"IDENTIFICATION OF OPPORTUNISTIC INFECTIONS CAUSED BY INTESTINAL PARASITES IN IMMUNOCOMPROMISED PATIENTS."

By

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Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"IDENTIFICATION OF OPPORTUNISTIC INFECTIONS CAUSED BY INTESTINAL PARASITES IN THE IMMUNOCOMPROMISED PATIENTS- ONE YEAR CROSS-SECTIONAL STUDY IN DR. PRABHAKAR KORE CHARITABLE HOSPITAL, BELAGAVI.",** is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee.

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LIST OF ABBREVIATIONS

ART	**Anti-retroviral treatment**
CA	**Carcinoma**
ESR	**Erythrocytic Sedimentation Rate**
HIV	**Human immunodeficiency virus**
IC	**Immunocompromised**
IS	**Immunosuppressed**
OI	**Opportunistic Infections**
PCR	**Polymerase Chain Reaction**
PLHIV	**Patient living with HIV**
ZN	**Ziehl- Neelsen**

ABSTRACT

Introduction:

Parasitic diseases remain a significant cause of morbidity and mortality worldwide. Conditions such as HIV/AIDS, the use of immunosuppressive medications, blood cancers, and other chronic illnesses increase patients' vulnerability to opportunistic infections. In a rising number of immunocompromised individuals, including transplant recipients and those undergoing immunosuppressive therapies, chronic diarrhoea is becoming more prevalent. Emerging pathogens such as Coccidia and Microsporidia play a crucial role in exacerbating this problem. This study aimed to investigate opportunistic intestinal parasites in immunocompromised patients.

Objectives:

1. To identify various Parasitic Opportunistic Infections (OIs) in immunocompromised patients/HIV patients.
2. To co-relate the OI with patient’s clinical manifestations.
3. To co-relate OIs with other laboratory parameters of the patients (complete blood picture), CD4 count, Erythrocytic Sedimentation rate (ESR), CD4 count, C-Reactive protein, D-dimer, and radiological investigations etc).

Materials and Methods:

In this study total of 150 patients attending ART, Oncology, Haemato-oncology departments of KLE’S Dr Prabhakar Kore Hospital and MRC, Belagavi were included.

Stool samples from study group are processed from period of January 2023 to December 2023. The samples were processed in Department of Microbiology, Jawaharlal Nehru Medical college, Belagavi. Wet mount preparation, faecal smear and concentrated smears were made, followed by Modified ZN stain with 1% H_2SO_4.

Results:

Among the 150 stool samples collected 9 were from patients on chemotherapy, 141 were from patients attending ART. The prevalence is 5.3% (8/150) the common parasite isolated being is Cryptosporidium.

Conclusion:

Among the 150 samples screened majority of participants were females than males and the age group is 41-50. Of the 150 samples screened 8 were positive for oocysts of Cryptosporidium seen in a total of 3.3% patients with diarrhea and 66% of patients with CD4 count <200cells/µL. This implies that the most common opportunistic parasitic intestinal parasite among the study group is Cryptosporidium with prevalence of 5.3%.

Key words: HIV, Immunocompromised patients, opportunistic intestinal parasites, Diarrhea, Cryptosporidium.

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INTRODUCTION

Parasitic illnesses persist as a substantial source of illness and death globally, affecting individuals regardless of their immune status. Roughly 340 parasite species have the potential to infect humans, with the bulk of the estimated 3 billion current infections concentrated in developing regions. Among these, enteric protozoan parasites stand out as the primary cause of morbidity and mortality, posing ongoing challenges. [(1)]

Conditions like HIV/AIDS, use of immune-suppressing medications, blood cancers, and various chronic illnesses elevate the susceptibility of patients to opportunistic infections. While there have been significant advancements in clinical outcomes for immunocompromised individuals, largely attributed to widespread antiretroviral therapy (ART) and other medical breakthroughs, diarrheal ailments persist as a common issue among HIV/AIDS patients. This problem is expected to become more prominent among an expanding population of immunocompromised individuals, including those undergoing hematopoietic and solid organ transplants, as well as immunosuppressive therapies. Chronic diarrhoea in these individuals can be caused by various microorganisms, with emerging opportunistic pathogens like coccidia and Microsporidia playing significant roles.[(2)]

Diarrhoea poses a common challenge for patients with weakened immune systems. The range of potential causes differs significantly from those with normal immune function, with opportunistic pathogens being particularly prevalent. It's crucial for accurate diagnosis and treatment to have a comprehensive understanding of the various factors that can contribute to diarrhoea in this patient population.[(3)]

This review examines the primary diarrhoea caused in immunocompromised patients, spanning infectious and non-infectious origins, using HIV infection for understanding similar conditions of immune suppression. It also addresses specific scenarios such as diarrhoea following organ or stem cell transplantation, drug-induced diarrhoea from immunosuppressive medications, and diarrhoea in inborn immunodeficiency syndromes. [3]

Gastrointestinal opportunistic infections are more commonly seen in severe form immunocompromised (e.g. PLHIV, haematological malignancies, organ transplant etc.). in them compared to healthy individual significant mortality and morbidity is seen. Impaired mucosal immune system is responsible for disease due to enteric pathogens like S*almonella* spp*, Shigella* spp, *Campylobacter*, *Mycobacterium tuberculosis*, *nontuberculous mycobacterium*, etc., [3] Parasites, that doesn't cause infection in healthy individuals include *Cryptosporidium* spp*, Isospora belli, Cyclospora cayetanensis.*[4]

In developing countries like India, these infections are common because of low level of sanitation and hygiene, consumption of contaminated food and water, open defecation or open disposal. [4]

Present statistics shows more than quarter of population have chronic infections to these parasites unlike in developed countries where less mortality and morbidity seen. Diarrhoea is most commonly seen in AIDS patients with distribution in developing countries with 90%, compared to developed countries at 30-60%. [5]

Antiretroviral therapy (ART) boosts the immunity in people living with HIV (PLHIV). Additionally, use of chemoprophylaxis can help in combating intestinal

parasites, thereby reducing their prevalence. As highly active antiretroviral therapy (HAART) becomes more widely available, there have been notable changes in the morbidity and mortality patterns among PLHIV. [6] This is evidenced by a decrease in opportunistic infections, including those caused by intestinal parasites. Despite the presence of ART, studies indicate a persistently high prevalence of intestinal parasitic infections among individuals with low CD4 cell counts.[7,8] Early and accurate diagnosis is crucial as many of these infections respond well to appropriate medication. Therefore, our objective was to investigate the occurrence of opportunistic intestinal parasitic infections among PLHIV attending ART of Dr Prabhakar Kore Hospital and MRC, Belagavi, examining their correlation with CD4+ cell counts and their association with diarrhoea and also patients attending oncology and haemato-Oncology.

OBJECTIVES

1. To identify various Parasitic Opportunistic Infections (OIs) in immunocompromised patients/HIV patients.
2. To co-relate the OI with patient's clinical manifestations.
3. To co-relate OIs with other laboratory parameters of the patients (complete blood picture), CD4 count, Erythrocytic Sedimentation rate (ESR), CD4 count, C-Reactive protein, D-dimer, and radiological investigations etc).

REVIEW OF LITERATURE

Intestinal parasites, helminths and protozoa species, are of major health threat especially among immunocompromised patients. According to statistics it is estimated that around 3.5 billion people are infected by these infections worldwide they are leading cause of mortality and morbidity in such patients. [9]

Definition Immunocompromised status:

Patients with weakened immune system due to few of chronic diseases or iatrogenic suppression are termed as **Immunocompromised** patients. The patients include people with HIV infection, on chemotherapy of cancer, patients on immunosuppressive agents following an organ transplant etc., [10]

In such cases microorganisms take advantage of the host immune system with an altered microbiota. Opportunistic infections can cause variability in pattern and extent of infection because of different pathogens (their exposure, virulence factors) especially for parasites. Difference and level of immunosuppression because of underlying condition also play a role for morbidity. [11]

Opportunistic infections (OI):

Opportunistic Infections (OIs): These are the infections seen as the micro-organisms that usually do not cause a disease, but causes when the body's immune system is lowered and are represented by unusual infections by common organisms causing morbidity.[12]

OIs are described in epidemiological surveys in population with a congenital or acquired lowered immune system. Pre-defined classifications of OI are not adopted in clinical trials of chemotherapeutic agents.[12]

HIV infection, steroids, immunosuppressive agents and transplantations causes deficient cell-mediated immunity. Relative Neutropenia is observed in patient undergoing treatment for tumours in first 1-7 days.[13]

Diarrhea is a condition in which the passage of >/=3 episodes loose or liquid stools per day. It is the most common manifestation seen because of immunocompromising states. The etiology of causative organisms differs from patients having normal immune status. The gastro intestinal tract plays a role in causing diarrhea with patients infected with HIV.[14] Compared to healthy hosts these infectious diseases are commonly seen in individuals with immunocompromised state causing morbidity and mortality. Impaired mucosal immune system causes variety of Enteric infections. [15]

Types of pathogens involved:

The most **common organisms** causing diarrhea in Immunocompromised HIV patients are *Salmonella* spp, *Cl. difficile, M. tuberculosis*, non-tuberculous mycobacterium, fungi and parasites. Parasites causing diarrhea in healthy individuals are *Giardia lamblia, E. histolytica, B. hominis,* and *S. stercoralis*. Parasites not causing disease in healthy individuals include *Cryptosporidium* spp, *Isospora belli, Cyclospora cayetanensis.* [14]

Among these several organisms causing Diarrhoea, the important and threatening pathogens include coccidian parasites and Microsporidia.[16]

Epidemiology:

The **prevalence and distribution** of opportunistic intestinal parasitic infections in immunocompromised populations vary widely across different regions and demographic groups worldwide. Several factors contribute to these variations, including socioeconomic status, access to healthcare, sanitation practices, and prevalence of underlying immunocompromising conditions such as HIV/AIDS or organ transplantation. [17]

