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The Use of Artificial Intelligence (AI) Combined with the Traditional Author Designed Questionnaires, Coproscopic, and Serological Methods as a Valuable Tool in Diagnosing Intestinal Human Parasites

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ABSTRACT

The diagnosis of intestinal human parasites using traditional coproscopic and serological methods faces persistent challenges, including operator dependence, variability in detection, and time-intensive processes. Coproscopic techniques, considered the gold standard, often struggle with low sensitivity in cases of light infections and require highly trained technicians. Similarly, serological methods, while effective in detecting antibodies or antigens, are limited by cross-reactivity and dependence on specialized equipment, which can hinder their broader application.

This thesis investigates the integration of artificial intelligence (AI) with a custom-designed questionnaire to enhance parasitology diagnostics. AI, powered by machine learning algorithms, offers the potential to analyze large datasets with precision and speed, identifying patterns that traditional methods may overlook. The questionnaire complements AI by capturing nuanced contextual data, including symptoms, environmental factors, and risk behaviors, enhancing the model's diagnostic accuracy for complex parasitic infections.

A structured database underpins the AI model, consisting of three primary tables (questions, parasites, and data) optimized for efficiency and binary encoding (0 for negative, 1 for positive) to facilitate seamless AI processing. While the model demonstrated high performance in identifying negative cases (91% accuracy and 99% sensitivity for negatives), it failed to detect positive cases, achieving 0% accuracy and sensitivity for the positive class. Despite the use of oversampling techniques like SMOTE to address data imbalance, these limitations highlight the need for advanced algorithms, balanced data collection, and threshold adjustments to improve sensitivity to minority classes.

The questionnaire was meticulously developed through a comprehensive review of parasitology literature, refined by expert input using the Delphi method, and validated through pilot testing. This rigorous process resulted in 112 variables, validated for reliability and relevance, ensuring its compatibility with AI and effectiveness as a diagnostic tool.

The combined method demonstrated notable strengths, including high accuracy for negative cases and significant time efficiency. Automated data processing, structured database design, and real-time performance metric computation reduced the time required for diagnosis compared to traditional approaches. However, the system's inability to classify positive cases accurately underscores the need for targeted refinements.

Future research should address these limitations by improving data balance, enhancing model sensitivity, and developing explainable AI tools to foster trust among healthcare practitioners. Seamless integration of AI into clinical workflows, alongside ethical considerations such as bias mitigation and data privacy, is critical for equitable adoption. Despite its challenges, the AI-questionnaire method holds transformative potential for parasitology diagnostics, paving the way for improved detection, patient care, and global health outcomes.

Key words

Artificial Intelligence (AI), intestinal parasites, parasitology diagnostics, data imbalance, questionnaire integration.

ABREVIATIONS

AAD : Antibiotic-associated diarrhea

ADD : Attention Deficit Disorder

ADHD : Attention deficit hyperactivity disorder

AI : Artificial Intelligence

AIDS : Acquired immunodeficiency syndrome

ANN : Artificial Neural Network

BC : Before Christ

Cm. : Centimeters

CNS : Central nervous system

CSF : Cerebrospinal fluid

CT : Computed tomography

CVI . Content Validity Index

ELISA : Enzyme-linked immunosorbent assay

Fa : source of argumentation

FBD : Foodborne diseases

FN : false negatives

FP : False Positives

GDPR : General Data Protection Regulation

HIV : human immunodeficiency virus

K : Competence Coefficient

Ka : Argumentation Coefficient

Kc : Knowledge Coefficient

MATLAB : Programming and numeric computing platform used by millions of engineers and scientists to analyze data, develop algorithms, and create models.

MCSVM : Multiclass support vector machine

MRI : Magnetic resonance imaging

NC : Neurocysticercosis

OLM : Ocular Larva Migrans

OTC : Over the counter

PANDAS : Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections

PANS : Pediatric acute-onset neuropsychiatric syndrome

PCOS : Polycystic ovary syndrome

PCR : Polymerase chain reaction

PI : Pertinence Index

PITAND : Pediatric infection triggered autoimmune neuropsychiatric disorders

RDBMS : Relational database systems

RI : Relevance Index

SIBO : Small intestinal bacterial overgrowth

SMOTE : Synthetic Minority Over-sampling Technique

SPSS : Statistical Package for the Social Sciences

STI : Sexually transmitted infection

TP : true positives

USA : United States of America

VLM : Visceral Larva Migrans

VVC : Vulvovaginal candidiasis

WHO : World Health Organization

XAI : Explainable AI

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I. INTRODUCTION

Parasites are organisms that live in association with other organisms, through which they obtain the means for their survival, generally by harming the host organism(1). They are classified as: a) ectoparasites: when they affect the external part of the host's body, for example lice, fleas, ticks, and other organisms with similar characteristics(2); b) endoparasites: when they reside inside the host's body, as in the case of tapeworms, nematodes, among others(3); and finally, c) haemoparasites: when they live in the bloodstream, as trypanosoma, plasmodium, etc.(4).

Parasites can have their life cycle exclusively in the blood, which makes them predominantly haematozoa, such as babesia (sporozoa) and plasmodium. They may also have part of their life cycle in the blood, such as filariae(5). In addition, some haemoflagellates such as Leishmania and Trypanosoma can also be visualized in peripheral blood smears and are therefore of clinical importance in differential diagnoses(6). Rarer parasites that can also be identified in smears include those causing filariasis and onchocerciasis(7).

However, it is important to note that parasitism is one of the possible symbiotic associations found in nature, along with commensalism (one of the symbiont organisms benefits from the other without causing harm) and mutualistic symbiosis (both symbiont species benefit from the symbiosis)(8). Unlike the latter two, parasitism is characterized by the antagonistic relationship between the parasite and the host, which provides the parasite with the means of subsistence to the detriment of the welfare of the other(9).

Based on these definitions, the distinction between parasites and commensals is made on the basis of pathogenicity (understood as the ability of a symbiont species to harm a host species)(10). Often, there is no clear

separation between the two, as the pathogenic potential of a parasite may require particular alterations in the host, such as nutritional deficiencies, breaks in the continuity of physical barriers or even a disruption of the immune system(9).

As a consequence, detecting a parasitic infection may entail the development of various analyses and clinical tests. Furthermore, a large part of Clinical Parasitology is traditionally reserved for parasitosis supported by Helminths and Protozoa. At the same time, within these two of the three conventionally defined branches of Parasitology (the third includes Entomology) a significant percentage is reserved for parasitoses of the gastrointestinal system (in a broad sense) and for parasitoses of the haematic, reticuloendothelial and systemic systems(11).

Particularly for these two groups of parasitoses of human interest, the diagnosis is purely laboratory-based and still relies on microscopic observation of specific slides or specimens. While pre-analytical steps are important and necessary, analytical routes and methods are also essential(12). From the proper preparation of the slides to the subsequent specific staining, as well as the recommended and advisable enrichments to the necessary preparatory procedures (even resorting to consolidated alternative methodologies) in order to finally reach a correct and complete morphological diagnosis by applying with care and skill, with attention and care, to the observations under the optical microscope, an instrument that is still irreplaceable(11).

Of course, both for the aforementioned tests and for other parasitosis it is, or may be, necessary to resort to other diagnostic techniques that, in any case, must be interpreted as complementary to the usual and routine ones. Although some of the innovative techniques are giving better and better results in the field of medicine, such as the use of Artificial Intelligence (AI), which is spreading rapidly in our society, at a pace probably faster than any optimistic forecast(13).

Every day, even unconsciously, we use AI-based technologies that facilitate more or less complex tasks of everyday life, ranging from navigating in a car, to finding a restaurant or suggesting the purchase of a particular consumer good(14).

The healthcare environment in general (and diagnostics in particular) is similarly undergoing this development process(14). For example, AI-based technologies already control large diagnostic imaging equipment (computed tomography, CT or magnetic resonance imaging, MRI), “standardizing acquisition protocols” and substantially reducing exam times, improving the patient comfort and compliance(15).

In a similar vein, we can find a series of algorithms capable of providing solid support to the specialist during the identification of pathologies (for example breast or lung nodules, bone fractures, pneumothorax, etc.), minimizing perception errors, or helping in the characterization of lesions (as may be the case of the detection of a cutaneous melanoma for a dermatologist), in order to improve the diagnosis. The latest scientific evidence also reports studies on the use of "intelligent" expert systems, capable of categorizing a patient's risk, determining a prognosis and supporting the physician in the subsequent therapeutic decision(13).

However, in the context of parasitology, the inclusion of such tools is not substantially widespread(16), which has led to the subject of analysis of this research, which focuses on the development of a specific tool (questionnaire) to determine the possible parasite infection of patients, which, together with IA, can reduce diagnosis times and help specialists to identify the corresponding type of infection. As a consequence, the following sections present the theoretical underpinnings of the proposed research, as well as an analysis of recent evidence on the use of IA in the field of clinical diagnosis.

II. STRUCTURE OF THE THESIS

In accordance with the premise described above, this thesis is structured into four comprehensive chapters, each addressing critical aspects of parasitic infections, their detection, and the integration of artificial intelligence (AI) into diagnostic processes. Together, these chapters provide a cohesive narrative, from understanding parasitic diseases and their impact to evaluating innovative diagnostic methodologies.

The first chapter lays the foundation by delving into the biological and clinical aspects of parasites and the diseases they cause. It begins with the definition of parasites and explores the wide-ranging impact of parasitic infections on the human body. The chapter provides an in-depth examination of 31 specific types of parasitic infections, including Giardia, Hookworms, Ascaris lumbricoides, and Toxoplasma gondii, among others. Each type is analyzed for its unique biological characteristics, epidemiology, symptoms, and implications for human health, providing a robust understanding of parasitology.

The chapter also investigates the interplay between diet and parasitic infections, highlighting the role of dietary practices and food handling in disease transmission. Further, the relationship between parasitic infections and domestic animals is explored, emphasizing zoonotic transmission. A critical component of this chapter is the overview of detection, diagnosis, and prevention techniques, culminating in a discussion on the role of new technologies. In particular, it emphasizes how AI is transforming traditional diagnostic approaches, offering greater precision and efficiency in detecting parasitic infections.

Chapter 2 outlines the research framework, detailing the objectives, hypotheses, and methodologies adopted for the study. The general objective focuses on integrating AI with a traditional questionnaire to enhance parasitology

diagnostics. Specific objectives include designing and validating the questionnaire, applying AI for data analysis, and evaluating the system's diagnostic effectiveness. This chapter is divided into two parts:

The first part describes the design and validation of the diagnostic questionnaire, detailing how the instrument was developed through literature reviews, expert validation using the Delphi method, and rigorous pilot testing. The process ensured that the questionnaire captures relevant symptoms, environmental factors, and behavioral risks essential for parasitology diagnostics.

The second part focuses on the application of AI within the diagnostic framework. It details the study's design, including sample size calculation, eligibility criteria, and ethical considerations. The methodology emphasizes using SQL for efficient data management and AI integration, highlighting its role in processing the vast dataset and supporting the diagnostic system.

The third chapter presents the study’s findings, focusing on the performance of the AI-questionnaire model. Key metrics such as recall (sensitivity), precision, and F1 Score are used to evaluate diagnostic effectiveness. The results highlight the system's strengths in detecting negative cases (class 0) but reveal significant limitations in identifying positive cases (class 1). These findings underscore the challenges of data imbalance, where the AI model demonstrates a pronounced bias toward the majority class, resulting in high accuracy for negatives but a failure to detect positives. The chapter also explores the outcomes for various parasitic infections, identifying correlations between dataset characteristics and diagnostic performance. These insights provide a basis for optimizing AI algorithms and improving sensitivity for underrepresented cases.

The final chapter synthesizes the findings and discusses their implications for parasitology diagnostics. It begins with a critical discussion of the study's strengths, including the validated questionnaire and the potential of AI to enhance diagnostic workflows. The limitations of the current system, particularly its inability to effectively detect positive cases, are examined in detail. Proposed solutions include data balancing strategies, threshold optimization, and algorithmic refinements to address biases and improve model sensitivity.

The chapter concludes by summarizing the study's contributions, emphasizing the potential of combining AI with traditional methods for improving diagnostic accuracy and efficiency. It also outlines future research directions, focusing on ethical considerations such as bias mitigation, data privacy, and accessibility. These recommendations aim to foster broader adoption of AI in clinical settings, ensuring equitable and effective healthcare outcomes. This chapter underscores the transformative potential of AI in advancing parasitology diagnostics, ultimately contributing to better global health outcomes.

CHAPTER 1: PARASITES, THEIR IMPACT ON THE ORGANISM AND THE DETECTION TECHNIQUES

Continuing with the line of argument of the research, this first chapter deals with the theoretical elements on the definition of parasites, identifying their essential characteristics, as well as their life cycle in the organism. This is followed by a presentation of parasitic infection in humans, with a brief historical overview of the research that has been carried out to consolidate the field of study.

Once the foundations of the academic context have been laid, the most common types of parasitic infection are discussed, determining the specific characteristics of each one, and the differences that can be found between countries. In this way, we move on to the study of the close relationship between diet and parasites, and conclude the chapter with an analysis of parasitic infection transmitted by domestic animals.

1.1. Definition of parasites

Intestinal parasites are divided into protozoa and metazoa, as in the case of unicellular and multicellular organisms(17). As such, protozoan parasites are small in size (usually in the order of a few μm), whereas metazoans have complex structures, tissue differentiation and much larger dimensions (from a few mm to several meters)(18).

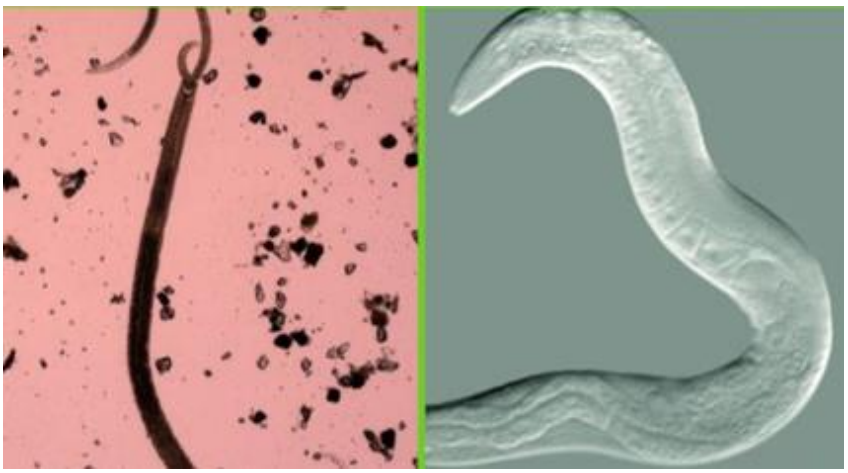

Figure 1. *Nematodes. Source(19)*

One of the metazoa of most interest for human medicine are the helminths (or intestinal worms), which are divided into roundworms (nematodes) as in the figure 1(20), and flatworms, like the figure 2. The latter are further divided into cestodes (ribbon-shaped and segmented) and trematodes (unsegmented)(21).

The most common intestinal parasites in the world are:

a) *Among the protozoa:* Balantidium coli, Cryptosporidium sp., Dientamoeba fragilis, Entamoeba histolytica, Giardia lamblia, and Isospora bell(22).

b) *Among the metazoans:* Ancylostoma duodenale, Ascaris lumbricoides (ascaridiasis), Enterobius vermicularis, Necator americanus, Trichuris trichiura, Schistosoma spp, Strongyloides stercoralis and Taenia spp. (Taenia solium - from pigs - tapeworm and Taenia saginata - from cattle)(23).

In this way, these parasites or micro-organisms cause intestinal infestations, which affect the digestive tract. This is why this type of disease is

also known as parasitosis. Despite their size, these micro-organisms are an endemic problem in some developing countries, where hygienic, climatic and environmental conditions favor their appearance and spread(24).

However, this problem is also present in developed countries, since even in Europe and in the more developed countries of the West, they are more widespread than might be thought, partly due to the current ease of intercontinental and transoceanic travel, and the increase in immigration phenomena(25).

The most common parasitoses in Europe are caused by worms of the Nematode group (Enterobius vermicolaris and Ascaris lumbricoides) and the Cestode group (Taenia solium and Taenia saginata). Giardiasis (Giardia lamblia) and amoebiasis (Entamoeba histolytica) are also widespread on the continent(26).

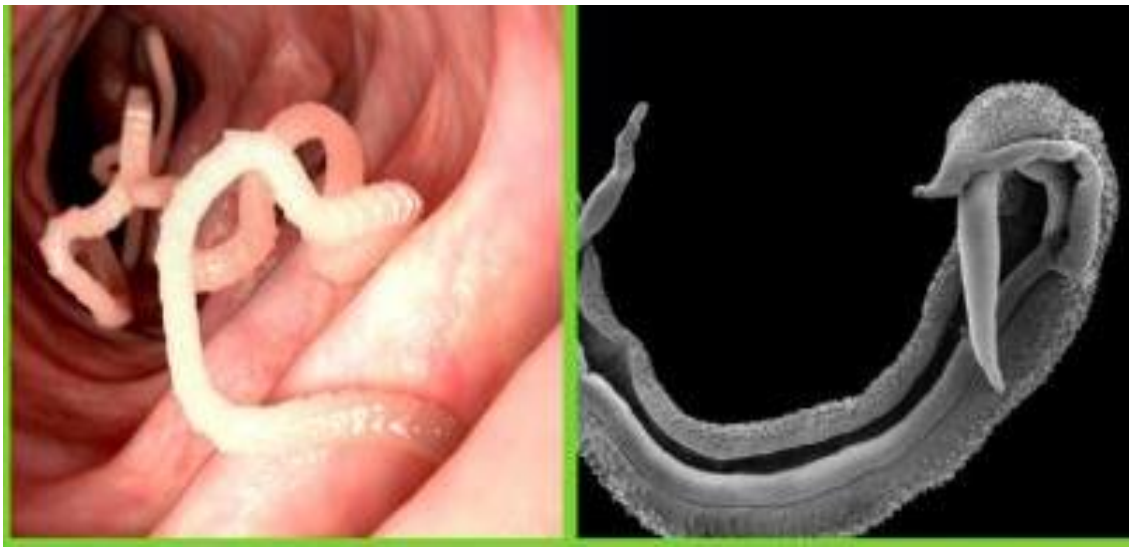

Figure 2. *Flatworms. Source(27)*

Parasitism (from the Greek *Părăsītus*, a Latin cultism taken from the Greek *παράσιτος* (parasites), literally meaning "one who eats at another's table")(28) is a biological interaction, mainly of a nutritional and trophic nature, between two organisms of different species, one of which is called a parasite and the other a host. Parasitism is a form of symbiosis, but not of the same mutualistic nature, in which the parasite gains an advantage, mainly of a nutritional and trophic nature, at the expense of the host, creating biological damage to the latter(29).

The characteristics of parasitism are that the parasite has no autonomous life and is dependent on the host, that it has a very simple anatomical and morphological structure in relation to the host, a life cycle that ends before the death of the host, and relationships with only one host, while the host may have relationships with several parasites(29).

However, different types of parasites can be found, such as the facultative parasite, which is able to live even autonomously, relying on forms of diet that are not conducive to parasitism. The endoparasite, also called endophagous parasite, which lives inside the body of the host organism (for example the tapeworm)(30). There is also the ectoparasite, also called the ectophagous parasite, which lives outside the body of the host, to which it is closely linked (like again, in the case of the tapeworm)(31). In this context, we can also appreciate the kleptoparasite, which aggressively acquires the food that the host is accustomed to eating (for example the Cuculus canorus and Nibbio fischiatore)(32).

In a similar framework, we can appreciate that there are several species of amoebae that can be found in the large intestine, such as Entamoeba coli, Entamoeba hartmanni, Iodamoeba butschlii, Endolimax nana, Entamoeba dispar and E. histolytica. However, the latter is the only one considered potentially

pathogenic to humans(33). Nevertheless, the various types of parasites will be discussed in more detail in later sections.

As expected, these parasites have a substantial impact on the hosts, which are usually humans, causing various problems in the organism, and which have led to the development of an extensive research framework, as described in detail in the following section of the thesis.

1.2. Parasite infection in humans

As might be expected, intestinal parasites are frequent infectious agents and are responsible for major public health problems in many countries. However, this situation is not unique to our time, as there are records of such infections dating back to ancient times. The first reports of intestinal infections date back to ancient Greece, when Hippocrates referred to a "fatal disease" in which individuals presented with symptoms of fever and dysentery(34).

More than a thousand years later, in the 19th century, Lambl observed micro-organisms in the feces of a child with dysentery, which was replicated by another expert (Cunningham) a few years later, who set out to isolate protozoa from the feces of patients with choleric diarrhea, establishing an association between the disease and the micro-organism(35).

In this way, and at the height of scientific progress, a few decades later, Lösch, through microscopic and clinical studies, made an association between dysentery and the presence of organisms in the feces and named them "Amoeba coli"(36). This research was continued by other experts, who mentioned clear evidence of tissue invasion by amoebae similar to those described in Lösch's studies(36).

Continuing with the advances in the field, Lafleur and Councilman described the pathological process of amoeba invasion of the intestine and liver, attributing the pathology to them, calling it "Entamoeba dysenterie". These authors were the first to use the terms amoebic dysentery and amoebic liver abscess specifically in their academic publications(37).

Only a couple of years later Roos and Quincke described cysts in feces as rounded structures with a well-defined cell wall and reported the presence of trophozoites(38). However, advances in the field continued and already in the early years of the 20th century, Schaudinn described another species of amoeba which he called "Entamoeba coli", considered non-pathogenic, and proceeded to rename "Entamoeba dysenterie" as "Entamoeba histolytica" due to its ability to lyse tissues, characterizing it as pathogenic(39).

However, as a consequence of this study, the period was marked by a specific controversy over the scientific name to be given to the amoeba. In this context, Craig's work in 1905 gave some priority to the nomenclature "Entamoeba dysenterie" over "E. histolytica". However, only five years later, Walker re-described the intestinal amoebae and distinguished the difference between "E. histolytica" and "Entamoeba coli" based on the number of cyst nuclei of the two species(40).

Thus, the pathogenicity of E. histolytica, like that of other parasites, has been extensively investigated, but to understand the historical development of this field, it is necessary to focus on the path of E. histolytica. On this premise, Dobell proposed the unifying theory that "E. histolytica", consisting of tetranucleated cysts, would not be pathogenic and could become virulent depending on the intestinal bacterial flora or unknown environmental factors(41).

Although Dobell's attempts were not accepted by the entire academic field, as the dualistic theory proposed by Brumpt in 1925 had significant support in the scientific community. This theory considered that there were two species of the genus Entamoeba with identical morphological characteristics, with "E. histolytica" being pathogenic and "Entamoeba dispar" only commensal(40).

Furthermore, the expert noted that many patients apparently infected with "E. histolytica" were asymptomatic and the infection resolved spontaneously without intervention or treatment(40). However, as at the time there was no adequate methodology to distinguish the two species, this theory was eventually rejected by the scientific community. This situation is evidence of the complexity of the field during the early years of the 20th century and how the lack of specific methods and protocols slowed down its development substantially.

Years later, Hoare presented the neodualist proposal, in which he stated that the pathogenic potential of "E. histolytica" would be very diverse, with degrees of virulence ranging from asymptomatic to severe forms of the disease(41). However, it was Diamond who first consolidated axenic cultures of E. histolytica, which allowed subsequent biochemical and immunological characterization studies(40).

In 1978, a series of studies were developed(42), in which pathogenic and non-pathogenic isolates of E. histolytica were differentiated by different electrophoretic patterns of isoenzymes, correlating them with clinical form and biological parameters, making the dualistic theory gain traction.

Years later, some experts(40), considering biochemical, immunological and genetic evidence that distinguished isolated amoebae from symptomatic and asymptomatic individuals, confirmed the existence of two morphologically

identical species, as proposed by Brumpt decades earlier, calling the non-pathogenic species "Entamoeba dispar"(43).

Near the end of the 20th century, at a seminar on amoebiasis held by the World Health Organization (WHO), researchers came to support the dualistic theory, however, they concluded that the diagnosis made with light microscopy should be recorded as "E. histolytica / Entamoeba dispar", because the size characteristic used in the taxonomy of "E. histolytica" would not be effective in differentiating it from "Entamoeba dispar"(44).

According to Talamás-Lara et al.(45):

> “The existence of two different species of *Entamoeba*, initially proposed by Brumpt in 1925, was approved at the XIII Seminar on Amebiasis, held in Mexico City. At present, unequivocal evidence for the existence of two morphologically similar and closely related species of *Entamoeba* in humans has been substantiated by immunological, genetic and molecular studies. As a result, *E. histolytica*, the invasive organism was formally redescribed to separate it from the noninvasive, and more common, *Entamoeba dispar*” (p.1)

This historical overview highlights the early problems faced by experts in determining the existence and diagnosis of parasites in humans, and it took more than a century of progress to consolidate the field as we know it today.

Therefore, based on the previously mentioned theoretical aspects, the following section will analyze the most common types of parasitic infections, their symptomatology and specific characteristics, as well as the prevalence and most affected populations.

1.3. Types of parasite infections

There are different types of parasitic infections, according to the number of parasites that can be found in different environments or elements with which human beings come into contact on a daily basis(46). However, it is important to take into consideration that some of these parasitic infections, which are quite frequent around the world, reach the European continent due to the geographical mobility of its inhabitants. The following sections will describe some of them.

1.3.1. The Giardia.

Giardia is a flagellated protozoan parasite that resides in the small intestine of mammals, birds, reptiles and amphibians. At least three species of Giardia are known(47):

I. *Giardia duodenalis:* which can infect mammals, including man, birds and reptiles.

II. *Giardia muris:* which can affect rodents, birds and reptiles.

III. Giardia agilis: which impacts amphibians.

Giardia is considered to be a direct life-cycle, monoxenic parasite. In humans, the most common form of infection is ingestion of mature specimens, via contaminated food and water, or from person to person via contaminated hands in crowded places, such as nurseries, nursing homes and so on. As a consequence, Giardia infection is a frequent disease in collective settings, where there is person-to-person contact and hygiene measures are difficult to implement or follow(48).

Giardia infects approximately 200 million people worldwide, and is responsible for causing acute diarrhea in children under 5 years of age, and has a substantial impact in developing countries, such as Africa, where infectious diarrhea is considered the leading cause of mortality among children under 5 years of age. This parasite is most prevalent among children aged 8 months to 10-12 years. High prevalence of these parasites is found in tropical and subtropical areas, with 4% to 30% among people of low economic status(49). Also, Giardiasis is the most common parasitic diseases in Poland.

In developed and developing countries, Giardia is one of the major parasites found in humans. Giardia lamblia is also one of the most common protozoan pathogens in the world, rarely causing severe and life-threatening diarrhea, but is a major contributor to malnutrition and stunting in children in developing countries. Intestinal protozoa, including Giardia, pose a public health risk as they are important aetiological agents of diarrhea, especially in children(49).

Giardia infections can occur in both adults and children, most often asymptomatic, some individuals may take up to six months to clear the parasites from their system. Symptomatic patients may present with acute self-limiting diarrhea or persistent diarrhea with evidence of malabsorption and weight loss; these infections can be difficult to treat, even in immunocompetent individuals(50).

In immunocompetent individuals, 30-50% may develop chronic diarrhea, accompanied by steatorrhea, weight loss and malabsorption problems, mainly of fats, fat-soluble vitamins (A, D, E, K), vitamin B12, iron, xylose and lactose. In children these nutritional deficiencies can have serious consequences. In adults, however, they are less severe. Some studies have suggested the development

of protective immunity to giardiasis, suggesting some resistance to subsequent infections(51).

Giardia parasites are resistant to chlorination, being frequently contracted through untreated (streams, dams, artesian wells, etc.) or insufficiently treated water from the public water supply, where chlorination is the only means of treatment. As a consequence, they are very resistant parasites, being able to survive in the environment, in good conditions of humidity and temperature for up to two months, surviving also under the nails for some time(51).

Despite being resistant to chlorination, they are destroyed at high temperatures such as boiling water, and it is advisable to boil the water before ingestion, especially in economically disadvantaged communities(52).

1.3.2. Hookworms

Hookworms are widely distributed throughout the world, with an estimated 500-700 million individuals infected and 80 million people suffering from severe infections as a result(53). The species A. duodenale, known as the "*Old World hookworm",* predominant in temperate zones, Europe and the Mediterranean, may also occur in tropical areas where the climate is less extreme. N. americanus, known as "New World hookworm", occurs in tropical regions with predominantly high temperatures, occurring in the Americas, sub-Saharan Africa and the Asia-Pacific islands(54).

The hookworm species, Necator americanus and Ancylostoma duodenale have a similar, cylindrical morphology, with A. duodenale being slightly larger (11-20 mm) than N. americanus (7-10 mm). Unlike A. lumbricoides and T. trichiura,

which are more common in children, hookworm infestation is more common in adults(55).

Hookworms have a direct life cycle, without the need for an intermediate host. The cycle has two phases: a free-living phase and an obligate parasitic phase. When hookworm eggs are deposited in the soil, under ideal conditions, they develop into non-infective first and second instar larvae, rhabditoids (L1) and (L2), which feed on soil microbiota(55).

However, the development of hookworm eggs and other geohelminths in soil is not observed in semi-arid climate regions. For their development they require ideal soil conditions, such as permeability, aeration, granularity (sandy), richness in organic matter and humidity (above 90%), which are usually found in the peridomestic environment. At the same time, temperature is a determining factor for the development of eggs and larvae, and ultraviolet rays are lethal for embryony(56).

Hookworm disease can occur in two different ways(56):

I. First: through active penetration of filarial larvae into the skin, conjunctivae and mucous membranes.

II. Second: passively through oral ingestion.

For N. americanus the most efficient way of infection is through the skin. For A. duodenale, in addition to oral and percutaneous infection there may be the possibility of transplacental infection(57).

Upon penetration of hookworm larvae, a "stinging sensation" may occur, followed by hyperemia, oedema and pruritus, resulting in an urticarial dermatitis.

This reaction, more common in N. Americanus infection, tends to cease after 10 days(57).

Other signs and symptoms may occur after the parasite reaches the intestine, such as: epigastric pain, poor appetite, indigestion, colic, indisposition, nausea, vomiting, flatulence, in some cases bloody diarrhea and constipation. In addition, with the onset of egg deposition, these symptoms can become more severe. Wakana syndrome is caused by oral infection with A. duodenale, characterized by symptoms of nausea, vomiting, pharyngeal irritation, cough, dyspnea and hoarseness(58).

One of the main symptoms of hookworm disease is anaemia, responsible for the jaundice, which characterizes the disease as “Yellow jaundice”. This condition results from the heavy consumption of host blood caused by the adult worms, which is most intense in A. duodenale, sucking 0.05 to 0.3 mV/day, while N. americanus sucks 0.01 to 0.04 ml/day. The most common anaemia associated with hookworms is iron deficiency anaemia(58).

1.3.3. Ascaris lumbricoides

Ascaris lumbricoides has a wide geographical distribution, occurring in more than 150 countries and territories, and is one of the most common helminth infections in the world. Although it is most prevalent in tropical and subtropical areas, it has variable frequencies depending on climatic and environmental conditions and, mainly, on the degree of socio-economic development of the country. Arid regions tend to have a low prevalence of ascaris, except during rainy periods, while in regions with warm and humid climates it is usually relatively high. It is considered the most common helminth in poor countries(59).

It is estimated that 30% of the world's population may be infected by A. lumbricoides, affecting more than 1.2 billion people. Furthermore, it is considered to be a cosmopolitan infection, impacting mainly Asian countries with a prevalence of 70%, followed by Africa (10%) and Latin America (less than 10%). Children are estimated to be the most affected by ascariasis, being most prevalent in the age group 2-10 years, with a decrease among adults(60). Also, ascariasis is the most common parasitic diseases in Poland.

A. lumbricoides infection occurs through ingestion of water or food contaminated with eggs. Insects such as flies and cockroaches, dust, rain and birds are also capable of mechanically carrying these eggs. The high ability of the eggs to adhere to surfaces, the complex removal of eggs by washing, as well as the high durability and the high number of eggs produced, contribute to the high prevalence of this parasitosis(61).

After infection with A. lumbricoides, white, rounded spots are often found on the face, trunk and arms, especially in children. A. lumbricoides is a single-host, monoxenic parasite. Adult worms are found in the small intestine, mainly in the jejunum and ileum, but may occupy the entire length of the intestine up to the rectum in cases of more intense infections(60).

Infection begins (after ingestion of eggs) in the small intestine, at which point the eggs are shed and larvae are released, penetrate the intestinal wall and migrate to the heart and lungs, via the bloodstream or lymphatic system, and occasionally may migrate to other sites such as the kidney and brain. Larvae usually reach the lung after 4 days of infection, mature within the alveoli for about 10 days, migrate to the bronchi and trachea and are swallowed. In the intestine they mature into adult worms, and after 2 to 3 months, adult females begin to lay eggs, completing the cycle(59).

Low intensity infections are defined as 3 to 4 adult worms in the intestine, usually without clinical manifestations. Moderate infections have 30-40 adult worms, while mass infections have more than 100 worms, resulting in liver and lung damage(59).

Most ascaris infections are asymptomatic, however, mass infections can lead to various complications in the body, especially in children, such as malnutrition, due to the great decomposing action of these worms, consuming large quantities of carbohydrates, proteins, lipids and vitamins A and C. The most common acute complication is intestinal obstruction(62).

Children are also the most prone to infection, due to the smaller size of the intestine, associated with the higher parasite load. The most serious complications occur when there is an ectopic location of these worms, which can lead to obstruction of the cecal canal, causing acute appendicitis and obstruction of the Wirsung's canal, leading to acute pancreatitis(61,63).

1.3.4. Trichuriasis Trichura

Trichuriasis Trichura infection is caused by the nematode T. trichiura and is cosmopolitan in nature, being widely distributed throughout the world, although it is more prevalent in regions with warm, humid climates and poor sanitary conditions. It is estimated that 1 billion people are infected worldwide, and is most prevalent in those under 15 years of age(64).

Trichuriasis Trichura is a long, slender, whip-shaped intestinal nematode, measuring 3-5 cm in adult stage, its geographical distribution and prevalence is similar to A. lumbricoides. In the biological cycle of Trichuriasis Trichura it is monoxenous, it is possible to establish a sexual distinction between male and

female worms, reproducing sexually, inhabiting the large intestine, releasing their eggs with the feces into the external environment. Contamination by this parasite occurs when the eggs are accidentally ingested through contaminated food and water. It is estimated that a small proportion of these eggs, 5-20%, develop into adult worms(65).

Trichuriasis Trichura infections are considered mild when fewer than 1000 eggs are found in feces, moderate when between 1000 and 9999 eggs are found, and severe when more than 10,000 eggs are found. The severity of Trichuriasis Trichura depends on the parasite load, host age and nutritional status. Generally speaking, mild infections are asymptomatic, while moderate infections show headache, epigastric pain in the lower abdomen, diarrhea, nausea and vomiting(65).

In severe infections, especially in children, there may be intermittent diarrhea with mucus and in some cases blood, abdominal pain with tenesmus, anaemia and severe malnutrition, characterizing Chronic Dysenteric Syndrome. In severe cases, rectal prolapse may occur(66).

1.3.5. Strongyloides stercoralis

S. stercoralis is a predominantly cosmopolitan parasite, with prevalence in tropical and subtropical areas, including the southeastern United States and tropical regions of Australia. Infestation occurs through exposure to soil contaminated with human feces and through autoinfection(67).

Strongyloides stercoralis is a parasite endemic to tropical and subtropical areas, and can coexist with the host asymptomatically, but in cases of immunosuppression, such as alcoholism or HIV, it can cause severe infections.

The most specific test to detect this parasite is the Baermann method, which is not always used by laboratories or requested by physicians(68).

Considering the high rate of people with a drinking habit in the world, about 24% of people could be infected by this parasite. Therefore, for diagnosis in endemic areas it is necessary to prevent asymptomatic infections, which can develop into severe forms of strongyloidiasis(68).

S. stercoralis contamination begins when rhabditoid larvae are excreted in feces and mature in soil to filarial larvae, the infective form, which are able to penetrate completely healthy human skin. Passage of the larvae through the skin can cause a traumatic, intensely itchy lesion. Another form of contamination is oral, through hands and food contaminated with filarial larvae, migrating directly to the intestine, where they may remain for the life of the carrier(69).

In strongyloidiasis some symptoms can be observed such as general malaise, urticaria, nausea, abdominal pain, loss of appetite, hemoptysis. Respiratory symptoms such as cough, sore throat, shortness of breath, fever, bloody sputum, secondary to the passage of larvae through the lung, may also appear(69).

1.3.6. Hymenolepis nana

Hymenolepis nana is the most common parasite of the cestoid class in humans (70). Predominantly cosmopolitan, it is estimated that more than 70 million people are affected by Hymenolepis nana worldwide. However, this prevalence is higher among individuals living in poor sanitary conditions and in crowded human settlements with fewer sanitary precautions (slums, kindergartens, etc.)(71).

The adult H. nana worm inhabits the small intestine of man, mainly in the ileum and jejunum. Eggs of the parasite are deposited in feces and cysticercoid larvae can be found in the intestinal villi of man or rodents (such as rats and hamsters). It has a heteroxenic and monoxenic life cycle (does not require an intermediate host). Both humans and rodents can be reservoirs of H. nana(72).

Transmission also occurs by ingestion of eggs deposited on hands or in contaminated food and water. However, infections are usually asymptomatic, and the onset of symptoms is usually associated with age and parasite load (more than 10,000 eggs per gram of stool). The main symptoms are agitation, insomnia, irritability, diarrhea, abdominal pain, and are more frequent in children(73)

1.3.7. Schistosoma mansoni

Schistosomiasis is an infection caused by trematode helminths of the genus Schistosoma, with five species parasitizing man(74):

- S. haematobium (Africa)

- S. japonicum (Japan, Southeast Asia, Western Pacific)

- S. Mansoni (Africa, Southwest Asia, Brazil and the Caribbean)

- S. Intercalatum (Africa)

- S. Mekongi (Mekong Basin) or Mekongi (Mekong Basin)

Approximately nearly 250 million people worldwide are affected by the disease and more than 500 million live at risk of infection, and it is endemic in

parts of Africa, South America, the Caribbean, the Middle East and Asia, spanning more than 70 countries(75).

The parasite's life cycle begins when feces contaminated with its eggs are deposited in freshwater. Under ideal conditions, these eggs develop into miracidia (free-living form), and these larvae migrate to find a snail of the genus Biomphalaria. These cercariae migrate into the water, entering the human system by penetrating directly through the skin. Once inside the body, they transform into schistosomula, spreading through the blood until they reach the portal system. In the liver they mature into adult forms, males and females' mate and migrate to the venous plexus of the intestine. Eggs are excreted in the feces, completing the parasite cycle(76).

The intensity of disease symptomatology is linked to several factors such as: parasite strain, acquired parasite load, age, nutritional status and individual immune response. In addition, there is a direct correspondence between parasite load (estimated by egg count per gram of stool on the Kato - Katz) and symptomatology(77).

In populations where the average number of eggs in feces is very high, the most frequent form is hepatosplenic and pulmonary. Skin (dermatitis) and liver disorders are influenced by the patient's immune response to schistosome and egg antigens. In addition to these lesions, intense spoliation of the host may occur due to high consumption of iron and glucose by the parasite(78). Other disorders can also be associated with chronic schistosomiasis infection, such as the risk of colon and liver cancer and chronic salmonellosis(79).

1.3.8. Enterobius vermicularis

Enterobiosis is a predominantly cosmopolitan infection, which is mainly related to personal hygiene problems rather than health and environmental problems, and is prevalent even in first world countries(80). Thus, it is highly prevalent in school-aged children. It is widely transmitted in the domestic environment or in closed collective environments (nurseries, old people's homes, children's wards, etc.)(81).

The most intense and common complaint of E. vermicularis infection is anal itching. The anal mucosa is congested, covered with mucus, containing eggs and sometimes whole females of the parasite. As a consequence, scratching of the anal area can damage the entire dermis of the area, allowing secondary bacterial infection(82).

Intense itching of the anal area can cause loss of sleep and nervousness, and one third of anal infections may be asymptomatic. In women, these infections can be seen in the genital organs and can lead to vaginitis, metritis, salpingitis and ovaritis(83,84).

Nevertheless, it is believed that enterobiasis (Enterobius vermicularis infestation) is far more common that ascariasis and giardiasis, since it is much more infectious. The point that the patients do not usually consult the doctors since the diagnosis is very often visual and the medicines to cure enterobiasis are (in some European countries like Poland) available with no medical prescription (OTC- medicines).

1.3.9. Amebiasis (Entamoeba histolytica infection)

This infection is also known as amoebic dysentery. Amoebiasis is an infection caused by a parasite that lodges in the patient's colon and is capable of causing various symptoms. Amebiasis is more common in environments where there is a clear lack of hygiene(85).

Amoebiasis is caused by the protozoan Entamoeba histolytica, whose eggs can be ingested through contaminated food and water or even by contact with contaminated people(86).

Amebiasis is usually asymptomatic in its early stages. It is only when it is advanced that symptoms of the disease become apparent. It is important to identify and treat the disease as soon as the first signs and symptoms of infection appear, because the disease can progress to the most severe stage of amoebiasis, which is characterized by extraintestinal complications and is called symptomatic extraintestinal amoebiasis(87).

In the latter scenario, the parasite can pass through the intestinal wall and reach the liver, causing abscess formation, and also the diaphragm, which can lead to pleuropulmonary amebiasis. As a consequence, the main symptoms of amoebiasis include: abdominal pain and cramps; tender abdomen; severe diarrhea; presence of blood and/or mucus in the stool; weight loss; fever(87).

