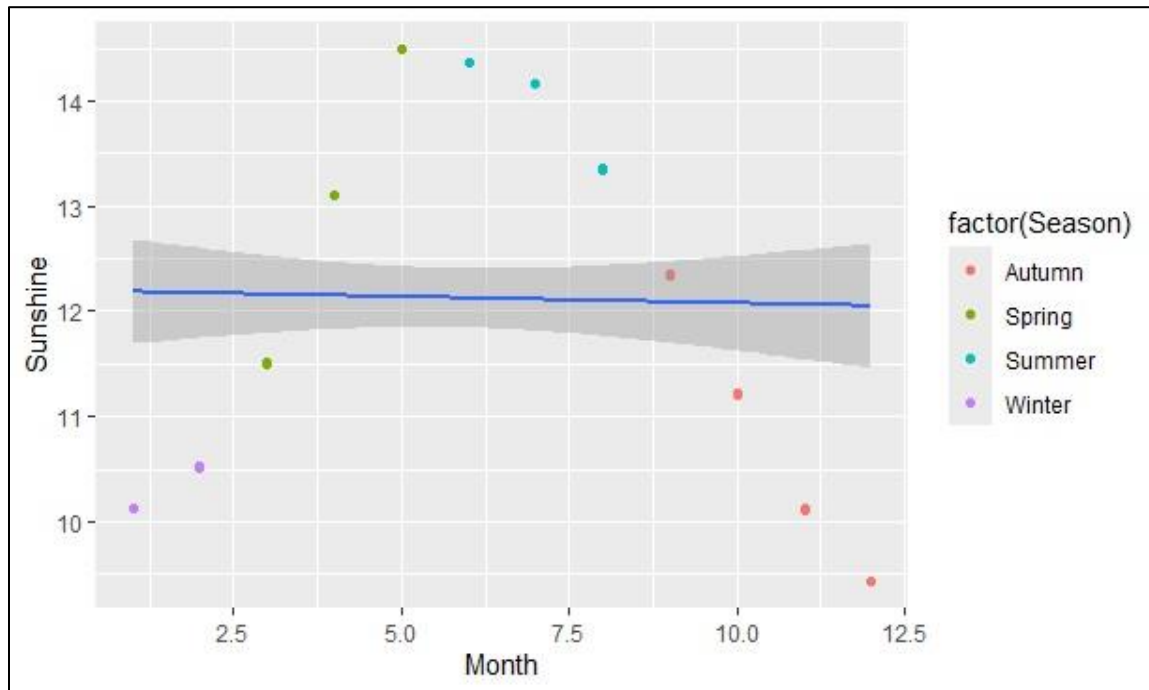


Figure 56: The correlation between the average sunshine duration (hours) and the number of infected patients according to the months and the season during the study period (2013-2023).

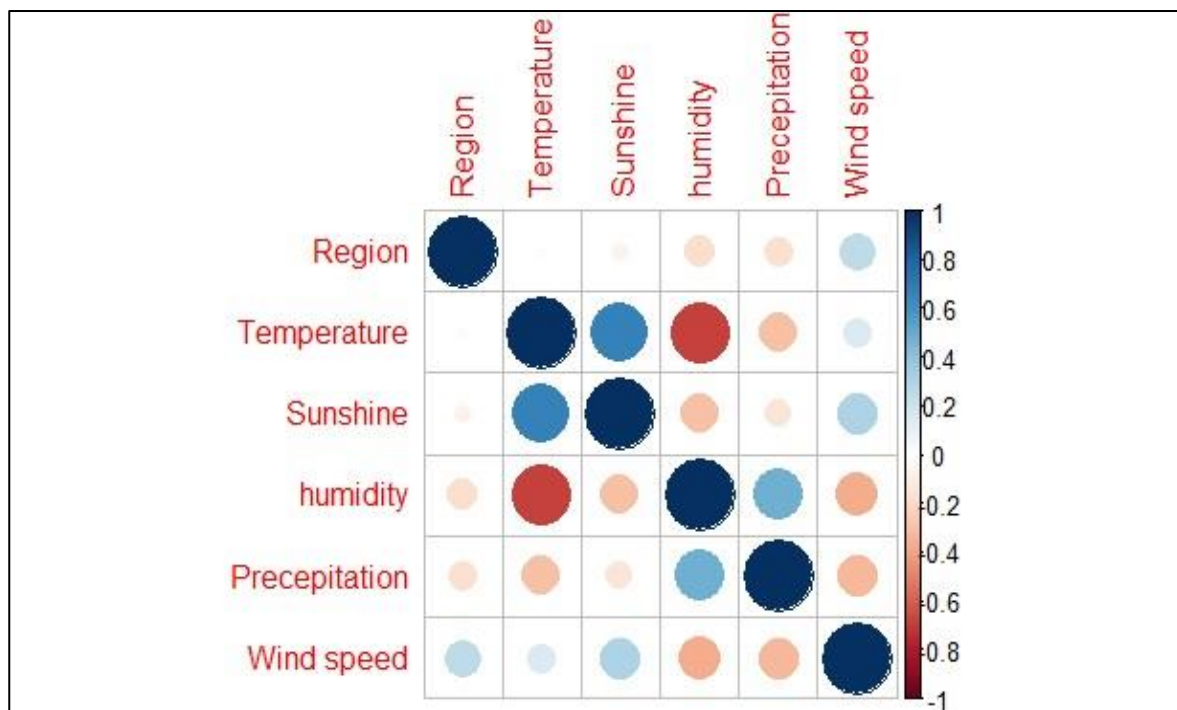


Figure 57: Correlation matrix applied between metrological parameters and number of cases of human giardiasis. Pearson correlation tests are given as correlation coefficients

5.4. Investigation Outcomes

In our laboratory work aimed at detecting *Giardia lamblia* within three regions of the dam water, comprehensive water analyses were conducted. The regions studied included Médious, Boudmaghe, and La Digue. Through detailed microscopic examination and morphological identification, *Giardia lamblia* was identified (**Fig 58 / 59**) in the form of cysts within the water samples collected from the third area which is Boudmaghe. This finding underscores the presence of this pathogenic protozoan in the Boudmaghe region.

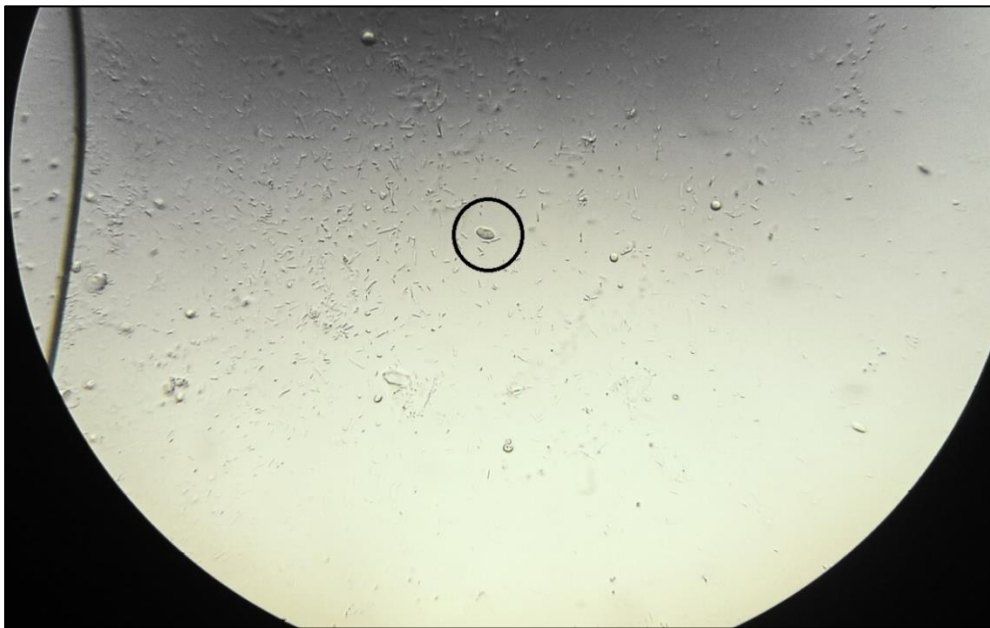


Figure 58: *Giardia lamblia* under microscope stained with lugol idiom (Objective X 40).
(Personal photo, 2024).