HIV/AIDS Epidemic: Historically, opportunistic intestinal parasitic infections have been closely associated with the HIV/AIDS epidemic. There were about 39.0 million PLHIV at the end of 2022, 2/3rd of whom are in the WHO African Region. [18]

In regions with high HIV prevalence, such as sub-Saharan Africa, parts of Asia, and certain areas of Latin America, the burden of opportunistic intestinal parasites is particularly significant. Protozoan parasites like *Cryptosporidium*, *Isospora*, and *Microsporidia* are common culprits in these populations. [18]

A study was done in 2013 among patients of HIV/AIDS from Peoples College of Medical Sciences and Research Centre, Bhopal. Results showed maximum *Isospora belli* infection (n=27; 35%) and the study concluded that the presence of intestinal parasitic infections was decreasing with improvement in CD4 cell count. [19]

A retrospective study among HIV patients of Guru Gobind Singh Hospital, Jamnagar, during 2009 to 2010 determine intestinal Parasites in patients with HIV/AIDS by microscopically examining using wet mount preparations and stained smears. The results identified parasites such as *Cryptosporidium* (135),

Isospora belli (42), *Cyclospora* (12), *Microspora* (02), *Entamoeba histolytica* (49), Hookworm (34). The conclusion was that the intestinal parasites were more in chronic diarrhoea than in acute diarrhoea in Western India.[20]

Organ Transplantation: Patients who have undergone organ transplantation are also at increased risk of opportunistic infections due to the immunosuppressive medications they must take to prevent organ rejection. This population may experience infections with parasites like *Strongyloides stercoralis*, which can cause severe disseminated disease in immunocompromised individuals. [21]

Chemotherapy and Immunosuppressive (IS) Therapies: Cancer patients undergoing chemotherapy and individuals receiving immunosuppressive therapies for autoimmune diseases or after organ transplantation are also susceptible to opportunistic intestinal parasites. The risk varies depending on the type and duration of immunosuppression.[21]

A study with control group was done between 2017 and 2018 among both patient group and controls consisting of 100 participants in each group, in patients with ca. stomach for these parasites. The results showed 14% of the ca. stomach, and in 2% of the controls along with identification of *Blastocystis* species in 11%, and most of the patients who were positive for parasites had diarrhoea. The study stated

that it is important to screen patients with ca. stomach if they mention the complaint of diarrhoea, for intestinal parasites. [22]

Geographic Distribution: Distribution of opportunistic intestinal parasites is influenced by geographic factors such as climate, environmental conditions, and sanitation standards. For example, parasitic infections like *Cyclospora cayetanensis* and *Giardia lamblia* are more prevalent in regions with poor sanitation and contaminated water sources. [23]

Urban vs. Rural: Disparities in healthcare access and infrastructure among different areas can also affect prevalence of opportunistic intestinal parasites. Rural populations may be at higher risk due to limited access to clean water, proper sanitation facilities, and healthcare services for diagnosis and treatment. [24]

Seasonal Variation: Some opportunistic parasites exhibit seasonal variation in their prevalence, with transmission rates peaking during certain times of the year. For example, *Cryptosporidium* infections are more common in warmer months when outdoor activities increase, leading to greater exposure to contaminated water sources. [25]

Demographic Factors: Certain demographic groups may be disproportionately affected by opportunistic intestinal parasitic infections. For instance, children, the elderly, and individuals with underlying malnutrition or other coexisting infections may be at higher risk of severe complications from these infections.[26]

Overall, while opportunistic intestinal parasitic infections affect immunocompromised populations worldwide, the prevalence and distribution patterns are complex and multifactorial. Understanding these variations is crucial for

implementing targeted prevention, screening, and treatment strategies to reduce the burden of these infections in vulnerable populations. [27]

Coccidian parasites are unicellular protozoans belonging to *Phylum Apicomplexa*. Members of this phyla live intracellularly and forms an apical complex with which it penetrates the host cells, hence included in Apicomplexa. [28]

The infections due to intestinal parasites are commonly seen in developing countries, in places of poor sanitation and with unhygienic practices especially in children and elderly and in immunosuppressed patients. [29]

Routes of transmission:

Opportunistic intestinal parasites are typically **transmitted** through various routes, with consumption of contaminated food and water being one of the primary modes of transmission.

Consumption of contaminated food and water: This is a common route of transmission for many opportunistic intestinal parasites. Parasite cysts, oocysts, or eggs can contaminate food or water sources through faecal contamination from infected individuals or animals. For example, protozoa like Cryptosporidium and Giardia can form environmentally resistant cysts that survive in water sources, allowing transmission through ingestion of contaminated water. [30]

Exposure to Infected Soil: Certain parasites have environmental stages in their life cycles that can persist in soil, increasing the risk of transmission through direct contact with contaminated soil. Helminths like *Strongyloides stercoralis* and hookworms have larvae that can penetrate the skin upon contact with contaminated soil, leading to infection. Individuals, particularly in rural or agricultural settings,

may acquire these infections through activities like walking barefoot or working in fields where soil contamination is prevalent.[31]

Direct Contact with Infected Individuals or Their Faces: Direct contact with infected individuals or their faeces can also facilitate transmission of opportunistic intestinal parasites. This can occur through poor hygiene practices, inadequate sanitation facilities, or close personal contact with infected individuals. For example, person-to-person transmission is a common route for parasites like *Enterocytozoon bieneusi*, which causes microsporidiosis, particularly in settings such as daycare centers, nursing homes, and institutions where individuals are in close proximity to one another. [32]

Fomite Transmission: In addition to direct contact with infected individuals, transmission can occur indirectly through fomites contaminated with parasite eggs, cysts, or oocysts. Fomites can include contaminated surfaces, objects, or utensils that come into contact with infected faeces. Without proper hygiene practices, individuals may inadvertently ingest parasite stages present on contaminated fomites, leading to infection.[33]

Understanding these transmission routes is essential for implementing effective prevention strategies, which may include improved sanitation practices, access to clean water sources, proper food hygiene measures, promotion of personal hygiene, and education on avoiding high-risk behaviours or environments that increase the likelihood of exposure to opportunistic intestinal parasites.

These parasites reproduce sexually in the intestinal epithelium and infection is transmitted through orofecal contamination of food or water with oocysts. Among the coccidian parasites Cryptosporidiosis is commonly seen and is an emerging infection in the world.[34]

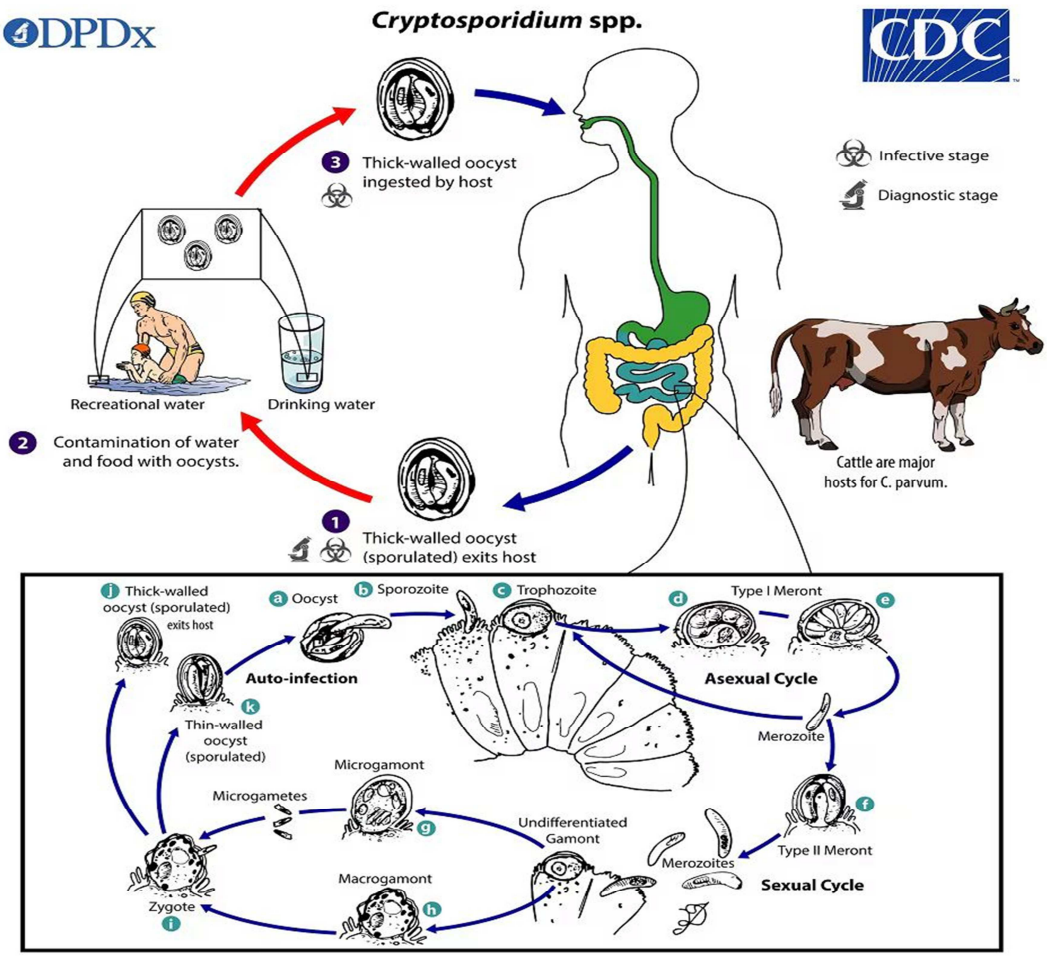
Life cycle of *Cryptosporidium* spp:

The ingested oocysts pass through gastric pH and settle in small intestine excyst and release into sporozoites. These sporozoites invade mucosal brush border and divides to growing stage trophozoites. Similar to other parasites of phyla Apicomplexa they have both sexual and asexual life cycle, through which oocysts are released in faeces.[35]

The parasite settles in extra cytoplasmic layer undergoes asexual or schizogony reproduction and produces 8 merozoites in Meront.[36] These merozoites enter the other intestinal cells and causes damage. At this stage it undergoes two lifecycles: asexual stage (production of oocyst) and sexual stage (type II meront) which differentiates to micro and macrogametocytes and form a zygote.