Regarding the progress of the infection, as the amebiasis progresses, ulcers and lumps may form inside the intestine, in addition to other secondary conditions such as anaemia. Thus, the diagnosis of amoebiasis can be made by a simple stool examination, but also by other procedures such as colonoscopy(88).

Transmission of the disease occurs through the consumption of water and food contaminated by the parasite, and is more frequent in regions or areas where there are no adequate sanitary systems or measures in place, which favors contact of food and water with feces. The initial diagnosis of amoebiasis is made by a general practitioner or gastroenterologist based on an assessment of the patient's presenting signs and symptoms(89).

However, it is important to mention that the infection can be treated in the short term. This is done by using drugs that kill the eggs and also the amoebae resulting from amoebiasis. The treatment of amoebiasis is determined by the physician according to the type of infection the person has, and may recommend the use of Iodoquinol, Paromomycin or Metronidazole depending on the recommendation based on observation of the patient. In case of extraintestinal amoebiasis, the doctor may recommend the combined use of Metronidazole and Tinidazole. However, throughout treatment, experts suggest the importance of maintaining hydration, as high fluid loss is common due to the diarrhea and vomiting that occur with amoebiasis(90).

1.3.10. Blastocystosis (Blastocystis spp. infection)

Blastocystosis infection results from the action of Blastocystis hominis in the body. Thus, B. hominis infection can cause significant clinical manifestations, which may persist for three to ten days or even for much longer periods, as it can remain in the intestine for weeks to years. However, the presence of the protozoan often does not cause any alterations, resulting in an asymptomatic picture(91).

However, the symptoms of affected individuals may range from those usually associated with gastroenteritis, the occurrence of abdominal pain, anal itching, flatulence, meteorism, nausea, vomiting and diarrhea of varying intensity, without the presence of leucocytes or blood in the stool(92).

It is important to mention that the finding of B. hominis in feces does not guarantee that it is responsible for a disease that is in the etiological elucidation phase, as doubts about its pathogenicity persist. It is therefore necessary to look carefully for the possible determinant action of another cause, through multiple analyses, in a really intensive work. Diarrhea and signs or symptoms of gastroenteritis depend on many causes, infectious or non-infectious. Thus, attributing blastocystosis to a given situation can be a particularly complex task(93).

The geographical distribution of blastocystosis is probably worldwide. However, it occurs most frequently in developing countries in tropical and subtropical regions, although it also occurs in developed countries. In addition, some cases have been detected in which tourists themselves have been affected, or in other settings where there is a large influx of children, such as in day-care centers(94).

1.3.11. Clonorchiasis (Clonorchis sinensis infection)

Clonorchis sinensis is a trematode of the family Opisthorchiidae, endemic to the Asian continent, limited to the area around the China Sea, including Japan, Korea and most of mainland China, as well as Taiwan. The conditions necessary for the life cycle of C. sinensis include, in addition to the definitive host, which may be man or various species of carnivorous mammals and even some birds,

two types of intermediate hosts. The first intermediate host is necessarily an operculate mollusk belonging mainly to the genera Parafossarulus, Bulimus, or Semisulcospira(95).

The second is represented by countless species of freshwater fish in Asia, in which cercariae, formed in the mollusks and released into the aquatic environment, become encysted and develop into metacercariae. Transmission to humans occurs through ingestion of raw or undercooked meat from these fish. Metacercariae hatch in the duodenum and reach the bile ducts where they mature after about 30 days(96).

Human involvement with C. sinensis causes alterations of varying intensity and severity, depending mainly on the number of worms present. In terms of symptomatology, clonorchiasis can be classified as mild, progressive or severe. In the first type, many patients are asymptomatic, while in more severe cases, there may be an association with cirrhosis(97).

The drug of choice in the treatment of clonorchiasis was for a long-time chloroquine diphosphate, administered at a dose of 250 mg, three times a day, for a period of six weeks. The occurrence of serious side effects, including optic neuropathy, and the not always satisfactory results of the treatment led to the choice of other drugs(98).

Praziquantel, a pyrazinoisoquinoline derivative, is a primarily hepatically metabolized drug with proven pharmacodynamic action against cestodes, against various schistosome and human agents and also in clonorchiasis(99).

Under the prevailing bioecological conditions of coastal environments, the finding of mollusks potentially susceptible to the evolution of C. sinensis, the occurrence of autochthonous outbreaks of clonorchiasis becomes possible if

there are suitable sources of infection, represented by infected humans shedding trematode eggs in their feces. This circumstance justifies epidemiological surveillance actions to prevent the entry of sources of infection(98).

1.3.12. Cysticercosis (neurocysticercosis/ neuro-Taenia- infection)

Human cysticercosis is caused by the presence of the larval form of Taenia solium in tissues and by parasite-host biological interactions. The central nervous system (CNS) location of the parasite, neurocysticercosis (NC), is the most studied form, due to the severity of symptoms and high lethality and morbidity. The adult parasite averages 2 to 3 metros in length, and can reach up to 9 meters(100).

It consists of a small, slightly quadrangular and globular scolex, 1 mm in diameter, with four protruding suckers that help it attach to the intestinal mucosa. The rostrum is poorly developed and armed with two sets of aculella, followed by a short, thinner neck and strobilus, with 800 to 900 proglottids. In the gravid proglottids, the uterus is full of eggs, 30 to 50 thousand in number, only partially mature. The uterine branches, 7 to 10 in number, are dichotomous, and the rings, two to three at a time, are passively shed along with the feces(101).

The rupture of the proglottids usually occurs in the external environment, with the release of the eggs. The egg is subspherical in shape, 30 to 40 m in diameter, greyish in color, containing within it a granular mass with three pairs of aculella, the hexacanth embryo or oncosphere. The larval form of T. solium consists of a semi-transparent fluid-containing vesicle with an invaginated scolex inside. The scolex forms a spiral canal and has a rostrum with four suckers surrounding a double crown with 20 to 30 aculella(100).

Man is the only host of the adult form of the parasite and maintains the parasite life - cycle by elimination of the eggs in the feces. The pig, an intermediate host, ingests eggs which reach the stomach, in this place are treated by gastric juice, initiating digestion of the shell and release of embryos into the “small intestine” by breaking the chitinous layer of the egg. The oncosphere, through its acts, it is travel to the intestinal mucosa and came into the circulatory system, reaching all tissues and organs, where it develops in about three months until it reaches the complete larval form. Completing the biological cycle, when humans ingest pork containing viable larvae, the adult worm develops in the “small intestine” (called taeniasis). A period of 2 to 3 months elapses from ingestion of the larval form to egg-laying(102).

In a similar vein, it is important to mention that the estimated longevity of the adult worm is up to two decades. Human cysticercosis occurs when humans are accidentally infected with the embryonic form, by ingesting water or food contaminated with eggs, or as a result of poor hygiene or as a result of anti-peristaltic movements or vomiting when carrying the adult worm. Only T. solium has been associated with the taeniasis-cysticercosis complex in humans, although the parasite may sporadically be present in other intermediate hosts, such as black bears (Ursus americanus)(103).

Human cysticercosis caused by T. crassiceps has also been reported in patients with acquired immune deficiency syndrome(104). The larval form of T. solium has shown a preference for the CNS, eyeball and skeletal muscles in man. The neurological form, neurocysticercosis, is the most frequent, accounting for 60% to 95% of reported cases, and is the most studied. In the human brain, the cysticercus is more spherical in shape than in muscle, with more abundant parenchyma, perhaps because of the better growth conditions found, where soft tissue does not offer difficulties for parasite development(105).

Cysticercosis is endemic, and several factors contribute to it consumption of infected pork, such as poor control of feces of individuals carrying taeniasis, the presence of vegetable gardens and plantations near pig farms, especially when irrigated with water contaminated or fertilized with human feces, water sources close to pig farms and toilets, poor environmental and personal hygiene habits, such as eating unwashed food or handling it with dirty or contaminated hands, lack of or inadequate inspection of meat and the establishments that sell it, and poor health education, among others(106).

The distribution of cysticercosis is universal, being prevalent in developing countries. That said, prevalence is high in Latin America, Africa, India and Asia, while in the United States and some European countries there are isolated reports due to immigration of individuals from endemic areas. Likewise, the prevalence of the disease does not show differences in race and sex. Most studies reveal an age range of 10 to 60 years, and among them, the highest frequency occurs in the 20 to 40 years age group(106).

The hospitalization period of patients affected by this infection is about one week to one month, and the case fatality of the disease in neurology and neurosurgery services is about 5% to 25%. Furthermore, the clinical manifestations of the disease are non-specific and depend on several factors, such as number, size, age, vitality, location, stage of evolution of the parasite and its reactional processes on the host, as well as the host's inflammatory and immune responses to the parasite. In human cysticercosis, the number of parasites found is small, with up to five parasites found in about 70% of cases(107).

Symptoms and eventual sequelae of infection usually appear months or even years after the onset of infection, when symptoms are mild or even absent, and are the result of immunobiological phenomena characteristic of the parasite-

host relationship, and are not pathognomonic of infection with the larval form of T. solium. The most common clinical symptoms are headache, seizures, intracranial hypertension, hydrocephalus, dementia, psychic syndromes, meningitis and paraparesis, isolated or associated. These symptoms may result from mechanical compression of the nerve parenchyma, obstruction of cerebrospinal fluid (CSF) drainage or an immuno-inflammatory reaction induced by cysticercus catabolites or degeneration products(107).

In general, intraparenchymal localization leads to seizures and mental disorders, while CSF drainage obstruction is implicated in intracranial hypertension, spinal cord compression and meningeal syndromes. The prognosis of the disease is directly related to the success of therapeutic measures to eradicate live parasites and to diminish the effects of dormant parasites on the brain parenchyma. The clinical course is variable, generally long, with pure convulsive forms having a better prognosis than hypertensive cases with or without seizures. However, the treatment used until the early 1980s was surgery, which has been replaced by the use of praziquantel in cases of active infection. Subsequent studies indicated a greater efficacy of albendazole in subarachnoid cysticercosis by reducing the number of eggs(104).

In this context, the use of anti-inflammatory drugs and corticosteroids in association with parasiticide treatment in the active phase of the disease is recommended to minimize the tissue damage induced by the host reaction. To diagnosticate the infection, is necessary a clinical, epidemiological and laboratory criteria. Clinical diagnosis of the disease is difficult due to polymorphic and non-specific symptoms, which can be confused with other pathologies affecting the CNS. Despite this diversity, there are signs and alterations in complementary examinations that are more frequent in Cysticercosis infection than in other

diseases affecting the CNS. Epidemiological data, such as the endemicity of the patient's area of origin, also help in the diagnosis(105).

In general, it has been envisaged that accurate diagnosis is possible through the simultaneous use of imaging studies and laboratory analysis of CSF, including cytomorphological, biochemical and immunological tests. CSF examination is a parameter really important in the evaluation and follow-up of individual with suspected Cysticercosis, although in some patients it may be normal or with insufficient alterations for the diagnosis of the disease. Alterations found in CSF include lymphomononuclear pleocytosis and the presence of specific antibodies. There is also an increase in the total protein level and alteration of the electrophoresis pattern (oligoclonal), with an increase in gamma globulins and a consequent decrease in the albumin/globulin ratio(108).

These latter alterations are related to the titer of specific antibodies in the CSF. In immunological diagnosis, technological advances have contributed to the detection of antibodies, thus increasing the diagnostic efficacy of CSF testing in Cysticercosis infection(108).

Immunological tests often contain a proportion of falsely non-reactive patients, which may be explained by the different degrees of disease evolution, the antigenic variation of the parasite, the immunosuppressive effects of anti-inflammatory treatment and the low sensitivity of some tests. Various immunological techniques have been studied and standardized for the diagnosis and study of Cysticercosis infection with antigen from the larval form of T. solium and with different levels of sensitivity and specificity in the search for IgG class antibodies, such as precipitation techniques, complement fixation, hemagglutination, indirect immunofluorescence, radioimmunoassay and enzyme-linked immunosorbent assays(109).

Most of the antibodies detected in CSF are synthesized intrathecally and, to a lesser extent, from peripheral blood by disruption of the blood-brain barrier(109).

1.3.13. Dientamoebiasis (Dientamoeba fragilis infection)

The species Dientamoeba fragilis was described at the beginning of 1900 by Jepps and Dobell(110), but its discovery can be attributed to the protozoologist C. Wenyon who, at the beginning of the 20th century, found this microorganism in his own feces, although he did not carry out the proper scientific communication. The original description of the species refers to its binucleated trophozoite form, which justifies its name, and for many years it was the only known form of the parasite, which raised doubts about its mode of transmission. Likewise, the precystic and cystic forms have only recently been described(111).

Dientamoeba fragilis was initially classified as belonging to the order Amoebida and was considered non-pathogenic, giving rise to a controversy that will accompany this species for a long time. However, between 1919 and 1923, there were already reports associating this protozoan with gastrointestinal alterations in naturally infected individuals. However, this did not mean that it could be guaranteed that such symptoms were due solely to infection by Dientamoeba fragilis(111).

Subsequent studies by Dobell in the 1940s have shown morpho functional similarities between Dientamoeba fragilis and flagellated protozoa, especially Histomonas meleagridis, a known parasite of birds. Research carried out after the advent of electron microscopy reinforced Dobell's hypothesis on the systematic position of Dientamoeba fragilis, definitively placing this species

among the flagellate protozoa that, capriciously, do not present visible flagella(112).

The trophozoites of Dientamoeba fragilis are typically binucleate and, as already noted, the name of the genus derives from this characteristic. However, about 20% of the forms have a single nucleus(112).

For many years it was considered that this protozoan did not form cysts, like other species of the same family. Recently, cysts were described, initially in experimental studies of rodent infections and later in the feces of naturally infected humans. These findings have radically changed the hypothesis on the transmission mechanisms of Dientamoeba fragilis, which were initially considered similar to those accepted for Histomonas meleagridis, for example, that its transmission would depend on trophozoites carried by eggs of some helminth species(113).

In the case of Histomonas meleagridis, the helminth involved is Heterakis gallinae, a roundworm that parasitizes poultry in high incidence, while Enterobius vermicularis eggs would be the carriers of Dientamoeba fragilis. However, the small number of cysts observed in the feces of humans infected with Dientamoeba fragilis casts doubt on the possibility that such structures are the only, or even the predominant, natural mode of transmission of this parasite(113).

The frequency of human infection with Dientamoeba fragilis is highly variable. Several studies reviewed by Stark et al(111) reveal varying rates, ranging from 0.2 to more than 80%, depending on the diagnostic technique used, the sample size tested and the population examined. In this series of studies analyzed, the fact that the highest frequencies occur in developed countries, such as Germany and the Netherlands, stands out, revealing a different

epidemiological profile from that observed for other entoparasites, whose incidence is lower in more developed regions.

Laboratory diagnosis of human infections with Dientamoeba fragilis is usually performed by parasitological examination of feces by light microscopy, using fixed fecal samples, due to the rapid degeneration of trophozoites and the rarity of finding cystic forms in feces(114).

Such samples must be stained and the most commonly used permanent staining techniques are ferric haematoxylin or trichrome. It is important to take into account the possibility of intermittent shedding of Dientamoeba fragilis trophozoites, which requires the examination of several fecal samples collected on different days to increase the sensitivity of this diagnostic method(115). Although this is a laborious technique, it is possible to culture Dientamoeba fragilis trophozoites isolated from the feces of infected patients. Loeffler's biphasic medium maintained at 42ºC in a microaerophilic atmosphere is the most commonly used for this purpose(116).

1.3.14. Diphyllobothriasis (Diphyllobothrium latum infection)

Diphyllobothriasis occurs in areas where lakes and rivers coexist with human consumption of raw, undercooked or smoked fish. These areas are found in Europe, Russia, North America and Asia. Only two countries in South America, Chile and Argentina, have reported cases of D. latum diphyllobothriasis. D. latum is endemic to unpolluted lakes and deltas in the northern hemisphere and D. pacificum occurs in South America(117).

As a consequence, a wide variety of parasites have been identified in raw fish. The increase in marine mammal populations, in particular seals and sea lions in the Pacific Ocean and North Atlantic, is related to the increased presence of parasites in fish. The increase in marine infections is also associated with the global distribution and growing popularity of raw seafood consumption(117).

Of the cestodes infecting fish and humans, the genus Diphyllobothrium is of greatest importance. This parasite develops sexual maturity in the intestinal tract of mammals. D. latum is found in the flesh of freshwater or saltwater fish that migrate to freshwater to reproduce; bears and humans are the definitive hosts of this parasite(118).

D. latum is a large, elongated cestode, ranging in length from one to two meters to ten meters. Like D. pacificum, it usually matures on seals or other marine mammals and is half the size of D. latum. Immature eggs are released in feces and, on contact with water, release choracids that are ingested by small crustaceans, where development of a procercid larva takes place. The fish ingest the arthropods and the plerocercoid larvae infest the fish's body(119).

In humans, the adult worm is located in the duodenum, jejunum or ileum and the immature eggs are released by the proglottids and excreted in the feces five to six weeks after infection. Humans are the definitive host and infection occurs through consumption of raw fish containing asparagines. Salmon is the most common carrier of diphyllobothriasis, which can also be transmitted by trout(119).

Diphyllobothriasis is rare in the United States, but is common in the Great Lakes region, where it is known as Jewish or Scandinavian housewife's disease because fish preparations are sampled before they are fully cooked. Cases have recently been reported on the west coast of the country(120). The first cases of

diphyllobothriasis appeared in North America in the 1930s. However, high levels of pleroceroids in fish flesh prevented the marketing of these products in Manitoba and northern Ontario (Canada), which reduced funding for the local fishing industry. D. latum is highly prevalent in Manitoba fish and has a half-life of four to five years(121).

Distribution and routes of infection have not changed for centuries and it is well established in natural hosts in the boreal regions of North America. Some researchers support the theory that D. latum was introduced into North America by European immigrants or by passage through the Bering Strait and then affecting susceptible hosts (immigrants from northern Europe)(122).

In Canada, more than 400 asymptomatic volunteers aged 1 to 70 years were evaluated and low rates of Diphyllobothrium infection were observed, lower than those found in northern Canada and Alaska(123). Likewise, in the Patagonian region of Argentina and in several regions of Chile, especially in the lake region of southern Chile, there are reports associated with the consumption of smoked raw fish meat, (named “ceviche”). A prevalence of almost 30% of Diphyllobobothrium latum and more than 55% of D. dendriticum was observed in fish from lakes in the southern region of Argentina(124).

Diphyllobothriasis is characterized by abdominal distension, flatulence, epigastric pain, anorexia, nausea, vomiting, asthenia, weight loss, eosinophilia and diarrhea after 10 days of consumption of raw or undercooked fish(125). A peculiar complication of this helminthiasis is the development of microcytic and megaloblastic anaemia, especially in genetically susceptible individuals, usually of Scandinavian origin, because the adult parasite has the ability to intensively absorb vitamin B12 in the intestine of the host with prolonged infestation. Most infections are asymptomatic(126).

Infection is diagnosed by finding operculated eggs in the feces of patients by microscopic examination. These eggs can be concentrated by sedimentation and are difficult to distinguish from eggs of Nanophyetus spp. The larvae of these parasites are found in fish flesh(127).

As preventive measures, visual inspection of fish and consumption after freezing at -20°C for 7 days or at -35°C for 15 hours, which inactivates the parasites and allows raw fish to be eaten, should be recommended. It is also important to prevent infection by proper cooking of fish, hygienic disposal of human excreta, inspection of fish and proper freezing of fish in slaughterhouses(119).

1.3.15. Dirofilariosis (Dirofilaria spp. infection)

Dirofilariasis, also called "heartworm disease", is a chronic emerging anthropozoonosis of dogs caused by nematodes of the genus Dirofilaria, where Dirofilaria immitis is the best-known species, being transmitted by mosquitoes of the genera Culex, Aedes and Anopheles. In humans, it is characterized by involvement of the lung parenchyma or subcutaneous nodules, however, in dogs, it manifests as lesions in the vascular endothelium and obstructions caused by the adult parasite, mainly found in the right ventricle of the heart. Cats, although they can be parasitized, are more resistant to infection compared to humans and dogs(128).

The first published description of the disease in dogs was in the USA by the physicist Osborne in 1847(129). Likewise, the first reports in cats were made by Travassos in 1921(130), while in 1850, J. Leidy classified the aetiological agent of this disease as Filaria immitis(129).

In 1979, due to the importance of this parasitic disease not only because of the damage it causes to infected animals, but also to the human population, and the close relationship of domestic animals with humans, the disease was considered a zoonosis by the WHO(131).

Dirofilariae are nematodes belonging to the superfamily Phylaroidea, family Filariidae, subfamily Dirofilarinae, genus Dirofilaria(132). The genus Dirofilaria has two subgenera, Dirofilaria (D. immitis) and Nochtiella (Dirofilaria tenuis, Dirofilaria repens and Dirofilaria ursi), which infect humans. D. immitis is a parasite of the circulatory system (heart and large vessels), lymphatic system, subcutaneous tissue, peritoneal cavity or mesentery of dogs, wild canids and, less frequently, cats, requiring an invertebrate host(133).

Adult male parasites are 120 to 200 mm long and 0.7 to 0.9 mm in diameter, and adult females are 250 to 310 mm long and 1 to 1.3 mm in diameter and are viviparous. Microfilariae are 298μm long and 7.3μm wide and have an oval anterior end and a straight posterior end(132).

Dogs, occasionally cats, and rarely humans are definitive hosts of D. immitis, while mosquitoes are the intermediate hosts. Transmission of this nematode has been described for Culex pipiens, Cx. quinquefasciatus, Aedes aegypti, Ae. albopictus, Anopheles maculipenis and Coquillettidia richiardii(134).

The geographical distribution of the disease is worldwide, with cases reported in Africa, Asia, Australia, Europe and South and North America, where it is estimated that nearly 50% of raccoons in south Florida may be infected by D. tenuis, the causative agent of nearly 85% of human infections in this region, as well as in Europe(135).

D. repens is a natural parasite of dogs and cats in Europe, Asia and Africa, localizing in the subcutaneous tissue and the eye, while D. tenuis parasitizes the subcutaneous tissue, lymph nodes, conjunctiva and spermatic cord of humans in southeastern Europe(136) and countries east of the Mediterranean(137), and the subcutaneous tissue of raccoons in southeastern North America(132). D. ursi, of little public health importance, infects perirenal and peritracheal tissues of bears in the United States(138).

Filariae require a hematophagous arthropod (mosquito) for their life cycle. Adult parasites, males and females, live in the tissues or organ cavities of definitive hosts. Females are viviparous, incubate their eggs in the uterus and release first instar larvae into the circulating blood, which lodge in the skin. The microfilariae are ingested by the arthropod during blood repassage(139).

Inside the mosquito or other arthropod, the microfilariae develop to the third larval stage, migrating rapidly to the mouthparts of the invertebrate host. During the third larval stage, they penetrate the skin of the new host through the mosquito bite site and, after several months of migration and maturation, reach the pulmonary arteries (133). Importantly, a single mosquito can transmit 10-12 microfilariae per bite(140).

Regarding the symptoms and health consequences of infection, inflammatory changes can be detected clinically before parasite death, either naturally or as a result of specific treatment, or before the discovery of the lesion, due to symptoms of pneumonitis and a radiopaque lung pattern(141).

Both dirofilarial antigens and derivatives of Wolbachia bacteria, an endosymbiont agent found inside dirofilarial, interact with the host organism during canine, feline and human infections and are involved in the development of pathology and in the regulation of the host immune response(142). For this

reason, Wolbachia bacteria have become a target for antibiotic treatments, which not only affect the fecundity and survival of the helminth, but also reduce the inflammatory pathology(143). In dirofilariasis, both innate and acquired immune responses occur, with the development of the acquired response depending on the host or parasite load(144).

Clinical manifestations depend on the type of immune response stimulated by the parasite. Eosinophil and neutrophil populations increase when the host immune system is stimulated by the third larval stage antigens or the adult parasite. Thus, neutrophils accumulate in the kidney and pulmonary artery walls during canine infection. The formation of a granulomatous reaction occurs, initiated when the parasite is present in the pulmonary arterial branches(145). In addition, some molecules secreted by the agent appear to stimulate the production of interleukin type 10 (IL-10) contributing to parasite survival, benefiting the host by inhibiting immune-mediated pathology(146). Death of the parasite causes an exacerbated inflammatory reaction with formation of granulomas that can obstruct pulmonary arteries.

D. immitis also develops in the dog's pulmonary artery and secondarily in the right ventricle. It almost always forms a tangle of numerous parasites. In more intense and long-lasting infections, live or dead filariae cause stenosis of the pulmonary vessels and impede blood flow. This eventually leads to right ventricular failure. Clinical signs in the dog may be absent or manifested by cough, exercise intolerance, dyspnea, heart and lung sounds, hepatomegaly, syncope, chronic cough and/or loss of vitality(136).

In severe forms, manifestations of right heart failure such as ascites, acute congestion of the liver and kidneys, hemoglobinuria and death within 24-72 hours may occur. Severity is closely related to the number of parasites, but is also exacerbated by exercise-related high blood flow stress. The classical description

of pulmonary syndrome is only induced in dogs with an exercise pattern that forces right ventricular hypertrophy resulting from increased cardiac output and pulmonary resistance(136).

During microfilaremia, a low and transient immune response develops against the third larval stage. The humoral response in cats is not only directed against the parasite, but also against Wolbachia, which can be massively eliminated after the death of the larva and/or the adult parasite(146). Traversa et al.(147) observed dyspnea and coughing in 50 cases studied. In these animals, the most common clinical signs in the acute phase were collapse, dyspnea, convulsions, diarrhea and vomiting, tachycardia, syncope and rarely death. In the chronic phase, coughing, vomiting, dyspnea, lethargy, anorexia, weight loss and chylothorax were observed.

Humans may remain asymptomatic or present with cough, hemoptysis, sore throat, wheezing, chills, fever, chest pain, exertional dyspnea, sweating, fatigue, syncope, weight loss and eosinophilia(129) and multiple bilateral pulmonary nodules with pleural effusion(148). Fragments of the dead parasite stimulate an intense granulomatous response. The central necrotic zone is surrounded by lymphocytes, plasma cells, epithelial cells and occasionally giant cells. In addition, the nodule is surrounded by fibrous tissue. The lung lesion is characterized on radiographic examination as a coin-shaped lesion, one to three centimeters in diameter at the periphery of the lung lobe, surrounded by normal lung parenchyma(149).

Diagnosis in animals is based on clinical signs of cardiovascular dysfunction and demonstration of microfilariae in blood by Giemsa-stained thick smears, modified Knott's concentration method or Millipore filters, with success in approximately more than half of infected dogs and less than 10% of infected cats. It may be supplemented by radiography, chest auscultation and pulmonary

angiography(150). Definitive diagnosis should be made by enzyme-linked immunosorbent assay (ELISA) for detection of adult parasite antigens or modified Knott's test. In humans, pulmonary radiography in association with biopsy of the wedged lesion is conclusive for diagnosis(151).

Due to the survival of microfilariae after adult death, a small percentage of dogs may have microfilaremia, without adult forms in the heart. Puppies with elevated microfilariae counts may have transient microfilaremia, which may not reach adulthood(152). Hidden infections (amicrofilaremic animals) may be caused by immature helminths in puppies less than six months of age, single helminth infections, single-sex helminth infections and host immune reactions against microfilariae. In these cases, antigen testing is necessary to determine the status of the dog in order to monitor the infection on a monthly basis(153).

In the case of D. immitis, the blood sample should preferably be obtained at night, when microfilaremia peaks. Microfilaremia for D. immitis appears six months after infection in dogs, however, in about one fifth of infected animals microfilaremia is not detected (occult dirofilariasis). In cases of immature parasites and low parasite loads, especially in the cat, results will be negative for antigen when parasites are present in the pulmonary arteries, even with clinical signs present(153).

Direct blood smear is a simple screening test, but is not recommended as a routine test. The animal needs to be infected with a large number of larvae for detection. Less than 20 to 50 microfilariae mL-1 of blood are not detected and in milder infections they will be absent(148). Likewise, puppies under six months of age should not be tested for dirofilariasis; however, puppies that are not undergoing preventive treatment for dirofilariasis should be tested directly for microfilariae. If negative, the microfilariae concentration test should be

performed, but less than 1% of dogs may be microfilariae positive and antigen negative(154).

In humans, imaging is of utmost importance in addition to hematological examinations. On radiological examination, the pulmonary arteries will be dilated, more prominent in the smaller lobes of the lung. It is the most commonly used diagnostic procedure, allowing confirmation and prognosis(128). The lesions are usually self-limited and calcified, coin-shaped, difficult to differentiate, being confused with neoplasia, sebaceous cyst, hematic cyst, other infectious diseases and granulomas(155).

When signs of initial pneumonitis and consequent granuloma formation are detected, identification of the coin-shaped lesion becomes possible only after clarification of the radiological pulmonary pattern. Associated with this fact, basophilia increases the suspicion of the disease. Definitive diagnosis by CT scan, MRI or aspiration cytology of the granuloma has been ineffective(156).

Serological tests by ELISA to detect antigen or antibodies have been performed, but do not yet have adequate sensitivity and specificity to be fully reliable as a diagnostic tool. The recent discovery of antibodies against a D. immitis specific protein may increase the specificity of ELISA in humans(157).

Differential diagnosis between D. immitis and D. repens for human infection can be made in the definitive host, but is more difficult to perform when the patient harbors immature or even degenerated nematodes(133). Polymerase chain reaction (PCR) has been successfully used to differentiate D. immitis and D. repens(158). In the occurrence of oral mucosal mass, the differential diagnosis should be made with epidermoid cyst, pleomorphic adenoma, neurofibroma and lipoma(159).

In humans, treatment of pulmonary dirofilariasis consists of identification and surgical resection of the parasite present in the lung lesions, differentiating it from tumors, when the diagnosis is confirmed by histopathological examination of the specimen. Resection of the nodule not only establishes the diagnosis but also the cure(160).

1.3.16. Dipilidiosis (Dipylidium caninum infection)

Dipylidium caninum is the scientific name for one of the most common worms in dogs and cats. In addition to companion animals, this parasite can accidentally infect humans, characterizing it as a zoonosis. Dipylidium caninum is a cestode (flatworm), commonly called the canine tapeworm or cucumber tapeworm, which attaches to the walls of the small intestine of its host and can reach a length of up to 60 cm. Its body is segmented into parts called proglottids that contain the worm's eggs(161).

The larval form of the parasite infects fleas and chewing lice (of the species commonly found in dogs), which are its intermediate hosts and act as vectors of diphylidiosis. Proglottids are shed both in the feces of infected animals and in the environment and the larval stages of fleas feed on these parasite segments and become infected(162).

Dogs and cats are the definitive hosts, and man is considered the accidental host. Transmission to the definitive host occurs when the animal accidentally ingests fleas or lice infected with Dipylidium cysticercoids larvae. This ingestion usually occurs when the dog or cat licks, licks or nibbles the skin in an attempt to relieve the itching caused by the flea (162).

Following ingestion of the Dipylidium larva, a prepatent period of approximately 3 weeks occurs, sufficient time for the transformation of the cysticercoid larva into an adult cestode. The adult worm attaches to the intestinal wall by hooks and begins to develop, eliminating the gravid proglottids, as it is hermaphroditic(163).

Infection rarely causes serious problems for the affected animal. In most cases there is anal itching, which is caused by the proglottids when they move. The presence of these rice-grain sized segments of the parasite in the feces or attached to the hair in the perianal region is what most attracts the attention of owners. Diarrhea, weight loss, constipation and growth retardation in young animals only occur in large infestations or severe cases(164).

As far as human infection is concerned, the proximity of pets to humans today has increased the risk of zoonotic disease transmission. Young children are the most susceptible due to their proximity and attraction to pets and their behavior of putting objects on the ground and in their hands in their mouths, favoring accidental ingestion of worm larvae. In the case of Dipylidium, there is also the possibility of accidental ingestion of the flea or louse. The clinical signs are usually the same as in animals(165).

In terms of treatment, once the infection has been identified, the treatment of choice is based on Praziquantel, at a dose of 5mg/kg. Praziquantel is a broad-spectrum anthelmintic, capable of eliminating larval and adult forms of the parasite after a single oral dose. However, a booster dose after 15 days is recommended(166).

Concomitant with the parasiticide treatment, it is necessary to completely eliminate the flea or lice infestation from the affected animal and possible contact animals in the residence. This process prevents reinfection and breaks the worm

cycle. It should be remembered that ectoparasites (such as fleas and lice) proliferate in the environment and climb on animals only to feed. Therefore, environmental control is also necessary and should be carried out by cleaning and fumigation of places to which the animals have access(167).

1.3.17. Echinococcosis (Echinococcus spp. infection)

Echinococcosis is a parasitic disease that occurs in two main forms: cystic hydatidosis (also known as echinococcosis) caused by Echinococcus granuloses and polycystic hydatidosis, caused by Echinococcus vogeli and Echinococcus oligarthrus. Dogs, foxes and other carnivorous animals harbor the adult worms in their intestines and pass the parasite eggs in their feces. If the eggs are ingested by humans, they develop into larvae in various organs, mainly in the liver and lungs(168).

Both hydatidosis (cystic and polycystic) are characterized by asymptomatic incubation periods that can last for many years before the parasite larvae evolve and trigger clinical signs. Both diseases can cause severe morbidity and death(169).

In terms of symptoms, after ingestion of Echinococcus eggs, they hatch in the digestive tract and the larvae migrate through the bloodstream to various organs where they concentrate and develop within cysts. In most cases of human infection, hydatid cyst development is asymptomatic, which means that people can carry the cyst throughout their lives without requiring medical attention and few develop severe disease. However, the clinical symptoms will be related to the physical state of the cyst, as well as its location and size, and may be found in the following places(170):

- Abdominal location: with the presence of pain, fistulas, palpable masses, jaundice, hepatomegaly or splenomegaly (the liver is preferentially affected).

- Pulmonary location: evidenced by cough, chest pain, haemoptysis (coughing up blood) or dyspnea (shortness of breath).

- Bone localization: problems linked to destruction of trabeculae, necrosis and spontaneous fracture.

In asymptomatic cases, the detection of a hydatid cyst may be the result of an occasional finding in a routine medical examination, a screening examination for another pathology or even a radiological study. In cases where the cyst ruptures, which could lead to complications such as anaphylactic shock and pulmonary oedema(169).

As the cyst(s) grow in the tissue, they can cause tissue destruction or organ compression. The main difference between cystic and polycystic hydatid disease lies in the more rapid and aggressive growth of the cyst, and the formation of multiple cysts in the case of polycystic hydatid disease(169).

In terms of transmission, a variety of herbivores and omnivores act as intermediate hosts for Echinococcus by ingesting parasite eggs in contaminated soil and developing parasitic larvae in their guts. Carnivores are the definitive hosts of the parasite, and can become infected by consuming the viscera of intermediate hosts harboring the parasite and also by disposal of infected carcasses. Humans are accidental intermediate hosts and cannot transmit the disease(171).

Transmission of cystic hydatidosis is mainly maintained in the dog-sheep-dog cycle, although other animals that come into contact with humans, such as

goats, pigs, horses and cattle, among others, may be involved. Transmission of polycystic hydatidosis generally occurs in a wild life cycle between canids and rodents-canids and felids. Among rodents, pacas and agoutis are the most frequent. In both forms of the disease, humans become infected by ingesting water or food contaminated with parasite eggs present in the feces or fur of carnivores, most commonly the domestic dog(171).

1.3.18. Endolimax nana infection

Endolimax is considered a non-pathogenic commensal protozoan parasite of the human colon. Its presence in feces can be an indicator of fecal contamination of a food or water source, and does not rule out the presence of other parasites. Most humans can carry E. nana without complications, but sometimes the tiny parasite combines with Blastocystis hominis to cause gastrointestinal symptoms(172).

Although much of the literature cites this parasite as non-pathogenic, there is every reason to believe that in some cases these "non-pathogenic" agents actually cause symptoms in patients, without other infections or conditions explaining their symptoms. Likewise, E. nana inhabits the colon and has also been found in the appendix. Trophozoites (8-10 μm) move by pseudopods and can reach a size of up to 30 μm during locomotion. They feed exclusively on bacteria and divide by binary fission(173).

In terms of transmission, Endolimax nana is transmitted by fecal-oral contamination of food or water. Endolimax cysts have been observed in drinking water from deep wells, in consumed raw vegetables and on banknotes, which have been suggested as potential fomites(172).

For a correct diagnosis of the disease, diagnosis is traditionally based on cyst microscopy, which can be direct or associated with a concentration procedure and different stains prior to analysis. Also, as far as treatment is concerned, the parasite seems to respond well to treatment with metronidazole and diphetarsone. In addition, doses of metronidazole, nitazoxanide and trimethoprim-sulfamethoxazole have been shown to treat Endolimax nana infections causing gastrointestinal discomfort such as acute diarrhea(173).

1.3.19. Fascioliasis (Fasciola hepatica infection)

Fasciolosis is a disease caused by two species of parasites, Fasciola hepatica and Fasciola gigantica, both belonging to the phylum Plathelminthes, class Trematoda and family Fasciolidae(174).

F. hepatica is a greyish-brown/reddish trematode, with an oblong, dorso-ventrally flattened body, which deforms continuously due to muscular movements and contractions, with dimensions ranging from 2-4 cm long by 1-2 cm wide and ends in a blunt tip, thus having the appearance of a plant leaf. F. gigantica is also an aetiological agent of fasciolosis and produces an anatomopathological picture similar to that produced by F. hepatica, but with some differences. F. gigantica is larger, rarer and found in tropical countries (Africa, Middle East and Asia), while F. hepatica is smaller and can infect humans in all continents except Antarctica(175).

The adult parasite inhabits the denser bile ducts of vertebrate hosts, which after some time become dilated and the walls hypertrophied, feeding on bile contents, the products of inflammation produced and necrotic material subsequently formed. Oviposition occurs in the biliary tract and with the bile, the

eggs reach the intestine and mix with the feces and thus reach the external environment, where in the absence of contamination, in suitable temperature, water and light they develop(176).

Eggs, in a shaded and humid environment, can remain viable for up to nine months. They hatch (between 9 and 25 days under 25 to 30°C or about three months at 10°C) ciliated larvae called miracidia, which are attracted by the mucus produced by the intermediate host and swim by cilia movements in the aquatic environment where they penetrate this, the mollusk of the genus Lymnaea. The development of a single miracidium in the intermediate host can give rise to more than 4,000 cercariae(175).

Cercariae take about two months to develop and hatch from eggs, which remain in the eggs and release them for up to 70 days after infection. The cercariae have a single tail and in the aquatic environment they swim for a few minutes and use their suckers to attach themselves to aquatic vegetation(177).

After this stage, either attached to vegetation or on the surface of the water in contact with oxygen, the cercariae body loses its tail and produces cystic layers that protect the parasite and make it infective. The vertebrate host ingests the metacercaria present on aquatic plants and other vegetation, in water or suspended in the soil(177).

In the duodenum of the definitive host, the metacercaria are excised and perforate the intestinal wall, invade the peritoneal cavity (in about 24 hours after infection) and move towards the liver, where they arrive after perforating the hepatic capsule (about 4 to 6 days) and after migrating through the hepatic parenchyma (acute phase), they become permanently lodged in the bile ducts (chronic phase) in about two months, with complete evolution and sexual maturation. The occurrence of fasciolosis in the lungs and other organs, including

infection of fetuses, is due to erratic migration of the parasites as they enter the hepatic veins and reach the systemic circulation(178).

The degree of susceptibility of animals varies according to species. Cattle show high resistance to F. hepatica infection and reinfection, and the main defense mechanism is fibrosis of the bile ducts, but depending on the degree of severity and parasitism, it can cause biliary cirrhosis and liver failure. With fibrosis, the fascioles can no longer feed and are forced to leave the liver. The reduction in the biological activities of the parasites is manifested by a decrease in the presence of eggs in the feces 4 to 6 weeks before the elimination of the adults, which occurs 16 to 30 weeks after infection(179).

In animals, pathogenesis varies according to parasite load and stage of parasite development. Penetration of F. hepatica into the abdominal wall causes only small foci of hemorrhage. Lesions caused by penetration into the liver depend on the parasite load; hemorrhage and rupture of the liver capsule may occur. Migration through the liver parenchyma causes hemorrhage and necrosis, and in cases of moderate to massive infection, liver enlargement, fibrosis and traumatic hepatitis occur(179).

Final settlement of the parasites in the bile ducts leads to chronic cholangitis and obstruction of the ducts. The clinical picture of the disease varies according to the degree of infection and the nutritional status of the animal, with the absence of alterations being more frequent in mildly to moderately parasitized animals. The large number of parasites migrating through the liver parenchyma can cause weight loss, decreased productivity, constipation, prostration, weakness and death of the animals(180).

Humans are described as occasional hosts of F. hepatica, and become infected when they ingest water or aquatic vegetables containing the

metacercaria. The acute phase of fascioliasis is accompanied by indigestion, nausea, vomiting, abdominal pain, high fever, and in some cases, there are allergic reactions (pruritus, urticaria) and anaemia. The chronic phase is usually asymptomatic, but in some cases, there may be localized pain below the thoracic region and weight loss. Erratic migration causes granulomas(175).

The geographical distribution of F. hepatica is wide and the trematode is present in Europe, Africa, Asia, Oceania and the Americas. The cycle and spatial distribution of the parasite depends on the existence of an ecosystem in which three key elements interact: the vertebrate host, the intermediate host and its habitat. The distribution of free-living stages and eggs of F. hepatica, as well as the intermediate host, also depends on climatic factors such as temperature and humidity(181).