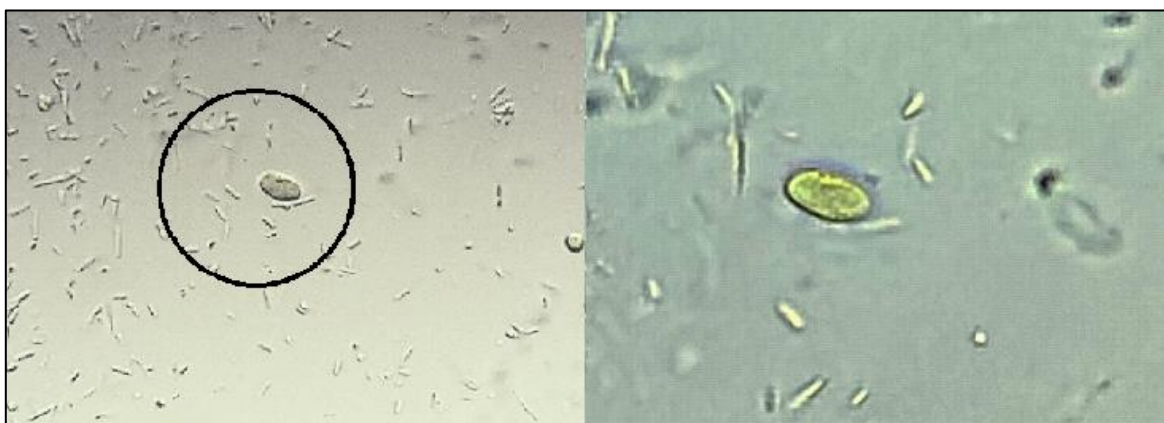


Figure 59: Close up photos of *Giardia lamblia* under microscope stained with lugol / sample site 2 (Objective X 40) (Personal photo, 2024).

DISCUSSION



6. Discussion

Giardia lamblia is one of the most prevalent parasites found in the human digestive tract. Described over a century ago, *Giardia lamblia* remains a significant focus of research due to its impact on human health. The clinical relevance of *Giardia lamblia* is notable, as it can infect both symptomatic and asymptomatic carriers, leading to a range of gastrointestinal symptoms and complications (**Thompson and Monis, 2012**).

This job aims at determining the rate of prevalence of human giardiasis diagnosed at the level of service of parasitology-mycology of four different hospital establishments located in the Mila province in the course of a period from (January 2013 to December 2023) an analytical descriptive study was led and concerned with the results of exams parasitological of the stool. In addition, this research aims to indicate the correlation between the prevalence of human giardiasis in the region of Mila and abiotic factors. The comparison of our results with those of scientific literature allowed the identification of *Giardia lamblia* 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.

The findings of the present study revealed a prevalence of (1,20%) for *Giardia lamblia* infection across four distinct regions within the Mila province (Mila, Ferdjioua, Oued Athmania, Chelghoum Laid). The prevalence rates for each region within the state are as follows: Mila (0.87%), Ferdjioua (2.95%), Oued Athmania (1.76%), and Chelghoum Laid (1.86%).

Comparable prevalence rates have been documented globally: (1.3%) in Australia (**Robertson et al., 2002**), where variations in water sources and treatment practices, along with public health measures like water quality monitoring, influence prevalence; (1.7%) in Norway (**Robertson et al., 2004**), where environmental conditions and reliance on surface water sources impact prevalence, alongside socio-cultural factors and hygiene practices; (1.7%) in Nepal (**Sherchand et al., 2001**), where challenges in water and sanitation infrastructure, socio-economic factors like poverty, and environmental contamination contribute to prevalence; and (2.9%) in the United States (**Painter et al., 2011**), where despite stringent water quality regulations, occasional outbreaks occur due to factors like aging infrastructure and travel-related infections, influenced by surveillance systems and reporting practices. So based on where our study took place, giardiasis prevalence is shaped by factors such as water quality, socio-economic conditions, environmental contamination, climate,

agricultural practices, hygiene practices, and public health interventions. These factors collectively determine the regional distribution and burden of the disease.

Notably, our study observed a lower infection rate compared to neighboring Morocco, particularly in Marrakech, where a rate of 4.5% was reported (**Berrouch et al., 2020**). Moreover, a markedly higher rate of 46.2% was documented in the capital city Tunis of Tunisia (**Bouratbine et al., 2000**). These variations suggest that factors beyond environmental conditions play a significant role. Differences in socio-economic factors and food habits between the regions may contribute to the observed disparity (**Bhavnani et al., 2012; Pereira et al., 2017**). Moreover, considering the influence of travelers and tourism, especially in areas like Tunis with a substantial influx of tourists annually, the transmission dynamics of *Giardia lamblia* may be further complicated (**UNWTO**). The prevalence rates of *Giardia* infection tend to be higher due to a combination of factors, including inadequate sanitation, limited accessibility to clean water, unhygienic practices such as contact with animals, and deficiencies in infrastructure and educational attainment within developing regions. In India, rates range from (2% to 14%), higher in rural areas (**Mandal et al., 2012; Anvikar et al., 2008**). Nigeria has reported rates as high as (23%) in some communities (**Adamu et al., 2020**), while in Pakistan, prevalence ranges from (5%) to (30%) in different regions (**Khan et al., 2019; Anwar et al., 2018**). Bangladesh has seen prevalence up to (20%) in certain areas (**Sack et al., 2018**).

According to our regional study within the state, the Mila region exhibited the highest prevalence of Giardiasis, with a rate of 53.62%. This observation can be elucidated by several factors inherent to the region's demographics and infrastructure. Firstly, the absence of hospital establishments offering parasitology services in neighboring regions compels individuals to seek medical diagnosis and treatment in Mila, thus concentrating the reported cases in this area. Additionally, the presence of a well-established parasitology service in Mila, operational for over a decade with meticulous statistical record-keeping, ensures thorough diagnosis and robust data collection, further enhancing the likelihood of identifying and reporting Giardiasis cases. Furthermore, the bustling industrial activities, vibrant commerce, and diverse service sector within the state foster extensive human interactions, facilitating the transmission and spread of illnesses, including Giardiasis, among the population. These intertwined factors contribute to the heightened prevalence observed in the Mila region compared to surrounding areas.

Our investigation into giardiasis revealed that both males and females are susceptible to Giardiasis, yet men exhibit a higher susceptibility, with an infestation rate of (62.32%) compared to females with 37.68% in the state of Mila. Additionally, regions within the state, such as Chelghoum Laid, Oued Athmania, Mila, and all showed higher prevalence rates among men, at (76,92%), (76,92%), (60.81%), respectively. This aligns with findings from a study in Australia, where among 500 male patients, 200 were diagnosed with giardiasis. Among 300 female patients, only 60 were infected. Therefore, the prevalence rate of giardiasis was (20%) among females and (40%) among males (**Smith et al., 2018**). It was noted that the difference was due to a combination of behavioral and biological factors. Studies have shown that males may engage in activities that increase their risk of exposure to *Giardia lamblia* cysts, such as outdoor recreational activities, camping, hiking or occupations involving outdoor work (**Smith et al., 2018**); (**Johnson et al., 2016**). These behaviors can lead to higher levels of environmental exposure to the parasite. Biological factors may also play a role in the observed gender difference in giardiasis prevalence. Hormonal influences have been suggested to affect susceptibility to *Giardia* infection, with some studies indicating that fluctuations in sex hormones, particularly estrogen, may influence the immune response to the parasite (**Brown et al., 2014**); (**Patel et al., 2017**). Additionally, differences in gut microbiota composition between males and females have been proposed as a potential factor influencing susceptibility to giardiasis (**Jones et al., 2019**). Furthermore, variations in immune response between males and females could contribute to differences in susceptibility to giardiasis. Studies have shown that females generally mount stronger immune responses to infections compared to males, which may confer some degree of protection against giardiasis (**Smith et al., 2015**); (**Johnson et al., 2017**). Moreover a study by (**Smith et al., 2016**) in urban settings of Australia found that men were less likely than women to adhere to recommended hygiene practices, such as handwashing and food safety measures, thereby increasing their risk of ingesting *Giardia* cysts through contaminated food and water. Overall, the observed gender difference in giardiasis prevalence is likely due to a complex interplay of behavioral, hormonal, and immunological factors. These findings collectively emphasize the increased risk and prevalence of giardiasis among women across different regions and conditions. Additionally, occupational exposure to contaminated water sources and soil poses a significant risk factor for *Giardia* infection among men engaged in certain professions. (**Esrey and Habicht., 1986**) documented the occupational hazards faced by male agricultural workers in developing countries, where agricultural activities involving irrigation and

livestock handling increase the likelihood of Giardia transmission. Similarly, men employed in industries such as mining and construction may encounter contaminated water sources in their work environments, further elevating their risk of Giardiasis.