Oocyst with 4 sporozoites are formed through sporogony and through formation of thick wall and are released into environment through faces. [36]

This infection is most commonly seen in developing countries because of lack of basic drinking water and sanitation unlike in industrialized countries. Outbreaks can be seen with contaminated water or through swimming pools. Although, there are many species present two among them *C. parvum* and *C. canis* are found commonly in humans. [37]



Pathogenesis of Coccidian parasites:

The diarrhoea seen in immunocompetent patients is usually self-limiting. Infection can be seen in severe bouts in patients whose immune system is weakened. The parasite is known to cause disruption of intestinal epithelial border, secretion of electrolytes thus causing diarrhea. In HIV/AIDS patients chronic form of infection is seen causing mortality. Fever, malabsorption are usually common, but rare forms of biliary tract diseases can occur.

So, it is considered as a matter of threat to treat the patients, especially the immunocompromised patients. [38]

Clinical manifestations:

Opportunistic intestinal parasitic infections can manifest with a range of **signs and symptoms** in immunocompromised patients. While the presentation can differ based on the parasite involved and amount of infection, symptoms often include [39]

Diarrhea: Chronic or persistent diarrhea is a hallmark symptom of many opportunistic intestinal parasitic infections. The diarrhea may be watery, voluminous, and sometimes contain blood or mucus. It can lead to dehydration and electrolyte imbalances if not managed promptly. [40]

Abdominal Pain: Patients may experience abdominal cramping, discomfort, or pain, often localized to the lower abdomen. The pain can be intermittent vs persistent and can differ based on the extent of inflammation of intestine or damage caused by the parasites. [40]

Weight Loss: Unintentional weight loss is a common consequence of chronic diarrhea and malabsorption associated with opportunistic intestinal parasitic infections. The parasites may interfere with nutrient absorption in the intestines, leading to malnutrition and weight loss over time. [41]

Malabsorption: Malabsorption syndrome can develop due to damage to the intestinal mucosa caused by parasitic infection. This can result in deficiencies of essential nutrients, vitamins, and minerals, leading to symptoms such as fatigue, weakness, anemia, and impaired immune function. [41]

Gastrointestinal Disturbances: In addition to diarrhea and abdominal pain, patients may experience other gastrointestinal disturbances such as nausea, vomiting, bloating, flatulence, and dyspepsia. These symptoms can contribute to overall discomfort and negatively impact quality of life. (42)

Systemic Symptoms: In severe cases or with disseminated infections, patients may present with fever, night sweats, and fatigue. These symptoms can indicate widespread dissemination of the parasites beyond the gastrointestinal tract and may require urgent medical attention.

Extra-intestinal Manifestations: Some opportunistic intestinal parasites have the potential to cause extra-intestinal manifestations, affecting organs outside the gastrointestinal tract. For example, disseminated *Strongyloides stercoralis* infection can lead to pulmonary symptoms, sepsis, or meningitis, particularly in immunocompromised individuals. (42)

Chronic Course: Opportunistic intestinal parasitic infections in immunocompromised patients often have a chronic or relapsing course, with symptoms persisting for weeks to months if left untreated. This chronicity can further exacerbate nutritional deficiencies and compromise the patient's overall health status. (43)

It's necessary to understand the clinical presentation in immunocompromised patients because of opportunistic intestinal infections by parasites can be nonspecific and overlap with other gastrointestinal conditions or opportunistic infections. Therefore, a high index of suspicion, appropriate history and investigations are essential for accurate diagnosis and timely management of these infections.

Laboratory diagnosis of Coccidian parasites:

Various **diagnostic techniques** are employed to identify opportunistic intestinal parasites in immunocompromised patients, each with its advantages and limitations. Here's a review of these diagnostic methods:

Stool Microscopy: Microscopic examination of stool samples remains one of the most widely used methods for diagnosing intestinal parasitic infections. Techniques such as direct wet mount, concentration methods (e.g., sedimentation, flotation), and staining (e.g., iodine, trichrome) are employed to visualize parasite cysts, oocysts, eggs, or larvae. Stool microscopy is particularly useful for detecting protozoan parasites like Cryptosporidium, Giardia, and Entamoeba histolytica, as well as helminth eggs and larvae.[44]

Antigen Detection Assays: Antigen detection assays utilize specific antibodies to detect parasite antigens in stool samples. Enzyme immunoassays (EIAs) and rapid diagnostic tests (RDTs) are commonly used antigen detection methods for detecting protozoan parasites such as Giardia and Cryptosporidium. These assays offer rapid results and are often more sensitive than microscopy, especially in cases of low parasite burden or intermittent shedding. [45]

Molecular Methods (PCR): Polymerase chain reaction (PCR) and other nucleic acid amplification techniques are highly sensitive and specific methods for detecting parasitic DNA or RNA in stool samples. PCR assays can target specific genetic markers unique to individual parasite species, allowing for accurate identification and differentiation. PCR is particularly valuable for detecting parasites with low

shedding rates, such as Cryptosporidium, Cyclospora, and microsporidia, as well as for confirming species identification in cases of mixed infections. [46]

Serological Tests: Serological tests measure the presence of specific antibodies against parasitic antigens in serum or other body fluids. While serology is not commonly used for diagnosing intestinal parasites directly, it can be valuable for detecting systemic or disseminated infections, particularly in cases where stool microscopy or antigen detection assays are negative or inconclusive. Serological tests are available for certain parasites like *Strongyloides stercoralis*, which may cause extra-intestinal manifestations in immunocompromised patients.

Histopathology: Invasive procedures such as endoscopy or biopsy may be required to obtain tissue samples for histopathological examination.

Histopathology allows for direct visualization of parasites within the intestinal mucosa or other tissues and can provide valuable diagnostic information, especially in cases of invasive protozoan or helminthic infections. Special stains (e.g., PAS) may be used to enhance parasite visualization in tissue sections.

Diagnostic Advances:

Molecular Diagnostics: The development of molecular diagnostic techniques, such as PCR assays targeting specific parasite genes, has improved the sensitivity and specificity of parasite detection, particularly for low-burden infections and species identification. [47]

Point-of-Care Testing: Advances in point-of-care testing technologies have enabled rapid and accurate diagnosis of intestinal parasitic infections at the bedside or in

resource-limited settings, facilitating timely initiation of treatment and reducing the risk of complications.[48]

Each Laboratory investigation has its plus points and limitations, and the choice of test includes as the suspected parasite, clinical presentation, patient's immune status, availability of laboratory resources, and cost-effectiveness. Combining multiple diagnostic approaches, such as stool microscopy with antigen detection assays or PCR, can improve the sensitivity and accuracy of parasite detection in immunocompromised patients with opportunistic intestinal infections.

Treatment:

The **treatment** of opportunistic intestinal parasitic infections in immunocompromised individuals involves a multifaceted approach aimed at eliminating the parasites, managing symptoms, preventing complications, and

addressing underlying immune deficiencies. Here's a discussion of the recommended treatment approaches:

Antimicrobial Therapy/Anti-parasitic Medications:

Protozoan Infections: Anti-parasitic medications are typically used to treat protozoan infections such as Cryptosporidium, Giardia, Cyclospora, and microsporidia. Drugs like nitazoxanide, metronidazole, tinidazole, and albendazole are commonly prescribed, depending on the specific parasite and its susceptibility profile. [49]

Helminthic Infections: Helminthic infections like *Strongyloides stercoralis* and other soil-transmitted helminths may require treatment with anthelmintic medications

such as ivermectin, albendazole, or mebendazole. In cases of disseminated strongyloidiasis or hyper infection syndrome, prolonged courses of treatment may be necessary to eradicate the parasite. [49]

Supportive Care:

Fluid and Electrolyte Management: Managing dehydration and electrolyte imbalances resulting from diarrhea is essential. ORS or IV fluids may be needed for electrolyte imbalance.

Nutritional Support: Malnutrition and weight loss are common complications of chronic intestinal parasitic infections. Nutritional supplementation and dietary counseling may be necessary to address deficiencies and support the patient's nutritional status. [50]

Management of Underlying Immune Deficiencies:

Antiretroviral Therapy (ART): In HIV/AIDS patients, initiating or optimizing antiretroviral therapy is crucial for restoring immune function and reducing the risk of opportunistic infections, including intestinal parasites. ART can improve CD4 cell counts and decrease viral load, thereby enhancing host immunity.

Immunosuppression Adjustment: For patients receiving immunosuppressive therapies for conditions such as organ transplantation or autoimmune diseases, adjustments to immunosuppressive regimens may be necessary to balance the risks of infection and rejection. Close monitoring of immune function and medication levels is essential to prevent opportunistic infections.[51]

Treatment Innovations:

Novel Therapeutics: Research into novel anti-parasitic drugs and therapeutic targets has led to the discovery of new treatment options for opportunistic intestinal parasitic infections, including compounds with improved efficacy, safety profiles, and resistance profiles.

Drug Combination Therapies: Investigating the use of combination therapies involving multiple anti-parasitic drugs or adjunctive agents has shown promise in overcoming drug resistance, enhancing treatment efficacy, and reducing the risk of treatment failure or recurrence.

One Health Approach: implementing one health approach which includes all three perspectives is critical for understanding the complex epidemiology of opportunistic intestinal parasitic infections and developing holistic strategies for disease control and prevention. [52]

By addressing these advancements and challenges through interdisciplinary research, collaboration, and innovation, we can improve our understanding of opportunistic intestinal parasitic infections and develop ample enough strategies for investigations, cure and prevention, thus decreasing burden of disease on affected Individuals.

Prevention of Infection:

Infection Control Measures: Implementing infection control measures to prevent transmission of parasites to other individuals is critical, especially in healthcare settings and institutions where susceptible individuals may be at increased risk of

exposure. This may include isolation precautions, hand hygiene practices, and environmental sanitation. [53]

Health Education: Providing education to patients and caregivers about proper hygiene practices, safe food and water consumption, and strategies to minimize exposure to parasites can help prevent reinfection and transmission within communities.