Periods of rainfall and river flooding are epidemiologically very important, because the habitat of the intermediate hosts expands and snails multiply rapidly, intensifying the F. hepatica cycle. In addition, extensive rearing of cattle and sheep in flooded areas, movement of animals between dry and wet pastures, and trade and transport of livestock across different regions, perpetuates fasciolosis among animals and creates new foci of the disease(175).

Pigs, horses, goats, camels, rabbits and deer can be hosts of F. hepatica, but only the ovine and bovine species are of epidemiological importance(182).

1.3.20. Filariasis (Filariaidae spp.infection)

Lymphatic filariasis (elephantiasis) is a chronic parasitic disease, considered one of the world's leading causes of permanent or long-term disability. It is caused by the nematode worm Wuchereria Bancrofti and is transmitted by

the bite of the Culex quiquefasciatus mosquito (the black-winged mosquito) infected with larvae of the parasite. Among the most important clinical manifestations of Lymphatic Filariasis are oedema (abnormal accumulation of fluid) of the limbs, breasts and scrotum, which can lead to disability(183).

The symptoms of lymphatic filariasis (Elephantiasis) are related to the process of development of the larvae that cause the disease and also to the site where the adult worms lodge(184).

However, as the clinical picture and symptoms are similar to those of other diseases, careful characterization by specific diagnosis is necessary. Thus, chronic symptoms of lymphatic filariasis may evolve into(185):

- Abnormal fluid accumulation (oedema) in the extremities, breasts and scrotum
- Enlargement of the testicles (hydrocele)
- Overgrowth or swelling of the limbs, breasts and scrotum.

In terms of treatment, it is recommended that, because Lymphatic Filariasis is being eliminated in much of the world, morphological identification of the parasite (classification of filarial species) is performed prior to specific treatment with diethylcarbamazine (DEC). The drug of choice is DEC in the form of 50mg tablets of the active drug, which is administered orally and has rapid absorption and low toxicity. This drug has a micro- and macro-filaricidal effect, with a rapid and profound reduction in the density of microfilariae in the blood(185).

The diagnosis of Lymphatic Filariasis (Elephantiasis) must be specific and detailed, to rule out the hypothesis of other diseases. Laboratory tests that demonstrate the presence of the parasitic worm causing the disease are(186):

- Direct examination on slides
- Positive haemoscopy
- Ultrasonography, which can demonstrate the presence of filariasis in the lymphatic channels.
- Other tests can be used for screening, especially card-support.

Regarding transmission, lymphatic filariasis is transmitted by the bite of the Culex quiquefasciatus mosquito (the black-winged mosquito) infected with larvae of the parasite. After entering the skin through the mosquito bite, the infecting larvae migrate to the lymph nodes (ganglia), where they develop into adults. If parasites of both sexes develop, they will also reproduce, shedding large numbers of microfilariae into the bloodstream, which will infect new mosquitoes, initiating a new cycle of transmission (185).

1.3.21. Opisthorchiasis (Opisthorchis felineus infection)

Opisthorchiasis is a disease caused by the trematodes Opisthorchis viverrini and Opisthorchis felineus that mainly affects cats and has very similar symptoms and course to clonorchiasis. Most infections cause common symptoms such as diarrhea, constipation and malaise, but in cases where the disease remains untreated for long periods, it can progress to complications such as malnutrition (due to damage to the digestive tract), severe liver inflammation and even cancer of the bile duct(187).

These liver flukes are transmitted through consumption of raw or undercooked fish contaminated with the parasites and are more common in Southeast Asian countries due to their dietary habits(188).

The opisthorchiasis parasite - opisthorchis felineus belongs to the flatworms (trematodes), a class of trematodes. It has an elongated body 8-14 mm long and 1-3.5 mm in diameter, and has two suckers (the oral sucker and the abdominal sucker). Opisthobranchs are hermaphroditic, and the eggs are pale yellow, almost colorless, with a smooth, two-contoured shell, which has a slightly narrowed dust cap and a slight thickening at the opposite end(188).

The causative agent of opisthorchis has a complex development cycle, as it has two intermediate and additional hosts in addition to the definitive host. In the definitive (basic) hosts, the helminth parasites in the sexually mature stage of development. From the bile ducts, gall bladder and pancreatic ducts man and carnivorous mammals (cats, dogs, foxes, domestic pigs and others)(189).

Eggs of the parasite with bile enter the intestine and are then released into the environment. Further development takes place in waters in which Opisthorchis remain viable for up to 6 months, and where it is ingested by the intermediate host (such as freshwater mollusks of the Codiella type). In the body of which there are a series of transformations: the miracidium emerges from the egg, forming a sporocyst, in which the redia are formed. These produce a large number of larvae of the next stage (cercariae). They then emerge from the mollusk and enter the muscles of the second intermediate host (for example, in carp, carp, barbel, sea bream), where the cercariae transform into metacercaria, which after 6 weeks become invasive. Fish that have ingested opisthorchus metacercariae are the source of infection for humans and many carnivores(189).

In the stomach and duodenum of the final host, cleavage of the metacercaria takes place. Under the influence of gastric juice, the fish tissue and connective tissue capsule are digested and, under the action of duodenal juice, the metacercaria are released from the inner shell. Possessing bile-positive chemotaxis, the parasites find holes in the bile duct and, through the common bile duct, penetrate into the galleries and gall bladder and sometimes into the pancreas(190).

After 3-4 weeks after infection, the helminths reach a mature stage and, after fertilization, begin to secrete eggs. The lifespan of opisthorchia is 15-25 years. In addition, the eggs are stable in the environment, as in freshwater they maintain viability for about one year. Opisthorch larvae die when fish is cooked in one piece after 20 minutes, and in the case of cut fish, this reaction occurs 10 minutes after the start of boiling. When fish is salted, the larvae die after 4-7 days. Hot smoke is disastrous for the pathogen and cold does not destroy it(190).

Following eating invading fish, metacercaria enter the stomach and duodenum and within 3-5 hours reach the intrahepatic bile ducts, the site of their primary habitat in the body of the final host. In 20-40% of infected individuals, opisthorchia are found in the ducts of the pancreas and gall bladder. In the process of migration and with further development, they secrete enzymes and metabolic products that exert a direct toxic and sensitizing effect on the organism(188).

In the dynamics of the invasive process with opisthorchiasis, two phases can be distinguished: early (acute) and late (chronic)(191):

- The pathogenesis of the early phase is based on toxic allergic reactions of the organism to the metabolites released by the larvae during migration and maturation, as well as to the antigens of the

larvae. In this phase, increased permeability of blood vessels of the liver and pancreas; productive vasculitis; eosinophilic infiltration of the stroma of the organ, its inflammation; proliferation and desquamation of the epithelium of the bile ducts are observed. Eosinophilic infiltrates form in the gastrointestinal tract (duodenum, liver, lungs, etc.).

- In the chronic phase: toxic and allergic reactions remain, but the main pathological change caused by the Opisthorchis vital functions that outbreaks and spines are irritating and detrimental effect on the wall of the bile ducts and pancreatic ducts, gall bladder, causing cholangitis reaction and periholangita inflammatory and regenerative-hyperplastic development, leading to fibrosis of organs. Accumulations of parasites and their eggs slow down the flow of bile and pancreatic juice. Hyperplastic and inflammatory processes lead to the development of strictures in the terminal portion of the common bile duct and cystic duct, favour the adhesion of bacterial infections and the formation of stones in the common bile duct and pancreatic duct. Prolonged infestation can lead to cirrhosis of the liver. It is often accompanied by gastroduodenitis (including erosive-ulcerative)(191).

Proliferative processes in opisthorchiasis, considered as a precancerous state, in combination with the action of exogenous carcinogens, may lead to the development of cholangiocarcinoma. In Western Siberia, where the level of opisthorchiasis is high, the incidence of cholangiocarcinoma is 10-15 times higher than in other populations(192).

The initial immune response when opisthorhoze accompanied by a 10-12-fold increase in total IgM levels with a maximum in 2-3 weeks and a reduction of

its concentration after 6-8 weeks, when the IgG content marks increase. In addition, the antibody concentration falls below threshold values, which creates conditions for prolonged re-invasion and parasitism of opisthorchiasis in the body(192).

The immunosuppression that accompanies invasion, reduces resistance to other infections, of course serious contributes Shigellosis and other intestinal infections often causes patients to suffer chronic typhoid fever, viral hepatitis aggravates severe cholestasis, frequent exacerbations and relapses(191).

1.3.22. Paragonimiasis (Paragonimus westernami infection)

Paragonimiasis (also known as pulmonary distomatosis or parasitic hemoptysis) is a non-contagious, zoonotic disease caused by trematodes of the genus Paragonimus, which has a chronic, limited course with predominantly pulmonary involvement, although extrapulmonary manifestations have been described(193).

One of the important aspects in this context is the similarity to pulmonary tuberculosis, the main differential diagnosis of the disease. The definitive host of helminthiasis is wild animals, but it is possible, under some circumstances, to infect humans and other domestic and peri - domestic animals, provided that they ingest the second intermediate host (freshwater crustacean) raw, undercooked or preserved (tanned in wine, vinegar or lemon)(194).

Helminths belonging to the genus Paragonimus are trematodes. The genus Paragonimus has approximately 49 species (eight identified as causing disease in man) that characteristically have a cavernous body. Specimens may

be small or large, usually curved (shell-shaped), distributed throughout the different endemic areas(195).

The evolutionary cycle begins when the eliminated eggs, together with sputum or feces, reach rivers, streams or other freshwater collections, under suitable temperature conditions. Within the egg, in about 21 days, the ciliated larval form, the miracidium, develops. It leaves the egg and actively swims in search of the first intermediate host, which is always a mollusk (freshwater snail). If it does not find it, it dies within about 24 hours, but if it does not, it penetrates it and develops into a sporocyst, inside which the first generation of rhabditids is formed. Each of these develops another generation of daughter rhabditids, which later form the cercariae (it takes about five months for this development)(195).

These specimens leave the first intermediate host and move slowly, without swimming, and may be ingested by the second intermediate host (freshwater crayfish or crayfish), where they develop into metacercaria (usually requiring three to five weeks), the infective form for the definitive host. The latter are preferentially located in the hepatopancreas, although they can be located in other regions, such as genital structures, gills and somatic muscles(196).

Definitive hosts (wild or domestic) ingest the metacercaria-infected second intermediate host, raw or undercooked, acquiring the helminth. In the digestive tract of the definitive host, and thanks to the action of digestive fluids, these metacercaria disassemble and acquire vitality, perforating the intestinal wall and falling into the abdominal cavity, crossing the diaphragm and reaching the lungs, where they develop until they reach their adult stage, after about sixty days(196).

There they remain inside a fibrous capsule containing purulent and bloody material containing the eggs. When these cysts rupture, this material is eliminated

in the sputum and can be dispersed in the environment, with the possibility of restarting the cycle(197).

The most important alterations in the host are in the lungs, characterized mainly by leukocyte infiltration and tissue necrosis, with formation of fibrous cysts, located in the deepest parts of the lung parenchyma. Inside the cysts are helminths, immersed in a chocolaty secretion(197).

With regard to the manifestations of the disease, in its pulmonary presentation, which is the most frequent, there are two phases(198):

- First phase: from ingestion of the metacercaria to the development of the adult helminth in the lung. There are usually no clinical manifestations and the etiological diagnosis is practically impossible, as it is basically due to the presence of eggs in sputum or feces.

- Second phase: characterized by the development of the adult helminth in the lung, with the appearance of the classic manifestations indicating lung parenchymal involvement, such as cough with bloody sputum, which can range from bloody sputum to frank haemoptysis.

However, the infection is relatively easy to diagnose, provided that a thorough clinical evaluation is performed on the subject, investigating the findings of current disease history and echo-epidemiological features (history of ingestion of the second intermediate host) related to the disease, and a careful request for complementary examinations is indicated(198).

Thus, the most common treatment is praziquantel, using a dose of 25mg/kg bw, three times daily, for three days. This regimen promotes a high cure

rate (95-100%), with a short regimen and few adverse effects (such as nausea, vomiting and malaise), which helps prevent treatment abandonment. Also, resistant pulmonary forms have been treated with triclabendazole (10 mg/kg, single dose, repeating 5 mg/kg after three days of treatment), which has been shown to be as effective as praziquantel, with good tolerability(197).

1.3.23. SIBO (small intestinal bacterial overgrowth)

Small intestinal bacterial overgrowth syndrome, also known by the acronym SIBO, is a condition in which there is an overgrowth of bacteria in the small intestine, reaching values similar to the number of bacteria present in the large intestine(199). Is important to mention that SIBO is not caused by parasites, but parasites stimulate very often the SIBO development, and since then, many parasitic infestations are associated with SIBO.

Although bacteria are important for the digestion of food and absorption of nutrients, when present in excess they can cause intestinal problems, resulting in symptoms such as excessive gas, constant bloating, abdominal pain and recurrent diarrhea among other symptoms. In addition, by altering nutrient absorption, it can lead to malnutrition in some people, even if the person is eating properly(200).

This syndrome is curable and can be treated, in many cases, with dietary and lifestyle changes, but may also include the use of antibiotics prescribed by a gastroenterologist(199).

Excessive presence of bacteria in the small intestine can cause symptoms such as(201):

- Pain in the stomach, especially after eating.
- Constant feeling of a bloated belly
- periods of diarrhea, interspersed with constipation
- Frequent feeling of poor digestion
- Excessive intestinal gas

Although the syndrome can cause periods of diarrhea and constipation, it is more common for the person to have chronic diarrhea.

In the most severe cases of SIBO, the intestine may lose some of its ability to absorb nutrients and so malnutrition may occur, even if the person is eating properly. When this happens, the person may experience excessive fatigue, weight loss and even anaemia(201).

The most commonly used way to confirm the diagnosis of SIBO is to perform a breath test, in which the amount of hydrogen and methane present in the exhaled air is assessed. This is because excess bacteria in the small intestine release these gases in a higher amount than is considered normal. Thus, the breath test is a non-invasive and non-direct way to identify a possible case of SIBS(202).

To perform this test, we must fast for 8 hours and then go to the health center to breathe into a tube. A special liquid must then be swallowed and drunk, after which further exhalations are collected in new tubes every 2 to 3 hours(203).

Typically, people with SIBO have an increase in the amounts of hydrogen and methane in the exhaled air over time. When this occurs, the result is considered positive. However, if the test is inconclusive, the doctor may ask for

further tests to be done, especially the removal of a sample of the fluid in the small intestine to assess the number of bacteria in a laboratory(203).

Some possible causes of SIBO include changes in gastric acid production, anatomical defects of the small intestine, pH changes in the small intestine, changes in the immune system, changes in gastrointestinal motility, changes in enzymes and commensal bacteria(204).

This syndrome may also be related to the use of some medications, such as proton pump inhibitors, antimotility agents and some antibiotics(204).

In addition, the syndrome may be related to some diseases, such as viral gastroenteritis, celiac disease, Crohn's disease, low stomach acid levels, gastroparesis, nerve damage, cirrhosis, portal hypertension, irritable bowel syndrome, bypass procedures or certain surgeries, for example(205).

As far as treatment is concerned, this syndrome should be guided by a gastroenterologist, however, the accompaniment of a nutritionist may also be necessary. In this way, we can find the following drugs or treatments:

- *Use of antibiotics:* The first step in the treatment of SIBO is to control the number of bacteria in the small intestine and thus the use of an antibiotic, prescribed by the gastroenterologist, but usually Ciprofloxacin, Metronidazole or Rifaximin, is necessary(206).

  Although in most cases the antibiotic can be used in tablet form, when the syndrome is causing malnutrition or dehydration, it may be necessary to stay in hospital for a few days to receive intravenous or parenteral feeding, which is given directly into the vein(206).

- *Dietary changes:* There is still no known diet that can cure SIBO, however, there are some nutritional changes that seem to alleviate symptoms, such as: a) Eating small meals throughout the day, avoiding large meals; b) Avoiding foods and drinks high in sugar; c) Avoiding foods that seem to worsen symptoms, such as those containing gluten or lactose(207).

  In addition, several doctors also suggest that following a diet, which eliminates foods that undergo fermentation in the gut and are therefore less absorbed, may be ideal for rapid symptom relief.

- *Taking probiotics*: Although more studies are still needed to prove their efficacy, taking probiotics seems to help the gut rebalance its natural flora, reducing excess bacteria(208).

  However, probiotics can also be ingested naturally through food, via fermented foods such as yoghurt, kefir or kimchi, among others.

1.3.24. Trichinellosis/ Trichinosis (Trichinella spp.infection)

Trichinosis is a disease caused by Trichinella infection. The parasite most commonly implicated is Trichinella spiralis, which is usually found in pigs and is transmitted to humans by ingestion of improperly cooked meat. Once ingested, the parasite grows and matures within the intestinal walls. Mating occurs in the adult stage and the larvae produced migrate through the bloodstream to the striated muscle(187).

Symptoms appear during larval migration. After consumption of infected meat, patients may manifest gastrointestinal symptoms within a few weeks and

systemic symptoms such as fever, chills, myalgia and periorbital oedema may appear later(209).

Diagnosis can be made by serological testing and confirmed by the presence of cysts or larvae in a muscle biopsy. Mild infections are self-limiting, but systemic disease is treated with antiparasitic and corticosteroids. Infection can be prevented by proper cooking and meat handling techniques(210).

Therefore, the parasite is an intestinal and tissue (morphologically cylindrical), intracellular nematode, the best-known species in the world being Trichinella spiralis. As for the causes of trichinosis, we can find its encapsulated or enclosed form (within the muscles of the host), as is the case with Trichinella britovi (found in Europe, Asia and North and West Africa)(211), Trichinella murelli (found in North America)(212), Trichinella natives (found in the Arctic)(213), and Trichinella nelsoni (common in East Africa)(214).

However, we can also see the unencapsulated form, such as Trichinella papuae (native to Papua New Guinea)(215), Trichinella pseudospiralis (found around the world)(216), and Trichinella zimbabwensis (from Tanzania)(217).

Regarding the epidemiology of the infection, it is estimated that 10,000 cases of trichinosis occur each year worldwide(218). Some reductions in the number of cases have been detected due to improved pig husbandry conditions. However, substantial prevalence has been detected in: China; Russia, Romania and other parts of Central Europe, Thailand and some South American countries (such as Argentina, Mexico, Bolivia)(219).

Regarding the life cycle of the parasite, it starts when the encysted larvae live inside the striated muscles of the host animal (some species are not encysted). Thus, the larvae are ingested when undercooked meat is consumed.

After contact with gastric acid and pepsin, the larvae are released from the cysts. The larvae then invade the mucosa of the small intestine, where they develop into adults(220).

Mating then occurs in the small intestine, as the females generate larvae, which are released into the circulation, and these larvae migrate to the striated muscles. The migratory phase can last up to one month, at which point symptoms appear(221).

Muscles that may be affected by the disease include the diaphragm, tongue, intercostal and extraocular muscles. In addition, larvae can reach the myocardium and brain and cause an inflammatory reaction in the heart and brain. Some tissue infiltration (by PMNs and eosinophils) and oedema may also be found as the larvae form cysts. After a period of months, the calcification phase follows, remaining in this state for years, although the symptomatology attenuates after a few months(222).

As a result, we are talking about an incubation period of 1-6 weeks, with the severity of symptoms depending on the number of larvae (infective dose) consumed. There are also two phases, intestinal and systemic(223).

The intestinal phase occurs 2-7 days after exposure and may be asymptomatic. In this phase we find abdominal pain (epigastric), diarrhea, nausea and vomiting. In a similar vein, the systemic phase occurs in the first week or two after ingestion, although it can persist for up to 8 weeks. In this phase we detect fever and chills, as well as periorbital or eyelid oedema (proptosis, chemosis, conjunctivitis). Myalgias (on the face and chest) may also occur. Other symptoms related to the infection are muscle weakness, dry cough, subungual filiform hemorrhages and/or retinal hemorrhages, petechial/urticarial rash, headache, and hepatomegaly(224).

As for the complications of infection, we can find at least five serious clinical pictures(225):

- Myocarditis: as transient passage leads to inflammatory cell infiltration. As well as life-threatening arrhythmias are the most frequent cause of death.
- Meningitis/encephalitis.
- Pulmonary impairment: as with secondary bacterial pneumonia, or respiratory myositis (affecting the diaphragm).
- Renal insufficiency.
- Thromboembolic disease.

To diagnose the disease, a series of clinical tests can be used, such as serological tests, although these only detect the infection after three weeks of incubation. Among the techniques used are ELISA, Western blotting, latex particle agglutination test, and indirect immunofluorescence test(226).

These tests allow a definitive diagnosis to be established, but are only performed in cases where initial tests are inconclusive. A biopsy of symptomatic muscle tissue can also be performed to identify an embedded Trichinella spiralis cyst(227).

In addition, complementary examinations can be performed, such as laboratory tests (hemogram, metabolic profile and muscle tests), as well as a simple X-ray of the intramuscular calcification(228).

Treatment varies according to the level of infection of the patient. That said, it is important to note that most infections are uncomplicated and self-limiting.

However, in infections with systemic involvement, treatment consists of: antiparasitic (Albendazole, Mebendazole, Corticosteroids especially in severe complications). In general terms, complete recovery can be achieved in 2 to 6 months, although there is an increase in morbidity when the patient has cardiac or CNS involvement(229).

Regarding the preventive measures that could be taken in this respect, one of the most important is the cooking of the meat, which has to be done at temperatures up to 77°C. Also, freezing of meat at -15°C is recommended (although not applicable to arctic species), proper handling of meat, or post-exposure prophylaxis with mebendazole can be effective if administered within 6 days of exposure(229).

1.3.25. Trichomoniasis (Trichomonas spp. infection)

It is a genital infection caused by the protozoan Trichomonas Vaginalis. It is transmitted through sexual intercourse or intimate contact with the secretions of an infected person. It can be transmitted from woman/man and woman/woman. In general, women are more affected(230).

Trichomonas vaginalis is a parasite that only infects humans; it usually lives in the vagina or urethra, but can also be found in other parts of the genitourinary tract. This protozoan causes microlesions inside the vagina and can lead to the development of other STIs(231).

Regarding the symptoms of the disease, in women, symptoms usually start during or after menstruation. However, in some cases, the disease can remain symptom-free for months, making it difficult to treat after its discovery(232).

The main symptoms to detect trichomoniasis are(233):

- Yellowish or greenish-yellow discharge.
- Itching
- Strong, unpleasant odour
- Vulvar irritation
- Pain
- Difficulty urinating

After recognizing the symptoms, the woman should see a gynecologist, who will order laboratory tests such as collection of vaginal discharge, culture of discharge or PCR, a blood test that assesses whether there is infection in the body. A Pap smear may also be performed(234).

Trichomoniasis is a disease caused by the protozoan Trichomonas vaginalis, which affects the female genital organ. It usually affects the external area of the vagina, such as the vulva and urethra. When observing some of these signs, it is advisable not to self-medicate, as only a doctor can correctly identify the disease and prescribe the necessary medication for its treatment(235).

The diagnosis of the disease is made on the basis of the evaluation of the symptoms in the analysis and the appearance of the vaginal discharge. Using a microscope, the doctor checks for the possible presence of protozoa(235).

If trichomoniasis is suspected, the gynecologist should be consulted as soon as possible, as he/she is the qualified professional to order the tests that will lead to the correct diagnosis and initiate treatment(236).

Trichomoniasis is considered a sexually transmitted infection (STI) that affects millions of people worldwide every year. The disease is caused by the protozoan Trichomonas vaginalis and is curable by drug treatment, although there are effective preventive measures against the disease(236).

Trichomoniasis is most commonly transmitted through sexual contact. Transmission through contaminated objects, such as toilet seats, is rare. The disease affects the external part of the female genital tract, such as the vulva and urethra, causing burning, itching, abdominal pain, pain during urination and sexual intercourse, and yellowish or greenish discharge with a foul odor. As a sexually transmitted disease, the best form of prevention is the use of condoms during all sexual intercourse(237).

The goal of treatment for trichomoniasis is to eradicate the causative agent. The first measure indicated is sexual abstinence, as it is necessary to rebalance the organism to avoid worsening, discomfort and the appearance of new diseases(238).

Antibiotics are also indicated, and joint treatment of the sexual partner is mandatory to avoid reinfection. In women, oral treatment is a single dose at the same time as topical treatment, with the use of vaginal cream. Avoidance of alcohol consumption is recommended to prevent nausea and vomiting(239).

To the list above, we must add some of the parasites that are frequently found on the European continent, such as the following three parasites described below:

1.3.26. Taeniasis (indefinite Taenia/ tapeworm infection)

Taeniasis is an intestinal infection caused by the flatworms Taenia solium and Taenia saginata(240). Humans are the definitive host for these species, and eating raw or undercooked meat from cattle (T. saginata)(241) or pigs (T. solium)(242) containing cysticerci leads to contamination by the parasites. After ingesting live cysticercoid, the larvae follow the path of digestion, until the tapeworm scolex attaches itself to the small intestine and the worm begins to develop, feeding on nutrients released in the digestive process(243).

Taeniasis is also known as tapeworm. After about 60 days, after settling in the human body, the first proglottids containing about 40,000 eggs each are released in human feces. These eggs survive for a long time in the external environment until they are ingested by a pig or a cow (intermediate hosts) and continue the tapeworm cycle(244).

Taeniasis may have no symptoms, but some of them are: appetite disturbance, abdominal pain, diarrhea or constipation, weight loss, dizziness, weakness, irritability, tiredness, nausea and vomiting. In addition, if the worm obstructs the appendix, common bile duct or pancreatic duct, taeniasis can lead to serious complications(245).

Diagnosis of taeniasis can be made by examining the proglottids and looking for eggs in the feces, or by the gummed tape technique in the perianal region. After confirming the presence of eggs, treatment with specific drugs should be initiated. Treatment of taeniasis is also carried out with drugs popularly known as anthelmintics. Among the most commonly used drugs are mebendazole and albendazole(246).

As has been seen, taeniasis is a disease closely related to animal husbandry conditions and basic sanitation in a region. Therefore, measures that can control taeniasis are considered(247):

- Construction of adequate sewage systems
- Improvement of animal husbandry conditions
- Promotion of sanitary inspection in slaughterhouses and butchers' shops.
- Investment in campaigns to raise public awareness of hygiene habits and the ways in which the worm is transmitted.

Taeniasis and cysticercosis are two vermin infections transmitted by Taenia solium and Taenia saginata, however, in each disease, the parasite is at a different stage of its life cycle. While in taeniasis the adult worm is present in the body, in cysticercosis the disease is caused by the larval form of the parasite in the tissues(248,249).

Cysticercosis occurs when we ingest the eggs of the parasite, which can develop into the larval form and settle in the tissues. The disease is potentially serious, as the cysticercosis can develop in the nervous system, a situation known as neurocysticercosis. It should be noted that some authors admit that T. saginata cysticercosis does not occur or is extremely rare(250). It is believed that *Taeniasis (Taenia infections)* are also far more common that the clinical data might suggest, but the sensitivity of the PCR and serological tests are circa 10%, and coprocospic test sensitivity does not exceed 20%.

1.3.27. Toxocariasis (Toxocara canis/ cati infection incl. Ocular Larva Migrans (OLM) & Visceral Larva Migrans (VLM)

Toxocariasis is an infection transmitted from animals to humans (zoonosis) caused by parasitic worms commonly found in the intestines of dogs (Toxocara canis) and cats (T. cati). However, the Toxocara parasite that most commonly affects humans is T. canis, which puppies often get from their mother before birth or through their mother's milk(251).

The larvae mature rapidly in the gut of the puppy; by 3 to 4 weeks of age, they begin to produce large numbers of eggs which contaminate the environment via the puppy's feces. Over a period of 2 to 4 weeks, infective larvae develop in the eggs. Toxocariasis is not transmitted by person-to-person contact, like a cold or flu(252).

Toxocariasis is an infection in people caused by parasitic worms found in the intestines of dogs and cats. It mainly affects young children who come into contact with contaminated dirt(253).

Toxocariasis is a parasitic disease caused by the larvae of two species of Toxocara nematodes: Toxocara canis from dogs and, less frequently, Toxocara cati from cats(253).

Toxocariasis is a type of parasitic infection that occurs when a person accidentally ingests dog or cat feces containing roundworm eggs. Ingested roundworm larvae spread through the human gastrointestinal tract, which can cause abdominal pain and nausea. In severe cases, the worms can cause more common problems in the lungs, eyes or brain(254).

Most infections are considered mild and do not require medical treatment. However, if toxocariasis causes organ damage or vision problems, the doctor may administer medication to eradicate the parasite and prevent further complications(255).

Dogs can carry an ascarid parasite known as Toxocara canis, while cats carry Toxocara cati. Either of these worms can cause toxocariasis in humans if the feces are ingested. The disease is most common in young children who do not wash their hands after playing in contaminated soil(256).

Eating unwashed garden vegetables or undercooked meat from animals raised with dogs or cats can also cause infection. Symptoms usually appear one to three weeks after ingestion, when the eggs begin to mature and hatch in the gastrointestinal tract(256).

Accordingly, doctors recognize three different types of toxocariasis infection. Most cases in children are called secretory, meaning that the symptoms are mild and short-lived. A person with secretory toxocariasis may experience mild abdominal pain, cough or headache(257).

Visceral larva migrans occurs when the eggs hatch and spread throughout the body, causing inflammation of the lungs, skin, heart or even the brain. Occasionally, the larvae can spread to the eyes in a condition called ocular larva migrans, which can cause blurred vision and redness(258).

If a person notices possible signs of toxocariasis in themselves or their child, they should see a doctor immediately for a proper diagnosis. The doctor may take a blood sample to check for increased levels of white blood cells and the presence of parasites. In addition, if visceral or ocular larva migrans is

suspected, the physician may perform a CT scan of the internal organs to analyze the extent of damage(258).

Treatment of toxocariasis depends on the type of infection and the severity of symptoms. Most cases of covert toxocariasis are not treated medically; the larvae pass naturally in the stool over a few weeks. However, if the worm larvae spread, the doctor may prescribe drugs called anthelmintics that seek out and destroy the parasites in the bloodstream(259).

Anti-inflammatory drugs can help relieve other symptoms related to the infection. Rarely, when parasites cause major damage to the eyes or organs, surgery may be necessary to repair or remove the affected body parts(259).

As for their impact on the body, if we ingest dirt contaminated with animal feces containing Toxocara eggs, we may contract toxocariasis. After swallowing the eggs, the larvae hatch in the intestines. The larvae pass through the intestinal walls and spread into the bloodstream. The larvae can affect almost every tissue in the body. The liver and lungs are most affected(260).

The larvae can live for months and cause damage as they move through the tissues, although the larvae do not mature until adulthood in humans(260).

Most cases of toxocariasis cause no symptoms. The worms will simply be passed with the feces at some point. However, if many Toxocara larvae have been consumed, they can migrate to different parts of the body(261).

That said, there are two main types of this disease:

- Visceral Toxocariasis: this occurs when Toxocara larvae move to various organs of the body. This can include the lungs, liver and

central nervous system. Another name for the infection is visceral larva migrans(262).

- Ocular toxocariasis: Ocular toxocariasis occurs when Toxocara larvae travel to the eye. It can cause irreversible vision loss. Another name for the infection is ocular larva migrans(263).

However, there is also occult toxocariasis, which occurs in children, and can cause a mild illness with fever. Other possible symptoms include cough, sleeplessness, headache and abdominal pain. Children may show signs of infected lymph nodes, swollen liver or wheezing in the chest. Occult toxocariasis is thought to be caused by prolonged exposure to migratory juvenile worms(263).

Visceral larva migrans is an inflammatory reaction caused by the death of larvae migrating through internal organs. It mainly affects children under five years of age. That said, there are a number of symptoms related to visceral larva migrans(264):

- Fatigue
- Weight loss
- Anorexia
- Fever
- Cough
- Pneumonia
- Bronchospasm

- Abdominal pain
- Headache
- Skin irritation
- Seizures (rare)

Liver inflammation, infected lymph nodes and wheezing may also occur. Serious complications such as pleural effusion, chronic urticaria, myocarditis and respiratory failure may occur(265). When larvae migrate to the eyes (ocular larva migrans), they can cause vision problems, red eyes or a white pupil appearance (leukocoria). Chorioretinitis (inflammation of the vascular lining and retina) and granulomas are typical findings on examination. Ocular larva migrans can cause vision loss, retinal fibrosis, retinoblastoma and retinal detachment(266). Ocular larva migrans is most common in children between five and ten years of age, and it can produce the following symptoms:

- Fever
- coughing
- Wheezing
- Extreme tiredness (fatigue)
- Abdominal pain
- Skin rash
- Enlarged liver
- Enlarged spleen

- Pneumonia

In addition, ocular toxocariasis usually affects only one eye, and symptoms of ocular toxocariasis may include(267):

- Pain and redness of the eye.
- Scarring and damage to the retina.
- Vision problems.

Toxocariasis infections in dogs are the most common. Puppies can contract a Toxocara parasite (T. canis) from their mother before birth or from her milk. Toxocara larvae grow rapidly in the puppy's intestine. A few weeks after birth, the worms in the puppy's intestine may start to produce eggs, and in the puppy's faeces, the larvae can be found. Two to four weeks later, infective larvae develop in the eggs(267). Is important to mention that the toxocariasis that is surely more common that the clinical results might suggest, but the serological tests are expensive in some countries, and many patients refuse to perform the tests.

As a consequence, the last of the parasitic infections to be found in Europe is Toxoplasmosis, which will be described in detail in the following section.

1.3.28. Toxoplasmosis (Toxoplasma gondii infection)

It is an infection caused by a parasite, Toxoplasma gondii. Reproduction of this parasite only occurs in the cells lining the intestine of cats. Therefore, the parasite's eggs are found in the cat's feces(268).

Toxoplasmosis is one of the most common parasitic diseases in cats, with a worldwide prevalence of between 6% and 97%. The parasite infects nearly one third of the world's population, although its prevalence in industrialized countries has been declining in recent decades, with incidence in 15–45-year-olds ranging from 10% to over 50%(269).

An estimated 60 million people are infected by this parasite in developed countries, while in Europe, some data suggest a prevalence of infection of around half of the population over 30 years of age(269).

Congenital toxoplasmosis can be asymptomatic or present severe and rapidly fatal symptoms. When signs appear, the most common are eye swelling, severe jaundice, easy bruising, seizures, and mental retardation. Very mild signs may appear soon after birth, but usually do not appear until months or years later(270).

Acquired toxoplasmosis rarely causes symptoms and is usually diagnosed when a blood test reveals antibodies to the parasite. In fact, in healthy people, this infection does not cause symptoms because the immune system prevents the parasite from causing the disease(271).

In milder cases, it can be similar to infectious mononucleosis, with swollen lymph nodes in the neck and armpits that are often insensitive to touch, a feeling of malaise, muscle pain and a low, fluctuating fever that can last for weeks or months. Mild anaemia, low blood pressure, low white blood cells, increased numbers of lymphocytes in the blood and abnormal liver function tests may also occur. Most of the time, people infected with toxoplasmosis have only a painless enlargement of the lymph nodes in the neck(272).

In chronic forms, ocular inflammation may occur and can be severe if not properly treated. Acute disseminated toxoplasmosis may cause rash, high fever, chills and exhaustion. In some cases, the infection can cause inflammation of the brain and its covering membranes, liver, lungs or heart. In an immunodeficient patient, as in HIV infection, toxoplasmosis tends to be generalized, with inflammation of the brain (encephalitis), seizures, tremors, headache, confusion or coma(272).

Diagnosis is almost always laboratory-based, with blood tests showing the presence of antibodies to the parasite. In some cases, a CT scan or MRI of the brain may be necessary(273).

Transmission of toxoplasmosis can occur by ingestion of raw or undercooked food containing the inactive (cyst) form of the parasite or by contact with soil with cat hooves containing eggs. If a pregnant woman is infected, the infection can be transmitted to the fetus through the placenta, which can lead to miscarriage or the development of toxoplasmosis in the child(274,275).

In most healthy people, recovery from toxoplasmosis occurs without treatment. When necessary, a combination of drugs such as spiramycin, sulfadiazine and pyrimethamine is given, often in combination. In AIDS patients, toxoplasmosis is so common that therapy must be maintained indefinitely. Treatment during pregnancy is debatable because the drugs may be toxic to the fetus. Therefore, each case must be evaluated individually(276).

As for possible preventive measures, these involve several combined actions(272):

- Proper cooking of food

- Freezing food before preparation
- Peel and/or wash fruits and vegetables thoroughly
- Wash hands and all cooking utensils thoroughly during food preparation
- Do not drink untreated or unsafe water
- Use gloves for gardening activities or activities involving contact with soil and wash hands after gardening (this care in regular hand washing should be passed on to children as a general principle).

For cats, whenever possible, keep litter boxes outside and/or covered, clean them daily, and feed them with specific commercially available feed or well-cooked food. For pregnant women, it is even more important not to come into contact with the litter box and, if necessary, to do so with gloves and wash hands immediately afterwards. During this period, cats should be kept indoors to avoid contamination outside(277).

At the same time, it is essential to mention the following infections that are not caused by parasites, but are often associated with parasitic infections, such as the following:

1.3.29. Candidiasis (Candida spp. infection)

Candidiasis is a fungal infection that mainly affects women, although it can also affect men. So, candidiasis is a fungal not a parasitic infection, and parasites

do stimulate very often the candidiasis development, and since then, most of the parasitic infestations are associated with candidiasis.

This infection occurs mainly on the mucous membranes and skin. Candidiasis is caused by the fungus Candida sp, an opportunistic fungus that can be present in the genital tract and gastrointestinal tract(278).

It is a Gram-positive, dimorphic fungus (for example, it occurs in different forms) and can occur as yeast (blastoconidia) in the saprophytic stage or as filaments (hyphae and pseudohyphae) especially in candidiasis. However, different species may be involved in the infection. Generally grouped into Candida albicans and Candida non-albicans (includes C. glabrata, C. tropicalis, C. krusei, C. parapsilosis)(279).

- *Candida albicans:* is the main protagonist of candidiasis episodes, accounting for more than 80% of cases(280).

- *Candida glabrata:* is the second most frequent(281).

Candida sp is a common fungus found in the genital tract as part of the local flora, and does not normally cause any discomfort or symptoms. However, in situations of imbalance, especially immunological imbalance, this fungus can proliferate in an exaggerated manner and thus cause candidiasis. In other words, having candida does not mean having candidiasis(282).

Candidiasis is therefore caused by an imbalance in the body, whether due to hormonal or metabolic changes, antibiotic therapy or immunosuppression, which allows Candida sp to proliferate excessively. Thus, certain risk factors may predispose to this imbalance and, consequently, to candidiasis. Examples of hormonal factors include pregnancy, as during pregnancy there is a major change in female sex hormones (mainly estrogen) (282).

Hormonal changes are also common in situations of use of combined oral contraceptives, hormone replacement therapy, especially when these drugs are rich in estrogen. Estrogen has a binding protein on fungal agents, and therefore facilitates the proliferation and infection of Candida sp(283).

In addition, female sex hormones increase the concentration of glycogen in the vagina, and glucose is a substrate for the bacilli present there, which, upon fermentation (or anaerobic respiration), produce lactic acid leading to acidification of the vagina, per example the lowering the pH, thus creating a favorable environment for Candida sp. to thrive(284).

Another contributing factor is diabetes mellitus, a metabolic factor leading to hyperglycemia, which is detrimental to the immune system, like in the case of neutrophils, affecting their function and their ability to phagocytose micro-organisms. Another factor in diabetes mellitus is the increase in acid proteinases that facilitate the adhesion of Candida sp to the epithelium. In addition, increased blood glucose serves as a nutrient for the fungus (284).

Antibiotic therapy is a very important factor, especially when broad-spectrum antibiotics are involved. This is because antibiotic treatment kills the bacteria, but does not act on the fungi. The normal vaginal flora contains several micro-organisms and they control each other. When bacteria are eliminated, fungi are free to proliferate and occupy the entire region. For example, the vaginal flora is rich in lactobacilli, which are bacteria. When antibiotics are used, there can be a significant suppression of lactobacilli, which are most involved in the defense against fungal growth(285).

Other factor is immunosuppression, in situations of use of drugs such as corticosteroids or other immunosuppressants, or immunosuppression, whether due to primary immunodeficiencies, stress, acquired immunodeficiency, such as

HIV. In addition, we can highlight the following external factors, such as the lack of local hygiene, the use of clothing that hinders ventilation (fungi like humid and warm environments) and the consumption of foods rich in sugar(286,287).

There are two forms of candidiasis, defined according to factors such as the form of presentation and the efficacy of treatment, such as the simple or uncomplicated form and the complicated form(288).

- *The simple (uncomplicated) form*: is a candidiasis that affects healthy women, and symptoms are sporadic and infrequent. The etiological agent is Candida albicans. Another important point is that this type of candidiasis is usually treatable with conventional antifungal therapy and cure rates are high (288).

- *The complicated form:* represents candidiasis that affects immunocompromised patients and may be caused by the less common Candida sp. species (such as Candida glabata or Candida krusei). Symptoms are recurrent and may recur 3 or more times a year (288).

At the same time, it is possible to identify four types of candidiasis that are more common in the general population, as described below:

- *Vulvovaginal candidiasis (female):* The main symptom of vulvovaginal candidiasis (VVC) is vulvovaginal pruritus (itching). In addition, there is significant burning and erythema in the region. Because of the pruritus, vulvar excoriations and fissures may also be found. Dysuria (painful urination) and dyspaurenia (pain during sexual intercourse) may also occur (288).

Another important symptom, which is a common complaint among women, is vaginal discharge. This discharge is white, lumpy, odorless and case-like ("curdled milk"). It sticks to the walls of the vagina (288).