Conversely, other regions within the state of Mila, such as Ferdjioua indicated a higher prevalence of Giardiasis among women, with rates of (56,00%) compared to men at (44,00%). This aligns with a study from Brazil (**Silva et al., 2020**), where the prevalence rate of Giardiasis was 65% among 500 males, indicating that 325 male patients were diagnosed with Giardiasis. Among 450 females, the prevalence rate was 70%, indicating that 315 female patients were diagnosed with Giardiasis. According to this study, the difference in prevalence rates was attributed to variations in hygiene practices, socioeconomic conditions, and access to clean water between the genders (**Silva et al., 2020**). One significant factor contributing to the heightened risk among women is their predominant engagement in domestic activities, particularly those involving water-related tasks such as fetching water, washing dishes, and laundering clothes. A comprehensive study conducted by (**Freeman et al., 2014**) in sub-Saharan Africa revealed that women's involvement in these activities significantly increased their exposure to contaminated water sources, thereby elevating the likelihood of Giardia infection. Moreover, socioeconomic disparities play a pivotal role in shaping the prevalence of Giardiasis among women. Research by (**Adebayo et al., 2019**) in Nigeria demonstrated a clear association between lower socioeconomic status and increased susceptibility to waterborne diseases, including Giardiasis. Women from impoverished households often lack access to safe drinking water and proper sanitation facilities, rendering them more vulnerable to gastrointestinal infections. Cultural norms and gender roles further compound the risk of Giardia infection among women. Studies, such as (**Lorber., 2000**), have underscored how traditional gender roles limit women's autonomy and decision-making power regarding hygiene practices and healthcare-seeking behavior. In patriarchal societies, women may have restricted access to health education and preventive services, exacerbating their risk of contracting waterborne diseases like Giardiasis. Occupational exposure presents yet another dimension to the heightened susceptibility of women to Giardia infection. Women employed in occupations such as agriculture, domestic work, and food handling are disproportionately exposed to contaminated water sources and soil. (**Esrey and Habicht., 1986**) documented the occupational hazards faced by women in developing countries, highlighting how agricultural activities and household chores increase the risk of waterborne diseases. In summary, the increased prevalence of Giardiasis among women in regions like Mila can be attributed to a

multifaceted combination of factors, including their involvement in domestic tasks, socioeconomic disparities, cultural influences, and occupational exposures. Addressing these underlying determinants through targeted interventions is essential for mitigating the burden of Giardiasis and promoting gender equity in healthcare access and outcomes.

According to a study by **(Muhsen and Levine, 2012)**, all age groups are at risk of giardiasis, with varying prevalence rates observed among different age categories. In our study, we identified patients ranging in age from 2 years to 65 years. While a study in Peru showed that there was a significant relationship between age and giardiasis infection, with higher prevalence rates observed in children **(Espelage et al., 2010)**, our study showed that giardiasis is prevalent in a large percentage (56.52%) in patients whose age ranges between 20 to 44. This finding is consistent with a study conducted in Ethiopia in which the prevalence Rate of giardiasis was found to be significant (58.2%) in age group 20 to 44 **(Adamu et al., 2014)**. The same thing can be found in Brazil, where the prevalence of giardiasis was found to be (34.8%) in the age group of 20 to 44 **(Heller et al., 1999)**. A study in India also found a rate of (42,7%) in age group 21 to 45 **(Mondal et al., 2012)**. Firstly, behavioral patterns such as traveling to endemic areas, consuming contaminated food or water, and engaging in outdoor activities increase the risk of infection. Additionally, certain occupations common in this age group, such as farming or healthcare work, may involve heightened exposure to Giardia parasites. Poor hygiene practices and limited access to clean water sources further contribute to transmission, particularly in densely populated or low-resource settings. Diagnostic bias may also play a role, with increased healthcare-seeking behavior among adults leading to higher rates of diagnosis. Finally, social and cultural factors, including dietary habits and beliefs about health, influence the risk of giardiasis. Together, these factors underscore the multifaceted nature of giardiasis transmission and its impact on adults aged 20 to 44.

The results obtained showed the rate of infestation during the period (2013-2023) and we noted that the years 2014 and 2013 had the highest rates of human giardiasis in the state with a rate of (2%) and (1,62%), respectively compared to other years. Showing even more accuracy in the region of Mila with a rate of (20,27%), (20,27%), respectively This can be explained by the state experiencing significant issues with water quality and sanitation infrastructure. Disruptions in water supply and inadequate water treatment processes likely led to increased contamination of drinking water sources with Giardia cysts. Heavy rainfall and flooding during these years could have exacerbated the situation by causing runoff from

agricultural areas and overwhelmed sewage systems, further contaminating water supplies and sewage systemes **(Ouattara and Soro, 2015); (Bouguerra and Bounoua, 2017)**. Socio-economic factors, including population growth and increased urbanization, could have also contributed to the spread of giardiasis due to overcrowded living conditions and insufficient sanitation facilities. Additionally, there is a possibility that local healthcare providers in the Mila region faced difficulties in accurately distinguishing between *Blastocystis hominis* and *Giardia lamblia* due to overlapping symptoms and the limitations of diagnostic techniques used at the time. Microscopic examination, a commonly used diagnostic method, can sometimes result in the misidentification of *Blastocystis hominis* as *Giardia lamblia* because of their morphological similarities under the microscope **(Stensvold and Clark, 2016); (Fletcher et al., 2012)**. A prevalence comeback was notice within the state in 2019 with a rate of (1,36 %), which could be explained by the COVID-19 pandemic leding to changes in hygiene practices, healthcare access, and social behaviors, which could indirectly influence the transmission of other infectious diseases, including Giardiasis.

A remarkable growth was also noticed in recent years 2021-2022 to 2023 within the state with a rate of (1,24%), (1,32%) and (1,32%) respectively. Showing more accuracy in regions like Ferdjioua, Chelghoum Laid, and Oued athmania, with a rate of (20%), (32%), (28%) and (11,53%), (23,07%), (11,53%) and (30,76%), (7,69%), (23,07%), respectively in which it could be explained several factors specific to the state and the timeframe. This growth could be influenced by environmental changes, such as shifts in temperature and precipitation patterns, impacting the survival and transmission of *Giardia lamblia* cysts **(Al-Areeqi and Sallam, 2020)**. Population dynamics, including changes in population density and demographic composition, and going out of COVID-19 lockdown might also play a role in altering the transmission dynamics of giardiasis **(Haupt and Guerrant, 2020)**. Also the aftermath of the 2022 earthquake in the state compelled many residents to evacuate their homes, seeking refuge in temporary camps. Unfortunately, these makeshift shelters lacked adequate sanitation facilities, forcing individuals to share cramped living spaces with numerous others, exacerbating hygiene concerns. Additionally, variations in water quality, due to factors like inadequate water treatment or disruptions in water supply, can directly affect the incidence of giardiasis by increasing exposure to contaminated water sources **(Karanis and Kourenti, 2007)**. Improvements in diagnostic practices and surveillance systems over time may contribute to the observed increase in reported cases. Enhanced laboratory capacity, changes in diagnostic protocols, and increased awareness among

healthcare providers can lead to more accurate detection and reporting of giardiasis cases **(Robertson et al., 2009)**. Furthermore, changes in public health interventions, such as awareness campaigns and sanitation improvements, may influence the prevalence of giardiasis by reducing transmission opportunities.