Follow-up and Monitoring:

Clinical Monitoring: Close clinical monitoring of patients during and after treatment is essential to assess treatment response, resolution of symptoms, and potential recurrence of infection. Follow-up stool examinations or other diagnostic tests may be indicated to confirm parasite clearance.

Immune Status Monitoring: Regular monitoring of immune function, such as CD4 cell counts in HIV/AIDS patients or immunosuppressive medication levels in transplant recipients, is important to ensure optimal management of underlying immune deficiencies and prevent opportunistic infections.[54]

By integrating these treatment approaches, healthcare providers can effectively manage opportunistic intestinal parasitic infections in immunocompromised individuals, minimize complications, and improve patient outcomes. Tailoring treatment plans to the specific parasite, host immune status, and clinical presentation is essential for optimal management of these infections.

Opportunistic intestinal parasitic infections in immunocompromised individuals can cause complications that can lead to mortality and morbidity. Here's

an overview of potential complications associated with these infections and their impact on prognosis:

1. Dehydration and Electrolyte Imbalances
2. Malnutrition and Weight Loss
3. Chronic Diarrhea and Gastrointestinal Disturbances
4. Dissemination of Parasites to Other Organs

Prognosis for affected individuals depends on various factors, including the underlying immune status, the severity of infection, timely intervention, and the presence of comorbidities. In IC patients, such as PLHIV or undergoing IS therapy, the prognosis may be poorer due to the increased risk of complications and reduced ability to mount an effective immune response against the parasites. However, with timely diagnosis, appropriate treatment, and supportive care, many opportunistic intestinal parasitic infections can be effectively managed, and the prognosis can be improved. Close monitoring of patients, follow-up, and usage of treatment regimens are essential for outcomes and preventing adverse effects in immunocompromised individuals with these infections.[55]

Preventive measure is important in reducing the risk of opportunistic intestinal parasitic infections in immunocompromised patients. Here's why they're essential:

1. Good Hygiene Practices
2. Safe Food and Water Consumption
3. Environmental Sanitation
4. Infection Control Protocols

5. Health Education and Awareness

By implementing these preventive measures, healthcare providers can help reduce the risk of opportunistic infections in IC patients, enhance patient safety, and improve overall health outcomes. Preventive strategies should be tailored to the specific needs and risk factors of individual patients, with a focus on promoting behaviors and practices that minimize the risk of exposure to parasites and prevent the spread of infection within healthcare facilities and communities.(56)

Recent advancements in the field of opportunistic intestinal parasitic infections have focused on improving diagnosis, treatment, and prevention strategies, while also addressing emerging challenges. Here's a summary of these advancements and areas for future research:

A study was conducted in Nehru Hospital attached to the PGIMER, Chandigarh, among 120 PLHIV to assess the presence of intestinal infections in PLHIV. A total of 186 stool samples were collected and the results showed 36 patients were found to harbor an intestinal parasite in which, *Cryptosporidium* (10.8%), *G.lamblia* (8.3%) then equal detection of *Cyclospora cayetanensis* and Blastocystis hominis (3.3%). Out of all these 36 patients, 27 had diarrhea. The study concludes that investigating for parasites in PLHIV emphasizing treating doctors regarding the presence of these parasites among the specific populations.(57)

In another study conducted in AIIMS, New Delhi to co-relate the relation for parasites and CD4 count in patients on retroviral therapy says that the existence of intestinal parasites was found to be 32%. In this study conducted, among the 400

patients (200 cases+200 controls) 132 patients were found to have intestinal parasitic infection. [58]

A conducted to see the co-relation between the microscopy of stool and level of immunosuppression in a medical college in Andhra Pradesh stated that prevalence of OI is common in the people with CD4 count <200cells/µL.[59]

METHODOLOGY

- **Study Centre:** Department of Microbiology, KAHER's J.N. Medical College, Belagavi.
- **Source of data:** Materials of the study are the clinical samples stool, gastric aspirates and other suitable samples shall be collected from immunocompromised 25patient's attending Dr. Prabhakar Kore Hospital, MRC, Belagavi.
- **Study design**: One-year Cross-sectional study
- **Study period**: One year-From January2023 – December2023
- **Study population**: All patients who attend ART, Oncology and Pediatrics Hematology of Dr. Prabhakar Kore Charitable Hospital, MRC, Belagavi.
- **Inclusion criteria**: All the Immunocompromised patients presenting with Diarrhea /dysentery, abdominal pain unexplained weight loss, patients showing features of malabsorption etc. to be suffering from intestinal parasitic infections.
- **Exclusion criteria**: Healthy individual with symptoms of diarrhea.
- **Sample size**: Single Proportion- Absolute Precision

  Expected proportion = 0.50

  Precision (%) = 8

  confidence level (%) = 95

  Sample size(n) = 150 should be taken

  Formula:

  $n=z^2pq/d2$

  Where, Z= Standard normal variate value (Z=1.96 at 5% alpha error)