White or greyish plaques may also be seen on the vaginal walls. The symptoms of VVC are usually aggravated in the premenstrual phase and improve with menstruation, as menstrual blood alkalinizes the vaginal area, causing this false improvement (288).

- *Penile candidiasis (male):* Candidiasis can also affect males and, in these cases, the main symptom is balanitis or balanoposthitis (inflammation of the glans and foreskin)(289).

  In the affected region, the glans, the most common symptoms are erythema (redness), oedema and pain. White plaques may also appear in this region (289).

  As with vulvovaginal candidiasis, there is significant itching in the region, which may lead to excoriations. In addition, the patient may experience dyspareunia and burning after intercourse (289).

  Discharge may also occur, being thick and with an unpleasant odor, especially in uncircumcised patients.

- *Oral candidiasis:* As the mouth is one of the habitats of Candida sp, in situations of imbalance there may be an exacerbated proliferation of the opportunistic fungus and result in the development of oral candidiasis(290).

There are several forms of oral candidiasis: pseudomembranous candidiasis, acute atrophic candidiasis, chronic hyperplastic candidiasis, chronic atrophic candidiasis, among others(290).

The main and most common is pseudomembranous candidiasis, the so-called "thrush", which mainly affects newborns. The lesion is characterized by white pseudo membranes, consisting of desquamated epithelial cells together with fibrin and fungal hyphae(291).

In general, some risk factors are extreme age (infants, children and the elderly), comorbidities such as diabetes mellitus, patients in a state of immunosuppression due to diseases such as HIV/AIDS, leukemia, use of broad-spectrum antibiotics, use of inhaled corticosteroids (for example the asthma sprays), among others(291).

- *Recurrent vulvovaginal candidiasis:* When there are 4 or more episodes of candidiasis treated and diagnosed within 12 months, the patient can be said to have recurrent candidiasis. Usually the symptoms are more persistent, and the main symptom is burning of the vulva and vestibule(292).

Clinical examination (history and physical examination) is usually sufficient for diagnosis, per example, the diagnosis of classical candidiasis can be defined by clinical criteria. Some tests may be requested mainly to evaluate recurrent cases in order to determine the micro-organism involved(293).

Specific vaginal cultures on media such as Microstix-candida or Sabourad, Nickerson can be performed to evaluate the micro-organism involved in recurrent

candidiasis, or when there is a high suspicion of vulvovaginal candidiasis due to Candida sp, but cytology is negative. Vaginal pH, which is usually less than or equal to 4.5 in cases of candidiasis, may be requested in the laboratory test(294).

1.3.30. Aspergillosis (Aspergillus spp. infection)

Aspergillosis is caused by the fungus Aspergillus fumigatus, which can be present in various everyday environments, such “as soil, plants, decaying material, or construction sites”(295). Even aspergillosis is not a parasitic infection, it may be associated with parasitic infestations.

Therefore, as the fungus may be found in a variety of habitats, people frequently come in contact with Aspergillus fumigatus, but not everybody develops the illness, as the fungus can grow most easily and causes symptoms to appear in people whose immune systems are most compromised by illnesses like HIV and lupus, transplantation of organs or use of medications. (296).

The primary way of Aspergillus is via inhalation, which allows it into the lungs and leads to such symptoms as cough, difficulty breathing and temperature, which can rapidly get worse and involve other areas of the human body, including the brain, heart and kidneys, particularly if antifungal treatment is not initiated(297).

The most common symptoms of aspergillosis are described below(298):

- “Fever above 38°C”
- “Coughing up blood or phlegm”
- “Shortness of breath”

- “Runny nose and difficulty in smelling odors”

In most cases, after inhaling Aspergillus fumigatus spores, the fungus can remain in the body without any signs or symptoms. If mild symptoms are detected, they are usually indicative of less severe aspergillosis, which can be easily treated with medication, and are more common in people with chronic lung diseases such as asthma or cystic fibrosis(298).

Aspergillosis can also be classified into a few main types depending on where the fungus is located and the severity of the infection. These include the following:

- *“Pulmonary aspergillosis”:* These are also very frequent, but often involve people with no previous history of pulmonary illness. Their symptoms are (299):

    - “Weight loss”
    - “Persistent cough”
    - “Coughing up blood”
    - “Excessive tiredness”
    - “Shortness of breath”

    If not treated adequately, the pulmonary infection may develop and extend to blood, spreading to other areas of the human body. Furthermore, in certain instances the fungus may colonize the lung cavities to form a mass of fungus, called an aspergilloma, which can continue growing and lead to a coughing up of blood, and it can also extend into the vessels and lead to an invasive aspergillosis(299).

- *"Invasive aspergillosis":* It is the most severe kind of infection, occurring when the fungus can multiply in the lungs and move to the blood. Symptoms of aspergillosis may include(300):
    - "Fever above 38°C"
    - "Chest pain"
    - "Persistent cough"
    - "Pain in the joints"
    - "Headache"
    - "Swelling of the face".

In addition, it is important to mention that this fungus has the ability to penetrate the blood vessels, spread more easily and promote vessel closure, leading to thrombosis(295).

Invasive The most common kind of invasive aspergillosis occurs when immune systems are very weakened and therefore its signs and symptoms can be hard to recognize, as they may be misinterpreted as signs of the illness that is at the root of the body's reduced defenses(301).

As for the contagion of the disease, it can occur by Inhalation of the environmental spores, but may also occur by inoculation of the spores into the cornea. While it can be breathed in by any person, the infection (particularly of the invasive type) develops most often in persons whose immune system is more compromised because of infectious and/or long-term diseases, including HIV and or lupus, have recently had an organ transplant or are on drugs that reduce the

immune system's activity, including corticosteroids, antibiotics, chemotherapy or immunosuppressants.(302).

Diagnosis of aspergillosis is made primarily by the infectious disease specialist, pulmonologist or general practitioner by assessing the person's presenting symptoms and signs, and health history(303).

Accordingly, to confirm fungal infection, microscopic observation of sputum or serological blood tests to detect fungus-specific antibodies, or culture of contaminated tissue, may be recommended. Depending on the results of these tests, confirmation of aspergillosis diagnosis and its seriousness is useful for the clinician in order to determine the most suitable treatment(303).

Treatment usually begins with antifungal medications, like itraconazole or amphotericin B, that help rid the body of excess fungus, helping the immune system to control the infection and relieve symptoms(304).

However, the doctor may also advise the use of corticosteroids, such as Budesonide or Prednisone, to relieve symptoms more quickly and enhance the effect of the antifungal, especially in people with very severe symptoms, such as asthmatics, for example(303).

In more severe cases of pulmonary or invasive aspergillosis, where a fungal mass, known as an aspergilloma, may develop, the doctor may recommend surgery to remove the most affected tissues and enhance the effect of antifungals(305).

1.3.31. Dysbiosis

Gut dysbiosis is a problem caused by a change in the number and distribution of bacteria in the microbiota. This imbalance is a problem because it risks causing inflammation or a decrease in the gut's ability to absorb nutrients, which in turn can lead to nutritional deficiencies(306). Even dysbiosis is not a parasitic infection, it may be associated with parasitic infestations.

There are also a number of situations that can cause the imbalance that leads to dysbiosis, such as the following(307):

- Feeding too much protein or sugar and too little fiber.
- Accidental ingestion of toxic substances such as pesticides in fruit peels that have not been properly washed.
- Frequent consumption of alcoholic beverages
- Recent antibiotic treatment
- Lack of proper oral hygiene
- High levels of stress and anxiety
- Unprotected sexual intercourse (not using condoms).

Often, the symptoms of this condition can be mild and even go unnoticed. Symptom manifestation depends very much on the types of bacteria that are out of balance and the severity of the problem, and may include(308):

- Abdominal pain (stomach or intestines).
- Belching

- Abdominal bloating
- Bad breath
- Nausea
- Constipation
- Diarrhea
- Difficulty urinating
- Vaginal or rectal itching
- Abdominal distention
- Chest pain
- Rash or redness of the skin
- Fatigue
- Problems with organization of thought or concentration
- Anxiety and depression.

Despite being a relatively simple problem to solve, lack of treatment makes dysbiosis a risk factor for the development of various problems. The main complications, which are also the most common, are lactose intolerance, coeliac disease (gluten intolerance), irritable bowel syndrome and immune system diseases such as arthritis, lupus and type 2 diabetes, among others. Other complications related to dysbiosis include(309):

- Colitis
- Candidiasis
- Obesity
- Polycystic ovary syndrome (PCOS)
- Skin diseases
- Liver disease
- Heart disease or heart failure
- Cancer of the colon or rectum.

It is also recommended to see a doctor immediately if, for example, we experience any unusual or persistent stomach pain or skin irritations. The earlier a doctor diagnoses the disease, the less likely it is that more serious complications will occur(310).

In terms of diagnosis, the medical assessment of intestinal dysbiosis is done by looking at symptoms, medical history and also by specific tests, including urinalysis, which looks at the first urine of the day or when the person has not urinated for at least 4 hours. As the name suggests, the test assesses the amount of Indican present in the sample(311).

A gut microbiome study can also be carried out, which is a type of stool examination that identifies the types and amounts of bacteria and other micro-organisms present in the gut flora. The aim is precisely to find out whether there is indeed a bacterial imbalance indicating dysbiosis(311).

The test is often indicated even when a diagnosis has already been made. This is because it helps the doctor to choose the most appropriate type of treatment. In addition, the test is also important to help the physician and/or dietician plan a diet that supports the treatment, which is in fact essential(312).

On the other hand, the exhaled hydrogen test, although less common, this type of test can also be requested to help diagnose dysbiosis. The test involves the patient drinking a sugar solution and breathing into a special balloon. The aim is to analyses the exhaled gases and identify the number of gases produced by bacteria. Indeed, a low number of certain gases may indicate a bacterial imbalance(312).

Regarding the treatment of the disease, first of all, it is important to remember that treatment is essential to avoid complications. Therefore, we already know that the first step is to follow medical recommendations exactly as indicated. Physicians usually prescribe treatment taking into account, first of all, the cause of the imbalance. If the problem is the result of an inadequate diet, for example, it will probably be necessary to change eating habits(313).

It is quite common for people with dysbiosis to have a constant craving for sugar. This sensation can be very similar to that felt by those who are chemically dependent on other substances such as cigarettes and alcohol, for example. This is due to an uncontrolled growth of bacteria that feed on sugar. This is undoubtedly one of the factors favoring complications such as pre-diabetes, diabetes and even obesity(314).

The good news is that by rebalancing the microbiota with the right treatment, the desire to eat sweets also tends to disappear, which helps to combat these complications more easily. Of course, there are no miracles to

make this happen, as it is necessary to adapt the diet and give up certain habits(314).

However, while diet is indeed the main pillar in combating dysbiosis, there are many other steps involved in the process, as stress and anxiety can also act as catalysts for dysbiosis symptomatology. That said, these factors interfere substantially with treatment, and certainly need attention when the patient is struggling with dysbiosis. The ideal is to try to identify and avoid (or learn to cope with) stressful situations. It is worth remembering that mental health professionals, such as psychologists and psychiatrists, can be of great help in this process(315).

In addition to therapy and professional monitoring, there are a number of ways to relieve stress to help combat dysbiosis(316):

- Practicing regular physical exercise
- Improving sleep quality with practices such as sleep hygiene
- Doing meditation exercises
- Set aside some free time to simply relax or even engage in pleasurable activities.
- Take short breaks during the day to rest the mind, avoiding working or studying for several hours without a break, for example.

It is also possible to resort to pharmacological treatment to combat the disease. Although it is necessary to have the opinion and guidance of a specialist in relation to the medication that can be prescribed or interrupted. Indeed, there are medications that interfere negatively with the balance of the intestinal flora.

Therefore, if this is one of the causes of dysbiosis, it may be necessary to interrupt or replace these medications until further notice(317). In addition, the doctor may also prescribe medications to help manage dysbiosis-related problems, such as antibiotics that treat intestinal infections, urinary tract infections or irritable bowel syndrome(317).

It is important to remember that many antibiotics cause changes in the gut flora, such as antibiotic associated diarrhea (AAD), and when taken for too long can lead to more serious changes in the body, such as the growth of drug-resistant pathogens(318).

Alternatively, dietary supplements or probiotics can also be used to address the problem. Probiotic supplementation can help by acting as an adjuvant in the treatment of dysbiosis, provided it is done under medical supervision. Medical advice is essential to ensure that these supplements are administered correctly. Maintain treatment for the recommended period of time for consistent results(319).

This type of supplement contains the right amount and types of good bacteria, such as lactobacilli and bifidobacterial, for example. As well as helping to balance the intestinal flora, they help to minimize common troublesome symptoms and improve the absorption of nutrients by the gut(320).

1.4. Relationship between diet and parasites

Zoonoses are associated with pathologies that are transmitted from animals to humans. Zoonoses have been known to exist since prehistoric times. However, it is in the Neolithic period (from eight thousand years BC) that conditions favorable to the transmission of transmissible disease agents between

vertebrate animals and humans increased, as it was at this time that the structuring of agriculture, the domestication of animals and the rise of organized urban life in villages began(321).

In Europe there are many animals used as food producers (bovine, bubaline, porcine, ovine, caprine and poultry), in the food production chain there can be failures in management practices, deficiencies in the dissemination and implementation of technology, as well as the appearance of transmissible diseases that compromise the activity and harm the international trade of animals and animal products(322).

It is estimated that there are more than 200 communicable diseases that meet the definition of zoonoses. Given the problems presented, improving the quality of these traded products has been the focus of discussions in recent years, due to the global concern with strategies that allow their control and, consequently, guarantee the placement of safe products in the consumer market(323).

Some of the pathologies associated with food are called zoonoses, for example they occur through the interaction between humans and animals, their products and environments, and can be viral, bacterial or parasitic in origin(324).

Diarrheal diseases, almost all caused by food or waterborne microbial pathogens, are the leading causes of illness and death in less developed countries, killing about 2 million people per year worldwide. Even in developed countries, it is estimated that up to one third of the population is affected by foodborne microbial diseases each year. Most of the pathogens causing this significant disease burden are now considered zoonotic(325).

The presence of some of these zoonotic pathogens seems to have increased significantly in recent years. The factors involved in such increases have not been well studied, but there is general agreement that they include changes in animal production systems and in the food production chain. Both types of changes may lead to corresponding changes in pathogen exposure patterns and in the susceptibility pattern of the human population(326).

As discussed above, there are a number of parasites causing such diseases. However, the most important emerging foodborne zoonotic pathogens are: Salmonella spp., Campylobacter spp., enterohaemorrhagic Escherichia coli, Toxoplasma gondii and Cryptosporidium parvum, which have been discussed in detail in the previous section(326).

According to this premise, foodborne diseases (FBDs) are those caused by the ingestion of contaminated food and/or water. There are more than 200 types of foodborne diseases in the world, most of which are infections caused by bacteria and their toxins, viruses and other parasites(323).

There have been changes in the epidemiological profile of foodborne diseases due to the expansion of consumer markets, economic globalization, changes in eating habits and increased consumption of processed or out-of-home foods. However, foodborne diseases remain a major cause of morbidity in countries around the world(327).

The occurrence of FBD is related to several factors, such as the development of inadequate sanitation and water quality for human consumption, as well as inadequate personal hygiene practices and consumption of contaminated food. In terms of meat consumption, the consumption of raw or undercooked meat is cited as one of the main factors associated with FBD(327).

Multiple factors that have contributed or are contributing to the changing trend of parasitic foodborne diseases are(328):

- Changing dietary habits, such as consumption of raw or undercooked foods.
- Demand for exotic foods, such as bushmeat
- Rapid population growth, concentrated in urban areas, which is increasingly consuming vegetables, fruits, meat, ethnic foods and even farm animals, some of which come from countries without adequate food security.

This global population and demographic shift is responsible for changes in food consumption, with a trend of demand for low and high protein foods as nations develop economically with a concomitant and global dependence on meat and fish products(329)

Another relevant factor is the improvement of transport logistics conditions, which allows actors to survive with food products and to reach the consumer in a viable way. Changes in agricultural practices, for example, have forced the intensification of cheaper food production or free or organic animal production to respond to consumer welfare concerns(329).

All these factors contribute to social and political upheavals that lead to altered veterinary control and modes of production, increase human intrusion into native wildlife habitats and generate climatic changes that favor the distribution of intermediate hosts, bringing new vectors into regions or changes in the level of contamination associated with temperature(330).

With increasing urbanization around the world, eating out is becoming more and more common. Despite the growth of the sector, there is no effective education or training of food handlers and no hygienic control of street food. Contamination of the food supply with pathogens and their persistence, growth, multiplication and/or toxin production has become a major public health problem. Foodborne diseases are a serious international problem and a major cause of reduced economic growth. As previously mentioned, it is a known fact that more than two hundred different diseases are transmitted by food. Despite this, only a fraction of all foodborne infections are officially diagnosed and reported, or can be traced back to a defined vehicle and a specific causative agent(330).

Food safety problems in the industrialized world differ considerably from those faced by developing countries. The latter use traditional methods for marketing fresh produce, whereas in industrialized countries there are standards for food processing and packaging. In developing countries, much of the ready-to-eat food is sold on the street. These practices can contribute to increased biological, chemical and physical hazards in relation to food safety and microbiological food quality(331).

Epidemiological and research data collected over the last century have shown that the following sequence of events must occur for people to acquire a foodborne illness(331):

a) The etiological agent must be present in the citizens of a community, in food animals, or in the environment where food is grown, harvested, processed or stored.

b) The agent itself or the organism producing the agent (if one of several toxins) must contaminate a food during the growing period or during harvesting, processing, storage or preparation.

Once these requirements are met, the following events must occur for infection to take place(330):

- The agents must be present in or on the contaminated food in sufficient numbers or concentrations to survive the remainder of the growing, storage and processing period and still cause disease.

- Bacteria present or in insufficient numbers to cause disease must multiply and reach sufficient numbers or produce toxins in sufficient quantities to cause disease.

- Micro-organisms, particularly bacteria, enter food preparation areas on or in raw food, where they are transferred to workers' hands or equipment surfaces, which if inadequately washed contaminate other food they subsequently touch (and therefore, if bacteria, multiply as described in

- Sufficient quantities of contaminated food containing sufficient agent to exceed a person's resistance-susceptibility threshold must be ingested.

Ingestion of contaminated food at this level can also lead to sporadic cases of the disease as outbreaks. Whether or not outbreaks are detected depends on the number of people who have ingested the contaminated food and the socio-cultural attitudes of the population to report illness and the efficiency of a health agency to determine that the illness is foodborne and epidemiologically linked to other cases. When the number of pathogens is insufficient due to ingestion of the disease, an infected individual can become a carrier and contaminate other food they touch(332).

Cross-contamination during food preparation has been identified as an important factor associated with foodborne illness. Food handlers play an important role in ensuring food safety throughout the production, processing, storage and preparation chain. Improper handling and non-compliance with hygiene measures on their part can lead to food contamination and its consequences. Foodborne diseases affect millions of people every year and an unknown but considerable proportion of these diseases could be prevented by measures taken by food handlers and consumers(332).

Close collaboration at local, national and international level with epidemiologists and authorities responsible for surveillance and prevention of foodborne diseases is important and should not be forgotten. The best way to reduce these risks is to take measures to train people involved in food handling(333).

Therefore, cleaning procedures for food contact surfaces should be evaluated and special attention should be paid to utensils used during processing, such as gloves, baskets, kitchen towels and hand utensils. It is recommended that food is prepared with as little hand contact as possible, with appropriate utensils and on surfaces that have been cleaned, rinsed and disinfected prior to use to avoid cross-contamination(334).

It is also necessary to assess the cleaning procedures of food contact surfaces and to pay special attention to utensils used during processing, such as gloves, baskets, kitchen towels and hand utensils. Another factor is that food should be prepared with as little hand contact as possible, with appropriate utensils and on surfaces that have been cleaned, rinsed and sanitized prior to use to avoid cross-contamination(334).

Many of these problems could be controlled by the efforts of food handlers, whether in a processing plant, a restaurant, among others. However, the high turnover of food workers makes effective training difficult. Trained microbiologists and food technologists must set an example in personal hygiene and food handling practices. We are constantly subjected to risks or hazards throughout our lives(26).

However, food may be safer today due to increased knowledge about microbes and sanitation, as well as increased regulatory process. On the other hand, due to large-scale, high-speed food processing, the disruption of traditional processing methods resulting in less control of micro-organisms, the proliferation of convenience foods for heating and eating, and national distribution with increased potential for mishandling, there is concern that foodborne disease outbreaks may occur worldwide(26).

Public health interventions to reduce foodborne disease transmission should focus on general hygiene measures in street food vending, for example hand washing with soap, proper food handling hygiene and frequent renewal of dishwater(335). However, there are practices or processes that take place in food handling that can lead to the spread of parasites and other infectious agents, such as the following:

1.4.1. Partitioning or splitting

This practice occurs when a large unit is divided into several small units. To describe the transmission dynamics of a pathogen along a food pathway in quantitative microbiological risk assessment, it is necessary to model several types of processes. Next to microbial processes, such as bacterial growth and inactivation, some food handling processes, such as partitioning and mixing of

food products, can be identified. Unlike most hazardous chemical compounds, the concentration of food pathogens changes during food processing, storage and preparation, making it difficult to estimate the number of microorganisms or the concentration of their toxins at the time of ingestion by the consumer(336).

These changes are attributed to microbial growth, survival and/or inactivation and should be taken into account when assessing exposure to a microbial hazard. The number of microorganisms may also change as a result of physical disposal, mixing of food ingredients, splitting of a food product or cross-contamination. Health hazards and spoilage of refrigerated and deep-frozen foods may be due to raw materials, for example pathogenic microorganisms originating from infected live animals or contaminating raw foods during the handling process(336).

Psychotropic organisms are of particular importance as pathogens or spoilage organisms, as they can also multiply during refrigeration due to improper processing. Abuse of temperature and inadequate temperature and humidity ratios can also lead to the growth of psychotropic organisms. The proper handling after refrigeration or storage of frozen foods ("thaw hygiene"), for example the initial contamination of raw products that are ready for consumption without further processing, also deserves special attention(337).

Recontamination after a thermal process is much more important and occurs before, during and after the application of cold. In these cases, again, a distinction has to be made between products that are(337):

2. Are ready for consumption without any process (bakery and confectionery products, ice cream, drinking milk).

3. Have to undergo a process that reduces the presence of bacterial load before the food is consumed (ready meals or other foods ready to reheat at home).

Therefore, the points of greatest hygienic risk in the case of food handling are the following(337):

- Lack of division of "clean" and "dirty" areas and processes.
- Sanitation and hygiene problems of personnel
- Packaging defects
- Leakages during aseptic filling.

However, this is not the only problem, as the interaction of people and their handling of food can also create a substantial problem and create an environment for the growth of parasites and other infectious agents.

1.4.2. Food handling

Handling practices in food preparation areas therefore provide an opportunity for cross-contamination of bacteria with ready-to-eat foods. Unhygienic food handling is a major public health hazard. The effect of food hygiene literacy on hygienic practices identifies specific areas that should be emphasized when developing and delivering effective food safety risk communication messages to consumers(338).

In a study on Salmonella typhimurium, Shigella flexneri and Staphylococcus aureus in street food in developing countries, samples were collected from street food, which is traded within eight to twelve hours. The

authors concluded that street food was highly contaminated with microorganisms and was a potential source of foodborne infections, and suggested that the health risks of street food could be significantly minimized by consuming it within four hours of preparation(339).

Another aspect that has become evident from the literature reviews is that, in most cases, when safety and hygiene practices were not respected, food became a real breeding ground for microorganisms, especially in temperate and tropical climates. In general, some results showed problems in the microbial quality of some foods that are not preheated (milk, fruit juice, vegetables, fruits) and in the case of foods that are not reheated after a long exposure time (dried meat and meat sticks)(340).

The presence of Salmonella and Shigella species in some foods represents a serious hazard to consumers. These aspects have been observed in most street food vendors and probably make street food the source of most diseases caused by bacteria and other micro-organisms(340).

However, parasitic diseases can also be caused by nearby items or animals, and do not necessarily involve the consumption of their meat, as is the case with infections related to companion or domestic animals, which are discussed in the following section.

1.5. Parasitic infection transmitted by domestic animals

In underdeveloped countries, the frequency of parasitic diseases is high, as they are related to socioeconomic levels and poor sanitation conditions, being one of the main causes of diseases in the population(341). However, in

developed countries there has been a significant growth of parasitic diseases with zoonotic potential, due to the close proximity of humans with domestic animals, especially dogs and cats(342).

Zoonotic diseases are diseases transmitted from animals to humans and caused mainly by protozoa and helminths. The main zoonotic agents include Toxocara canis, Ancylostoma sp, Giardia sp, Strongyloides stecoralis among others. They can cause serious health damage such as intestinal obstructions, anaemia, diarrhea and malnutrition(342).

Intestinal parasitosis are important indicators of socio-economic development(343). One of the main routes of infection is the intimate and constant contact of animals with their owners(344), however, they can also be transmitted by water, food, soil and contact with contaminated feces(345).

The barriers and lack of investment in some countries in relation to parasitic diseases is an impediment to public awareness and preventive control actions for human and animal health(345). Studies conducted, in some countries, reported the prevalence of parasitic diseases in dogs and cats in which China showed positivity for 100% of the dogs studied(346), Italy 55%(347), and Brazil with 17%(348), showing that this problem is real and cosmopolitan(345).

The interaction, coexistence, between humans and companion animals has increased in recent years. This interaction is beneficial for both, but it can also be a risk factor for the transmission of zoonotic parasites through the direct contact of playing, kissing, hugging the pet or even walking with it on contaminated soil(345).

A study conducted to verify parasitosis in children under 10 years of age, found the occurrence of Giardia sp in more than one third of the more than 200

children sampled. In another study with a similar sample, the prevalent protozoan was also Giardia sp (with almost 10% occurrence). These data may be indicative of possible zoonotic transmission(349).

The fact that Giardia sp was the most prevalent parasite may be explained by the easy contact with soil, ingestion of food and water contaminated by parasites, as it has been shown that even treated water is at risk of parasitic contamination(349).

For intestinal coccidia in dogs, the presence of an intermediate host is obligatory in the evolutionary cycle of Sarcocystis, Neospora and Hammondia species, facultative in the case of Isospora and absent in the Cryptosporidium cycle (350). The morphological characteristics of some coccidial cysts with the dog as definitive host may not be sufficient for generic identification of these parasites, as is the case for Neospora and Hammondia.

Dogs are definitive hosts for a large number of Sarcocystis species, whose intermediate hosts include domestic and wild mammals as well as birds. Sarcocystis species that have the dog as a definitive host are considered apathogenic to this host(351).

At the same time, Cryptosporidium spp. is an intestinal coccidium that affects several species of domestic animals as well as humans. Thirteen species of mammalian parasitic Cryptosporidium are considered valid, with more than 30 different genotypes identified(352). According to the most recent molecular studies, two genotypes are most commonly found in humans: the human genotype (or type I; recognized as Cryptosporidium hominis) and the bovine genotype (or type II; Cryptosporidium parvum).

However, it appears that the human genotype is limited to humans, but the bovine genotype, in addition to infecting humans, is infectious to other mammalian species(353). Consequently, infected dogs can be a source of infection for humans, especially for immunocompromised individuals(354). In addition, other Cryptosporidium species, including C. canis, have been found in clinical samples from immunocompetent and immunocompromised individuals. According to Thompson (353) the frequency of these infections is lower and affects individuals with concomitant diseases or living in conditions that increase susceptibility to these infections.

Although most gastrointestinal parasites detected in companion animals have a cosmopolitan distribution, they tend to be more prevalent in tropical and subtropical countries (355) and in countries where the socio-economic conditions of the population are precarious (356). However, even in developed countries, parasitic zoonoses in companion animals have been a cause for concern.

Immunodeficiencies in general, and AIDS in particular, as well as the increasingly diverse and widespread inclusion of diverse animals as pets in society, have led to the search for new ways to prevent and detect these infections, such as the development of technologies that have improved diagnostic and research conditions, and have highlighted, especially in the last 20 years, some parasitic diseases that have been nominated as "emerging" or "re-emerging" (357).

In analyzing this situation, Romiti et al. (358) pointed out that it is up to veterinarians in this new millennium to diagnose, to structure a system of research and epidemiological surveillance and to transfer knowledge through health education so that these parasitosis can be properly controlled.

In some developed countries such as Australia (359) reported that veterinary surgeons' involvement with parasitic diseases in small animal clinics is limited to the prescription of broad spectrum anti-parasitic drugs, reflecting their mistaken belief that control of parasitic infections in companion animals can be limited to the use of drugs. In addition to having undesirable consequences for animal health, the unnecessary use of parasiticides is considered to be the main cause of the development of resistance. Likewise, it seems that in the rest of the world, lack of interest in ordering and performing coprological tests and indiscriminate prescription of parasiticides is also the norm.

However, in addition to the concerns already mentioned, it is necessary to realize that the costs of this attitude are far greater for society at large. Perhaps the worst of these is the false sense of security that causes many veterinarians to refrain from learning about the evolutionary cycle of parasites, their epidemiology and prophylaxis and, above all, refrain from their role as public health promoters responsible for educating animal owners about their health (357).

Thus, it is undeniable that parasite infections are frequent in various regions of the world, firstly because of the mobility that people have nowadays, which allows parasites to travel more quickly across borders, but also, pets can be carriers of these parasites. This situation has led to the search for and development of various detection techniques, which is the subject of the next section of the thesis.

1.6. Detection, diagnosis, prevention and new technologies

Continuing with the line of argument proposed for the development of the research, this chapter deals with the detection, diagnosis and prevention of

parasite infections. Therefore, to begin the section, the tools and systems for the detection and diagnosis of infections are presented. Furthermore, an analysis of prevention measures for parasitic diseases is also presented.

On the other hand, and in order to contextualize the research that is contemplated in this thesis, the main innovations regarding the new technologies used for the diagnosis and detection of parasitic infections are established, as well as the role that artificial intelligence plays in the diagnosis of parasitic infections.

1.6.1. Detection and diagnostic tools and systems

At present, despite the availability of immunological and molecular techniques for the diagnosis of some human intestinal parasites, two restrictive aspects for the use of these techniques should be noted. Firstly, the availability of many of these techniques is still limited to research laboratories; secondly, in many cases there is no justification for replacing conventional coproparasitological techniques, which in many situations remain the techniques of choice because of their simplicity, sensitivity and low cost (360). Although the identification of some emerging parasites has only been possible through the use of molecular methods, the abolition of effective conventional methods is not justified (361). Microscopic examination of feces remains the basis for the diagnosis of a large number of parasitic diseases, as it allows the detection of evolutionary forms of helminths and protozoa with 100% specificity.

As there are different methods of processing fecal samples for microscopic examination, it is necessary to observe certain characteristics such as sensitivity, ease of execution and cost (362). In addition to the intrinsic sensitivity of the

method, it is necessary to consider extrinsic factors that may influence the efficacy of a given diagnostic method. The volume of the sample examined, the time of collection, the use of preservative liquids and the conditions under which the sample is taken are all factors that may influence the efficacy of a given diagnostic method (362).

In addition to the intrinsic sensitivity of the method, it is necessary to consider extrinsic factors that may influence the efficacy of a given diagnostic method. The volume of the sample examined, the time of collection, the use of preservative liquids and the conditions under which it is sent to the laboratory may also influence the test results; old or poorly preserved samples are frequent causes of diagnostic errors (363).

The physical characteristics of feces are the first aspect to be observed, as they may suggest the presence of pathogenic organisms. As far as parasites are concerned, in general it can be said that diarrhea feces are dominated by protozoan trophozoites, while formed feces are dominated by cysts; helminth eggs and larvae can be found in feces of any consistency (363).

Fecal samples to be processed by the different coproparasitological methods can be used fresh or preserved in fixing substances. The different kinds of fixative substances available have the function of preserving the morphological characteristics of the parasites, increasing the possibility of their detection and correct identification (364).

Microscopic examination of feces consists of recognizing the typical evolutionary forms of parasites, distinguishing them from the other constituents of fecal matter. Thus, irrespective of the coproparasitological technique used, microscopic examination has a positive predictive value equal to unity (= 1), for example it does not detect false positives. On the other hand, the analytical

sensitivity of the test depends on the technique used to obtain the microscopic preparations (364).

The most commonly used methods for processing fecal samples include dilution of a small amount of feces for direct examination and the use of flotation and sedimentation to concentrate parasite elements (365). Considering the diversity of parasites shedding evolutionary forms in feces, it is easy to understand why none of these methods, when used alone, is sensitive enough to detect all possible parasites infecting a given host. It is therefore important to be aware of the uses and limitations of the available methods.

Direct examination of fresh feces is mainly used to detect motile forms of parasites, such as protozoan trophozoites and helminth larvae. According to Carrasco et al. (366), it is a simple and rapid method whose positive results are as valid as those obtained by concentration methods. The main limitations of the direct examination are the fact that it evaluates a very small amount of feces and the difficulty of examining samples packed with preservatives, as the parasites die and thus lose their motility (367). Although it is possible to detect helminth eggs and/or larvae, cysts or oocysts of protozoa, these are more frequently found when concentration techniques are used. For these reasons, co-proparasitological techniques using concentration procedures have replaced direct examination of fresh feces in routine diagnosis.

Flotation concentration methods (gravity or centrifugation) are based on the difference in specific gravity (SD) between the evolved forms of parasites, fecal debris and the solution used for flotation (367). According to these authors, the most commonly used solutions for flotation are: sodium chloride, sugar (Sheather's solution), sodium nitrate, magnesium sulphate and zinc sulphate.

Since the specific gravity of most evolutionary forms of parasites is between 1.05 and 1.23 (368), the use of solutions with a high specific gravity allows the separation of protozoan cysts, coccidial oocysts and helminth eggs and larvae, so that they can be recovered at the surface while the debris remains at the bottom of the tube. As a result, the preparation examined under the microscope is cleaner than that obtained by sedimentation concentration. Preparations obtained by flotation methods must be examined quickly because the solutions used produce distortions in the parasitic forms, making identification difficult. The most commonly used methods for detecting parasite eggs and cysts in small animal feces are those using flotation as the concentration method (369)

In addition to requiring rapid examination of the preparations, another limitation of flotation concentration methods is their low sensitivity for detecting evolutionary forms of parasites that have an ED higher than that of the saturated solutions used. Eggs of physaloptera sp. and Taenia spp. whose specific densities may not float on these preparations, giving false negative results. In these cases, sedimentation techniques may be more appropriate (369).

Techniques using sedimentation to concentrate the evolutionary forms of parasites are particularly recommended for detecting helminth infections that eliminate heavy (operculate or very dense) eggs, but also allow the recovery of cysts and oocysts of protozoa and helminth larvae (369). Sedimentation can also be carried out naturally (gravitational), by centrifugation or by chemical means (370).

Gravitational sedimentation has been used especially to detect opercula and eggs of trematodes such as Schistosoma spp (370). Advantages of the method include its simplicity and the fact that it does not alter the viability of the eggs. Limitations include the production of a preparation containing a large number of debris (which makes identification of the parasites difficult) and the

long time required for spontaneous sedimentation. Sedimentation by centrifugation only eliminates the latter drawback.

Pre-filtering the samples and adding formaldehyde-ether or ethyl acetate to the stool samples facilitates microscopic examination of the sediment, as it produces a cleaner preparation. The addition of formaldehyde-ether does not distort the organisms and even allows the identification of evolutionary forms of helminths and protozoa. Ethyl acetate has been used to replace formaldehyde-ether, with the advantage of being less flammable and less hazardous (364).

At the same time, it is important to highlight the real-time PCR technology which is an evolution of the PCR (polymerase chain reaction) method. Its principle is based on the duplication of DNA strands "in vitro" which can be repeated several times, generating sufficient DNA for various analyses. Millions of copies can be reproduced from a single DNA fragment (371).

PCR was developed in the late 1980s by Kary Mullis, who later won the Nobel Prize in Chemistry for this feat. In addition to revolutionizing molecular genetic techniques, its application covers a variety of fields such as genetic identification, forensic medicine, clinical analysis, disease diagnosis and industrial quality control, among others (371).

Real-time PCR assays are much more sensitive, specific and rapid, especially compared to conventional tests, which take 2-3 hours to produce a result. Diagnostically, they are widely used in clinical infectiology for pathogen detection, identifying viral and bacterial infections where culture of the causative agents may be very difficult or even impossible. This method does not depend on the isolation or growth of the pathogen or the detection of an immune response against the agent (372).

Instead, it is the nucleic acid sequences of the pathogens that are detected in the assays. Examples of its application include the diagnosis of dengue fever, respiratory infections, STIs, formerly known as STDs, and meningitis. It is also used in the diagnosis of genetic diseases, identifying mutations and genetic predisposition to certain diseases, such as thrombosis. In addition, this molecular technique is also used in oncology to identify the Philadelphia chromosome, a cytogenetic alteration associated with some forms of leukemia (372).

However, to understand what PCR is, we first need to understand the process of the test, which consists of three steps(373):

a) Denaturation: The genomic DNA containing the sequence to be amplified is denatured. In other words, the double stranded DNA is separated.

b) Ringing or hybridization: primers or primers bind to the DNA strand to be amplified. One primer is complementary to the sequence of one strand of the DNA double helix and the other is complementary to the sequence of the other strand. They will identify/mark which stretch of the DNA of interest to copy.

c) Extension or polymerization: with the starting point already identified, Taq polymerase binds to the strand marked by the primer, complementing it. Extension of the new DNA fragment begins, forming a new double-stranded DNA.

This cycle is performed countless times until millions of copies are made. In conventional PCR, the amplification product is usually detected by agarose gel electrophoresis. After staining, the DNA under investigation is visualized (374).

However, the process in real-time PCR differs from traditional PCR in that the result is visualized immediately, without the need for electrophoresis. This is made possible by the addition of fluorescent probes to the PCR reactions. The amplification of the target DNA is monitored during the real-time PCR process. As the DNA is amplified, the level of fluorescence increases proportionally (374).

The equipment is able to detect any fluorescence produced by the sample, so the technique allows the reaction to be monitored and results to be presented in real time. In addition, by controlling the rate of increase of fluorescence in the PCR reaction, it is possible to accurately determine the amount of target DNA present in the original sample. Real-time PCR can be used to assess the presence of a pathogen in a sample and can be a qualitative or quantitative test (375).

That said, the high sensitivity of this technique allows for excellent results, but it is also subject to the presence of inhibitors and contamination that can affect its efficacy. Real-time PCR plasticizers have a major impact on clinical trials and need special treatment in order not to interfere with the reaction, especially when reading the fluorescent signal. This can lead to incorrect results, low concentrations or even make the process unfeasible.

1.6.2. Preventive measures for parasitic diseases

Intestinal parasitosis remains one of the most serious public health problems in several countries. It results from the presence of macro parasites (helminths) and/or microparasites (protozoa) in the intestine and affects about 25% of the world's population heterogeneously. The frequency of parasites is

directly related to socio-economic conditions, with increased prevalence being a direct consequence of the impoverishment of the population(376).

It also depends on climatic aspects, soil characteristics, dietary and hygienic habits and community sanitary conditions. Parasitosis mainly affects pre-school and school children and is correlated with malnutrition and deficits in physical, psychosomatic and social development. The parasite invades through the skin (direct contact with contaminated soil/water) and/or through the mouth (ingestion of contaminated water and/or food, fomites, or sexual practices)(377).

As a consequence, to control these diseases, it is necessary to change the behavior of the population at risk in order to reduce environmental contamination and re-infection of the population(377). General health education and sanitation measures should ensure the implementation of general and individual measures such as:

- Use of adequate sanitary facilities, with proper waste treatment, to actually prevent contamination of surfaces and soils(378).
- Proper water treatment(379).
- Sanitary inspection of meat to reduce the consumption of contaminated meat(378).
- Prevent gardens and orchards from being irrigated with water from rivers and streams receiving sewage or other contaminated water sources(379).
- Preventing pigs, livestock or pets from having access to human feces or fecal contaminated food and water(380).

- Treat the sick and any other family members, if necessary. Prevent them from becoming sources of infection(381).
- Educate the public about intestinal parasites and how they are transmitted(382).
- Wash hands thoroughly before eating or handling food, after defecating or after contact with soil(383).
- Thoroughly wash fruits and vegetables that are to be eaten raw (soak them in a chlorine or sodium hypochlorite solution - one tablespoon per liter of water and leave the food for 20 minutes)(383).
- Protect food from dust, insects or other animals that can serve as a mechanical vector for these parasites(383).
- Wear appropriate footwear(384).
- Keep fingernails trimmed to avoid accumulation of contaminated material(385).
- Avoid scratching the uncovered anal area and putting hands in mouth (82).
- Wash and properly care for underwear, bath towels and bed linen (385).
- Cook pork thoroughly or keep refrigerated at - 20°C for 12 to 24 hours(386).
- Do not enter water suspected of being contaminated (387).

However, the success of these measures requires the cooperation of various sectors of the population and civil society, such as the government, the provision of appropriate urban infrastructure, collaboration of educators, health professionals and the community (382).

It is also important to bear in mind that new and more effective therapeutic resources are regularly made available to the medical profession, justifying a more optimistic expectation of a possible reduction in the number of individuals infected by parasitic diseases(382). However, population growth, especially in areas with fewer cultural and hygienic resources, encourages the emergence of new cases. It is therefore justified to try to implement more and more measures to prevent these diseases, and also to have new technologies that allow faster and more effective detection and diagnosis.

1.6.3. New technologies applied in detection and diagnosis

What the human eye does not perceive, can be improved and even more detailed with the help of electronic tools for this purpose. This relationship, although rather simplified, helps to understand the advances in the systems developed by various researchers, which are based on the inclusion of new technologies for the automatic detection of intestinal parasites in humans(388).