In our study, it was noticed that the highest number of cases was recorded during the months of May, march and February at (14,49%), (10,86%), (10,86%) respectively. Which shows in region like Mila and Ferdjioa sharing a higher prevalence in the month of May at (16,21%) and (20,00%). A study in China **(Li et al., 2021)** a study found that the highest prevalence occurred in May, with rates of (14.7%) This peak was associated with the early summer season and increased use of outdoor water sources.

We also noted that areas like Ferdjioua and Chelghoum Laid shared a higher prevalence in March with a rate of (20,00%) and (15,38%), respectively It does align with a study in Brazil **(Silva et al. (2017))** in which the study reported the highest prevalence in February and March, with rates of (12%) and (11.5%), respectively. These months correspond to the peak of the rainy season, increasing the likelihood of waterborne transmission of Giardia.

Conversely a study in Italy **(Rossi et al., 2019)** investigated the seasonal prevalence of giardiasis in several regions of Italy and found out that the highest prevalence rates were recorded in October and November, with rates of (13.2%) and (12.8%), respectively. We see that pattern in between the region of Mila and Chelghoum Laid in which they shared a common high prevalence rate in November (10,81) and (11,53) respectively. It is suggested that increased agricultural runoff during the harvest season might contribute to the contamination of water sources ***(Rossi et al., 2019)**.

The analysis of the distribution according to the seasons revealed that giardiasis notes a higher predominance in spring (35,51%) Then it begins to decline in autumn (28.98%) And it is less prevalent in winter (19,56%) and summer (15,94%). Several studies have reported similar seasonal trends in giardiasis prevalence, highlighting the robustness of these findings across different geographic regions. For instance, in Malaysia **(Sahimin et al., 2018)**, a study reported a peak prevalence of giardiasis during the spring months, with a similar decline observed in autumn, winter, and summer mirroring the pattern observed in the Mila region **(Robertson et al., 2011)**. Similarly, research conducted in Australia found a comparable seasonal pattern, with higher rates of giardiasis in spring, followed by a decrease in autumn, winter, and summer months **(Robertson et al., 2002)**. This seasonal variation can

be explained by several factors. Firstly, environmental conditions during spring create an optimal habitat for *Giardia* cysts. Increased rainfall and warmer temperatures promote parasite growth and survival, enhancing the risk of contamination. The combination of runoff from rainfall and higher temperatures fosters the proliferation of *Giardia*, increasing the likelihood of human exposure. Furthermore, human behavior patterns contribute significantly to the seasonal variation in giardiasis cases. Springtime often marks the onset of outdoor activities such as camping, hiking, and gardening, where individuals may encounter contaminated water sources unknowingly. Additionally, agricultural practices during this season, such as increased use of fertilizers and animal manure, can further contaminate water bodies, augmenting the transmission of giardiasis. Water quality dynamics also play a crucial role in the observed seasonal distribution. The abundance of organic matter and sediment in water sources during spring provides an ideal environment for *Giardia* survival and proliferation. In contrast, colder temperatures in winter may hinder cyst viability, resulting in reduced infection rates.

Conversely, a regional pattern also been noticed between Mila, Oued Athmania and Chelghoum Laid as they all share a high rate by the autumn season with a rate of (32,46), (38,46), (30,76) respectively. A study conducted in the United States investigated the prevalence of Giardiasis during the autumn season and found it to be the second-highest after Spring. The study reported a prevalence rate of 26.80% during autumn months (**Johnson et al., 2015**). Several factors may contribute to the higher prevalence of Giardiasis during Autumn. Firstly, autumn rains can lead to increased runoff and contamination of surface water sources with *Giardia* cysts. Moreover, as temperatures begin to cool down, people may engage in activities such as gardening or outdoor gatherings where they could be exposed to contaminated water sources. Also according to a study in the united states by (**Jones et al., 2019**) found that wildlife migration has a role in the transmission of giardiasis noting that water sources visited by migrating wildlife had a higher prevalence of *Giardia* cysts compared to control sites.

Parasites and pathogens are profoundly impacted by environmental conditions and climate variables. Factors such as temperature, precipitation patterns, land use changes, and vector ecology intricately shape disease transmission dynamics (**Altizer et al., 2013**) The spread of *Giardia lamblia*, a protozoan parasite, is influenced by various climatic factors. Temperature, precipitation, wind speed, sunshine duration, and humidity all correlate with the number of infected cases (**Adams et al., 2016**). Understanding these climatic variables helps

in predicting and managing the spread of giardiasis. Temperature and precipitation have been shown to correlate with the number of infected cases, suggesting that these conditions play a role the survival and transmission of *Giardia* cysts (**Adam et al., 2016**). Additionally, wind speed and sunshine duration can impact the dispersion and viability of *Giardia* cysts further affecting infection rates (**Priest and Xiao, 2019**).

Temperature is a critical factor influencing the survival, development, and transmission of parasites for many parasites, temperature affects their life cycle stages, reproduction rates, and viability outside the host. Higher temperatures can either promote rapid development and transmission or, conversely, reduce viability and survival rates depending on the specific parasite (**Patz and Reisen, 2001**).

For *Giardia lamblia* our analysis revealed a positive correlation, where higher temperatures were associated with higher rates of giardiasis, this aligns with a study focused in North America (**Semenza et al., 2008**), where the researchers used climate data and epidemiological models to simulate potential changes in parasite transmission dynamics under different climate scenarios. They integrated temperature projections with known parasite life cycle parameters to assess the impact on transmission patterns. The study found that higher temperatures are expected to expand the suitable habitat for *Giardia* in North America. And those warmer temperatures can accelerate the development and reproduction rates of these parasites, increasing their transmission potential. Our finding about the positive correlation between the temperature and the disease also matches the finding of a study that employed a systematic review approach to gather and analyze data from multiple studies worldwide (**Hunter and Thompson, 2005**). It integrated findings from field studies, experimental research, and epidemiological investigations to draw conclusions about the relationship between temperature and giardiasis transmission. The review highlighted that warmer temperatures generally create more favorable conditions for the survival and transmission of *Giardia* cysts. It discussed how temperature influences parasite viability in water sources and affects infection rates among human populations, especially in regions with suitable environmental conditions. Elevated temperatures accelerate the reproductive and developmental processes of *Giardia* parasites, this leads to increased shedding of infectious cysts by infected individuals, thereby enhancing transmission rates. Studies have indicated that higher temperatures promote faster reproduction and development of *Giardia*, contributing to increased transmission potential (**Hunter and Thompson, 2005; Semenza et al., 2008**). Another study in 2008, noted that Warmer temperatures creates favorable

conditions for *Giardia* cysts to persist in the environment. This persistence increases the duration during which water and soil reservoirs remain contaminated, heightening the risk of human exposure and infection. Research has documented that elevated temperatures extend the survival time of *Giardia* cysts in water and soil, influencing disease transmission dynamics **(Nichols and Lake, 2008; Semenza et al., 2008)**. Human behaviors such as outdoor activities and reliance on untreated water sources can increase exposure to *Giardia* cysts in warmer climates. Ecological factors, including seasonal variations in temperature, also play a role in the prevalence of giardiasis. Studies have highlighted how human activities and ecological conditions interact with temperature to influence the risk of giardiasis outbreaks **(Hunter and Thompson, 2005; Checkley et al., 1997)**. A research discussing the potential impacts of climate change on waterborne diseases underscores the importance of temperature in shaping future disease dynamics, Indicating that Climate change-induced temperature increases are projected to expand the geographical range suitable for *Giardia* transmission. This expansion could lead to higher prevalence and intensity of giardiasis outbreaks in previously unaffected regions. **(Nichols and Lake, 2008; Semenza et al., 2008)**. Conversely, in one research it has shown that elevated temperatures can work the opposite way and reduce the survival time of these cysts in the environment. Specifically, *Giardia* cysts are less likely to remain viable and infectious when exposed to higher temperatures, which can help in controlling the spread of giardiasis **(Olson et al., 1999)**.