d= Margin of error =8%

p=50%, q= 100-50=50%

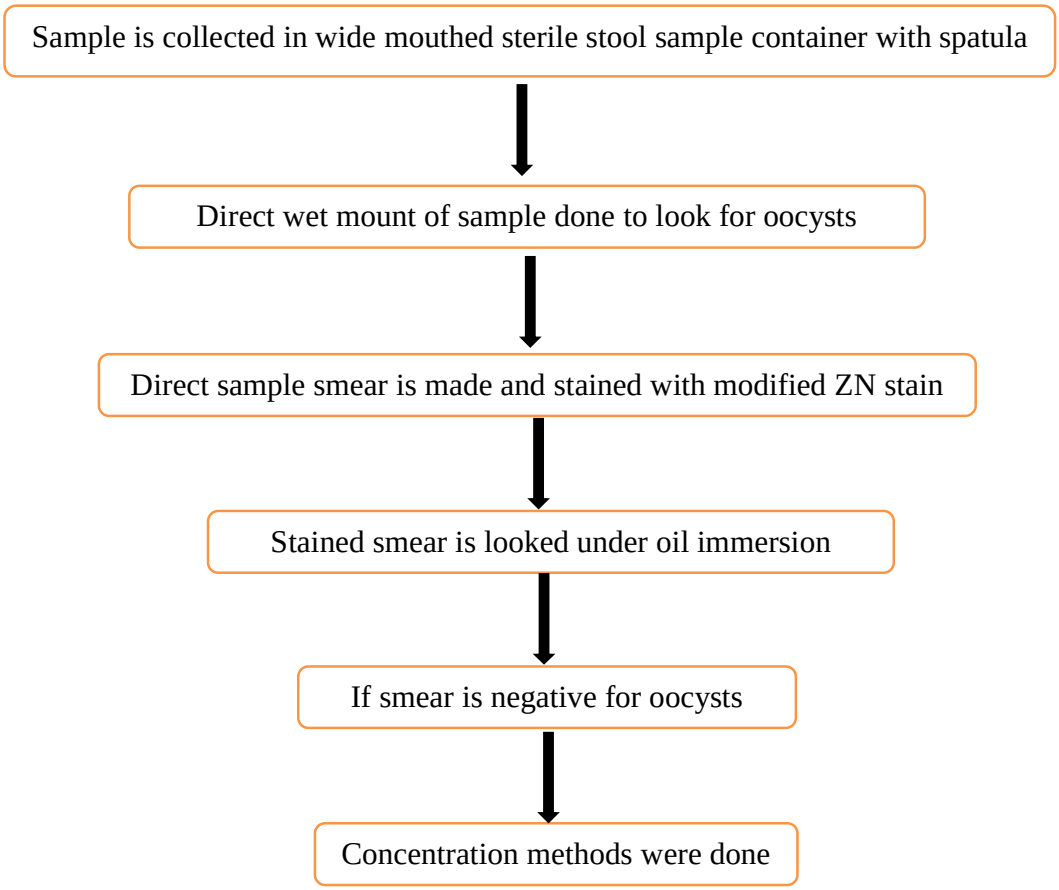
$n=z^2pq/d2$

= 1.96 x 1.96 x 50 x 50/8 x 8

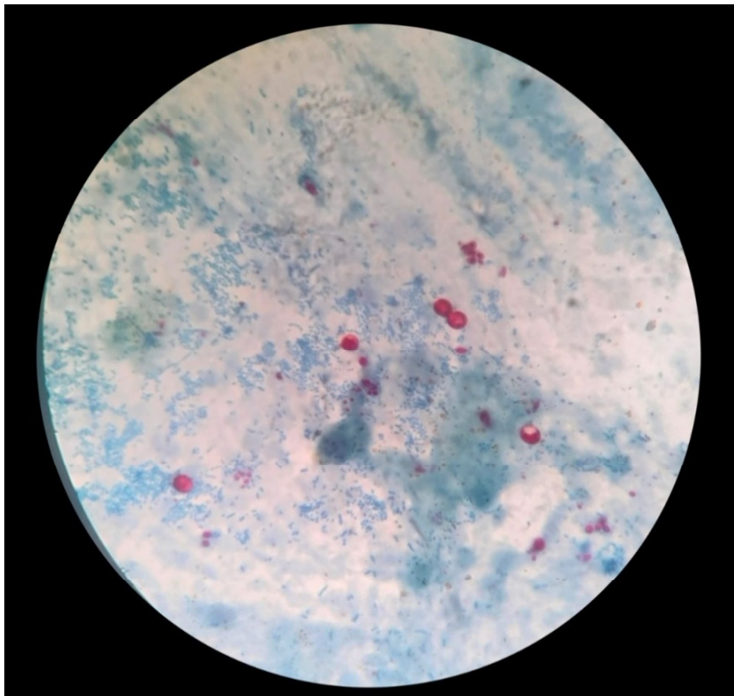
= 150.0625

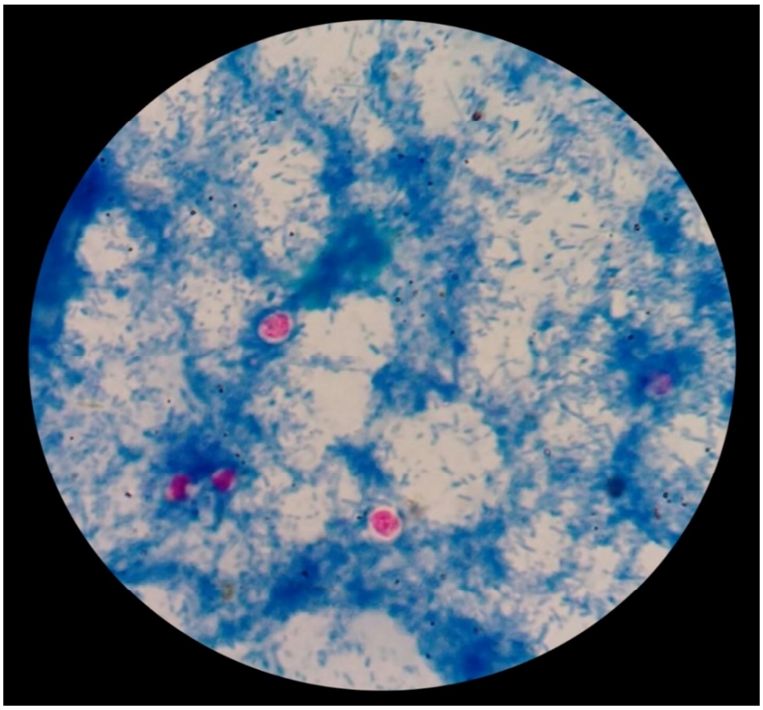
- **Statistical analysis**: Prevalence was calculated and expressed in percentage
- **Instruments used for data collection**: Proforma and checklist

Processing of Stool Sample:



PHOTOGRAPHS





Photograph 1,2: showing oocysts of Cryptodporidium.

Photograph: 3: Formol ether sedimentation technique.

RESULTS:

Stool samples were collected from patients attending ART, Oncology department and Haemato-oncology department of Dr Prabhakar kore Hospital and MRC, Belagavi. Direct fecal smears were made followed by formol-ether sediment smears were made and were subjected to Modified ZN stain.

In the present study among the patients attending ART, Oncology, Haemato-oncology cases females were predominant in number 88 (58.66%) compared to males 62(41.33%). Female: male ratio is 0.59:0.41

TABLE NO. 01: Age and gender distribution of Immunocompromised patients.

Age Group	Males		Females		Total	
	No.	Percentage	No.	Percentage	No.	Percentage
0-10	1	0.66%	1	0.66%	2	1.33%
11-20	6	4%	4	2.66%	10	6.67%
21-30	7	4.66%	16	10.66%	23	15.3%
31-40	11	7.33%	22	14.66%	33	22%
41-50	17	11.33%	23	15.33%	40	26.6%
51-60	11	7.33%	16	10.66%	27	18%
61-70	9	6%	5	3.33%	14	9.3%
>70	-	-	1	0.66%	1	0.66%
Total	62	41.33%	88	58.66%	150	100%

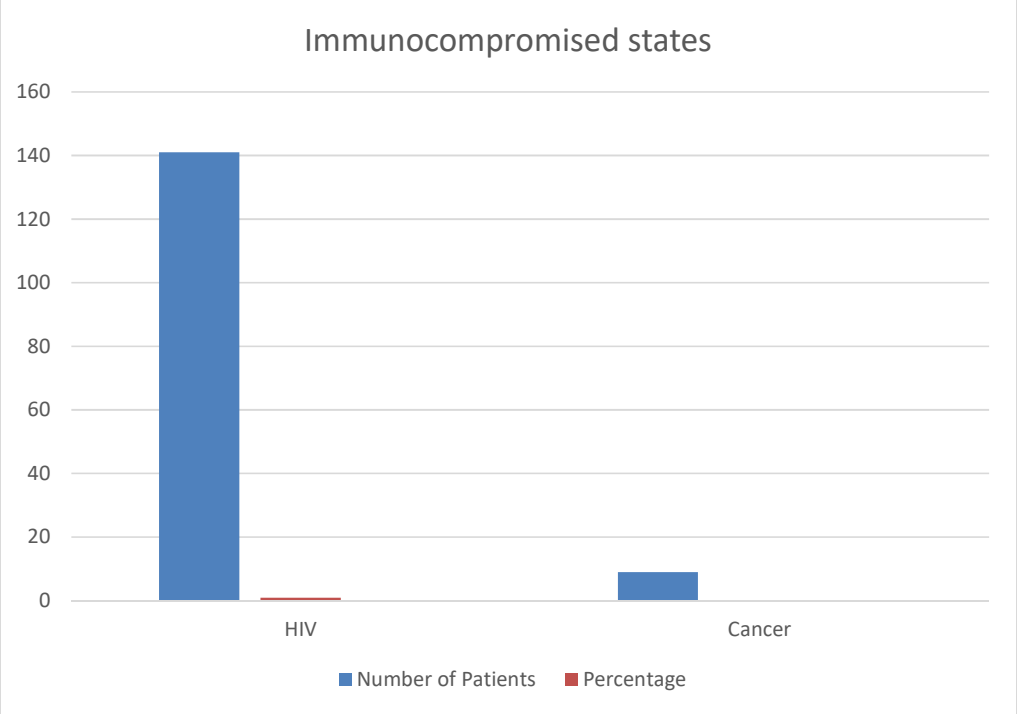
Maximum No. of samples were collected from patients of age group 41-50 with a percentage of 26.6% followed by 31-40 which is 22%. Among them Females were more in number.

GRAPH NO. 1 :**Age and gender wise distribution**

TABLE NO.02: Cause of Immunocompromised status in study group:

Diagnosis	Number of Patients	Percentage	Positive for Parasites
HIV	141	94%	8(5.3%)
Cancer	09	6%	0

In our study Among the 150 samples collected 141 (94%) were from patients attending ART, 9(6%) were from patients attending Oncology unit.

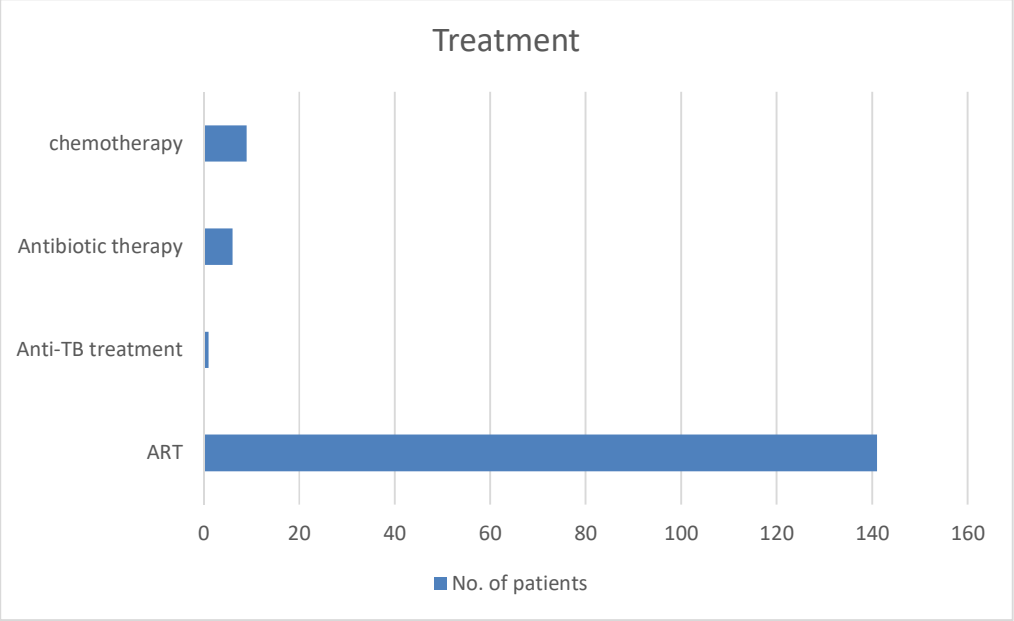


GRAPH NO. 2: Cause of Immunocompromised status in study group

TABLE NO. 03: Patients undergoing Treatment

Type of medication	Number of Patients	Percentage
ART	141	94%
Anti-TB medication	01	0.66%
Antibiotic Therapy	06	4%
Chemotherapy	09	6%
Corticosteroid therapy	09	6%

The table and graph depict the patients undergoing various treatment as mentioned.Of them 141(94%) were on ART, 1 (0.66%) patient was on Anti-Tb medication, 6 (4%) were on Antibiotics, 9(6%) on chemotherapeutic agents, 9(6%) were on corticosteroid treatment.

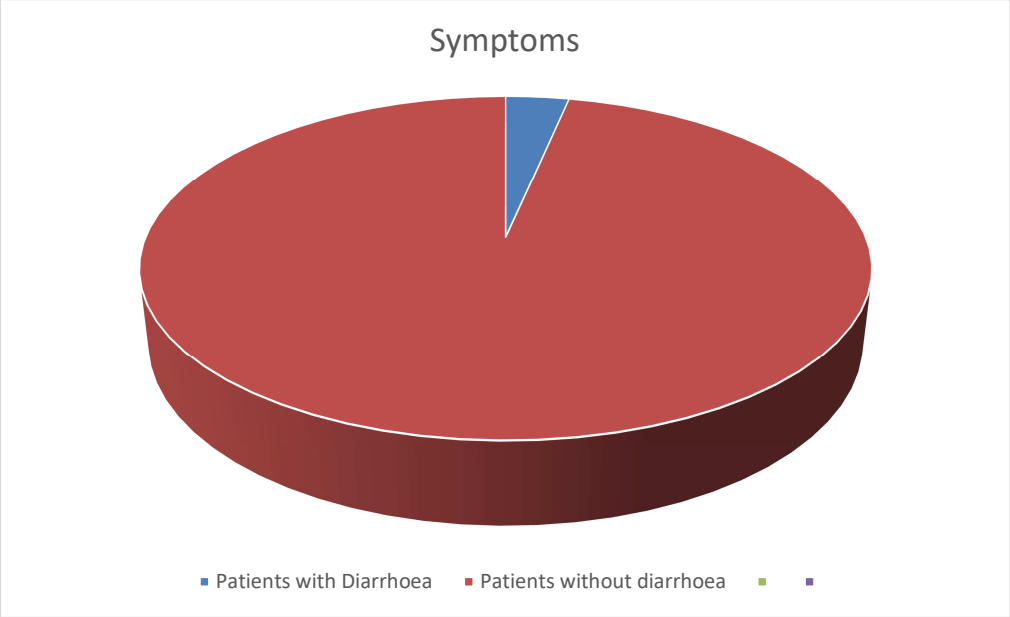


GRAPH NO. 3: Patients undergoing Treatment

TABLE NO. 04: individuals with history of Diarrhea

Patients with diarrhea	Patients without diarrhea
05 (3.33%)	145 (96.6%)

Out of the 150 patients from which samples were collected 5 (3.33%) mentioned the history of Diarrhea and remaining 145(96.6%) did not mention about having Diarrhea.