These new advances allow a diagnosis to be made with an average sensitivity of 97%, which is a substantial advance over the methods that have traditionally been used in the health sector (which range from 48% to 76%) (389).

As a consequence, this wide margin of improvement in the efficacy of the instrument makes it even more important, especially considering the prevalence rates or new cases of intestinal parasite infections in recent years (389).

Thus, studies have focused on the search for a diagnostic system that, with the help of computers, would provide a more efficient and safer alternative for testing fecal samples. When using conventional methods and commercial kits, the procedures are performed manually and are subject to a number of situations and biases that can interfere with the final test result, such as misinterpretation. In this case, a technician prepares the slide with the processed fecal sample and then analyses it under the microscope. Normally, the technician makes an initial observation, magnifying the image 100 times, which allows him to identify certain types of parasites with some ease(390).

However, another group, protozoa, which are much smaller, can only be detected if the image is magnified 400 times. Although the protocol states that the examination should be performed at both magnifications, the procedure is not always adopted in practice. Due to the heavy workload in laboratories, it is relatively common for technicians to scan the image at 100x magnification and only magnify it to 400x if they have any doubts about the presence of parasites (391).

Likewise, the sensitivity of conventional diagnostics may also be affected by human error. In addition, it is also possible for the slide to become loaded (or soiled) during preparation with conventional techniques, which can obscure parasites and distort the result, especially in cases of medium and low intensity parasite infection(392).

To overcome these problems and to make fecal parasitological tests more reliable, new diagnostic systems have been developed, which use a computer instead of the human eye to analyze images of fecal samples. Initially, in addition to the computer, the systems consist of a peristaltic pump, an optical microscope, a motorized plate, a focus controller and a camera. However, in some of the new methods, the fecal sample is sucked by the peristaltic pump onto the slide

attached to the microscope. The equipment scans the slide completely, with the image magnified 400 times, and from several focal points (392).

These images, in turn, are captured by the camera and transferred to the computer. A specially developed computer program then identifies the presence of parasites by comparing these images with a database containing information on almost 80% of the pathogenic parasite species present in the countries. However, despite the high effectiveness of these detection methods, they only focus on detecting whether or not parasites are present in a given sample, so the next logical step would be to refine the system so that it also identifies which parasite species has been detected(393).

As a result, in the last decade, several authors have explored ways to automate this process of finding and classifying parasites and their larvae in fecal samples using a microscope. Most of the work carried out to date is based on the application of digital image processing and pattern recognition methods, trying to find specific characteristics of each type of parasite larva in order to classify it correctly (393).

Therefore, several examples can be found in this context, such as the work of Alva et al.(394) who proposed an algorithm using digital image processing techniques and an Artificial Neural Network (ANN). Morphometric features of human parasite larvae in fecal samples were extracted from microscopic images using digital image processing, while an ANN classified parasite species based on these features.

The authors (394) selected four morphometric features based on three morphological characteristics representing shape, shell smoothness and size, while the results obtained showed an average correct classification rate of 86.1% for ANN-1 and 90.3% for ANN-2.

Another case can be found in the study by Avci y Varol (395), who proposed a technique based on the invariant moments of an image, which allows the area of an object to be calculated, even if it has changed size or has been repositioned. Some digital image processing methods, such as noise reduction, contrast enhancement, segmentation and morphological processing, were applied and used in the feature extraction stage of the approach used in this study. The technique presented in this work allows the classification of 16 different parasite larvae or eggs from their microscopic images. The simulations were carried out on a standard computer in the MATLAB environment and the overall success rate was around 95%.

In a similar vein, Kaur et al., (396) also propose a methodology based on invariant moments, combining it with the multiclass support vector machine (MCSVM). The proposed method consists of four stages: a) pre-processing, b) feature extraction, c) classification and d) testing. MATLAB software was used to estimate the classification success rate of the proposed approach. For this purpose, the method was evaluated using data from the test set. At the end of the evaluation, an overall success rate of 97.70% was obtained.

On the other hand, it is possible to identify the study of Osaku et al. (397) who proposed a method capable of segmenting and classifying more than a dozen of the most common species of protozoan cysts, helminth eggs and larvae from microscopy images containing fecal impurities. The developed approach exploits the shape of ellipsoidal objects and the image-forest transform for image segmentation. The results indicate that the implemented method is a promising approach for the complete automation of entoparasite diagnosis.

Likewise, Yang et al. (398) mention that, although there are some methods for automatic identification of parasite eggs, there are still some species that have not reached an acceptable rate of accuracy, so the authors propose a solution

that aims to automate the diagnosis of the most common species of entoparasites in the area, using a sensitive parasitological technique, a motorized microscope with a digital camera for automatic image acquisition and focusing, and image analysis methods. The results indicate that the proposed solution is efficient and suitable for laboratory routines, where the examination can be completed in a few minutes.

In the same framework, Osubor & Chiemeke(399) present a computer-based system that automatically analyses microscopic images and can classify intestinal parasites. The approach is based on segmenting and training a classifier using a combination of a neuro-fuzzy system and an ANN. The parasite is first localized using the circular Hough transform and then extracted for segmentation. The results show a classification with a 100% recognition rate for each of the 20 parasite classes.

Only one year later we can find the study by Tchinda et al.(400), who implemented an automated medical expert system useful for diagnosing human intestinal parasitosis for more than twenty species. The software was developed based on a decision algorithm, and a knowledge base was built with information obtained from books and doctors, with information about each disease caused by a parasite.

In this way, the user interacts with the system by answering a series of questions, while the information obtained is cross-checked with a second method using the circular Hough transform and a trained neuro-fuzzy classifier. The system was evaluated with 60 cases of infection and compared with the diagnosis of two medical specialists, obtaining more than 96% accuracy in this respect (400),

Finally, we can highlight the work of Ghazali et al. (401) who offered a solution to automate the diagnosis of intestinal parasites using images obtained from a microscope directly connected to a computer. The approach exploits contour detection to detect the parasite. In this way, the active contours are combined with the Hough transform to segment and extract the parasite image.

The classification tool is also based on a probabilistic neural network. The developed algorithm was tested on almost 1000 microscopic image samples of almost two dozen different species of intestinal parasites, showing a 100% recognition success rate (1).

However, as might be expected in today's environment, artificial intelligence is also playing a substantial role in various aspects of medicine, with parasitology being a field it is gradually entering, as described in detail in the next section of the thesis.

1.6.4. Artificial intelligence and its role in disease diagnosis

Artificial intelligence (AI) has made significant advances in a number of fields, such as health, finance and manufacturing. One of the lesser-known fields where AI is changing the rules of the game is parasitology, the study of parasites and their interactions with host organisms. Harnessing the power of AI, researchers and scientists are developing robotic systems that can help detect, diagnose and treat parasitic infections (402).

One of the main challenges in parasitology is the identification and classification of parasites. Traditionally, this process has been manual, time-consuming and prone to human error. However, AI image recognition systems are being developed to automate this task. These systems can analyze

microscopic images of parasites and accurately identify them based on their unique morphological characteristics. This not only saves time and resources, but also ensures a higher degree of accuracy in parasite identification (403).

In addition to identification, AI is also being used to predict the risk of parasitic infections in specific geographic regions. By analyzing large datasets of environmental factors such as temperature, humidity and rainfall, AI algorithms can identify patterns and correlations that can help predict the likelihood of parasite outbreaks. This information can be of great value to public health authorities in different countries, who can use it to implement targeted prevention and control measures in high-risk areas (404).

Robotic systems are also being developed to help treat parasitic infections. One example is the use of AI-powered robotic systems for targeted delivery of anti-parasitic drugs. These systems can be programmed to navigate the body of the host organism and deliver drugs directly to the site of infection, minimizing the risk of side effects and improving the overall effectiveness of treatment (405).

Another promising application of AI in robotic parasitology is the development of robotic surgical systems to remove parasites. In some cases, surgical intervention may be necessary to remove large or complex parasitic infections. Robotic systems with AI can help surgeons perform these delicate interventions with greater precision and control, reducing the risk of complications and improving patient outcomes (406).

In addition, AI is playing a crucial role in the development of new diagnostic tools for parasitic infections. For example, researchers are using machine learning algorithms to analyze the genetic sequences of parasites, which can help identify new species and develop more effective treatments. AI is also being used to analyze the host immune response to parasitic infections, which can provide

valuable insights into the mechanisms of parasite-host interactions and contribute to the development of new therapeutic strategies (407).

In conclusion, the integration of AI into robotic parasitology is opening up new avenues for the research, diagnosis and treatment of parasitic infections. By automating parasite identification and classification, predicting the risk of outbreaks, and contributing to the development of new diagnostic tools and treatments, AI is revolutionizing the field of parasitology. As AI technology continues to advance, we are likely to see even more innovative applications in this field, leading to better prevention, control and treatment of parasitic infections worldwide (408).

These findings support the development of the research reported in this PhD thesis, as it aims to provide an AI-based alternative to improve the diagnosis of parasitic infections, facilitating the care, identification and treatment of parasitic infections in the short term.

CHAPTER 2: MATERIALS AND METHODS

2.1. Objectives and hypotheses

2.1.1. General objective

Based on the academic evidence previously reviewed, and considering the object of study of this doctoral thesis, the general objective is to demonstrate the applicability and effectiveness of IA, combined with a traditional method specially designed for this research (questionnaire) in the framework of parasitology diagnosis.

2.1.2. Specific objectives

In line with the general objective, the following specific objectives have been formulated:

2. To design a specific questionnaire to enable joint work with AI to facilitate diagnosis.

3. To analyze the effectiveness of the proposed method

4. To identify the possible correlations in the diagnoses facilitated by the method.

5. To test the reduction in time involved in using the proposed method.

2.1.3. Hypotheses

In a similar context, and following the specific objectives, the following hypotheses have been formulated and will be tested throughout the research:

6. *Hypothesis 1:*

"The design of a specific questionnaire to work with IA, facilitates the diagnosis of parasite infection."

7. *Hypothesis 2:*

"The proposed method (IA + questionnaire) is highly effective for diagnosis"

8. *Hypothesis 3:*

"The proposed method (IA + questionnaire) allows identifying correlations in diagnostics much more quickly and effectively."

9. *Hypothesis 4:*

"A statistically significant time reduction was detected when using the proposed method (IA + questionnaire)."

2.2. First part: Design and validation of the instrument

2.2.1. Design of the instrument

For the design of the questionnaire, the various parasitic infections and the symptoms they present in patients were considered. In this way, and in order to

adjust the symptomatology as much as possible, a review of the academic literature on the subject was carried out, classifying the evidence into the different parasitic infections described in the theoretical section of this research, a detailed list of which is given in appendix 1.

The information compiled during the literature search focused on the most frequent symptomatology in these infections, so that a list of more than 300 symptoms or variables that can affect the development of infections was developed. Subsequently, duplicated variables and symptoms that were far from the subject of study, or that were infrequent in the populations that made up the theoretical evidence, were eliminated and unified.

This resulted in a total of 112 variables and symptoms common to the infections analyzed, which would become part of the instrument used in the pilot test. Likewise, the inclusion of the variable "sex" was considered, as it has an impact on subsequent symptoms. A dichotomous variable (0=No, 1=Yes) was chosen for the responses, in order to facilitate the introduction of the information into the subsequent computer program.

2.2.2. Selection of the expert committee

Once the first version of the instrument was ready, the experts who would form part of the committee were selected and the conventional Delphi method was developed (409,410). As a result, in order to respond to the analysis in question, a sample of potential participants was formed by searching for information on the possible profiles that could fit the study in question, reviewing scientific databases to identify the professionals, and using the contact information published by them.

Therefore, the following eligibility criteria for committee members were defined:

a) They should have more than five years of experience in the hospital setting (professionals who were part of different units and hospitals).

b) Previous participation in other publications or research studies in the field in question (parasitology).

c) They were part of the parasitology units of their institutions or workplaces.

d) (d) Who had the capacity to provide opinions and recommendations

e) That they agree to participate in the study and show motivation for the development of the study.

Once the eligibility criteria had been defined, and the profiles that could fit our study had been chosen, we proceeded to invite 20 professionals from various hospitals and healthcare centers, using telephone and e-mail contact methods, and sending a report on the project to be carried out (objectives, type of participation, data processing, etc.).

However, it is important to mention that the profiles selected were not aware of the rest of the members who would make up the committee of experts (411,412), since at all times they worked with total anonymity and confidentiality of the personal data processed in the study.

It should also be noted that the selection of the committee members was carried out by assessing the suitability of its participants, working with the so-

called Competence Coefficient (K), which is represented by the following formula (413):

$$(K) = 0.5\ (Kc + Ka)$$

In the aforementioned expression, (Kc) corresponds to the Knowledge Coefficient, which is defined as the self-assessment of each of the experts and the level of information and knowledge they possess on the subject of the thesis. At the same time, the value (Ka) is defined as the Argumentation Coefficient, and is translated into the different arguments (or sources) that serve to guarantee the knowledge of the experts (413,414), as shown in the following table to weight the value of Ka of each expert:

Table 1. *Scoring of the sources of argumentation to obtain the "coefficient of argumentation" (Ka)*

Source of argumentation	**High**	**Medium**	**Low**
Theoretical studies you have carried out on parasitology and parasitic infections.	0.3	0.2	0.1
Assessment of your professional experience	0.5	0.4	0.2
Participation in the analysis of research studies in relation to parasitology and parasitic infections by national authors.	0.05	0.05	0.05
Participation in the analysis of research studies related to parasitology and parasitic infections by international authors.	0.05	0.05	0.05
Own knowledge of parasitology and parasitic infections	0.05	0.05	0.05

Own intuition on parasitology and parasitic infections	0.05	0.05	0.05

Note. Own elaboration, based on Cabero & Barroso (413)

After assessing the Competence Coefficient (K) of the 30 experts, 10 experts with the highest coefficients were finally selected, as described in the following table:

Table 2. *Competence Quotient (K) Expert Panel Result*

Expert	**Knowledge or information coefficient (Kc)= nc* (0,1)**	**Argumentation coefficient (Ka)=fa*1+fa2+fa3+fa4+fa5+fa6**	**Competence coefficients (K) = 0.5 (Kc + Ka))**	**Expert Committee**
Expert 1	**0,1**	**0,8**	**0,45**	**Excluded - Low K**
Expert 2	0,9	0,9	0,9	Included - High K
Expert 3	0,8	0,7	0,75	Included - Medium K
Expert 4	**0,3**	**0,6**	**0,45**	**Excluded - Low K**
Expert 5	0,6	0,7	0,65	Included - Medium K
Expert 6	**0,1**	**0,85**	**0,475**	**Excluded - Low K**

Expert 7	**0,1**	**0,8**	**0,45**	**Excluded - Low K***
Expert 8	0,8	0,9	0,85	Included - High K
Expert 9	**0,4**	**0,5**	**0,45**	**Excluded - Low K**
Expert 10	**0,4**	**0,5**	**0,45**	**Excluded - Low K**
Expert 11	0,8	0,7	0,75	Included - Medium K
Expert 12	0,9	0,9	0,9	Included - High K
Expert 13	0,6	0,5	0,55	Included - Medium K
Expert 14	**0,2**	**0,5**	**0,35**	**Excluded - Low K**
Expert 15	0,7	0,8	0,75	Included - Medium K
Expert 16	**0,3**	**0,5**	**0,4**	**Excluded - Low K**
Expert 17	**0,3**	**0,6**	**0,45**	**Excluded - Low K**
Expert 18	0,8	0,9	0,85	Included - High K
Expert 19	0,9	1	0,95	Included - High K
Expert 20	**0,4**	**0,5**	**0,45**	**Excluded - Low K**

nc*= experts' self-assessment of their level of knowledge on a scale of 1 to 10
fa* = source of argumentation for items 1 to 6
High K = 0.8 < K < 1.0
Medium K* = 0.5 < K < 0.8
Low K*= 0.0< K < 0.49

Note. Own elaboration

2.2.3. Procedure

Once the calculations of the Competence Coefficient (K) value had been made, 10 experts participated and became part of the expert committee. Each expert was given a table with 112 variables (appendix 2) on which they had to give their judgement, first on the items independently, and then on the instrument in general. Likewise, and in line with other studies carried out in this field, it has been considered that the number of experts for the development of the instrument allows content validity to be supported (415,416).

The assessment by the experts was carried out in two rounds, the first being dedicated to the understanding of each item (with the agreement of at least two thirds of the experts). The second round focused on the assessment of the Relevance Index (RI) and the Pertinence Index (PI) of each of the variables and symptoms of the scale, considering the values of 0= not relevant/not pertinent and 1= pertinent / relevant, and applying the following formula in this regard:

$$\frac{No.of\ experts\ scoring\ 1}{total\ number\ of\ experts\ who\ assessed\ the\ item}$$

Finally, the Content Validity Index (CVI) was calculated for all items as a whole, which is calculated by averaging the IR/IP of all items together. Therefore, content validity was considered as accepted if it had a value greater than 0.8 for each of the items, as was considered for the scale as a whole (415–417).

At the same time, during this phase of the study, the various qualitative observations of the experts were collected in order to improve the instrument designed prior to the pilot test.

2.2.4. Pilot Test and data analysis

In conjunction with the development of the scale, a preliminary pilot study was undertaken, engaging 20 participants, aimed at evaluating the test-retest reliability of the instrument. This investigative phase involved the application of a range of evaluation metrics, including the Kappa index (418), the Intraclass Correlation Coefficient (ICC) (419), and the general concordance index. Each metric was tailored to suit the specific nature and attributes of the variable under scrutiny.

To ensure the robustness and reliability of the findings, a deliberate time frame of 15 days was instituted between the initial administration of the test and its subsequent retesting. This strategic approach facilitated the observation of potential fluctuations or variations in participants' responses over time, enabling a comprehensive and nuanced assessment of the scale's stability and consistency across diverse contexts and conditions.

Therefore, this preliminary pilot study serves as a cornerstone in the validation process of the scale, offering invaluable insights into its reliability and efficacy in consistently measuring the constructs or phenomena of interest within

the designated target population. Such insights are crucial for informing future research endeavors and guiding the development of effective assessment tools and methodologies.

Appendix 3 provides an intricate breakdown of the item composition following the exhaustive content validity analysis detailed in the preceding section. Through this rigorous process, we meticulously scrutinized each item to ensure its alignment with the intended constructs and objectives of the questionnaire. The results confirm that all items have surpassed the threshold of an RI value exceeding 0.8, denoting an exceptional level of appropriateness and relevance in the context of the study. Furthermore, the PI scores for all items have consistently exceeded 0.80, indicating a high level of agreement among experts regarding the relevance and significance of the questionnaire items.

This substantial and reassuring finding has led us to conclude that no symptoms warrant removal from the original pool of 112 items included in the questionnaire. This decision underscores our confidence in the comprehensive coverage and representativeness of the instrument as a whole. Consequently, we are poised to advance with the implementation of the pilot test, fortified by our unwavering belief in the quality and relevance of the questionnaire items. This pivotal step marks a significant milestone in the validation process, heralding the instrument's potential utility in future studies and research endeavors.

Moreover, the calculated CVI for the entire scale further underscores its robustness, with a value of RI = 0.865 and PI = 0.935, affirming its credibility and effectiveness in capturing the intended constructs and phenomena of interest. This comprehensive validation process sets a solid foundation for the instrument's deployment and underscores its potential value in informing clinical practice and research initiatives in the future.

2.3. Second part: Application of the instrument using artificial intelligence (SQL)

In this subsequent phase of the study, our focus shifts towards presenting a comprehensive overview of the methodology employed, which involves integrating the previously validated questionnaire with the utilization of the artificial intelligence program SQL. The section encompasses an in-depth discussion of the selected study type, the methodology for sample calculation design, the establishment of specific eligibility criteria, the comprehensive collection of variables, the ethical principles upheld, the procedural framework implemented, and the robust data analysis techniques employed to conduct the study.

This detailed exposition of the methodology serves as a foundational framework for comprehending the execution of the study, offering insights into the intricacies of the research process. By providing a thorough examination of each aspect of the methodology, this section facilitates a nuanced understanding of the study's design and implementation. Moreover, it lays the groundwork for a critical evaluation and interpretation of the results obtained, enabling stakeholders to derive meaningful insights and draw informed conclusions from the study findings.

2.3.1. Type of study

A descriptive cross-sectional observational study was undertaken, a research design delineated by Cvetkovic-Vega et al. (420) as follows:

> “Traditionally, cross-sectional studies have been considered useful for determining the prevalence of a condition, hence they are also known as “prevalence studies”. However, they can also evaluate the association between two or more variables, meaning having an analytical approach. This is an attractive alternative for exploring associations in advance or in scenarios with limited resources.” (p. 180)

The study focuses on the application of a previously validated questionnaire, whose answers were adapted to a technological environment by translating them into SQL language, facilitating their processing and analysis with the artificial intelligence software Program Xojo which was used, along with a MySQL database. The AI was processed using Apple's CreateML application, as well as proprietary algorithms developed in Python, utilizing libraries such as TensorFlow. This technological integration allows for a more efficient and accurate evaluation of the data collected, thus optimizing the research process.

The research was carried out during the following period: 05/2018-09/2024. During this period, direct contact was made with participants who attended medical consultations and presented with symptoms associated with parasite infection. This direct and selective approach to participant identification ensures the relevance and representativeness of the sample, as well as the quality of the data collected for a comprehensive analysis.

Through this meticulous process of data collection, we have sought to gain a deeper understanding of the factors related to parasite infection and patient perceptions in the clinical setting. This knowledge will be fundamental to the design of more effective prevention, diagnosis and treatment strategies in the future.

2.3.2. Sample size calculation

To calculate the sample, we followed the guidelines proposed by Cvetkovic-Vega et al. (420), who mention the use of the following formula for sample calculation when the size of the total population is unknown (because we do not know the number of patients who may have a parasitic infection):

$$n = \frac{Z^2_{\propto} X\ p\ x\ Q}{d^2}$$

N: Sample size

P: prevalence of the study event

Q: 1-p

Zα: When α: 0.05, value in the Gaussian distribution equals 1.96

D: error tolerance (If 95%, its value is 5%)

As a consequence, by applying this formula, the sample calculation carried out has defined the sample to be made up of 2503 patients, determined by the eligibility criteria described in the following section.

2.3.3. Eligibility criteria

In relation to eligibility criteria, the following criteria have been outlined for the selection of study participants:

- Participants have been required to be of legal age, which ensured that they had the legal capacity to give informed consent and participate autonomously in the study.

- We looked for people who presented for medical consultation due to the manifestation of symptoms related to parasitic infections. This criterion ensured that participants were directly affected by the condition of interest in the study.

- It was essential that participants voluntarily agreed to take part in the study, which implied a full understanding of the objectives, procedures and possible risks involved.

- In the case of patients who were minors, the explicit consent of their legal guardians was required for their participation in the research. This requirement ensured the protection and well-being of minors, as well as compliance with ethical and legal regulations related to research involving pediatric populations.

These criteria have been carefully established to ensure the selection of appropriate and ethically responsible participants, as well as to safeguard the integrity and safety of all those involved in the study.

2.3.4. Study variables

Taking into consideration the questionnaire carried out and the various symptoms and habits collected in the questionnaire, it is possible to identify 8 groups of main variables:

a) *Demographic Variables (Gender -Male/Female-, Age):* As a consequence, it is possible to state that the demographic variables considered in the study include gender, which is defined as male or female, and age, which will be used to categorize participants into specific groups according to their age range.

b) *Physical Symptoms (Complexion -Pale or sallow-, Presence of circles and bags under the eyes, Skin disorders (Psoriasis-type, Boils, Seborrhoeic dermatitis, etc., Itching and dermatitis symptoms, Cracked heels, flaking nails, brittle nails):* In terms of physical symptoms, aspects such as complexion, which may be pale or yellowish, and the presence of dark circles and bags under the eyes, which could indicate certain health problems, will be assessed. In addition, skin disorders such as psoriasis, boils and seborrheic dermatitis will be analyzed, along with symptoms such as itching and general dermatological problems such as peeling skin and brittle nails.

c) *Digestive and Intestinal Symptoms (Intestinal noise, Constipation, Diarrhoea, Sweet cravings, sugar cravings, Anxiety related to certain foods, Regular headaches, Anal and genital itching, Grinding teeth, Sleep disturbances and nightmares):* Digestive and intestinal symptoms focus on the functioning of the gastrointestinal system and include aspects such as bowel sounds, constipation, diarrhea, sugar cravings and cravings related to certain foods such as flour and sweet desserts. Regular headaches, anal and genital itching and teeth grinding are also considered.

d) *Respiratory and Allergy Symptoms (Dyspnoea, chronic cough, Chronic cold, Recurrent urinary tract infections, Recurrent mycosis*

of the genitourinary system, Rhinorrhoea, Throat clearing and expectoration): In terms of respiratory symptoms and allergies, problems such as dyspnea, chronic cough, recurrent colds, urinary tract infections, recurrent genitourinary mycoses, rhinorrhea and throat clearing and expectoration are examined.

e) *Behavioral and Psychiatric Symptoms (Hyperactivity, Drowsiness/apathy, Anxiety disorders, Depression, Eating disorders (Anorexia, Bulimia), Attention deficit disorders (ADD, ADHD), Autism spectrum disorders (Autism, Asperger Syndrome), Psychomotor developmental delay):* Behavioural and psychiatric symptoms include a variety of emotional and psychological disorders, such as hyperactivity, sleepiness, anxiety disorders, depression, eating disorders, ADHD, autism and delayed psychomotor development.

f) Medical History and Diagnostic Groups (Family medical history, Diagnosed groups (Allergological, Hematological, Gastroenterological, etc.): In relation to medical history and diagnostic groups, family medical history and diagnostic groups to which participants belong, such as allergies, hematological disorders, gastrointestinal disorders, among others, are considered.

g) *Environmental and Lifestyle Factors (Pet ownership, Lunar cycle correlation with symptoms, Consumption of certain meats (grilled, venison), Travel history, Residential environment (wetlands, floodplains), Personal habits (finger sucking, nail biting), Food consumption habits (seafood, unwashed fruits/vegetables):* Environmental and lifestyle factors include pet ownership,

correlation of symptoms with moon phases, consumption of certain meats, travel history and residential environment, as well as personal and food consumption habits.

h) *Hygiene and Social Habits (Use of public facilities (swimming pools, hot tubs, rehabilitation centers), Hygiene practices (washing fruits/vegetables, water consumption, hand hygiene):* Finally, hygiene and social habits are assessed, including use of public facilities, personal hygiene practices and food consumption habits. These variables provide a comprehensive picture of factors that could influence the health and symptoms of study participants.

Thus, the above variables have allowed several important relationships with parasitic infections to be established:

- Prevalence of Characteristic Physical Symptoms: parasitic infections are often associated with a number of specific physical symptoms, such as anal or genital itching, skin disorders, gastrointestinal disorders (diarrhea, constipation) and other symptoms that may be present in participants. By collecting detailed information on these symptoms, we can identify patterns suggestive of parasitic infections.

- Exposure to Risk Factors: Variables related to exposure to risk factors, such as consumption of contaminated food, presence of pets in the household, hygiene habits and travel history, can help identify possible sources of parasitic infections. For example, consumption of raw or undercooked food, contact with infected animals or exposure to contaminated environments may increase the risk of infection.

- Assessment of Associated Psychological and Behavioural Disorders: Some psychological and behavioural disorders, such as anxiety,

depression and attention deficit disorders, may influence hygiene habits and behaviours that affect susceptibility to parasitic infections. For example, anxiety about contamination or inattention to hygiene practices may increase the risk of infection.

- Medical History and Related Diagnostic Groups: Medical history and related diagnostic groups may provide information on pre-existing medical conditions that may increase susceptibility to parasitic infections or affect the severity of symptoms. For example, conditions that affect the immune system may predispose people to more severe parasitic infections.

These variables have allowed us to explore the relationship between a wide range of factors and the presence, severity and susceptibility to parasitic infections, which in turn can inform more effective prevention, diagnosis and treatment strategies.

2.3.5. Ethical aspects of the research

In terms of ethical aspects, the stipulations of the applicable regulations (GDPR) have been considered, establishing as a requirement for participation, the signing of the informed consent form, which is included in annex 4. Thus, the informed consent process described in the document provided involves several key ethical considerations:

The participant has the right to be fully informed about the nature, purpose and procedures of the study. This includes understanding the research topic, its objectives, and the methods involved in data collection and analysis. Providing full information ensures transparency and allows individuals to make informed decisions about their participation.

The participant acknowledges that their participation in the study is voluntary. This means that they are free to choose whether or not to participate without any coercion or pressure from researchers or other parties. Ensuring voluntary participation upholds the principle of respect for individual autonomy and personal choice.

Participants are also informed of their right to withdraw from the study at any time without facing negative consequences. This includes the freedom to withdraw without having to give a reason or explanation for their decision. Respecting participants' autonomy to withdraw reinforces the principle of beneficence and non-maleficence by prioritizing their welfare and interests.

It also provides that participants are assured that their personal data will be kept confidential and protected in accordance with applicable laws and regulations. This includes safeguarding confidential information and ensuring that data is not shared with third parties or institutions without explicit consent. Protecting privacy and confidentiality is essential to maintain trust and uphold the principle of respect for the privacy rights of individuals. In this way, each participant has been assigned an alphanumeric identification code, thus avoiding the handling of sensitive personal information.

By obtaining informed consent from participants, researchers demonstrate their commitment to conducting ethical research and respecting the rights and welfare of participants. It establishes a foundation of trust and transparency between researchers and participants, fostering an ethical and collaborative research environment.

In addition, consideration has been given to the need for parents of minor participants to also complete and sign an informed consent form for participation, which is described in Annex 5.

2.3.6. Procedure

The process of conducting this qualitative study commenced with an extensive examination of the existing body of knowledge pertaining to the detection of parasitic infections in humans. This involved not only delving into previously utilized methodologies but also identifying areas where advancements, particularly through the integration of artificial intelligence (AI), could be made to address unmet needs and fill existing gaps in research. This thorough exploration served as the cornerstone upon which the study's conceptual framework was built, providing a robust foundation for the delineation of research objectives aimed at tackling the challenges inherent in detecting these infections.

Moreover, a meticulous scrutiny of the pertinent scientific literature was undertaken to glean insights into methodological approaches previously adopted and to distill best practices prevalent in the field of study. This exhaustive review not only furnished us with a comprehensive understanding of past research endeavors but also enabled us to situate our study within the broader scientific discourse. By synthesizing and contextualizing previous advancements and discoveries, we were able to leverage existing knowledge and build upon it, thereby enhancing the relevance and significance of our study within the contemporary scientific landscape.

Upon recognizing the imperative need for conducting the study, a meticulously detailed project report was meticulously drafted. This comprehensive document served as the blueprint for the study, encompassing a thorough delineation of the study's specific objectives, the intricacies of the proposed methodology, the planned utilization of software and AI applications,

and the meticulous protocols devised for the ethical handling of patients' personal data.

Every aspect of the study, from its overarching goals to the minutiae of data collection and analysis, was meticulously outlined in the project report. Special attention was paid to ensuring clarity and precision in articulating the study's aims and methodologies, laying the groundwork for a robust and rigorously executed research endeavor.

Following the exhaustive compilation of the project report, it was diligently submitted to the management of the pertinent healthcare center. This submission marked the commencement of the rigorous process of review and approval by the ethics committee. The involvement of the ethics committee was paramount, as it served as a safeguard to ensure that the study adhered to the highest ethical standards and respected the rights and privacy of the study participants.

The ethical review process, as described by Alfonso et al.(421)*(Own translation)* was integral to the study's ethical conduct:

> “Any research conducted on human subjects must be ethically justified, which implies that the potential benefit to be obtained, for the participating subjects or for society as a whole, outweighs the discomfort and/or risks arising from participation. At the same time, it must respect universally recognized ethical principles, in particular the principles of autonomy, beneficence, non-maleficence and justice, which research ethics committees must ensure are complied with” (p. 352)

It entailed a thorough examination of the project report to assess the proposed methodologies and ensure compliance with established ethical and legal norms in the field of medical research. By subjecting the study to rigorous

ethical scrutiny, the aim was to mitigate potential risks to participants and uphold the principles of beneficence, non-maleficence, autonomy, and justice.

Ultimately, the meticulous preparation and ethical review of the project report were essential steps in laying the foundation for a study that not only advanced scientific knowledge but also prioritized the welfare and rights of its participants. Through adherence to rigorous ethical guidelines and meticulous attention to detail, the study aimed to uphold the highest standards of integrity and ethical conduct in medical research.

Following the successful acquisition of ethical approval, a pivotal meeting convened with the professionals comprising the unit. The primary objective of this gathering was to apprise them of the forthcoming program's development and furnish them with comprehensive insights into the study's intricacies and the prescribed protocol to be adhered to. During this collaborative session, an array of effective communication strategies was deployed, including the dissemination of informative posters, strategically aimed at heightening awareness regarding the study among both the medical personnel and potential participants.

This multifaceted approach served to ensure that all stakeholders were thoroughly acquainted with the study's objectives, methodologies, and anticipated outcomes. By fostering an environment of transparency and clarity, it facilitated informed decision-making and voluntary engagement from all parties involved.

In alignment with this overarching objective, a meticulously crafted protocol governing participant selection was established. This protocol was meticulously formulated based on discernible criteria derived directly from the study's objectives and the desired demographic profile of the participants. Its primary aim was to guarantee the meticulous selection of a sample cohort that

accurately represented the broader target population. By meticulously adhering to this protocol, the study aimed to ensure the generalizability and applicability of its findings to a wider demographic context, thereby enhancing the robustness and reliability of the research outcomes.

Each eligible individual was extended a voluntary invitation to participate, concomitant with their traditional diagnosis, after receiving a comprehensive overview of the study's objectives. Informed consent was diligently obtained from each participant prior to their inclusion in the study, ensuring that they were fully apprised of the study's aims, methodologies, and any potential risks associated with participation. A similar process was employed for parents, relatives, or legal guardians of minors eligible for inclusion in the study, thereby guaranteeing that all relevant stakeholders were adequately informed and consented to participation.

This meticulous approach fostered an environment conducive to informed and autonomous decision-making among participants, empowering them to actively engage in the study based on a thorough understanding of its objectives and procedures. By prioritizing transparency and clarity in the recruitment process, the study endeavored to uphold the integrity and ethical principles of research conduct.

Furthermore, the implementation of a clear and transparent protocol for participant selection bolstered the validity and representativeness of the data collected. By adhering to predetermined criteria for participant inclusion, the study aimed to mitigate potential biases and ensure the reliability of its findings. This methodical approach contributed to the overall robustness and credibility of the study results, enhancing its potential impact and relevance in the field of research.

Once the participants expressed their willingness to partake in the study, the progression ensued with the requisite diagnostic procedures conducted in strict adherence to the established protocol. This encompassed not only the administration of the study-specific questionnaire but also the provision of comprehensive support and guidance by the research professional throughout the healthcare consultation. This personalized approach ensured that participants comprehended the study's objectives fully and were adept at accurately completing the questionnaire, thereby enhancing the quality and reliability of the data collected.

Subsequently, the data acquired through the questionnaire underwent meticulous analysis employing advanced artificial intelligence techniques. This analytical phase facilitated the discernment of latent patterns and trends inherent in the data, thereby affording a deeper insight into the myriad factors that could potentially influence the study outcomes. Moreover, leveraging artificial intelligence enabled a comprehensive exploration of the amassed information, thereby yielding a more exhaustive and intricate understanding of pertinent aspects germane to the study of parasitic infections in humans.

In order to validate the results obtained, the processed data underwent further analysis using statistical software such as SPSS. This pivotal step was indispensable for conducting a comparative analysis between the outcomes derived from the questionnaire and those obtained through traditional diagnostic tests, a detailed exposition of which is provided in the subsequent section. By undertaking this comparative evaluation, our aim was twofold: firstly, to corroborate the accuracy and reliability of the data gleaned from the questionnaire, and secondly, to discern any potential disparities or avenues for enhancement in the realm of parasitic infection detection.

This holistic approach to data collection, analysis, and validation underscored the thoroughness and credibility of the study's findings. Furthermore, it engendered a profound and nuanced comprehension of parasitic infection detection in humans, thereby furnishing invaluable insights that could significantly inform the development of more efficacious prevention, diagnostic, and treatment modalities in the realm of public health.

The culmination of the comprehensive research endeavor was marked by a meticulous interpretation of the results obtained, a process that entailed delving deep into the data to discern intricate patterns and trends. This in-depth analysis facilitated a thorough evaluation of the similarities and differences among the various diagnostic methods employed throughout the study. By scrutinizing the effectiveness and applicability of integrating the questionnaire with artificial intelligence processing for the identification of parasitic infections, the study aimed to shed light on novel insights and potential avenues for improvement in diagnostic approaches.

Moreover, the interpretative phase served as a catalyst for critical reflection, as it prompted the identification of possible areas for refinement or optimization in the diagnostic protocols utilized. This reflective exercise was instrumental in fostering a culture of continuous improvement, ensuring that the study's outcomes contributed meaningfully to the advancement of diagnostic practices in the field of parasitic infection detection.

In addition to the internal examination of the study's findings, a concerted effort was made to disseminate the results widely within the medical community. This dissemination strategy encompassed the publication of research findings in reputable scientific journals renowned for their rigorous peer-review process and adherence to academic standards. Furthermore, the study's outcomes were

showcased through presentations at prominent academic conferences, providing an opportunity for scholarly exchange and collaboration among experts in the field.

By actively sharing the findings through multiple channels, the study aimed to catalyze knowledge dissemination and uptake among healthcare professionals, researchers, and policymakers. This proactive approach to dissemination not only ensured broad access to the study's insights but also facilitated their translation into evidence-based practices and informed decision-making in the realm of public health. Ultimately, the concerted efforts to disseminate the study's findings would contribute significantly to advancing the frontiers of knowledge in parasitic infection detection, ultimately benefiting global health outcomes.

The impact of this dissemination would not only be limited to academia, but would also have practical implications for improving health care and prevention of parasitic diseases. The study's findings could be used to inform the design and implementation of more effective strategies for early diagnosis, timely treatment and effective prevention of parasitic infections in humans, ultimately benefiting affected communities and contributing to the promotion of public health in general.

2.3.7. Data analysis

Upon compiling the questionnaire responses from each participant, they were meticulously transcribed into the database of an AI program, utilizing a specialized software known as MySQL database and the Random Forest algorithm.

MySQL is one of the most widely used relational database systems (RDBMS) in the world, known for its reliability and flexibility. Originally developed by Michael Widenius, MySQL combines the ease of SQL (Structured Query Language) with efficient performance. It has established itself as a fundamental tool for many web and business applications due to its ability to adapt to different environments and requirements (422).

One of the great advantages of MySQL is its ability to operate on a variety of operating systems, such as Windows, Linux, macOS and other UNIX systems. This feature allows the system to be integrated into a variety of technological infrastructures, providing flexibility for both development and production. In addition, as open-source software under the GPL license, it can be used freely in many projects, although it also has commercial versions supported by Oracle (422).

MySQL is commonly integrated into Linux environments, being part of the well-known LAMP (Linux, Apache, MySQL, PHP/Python/Perl) stack, which offers a robust solution for web applications. Also, its use on Windows servers allows it to cover a broader spectrum of business needs. In addition, the MySQL engine is based on SQL, a widely used language for managing relational databases. SQL allows a variety of operations such as inserting, modifying, deleting and querying data to be performed easily and efficiently. This approach allows developers to focus on the needs of the application without worrying about the internal processes of data manipulation, thus optimizing development time. In addition, MySQL offers robust tools for the creation and management of database schemas, allowing for flexible configuration tailored to the needs of each project. This includes the ability to create tables, define indexes and manage advanced structures such as stored procedures and triggers (423).

An essential aspect of MySQL is its ability to support multiple concurrent users without compromising performance or data integrity. Through the use of transactions and locking systems at the table and row level, MySQL ensures that concurrent operations are performed in a consistent and error-free manner. The ACID (Atomicity, Consistency, Isolation, Isolation and Durability) system it implements ensures that all transactions are processed securely and reliably (423).

MySQL also offers an advanced permissions management system, which allows different levels of access to be assigned to users, ensuring that only authorized users can modify or view data as required (423).

As a result, MySQL is a scalable system that can grow with the needs of the application. Both vertically scalable (increasing resources on a single server) and horizontally scalable (distributing data across multiple servers), MySQL is able to handle increasing volumes of information without the need to modify the underlying infrastructure. This is particularly useful in projects that start with small databases and, over time, experience a considerable increase in traffic or in the amount of data processed (422).

The replication system offered by MySQL allows copies of the database to be created on different servers, ensuring data availability and increased resilience to failures. One of the key reasons why MySQL is so popular is its efficiency in processing queries and handling transactions. The default InnoDB storage engine is optimized to handle large amounts of data without significantly impacting performance. In addition, the query optimizer analyses each SQL statement and decides the most efficient way to execute it, thus improving overall system performance. Transaction support ensures that operations are performed

consistently, even in complex situations, and that failures do not affect data integrity (422).

MySQL is therefore a fundamental pillar in the development of dynamic web applications, due to its ability to handle large amounts of data and concurrent users. Its use in platforms such as social networks, e-commerce systems and online services has established it as an indispensable tool in the web development ecosystem. Integration with popular programming languages such as PHP, Python and Java, along with administration tools such as MySQL Workbench, greatly facilitates its management and maintenance (422).