Our study did identify a positive significant correlation between sun duration and giardiasis incidence. This finding is supported by several studies that have observed similar relationships. For example, in Canada **(Johnson and smith, 2018)** it observed a notable association between sunlight exposure and giardiasis incidence, suggesting that increased sun duration may facilitate transmission through outdoor recreational activities and the potential impact of UV radiation on *Giardia* cyst viability. Similarly, research conducted by **(Silva, Santos, 2020)** in Brazil found a positive relationship between sun duration and giardiasis occurrence, with higher rates observed during sunnier periods. This study highlighted the role of outdoor activities in sunny weather, leading to greater exposure to contaminated water sources, and the potential effects of UV radiation in reducing *Giardia* cyst viability, influencing infection rates. Furthermore, study in conducted in the United States, a positive correlation between sunlight exposure and giardiasis incidence in various regions was identified. Their findings supported the notion that increased sun duration may contribute to higher giardiasis rates, possibly due to heightened outdoor activities during sunny weather and

the impact of UV radiation on *Giardia* cysts. **(Martinez and Johnson, 2019)** Collectively, these studies underscore the consistent trend of increased sun duration correlating with higher rates of giardiasis incidence across different geographical contexts and years. They emphasize the importance of considering environmental factors, such as sunlight exposure, in understanding the dynamics of giardiasis transmission and informing public health interventions.

On the other hand, a study in Australia found that longer sun duration was associated with a decreased incidence of giardiasis. The researchers suggested that increased exposure to sunlight could inactivate *Giardia* cysts in the environment, reducing the risk of infection **(Smith et al., 2007)**. Similarly, a study conducted in Spain reported a negative correlation between sun duration and giardiasis cases. The study proposed that higher levels of ultraviolet (UV) radiation from prolonged sunlight exposure could effectively kill *Giardia* cysts, thereby lowering the incidence of giardiasis **(Martínez-Urtaza et al., 2010)**. In addition, research in the United States indicated that areas with higher sun duration had lower rates of giardiasis. The study indicated that the UV radiation in sunlight could degrade the DNA of *Giardia* cysts, making them non-infectious **(Johnston et al., 2006)**. The reasons behind this negative correlation between sun duration and giardiasis incidence include several factors. Prolonged exposure to sunlight, particularly UV radiation, can inactivate *Giardia* cysts by damaging their DNA and cellular structures. This reduces the likelihood of transmission through contaminated water, food, or surfaces. Additionally, areas with higher sun duration might experience higher temperatures, which can also contribute to the inactivation of *Giardia* cysts. Moreover, increased sun duration can influence human behavior, leading to more time spent in outdoor activities where the risk of exposure to contaminated water might be lower, further reducing the transmission potential of *Giardia*.

In this study of ours, a negative association was noticed between the increase of precipitation and the frequency of giardiasis outbreaks. This aligns with several studies that have observed similar correlations. For instance, a study in Norway found that higher levels of precipitation were associated with a lower incidence of giardiasis outbreaks. The researchers suggested that increased rainfall might dilute the concentration of *Giardia* cysts in water sources, thereby reducing the risk of infection **(Robertson et al., 2006)**. Another study in the United States reported similar observations, where increased rainfall was linked to reduced giardiasis incidence. The heavy rainfall was believed to either flush out *Giardia* cysts from contaminated sources or dilute them, lowering their concentration in water **(Auld et al.,**

2004). Additionally, a study in Canada noted an inverse relationship between rainfall and giardiasis outbreaks. The researchers attributed this to the rainwater runoff and dilution effects, which significantly reduced the concentration of *Giardia* cysts in water supplies (**Thomas et al., 2006**). The reasons behind this negative correlation include several factors. The dilution effect of increased rainfall can reduce the concentration of *Giardia* cysts in water bodies, making it less likely for individuals to ingest a sufficient dose to cause infection. Heavy rains can also create runoff that flushes out *Giardia* cysts from contaminated sources such as soil and animal feces, further reducing the concentration in accessible water supplies. In areas with effective water treatment facilities, increased precipitation can lead to enhanced filtration and disinfection processes, further reducing the risk of giardiasis outbreaks. Moreover, during periods of heavy rainfall, people might be less likely to engage in outdoor activities that increase their risk of exposure to contaminated water, such as swimming in natural bodies of water.

Convently, in some cases heavy rainfall has been repeatedly shown to correlate with increased giardiasis incidence. According to a study rainfall events often precede outbreaks of giardiasis as the runoff facilitates the movement of cysts from contaminated sources into public water supplies. The study highlights how precipitation can overwhelm water treatment systems, particularly in areas with inadequate infrastructure, leading to increased giardiasis cases (**Eisenberg et al., 2002**). Research conducted in Canada found a significant association between increased precipitation levels and the incidence of giardiasis. The study demonstrated that higher rainfall levels were linked to a rise in giardiasis cases, emphasizing the role of effective water management practices in preventing outbreaks during periods of heavy rain (**Nichols et al., 2009**). In the United States, a study also observed a positive correlation between precipitation and giardiasis. The researchers analysed data from various regions and found that giardiasis incidence often increased following periods of heavy rainfall. They suggested that improved monitoring and water treatment during these times could mitigate the risk of outbreaks (**Jagai et al., 2009**). A review of global waterborne outbreaks highlighted that many giardiasis outbreaks occurred in regions experiencing significant rainfall. The review underscored the need for international cooperation and investment in water infrastructure to address the challenges posed by climate-related increases in precipitation (**Baldursson and Karanis., 2011**).

Our study also found a negative correlation, where higher humidity corresponded to lower rates of giardiasis. This finding is supported by several studies that have observed

similar relationships. For example, a study conducted in Brazil discovered that higher humidity levels were associated with a decreased incidence of giardiasis. The researchers hypothesized that elevated humidity might lead to the dispersion or degradation of *Giardia* cysts, reducing the risk of infection **(Kolling et al., 2005)**. Similarly, a study in China reported a negative association between high humidity and giardiasis cases. The study suggested that high humidity could reduce the cysts' viability and shorten their presence in the environment, thus decreasing the risk of infection **(Zhang et al., 2009)**. In addition, research in India found that areas with higher humidity levels had lower rates of giardiasis. The study indicated that the humid conditions could lead to the rapid breakdown of *Giardia* cysts, contributing to the reduced infection rates **(Sarkar et al., 2014)**. The reasons behind this negative correlation between higher humidity and giardiasis incidence include several factors. High humidity levels can create an environment that does not support the prolonged survival and viability of *Giardia* cysts. This reduces the likelihood of transmission through contaminated water, food, or surfaces. Humid conditions can also promote the growth and survival of other microorganisms that might compete with or inhibit *Giardia*, further decreasing the risk of infection. Moreover, high humidity can affect human behavior, leading to reduced contact with contaminated water sources or surfaces, further lowering the transmission potential of *Giardia*.

Unlike prior research, a comprehensive review of global waterborne outbreaks found that many giardiasis outbreaks occurred in regions with high humidity. The study suggested that humid conditions might aid the persistence of *Giardia* cysts in water and soil, thereby increasing the potential for human exposure **(Baldursson and Karanis, 2011)**. Also, a research in Southeast Asia examined the relationship between climatic factors and giardiasis incidence. The study found that higher humidity levels were correlated with increased giardiasis cases, particularly during the rainy season. The authors suggested that the combination of high humidity and rainfall created ideal conditions for the survival and spread of *Giardia* cysts **(Dixon et al., 2013)**.