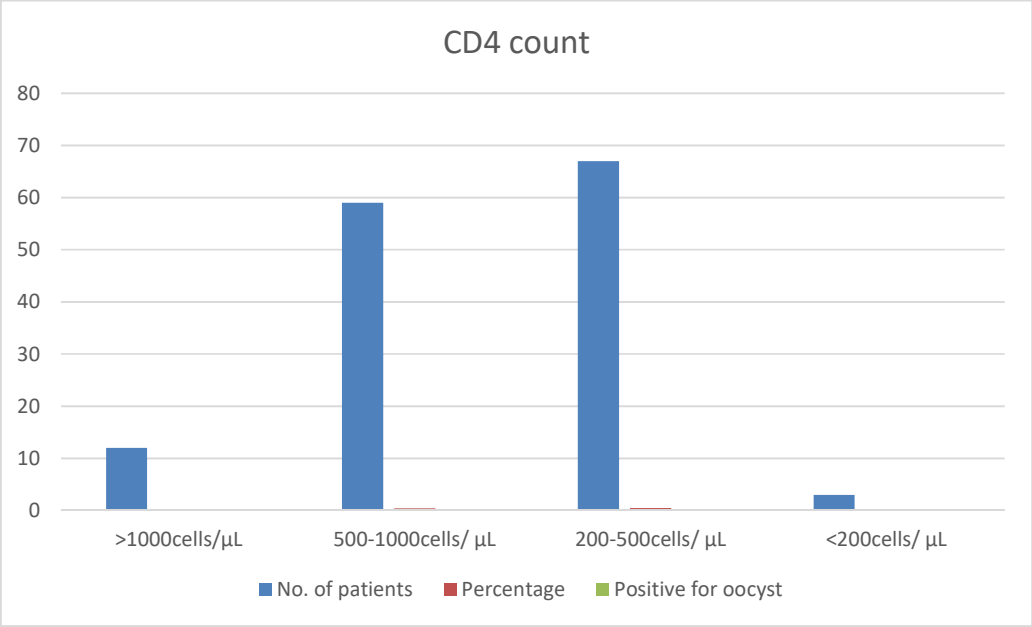


GRAPH NO. 4: Symptoms

TABLE NO.05: CD4 count among the patients (n=141)

CD4 count	No. of patients	Percentage	Positive for oocyst
>1000cells/µL	12	9%	0
500-1000cells/ µL	59	42%	0
200-500cells/ µL	**67**	**48%**	**5(3.54%)**
<200cells/ µL	03	1.42%	3(2.12%)

Out of the 141 patients attending ART, patients with maximum CD4 count was in the range of 200-500cells/ µL, 64 patients (48%), followed by 500-1000cells/ µL, 59 patients (42%), >1000cells/µL, 12 patients (9%), and <200cells/ µL, 3 patients (1.42%).



GRAPH NO. 5 CD4 count among the patients

TABLE NO.06: Modified ZN stain for detection of Oocysts

Opportunistic parasites	Number of patients	Percentage
Cryptosporidium spp	8	5.3%
Isospora	0	0
Cycloisospora	0	0

In our study, out of the positive 8/150 (5.3%)stool samples the isolates were Cryptosporidium spp.

DISCUSSION

OI of intestines are important cause of mortality and morbidity in immunocompromised patients.

In our study, 150 stool samples were collected from patients attending ART, Oncology department and Haemato-oncology department of Dr Prabhakar kore Hospital and MRC, Belagavi.

Age and gender wise distribution:

In present study among the patients attending ART, Oncology, Haemato-oncology cases females were predominant in number 88 (58.66%) compared to males 62 (41.33%). Female: male ratio is 0.59:0.41.

Opportunistic infections are present irrespective of the gender.

Intestinal parasitic infections can affect anyone, regardless of age or gender, due to factors like contaminated food/water, poor hygiene, close contact, environmental conditions, weakened immunity, travel to high-risk areas, and certain occupations.

Another study done on presence of OI due to coccidian parasites among IC individuals in Iran showed that occurrence is more in females (53.9%) than in males (34.8%).[60]

Another study conducted by Yampa Kamki, Rebachandra Singh H,et al.says of the 170 stool samples collected from patients the maximum number of participants

in the study were females118(69.4%) and males were 52(31%). Age group of 41-50 years in majority were included which is matching with our study. [61]

A study conducted on Intestinal parasitic infections in patients attending a tertiary care centre in New delhi showed that among 57 patients included in study the predominant patients were females were 34(60%) and males were 23(40.4%). The maximum number of participants were of 31-40 years age group. [62]

Cause of Immunocompromised status in study group:

In our study Among The 150 samples collected 141 (94%) were from patients attending ART, 9(6%) were from patients attending Oncology unit.

A study conducted by Yompe Kamki, Rebachandra Singh H,et al mentioned that the maximum No.of PLHIV [142(83.53%)], followed by some type of malignancy [12 (7.06%)].[63]

A study conducted on parasitic diseases in patients visiting a care centre in New Delhi showed that among 57 patients included 57% were from HIV patients, 43% were from other immunocompromised status.

In these studies, they included the study population similar to our study.

Patients undergoing Treatment:

Patients undergoing various treatment as mentioned. Of the 150 patients, 141(94%) were on ART, 1 (0.66%) patient was on Anti-Tb medication, 6 (4%) were on Antibiotics, 9(6%) on chemotherapeutic agents, 9(6%) were on corticosteroid treatment, similar to a study report conducted by Izadi S et.al among 204 patients HIV are 32.7%, Lymphoma is 39.6%, leukemia is 46.2% (31). And in other study by

Yompe Kamki,et al among the 170 patients they included,143(82%) were of HIV, 12(7.06%) were of cancer.[63]

Patients with history of Diarrhea

In present study, among the patients included 5(3.3%) have mentioned history of diarrhoea, while the rest 145(96.8%) have not mentioned the history of Diarrhea.

The prevalence rate of this parasites are more in the patients who complained for diarrhea, like that of a study by Jain S et ..al in Bhuvaneshwar whose prevalence of parasites is 7.2% [64]

In another study by G. Rathod P, Mishra B, et al in mentioned that the prevalence of positivity if 17.2% among the patients suffering from Diarrhea. [62]

CD4 count among the patients and positivity:

The enteric parasites were found in 2 (66%) (n=3) of the patients with CD4 count <200cells/µL as like with 6(8.9%) (n=67) CD4 count >200cells/ µL in the seropositive patients.

Which is like that study conducted by Gupta K et…al of VMMC hospital who showed prevalence of enteric parasites among the patients with CD4 count rate is <200cells/µL is 59.3% and >200cells/ µL is 23.5%. [65]

Another study done on identification of OI in HIV patients in Madhurandagam showed maximum no. of positivity of enteric parasites were among the patients with CD4 count <200cells/µL with cryptosporidium is the maximum isolated parasite. **[66]**

CONCLUSION

A total of 150 stool samples were collected from patients attending ART, Oncology, Haemato-oncology of Dr Prabhakar kore Hospital and MRC, Belagavi.

The majority of participants are females with 58.6% and males were of 41.3%. and the age group is 41-50(26.6%) followed by 31-40(22%).

Stool samples were collected in a container with spatula, wet mount preparation fecal smear and formol-ether sediment smear is done and stained by Modified-ZN staining.

Of the 150 samples screened 8(5.3%) were positive for oocysts of Cryptosporidium seen in a total of 3.3% patients with diarrhea and 66% of patients with CD4+ count <200cells/µL.

This implies that common opportunistic parasitic intestinal parasite among the study group is Cryptosporidium with prevalence of 5.3%.

SUMMARY

The present study entitled **"Identification of opportunistic infections caused by intestinal parasites in Immunocompromised patients"** is a one-year cross-sectional study was conducted in the Department of Microbiology, Jawaharlal Nehru Medical College, Belagavi and Dr. Prabhakar Kore Hospital and MRC, Belagavi from January 2023 – December 2023. This study included 150 immunocompromised patients attending ART, oncology, Haemato-oncology. The stool sample visualized for opportunistic intestinal parasites.

- Parasitic infection are still a major cause of mortality and morbidity globally among the immunocompromised patients.
- Maximum number of samples were collected from patients of age group 41-50 with a percentage of 26.6% followed by 31-40 which is 22%. Among them Females were Predominant participants.
- Among the 150 samples collected 141 (94%) were from patients attending ART, 9(6%) were from patients attending Oncology unit.
- 5 (3.33%) patients mentioned the history of Diarrhea and remaining 145(96.6%) did not mention about having diarrhea.
- 141(94%) were on ART, 1 (0.66%) patient was on Anti-Tb medication, 6 (4%) were on Antibiotics, 9(6%) on chemotherapeutic agents, 9(6%) were on corticosteroid treatment.
- Out of the 141 patients attending ART, patients with maximum CD4 count were in the range of 200-500cells/ µL, 64 patients (48%), followed by 500-

1000cells/ µL, 59 patients (42%), >1000cells/µL, 12 patients (9%), and <200cells/ µL, 3 patients (1.42%).