Also, in the development of the parasitic prediction application, MySQL has integrated the SMOTE (Synthetic Minority Over-sampling Technique) technique to correct the class imbalance present in the datasets. This technique, widely used in machine learning, is essential when one class is significantly under-represented compared to the others, which can lead to prediction models being biased towards the dominant class, ignoring examples of the minority class. The use of SMOTE allows balancing the data without simply duplicating instances, as in other oversampling methods, but by generating new synthetic examples of the minority class (424).

Class imbalance is a common problem in many datasets, especially in classification datasets. For example, in the medical field, when seeking to diagnose a rare disease, it is possible that only a small percentage of the cases are positive (the patients with the disease), while the majority are negative (the healthy patients). Without proper tuning, learning algorithms tend to misclassify the minority class of examples, which biases the model and reduces its ability to correctly predict rare cases. This imbalance can lead to major problems, such as

biased classifiers and misleading evaluations, where overall accuracy appears high, but performance in the minority class is poor (425).

SMOTE solves this problem by generating new synthetic examples by interpolating between nearest neighbors in the minority class. This process involves several steps: first, the minority class is identified and an instance of that class is selected; then, the k nearest neighbors of that instance is searched and a new synthetic example is generated by interpolating between the original instance and one of its nearest neighbors. This process is repeated until the dataset is balanced. The main advantage of SMOTE is that it allows keeping all the information of the majority class without reducing it, while introducing more variety in the minority class, which improves the performance of classification models in metrics such as recall and F-score (425).

Despite its advantages, SMOTE is not without its limitations. One of the main risks is the generation of noise in the data, especially if the minority class examples contain outliers, which can lead to the creation of unrepresentative synthetic instances. In addition, SMOTE may not generate realistic examples in certain cases, especially when the data has complex structures or non-linear relationships. It is also important to note that SMOTE does not address internal imbalance within subclasses of the minority class and that its use is most effective on continuous numerical variables, which may limit its application on datasets with categorical variables (425).

To overcome some of these limitations, variants of SMOTE have been developed, such as Borderline-SMOTE, which generates synthetic examples at decision boundaries between classes, or ADASYN, which adapts the process according to the classification difficulty of each instance. Other variants, such as SMOTE-NC, are specifically designed to handle mixed datasets with both

numerical and categorical features. In addition, SMOTEENN and SMOTETomek combine SMOTE with data cleaning methods, removing noisy or problematic examples after the generation of synthetic examples (426).

The Random Forest algorithm is a supervised learning technique used for both classification and regression, based on the idea of building multiple decision trees and combining their results. This strategy not only improves the accuracy of the model, but also reduces the risk of overfitting that can occur when using a single tree (427).

At its core, Random Forest is based on two key concepts: decision trees and the technique of Bagging (Bootstrap Aggregating). A decision tree is a model that classifies or predicts an outcome by dividing the dataset into smaller subsets, using specific criteria such as information gain. Bagging, on the other hand, involves generating multiple versions of a model (in this case, decision trees) trained on different subsets of data obtained by sampling with replacement. In this way, the variance is reduced and the robustness of the model is increased (428).

The process of constructing a Random Forest begins by selecting several subsets of the original data set by random sampling with replacement. These subsets have the same size as the original set, although some observations may be repeated, while others may be excluded. On average, each subset will include approximately 63% of the original set. Decision trees are then constructed for each subset, but instead of using all available features at each node, a subset of features is randomly selected. For classification problems, the square root of the total number of features is usually chosen, while for regression, one third of the features are considered. Each tree grows until it meets a stopping criterion, such

as reaching a maximum depth or having a minimum number of samples per node (427).

When the trees are constructed, Random Forest makes predictions by combining the results of all of them. In the case of classification, each tree votes for a class, and the class with the most votes becomes the final prediction. For regression, instead of voting, the numerical predictions of the trees are averaged to obtain the final value (427).

The use of Random Forest has several advantages. First, it is very effective in reducing overfitting, as combining multiple trees trained on different subsets decreases the likelihood that the model will overfit the training set. Furthermore, it is a robust model against noise and irrelevant features, thanks to randomization in the selection of features and subsets of data. Finally, Random Forest offers stability, as by averaging or voting across multiple trees, individual decisions are smoothed, resulting in a more consistent model (429).

However, it also has some disadvantages. Its main drawback is computational complexity, as building multiple trees requires more time and resources than training a single decision tree. In addition, while individual decision trees are easily interpretable, the set of trees in a Random Forest can be difficult to interpret as a whole (429).

Random Forest performance can be tuned by several key hyperparameters. The number of trees (n_estimators) influences performance: a larger number of trees usually improves accuracy, but also increases computational cost. The number of features considered in each split (max_features) affects the balance between bias and variance. The maximum depth of the trees (max_depth) is another way to control overfitting, as is the

minimum number of samples required to split a node (min_samples_split) or to be in a leaf (min_samples_leaf), which limit the complexity of the tree (429).

Random Forest is widely used in various applications. In classification, it is useful for pattern recognition, medical diagnosis and spam filtering, while in regression, it is used to predict house prices or financial risk estimates. In summary, Random Forest is a powerful and versatile technique that improves the accuracy and robustness of predictions by combining multiple decision trees, making it a valuable tool for both classification and regression tasks (429).

To streamline the analysis process, the responses were encoded into a binary system (0-1), facilitating a concise and efficient representation of the data for subsequent interpretation.

This transformative process enabled the AI program to autonomously generate individualized diagnoses for each patient, which were meticulously documented on personalized tracking sheets for every participant enrolled in the study. These meticulously maintained records served as a comprehensive repository, providing a detailed chronicle of each participant's journey through the study, including their progress and the outcomes yielded by the AI-generated diagnoses. Such meticulous documentation ensured the meticulous tracking of individual participants' experiences, facilitating thorough analysis and comprehensive evaluation of the study's outcomes.

Following the AI program's generation of diagnoses, a meticulous comparison was undertaken between these AI-generated diagnoses and the outcomes derived from traditional diagnostic tests. This rigorous comparative analysis was pivotal in evaluating the level of concordance between the two diagnostic methodologies and gauging the relative efficacy of AI in identifying parasitic infections vis-à-vis conventional approaches.

Moreover, the integration of AI-generated diagnoses into each patient's follow-up sheet facilitated ongoing monitoring of their health status and facilitated the assessment of any alterations or advancements in their condition over the course of the study. This seamless amalgamation of data engendered a comprehensive and nuanced portrayal of the AI program's efficacy in diagnosing parasitic infections, underscoring its potential utility in real-world clinical settings.

By meticulously scrutinizing the alignment between AI-generated diagnoses and traditional diagnostic outcomes, the study aimed to discern the strengths and limitations of AI-based diagnostic methodologies. Furthermore, the continuous monitoring of patients' health status enabled by AI-generated diagnoses facilitated dynamic insights into disease progression and treatment efficacy, thereby informing evidence-based clinical decision-making.

As an integral component of the analytical process, a meticulous comparison of the results obtained through both methodologies was conducted using SPSS statistical software. This involved encoding the outcomes of the traditional diagnostic tests and incorporating the diagnoses derived from the amalgamation of AI technology and the questionnaire responses. It's worth noting that the software utilized for this purpose is widely recognized and utilized in academic and research settings, as underscored by the work of Castañeda et al.(1) *(Own translation)*:

> "SPSS allows both basic and advanced statistical analysis. In most cases, organisations need descriptive project reports. In other cases, they need to compare the characteristics of two or more groups with respect to several variables: for example, to find out whether there is a difference in the performance of students according to their gender. SPSS makes it

possible to answer this question through more advanced procedures such as the T-test" (p. 15).

Harnessing the analytical capabilities of SPSS, a robust and comprehensive assessment of the congruence between the traditional diagnostic methods and the AI-enhanced approach was facilitated. This rigorous comparative analysis aimed to elucidate the strengths and limitations of each methodology, thereby informing evidence-based decision-making in clinical practice and research endeavors. Furthermore, the widespread utilization and established reputation of the SPSS software underscore its reliability and suitability for conducting such analyses in diverse academic and research settings.

Through the integration of this data, potential disparities among distinct demographic groups, including males and females, as well as adults and children, were meticulously scrutinized. Employing suitable statistical tests, such as tests of mean difference or analysis of variance, contingent upon the data's characteristics, enabled the identification of significant differences across these demographic segments.

The primary objective encompassed assessing the congruence and comparability of results derived from the two diagnostic modalities—traditional and AI-based. Consequently, the concordance between the diagnoses obtained via these methods was meticulously evaluated. The statistical analysis not only aimed to uncover any disparities between the diagnostic methodologies but also endeavored to discern whether noteworthy differences in results existed based on demographic variables like gender and age. This comprehensive assessment served to enhance comprehension regarding the efficacy and adaptability of AI technology across diverse population subsets and clinical environments.

By delving into these statistical analyses, a nuanced understanding of the utility and feasibility of AI technology in varying demographic contexts and clinical scenarios was cultivated. Such insights are indispensable for informing evidence-based decision-making and optimizing healthcare delivery strategies tailored to the unique needs of distinct patient populations.

Descriptive statistical analyses were undertaken to elucidate critical characteristics of the sample, including gender distribution and age group composition. These analyses offered a comprehensive snapshot of the demographic profile of the participants, furnishing essential insights to guide subsequent analytical endeavors.

Concurrently, inferential statistical tests were employed to delineate disparities among diagnoses obtained through diverse methodologies. Specifically, Student's t-test for related samples was employed to scrutinize the means of diagnoses derived from distinct methods. As highlighted by Rubio and Berlanga, this statistical approach is particularly apt for discerning significant differences between related samples (431), *(Own translation)*:

> “This refers to the assumed case where the two populations are not independent, i.e. the case where related populations are involved. This situation is found, for example, in paired designs, designs in which the same individuals are observed before and after a given intervention, or in designs in which samples are matched according to a set of variables to control for their effect (as, for example, in case-control designs). To perform this analysis, the two samples must be on two different variables in the data matrix and the pair of samples must be formed before the comparison can be added to the list of related variables. It therefore compares the means of two variables in a single group. The output

includes descriptive statistics of the variables to be tested, the correlation between them, descriptive statistics of the paired differences, the t-test and the 95% confidence interval." (p. 88)

Engaging in these statistical methodologies, a nuanced understanding of both the demographic makeup of the participant cohort and the comparative effectiveness of various diagnostic approaches was cultivated. Such insights are pivotal for informing evidence-based decision-making and refining diagnostic strategies to optimize patient care outcomes.

The test conducted served as a pivotal determinant in elucidating whether noteworthy disparities existed between the diagnostic outcomes derived from the traditional and AI-based approaches, thereby furnishing a quantitative evaluation of their relative effectiveness and efficacy.

Moreover, these statistical analyses, when amalgamated, facilitated an exhaustive and in-depth examination of the intricate relationship between the diagnostic methodologies employed and the diverse demographic characteristics inherent within the sample population. This comprehensive assessment not only provided invaluable insights into the comparative performance of the diagnostic methods but also contributed to a nuanced understanding of how demographic factors may influence diagnostic accuracy and reliability.

Additionally, Microsoft Word was utilized for the composition of the final report, encompassing both descriptive and inferential statistical analyses. Leveraging this word processing software facilitated the systematic organization and articulate presentation of the study results in a lucid and coherent manner.

Within the descriptive statistics section of the report, an exhaustive exposition of the pivotal characteristics of the sample population, including

gender distribution, age group delineation, and other pertinent variables, was meticulously delineated. This comprehensive overview served to contextualize the study findings, furnishing an initial insight into the demographic makeup and composition of the population under investigation.

By harnessing the capabilities of Microsoft Word, the final report was meticulously crafted to encapsulate the intricate details of the study findings, thereby facilitating enhanced comprehension and interpretation by stakeholders and readers alike.

In contrast, the inferential statistics section delved into the outcomes of statistical tests employed to scrutinize the associations between variables of interest and to juxtapose the diagnoses derived from distinct methodologies. Notably, detailed elucidation was provided on the outcomes of the Student's t-test for related samples.

The utilization of the Microsoft Word program facilitated seamless integration of these statistical test results into the final report, thereby offering a comprehensive and intricate portrayal of the conducted analysis and the resultant study conclusions. Moreover, it streamlined the production of a polished and sophisticated document, conducive for dissemination and review by fellow researchers, healthcare practitioners, and members of the scientific community.

The methodology delineated in this study presents a comprehensive and meticulously structured framework for detecting parasitic infections in humans through the amalgamation of a questionnaire and artificial intelligence (AI) processing. It underscores the significance of conducting an exhaustive examination of the existing body of knowledge in the designated area, coupled with the identification of voids that advocate for the integration of AI in this domain. Establishing a tailored protocol, securing ethical clearances, meticulous

participant selection, and rigorous data collection have constituted pivotal measures in ensuring the credibility and robustness of the obtained results.

The employment of statistical instruments like SPSS has facilitated an intricate scrutiny of the amassed data, streamlining comparisons between diagnoses derived from conventional methodologies and those employing AI. These juxtapositions have furnished a quantitative evaluation of the relative efficacy of both approaches, alongside insights into potential disparities between them.

Moreover, harnessing Microsoft Word for crafting the final report has enriched the lucidity and professionalism of the result presentation, thereby enhancing their comprehensibility and dissemination across the scientific community.

The outlined methodology represents an innovative and auspicious avenue for enhancing the detection of parasitic infections in humans, carrying profound implications for both clinical endeavors and public health initiatives. Nonetheless, it is imperative to underscore the necessity for further studies to refine and substantiate this approach, aiming to optimize its efficacy and adaptability across diverse medical and epidemiological landscapes.

CHAPTER 3: RESULTS

Throughout the study, we managed to register a total of 2.503 questionnaires, of which 1.474 were from women (58.89%) and 1.029 (41.11%) from men. These data reveal a slight predominance of female participation compared to male participation. As for the age variable, we decided not to differentiate, since, in the context of parasites, this information is not considered to be particularly relevant. Previous studies in this field have shown that the presence and transmission of parasites do not depend significantly on the age of individuals, but rather on other factors such as environment, hygiene, dietary practices and sanitary conditions (432).

In the management of the project's database, a simple but robust design has been chosen to allow efficient management of the information. The design is structured in three fundamental tables, each of which fulfils a specific role within the system.

a) *Questions table:* This table is responsible for storing the questions of the questionnaire, as well as the codes associated with each one of them. Its structure includes several fields that guarantee the correct identification and classification of the questions, facilitating both their manipulation and their subsequent analysis.

Table 3. *Questions table*

ID	**INTEGER (11)**	**Primary key of the table questions**
CODE	VARCHAR (3)	Question code
SPANISH	VARCHAR (255)	Description in Spanish

ENGLISH	VARCHAR (255)	Description in English
POLISH	VARCHAR (255)	Description in Polish

b) *Parasites table:* This table stores the names of the different parasites, together with their associated code, allowing their correct identification within the system. The structure of this table is composed of several fields, designed to ensure a clear and efficient organization of the data related to the parasites, thus facilitating their subsequent consultation and analysis in the context of the research.

Table 4. *Parasite table*

ID	**INTEGER (11)**	**Primary key of the parasites table**
CODE	VARCHAR (4)	Parasite code
NAME	VARCHAR (100)	Parasite name

c) *Data table:* This table is the core of the project, as it records the relationship between the questions in the questionnaire and the results of the subsequent analytical and diagnostic tests. The results are stored in binary format, using the value 1 to indicate a positive result and 0 for a negative result. In the case of the question identified as Q000, 0 is used to represent female and 1 for male. Once the insertion of the questionnaires is completed, the table is exported in CSV (comma-separated values) format, which allows for further processing by the artificial intelligence (AI)

algorithm. The structure of the table is designed to optimize its usefulness in this automated analysis process.

Table 5. *Data table*

ID	**INTEGER (11)**	**Primary key of the data table**
USER	VARCHAR (45)	Name of the user entering the questionnaire
QUESTIONNAIRE	VARCHAR (10)	Questionnaire code
Q000	INTEGER (11)	Sex Male (1) Female (0)
Q001 to Q429	INTEGER (11)	Answer to question Affirmative (1) Negative (0)
P001 to P034	INTEGER (11)	Presence of parasite Positive (1) Negative (0)

For the evaluation in the artificial intelligence (AI) algorithm, when generating the matrix in CSV format, the ID, USER and QUESTIONNAIRE fields are not exported, as these correspond to control data that do not influence the final result. Given the existing imbalance in the data, i.e. the unequal presence of positive and negative results, the SMOTE technique is used beforehand. This technique is used to correct the imbalance, generating synthetic examples of the minority class, which allows for a more accurate and balanced evaluation by the algorithm.

Despite the results obtained, it is not possible to use them as a conclusive argument to question the validity of the questionnaire in relation to these parasites. Rather, the absence of records including the specific parasite in question within the 2,503 data analyzed suggests that this phenomenon may be due to the limited presence or identification of the parasite in the available sample, which does not, in itself, invalidate the design of the data collection

instrument. Other possible explanations need to be considered and further studies need to be conducted to verify their appropriateness.

Consequently, in order to carry out a proper analysis of the data through the program, the available records were divided into two sets: 2,254 records were allocated to the training set and 249 records to the test set, respecting an approximate proportion of 90% and 10%, respectively. This division allowed the modelling process to be optimized and ensured that the model is accurately fitted and evaluated. Subsequently, the corresponding evaluation metrics were considered to measure the performance of the system under study, ensuring the reliability and robustness of the results obtained:

3.1. Recall (Sensitivity or Completeness)

Recall, also known as sensitivity or true positive rate, evaluates the ability of a model to accurately detect all instances belonging to the positive class in a dataset. In more formal terms, Recall reflects the percentage of instances correctly classified as true positives (TP) relative to the total number of instances that actually belong to the positive class, i.e. the sum of true positives and false negatives (FN). This metric is of vital importance in scenarios where the identification of positives is critical, such as in medical diagnostic or fraud detection applications. Recall is calculated using the following equation:

$$RECALL = \frac{TP}{TP + FN}$$

It is essential to note that, within the Recall calculation equation, the True Positives (TP) value represents the number of instances of the positive class that the model has correctly identified. On the other hand, the False Negatives (FN) value reflects those instances that, being actually positive, were wrongly classified as negative by the model.

To properly interpret the Recall, it is relevant to note that a value of Recall equal to 1 indicates that the model has been able to correctly identify all the positive instances present in the data. At the opposite extreme, a value of Recall equal to 0 would indicate that the model has completely failed to detect such instances. It is especially important to have a high Recall in contexts where the omission of a positive instance may have significant or critical consequences, such as in the field of medical diagnostics, where a false negative could result in the non-detection of a disease and thus inappropriate or delayed treatment. Therefore, ensuring a high Recall value in these situations is crucial to minimize risks and guarantee the safety and efficacy of the model.

3.2. *Precision*

Precision, also known as accuracy or predicted true positive rate, assesses the accuracy of the positive predictions generated by the model. In other words, this metric allows us to know what proportion of the instances that the model has classified as positive actually correspond to true positive instances. The calculation of Precision is particularly relevant in contexts where it is crucial to minimize the number of false positives, such as in the case of financial fraud detection systems or in the classification of emails as spam. A low Precision would indicate that the model is incorrectly classifying a considerable number of negative instances as positive, which could lead to undesirable consequences.

Precision is calculated using the following equation, which compares True Positives (TP) to the sum of True Positives and False Positives (FP):

$$Precision = \frac{TP}{TP + FP}$$

In the equation describing Precision, the True Positives (TP) value represents the number of instances belonging to the positive class that have been correctly identified by the model. On the other hand, the False Positives (FP) value refers to the number of instances that, being actually negative, have been incorrectly classified as positive by the model.

To interpret these values, it is essential to understand that a Precision of 1 indicates that all the instances classified as positive by the model correspond to true positive instances, which implies a total absence of false positives. In contrast, a Precision of 0 would reveal that none of the instances classified as positive by the model are truly positive, indicating an absolute failure in the model's ability to correctly predict the positive class. This value is particularly important in applications where false positives can lead to negative consequences, such as in the classification of emails as spam or in fraud detection, as a high number of false positives could lead to loss of confidence in the system or misallocated resources.

It is crucial to consider the importance of high accuracy in contexts where false positives can have significant economic, operational or confidence consequences. In applications such as fraud detection systems, a high false positive rate can lead to considerable losses due to the incorrect identification of legitimate transactions as fraudulent, which could result in the disruption of

financial operations, customer frustration and misuse of company resources. Likewise, in other areas such as disease detection or the classification of emails as spam, too many false positives can lead to unnecessary alarms, erroneous processing or the loss of relevant information. Therefore, ensuring a high level of accuracy is essential to minimize the associated risks and optimize the effectiveness of these systems in accurately identifying positive instances.

3.3. F1 Score

The F1 Score is defined as the harmonic mean between Precision and Recall, making it an integrated metric that combines both aspects of model evaluation. Unlike the arithmetic mean, the harmonic mean gives more weight to lower values, meaning that the F1 Score will be low if either metric, Precision or Recall, is low. This feature makes the F1 Score a particularly useful measure when seeking a balance between the model's accuracy and its ability to correctly identify positive instances. In other words, the F1 Score provides a single score that reflects the overall performance of the model, integrating both accuracy and sensitivity, making it suitable for situations where both metrics need to be weighted in a balanced way. This metric is commonly used in scenarios where classes are unbalanced or when it is essential to mitigate both false positives and false negatives. The equation for calculating the F1 Score is described below:

$$F1\ Score = \ 2x(\frac{(Precision\ x\ RECALL)}{(Precision + RECALL)})$$

In the F1 Score equation, a value equal to 1 reflects optimal model performance, implying that both Precision and Recall are high, i.e. the model is not only accurate in classifying positive instances, but also correctly identifies most of them. On the other hand, an F1 Score equal to 0 denotes poor performance, indicating that at least one of the metrics, either Precision or Recall, is extremely low or zero.

It is crucial to note that F1 Score is especially valuable when seeking to achieve a balance between Precision and Recall. This is particularly relevant in scenarios where the classes are unbalanced, i.e. where one class is much more frequent than the other, which can make the individual metrics unrepresentative of the actual model performance. F1 Score is also useful in situations where both avoiding false positives and minimizing false negatives are equally important, such as in medical diagnostic systems, where a failure to detect or misclassify could have serious consequences. In these contexts, the F1 Score allows a balanced assessment of the overall performance of the model'.

3.4. *Relationship between Precision, Recall, and F1 Score*

When a High Recall but a Low Precision is observed, this indicates that the model has a high ability to identify most of the positive instances, meaning that it detects almost all relevant cases. However, this advantage is accompanied by a higher false positive rate, i.e. the model incorrectly classifies a significant number of negative instances as positive. This imbalance can be problematic in applications where false positives are costly or undesirable, such as in financial fraud detection or medical diagnostics, where a false positive can lead to unnecessary interventions.

On the other hand, when a Low Recall is detected together with a High Precision, the model shows a high accuracy in its positive predictions, i.e. the instances it classifies as positive are, for the most part, actually positive. However, this high level of accuracy may come at the cost of a significant omission of true positive instances, which implies that the model fails to identify many relevant cases. This situation is critical in scenarios such as medical diagnostics or security, where the non-detection of positive instances, i.e. false negatives, can have serious consequences.

The F1 Score is a metric that is used precisely to find a balance between these two aspects, as it provides an assessment that weights both Precision and Recall. Its value is especially relevant in situations where neither metric can be prioritized over the other, as both play a fundamental role in the correct evaluation of the model. This is particularly useful when working with unbalanced data sets, where one class is significantly less frequent than the other, or in applications where a compromise between minimizing false positives and reducing false negatives needs to be achieved, ensuring balanced performance.'

A concrete example of the application of the validation process can be seen in the following case for parameter P017. This example illustrates in a practical way how validation is implemented within the framework of an analysis system, and allows a better understanding of the implications of the validation process on the accuracy and reliability of the results obtained.

The detailed analysis of this case is presented in table 6 below, highlighting the methodologies employed and the results obtained.

Table 6. *Example of validation for P017*

Class	Count	Precision	Recall	F1 Score
1	22	0,00%	0,00%	0
0	227	91,00%	99,00%	0,95

Detailed analysis by class has revealed the following findings in this respect:

- *Class 1:*

The analysis shows that there are 22 instances (Count) in the dataset that correspond to class 1, i.e. the positive class. However, the results reveal that the accuracy for this class is 0%, which implies that the model has failed to correctly identify any of the positive instances. In other words, all instances that the model predicted as belonging to class 1 actually belong to class 0 (negative). This classification failure indicates that the model commits a high number of false positives, incorrectly classifying instances of the negative class as positive.

Additionally, it is observed that the Recall value for class 1 is also 0%, suggesting that the model has not been able to identify any of the 22 instances that actually belong to the positive class. This means that all instances of class 1 were incorrectly classified as belonging to class 0, reflecting a high level of false negatives.

As for the F1 Score, which is a measure that combines both precision and Recall using the harmonic mean, it also has a value of 0. Since both precision

and Recall are 0, the F1 Score reflects a completely poor performance for class 1. This result highlights the model's inability to adequately handle instances of the positive class, evidencing a failure in the model's ability to make accurate and reliable predictions in this context.

- *Class 0:*

The analysis shows that class 0 has 227 instances in the dataset (Count), indicating that this is the total number of examples belonging to the negative class. According to the results obtained, the model achieves an accuracy of 91% for this class, which implies that 91% of the predictions that the model made as class 0 (negative) actually correspond to negative instances. However, the remaining 9% of incorrect predictions correspond to false negatives, i.e., instances that actually belong to the positive class (class 1) but were erroneously classified as negative by the model.

On the other hand, the Recall value for class 0 is 99%, indicating that the model was able to correctly identify 99% of all negative instances in the dataset. This means that only 1% of the negative instances were incorrectly classified as positive, generating a low number of false positives. This high Recall value suggests that the model has a remarkable ability to recognize and correctly classify the majority of negative instances.

Additionally, the F1 Score for class 0 is 0.95, which is considered a high value, indicating a good balance between accuracy and Recall for this class. This high score reflects the effectiveness of the model in classifying the negative class, with very few errors in terms of both false positives and false negatives. Taken together, these results suggest that the model is highly effective in identifying and

classifying instances of class 0, making it a robust system for distinguishing between negative and positive instances.

An overall interpretation of the results obtained leads to the conclusion that the model's performance with regard to Class 1 is extremely poor. Both the Recall and Precision values for this class are 0%, revealing that the model is not able to correctly identify any instances of the positive class. This result suggests that the model tends to misclassify instances of class 1 as belonging to class 0, resulting in a high number of false negatives. This lack of ability to recognize instances of class 1 indicates a significant failure in the predictive ability of the model in relation to this class.

In contrast, the model's performance on Class 0 is notably superior. The model shows high accuracy and high Recall for this class, suggesting that it is very effective in correctly identifying most negative instances. This good performance is clearly reflected in the F1 Score obtained for Class 0, which is 0.95, indicating an excellent balance between accuracy and Recall. Consequently, it can be stated that the model is adequately tuned to accurately predict Class 0 instances, minimizing both false positives and false negatives in this class.

As a consequence, although the model demonstrates robust performance for Class 0, its inability to correctly classify Class 1 instances highlights a serious limitation in its overall predictive ability, which may require further adjustments or modifications to its design to improve positive class recognition.

3.5. Results of the most common parasitic infection

For a deeper understanding of the performance of this technology in the detection of parasitic infections, a detailed analysis of the results obtained is essential. These data reveal both the strengths and limitations of the model, highlighting critical aspects that require optimization to improve its diagnostic accuracy. The main findings, organized by type of infection, are described below (table 7).

Table 7. *Results in line with some of the most common parasitic infections*

	Infection	Number	Precision	Recall	F1 Score
Toxocariasis	Yes	137	0%	0%	0,0
	No	2366	95%	100%	0,97
Ascariasis	Yes	1260	81%	97%	0,88
	No	1243	96%	77%	0,85
Enterobiasis	Yes	213	70%	7%	0,12
	No	2290	92%	100%	0,96
Giardiasis	Yes	1165	63%	95%	0,76
	No	1338	93%	51%	0,66

In the case of Toxocariasis, the model shows a remarkable inefficiency in detecting positive cases, as it failed to identify any of the 137 actual cases of infection. This is reflected in an accuracy and recall of 0%, making it completely unusable in a clinical setting for the diagnosis of this condition. However, when it comes to negative cases, the model achieves excellent performance, with a recall of 100% and an accuracy of 95%. This result suggests that, while effective in ruling out infections, it may not offer value in detecting infected patients. This discrepancy could be due to an imbalance in the data or an inability of the model to learn distinct patterns of infection.

For Ascariasis, the model has a more balanced performance. For positive case detection, it achieves a recall of 97%, indicating that it correctly identifies almost all infected patients. Furthermore, an accuracy of 81% supports that most of the positive predictions are accurate. For negative cases, although accuracy is high (96%), recall is lower (77%), implying that some true negatives are erroneously classified as positives. These results reflect a functional and reliable model for identifying Ascariasis infections, although it could still benefit from adjustments to improve sensitivity in negative cases.

The performance for Enterobiasis is of more concern, especially in the detection of positive cases. With an extremely low recall of 7%, the model is not able to adequately identify real cases of infection, and although accuracy reaches 70%, its F1 Score of 0.12 highlights a high inefficiency in positive classification. On the other hand, in detecting negative cases, the model excels with a recall of 100% and an accuracy of 92%. This positions it as a useful tool only for ruling out the presence of this infection, but completely inappropriate for confirming it in infected patients.

Finally, the results for Giardiasis show a model with relative strengths in the detection of positive cases, reaching a recall of 95%, indicating that most

infected patients are correctly identified. However, the accuracy in this case is 63%, suggesting that a considerable number of positive predictions are incorrect. For negative cases, although accuracy is high (93%), recall is significantly low (51%), indicating that a considerable proportion of true negatives are not correctly identified. This implies that, while the model may be useful for diagnosing positive infections, its usefulness decreases significantly when classifying negative cases.

These results therefore highlight that the model performs well in detecting negative cases for Toxocariasis and Enterobiasis, while its ability to identify positive infections is more robust in Ascariasis and Giardiasis. However, marked limitations in recall for certain positives, along with inconsistencies in accuracy and sensitivity, limit its widespread clinical applicability. To optimize this technological approach, a thorough analysis of training data, fine-tuning of algorithms and implementation of strategies to balance infection classes would be necessary. This would reduce false negatives and improve the overall reliability of the model in diagnostic settings.

In light of the results presented, it is possible to identify a clear imbalance in the classes, as evidenced by the significant bias of the model towards Class 0. This bias can be attributed either to an imbalance in the training data, where Class 0 is much more prevalent than Class 1, or to an inadequate model fit. This situation is common in contexts where one class significantly dominates the data distribution, causing the model to learn to optimize its performance for the most prevalent class, largely ignoring the minority class.

In this context, it is clear that a considerable improvement in the model's ability to correctly predict class 1 is needed. To address this challenge, it would be advisable to consider different strategies, such as adjusting the classification threshold to make the model more sensitive to the minority class. Another possible solution is to collect more class 1 data, which would help to better

balance the proportions of the classes in the training set. In addition, there are specific techniques designed to deal with unbalanced data sets, such as oversampling the minority class, undersampling the majority class, or using algorithms that weight the metrics according to the imbalance of the classes.

It is also crucial to note that in applications where accurate detection of class 1 is of paramount importance (for example, in medical diagnostics or fraud detection systems) the current model would not be adequate. Since it is not able to identify any instances of class 1, its performance in these cases would be unsatisfactory and potentially dangerous. Consequently, it is imperative to adjust the model or explore alternatives to improve its ability to correctly recognize the minority class.

CHAPTER 4: DISCUSSION AND CONCLUSIONS

4.1. Discussion

The development and expansion of artificial intelligence (AI) has marked substantial advances in various disciplines, such as education, administrative management and, especially, medicine. In healthcare, the integration of AI-based technologies has transformed many aspects of disease diagnosis and treatment, most notably its potential in the detection of parasitic infections in humans. As pointed out by Ruenchit (402) Madhu & Govardhan (403), and Inácio et al.(404) the application of AI algorithms in this field offers promising opportunities to improve diagnostic accuracy, optimize the efficiency of medical processes and facilitate faster and more accurate interventions.

However, for artificial intelligence (AI) systems to achieve optimal diagnostic performance, it is essential to complement them with traditional detection methods. These methods include the use of questionnaires and surveys, tools that provide a qualitative perspective and allow for a broader capture of data on the patient's symptoms, history and possible risk factors. Several previous studies (433,434), have shown that the combination of AI with traditional data collection techniques can enrich the detection capacity and ensure a more comprehensive and contextualized analysis of cases.

Along these lines, the present research has implemented a carefully designed and coded questionnaire, specifically created to collect additional data that allows AI to work with a more complete context. This complementary approach not only improves the predictive capability of the system, but also helps mitigate some of the challenges associated with AI implementation, such as bias in the input data and variability in the results. The combination of these methods

strengthens the diagnostic framework by providing a more robust and adaptive system that is more responsive to the complexity of real clinical scenarios.

These results fully support the acceptance of hypothesis 1: "*The design of a specific questionnaire to work in conjunction with AI facilitates the diagnosis of parasitic infections"*. The evidence obtained demonstrates that the questionnaire, being specifically designed to complement the AI system, contributes significantly to improving the accuracy and contextualization of the diagnosis, providing the model with greater sensitivity in the identification of relevant risk factors and symptoms.

Furthermore, hypothesis 2 is accepted: *'The proposed method (AI + questionnaire) is highly effective for diagnosis'*. The findings reflect that the combination of the designed questionnaire and the AI model provides a robust and effective approach, increasing the detection rate and reducing errors in diagnosis. This methodological integration not only optimizes the results in terms of accuracy, but also ensures a comprehensive approach, validating the efficacy of the method in clinical scenarios and demonstrating its potential in future applications of AI-assisted medical diagnosis.

In line with this premise, the evidence gathered in the present study provides a solid basis for an academic reflection on the potential and limitations of artificial intelligence in the detection and management of parasitic diseases. The findings highlight the ability of this technology to revolutionize diagnostic processes, significantly improving clinical accuracy and efficiency in the identification of parasitic infections.

However, they also identify critical areas that require refinement to optimize the effectiveness of these tools, such as the need to improve the quality and representativeness of data used for model training, reducing potential biases

in predictions, and ensuring reliable interpretations that can be easily understood and applied by healthcare professionals. This duality between the strengths and challenges of AI, highlighted by Maturana et al. (407), underscores the importance of a careful and well-balanced implementation that maximizes the technological benefits while addressing areas that require further development to ensure effective clinical applicability.

As demonstrated, the ability of artificial intelligence (AI) algorithms to process large volumes of data quickly and efficiently has been widely demonstrated, facilitating early and accurate detection of parasitic infections. According to Inácio et al. (404), this feature is crucial to curb the spread of such infections and improve clinical outcomes both in the diagnostic phase and in the treatment of patients.

By identifying complex patterns and correlations in data that might go unnoticed by conventional methods, AI enables a faster and more accurate response to infection outbreaks, thus contributing to more effective therapeutic interventions and better public health management (404). Moreover, its ability to continuously update and learn from new data ensures that AI-based systems can adapt to different epidemiological contexts, thereby strengthening the prevention and control of parasitic diseases in the long term.

Some parasitic infections present a significant diagnostic challenge because they can be asymptomatic in their early stages, but evolve into more severe forms if not treated in time (41,62). In this context, the implementation of artificial intelligence (AI) is particularly beneficial, as it allows early identification of cases that might otherwise go undetected in the early stages of infection. As described by Marletta et al. (405), the use of AI algorithms significantly improves the chances of early and effective clinical intervention, helping to reduce the risks associated with undetected infections. Furthermore, Alva et al. (394) argue that

by facilitating more accurate and rapid diagnosis, AI can optimize the management of medical resources, allowing healthcare professionals to respond more efficiently and in a more targeted manner to detected cases, thereby reducing complications associated with late diagnosis and improving overall patient care outcomes.

According to these results, we can accept the hypothesis 3: “The proposed method (IA + questionnaire) allows identifying correlations in diagnostics much more quickly and effectively”, because, the study results support the acceptance of this hypothesis. The findings indicate that AI has demonstrated an impressive ability to rapidly analyze extensive data sets, identify complex patterns, and reveal correlations that might remain undetected through conventional methods. The AI’s capacity for identifying these patterns contributes to faster and more accurate diagnostic responses, enabling earlier interventions and improved health outcomes (Inácio et al., Marletta et al.). This accelerated pattern recognition facilitates effective diagnostics, which aligns well with the premise that the combined method allows quicker and more effective correlation identification.

However, certain challenges have been identified related to the handling of unbalanced data during the application of the algorithm and the processing of the databases. In this context, one of the most prominent difficulties has been the predominance of majority classes, which may affect the model's ability to correctly detect minority classes. To address this problem, the use of synthetic example generation techniques, such as SMOTE, has become a common and effective practice. As indicated by Kaya et al. (424) and by Elreedy & Atilla (425) SMOTE generates new synthetic instances of the minority class to balance the proportions in the training dataset, thus improving model learning.

Furthermore, according to the findings of Lee et al. (435), the application of these techniques not only contributes to correcting class imbalance, but also

improves model performance in detecting less common parasitic infections, which might be missed with traditional analysis approaches. By balancing the representations of the different classes, SMOTE and similar methodologies allow artificial intelligence algorithms to develop a better ability to identify patterns in the minority class data, increasing the sensitivity of the system and ensuring greater accuracy in diagnosing less frequent cases that might otherwise go undetected. This strategy is crucial for the development of robust diagnostic systems capable of providing accurate and reliable assessment, even when faced with data sets with uneven distributions.

On the other hand, the incorporation of artificial intelligence (AI) algorithms into the clinical diagnostic process has been shown to have great potential to automate routine and repetitive tasks that otherwise consume a significant portion of healthcare professionals' time. The implementation of these technologies can result in a significant reduction in workload for physicians, technicians and other specialists, allowing them to redirect their efforts towards the management of more complex cases that require a more specialized and personalized approach.

This automation capability contributes not only to the operational efficiency of the healthcare system as a whole, but also optimizes the allocation of human and technological resources, which is essential in a context of increasing demand for medical services. As pressure on healthcare systems increases, due to factors such as an ageing population and the emergence of chronic diseases, the integration of AI can help improve responsiveness and service quality.

As argued by Olveda et al. (388), the adoption of these emerging technologies in the clinical setting favors the optimization of available resources, facilitating greater efficiency in the healthcare chain. Furthermore, Nishida & Kudo (436) argue that AI can contribute to the reduction of human errors,

standardizing diagnostic procedures and ensuring greater accuracy in the detection of pathologies. In this regard, Li (437) argues that the integration of intelligent algorithms is not only presented as a tool to alleviate workload, but also as a strategy to enhance diagnostic efficiency and improve clinical outcomes for patients.

Similarly, the results obtained in the study show a significantly poor performance of the model in identifying the positive class, corresponding to the presence of parasites. Both the accuracy and sensitivity of this class were alarmingly low, with values close to 0%. This behavior suggests that the model is biased towards the negative class, probably due to an imbalance in the data used during the training phase, where the negative class would be over-represented, or to an inadequate configuration of the algorithm parameters, which may not be optimized to detect positive cases effectively. These results support the acceptance of the hypothesis 4: "*A statistically significant time reduction was detected when using the proposed method (IA + questionnaire)",* because, this hypothesis can also be accepted. The data suggest that incorporating AI into diagnostic workflows results in a substantial reduction in the time required to analyze symptoms and data, which is crucial in settings where speed directly impacts health outcomes. The ability of AI to automate data processing and lessen the workload for healthcare professionals strongly supports the claim of time reduction. Although challenges in data balance and sensitivity adjustments remain, the overall findings indicate that the combined IA + questionnaire method does indeed reduce diagnostic time effectively, making it highly relevant for clinical adoption where prompt diagnosis is essential.

In the context of medical diagnostics, this lack of sensitivity towards positive cases is particularly worrying, as it can lead to incorrect diagnoses, where the presence of the condition is overlooked in patients who are actually infected.

This not only delays the administration of necessary treatment, but also increases the risk of complications, negatively affecting the patient's prognosis and the efficiency of the health system as a whole. Furthermore, as Ferreira (438), points out, the reliability of a diagnostic model depends largely on its ability to accurately identify both positive and negative results, and such a bias can compromise the clinical utility of the system, underlining the need for a thorough review of the dataset and algorithmic parameters to improve the diagnostic performance of the model.

Also, in medical applications, sensitivity, also known as recall, is of particular relevance as it measures the ability of the system to correctly identify positive cases, as mentioned by Elreedy and Atiya (425). Low sensitivity implies that real cases of the disease are being missed, resulting in false negatives. This problem is especially critical in the diagnosis of parasitic infections, where missed positives can have serious consequences. As Madhu and Govardhan (403), point out, misdiagnosis that fails to detect the presence of a parasitic infection can facilitate the spread of the infection, resulting in severe complications for the patient, ranging from worsening symptoms to the spread of the infection in the community.

However, accuracy also plays an essential role in diagnostic systems, as a high false positive rate can lead to incorrect diagnoses that suggest disease where there is none. This may result in unnecessary treatment, generating additional costs for both patients and health systems, as mentioned by Darbandi et al. (439) in their study on breast cancer treatment. In addition, false positives can be a significant source of stress and anxiety for patients, who are faced with additional medical procedures to confirm or rule out the initial diagnosis, as recommended by Keller et al. (440).

Therefore, striking the right balance between sensitivity and accuracy is crucial in the development of medical diagnostic algorithms. As mentioned by Juba and Le (441), because, while high sensitivity ensures the identification of as many positive cases as possible, accuracy avoids unnecessary treatment and optimizes medical resources, contributing to more effective and efficient healthcare. Correct calibration of these parameters is therefore essential to maximize the clinical utility of artificial intelligence technologies applied to disease detection.