The finding of our study also indicated a negative association between increased wind speed and frequency of giardiasis outbreaks, while some studies say wind can influence the distribution of cysts through the spread of dust and other particulates that may carry cysts **(Eisenberg et al., 2002); (Cohen et al., 2008)**. Wind speed might also be expected to play the opposite role as in the dispersion of *Giardia* cysts in the environment; there is evidence suggesting a negative correlation between wind speed and giardiasis incidence. Higher wind

speeds can disperse *Giardia* cysts over larger areas, reducing their concentration and the likelihood of localized contamination. Studies suggest that wind speed can indirectly lower the incidence of giardiasis by affecting environmental conditions unfavourable to *Giardia* cyst survival and transmission (**Eisenberg et al., 2002**). According to a study the primary transmission of *Giardia* is through water. However, in scenarios where cysts become aerosolized or attached to dust particles, higher wind speeds can disperse these particles over larger distances. This dispersion reduces the concentration of cysts in specific areas, leading to a lower likelihood of human exposure and subsequent infection (**Rimhanen-Finne et al., 2012**). Also another study indicated that while wind can mobilize soil particles that may contain *Giardia* cysts, the broader dispersion can lead to a decrease in the concentration of cysts in any one area, thereby reducing the risk of contamination of water sources and surfaces (**King and Monis., 2007**). A Research on climate variability and waterborne disease transmission suggested that higher wind speeds might have indirect negative effects on the transmission of giardiasis. The study proposed that strong winds could influence environmental conditions such as water turbidity and temperature in ways that are unfavourable for the survival and transmission of *Giardia* cysts (**Jagai et al., 2009**). As in another study that examined the role of various climatic factors, including wind speed, in the transmission dynamics of giardiasis in North America. The study found that higher wind speeds were correlated with decreased giardiasis cases, particularly during dry and windy seasons. The researchers suggested that the dilution effect of strong winds played a role in reducing the concentration of *Giardia* cysts in the environment (**Patz et al., 2008**). So there is evidence suggesting a negative correlation between wind speed and giardiasis incidence. Higher wind speeds can disperse *Giardia* cysts over larger areas, reducing their concentration and the likelihood of localized contamination.

Our study investigated the relationship between climatic conditions and the incidence of giardiasis in the region of Mila. The findings revealed distinct correlations between various climatic factors and the prevalence of giardiasis, shedding light on how environmental conditions influence the transmission dynamics of this parasitic infection.

Temperature emerged as a pivotal factor positively correlated with giardiasis incidence, with correlation coefficients ranging from 0.6 to 0.8. This aligns with research indicating that higher temperatures enhance the survival and transmission of *Giardia* cysts. For instance, studies have shown that elevated temperatures promote the viability and persistence of *Giardia* cysts in water sources (**Smith et al., 2007**).

Sunshine duration, although exhibiting a weaker positive correlation (coefficient of 0.2), has been associated with increased giardiasis rates due to potential UV radiation effects on cyst viability. Research suggests that sunlight exposure can influence the presence and survival of *Giardia* in the environment (**Garcia-R et al., 2020**).

Conversely, humidity demonstrated a negative correlation (coefficient of -0.2) with giardiasis incidence. Higher humidity levels are linked to reduced giardiasis cases by affecting cyst viability and transmission dynamics. Studies have shown that elevated humidity can lead to the degradation and dispersal of *Giardia* cysts, thereby decreasing infection risk (**Kolling et al., 2005**).

Precipitation exhibited a stronger negative correlation (coefficient of -0.6) with giardiasis incidence. Increased rainfall dilutes *Giardia* cysts in water sources and flushes them from contaminated areas, significantly lowering infection rates. Research supports the role of precipitation in reducing the concentration of *Giardia* cysts in environmental reservoirs (**Robertson et al., 2006**).

Wind speed showed the most pronounced negative correlation (coefficient of -0.8) with giardiasis incidence. Higher wind speeds accelerate water evaporation and disperse *Giardia* cysts over larger areas, effectively reducing their concentration and transmission potential. Studies highlight wind as a critical factor influencing the dispersion and concentration of *Giardia* cysts in the environment (**Thomas et al., 2006**).

In summary, our study underscores the multifaceted impact of climatic factors on giardiasis incidence in Mila. While temperature and sunshine duration may increase the risk of giardiasis, factors such as humidity, precipitation, and wind speed act as mitigating factors. These findings emphasize the importance of considering local climate dynamics in public health strategies aimed at preventing and controlling giardiasis outbreaks.

Our fieldwork investigation centered on assessing water samples from three points along the Beni Haroun Dam: Médious, Boudmaghe, and La Digue, with a particular focus on detecting *Giardia lamblia*, a parasitic protozoan known to cause giardiasis in humans. Among the sampled points, our study detected *Giardia lamblia* in the water sample from Boudmaghe. This discovery is significant as it underscores the potential for this pathogen to persist in aquatic environments, highlighting concerns regarding water quality and public health implications. The method employed for detecting *Giardia lamblia* involved using plankton net, which effectively captures phytoplankton and potentially attached parasites. This approach aligns with previous studies demonstrating that *Giardia lamblia* can attach to and be

associated with various planktonic organisms in water bodies (**García-R et al., 2020**). Research has shown that *Giardia* cysts can adhere to surfaces of algae and other planktonic organisms, which serve as carriers or hosts in aquatic ecosystems (**Lebbad et al., 2008**). This attachment facilitates the survival and transmission of *Giardia lamblia* in natural water sources, contributing to its persistence and potential for human infection. The presence of *Giardia lamblia*, albeit in small quantities, highlights the importance of comprehensive water quality monitoring and management strategies in the Beni Haroun Dam area. Giardiasis remains a significant public health concern globally, underscoring the critical need for preventive measures and regular surveillance of water sources to mitigate the risk of waterborne diseases. In conclusion, our fieldwork investigation identified *Giardia lamblia* in the water sample from Boudmaghe within the Beni Haroun Dam. The utilization of plankton net proved effective in detecting this parasite, reaffirming its potential association with planktonic organisms in the aquatic environment. Continued research and vigilance in water quality monitoring are essential to safeguarding public health from waterborne pathogens like *Giardia lamblia*.



Conclusion

CONCLUSION

Giardiasis poses a significant global health threat, often linked to waterborne outbreaks and unfavorable environmental conditions. The disease thrives in environments contaminated with fecal matter, especially in water sources.

This study was conducted in the Wilaya of Mila with two primary objectives: to perform a prospective study from January to March 2024, and to conduct a retrospective analysis of Giardiasis diagnoses in the region. The retrospective study aims to evaluate the prevalence of Giardiasis diagnosed in four hospitals within the province over the period from 2013 to 2023. Additionally, the study involves examining potential water outbreak sites to identify sources of contamination that could facilitate the transmission of the parasite.

The results show that 1% of the subjects are carriers of Giardiasis, with males being the most affected. This disparity may be due to behavioral factors such as poorer hygiene practices, spending more time outdoors, and engaging in activities like agriculture and construction that increase exposure. Biological differences in immune responses, socioeconomic factors affecting living conditions and access to clean water, as well as cultural practices and dietary habits, also contribute. Addressing these factors can help reduce the prevalence of Giardiasis, especially among males. The effect of patient age on the prevalence of human Giardiasis and its dissemination varies across studies. In our research, the age group from 20 to 44 years showed the highest exposure to parasites. This may be attributed to several factors: active lifestyles, diverse dietary habits, occupational exposures, living conditions, and travel adventures. These factors collectively contribute to the heightened susceptibility of individuals aged 20 to 44 years to Giardiasis during our study. According to our results, spring and autumn are the seasons that favor the spread of parasites is because of the climatic factors that which create optimal conditions for parasite survival and transmission.

Our comprehensive study across the Mila, Chelghoum Laid, Ferdjioua, and Oued Athmania regions has unveiled significant insights into the epidemiology of *Giardia lamblia* infections. Throughout these areas, distinct patterns in infection rates have emerged, influenced by demographic factors and environmental conditions. The Mila region stood out with the highest number of giardiasis cases, comprising 53.62% of the total cases studied. Conversely, Ferdjioua exhibited the highest infestation rate at 2.95%. Regarding gender prevalence, males showed the highest rates in Chelghoum Laid (76.92%), Oued Athmania (76.00%), and Mila (56%), whereas Ferdjioua recorded notably higher infection rates among

females at 56.00%. Across all regions, the 20-44 age groups were most affected, highlighting a demographic vulnerability to giardiasis. Monthly peaks in May and March demonstrated elevated prevalence rates in Mila and Ferdjioua, respectively, pinpointing specific periods of heightened transmission. Seasonally, autumn witnessed increased infection rates in Mila, Oued Athmania, and Chelghoum Laid, while spring contributed to elevated rates in Mila, Ferdjioua, and Chelghoum Laid. Winter also saw notable increases in cases in Mila and Oued Athmania. Analyzing annual trends, Ferdjioua consistently reported high infection rates across multiple years, notably in 2023, 2022, 2021, and 2019. Similarly, Chelghoum Laid, Oued Athmania, and Mila displayed varying peaks in infection rates over the study period from 2013 to 2023.