- out of the positive 8/150 (5.3%)stool samples the isolates were Cryptosporidium spp.

LIMITATIONS OF STUDY:

- Immunocompromised patients often have atypical presentations or may not show typical symptoms of infection, making it challenging to diagnose intestinal parasite infections solely based on clinical symptoms.
- Fluorescent microscopy of stool specimen was not performed as we could not procure fluorescent tagged antibodies directed to stool sample.
- ELISA for confirmation of antigen of oocysts could not be performed.
- We could not collect the details of ESR, C-Reactive protein and D-dimer as the patients were attending ART.

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ANNEXURE I

KAHERs JNMC BELAGAVI

INFORMED CONSENT FORM

"IDENTIFICATION OF OPPORTUNISTIC INFECTIONS CAUSED BY INTESTINAL PARASITES IN THE IMMUNOCOMPROMISED PATIENTS".

Name of Student/Principal Investigator: _____

Name of Guide/Co Investigators: _____

Need of study: Gastrointestinal parasitic infections are common in Immunocompromised states such as HIV/AIDS, patients undergoing immunosuppressive therapy, corticosteroid therapy. Patients may have unexplained weight loss, may cause malnourishment. So early Identification of intestinal parasites can decrease the Mortality and Morbidity of such Patients.

Objective: 1) To identify various Parasitic OIs in immunocompromised patients. 2) To co-relate the OI with patient's clinical manifestations. 3)To co-relate OIs with other laboratory parameters of the patients (complete blood picture), CD4 count, Erythrocytic Sedimentation rate (ESR), CD4 count, C-Reactive protein, D-dimer, and radiological investigations etc).

Explanation of procedure: Stool sample is collected from immunocompromised patients in sterile wide mouthed leak proof container.

Withdrawal from participation in the study: Participation in this study is voluntary. You will be free to decide whether to participate in this study or continue participation

once enrolled. In case you decide to withdraw your participation, you are free to do so. However, please convey the decision to the principal investigator.

Possible benefits from participating in the study: You will know the causative organism by participating in this study, so appropriate treatment for opportunistic infection is given. The data gathered will help the population at large.

Possible risks from participating in the study: There are no risks involved in participating in this study.

Privacy and confidentiality: The information collected from you will be coded, to prevent any person from identifying you. Your identity will never be revealed. The data collected from you will be kept confidential and only processed or aggregated data will be used for publication.

Financial incentives: You will not receive any payment for participating in this study.

Authorization for publication of aggregated data: Results obtained after processing of the aggregated data will be published for scientific purposes and or presented to scientific groups. However, your identity will never be revealed.

Questions: If you have any question or complaints with regard to your right as study participant you may contact **Dr Harsha Hegde,** Chairperson, Ethical committee of JNMC, 0831-2473777 Extension 4052.

Legal rights: By signing this consent form, we are not waving any of your legal rights.

CONSENT STATEMENT

I am making a voluntary decision to participate in the study **"IDENTIFICATION OF OPPORTUNISTIC INFECTIONS CAUSED BY INTESTINAL PARASITES IN THE IMMUNOCOMPROMISED PATIENTS"**. My signature below indicates that I have decided to participate and I have read the information provided above or the information provided above has been read to me in the language that I understand best. I was given the opportunity to ask questions and that they have been answered to my satisfaction.

Name of the participant:

Signature or left thumb impression of the participant:

Name of the witness:

Signature or left thumb impression of the witness:

Name of the investigator:

Signature of the investigator:

ANNEXURE II

QUESTIONNAIRE (PROFORMA)FOR COLLECTING DATA

Name: IP/OP No:

Age: DOP:

Sex: Nature of sample:

Address:

Diagnosis:

IMMUNOCOMPROMISED STATES	YES	NO
HIV STATUS		
MALIGNANCY		
IMMUNOSUPPRESIVE THERAPY		
CORTICOSTEROIDS		

PRESENTING ILLNESS:

PRESENTING COMPLAINTS	YES	NO
DIARRHOEA		

H/O PRESENTING ILLNESS:

H/O PRESENTING ILLNESS	YES	NO
FREQUENCY		
ASSOC. WITH FRANK BLOOD		
ASSOC.WITH MUCUS/PUS		
WEIGHT LOSS		

H/O MEDICATIONS:

	YES	NO
ART		
ANTI-TB MEDICATION		
ANTIBIOTIC THERAPY		
IMMUNOSUPRESSIVE THERAPY		
CORTICOSTEROID THERAPY		

CD4 COUNT:

VIRAL LOAD:

H/O OF ANY OTHER RELEVANT INVESTIGATIONS

ANNEXURE III

MEDIA AND REAGENTS

PREPARATION OF ZIEHL-NEELSEN STAIN:

Reagents:

Carbol fuschin- primary dye

Sulphuric acid-1%

Methylene blue

Carbol fuschin:

Distilled water: ml

Basic fuschin: 1g

Ethyl alcohol(100%): 10ml

Phenol crystals: 5ml

0.25% Methylene blue in 1%acetic acid:

Methylene blue: 0.25g

Distilled water: 99 ml

Acetic acid: 1ml

1% Sulphuric acid:

Distilled water: 99ml

Sulphuric acid: 1ml

Procedure of staining:

1. On a sterile clean slide microscopic slide make this smear of the sample and heat fix the smear over blue flame.
2. Over this Smear pour and flood this smear with carbon fuschin and heat gently until it produces fumes.
3. Allow it to stand for 5 minutes and wash it off with gently flowing tap water.

4. Add 1 % H_2SO_4 and leave it for 1 to 2 minutes repeat this step until this smear appears pink in color.
5. Wash of the acid with water.
6. Flod the smear with methylene blue dye and leave it for two to 3 minutes and wash it with water.
7. Air dry the slide and look under oil immersion.

FORMOL-ETHER SEDIMENTATION TECHNIQUE

1. About half teaspoonful (4g) of feces is transferred to a tube containing 10 mL of 5–10% formalin, mixed thoroughly and allowed to stand for 30 minutes.
2. Then the mixture is filtered into a 15 mL conical centrifuge tube covered with two layers of gauze. About 8 mL of the filtrate is collected (3–4 mL for formalin persevered stool)
3. 0.85% saline (or 5–10% formalin) is added almost to the top of the tube containing the filtrate and centrifuged for 10 minutes.
4. The supernatant is discarded and 0.5–1 mL of the sediment is resuspended in saline or formalin (filled up to the top of the tube) and centrifuged again for 10 minutes.
5. The sediment is resuspended in 5–10% formalin (filled half of the tube) and centrifuged. This step may be eliminated if the supernatant fluid is clear after the first wash.
6. 4–5 mL of ether (or ethyl acetate) is added and the tube is closed with a stopper and shaken vigorously to mix well. The stopper is removed and the tube is centrifuged for 10 minutes.
7. Four layers are formed. Top layer consists of ether, second is a plug of debris, third is a clear layer of formalin and the fourth is the sediment.

8. The debris is removed from the side of the tube with the help of a glass rod and supernatant is discarded.
9. With a pipette, the sediment is removed and the saline or iodine mount is made and examined under the microscope.
10. Smear is made with sediment and modifies acid fast stain is done and looked under oil immersion.

ANNEXURE IV MASTER CHART

sl no	IP/OP no	Age	sex	Diagnosis	HIV status	Malignancy	Immunosuppresive therapy	Corticsteroids	Diarrhoea	Frequency of diarrhoea	wt loss	ART	Anti-TB medication	Antibiotic therapy	chemotherapy	Corticosteroid therapy	CD4 count	Viral load	Modified ZN	Concentration
1	ART-KA-BE-07-	28	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	429	TND	-	-
2	ART-KA-BE-07-319	22	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	276	TND	-	oocyst of Cryptosporidium
3	ART-KA-BE-07-320	54	F	HIV	Reactive	No	No	No	Yes	2	No	Yes	No	NO	No	No	364	TND	-	-
4	ART-KA-BE-07-321	64	M	HIV	Reactive	No	No	No	Yes	-	No	Yes	No	NO	No	No	420	TND	-	-
5	ART-KA-BE-07-327	65	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	542	TND	-	-
6	ART-KA-BE-07-245	66	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	341	TND	-	-
7	ART-KA-BE-07-255	68	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	336	TND	-	-
8	ART-KA-BE-07-269	51	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	674	TND	-	-
9	ART-KA-BE-07-272	48	F	HIV	Reactive	No	No	No	Yes	-	No	Yes	No	NO	No	No	432	TND	-	-
10	ART-KA-BE-07-257	42	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	267	TND	oocyst of cryptosporidium	-
11	ART-KA-BE-07-277	43	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	212	TND	oocyst of cryptosporidium	-
12	ART-KA-BE-07-279	20	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	560	TND	-	-
13	ART-KA-BE-07-	29	F	HIV	Reactive	No	No	No	No	-	No	Yes	Yes	NO	No	No	361	TND	-	-
14	ART-KA-BE-07-249	28	F	HIV	Reactive	No	No	No	Yes	-	No	Yes	No	NO	No	No	249	TND	-	-
15	ART-KA-BE-07-	52	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	437	TND	-	-
16	ART-KA-BE-07-224	36	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	784	TND	-	-
17	ART-KA-BE-07-231	33	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	346	TND	-	-
18	ART-KA-BE-07-232	42	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	623	TND	-	-
19	ART-KA-BE-07-233	54	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	374	TND	-	-
20	ART-KA-BE-07-235	38	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	165	TND	oocyst of Cryptosporidium	-
21	ART-KA-BE-07-236	30	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	248	TND	-	-
22	ART-KA-BE-07-	31	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	609	TND	-	-
23	ART-KA-BE-07-161	65	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	231	TND	-	-
24	ART-KA-BE-07-169	60	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	533	TND	-	-
25	ART-KA-BE-07-172	61	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	330	TND	-	-
26	ART-KA-BE-07-176	42	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	660	TND	-	-
27	ART-KA-BE-07-184	47	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	258	TND	-	oocyst of cryptosporidium
28	ART-KA-BE-07-185	44	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	371	TND	-	-
29	ART-KA-BE-07-189	45	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	623	TND	-	-
30	ART-KA-BE-07-191	31	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	638	TND	-	-
31	ART-KA-BE-07-192	30	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	893	TND	-	-
32	ART-KA-BE-07-196	38	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	1309	TND	-	-
33	ART-KA-BE-07-197	76	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	406	TND	-	-
34	ART-KA-BE-07-198	25	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	376	TND	Oocysts of Cryptosporidium	-
35	ART-KA-BE-07-201	39	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	437	TND	-	-
36	ART-KA-BE-07-204	40	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	799	TND	-	-
37	ART-KA-BE-07-207	52	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	913	TND	-	-