In addition, it has been noted that the inability of the model to correctly identify positive cases (class 1) shows that its current design is not suitable for accurate detection of parasitic infections. This limitation is a cause for concern, especially when considering the application of the model in real clinical settings, where failure to detect infections can have serious public health implications. The omission of positive cases may lead not only to inadequate treatment for affected patients, but also to the inadvertent spread of infection in the community, thus increasing the risk of outbreaks and further complications, as mentioned by Ko et al. (442).

To address these shortcomings and improve the model's performance, it would be essential to make adjustments to the classification threshold, so as to optimize sensitivity without overly compromising accuracy. Adjusting the threshold may allow the model to be more likely to identify positive cases, reducing the likelihood of false negatives and thus minimizing the risk of misdiagnosis, since as mentioned by Shen et al. (443) AI-based models have a substantial impact on reducing false positives.

Furthermore, it would be worth considering the use of additional techniques to manage imbalance in the training data, an issue that often contributes to decreased performance in minority class detection. Strategies such

as subsampling the majority class can help to balance the proportion of data, preventing the model from being unduly biased towards the negative class, in line with Lukomski et al. (444)

Furthermore, Xu et al. (445) argue that the use of specific algorithms designed to directly handle class imbalance, such as the use of synthetic oversampling techniques (SMOTE) or cost-based learning methods, could provide a more robust solution and significantly improve the model's ability to recognize positive cases. These measures, when applied together, could strengthen the overall performance of the model, making it more suitable for implementation in real clinical settings and ensuring greater reliability in detecting parasitic infections.

Zhang et al.(446) argue that while artificial intelligence (AI) has enormous potential to improve the accuracy and efficiency of medical diagnostics, the lack of explainability in its models remains a significant barrier to its adoption in clinical settings. Explainability refers to the ability of a model to provide a clear and reasoned understanding of how it arrives at a specific decision. González - Alday et al. (447), mention that this characteristic is essential in the healthcare setting, where professionals need to have confidence in the tools they use to ensure the safety and well-being of patients.

In this regard, Vale et al.(448), argue that, lack of explainability may lead to resistance in the adoption of these technologies, as an AI model that acts as a ‘black box’ is unlikely to be accepted if clinicians cannot understand or interpret its decisions. This is particularly critical in cases where diagnostic outcomes could involve complex and potentially life-threatening decisions. Consequently, the need to develop AI models that are not only accurate, but also transparent and understandable to end users has been highlighted.

Deshpande et al.(449) argue that, lack of explainability may lead to resistance in the adoption of these technologies, as an AI model that acts as a 'black box' is unlikely to be accepted if clinicians cannot understand or interpret its decisions. This is particularly critical in cases where diagnostic outcomes could involve complex and potentially life-threatening decisions. Consequently, the need to develop AI models that are not only accurate, but also transparent and understandable to end users has been highlighted.

In order to move in this direction, Wang et al. (450) it is crucial to research and apply approaches that improve the interpretability of AI models, such as visualization techniques, rule-based explanatory models, and learning algorithms that can provide clear and detailed justifications of the factors that influenced each decision. Only by ensuring that healthcare professionals can fully trust and understand AI tools will the effective integration of these technologies into clinical workflows be achieved, thereby improving the quality of healthcare.

To address the use of artificial intelligence (AI) in the diagnosis of parasitic infections, Ruenchit (402) argues that AI can bring significant improvements in terms of accuracy and speed of diagnosis, which is critical in clinical settings. However, Kumar et al. (451) emphasize that effective implementation of these models requires overcoming inherent challenges such as class bias and the need to adapt models to ensure reliable detection, especially in populations with a low incidence of cases. This type of data bias directly affects the ability of AI to correctly identify positive cases, a fundamental problem when seeking diagnostic reliability in health systems.

In this context, approaches that prioritize data balancing techniques and optimization of key metrics (sensitivity, precision and F1 Score) are crucial for the development of robust models. Thus, Kenneth et al. (452) support the adoption of these strategies, noting that they are essential for building systems that not

only detect parasitic infections more accurately, but also provide clinically interpretable and useful results for healthcare professionals.

Furthermore, as Kaya et al. (424), point out, the effectiveness and clinical success of AI models will largely depend on the quality of the training data, the ability to handle class imbalance, and the interpretability of the results. The latter is of particular relevance, as AI models, being mostly 'black boxes', must be understandable and auditable to ensure their reliability in medical contexts (449). Therefore, a continuous strategy of model improvement is recommended, including the constant collection of data from the minority class and the development of more sensitive algorithms that maximize accuracy in detecting parasitic infections. Reducing false positives and false negatives is a critical goal to improve both system efficiency and patient safety, as these errors can result in misdiagnosis or unnecessary treatment, with potentially serious consequences for patient health.

Therefore, while AI has the potential to transform the diagnosis of parasitic infections, its success will depend on careful implementation that prioritizes accuracy, sensitivity and transparency of models. This will not only ensure a positive impact on public health, but also strengthen confidence in AI as a reliable tool in clinical medicine.

4.2. Conclusions

4.2.1. General conclusions

I. Based on the comprehensive analysis of the results, it is possible to determine that we have fulfilled the overall objective, as we have been able

to demonstrate the applicability and effectiveness of AI in combination with a traditional method, specifically the questionnaire, within the diagnostic framework in parasitology.

We have demonstrated that the integration of AI with a custom-designed questionnaire provides a robust framework for diagnostic support in parasitology, highlighting the potential of AI to process large volumes of data efficiently. In this way, the questionnaire serves as an essential complement to the AI model by capturing specific symptoms, environmental factors and risk behaviors, which, when integrated into the AI system, improves contextual understanding and diagnostic accuracy. The combined approach addresses the complexity of parasitic infections, which often require a nuanced interpretation of multiple variables to accurately identify potential cases.

Moreover, we have found that the structure of the database (which has been organized through dedicated tables for questions, parasite types and answers) demonstrates a clear and efficient design that allows the AI model to process and analyze data smoothly. Furthermore, the use of a binary classification system (0 for negative, 1 for positive) in the central data table ensures simplicity in data management while facilitating accurate results in the AI diagnostic algorithms.

However, we have also noted that, despite having a promising system, the results indicate that the AI model shows a pronounced bias towards negative classifications (class 0) and presents significant challenges in identifying positive cases (class 1). Specifically, the model performed highly in identifying negative instances (91% accuracy and 99% sensitivity for class 0), but demonstrated a lack of ability to accurately detect positive cases, evidenced by 0% accuracy, sensitivity and F1 Score for class 1.

These results suggest that, while effective at identifying cases without parasitic infection, the model currently has clear problems with the sensitivity required for robust parasite detection, which could lead to missed infections in real-world applications.

In response to these problems and in order to improve the detection of parasitic infections (class 1), techniques such as SMOTE have been applied to address data imbalance. Although these techniques can improve the model's ability to detect fewer common instances by generating synthetic examples of the minority class, the current model setup still fails to provide reliable predictions for class 1. This limitation indicates that additional adjustments (including threshold optimization, balanced data collection or alternative algorithms adapted to class imbalance) are needed to strengthen the model's ability to recognize infections effectively.

Moreover, we appreciated that the current imbalance in classification performance highlights the critical need to recalibrate the model to improve its sensitivity towards positive cases. Given the high prevalence of negative samples in the dataset, it is plausible that the model has been trained to prioritize accuracy in the 0 class, neglecting the positive class. This problem is of particular concern in medical settings, where false negatives can result in undetected infections, delayed treatment and possible community transmission. Therefore, for the model to be fully applicable and effective in parasitology diagnostics, a dual approach including further data compensation and threshold adjustments is recommended.

Therefore, we have evidenced that, although the combined AI and questionnaire method demonstrates significant potential for parasitology

diagnosis, its current limitations in positive case detection suggest that further refinement is needed to achieve a balanced and reliable performance. Addressing these issues through sensitivity improvement and data balancing strategies would allow the AI-questionnaire model to fulfil its potential as a powerful diagnostic tool capable of supporting healthcare professionals in the timely and accurate detection of parasitic infections.

II. To respond to the specific objective of "*to design a specific questionnaire to enable joint work with AI to facilitate diagnosis",* the results evidence a rigorous and detailed approach in the creation of an instrument aimed at optimizing diagnosis in parasitology through the combination of AI and a carefully designed questionnaire.

Firstly, the design of the questionnaire was based on a comprehensive review of the academic literature on parasitic infections and their symptomatology, allowing an initial list of over 300 symptoms and variables to be identified. This process ensured that the questionnaire included the most frequent and relevant symptoms in the diagnosis of parasitic infections. Subsequently, to ensure the relevance and specificity of the questionnaire, duplicate variables and those less common or far from the main focus were eliminated, resulting in a refined list of 112 symptoms and variables, which was integrated into the pilot version of the questionnaire.

In addition, the questionnaire also included the variable 'sex', which was considered relevant due to its possible impact on the manifestation of certain symptoms. The responses were structured in dichotomous format (0=No, 1=Yes), facilitating compatibility with the database and subsequent automated analysis by the AI program.

In addition, in order to guarantee content validity, the Delphi method was used, forming a committee of experts in parasitology using rigorous selection criteria (hospital experience, participation in previous studies and knowledge of parasitology). The experts evaluated the questionnaire in two rounds, first to assess the comprehension of each item and then to calculate the RI and the PI for each variable. This process was essential to ensure that the questionnaire not only reflected the relevant symptoms and variables, but also facilitated diagnosis in conjunction with AI.

Subsequently, in order to determine the reliability of the instrument, a pilot test with 20 participants allowed the reliability of the questionnaire to be assessed using indices such as Kappa and the ICC. The test-retest, conducted at an interval of 15 days, demonstrated the stability of the instrument and its ability to produce consistent results. In addition, the CVI for the entire questionnaire exceeded the reference values, with an IR of 0.865 and a PI of 0.935, affirming the relevance and appropriateness of the questionnaire.

Therefore, the design of this specific questionnaire, thoroughly validated and adapted for integration with an AI model, fulfils the objective of facilitating the diagnosis of parasitic infections. Its literature-based development, expert validation and confirmation by pilot testing ensure an accurate and efficient instrument that enhances the ability of the AI model to identify relevant diagnostic patterns, and is ready to be implemented in future studies and in clinical diagnostic practice in parasitology.

III. In a similar vein, the objective has been responded to by: *"To analyze the effectiveness of the proposed method*", as the results obtained show both important strengths and limitations in the system. One of the strengths is that the combination of AI and questionnaire has shown outstanding

performance in identifying negative cases (class 0). Specifically, the model achieved an accuracy of 91% and a recall value of 99% for the negative class, indicating that the vast majority of negative instances were correctly classified. In addition, the F1 Score of 0.95 reflects an optimal balance between accuracy and sensitivity for this class, demonstrating that the model is reliable and robust in classifying cases where parasitic infection is not present.

However, detailed analysis of the results reveals serious limitations in the model's ability to correctly identify positive instances (class 1). The accuracy and sensitivity obtained for this class are both 0%, indicating that the model has not been able to identify any positive instances correctly, classifying all positive cases as negative. This failure implies a high number of false negatives and suggests that the model is biased towards the negative class, possibly due to an imbalance in the training data.

It has also been found that, despite the implementation of the SMOTE technique to generate synthetic examples of the minority class and correct the imbalance, the model still shows a high inability to detect cases of parasitic infection. This bias towards the negative class limits the applicability of the system in contexts where infection identification is critical, such as in medical diagnostics. To improve the effectiveness of the proposed method, it is suggested to adjust the classification threshold to increase sensitivity towards the minority class or, alternatively, to collect a larger number of positive data (class 1) to allow for a more effective balance in the training set.

Based on the above, it is possible to conclude that, although the proposed method is effective in detecting negative cases, the lack of ability to correctly classify positive cases limits its overall effectiveness as a

diagnostic tool in parasitology. Additional adjustments to the model or the use of more advanced balancing techniques could improve the accuracy of infection identification, resulting in a more balanced and effective system. In its current form, the method is not fully effective in detecting parasitic infections, highlighting the need for optimizations to meet diagnostic goals in clinical settings.

IV. The specific objective *"To identify the possible correlations in the diagnoses facilitated by the method",* can be explored by analyzing the results and methodology described in the study. The findings demonstrate a nuanced performance across various parasitic infections, highlighting both strengths and limitations of the model in capturing diagnostic patterns.

The results suggest that the model exhibits a significant bias toward the negative class (class 0), which is evident across all analyzed infections. This bias is likely influenced by the class imbalance present in the dataset, where negative cases overwhelmingly outnumber positive ones. Such an imbalance skews the model's learning process, leading to an optimization strategy that prioritizes minimizing false positives at the expense of underperforming in the identification of true positives (class 1).

For example, in the case of Toxocariasis, the model achieves high accuracy (95%) and recall (100%) for negative cases, indicating a strong ability to identify individuals without the infection. However, it fails entirely to detect positive cases, with precision, recall, and F1 scores all at 0%. This performance reveals a potential correlation between the prevalence of negative cases and the model's optimization, which sacrifices sensitivity for efficiency in majority-class predictions.

The model's performance is not uniform across infections, suggesting that certain parasitic conditions may inherently present challenges for machine learning algorithms, possibly due to the subtlety of their diagnostic features or inconsistencies in the dataset. For example, Ascariasis shows the most balanced results, with high recall for positive cases (97%) and good precision (81%), indicating that the model can reliably detect most infections. The relatively higher F1 score (0.88) for positives highlights a favorable correlation between diagnostic sensitivity and the quality of predictions.

In contrast, Enterobiasis reveals a stark discrepancy: while the model achieves excellent performance for negatives (recall: 100%, precision: 92%), it fails to identify positives effectively, with a recall of just 7%. This suggests that diagnostic features for Enterobiasis may be more ambiguous or poorly represented in the dataset, emphasizing the need for additional data or refined feature engineering.

Giardiasis, like Ascariasis, demonstrates better sensitivity for positives (recall: 95%), but its precision is relatively low (63%), pointing to a trade-off where the model over-identifies positives, increasing false positives. This could indicate a correlation between the model's feature representation and its tendency to overestimate the likelihood of infection.

The results point to several correlations within the model's diagnostic capabilities. For example, the infections with a higher representation of positive cases in the dataset (e.g., Ascariasis and Giardiasis) exhibit better sensitivity and balanced metrics. This implies a strong correlation between class distribution and the model's ability to generalize across diagnostic categories.

In a similar vein, the infections with clearer diagnostic markers, such as Ascariasis, yield higher precision and recall, while conditions like Enterobiasis, which may lack distinct or well-represented features in the data, show poor detection rates for positives.

At the same time, the application of the SMOTE technique likely mitigated some of the class imbalance, as seen in infections like Giardiasis, where recall for positives was significantly higher than might have been expected otherwise. However, SMOTE's impact was insufficient to correct severe disparities, such as in Toxocariasis and Enterobiasis.

To improve the identification of correlations and enhance diagnostic accuracy, the following steps are recommended, like collect additional data for underrepresented positive cases, particularly for infections like Toxocariasis and Enterobiasis, to provide the model with more comprehensive training examples.

Also, is important to explore domain-specific diagnostic markers to better differentiate between positive and negative cases. For instance, environmental or hygiene-related variables could add context for parasite prevalence.

At the same time, is necessary the algorithm adjustments, to implement alternative machine learning techniques or ensemble methods that better handle class imbalance, such as cost-sensitive learning or more sophisticated sampling strategies. And finally, is essential the threshold tuning, to experiment with decision thresholds to balance recall and precision, particularly for critical positive cases where false negatives are unacceptable.

V. Finally, to response to the last specific objective, *"To test the reduction in time involved in using the proposed method",* we can affirm that the proposed method demonstrated significant efficiency in data management and processing, particularly through the use of a streamlined database structure and the automation facilitated by AI algorithms. By employing these tools, the method effectively reduced the time traditionally required for collecting, organizing, and analyzing data related to parasitic infections. The following analysis highlights key aspects of the time-saving benefits observed, like the efficient data organization. The database design played a pivotal role in reducing time spent on data management. Structured across three key tables—questions, parasites, and data—the system allowed for streamlined storage, retrieval, and classification of information. The binary format used for recording results (1 for positive, 0 for negative) and the structured inclusion of questionnaire responses ensured minimal manual intervention during data entry and facilitated rapid query execution. Exporting data in CSV format further optimized integration with AI tools, eliminating the need for intermediate data formatting or manual preprocessing.

Also, we can identify the automation of analytical processes. By leveraging AI for analysis, the time-intensive manual evaluation of the 2,503 questionnaires was replaced with automated modeling. The algorithm efficiently processed 429 questionnaire items and parasite-related data (P001–P034) per participant. This approach drastically reduced the diagnostic timeline, which would otherwise involve labor-intensive tasks such as manual correlation and cross-referencing of variables. The SMOTE technique used for balancing data further highlights the sophistication of automation, as it corrected class imbalances without requiring manual data augmentation.

In a similar vein, we can highlight the optimized workflow for training and testing. The division of records into 90% training (2,254 records) and 10% testing (249 records) sets exemplifies a rational approach to model training. This split ensured that the algorithm could be trained on a large dataset while retaining sufficient data for validation, reducing the iteration time needed for model adjustments. Traditional methods of analysis might have required repeated cycles of sampling and validation, which the proposed workflow significantly streamlined.

At the same time, it is essential the reduction in error-driven iterations. The method's incorporation of metrics like Precision, Recall, and F1 Score allowed for rapid identification of model shortcomings, directing targeted optimizations. For example, insights into the model's performance for positive and negative cases of specific parasites (e.g., high recall for negative Toxocariasis cases but complete failure in positives) enabled focused troubleshooting without exhaustive manual evaluations.

we can also highlight the impact on diagnosis turnaround. While specific metrics for time reduction were not provided, the method's ability to handle large datasets and produce immediate diagnostic insights suggests a considerable reduction in turnaround time compared to traditional diagnostic approaches. In contexts such as clinical settings, where rapid diagnosis is critical, the efficiency of this system represents a transformative improvement.

The proposed method for diagnosing parasitic infections has demonstrated a significant reduction in time compared to traditional diagnostic approaches. This improvement is attributable to a combination of technological advancements and a well-structured data management system. The findings underscore the potential of this method to

revolutionize diagnostic workflows, particularly in scenarios requiring the analysis of large datasets or rapid decision-making.

One of the most critical contributors to the observed time efficiency is the automated data processing enabled by AI algorithms. By automating the analytical processes, the system eliminates the need for manual data review and correlation, drastically reducing the time required to generate insights. The AI models not only streamline the processing of complex datasets but also deliver consistent and scalable performance across the entire sample size of 2.503 questionnaires.

Another cornerstone of the method's efficiency is the streamlined and structured database design, which minimizes complexities in data management. The division into three fundamental tables—questions, parasites, and data—provides a logical framework that simplifies data storage, retrieval, and organization. This structure ensures that data handling is not only efficient but also error-resistant, allowing researchers to focus on higher-level analysis rather than administrative tasks.

A further enhancement to time efficiency is the elimination of manual preprocessing steps through binary coding and CSV integration. By encoding responses and results in a binary format (1 for positive, 0 for negative), the system reduces the potential for human error and expedites the transition from raw data to machine-readable formats. The export of this data as CSV files further facilitates seamless integration with AI algorithms, eliminating the need for intermediate formatting or adjustments.

The method also demonstrates proficiency in handling data imbalances using automated techniques like SMOTE. Correcting class imbalances is

a time-consuming task in traditional workflows, often requiring manual intervention to augment minority class data. SMOTE automates this process by generating synthetic examples, ensuring a more balanced dataset that allows for accurate algorithm training and validation. This automation not only enhances model performance but also saves substantial time that would otherwise be spent on data manipulation.

Lastly, the approach delivers faster diagnostic insights by leveraging real-time computation of model performance metrics. Metrics such as Precision, Recall, and F1 Score are computed dynamically, enabling researchers to identify areas of model improvement without extensive manual evaluations. This capability accelerates the iterative process of refining the model and ensures that diagnostic outcomes are available promptly, which is especially critical in clinical or high-stakes environments.

4.2.2. Limitations

The investigation into the application of AI for diagnosing parasitic infections has revealed several critical limitations that impact the study's conclusions, model reliability, and potential clinical applicability. These limitations span the scope of the dataset, the performance of the model, methodological constraints, and broader generalizability issues. An extended discussion of these limitations is provided below, along with their potential implications.

One of the most significant limitations is the pronounced imbalance in the dataset. Positive cases are underrepresented for certain parasitic infections, leading to a bias in model training and evaluation. For example, the model

completely failed to detect positive cases for Toxocariasis (precision and recall both 0%) and demonstrated extremely poor performance for Enterobiasis positives, with a recall of only 7% and an F1 score of 0.12. This imbalance skews the model's optimization toward the majority class (negative cases), which compromises its ability to identify minority class instances, often the primary objective in diagnostic contexts.

Moreover, the demographic composition of the dataset shows a predominance of female respondents (58.89% women versus 41.11% men), which may introduce additional biases. While the study excludes age as a variable, arguing it has minimal relevance to parasitic infections, this decision may overlook potentially meaningful demographic patterns. By not addressing these disparities, the dataset may fail to capture the variability needed for robust, generalizable insights.

The absence of sufficient records for certain parasites within the 2.503 analyzed questionnaires suggests limitations in sample diversity. This absence may result from the low prevalence of certain parasites in the sampled population or inadequate detection methods in the data collection process. Regardless of the cause, this limitation restricts the model's applicability to a narrower range of infections, reducing its utility in regions or populations where these parasites are more prevalent.

This issue is compounded by the reliance on synthetic data generation (via SMOTE) to address class imbalance. While SMOTE creates artificial examples to augment the minority class, these synthetic data points may not fully replicate the complexities and nuances of real-world cases, potentially limiting the model's robustness and contributing to overfitting during training.

The model demonstrated consistently poor performance in detecting positive cases across multiple parasitic infections, reflecting its bias toward the negative class. For example, a) The toxocariasis failed completely to identify positive cases (recall and precision 0%), b) The enterobiasis is very low recall for positives (7%), despite excellent metrics for negatives (recall 100%, precision 92%), c) In the case of the giardiasis, the higher recall for positives (95%) but relatively low precision (63%), indicating frequent false positives.

This inability to reliably detect true positives raises concerns about false negatives, which can have severe implications in medical diagnostics. False negatives mean undiagnosed infections, delayed treatment, and potentially worsened health outcomes. This limitation significantly undermines the model's utility in clinical or public health settings.

At the same time, the model's strong performance in detecting negative cases (for example, Toxocariasis negatives with precision 95% and recall 100%) highlights a structural bias toward the majority class. While this ensures accurate identification of uninfected individuals, it does little to fulfill the diagnostic goal of identifying infected patients. The overemphasis on the negative class is problematic in contexts where the primary concern is the detection of rare but critical positive cases.

Also, the database design, while optimized for efficiency, may introduce limitations in capturing nuanced relationships between variables. The reliance on binary encoding for questionnaire responses and diagnostic results (For example, the 1 for positive, 0 for negative) simplifies data representation but may obscure gradients or complexities in the data. For instance, responses with subtle variations or borderline cases may not be adequately reflected in the binary format, potentially leading to misclassification.

Furthermore, while the binary data and CSV export streamline AI integration, they limit the potential for richer feature engineering. Advanced preprocessing techniques could have allowed for the inclusion of derived variables, interaction terms, or multi-dimensional feature representations, which might have improved the model's performance and interpretability.

The exclusion of potentially relevant variables, such as age, geographic location, or socioeconomic status, limits the study's ability to contextualize findings. While age is deemed irrelevant for parasitic infections based on prior studies, other demographic or environmental factors could play critical roles in understanding parasite prevalence and transmission. Ignoring these variables risks oversimplifying the problem and reduces the model's applicability in diverse settings.

The study's findings may not generalize well to populations or settings outside the sample used. The reliance on a single dataset, with inherent biases and imbalances, constrains the ability to evaluate the model's effectiveness across different regions, demographic groups, or healthcare contexts. For instance, a dataset that includes underrepresented geographic regions, rural populations, or varying sanitary conditions could reveal different performance patterns and challenges.

While SMOTE was employed to address the dataset's imbalance, its use introduces limitations. Synthetic data may not fully encapsulate the complexity of real-world cases, leading to potential overfitting or reduced generalizability. Moreover, SMOTE does not address underlying issues in data collection, such as sampling biases or the need for better diagnostic instrumentation. This dependency underscores the need for improved data acquisition methods rather than over-reliance on post-hoc balancing techniques.

In a similar vein, the study does not provide evidence of the model's performance in real-world clinical settings, where diagnostic accuracy and timeliness are critical. Metrics such as recall and precision, while useful for evaluation, do not fully capture the practical challenges and consequences of deploying the model in healthcare environments. For example, high false negative rates in clinical diagnostics could lead to undiagnosed infections, while false positives might result in unnecessary treatments or interventions. Without clinical validation, the applicability of the model remains uncertain.

To address the limitations identified in this study, several targeted actions are recommended to improve the robustness, reliability, and applicability of the proposed diagnostic method. These recommendations focus on enhancing the dataset, refining preprocessing and model training techniques, and ensuring broader validation and applicability.

A priority should be to expand and diversify the dataset, particularly by collecting more data for underrepresented positive cases and rarely encountered parasites. This approach would reduce reliance on synthetic data generation techniques, such as SMOTE, which, while effective in addressing class imbalances, may not fully replicate the complexities of real-world cases. A richer, more representative dataset would enable the model to better capture diagnostic patterns and improve both training and evaluation outcomes.

Refining preprocessing techniques is another critical step. By incorporating richer feature representations and exploring alternative encoding methods, the system can capture more nuanced relationships between variables. For example, rather than relying solely on binary encoding, which may oversimplify data, employing advanced encoding techniques or engineered features could improve the model's ability to detect subtle patterns and relationships, particularly for difficult-to-classify cases.

Improvements in model training are also essential to enhance performance, especially for underrepresented positive cases. Algorithms designed to handle class imbalance, such as cost-sensitive learning or ensemble methods, should be explored to address the bias toward the negative class observed in the current results. Additionally, tuning classification thresholds to optimize sensitivity for positive cases would help reduce the high false-negative rates that compromise clinical utility.

A broader variable scope should be considered to increase the model's applicability and interpretability. Including additional demographic, environmental, and contextual factors (such as geographic location, socioeconomic status, and hygiene practices) could provide a more comprehensive understanding of parasite prevalence and transmission. This expanded scope would enhance the model's ability to adapt to different populations and settings.

Clinical validation is critical to test the model's performance in real-world healthcare scenarios. Evaluating the system under practical conditions would provide insights into its effectiveness in diagnosing infections and supporting clinical decision-making. Such validation would also highlight operational challenges, such as response times, integration with existing systems, and the impact of false negatives or false positives on patient outcomes.

Finally, improving sampling strategies would ensure that data collection encompasses a diverse range of populations, geographic regions, and health conditions. This diversity would enhance the model's generalizability and robustness, allowing it to perform reliably across different contexts and reduce biases introduced by narrowly defined datasets.

Therefore, while the study provides valuable insights into the potential of AI in diagnosing parasitic infections, its limitations underscore the need for further

refinement. Addressing these issues through expanded data collection, methodological improvements, and clinical validation will be essential for realizing the full potential of this technology in medical diagnostics.

While this study provides valuable insights into the capabilities and limitations of AI in diagnosing parasitic infections, it also underscores the need for refinement and further research. Addressing these limitations through enhanced data collection, methodological improvements, and real-world validation will be critical for transforming this promising technology into a robust and reliable tool for medical diagnostics.

4.2.3. Future research

The findings and discussions presented highlight critical areas where further research is necessary to enhance the efficacy, reliability, and applicability of AI in diagnosing parasitic infections. These areas encompass data quality, model performance, clinical integration, and broader ethical and societal concerns, each of which can significantly influence the success of AI-based diagnostic tools in healthcare.

One of the most critical challenges identified in the application of AI for diagnosing parasitic infections is the imbalance inherent in the training datasets. This issue is particularly evident in the underrepresentation of minority classes, such as positive cases of parasitic infections, which significantly impacts the model's ability to detect and accurately classify these instances. Models trained on imbalanced datasets often exhibit bias toward the majority class, leading to a higher rate of false negatives in scenarios where detecting positive cases is crucial. This limitation not only undermines the clinical utility of AI systems but

also poses a significant risk to patient outcomes by potentially delaying or missing necessary treatments.

To address this challenge, future research should prioritize targeted data collection strategies designed to capture a more representative and diverse range of cases. Efforts could include conducting focused sampling in endemic regions, where the prevalence of parasitic infections is higher, or within underrepresented populations that might not be adequately included in current datasets. By diversifying the data collection process, researchers can ensure that the datasets used for training AI models more accurately reflect the complexity and variability of real-world infection patterns.

In addition to improving data collection practices, advanced oversampling techniques such as the SMOTE should be further refined and applied. SMOTE works by generating high-quality synthetic examples of minority classes, effectively augmenting the dataset to provide the AI model with more balanced training data. This approach helps to mitigate the skewed class distribution and enhances the model’s ability to identify and classify positive cases more effectively.

Another promising avenue for improvement is the exploration of dynamic data augmentation techniques. These methods involve generating diverse synthetic datasets in real-time during the model training process. Dynamic augmentation can adapt to the evolving learning needs of the model, providing tailored synthetic examples that enhance robustness and improve the AI system's adaptability to various clinical scenarios. By employing these advanced techniques in combination with more representative data collection practices, future research can address the challenge of data imbalance comprehensively, paving the way for AI systems that are both accurate and reliable in detecting parasitic infections across diverse populations and settings.

Achieving an optimal balance between sensitivity and precision is a fundamental challenge in medical diagnostics, particularly in the context of AI. Both false positives and false negatives carry potentially severe implications. False negatives, where positive cases are missed, can delay necessary treatment, worsen patient outcomes, and increase the risk of disease transmission in the community. Conversely, false positives can lead to unnecessary treatments, financial burdens, and emotional stress for patients. Striking the right balance between these metrics is, therefore, essential to ensure the efficacy and reliability of AI-based diagnostic systems.

Future research should focus on exploring dynamic threshold optimization methods that allow AI models to adapt in real-time to the specific requirements of the diagnostic context. For example, in situations where the detection of positive cases is paramount (such as the early identification of parasitic infections to prevent outbreaks) models can be adjusted to prioritize sensitivity. This dynamic approach ensures that while the model identifies more positive cases, precision is not entirely sacrificed, maintaining an acceptable level of accuracy in its predictions. Such threshold adjustments could be tailored to specific scenarios, ensuring that the model remains flexible and context-aware.

In addition to threshold optimization, the development and application of hybrid models present a promising avenue for improving diagnostic accuracy. These models combine the predictive power of AI with traditional statistical methods, leveraging the strengths of both approaches. Traditional methods can provide stability and robustness to the predictions, while AI contributes advanced pattern recognition capabilities, particularly for complex datasets. The integration of these methodologies can result in a synergistic effect, producing models that are more accurate, reliable, and adaptable to diverse diagnostic challenges.

Furthermore, refining model architectures is a critical area for future exploration. Techniques such as ensemble learning, which combines multiple models to improve overall performance, and cost-sensitive algorithms, which assign different weights to errors based on their clinical significance, can effectively address class imbalance issues. These methods allow the model to focus more on minimizing critical errors, such as false negatives in medical diagnostics, thereby enhancing its overall utility.

By prioritizing these research directions, the field can advance toward creating diagnostic systems that are not only highly sensitive and precise but also adaptable to varying clinical needs. Such advancements would significantly improve the reliability of AI in healthcare, ensuring better outcomes for patients and greater confidence in these technologies among healthcare professionals.

The successful adoption of AI in clinical settings relies heavily on the trust and confidence of healthcare professionals, which, in turn, depends on their ability to understand and interpret the AI's decision-making processes. Without transparency and interpretability, even the most advanced AI systems may face resistance, as healthcare practitioners need clear explanations to validate the recommendations and ensure patient safety. This underscores the critical importance of focusing on explainable AI (XAI) in future research and development.

Efforts to enhance explainability should prioritize the creation of tools and methodologies that make AI outputs transparent and interpretable. Visualization tools that graphically represent the factors influencing AI decisions can offer healthcare providers an accessible way to understand complex data relationships. Similarly, rule-based explanatory models, which break down AI decisions into logical, step-by-step reasoning, can provide clarity, particularly in high-stakes medical scenarios. Interpretable neural network architectures,

designed to reveal the underlying mechanics of AI predictions, represent another promising approach to demystify the so-called “black box” of AI.

Equally important is the user-centric design of AI interfaces. Diagnostic results and insights generated by AI systems should be presented in a format that is intuitive, actionable, and tailored to the workflows of healthcare practitioners. This includes simplifying technical jargon, highlighting critical findings, and offering clear recommendations for next steps. Such design considerations not only enhance usability but also empower clinicians to make informed decisions with greater confidence in the system's reliability.

Establishing robust standards for auditing and validating AI decisions is another essential step in fostering trust and accountability. These standards should outline processes for assessing the accuracy, reliability, and fairness of AI systems, as well as mechanisms for identifying and addressing potential biases. Regular audits and validations will ensure that AI models perform consistently across diverse clinical settings and populations, providing healthcare professionals with an added layer of assurance.

If we focus on explainability, usability, and accountability, future research can address the barriers to AI adoption in clinical environments. Transparent and interpretable AI systems will not only improve diagnostic accuracy but also strengthen the partnership between technology and healthcare providers, ultimately enhancing patient care and safety.

The seamless integration of AI into clinical workflows is vital to fully harness its potential in improving healthcare outcomes. For AI systems to be truly impactful, they must blend effortlessly with existing processes, providing value to healthcare professionals without introducing unnecessary complexity or disruptions. This calls for a concerted research effort to develop AI-assisted

decision support systems that can deliver real-time insights and actionable recommendations tailored to the needs of clinical practice.

Future advancements should focus on creating systems capable of incorporating multi-modal data, combining patient history, environmental factors, genetic predispositions, and other relevant information. By synthesizing diverse data sources, AI models can achieve a richer contextual understanding, which is essential for producing more accurate and comprehensive diagnoses. For instance, integrating information about environmental exposures with genetic markers might help identify risk factors for certain diseases more effectively than relying on isolated datasets. This holistic approach would allow AI systems to provide nuanced insights, better reflecting the complexity of real-world medical conditions.

Collaboration will play a pivotal role in achieving this integration. Close cooperation between AI researchers, epidemiologists, and healthcare professionals is essential to design systems that not only address theoretical challenges but also meet the practical demands of clinical and public health settings. Epidemiologists can provide expertise in disease patterns and population health, while clinicians can offer critical feedback on how AI tools fit into their workflows and decision-making processes. These interdisciplinary partnerships will ensure that AI systems are both scientifically robust and operationally viable.

Additionally, it is crucial to align the development of AI systems with the realities of clinical practice and epidemiological contexts. AI tools must be adaptable to varying healthcare environments, from resource-limited settings to advanced medical institutions, ensuring their utility across diverse populations. Addressing these challenges requires iterative testing in real-world scenarios, allowing developers to refine AI systems based on feedback from end-users.

By focusing on real-time decision support, multi-modal data integration, and interdisciplinary collaboration, future research can pave the way for AI systems that are not only highly accurate but also practically indispensable. These advancements will ensure that AI becomes a trusted ally in clinical workflows, enhancing the quality of care, improving patient outcomes, and advancing public health on a global scale.

Standard metrics such as sensitivity, precision, and F1 Score, while foundational to evaluating AI models, often fall short of addressing the nuanced demands of medical diagnostics. These general metrics may not fully capture the complexity of real-world healthcare scenarios, where the stakes of false positives and false negatives can vary dramatically depending on the condition being diagnosed. To better meet the specific needs of medical applications, future research should prioritize the development and standardization of tailored evaluation metrics that emphasize patient safety, clinical relevance, and practical utility.

One promising direction involves redefining metrics like the F1 Score to better reflect the priorities of specific medical contexts. For example, weighted F1 Scores that assign greater importance to sensitivity in the case of life-threatening or highly transmissible infections could provide a more accurate and contextually appropriate measure of AI performance. By emphasizing sensitivity, such metrics ensure that the model prioritizes the identification of as many true positive cases as possible, even at the potential cost of slightly reduced precision. This approach is particularly valuable in applications like parasitic infection diagnostics, where missing a positive case could lead to severe patient outcomes and increased disease spread.

In addition to redefining metrics, the use of trade-off analyses between false positives and false negatives tailored to specific diseases could guide the

optimization of AI systems for diverse clinical applications. For instance, while minimizing false positives may be critical in conditions where unnecessary treatment poses significant risks or costs, prioritizing the reduction of false negatives is essential for infections that require prompt intervention. Tailoring the balance of these errors based on the specific implications of each condition ensures that AI systems are optimized for their intended medical use, maximizing their value and effectiveness in clinical practice.

Future efforts should also focus on standardizing these application-specific metrics across the field to facilitate consistency and comparability. By establishing clear benchmarks that align with the unique demands of various medical contexts, researchers and practitioners can more accurately evaluate and trust the performance of AI systems. Additionally, the development of tools and methodologies to incorporate these tailored metrics into model design and validation processes will be crucial in advancing the adoption of AI in healthcare.

Through the creation and adoption of customized evaluation metrics, researchers can ensure that AI systems are not only technically robust but also aligned with the practical realities of medical diagnostics. This approach will enable more effective and reliable deployment of AI technologies, ultimately improving patient outcomes and enhancing the role of AI as a transformative tool in modern healthcare.

In a similar vein, the AI models designed for medical diagnostics must demonstrate robustness and adaptability to function effectively across a wide range of epidemiological and clinical contexts. In healthcare, diseases manifest differently based on geographic, demographic, and environmental factors, making it essential for AI systems to account for such variability. To ensure their broad applicability and reliability, future research should prioritize the development of models trained on geographically and demographically diverse

datasets. This approach will help create AI systems that are not only accurate but also equitable, catering to the unique needs of various populations.

Training AI models on diverse datasets ensures that they are equipped to handle the complexities and nuances of different clinical scenarios. For instance, incorporating data from regions with varying prevalence rates of specific infections or from populations with differing genetic predispositions can enhance the model's ability to generalize its predictions. This strategy reduces the risk of bias, making the models more inclusive and effective in addressing global health challenges.

Integrating real-time learning capabilities into AI systems is another critical avenue for future research. Dynamic adaptability enables models to evolve with new data, allowing them to recognize emerging patterns of infection or adapt to sudden shifts in disease epidemiology, such as those caused by outbreaks or environmental changes. This real-time adaptability is particularly valuable in the context of rapidly evolving healthcare landscapes, where early recognition of new trends can significantly improve public health responses and patient outcomes.

Furthermore, the development of context-specific training methodologies can enhance the performance of AI models in diverse clinical environments. Tailoring model training to the specific conditions of a region or healthcare setting ensures that the AI system aligns with local diagnostic needs and practices. For example, models designed for resource-limited settings might focus on detecting diseases prevalent in those areas while being optimized to work with limited data and infrastructure. Conversely, models for advanced healthcare systems could leverage more sophisticated data inputs and analytics for precise, high-stakes diagnostics.

If we focus on geographic diversity, real-time learning, and context-specific training, future research can produce AI systems that are robust, adaptable, and ready to meet the diverse needs of global healthcare. These advancements will not only enhance the diagnostic capabilities of AI but also contribute to the development of equitable healthcare solutions that address the needs of underserved populations, ultimately improving health outcomes worldwide.

The ethical implications of AI in healthcare are a critical area of concern, particularly when addressing issues such as bias, accessibility, and patient privacy. These considerations are fundamental to ensuring that AI technologies are not only effective but also equitable and trustworthy. As AI becomes increasingly integrated into medical diagnostics, it is essential to prioritize research that identifies and mitigates biases, promotes accessibility, and safeguards patient data.

Bias in AI models poses a significant ethical challenge, as it can lead to inequitable diagnostic outcomes. For instance, models trained on datasets that disproportionately represent certain populations may fail to perform accurately for underrepresented groups, exacerbating existing healthcare disparities. Future research must focus on developing methods to identify and mitigate such biases. This includes strategies like diversifying training datasets to ensure representation across geographic, demographic, and socio-economic groups, as well as employing algorithms designed to detect and correct imbalances in the decision-making process. By addressing these biases, researchers can create AI systems that deliver equitable and reliable diagnostic results for all patients, regardless of their background.

Another vital ethical consideration is accessibility, particularly in resource-limited settings where the burden of parasitic infections is often highest. AI diagnostic tools must be designed to function effectively in these environments,

taking into account constraints such as limited internet connectivity, minimal infrastructure, and a lack of specialized healthcare professionals. Future advancements should aim to develop cost-effective, user-friendly AI systems that can operate offline and require minimal training, making them accessible to underserved communities. Ensuring accessibility not only improves health outcomes in these areas but also fosters global equity in the fight against parasitic diseases.

Protecting patient privacy is equally critical in the deployment of AI in healthcare. The collection and processing of sensitive medical data during AI training and implementation raise concerns about potential breaches of confidentiality and misuse of information. To address these risks, robust mechanisms for safeguarding patient data must be developed. This includes employing advanced encryption techniques, anonymizing datasets, and adhering to stringent privacy regulations such as the General Data Protection Regulation (GDPR). Transparent policies and practices for data handling are essential to building public trust and ensuring the ethical use of AI technologies in medical settings.

If we focus on mitigating bias, enhancing accessibility, and protecting patient privacy, future research can ensure that AI technologies are ethically sound and universally beneficial. Addressing these ethical challenges is not only a moral imperative but also a practical necessity for the widespread adoption of AI in healthcare. Such efforts will pave the way for AI systems that uphold the principles of fairness, inclusivity, and trust, ultimately improving healthcare delivery and patient outcomes on a global scale.