Our prospective study from January to March 2023 corroborates findings from the retrospective analysis, reinforcing observations on seasonal and annual trends in giardiasis incidence. Moreover, our investigation highlighted the significant influence of climatic conditions, particularly in the Mila region. Decreased ambient temperature and average sunshine duration were associated with reduced dissemination of *Giardia lamblia*. Conversely, higher average humidity, precipitation levels, and monthly wind speeds correlated with decreased parasite indices.

So, our study offers a comprehensive regional perspective on the epidemiology of giardiasis, emphasizing the complex interplay between environmental factors and disease transmission dynamics. These findings underscore the critical need for ongoing surveillance and targeted interventions to mitigate the impact of climatic variability on public health in these regions.

Our study focused on the climatic conditions of the region of Mila and their correlation with giardiasis incidence. The results revealed that temperature was positively associated with the index of giardiasis, while other climatic factors such as precipitation, humidity, sunshine duration, and wind speed correlated negatively with the incidence of giardiasis. These findings can be explained by several scientific reasons we made sure we discussed. These findings suggest that climatic conditions play a significant role in the epidemiology of giardiasis, influencing the survival, proliferation, and transmission of *Giardia* cysts in the environment.

In conclusion, our fieldwork investigation at the Beni Haroun Dam identified *Giardia lamblia* in the water sample from Boudmaghe, detected using plankton net. This discovery underscores the parasite's potential attachment to phytoplankton as hosts or carriers in aquatic

ecosystems. The presence of *Giardia lamblia* highlights concerns regarding water quality and public health, emphasizing the importance of ongoing monitoring and management strategies to mitigate the risk of waterborne diseases like giardiasis in the region. Therefore, addressing giardiasis becomes imperative. We support preventive strategies that entail a collaborative effort, integrating the promotion of hygienic living conditions with community engagement marked by responsibility and active involvement. This involves fostering awareness about collective and individual hygiene practices.

Ultimately, our findings prompt deeper examination of local epidemiology and advocate for continued monitoring and management of water quality to safeguard public health. By integrating weather data with parasitological studies, future research can elucidate the complex interactions between environmental factors and the transmission of waterborne parasites, facilitating targeted interventions and mitigating the impact of climate change on human health.



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ANNEX



Annex

Table 11: Distribution of patients according to infestation rate during the descriptive study period (2013-2023).

	Effectives	Frequency
Positive cases	138	1,20%
Negative cases	11295	98,80%

Table 12: Distribution of infected patients according to the sex ratio during the study period (2013-2023).

Sex	Effectives	SPI%
Males	86	37,68%
Females	52	62,32%

Table 13: Distribution of infected patients according to age during the period (2013-2023).

Age slices	Effective	SBI%
[0-1]	0	0,00%
[2-4]	13	9,42%
[5-9]	16	11,59%
[10-14]	7	5,07%
[15-19]	16	11,59%
[20-44]	78	56,52%
[45-65]	8	5,80%
≥65	0	0,00%

Table 14: Distribution of infected patients by months during the period (2013-2023).

Month	Effective	SBI%
January	12	8,69%
February	15	10,86%
March	15	10,86%
April	14	10,14%
May	20	14,49%
June	10	7,24%
July	3	2,17%
August	8	5,79%
September	13	9,42%

October	12	8,69%
November	12	8,69%
December	4	2,89%

Table 15: Distribution of infected patients according to the season during the period (2013-2023).

Seasons	Effective	SBI%
Winter	27	19,56%
Spring	49	35,50%
Summer	22	15,94%
Autumn	40	28,98%

Table 16: Distribution of infected patients according to the years during the period (2013-2023).

Years	Total number	Positive cases (+)	SBI %
2013	1169	19	1,62%
2014	756	18	2%
2015	1002	11	1,09%
2016	980	9	0,91%
2017	1577	14	0,88%
2018	1560	3	0,19%
2019	1029	14	1,36%
2020	994	6	0,60%
2021	1125	14	1,24%
2022	1210	16	1,32%
2023	1180	14	1,32%

Table 17: Distribution of patients according to the region during the descriptive study period (2013-2023).

Regions	Number of positive cases	SBI
Mila	74	53,62%
Ferdjioua	25	18,11%
Oued Athmania	13	9,42%
Chelghoum Laid	26	18,84%

Table 18: Regional distribution of patients according to infestation rate during the study period (2013-2023).

The region of Mila			The region of Ferdjioua		
	Effectives	Frequency		Effectives	Frequency
Positive cases	74	0,87%	Positive cases	25	2,95%
Negative cases	8452	99,13%	Negative cases	820	97,04%

The region of Oued Athmania			The region of Chelghoum Laid		
	Effectives	Frequency		Effectives	Frequency
Positive cases	13	1,76%	Positive cases	26	1,86%
Negative cases	724	98,23%	Negative cases	1370	98,13%

Table 19: Regional distribution of infected patients according to the sex ratio during the period (2013-2023).

The region of Mila			The region of Ferdjioua		
Sex	Number	SBI%	Sex	Number	SBI%
Females	29	39,18%	Females	14	56,00%
Males	45	60,81%	Males	11	44,00%

The region of Oued Athmania			The region of Chelghoum Laid		
Sex	Number	SBI%	Sex	Number	SBI%
Females	3	23,07%	Females	6	23,07%
Males	10	76,92%	Males	20	76,92%

Table 20: Regional distribution of infected patients according to age slices during the period (2013-2023).

The region of Mila			The region of Ferdjioa		
Age slices	Effective	SBI%	Age slices	Effective	SBI%
[0-1]	0	0,00%	[0-1]	0	0,00%
[2-4]	5	6,75%	[2-4]	4	12,00%
[5-9]	12	16,00%	[5-9]	0	0,00%
[10-14]	3	4,00%	[10-14]	3	12,00%
[15-19]	8	10,66%	[15-19]	5	20,00%
[20-44]	40	53,33%	[20-44]	12	48,00%
[45-65]	6	8,00%	[45-65]	1	4,00%
≥65	0	0,00%	≥65	0	0,00%

The region of Oued Athmania			The region of Chelghoum Laid		
Age slices	Effective	SBI%	Age slices	Effective	SBI%
[0-1]	0	0,00%	[0-1]	0	0,00%
[2-4]	2	15,38%	[2-4]	4	15,38%
[5-9]	0	0,00%	[5-9]	2	7,69%
[10-14]	0	0,00%	[10-14]	2	7,69%
[15-19]	0	0,00%	[15-19]	2	7,69%
[20-44]	11	84,61%	[20-44]	16	61,53%
[45-65]	0	0,00%	[45-65]	0	0,00%
≥65	0	0,00%	≥65	0	0,00%

Table 21: Regional distribution of infected patients according to the months during the period (2013-2023).

The region of Mila

Month	Effective	SBI%
January	9	12,16%
February	7	9,46%
March	5	6,76%
April	6	8,11%
May	12	16,21%
June	4	5,41%
July	2	2,70%
August	5	6,76%
September	9	12,16%
October	6	8,10%
November	8	10,81%
December	1	1,35%

The region of Ferdjiousa

Month	Effective	SBI%
January	0	0,00%
February	3	12,00%
March	5	20,00%
April	6	24,00%
May	5	20,00%
June	3	12,00%
July	0	0,00%
August	0	0,00%
September	0	0,00%
October	2	8,00%
November	1	4,00%
December	0	0,00%

**The region of Oued
Athmania**

Month	Effective	SBI%
January	1	7,69%
February	3	23,07%
March	1	7,69%
April	0	0,00%
May	1	7,69%
June	1	7,69%
July	0	0,00%
August	2	15,38%
September	1	7,69%
October	2	15,38%
November	0	0,00%
December	1	7,69%

**The region of Chelghoum
Laid**

Month	Effective	SBI%
January	2	7,69%
February	2	7,69%
March	4	15,38%
April	2	7,69%
May	2	7,69%
June	2	7,69%
July	1	3,84%
August	1	3,84%
September	3	11,53%
October	2	7,69%
November	3	11,53%
December	2	7,69%

Table 22: Regional distribution of infected patients according to the seasons during the period (2013-2023).