38	ART-KA-BE-07-208	25	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	780	TND	-	-
39	ART-KA-BE-07-210	22	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	573	TND	-	-
40	ART-KA-BE-07-213	34	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	1206	TND	-	-
41	ART-KA-BE-07-214	26	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	484	TND	-	-
42	ART-KA-BE-07-215	28	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	854	TND	-	-
43	ART-KA-BE-07-216	29	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	897	TND	-	-
44	ART-KA-BE-07-220	22	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	475	TND	-	-
45	ART-KA-BE-07-223	27	F	HIV	Reactive	No	No	No	Yes	-	No	Yes	No	NO	No	No	249	TND	Oocysts of Cryptosporidium	-
46	ART-KA-BE-07-225	28	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	683	TND	-	-
47	ART-KA-BE-07-227	38	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	1064	TND	-	-
48	ART-KA-BE-07-228	46	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	437	TND	-	-
49	ART-KA-BE-07-232	68	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	623	TND	-	-
50	ART-KA-BE-07-231	22	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	346	TND	-	-
51	ART-KA-BE-07-235	35	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	165	TND	Oocysts of Cryptosporidium	-
52	ART-KA-BE-07-189	28	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	457	TND	-	-
53	ART-KA-BE-07-190	36	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	623	TND	-	-
54	ART-KA-BE-07-186	27	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	554	TND	-	-
55	ART-KA-BE-07-173	44	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	481	TND	-	-
56	ART-KA-BE-07-171	46	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	594	TND	-	-
57	ART-KA-BE-07-163	48	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	814	TND	-	-
58	ART-KA-BE-07-169	35	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	533	TND	-	-
59	ART-KA-BE-07-166	65	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	420	TND	-	-
60	ART-KA-BE-07-165	52	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	619	TND	-	-
61	ART-KA-BE-07-175	55	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	312	TND	-	-
62	ART-KA-BE-07-236	36	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	451	TND	-	-
63	ART-KA-BE-07-139	30	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	No	No	No	245	TND	-	-
64	ART-KA-BE-07-120	45	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	312	TND	-	-
65	ART-KA-BE-07-	45	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	391	TND	-	-
66	ART-KA-BE-07-111	42	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	221	TND	-	-
67	ART-KA-BE-07-274	69	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	281	TND	-	-
68	ART-KA-BE-07-237	40	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	511	TND	-	-
69	ART-KA-BE-07-211	35	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	347	TND	-	-
70	ART-KA-BE-07-210	39	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	527	TND	-	-
71	ART-KA-BE-07-532	31	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	654	TND	-	-
72	ART-KA-BE-07-524	37	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	735	TND	-	-
73	ART-KA-BE-07-684	40	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	No	No	No	406	TND	-	-
74	ART-KA-BE-07-623	28	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	457	TND	-	-
75	ART-KA-BE-07-606	39	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	1028	TND	-	-
76	ART-KA-BE-07-131	18	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	1049	TND	-	-
77	ART-KA-BE-07-132	48	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	1074	TND	-	-
78	ART-KA-BE-07-698	49	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	728	TND	-	-
79	10045106	14	F	Pre-B ALL	Non-Reactive	Yes	Yes	Yes	No	-	No	Yes	No	NO	Yes	No	-	-	-	-
80	10045895	13	F	T-ALL	Non-Reactive	Yes	Yes	Yes	No	-	No	No	No	No	Yes	No	-	-	-	-
81	10045395	4	M	Pre-B ALL	Non-Reactive	Yes	Yes	Yes	No	-	No	No	No	NO	Yes	No	-	-	-	-
82	10032928	7	F	Pre-B ALL	Non-Reactive	Yes	Yes	Yes	No	-	No	No	No	Yes	Yes	Yes	-	-	-	-
83	10043254	13	M	Relapsed ALL	Non-Reactive	Yes	Yes	Yes	No	-	No	No	No	Yes	Yes	Yes	-	-	-	-
84	10045975	13	M	AML 18:21 translocation positive	Non-Reactive	Yes	Yes	Yes	No	-	No	No	No	Yes	Yes	Yes	-	-	-	-
85	10042767	11	F	Pre-B ALL	Non-Reactive	Yes	Yes	Yes	No	-	No	No	No	Yes	Yes	Yes	-	-	-	-
86	10041776	14	F	Extrarenal Rhabdoid tumor	Non-Reactive	Yes	Yes	Yes	No	-	No	No	No	Yes	Yes	Yes	-	-	-	-
87	10039624	11	M	Pre-B ALL	Non-Reactive	Yes	Yes	Yes	No	-	No	No	No	Yes	Yes	Yes	-	-	-	-
88	ART-KA-BE-07-481	22	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	281	TND	-	-

89	ART-KA-BE-07-482	56	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	511	TND	-	-
90	ART-KA-BE-07-483	39	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	347	TND	-	-
91	ART-KA-BE-07-484	35	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	527	TND	-	-
92	ART-KA-BE-07-485	12	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	654	TND	-	-
93	ART-KA-BE-07-486	44	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	735	TND	-	-
94	ART-KA-BE-07-472	35	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	406	TND	-	-
95	ART-KA-BE-07-473	46	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	457	TND	-	-
96	ART-KA-BE-07-474	48	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	1028	TND	-	-
97	ART-KA-BE-07-475	45	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	1049	TND	-	-
98	ART-KA-BE-07-476	55	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	1074	TND	-	-
99	ART-KA-BE-07-477	52	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	728	TND	-	-
100	ART-KA-BE-07-478	55	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	554	TND	-	-
101	ART-KA-BE-07-479	60	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	481	TND	-	-
102	ART-KA-BE-07-480	53	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	594	TND	-	-
103	ART-KA-BE-07-466	33	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	814	TND	-	-
104	ART-KA-BE-07-443	60	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	533	TND	-	-
105	ART-KA-BE-07-444	55	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	420	TND	-	-
106	ART-KA-BE-07-441	45	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	619	TND	-	-
107	ART-KA-BE-07-445	45	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	312	TND	-	-
108	ART-KA-BE-07-446	63	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	451	TND	-	-
109	ART-KA-BE-07-447	45	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	245	TND	-	-
110	ART-KA-BE-07-448	48	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	312	TND	-	-
111	ART-KA-BE-07-449	26	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	391	TND	-	-
112	ART-KA-BE-07-450	52	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	249	TND	-	-
113	ART-KA-BE-07-451	54	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	683	TND	-	-
114	ART-KA-BE-07-440	37	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	1064	TND	-	-
115	ART-KA-BE-07-452	64	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	437	TND	-	-
116	ART-KA-BE-07-453	64	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	623	TND	-	-
117	ART-KA-BE-07-454	54	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	346	TND	-	-
118	ART-KA-BE-07-455	60	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	165	TND	-	-
119	ART-KA-BE-07-456	37	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	457	TND	-	-
120	ART-KA-BE-07-457	42	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	623	TND	-	-
121	ART-KA-BE-07-458	51	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	554	TND	-	-
122	ART-KA-BE-07-459	24	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	481	TND	-	-
123	ART-KA-BE-07-460	46	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	594	TND	-	-
124	ART-KA-BE-07-422	57	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	814	TND	-	-
125	ART-KA-BE-07-423	60	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	533	TND	-	-
126	ART-KA-BE-07-424	48	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	420	TND	-	-
127	ART-KA-BE-07-425	35	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	550	TND	-	-
128	ART-KA-BE-07-426	44	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	1021	TND	-	-
129	ART-KA-BE-07-427	48	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	552	TND	-	-
130	ART-KA-BE-07-428	45	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	554	TND	-	-
131	ART-KA-BE-07-429	37	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	481	TND	-	-
132	ART-KA-BE-07-430	47	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	594	TND	-	-
133	ART-KA-BE-07-340	56	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	814	TND	-	-
134	ART-KA-BE-07-412	40	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	533	TND	-	-
135	ART-KA-BE-07-413	46	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	420	TND	-	-
136	ART-KA-BE-07-414	51	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	619	TND	-	-
137	ART-KA-BE-07-415	45	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	312	TND	-	-
138	ART-KA-BE-07-416	45	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	451	TND	-	-
139	ART-KA-BE-07-417	46	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	245	TND	-	-
140	ART-KA-BE-07-418	68	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	312	TND	-	-
141	ART-KA-BE-07-419	52	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	391	TND	-	-

142	ART-KA-BE-07-420	45	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	623	TND	-	-
143	ART-KA-BE-07-421	46	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	638	TND	-	-
144	ART-KA-BE-07-402	40	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	893	TND	-	-
145	ART-KA-BE-07-403	38	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	1309	TND	-	-
146	ART-KA-BE-07-404	49	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	406	TND	-	-
147	ART-KA-BE-07-405	42	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	376	TND	-	-
148	ART-KA-BE-07-406	58	M	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	437	TND	-	-
149	ART-KA-BE-07-407	65	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	799	TND	-	-
150	ART-KA-BE-07-408	31	F	HIV	Reactive	No	No	No	No	-	No	Yes	No	NO	No	No	913	TND	-	-
	10045764	61	M	ca. stomach	Non reactive	Yes	Yes	Yes	No	-	No	No	No	Yes	Yes	Yes	-	-	-	-
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