Extensive validation and benchmarking of AI models are essential to guarantee their reliability, effectiveness, and applicability in real-world clinical settings. While AI has shown immense potential in revolutionizing diagnostics, its

successful integration into routine medical practice depends on rigorous evaluation processes that assess its performance under diverse and practical conditions. Future research must prioritize comprehensive strategies to validate AI-based diagnostic tools, ensuring they meet the stringent demands of clinical reliability and accuracy.

A critical step in this process involves conducting large-scale clinical trials that compare the performance of AI-based diagnostics with traditional diagnostic methods. These trials should encompass a wide range of patient populations, capturing variability in demographic, geographic, and epidemiological factors. Such an approach ensures that AI models are not only effective for the specific datasets on which they were trained but also generalizable across diverse clinical scenarios. By directly comparing AI-driven tools with established diagnostic practices, researchers can identify areas where AI outperforms or complements traditional methods, as well as uncover potential limitations that require further refinement.

The establishment of international benchmarks and standardized datasets for parasitic infection diagnostics represents another pivotal avenue for advancing the field. Uniform benchmarks enable researchers and practitioners to evaluate AI models against a consistent set of performance criteria, ensuring comparability and reliability across different studies. Standardized datasets, curated to reflect the diversity and complexity of real-world cases, provide a common foundation for training and testing AI systems. These resources will not only accelerate the development of robust AI solutions but also foster collaboration and knowledge-sharing among researchers worldwide.

Moreover, benchmarking should extend beyond accuracy metrics, such as sensitivity and precision, to include practical considerations like computational efficiency, ease of implementation, and adaptability to various healthcare

settings. This holistic approach ensures that validated AI systems are not only technically robust but also feasible for deployment in diverse environments, including resource-limited settings where parasitic infections are most prevalent.

Validation and benchmarking are indispensable for the successful deployment of AI in clinical diagnostics. By conducting large-scale trials, establishing international benchmarks, and creating standardized datasets, future research can ensure that AI models are both scientifically rigorous and practically viable. These efforts will pave the way for reliable, scalable, and globally impactful AI-driven diagnostic solutions, significantly advancing the fight against parasitic infections and improving patient outcomes.

The AI holds transformative potential in revolutionizing the early detection and prevention of parasitic infections, addressing challenges that traditional diagnostic and surveillance methods often fail to meet. With its ability to process vast amounts of data and detect subtle patterns, AI offers a unique opportunity to shift the focus of healthcare from reactive treatment to proactive prevention. This is particularly crucial for parasitic infections, which can remain asymptomatic for extended periods before progressing to more severe stages, making early intervention vital.

Future research should prioritize the development of AI-powered screening tools specifically designed for asymptomatic cases. These tools could leverage machine learning algorithms to analyze complex data sets, including patient history, environmental exposures, and even genomic markers, to identify individuals at risk before clinical symptoms manifest. By focusing on early detection, these systems can enable timely medical interventions that prevent the progression of the disease and reduce transmission rates. Special attention should be given to tailoring these tools for use in endemic regions and among vulnerable populations, where the burden of parasitic infections is often highest.

This targeted approach would maximize the impact of AI technologies on the communities that need them most.

Another promising application of AI lies in predictive epidemiology models. By analyzing epidemiological, environmental, and socio-economic data, AI can uncover trends and potential outbreak patterns that might otherwise go unnoticed. These predictive models could be used to forecast infection hotspots, identify emerging threats, and evaluate the potential impact of various intervention strategies. Such insights would allow public health authorities to allocate resources more effectively, deploying targeted preventive measures such as mass drug administration, vector control programs, or public awareness campaigns in areas at greatest risk.

The integration of AI in early detection and prevention strategies can also enhance the global response to parasitic infections. Collaborative research efforts should aim to create shared platforms and databases that consolidate information from diverse regions, enabling AI models to learn from and adapt to varied epidemiological contexts. This global perspective would not only improve the accuracy and relevance of AI-driven predictions but also foster international cooperation in combating parasitic diseases.

If we focus on the development of proactive screening tools and predictive epidemiology models, future research can harness the full potential of AI to combat parasitic infections more effectively. These advancements will not only improve early detection and intervention but also contribute to the broader goals of disease prevention and public health management, reducing the global burden of parasitic diseases and improving the quality of life for affected populations.

The future of the AI in diagnosing parasitic infections rests on overcoming current challenges while leveraging its transformative potential to enhance

healthcare. To achieve this, researchers must address key areas such as data quality, diagnostic sensitivity and precision, model explainability, and seamless integration into clinical workflows. By focusing on these priorities, the reliability and utility of AI-driven diagnostic tools can be significantly enhanced, positioning them as indispensable assets in the fight against parasitic diseases.

One critical area for future research is improving the quality and representativeness of the data used to train AI models. Diverse and comprehensive datasets, encompassing a wide range of geographic regions, demographic groups, and infection patterns, are essential for developing robust models capable of accurate diagnostics across various clinical contexts. Efforts to reduce data imbalances and ensure that both minority and majority classes are well-represented will be pivotal in addressing the shortcomings of existing AI systems.

Another vital focus is optimizing the sensitivity and precision of AI diagnostics. False negatives can result in missed diagnoses and delayed treatments, while false positives can lead to unnecessary interventions and increased patient anxiety. Future advancements should aim to strike an optimal balance between these metrics, tailoring AI models to the specific demands of parasitic infection diagnostics. Techniques such as dynamic threshold adjustments, cost-sensitive learning algorithms, and hybrid models that combine AI with traditional diagnostic approaches hold promise in achieving this balance.

At the same time, the explainability is another cornerstone of future AI development in healthcare. To gain widespread acceptance among clinicians and healthcare providers, AI models must be transparent and interpretable. This entails developing XAI systems that provide clear insights into the decision-making process, empowering medical professionals to trust and effectively utilize

these tools. User-centric design, coupled with rigorous validation and auditing processes, will further strengthen confidence in AI technologies.

Equally important is the seamless integration of AI into clinical workflows. For AI systems to maximize their impact, they must align with existing healthcare practices and be designed with the end-user in mind. This requires close collaboration between AI researchers, clinicians, and healthcare administrators to ensure that these tools address real-world challenges and enhance operational efficiency. Integration efforts should also prioritize accessibility, particularly in resource-limited settings where parasitic infections are most prevalent.

Addressing ethical considerations and validating AI models in real-world settings are essential steps toward their broader acceptance and effectiveness. Transparent mechanisms for safeguarding patient privacy, mitigating biases, and ensuring equitable diagnostic outcomes are critical to fostering trust in these technologies. Rigorous validation processes, including large-scale clinical trials and the establishment of international benchmarks, will provide the evidence needed to support the adoption of AI in clinical practice.

By advancing research in these directions, the field can unlock the full potential of AI to revolutionize the diagnosis and management of parasitic infections. These efforts collectively pave the way for a future where AI serves as a central tool in combating these diseases, improving patient outcomes, and advancing global healthcare. The integration of AI into medical diagnostics holds the promise of not only enhancing efficiency and accuracy but also addressing disparities and fostering equity in healthcare delivery worldwide.

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INDEX

APPENDICES

Appendix 1. List of parasites

Parasites	Disease
Amebiasis	Entamoeba histolytica infection
Ancylostomiasis	Ancylostoma duodenale infection
Ascariasis	Ascaris lumbricoides infection
Aspergillosis	Aspergillus spp. Infection
Blastocystosis	Blastocystis spp. Infection
Candidiasis	Candida spp. Infection
Clonochiasis	Clonorchis sinensis infection
Cysticercosis	Neurocysticercosis / neuro- taenia infection
Dientamebiasis	Dientamoeba fragilis infection
Diphyllobothriasis	Diphyllobothrium latum infection
Dirofilariasis	Dirofilaria spp. Infection
Dipylidiosis	Dipylidium caninum infection
Dysbiosis	Dysbiosis
Echinococcosis	Echinococcus spp. Infection

Enfolimax	Endolimax nana infection
Entamebiasis	Entamoeba coli infection
Enterobiasis	Enterobius vermicularis infecti
Facioliasis	Fasciola hepatica infection
Fasciolopsiasis	Fasciolopsis buski infection
Filariasis	Filariaidae spp infection
Hymenolepiasis	Hymenolepis nana infection
Opisthorchiasis	Opisthorichis felines infection
Paragonimiasis	Paragonimus weternami infection
Schistosomiasis	Schitosoma mansoni, S. haematobium or S. Japonicum infection
SIBO	Samal intestinal bacterial overgrowth
Strongyloidiasis	Strongyloides stercoralis infection
Taeniasis	Indefinite taenia / tapeworm infection
Toxoplasmosis	Tocoplasma gondii infection
Trichinellosis / Trichinosis	Trichinella spp. Infection
Trichomoniasis	Trichomonas spp. Infection

Appendix 2. Table of assessment

Item	P (1)	NP (0)	R (1)	NR (0)
1. Male / Female				
2. Pale or sallow complexion				
3. Circles and bags under the eyes				
4. Psoriasis-type skin disorders				
5. Boils				
6. Granulomas or lipomas				
7. Seborrhoeic dermatitis and/or cradle cap				
8. Itching and/or atopic dermatitis symptoms				
9. Cracked heels				
10. Flaking nails				
11. Brittle nails				
12. Intestinal noise				
13. Constipation				
14. Diarrhoea				
15. Hyperactivity				
16. Drowsiness/apathy				
17. Sweet cravings, sugar cravings				
18. Anxiety about flour				

19. **Anxiety about sweet desserts derived from milk or dairy products or cheese**
20. **Regular headaches (headaches)**
21. **Anal and genital itching at night or during the day**
22. **Grinding teeth at night or possibly during the day as well**
23. **Sleep disturbances (short or long sleep) and/or nightmares**
24. **Dyspnoea, bronchial obstruction, chronic or recurrent bronchial asthma type cough**
25. **Chronic cold**
26. **Adenoid hypertrophy (the third tonsil)**
27. **Recurrent urinary tract infections**
28. **Recurrent mycosis of the genitourinary system**
29. **Blinking**
30. **Lip trembling**
31. **Shake your head**
32. **Nystagmus**
33. **Frowning**
34. **Shrugging**
35. **Onychophagia (nail biting)**

36. The habit of sucking fingers or putting objects in the mouth
37. Habit of moving hands or fingers in the area around the mouth
38. Throat clearing and/or expectoration (in the absence of infection or allergy)
39. Rhinorrhoea (runny nose in the absence of infection or allergy)
40. The habit of involuntarily saying some words or sounds
41. **The habit of washing hands every few minutes**
42. Scratching or maneuvering hands in the anal area
43. Dwarfism or stunting/slow growth
44. **Emaciation, obesity or low body mass weight growth**
45. Premature ageing
46. **The length of the fingers is shorter than the length of the palm of the hand.**
47. The ring finger is longer than the middle finger.
48. Recurrent or difficult to treat anaemia due to iron deficiency
49. **Vitamin B12 deficiency anaemia**

50. Infertility
51. Loss of libido
52. Bipolar disorder
53. Schizophrenia
54. Depression
55. Anorexia
56. Bulimia
57. **ADD**
58. ADHD
59. **Autism**
60. Asperger Syndrome
61. PANDAS
62. PANS
63. PITAND
64. Tourette Syndrome
65. Epilepsy
66. Dementia
67. Pathological jealousy
68. Cranial nerve palsies
69. Eyesight disorders
70. Retinitis

71. **Choroiditis**
72. **Nocturnal enuresis**
73. **Psychomotor developmental delay**
74. **Childhood onanism**
75. **Leucorrhoea (Vaginal discharge)**
76. **Irregular or frequent menstruation**
77. **Menorrhagia (heavy menstrual bleeding)**
78. **Uterine myoma**
79. **Cervical carcinoma (non-HPV)**
80. **Fibrocystic mastopathy**
81. **Prostate pain/inflation**
82. **Testicular pain/swelling**
83. **Breast enlargement (gynaecomastia)**
84. **Do you have dogs, cats, rabbits, hamsters, guinea pigs, chinchillas and/or gerbils?**
85. **Do your symptoms appear or intensify with the new or full moon?**
86. **Have you consumed grilled meat or other non-tested beef or pork?**
87. **Have you consumed grilled venison or other venison that has not been tested?**
88. **Have you travelled in Africa, Asia and/or South or Central America?**

89. You live in wetland areas, floodplains or areas that flood
90. You put your fingers in your mouth or bite your nails
91. You have a sandpit for children or have used one in a public area.
92. You drink unboiled tap or well water
93. You have consumed seafood or raw fish dishes such as sushi.
94. Have you consumed vegetables fertilized with sludge or compost?
95. You have eaten vegetables or fruits straight from the ground or from the tree without washing them.
96. You have consumed unwashed blackberries
97. One of your family members has been diagnosed with a parasitic disease or has other similar symptoms.
98. In the city or area where you live there are many people infected with parasites.
99. You have a habit of sucking blades of grass and eating sorrel.
100. Your children have played in ball pools
101. Do you use public swimming pools and hot tubs on a regular basis?

102. Do you benefit from public rehabilitation?
103. Have you been diagnosed as an allergological group?
104. Have you been diagnosed as a haematological group?
105. Have you been diagnosed as a gastroenterological group?
106. Have you been diagnosed as a lung group?
107. Have you been diagnosed as a dermatological group?
108. Have you been diagnosed as a cancer group?
109. Have you been diagnosed as an immune group?
110. Have you been diagnosed as a neurological group?
111. Have you been diagnosed as a urological group?
112. Have you been diagnosed as a gynaecological group?

Appendix 3. Content Validity Index (CVI) for all the instrument

Item	RI	PI	RS
1. Male / Female	0.81	1	1
2. Pale or sallow complexion	0.81	1	0.83
3. Circles and bags under the eyes	0.81	0.90	0.859
4. Psoriasis-type skin disorders	1	1	0.835
5. Boils	1	1	0.84
6. Granulomas or lipomas	1	1	1
7. Seborrhoeic dermatitis and/or cradle cap	0.81	0.81	1
8. Itching and/or atopic dermatitis symptoms	0.90	0.90	0.914
9. Cracked heels	0.90	0.90	0.927
10. Flaking nails	0.81	1	1
11. Brittle nails	0.81	1	1
12. Intestinal noise	1	1	0.835
13. Constipation	0.81	1	0.83
14. Diarrhoea	1	1	0.84
15. Hyperactivity	0.9	0.9	1
16. Drowsiness/apathy	0.9	0.90	0.927
17. Sweet cravings, sugar cravings	0.81	1	0.83
18. Anxiety about flour	0.81	0.90	0.859

19. Anxiety about sweet desserts derived from milk or dairy products or cheese	0.81	0.81	1
20. Regular headaches (headaches)	0.81	0.90	0.859
21. Anal and genital itching at night or during the day	0.81	1	0.83
22. Grinding teeth at night or possibly during the day as well	0.9	0.90	0.928
23. Sleep disturbances (short or long sleep) and/or nightmares	1	1	0.84
24. Dyspnoea, bronchial obstruction, chronic or recurrent bronchial asthma type cough	0.81	1	1
25. Chronic cold	0.81	1	0.83
26. Adenoid hypertrophy (the third tonsil)	0.9	0.90	0.927
27. Recurrent urinary tract infections	0.9	0.90	0.928
28. Recurrent mycosis of the genitourinary system	0.9	0.9	1
29. Blinking	1	1	0.835
30. Lip trembling	0.81	1	0.83
31. Shake your head	0.9	0.9	1
32. Nystagmus	0.81	1	1
33. Frowning	0.81	0.90	0.859
34. Shrugging	0.81	1	0.83
35. Onychophagia (nail biting)	0.81	0.81	1
36. The habit of sucking fingers or putting objects in the mouth	0.81	1	1

37. Habit of moving hands or fingers in the area around the mouth	0.81	0.90	0.859
38. Throat clearing and/or expectoration (in the absence of infection or allergy)	1	1	0.835
39. Rhinorrhoea (runny nose in the absence of infection or allergy)	0.9	0.90	0.927
40. The habit of involuntarily saying some words or sounds	0.9	0.81	0.928
41. The habit of washing hands every few minutes	0.81	0.81	1
42. Scratching or maneuvering hands in the anal area	0.81	1	0.83
43. Dwarfism or stunting/slow growth	0.9	0.9	1
44. Emaciation, obesity or low body mass weight growth	0.81	1	1
45. Premature ageing	0.81	0.90	0.859
46. The length of the fingers is shorter than the length of the palm of the hand.	0.81	1	1
47. The ring finger is longer than the middle finger.	0.81	1	0.83
48. Recurrent or difficult to treat anaemia due to iron deficiency	0.9	0.90	0.928
49. Vitamin B12 deficiency anaemia	0.9	0.9	1
50. Infertility	1	0.90	1
51. Loss of libido	0.9	0.90	0.927

52. Bipolar disorder	0.90	0.90	0.914
53. Schizophrenia	1	0.90	1
54. Depression	0.81	0.90	0.859
55. Anorexia	1	1	0.835
56. Bulimia	0.81	1	0.83
57. ADD	0.81	1	0.83
58. ADHD	0.90	0.90	0.914
59. Autism	0.9	0.9	1
60. Asperger Syndrome	1	1	0.84
61. PANDAS	0.9	0.90	0.928
62. PANS	0.9	0.90	0.927
63. PITAND	0.81	1	1
64. Tourette Syndrome	1	1	0.835
65. Epilepsy	0.81	1	0.83
66. Dementia	0.81	0.90	0.859
67. Pathological jealousy	0.9	0.81	0.928
68. Cranial nerve palsies	0.81	0.81	1
69. Eyesight disorders	0.81	1	0.83
70. Retinitis	0.9	0.9	1
71. Choroiditis	1	0.90	1
72. Nocturnal enuresis	0.81	1	1

73. Psychomotor developmental delay	0.90	0.90	0.914
74. Childhood onanism	0.81	1	1
75. Leucorrhoea (Vaginal discharge)	0.81	0.90	0.859
76. Irregular or frequent menstruation	0.81	0.91	1
77. Menorrhagia (heavy menstrual bleeding)	0.81	1	0.83
78. Uterine myoma	0.81	1	1
79. Cervical carcinoma (non-HPV)	1	0.90	1
80. Fibrocystic mastopathy	1	0.90	1
81. Prostate pain/inflation	0.81	1	0.83
82. Testicular pain/swelling	0.81	0.90	0.859
83. Breast enlargement (gynaecomastia)	0.9	0.81	0.928
84. Do you have dogs, cats, rabbits, hamsters, guinea pigs, chinchillas and/or gerbils?	0.81	0.81	1
85. Do your symptoms appear or intensify with the new or full moon?	0.81	1	1
86. Have you consumed grilled meat or other non-tested beef or pork?	0.81	1	0.83
87. Have you consumed grilled venison or other venison that has not been tested?	0.81	0.90	0.859
88. Have you travelled in Africa, Asia and/or South or Central America?	0.81	1	1
89. You live in wetland areas, floodplains or areas that flood	0.9	0.90	0.927

90. You put your fingers in your mouth or bite your nails	1	0.90	1
91. You have a sandpit for children or have used one in a public area.	0.9	0.9	1
92. You drink unboiled tap or well water	0.81	0.90	0.859
93. You have consumed seafood or raw fish dishes such as sushi.	0.81	1	0.83
94. Have you consumed vegetables fertilized with sludge or compost?	1	1	0.84
95. You have eaten vegetables or fruits straight from the ground or from the tree without washing them.	0.81	0.91	1
96. You have consumed unwashed blackberries	0.90	0.90	0.914
97. One of your family members has been diagnosed with a parasitic disease or has other similar symptoms.	0.9	0.9	1
98. In the city or area where you live there are many people infected with parasites.	1	1	0.84
99. You have a habit of sucking blades of grass and eating sorrel.	0.9	0.90	0.927
100. Your children have played in ball pools	0.81	1	1
101. Do you use public swimming pools and hot tubs on a regular basis?	0.81	0.90	0.859
102. Do you benefit from public rehabilitation?	0.9	0.9	1
103. Have you been diagnosed as an allergological group?	0.81	1	0.83

104. Have you been diagnosed as a haematological group?	0.9	0.81	0.928
105. Have you been diagnosed as a gastroenterological group?	1	0.90	1
106. Have you been diagnosed as a lung group?	0.81	1	1
107. Have you been diagnosed as a dermatological group?	0.81	0.90	0.859
108. Have you been diagnosed as a cancer group?	0.81	0.81	1
109. Have you been diagnosed as an immune group?	0.9	0.90	0.927
110. Have you been diagnosed as a neurological group?	1	1	0.835
111. Have you been diagnosed as a urological group?	0.81	1	0.83
112. Have you been diagnosed as a gynaecological group?	1	1	0.835

Appendix 4. Informed consent (adults)

INFORMED CONSENT

Mr/Mrs ....., of legal age, ... years of age, hereby declare that I have been informed about the study, “The use of Artificial Intelligence (AI) combined with the traditional author designed questionnaires, coproscopic and serological methods as a valuable tool in diagnosing intestinal human parasites”, conducted by researcher Montserrat Gras Graupera, Faculty of Natural Health Science at Selinus University of Sciences and Literature

1. I have received sufficient information about the study.
2. I have been able to ask as many questions about the study as I felt appropriate and have had them answered satisfactorily.
3. I understand that my participation is voluntary.
4. I understand that I may withdraw from the study and revoke this consent:
   a) I At any time
   b) Without having to give any explanation and without any consequences of any kind.

I have also been informed that my personal data will be protected and subject to the guarantees provided for in the applicable law and that my data will NEVER be passed on to third parties or institutions.

Taking this into consideration, I hereby GIVE my CONSENT to participate in this study for the specified purposes.

Signature of participant: Signature of researcher:

Name and date: Name and date:

Appendix 5. Informed consent (children and adolescents)

INFORMED CONSENT

Mr/Mrs ....., father, mother or legal tutor of the minor, ..., hereby declare that I have been informed about the study, “The use of Artificial Intelligence (AI) combined with the traditional author designed questionnaires, coproscopic and serological methods as a valuable tool in diagnosing intestinal human parasites”, conducted by researcher Montserrat Gras Graupera, Faculty of Natural Health Science at Selinus University of Sciences and Literature

I have received sufficient information about the study.

1. I have been able to ask as many questions about the study as I felt appropriate and have had them answered satisfactorily.
2. I understand that the participation of my daughter / son is voluntary.
3. I understand that I may withdraw my daughter / son from the study and revoke this consent:
   c) At any time
   d) Without having to give any explanation and without any consequences of any kind.

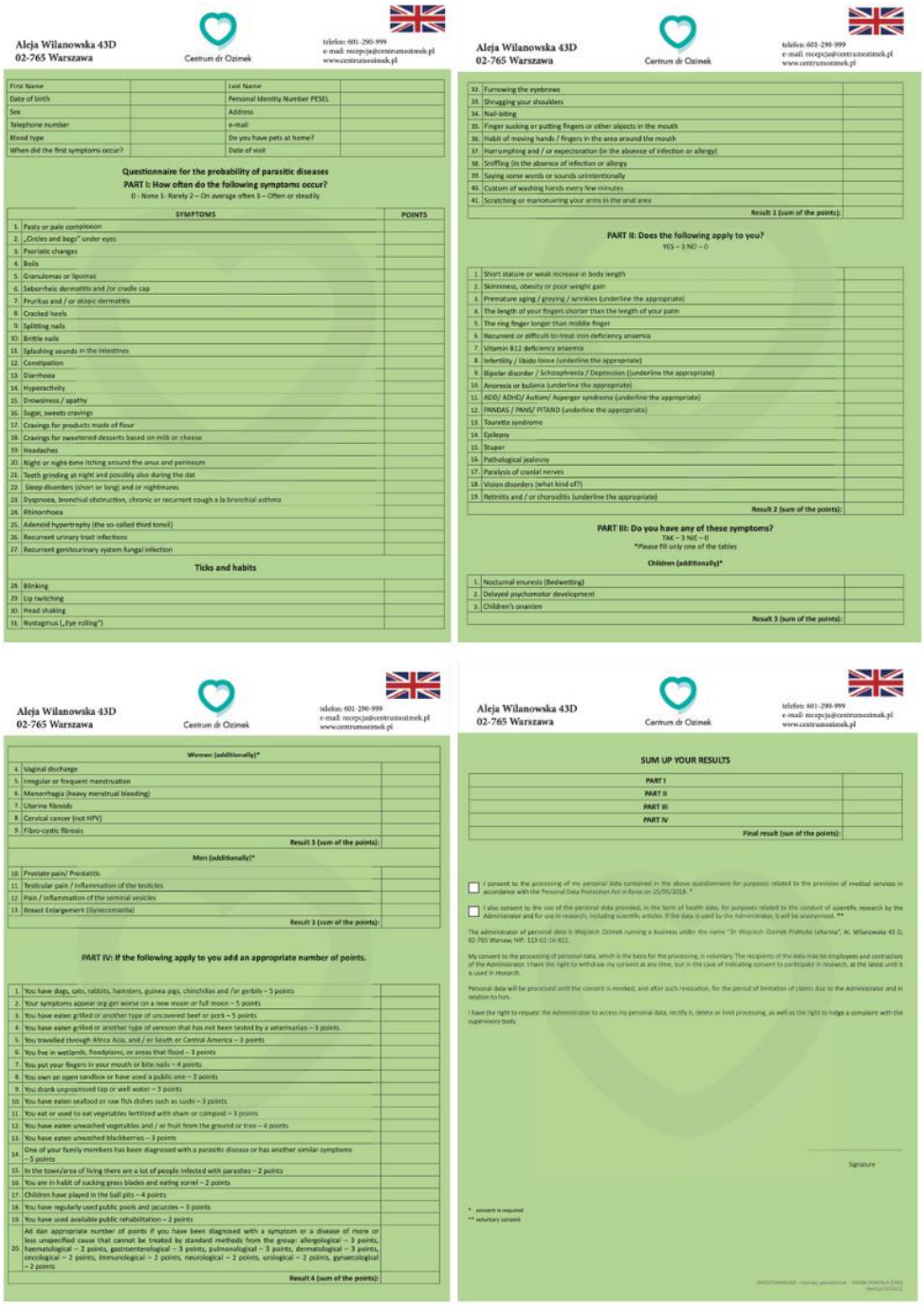
I have also been informed that my personal data will be protected and subject to the guarantees provided for in the applicable law and that my daughter / son data’s will NEVER be passed on to third parties or institutions.

Taking this into consideration, I hereby GIVE my CONSENT to my daughter / son participate in this study for the specified purposes.

Signature of legal tutor of the minor: Signature of researcher:

Name and date: Name and date:

Appendix 6. Patient's questionnaire (adults)



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First Name		Last Name	
Date of birth		Personal Identity Number PESEL	
Sex		Address	
Telephone number		e-mail	
Blood type		Do you have pets at home?	
When did the first symptoms occur?		Date of visit	

Questionnaire for the probability of parasitic diseases

PART I: How often do the following symptoms occur?

0 - None 1- Rarely 2 – On average often 3 – Often or steadily

	SYMPTOMS	POINTS
1.	Pasty or pale complexion	
2.	„Circles and bags" under eyes	
3.	Psoriatic changes	
4.	Boils	
5.	Granulomas or lipomas	
6.	Seborrheic dermatitis and /or cradle cap	
7.	Pruritus and / or atopic dermatitis	
8.	Cracked heels	
9.	Splitting nails	
10.	Brittle nails	
11.	Splashing sounds in the intestines	
12.	Constipation	
13.	Diarrhoea	
14.	Hyperactivity	
15.	Drowsiness / apathy	
16.	Sugar, sweets cravings	
17.	Cravings for products made of flour	
18.	Cravings for sweetened desserts based on milk or cheese	
19.	Headaches	
20.	Night or night-time itching around the anus and perineum	
21.	Teeth grinding at night and possibly also during the dat	
22.	Sleep disorders (short or long) and or nightmares	
23.	Dyspnoea, bronchial obstruction, chronic or recurrent cough a la bronchial asthma	
24.	Rhinorrhoea	
25.	Adenoid hypertrophy (the so-called third tonsil)	
26.	Recurrent urinary tract infections	
27.	Recurrent genitourinary system fungal infection	
	Ticks and habits	
28.	Blinking	
29.	Lip twitching	
30.	Head shaking	
31.	Nystagmus („Eye rolling")	

32.	Furrowing the eyebrows	
33.	Shrugging your shoulders	
34.	Nail-biting	
35.	Finger sucking or putting fingers or other objects in the mouth	
36.	Habit of moving hands / fingers in the area around the mouth	
37.	Harrumphing and / or expectoration (in the absence of infection or allergy)	
38.	Sniffling (in the absence of infection or allergy	
39.	Saying some words or sounds unintentionally	
40.	Custom of washing hands every few minutes	
41.	Scratching or manoeuvring your arms in the anal area	
	Result 1 (sum of the points):	

PART II: Does the following apply to you?

YES – 3 NO – 0

1.	Short stature or weak increase in body length	
2.	Skininess, obesity or poor weight gain	
3.	Premature aging / greying / wrinkles (underline the appropriate)	
4.	The length of your fingers shorter than the length of your palm	
5.	The ring finger longer than middle finger	
6.	Recurrent or difficult-to-treat iron deficiency anaemia	
7.	Vitamin B12 deficiency anaemia	
8.	Infertility / libido loose (underline the appropriate)	
9.	Bipolar disorder / Schizophrenia / Depression (underline the appropriate)	
10.	Anorexia or bulimia (underline the appropriate)	
11.	ADD/ ADHD/ Autism/ Asperger syndrome (underline the appropriate)	
12.	PANDAS / PANS/ PITAND (underline the appropriate)	
13.	Tourette syndrome	
14.	Epilepsy	
15.	Stupor	
16.	Pathological jealousy	
17.	Paralysis of cranial nerves	
18.	Vision disorders (what kind of?)	
19.	Retinitis and / or choroiditis (underline the appropriate)	
	Result 2 (sum of the points):	

PART III: Do you have any of these symptoms?

TAK – 3 NIE – 0

*Please fill only one of the tables

Children (additionally)*

1.	Nocturnal enuresis (Bedwetting)	
2.	Delayed psychomotor development	
3.	Children's onanism	
	Result 3 (sum of the points):	

	Women (additionally)*	
4.	Vaginal discharge	
5.	Irregular or frequent menstruation	
6.	Menorrhagia (heavy menstrual bleeding)	
7.	Uterine fibroids	
8.	Cervical cancer (not HPV)	
9.	Fibro-cystic fibrosis	
	Result 3 (sum of the points):	
	Men (additionally)*	
10.	Prostate pain/ Prostatitis	
11.	Testicular pain / Inflammation of the testicles	
12.	Pain / inflammation of the seminal vesicles	
13.	Breast Enlargement (Gynecomastia)	
	Result 3 (sum of the points):	

PART IV: If the following apply to you add an appropriate number of points.

1.	You have dogs, cats, rabbits, hamsters, guinea pigs, chinchillas and /or gerbils – 5 points	
2.	Your symptoms appear/ org get worse on a new moon or full moon – 5 points	
3.	You have eaten grilled or another type of uncovered beef or pork – 5 points	
4.	You have eaten grilled or another type of venison that has not been tested by a veterinarian – 3 points	
5.	You travelled through Africa /usa, and / or South or Central America – 3 points	
6.	You live in wetlands, floodplains, or areas that flood – 3 points	
7.	You put your fingers in your mouth or bite nails – 4 points	
8.	You own an open sandbox or have used a public one – 3 points	
9.	You drank unprocessed tap or well water – 3 points	
10.	You have eaten seafood or raw fish dishes such as sushi – 3 points	
11.	You eat or used to eat vegetables fertilized with sham or compost – 3 points	
12.	You have eaten unwashed vegetables and / or fruit from the ground or tree – 4 points	
13.	You have eaten unwashed blackberries – 3 points	
14.	One of your family members has been diagnosed with a parasitic disease or has another similar symptoms – 5 points	
15.	In the town/area of living there are a lot of people infected with parasites – 2 points	
16.	You are in habit of sucking grass blades and eating sorrel – 2 points	
17.	Children have played in the ball pits – 4 points	
18.	You have regularly used public pools and jacuzzies – 3 points	
19.	You have used available public rehabilitation – 2 points	
20.	Ad dan appropriate number of points if you have been diagnosed with a symptom or a disease of more or less unspecified cause that cannot be treated by standard methods from the group: allergological – 3 points, haematological – 2 points, gastroenterological – 3 points, pulmonological – 3 points, dermatological – 3 points, oncological – 2 points, immunological – 2 points, neurological – 2 points, urological – 2 points, gynaecological – 2 points	
	Result 4 (sum of the points):	

SUM UP YOUR RESULTS

PART I	
PART II	
PART III	
PART IV	
Final result (sum of the points):	

☐ I consent to the processing of my personal data contained in the above questionnaire for purposes related to the provision of medical services in accordance with the Personal Data Protection Act in force on 25/05/2018. *

☐ I also consent to the use of the personal data provided, in the form of health data, for purposes related to the conduct of scientific research by the Administrator and for use in research, including scientific articles. If the data is used by the Administrator, it will be anonymised. **

The administrator of personal data is Wojciech Ozimek running a business under the name "Dr Wojciech Ozimek Praktyka Lekarska", Al. Wilanowska 43 D, 02-765 Warsaw, NIP: 113-02-16-822.

My consent to the processing of personal data, which is the basis for the processing, is voluntary. The recipients of the data may be employees and contractors of the Administrator. I have the right to withdraw my consent at any time, but in the case of indicating consent to participate in research, at the latest until it is used in research.

Personal data will be processed until the consent is revoked, and after such revocation, for the period of limitation of claims due to the Administrator and in relation to him.

I have the right to request the Administrator to access my personal data, rectify it, delete or limit processing, as well as the right to lodge a complaint with the supervisory body.

Signature

* consent is required
** voluntary consent

Appendix 7. Patient's questionnaire (children and adolescents)

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First Name		Last Name	
Date of birth		Personal Identity Number PESEL	
Sex		Address	
Telephone number		e-mail	
Blood type		Do you have pets at home?	
When did the first symptoms occur?		Date of visit	

Questionnaire for the probability of parasitic diseases
PART I: How often do the following symptoms occur?
0 - None 1- Rarely 2 – On average often 3 – Often or steadily

	SYMPTOMS	POINTS
1.	Pasty or pale complexion	
2.	„Circles and bags" under eyes	
3.	Psoriatic changes	
4.	Boils	
5.	Granulomas or lipomas	
6.	Seborrheic dermatitis and /or cradle cap	
7.	Pruritus and / or atopic dermatitis	
8.	Cracked heels	
9.	Splitting nails	
10.	Brittle nails	
11.	Splashing sounds in the intestines	
12.	Constipation	
13.	Diarrhoea	
14.	Hyperactivity	
15.	Drowsiness / apathy	
16.	Sugar, sweets cravings	
17.	Cravings for products made of flour	
18.	Cravings for sweetened desserts based on milk or cheese	
19.	Headaches	
20.	Night or night-time itching around the anus and perineum	
21.	Teeth grinding at night and possibly also during the dat	
22.	Sleep disorders (short or long) and or nightmares	
23.	Dyspnoea, bronchial obstruction, chronic or recurrent cough a lo bronchial asthma	
24.	Rhinorrhoea	
25.	Adenoid hypertrophy (the so-called third tonsil)	
26.	Recurrent urinary tract infections	
27.	Recurrent genitourinary system fungal infection	
	Ticks and habits	
28.	Blinking	
29.	Lip-twitching	
30.	Head shaking	
31.	Nystagmus („Eye rolling")	

32.	Furrowing the eyebrows	
33.	Shrugging your shoulders	
34.	Nail-biting	
35.	Finger sucking or putting fingers or other objects in the mouth	
36.	Habit of moving hands / fingers in the area around the mouth	
37.	Harrumphing and / or expectoration (in the absence of infection or allergy)	
38.	Sniffing (in the absence of infection or allergy	
39.	Saying some words or sounds unintentionally	
40.	Custom of washing hands every few minutes	
41.	Scratching or manoeuvring your arms in the anal area	
	Result 1 (sum of the points):	

PART II: Does the following apply to you?
YES – 3 NO – 0

1.	Short stature or weak increase in body length	
2.	Skinniness, obesity or poor weight gain	
3.	Premature aging / greying / wrinkles (underline the appropriate)	
4.	The length of your fingers shorter than the length of your palm	
5.	The ring finger longer than middle finger	
6.	Recurrent or difficult-to-treat iron deficiency anaemia	
7.	Vitamin B12 deficiency anaemia	
8.	Infertility / libido loose (underline the appropriate)	
9.	Bipolar disorder / Schizophrenia / Depression ((underline the appropriate)	
10.	Anorexia or bulimia (underline the appropriate)	
11.	ADD/ ADHD/ Autism/ Asperger syndrome (underline the appropriate)	
12.	PANDAS / PANS/ PITAND (underline the appropriate)	
13.	Tourette syndrome	
14.	Epilepsy	
15.	Stupor	
16.	Pathological jealousy	
17.	Paralysis of cranial nerves	
18.	Vision disorders (what kind of?)	
19.	Retinitis and / or choroiditis (underline the appropriate)	
	Result 2 (sum of the points):	

PART III: Do you have any of these symptoms?
YAE – 3 N/E – 0
*Please fill only one of the tables

Children (additionally)*

1.	Nocturnal enuresis (Bedwetting)	
2.	Delayed psychomotor development	
3.	Children's onanism	
	Result 3 (sum of the points):	

	Women (additionally)*	
4.	Vaginal discharge	
5.	Irregular or frequent menstruation	
6.	Menorrhagia (heavy menstrual bleeding)	
7.	Uterine fibroids	
8.	Cervical cancer (not HPV)	
9.	Fibro-cystic fibrosis	
	Result 3 (sum of the points):	
	Men (additionally)*	
10.	Prostate pain/ Prostatitis	
11.	Testicular pain / inflammation of the testicles	
12.	Pain / inflammation of the seminal vesicles	
13.	Breast Enlargement (Gynecomastia)	
	Result 3 (sum of the points):	

PART IV: If the following apply to you add an appropriate number of points.

1.	You have dogs, cats, rabbits, hamsters, guinea pigs, chinchillas and /or gerbils – 5 points	
2.	Your symptoms appear org get worse on a new moon or full moon – 5 points	
3.	You have eaten grilled or another type of uncovered beef or pork – 5 points	
4.	You have eaten grilled or another type of venison that has not been tested by a veterinarian – 3 points	
5.	You travelled through Africa Asia, and / or South or Central America – 3 points	
6.	You live in wetlands, floodplains, or areas that flood – 3 points	
7.	You put your fingers in your mouth or bite nails – 4 points	
8.	You own an open sandbox or have used a public one – 3 points	
9.	You drank unprocessed tap or well water – 3 points	
10.	You have eaten seafood or raw fish dishes such as sushi – 3 points	
11.	You eat or used to eat vegetables fertilized with sham or compost – 3 points	
12.	You have eaten unwashed vegetables and / or fruit from the ground or tree – 4 points	
13.	You have eaten unwashed blackberries – 3 points	
14.	One of your family members has been diagnosed with a parasitic disease or has another similar symptoms – 5 points	
15.	In the town/area of living there are a lot of people infected with parasites – 2 points	
16.	You are in habit of sucking grass blades and eating sorrel – 2 points	
17.	Children have played in the ball pits – 4 points	
18.	You have regularly used public pools and jacuzzies – 3 points	
19.	You have used available public rehabilitation – 2 points	
20	Ad dan appropriate number of points if you have been diagnosed with a symptom or a disease of more or less unspecified cause that cannot be treated by standard methods from the group: allergological – 3 points, haematological – 2 points, gastroenterological – 3 points, pulmonological – 3 points, dermatological – 3 points, oncological – 2 points, immunological – 2 points, neurological – 2 points, urological – 2 points, gynaecological – 2 points	
	Result 4 (sum of the points):	

SUM UP YOUR RESULTS

PART I	
PART II	
PART III	
PART IV	
Final result (sun of the points):	

I declare, I am the legal guardian of the child whose data is indicated above and I am entitled to full parental custody of the child.

☐ I consent to the processing of my personal data contained in the above questionnaire for purposes related to the provision of medical services in accordance with the Personal Data Protection Act in force on 25/05/2018. *

☐ I also consent to the use of the personal data provided, in the form of health data, for purposes related to the conduct of scientific research by the Administrator and for use in research, including scientific articles. If the data is used by the Administrator, it will be anonymised. **

The administrator of personal data is Wojciech Ozimek running a business under the name "Dr Wojciech Ozimek Praktyka Lekarska", Al. Wilanowska 43 D, 02-765 Warsaw, NIP: 118-02-16-822.

My consent to the processing of personal data, which is the basis for the processing, is voluntary. The recipients of the data may be employees and contractors of the Administrator. I have the right to withdraw my consent at any time, but in the case of indicating consent to participate in research, at the latest until it is used in research.

Personal data will be processed until the consent is revoked, and after such revocation, for the period of limitation of claims due to the Administrator and in relation to him.

I have the right to request the Administrator to access my personal data, rectify it, delete or limit processing, as well as the right to lodge a complaint with the supervisory body.

Signature

* consent is required
** voluntary consent

ACKNOWLEDGMENTS

To my dear husband, Wojciech Ozimek, I owe a profound debt of gratitude for his exceptional dedication as a physician and outstanding specialist in parasitology. Without his expertise and unwavering support, the development of the questionnaires and the collection of results would not have been possible. His insight and collaboration have been pivotal to the realization of this PhD, and I am forever thankful for his partnership in both life and science.

I extend my heartfelt gratitude to Joan Montlló for inspiring me to utilize my questionnaires as the foundation for my PhD. Your invaluable guidance and support, particularly in navigating the technical and informatics aspects, have been crucial to the successful completion of this work. Your encouragement and expertise have truly made a lasting impact on my academic journey.