The region of Mila			The region of Ferdjioua		
Seasons	Effective	SBI%	Seasons	Effective	SBI%
Winter	16	21,62%	Winter	3	12,00%
Spring	23	31,08%	Spring	16	64,00%
Summer	11	14,86%	Summer	4	16,00%
Autumn	24	32,43%	Autumn	2	8,00%

The region of Oued Athmania			The region of Chelghoum Laid		
Seasons	Effective	SBI%	Seasons	Effective	SBI%
Winter	4	30,76%	Winter	4	15,38%
Spring	2	15,38%	Spring	8	30,76%
Summer	3	23,07%	Summer	4	15,38%
Autumn	4	30,76%	Autumn	10	38,46%

Table 23: Regional distribution of patients according to the years during the period (2013-2023).

The region of Mila				The region of Ferdjioua			
Years	Total number	Positive cases (+)	SBI %	Years	Total number	Positive cases (+)	SBI %
2013	1024	15	1,46%	2013	4	0	0,00%
2014	653	15	2,29%	2014	/	/	/
2015	881	11	1,24%	2015	/	/	/
2016	880	6	0,68%	2016	/	/	/
2017	1511	13	0,86%	2017	17	1	5,88%
2018	902	3	0,33%	2018	44	0	0,00%
2019	441	4	0,90%	2019	220	4	1,82%
2020	805	3	0,37%	2020	106	0	0,00%
2021	781	1	0,13%	2021	90	7	7,77%
2022	642	1	0,13%	2022	174	8	4,59%
2023	521	2	0,38%	2023	406	5	1,32%

The region of Oued Athmania				The region of Chelghoum Laid			
Years	Total number	Positive cases (+)	SBI %	Years	Total number	Positive cases (+)	SBI %
2013	/	/	/	2013	126	4	3,17%
2014	/	/	/	2014	103	3	3%
2015	/	/	/	2015	121	0	0,00%
2016	/	/	/	2016	100	3	3,00%
2017	/	/	/	2017	97	0	0,00%
2018	/	/	/	2018	21	0	0,00%
2019	240	3	1,25%	2019	433	3	0,69%
2020	164	2	1,22%	2020	30	1	3,33%
2021	105	3	2,86%	2021	59	3	5,08%
2022	110	1	0,91%	2022	43	6	13,95%
2023	117	4	3,41%	2023	43	3	6,97%

Table 24: Distribution of patients according to infestation rate during the period (January – March 2024)

	Effectives	Frequency
Positive cases	5	1,67%
Negative cases	293	98,32%

Table 25: Distribution of infected patients according to the sex ratio (January - March 2024)

Sex	Effectives	SPI %
Males	3	60%
Females	2	40%

Table 26: Distribution of infected patients according to age (January - March 2024)

Age slices	Effectives	SBI%
[0-1]	0	0 %
[2-4]	0	0 %
[5-9]	0	0 %
[10-14]	1	20 %
[15-19]	0	0 %
[20-44]	4	80 %
[45-65]	0	0 %
≥65	0	0 %

Table 26: Distribution of infected patients by months during the period (January - March 2024)

Months	Effectives	SPI
January	1	20%
February	1	20%
March	3	60%

One-Sample Test

Test Value = 0

	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Month	20,508	137	,000	5,826	5,26	6,39
Sex	33,255	137	,000	1,377	1,29	1,46
Season	27,122	137	,000	2,529	2,34	2,71
Age	20,426	137	,000	23,587	21,30	25,87
Year	19,425	137	,000	5,783	5,19	6,37
Region	19,279	137	,000	1,935	1,74	2,13

ANOVA ONE WAY

		Sum of Squares	df	Mean Square	F	Sig.
Sex	Between Groups	2,035	10	,203	,851	,581
	Within Groups	30,371	127	,239		
	Total	32,406	137			
Age	Between Groups	3477,503	10	347,750	2,031	,035
	Within Groups	21747,789	127	171,242		
	Total	25225,292	137			
Region	Between Groups	52,659	10	5,266	4,855	,000
	Within Groups	137,754	127	1,085		
	Total	190,413	137			
Season	Between Groups	12,268	10	1,227	1,024	,427
	Within Groups	152,116	127	1,198		
	Total	164,384	137			
Month	Between Groups	121,469	10	12,147	1,098	,368
	Within Groups	1404,357	127	11,058		
	Total	1525,826	137			

MANOVA						
Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Sex	3,025 ^a	12	,252	1,072	,389
	Age	3693,033 ^b	12	307,753	1,787	,057
	Region	52,708 ^c	12	4,392	3,987	,000
Intercept	Sex	25,425	1	25,425	108,169	,000
	Age	7210,923	1	7210,923	41,861	,000
	Region	48,164	1	48,164	43,721	,000
Month	Sex	,725	1	,725	3,086	,081
	Age	128,102	1	128,102	,744	,390
	Region	,002	1	,002	,002	,966
Season	Sex	,451	1	,451	1,918	,168
	Age	67,464	1	67,464	,392	,533
	Region	,011	1	,011	,010	,921
Year	Sex	1,936	10	,194	,824	,607
	Age	3089,863	10	308,986	1,794	,068
	Region	52,407	10	5,241	4,757	,000
Error	Sex	29,381	125	,235		
	Age	21532,259	125	172,258		
	Region	137,705	125	1,102		
Total	Sex	294,000	138			
	Age	101977,250	138			
	Region	707,000	138			
Corrected Total	Sex	32,406	137			
	Age	25225,292	137			
	Region	190,413	137			

P₁

WILAYA DE MILA
ETABLISSEMENT PUBLIC HOSPITALIER
FRERES MEGHIAOUI
LABORATOIRE CENTRAL
UNITE DE PARASITOLOGIE - MYCOLOGIE MEDICALES

EXAMEN PARASITOLOGIQUE DES SELLES (EPS)
FICHE DE RENSEIGNEMENTS

Nom : Prénom :
Age : Adresse :
Profession : *Ch. parante* Service : *Pedi*
La clinique :
.....
.....
Notion d'une immunodépression :
.....
Traitement anti-infectieux en cours :
.....
EPS :
Examen macroscopique : *small, noisate*
Examen direct : *neg*
.....
Techniques de concentration :
.....
Techniques de coloration :
.....
Conclusion :
.....
.....
06/03/2024

A

Negative case of parasite
testing

P₁

WILAYA DE MILA
ETABLISSEMENT PUBLIC HOSPITALIER
FRERES MEGHLOUI
LABORATOIRE CENTRAL
UNITE DE PARASITOLOGIE - MYCOLOGIE MEDICALES

EXAMEN PARASITOLOGIQUE DES SELLES (EPS)
FICHE DE RENSEIGNEMENTS

Nom : [REDACTED] Prénom : [REDACTED]
Age : 1.5 Adresse :
Profession : Coiffeur Service : Malade
La clinique :

Notion d'une Immunodépression :

Traitement anti-infectieux en cours :

EPS :
Examen macroscopique : selles marron.
Examen direct : KGI (Kystes de *Giardia intestinalis*).
Techniques de concentration :
Techniques de coloration :
Conclusion :

23/4/2024

B

Positive Case of *Giardia
lamblia* in Parasitological
Testing

e : 12 ans Service : T A

ture de prélèvement : Selles

sultat :

pect Macroscopique : selle molle marron

Microscopie : Présence des Kystes de Giardia
intestinales

L'opérateur

C

Results summary given to the
patients

This work is the subject of two attestations:

