STUDY OF OPPORTUNISTIC PARASITIC INFECTIONS IN HIV / AIDS PATIENTS PRESENTING WITH DIARRHEA & ITS CO-RELATION WITH CD4 COUNT.

By

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Dissertation submitted to

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In partial fulfillment of the
requirements for the degree of

DOCTOR OF MEDICINE
In
MICROBIOLOGY

Under the guidance of

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2013

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LIST OF ABBREVIATIONS

HIV	: Human immunodeficiency virus
AIDS	: Acquired immunodeficiency syndrome
CD4	: Cluster of differentiation 4
UNAIDS	: United Nations Programme on HIV/AIDS.
WHO	: World health organization
NACO	: National AIDS control organization
NACP	: National AIDS control program
NGO	: Non-governmental organization
FSW	: Female sex worker
HTLV	: Human T-Lymphotropic Virus
RNA	: Ribonucleic acid
DNA	: Deoxyribonucleic acid
MHC	: Major histocompatibility complex
ART	: Anti-retroviral therapy
HAART	: Highly active anti-retroviral therapy
TB	: Tuberculosis
ITP	: Idiopathic thrombocytopenic purpura
CMV	: Cytomegalovirus
ELISA	: Enzyme linked immunosorbent assay
EIA	: Enzyme immune assay

INF	: Interferon
TNF	: Tumor necrosis factor
ICT	: Immunochromatographic test
IFA	: Immunofluorescent assay
PCR	: Polymerase chain reaction
TMP- SMZ	: Trimethoprim - Sulphamethoxazole
CMI	: Cell mediated immunity
VCTC	: Voluntary Counseling and Testing Centre
RBC	: Red blood cells
FITC	: Fluorescein Isothiocyanate
CI. Interval	: Confidence interval
Cr.	: Cryptosporidium parvum
Cy.	: Cyclospora cayetanensis
I.	: Isospora belli
SWM	: Saline Wet Mount
IWM	: Iodine Wet Mount
LPCB	: Lactophenol Cotton Blue Mount
MKAFS	: Modified Kinyoun’s Acid Fast Staining

ABSTRACT

OBJECTIVE – The present study was undertaken to detect opportunistic parasites in HIV Seropositive patients with diarrhoea and correlation with different levels of immunity (CD4 Count).

MATERIALSANDMETHODS – The study was carried out at BLDEA's Sri B.M .Patil Medical College Hospital & Research Centre, Bijapur from DEC. 2011 to MAY 2013 among consecutively enrolled 110 HIV patients presenting with diarrhoea. Stool samples were collected and examined for parasites by direct microscopic examination of feces i.e. Saline wet mount, Iodine wet mount, Lactophenol cotton blue mount, Modified Kinyoun's acid fast stain.CD4 counts of these patients was monitored and noted.

RESULTS – Opportunistic parasites were detected in 44.54% patients i.e. 49 of 110 patients studied. Majority of the study population belonged to the age group of 31 – 50 years (68.19%) with male preponderance accounting for 55.45%, and Male to female ratio 1.2:1. Chronic diarrhoea had higher percentage of parasite positivity 80.9% (47/110).Cryptosporidium was identified in maximum number of positive cases (32.73%) followed by mixed infection (9.09%) and Cyclospora infection (2.72%). Modified Kinyoun's acid-fast staining could identify maximum number of Isospora, Cyclospora and Cryptosporidium. Cryptosporidium were seen only by this method. Maximum patients which were positive for parasites had CD4 counts <100 i.e. 24 (50%), followed by CD4 counts 100 - 200 i.e. 22 (44.9%), whereas only 3 (6.1%) had CD4 counts 200 - 500. No pathogenic parasites were isolated in patients with CD4 counts > 500 cell/mm3. 87.3% of our study group was on HAART and in maximum (55.2%) cases on treatment no parasite was identified.

CONCLUSION – The present study shows that diarrhoea in HIV/AIDS was mostly due to opportunistic coccidian parasites associated with low CD4 count. Thus Detection of etiologic pathogens might help clinicians to decide appropriate management strategies and reduce morbidity and mortality in such individuals.

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INTRODUCTION

INTRODUCTION

Human immunodeficiency virus (HIV) infection has become a global epidemic far more extensive than what was predicted even a decade ago. The global spread has been so swift that no country has been spared and the pace of the epidemic is increasing in India.[1]

Globally, 34.0 million (31.4 million–35.9 million) people were living with HIV at the end of 2011.[2] India has the third largest number of people living with HIV/AIDS. As per the 2008-09 HIV estimates, there are an estimated 23.9 lakh people currently living with HIV/AIDS in India with an adult prevalence of 0.31% in 2009.[3]

HIV infection is marked by a progressive decrease in the number of circulating CD4 T-helper cells, which, over a period of years, leads to immunologic decline and death due to opportunistic infections and neoplasms. The spectrum of opportunistic infections differs from region to region.[1]

Gastrointestinal infections are very common in patients with HIV infection or AIDS. Diarrhoea is a common clinical presentation of these infections. Reports indicate that diarrhoea occurs in 30-60 per cent of AIDS patients in developed countries and in about 90 per cent of AIDS patients in developing countries.[4] In Africa this disorder has been associated with “Slim disease” a term that describes the great loss of weight that accompanies diarrhoea. [5]

Diarrhoea is defined as the passage of three or more loose stools/day. Diarrhoea associated with HIV infection may be acute or chronic. Acute diarrhoea is defined as diarrhoea of <14 days duration and chronic diarrhoea is defined as diarrhoea lasting for >14 days to 4 months or diarrhoea for more than a month.[6, 7]

Studies have shown that AIDS patients with chronic diarrhoea and occult

enteropathogens often have greater mean weight loss and a significantly shorter survival than those without pathogens. Chronic diarrhoea is an independent marker of poor prognosis in patients with AIDS. In addition to substantial work loss and a markedly decreased quality of life, such patients also frequently have annual health costs that are 50% higher than comparable patients without diarrheal symptoms. [8]

The etiologic spectrum of enteric pathogens causing diarrhoea includes bacteria, parasites, fungi and viruses,[9] though that of parasitic origin is prominent in patients with AIDS in developing countries. Of these, protozoan parasitic infections are the most serious ones causing severe morbidity and mortality.[10]

The presence of opportunistic parasites Cryptosporidium parvum, Cyclospora cayetanensis, Isospora belli and Microsporidia are documented in patients with AIDS. Non opportunistic parasites such as Entamoeba histolytica, Giardia lamblia, Ascaris lumbricoides, Strongyloides stercoralis, Blastocystis hominis and Ancylostoma duodenale are frequently encountered in developing countries but are not currently considered opportunistic in AIDS patients.[11], [4]

The degree of immune-suppression, as defined by the CD4+ T-cell count, determines to a large extent when individuals with HIV infection will develop opportunistic infections. The incidence and outcome of many of these complications, however, can be altered by preventive measures, in particular primary and secondary prophylaxis.[12]

At present, the initiation of primary prophylactic therapies for opportunistic infections is based chiefly on the absolute CD4+ T-cell count which has been shown to be an excellent predictor of the short term overall risk of developing AIDS among HIV-infected patients. A decrease in CD4+ T-cells counts is responsible for the profound immunodeficiency that lead to various opportunistic infections in HIV-

infected patients.

There have been reports on frequency of various pathogens causing diarrhoea from different parts of India. . The incidence and prevalence of infection with a particular enteric parasite in HIV/AIDS patients is likely to depend upon the endemicity of that particular parasite in the community. However, there is a paucity of data on correlation of CD4+ T-cell counts and the etiology of diarrhoea among the HIV patients in this part of India. Thus, this study was conducted to identify the opportunistic parasitic infection in HIV/AIDS patients presenting with diarrhoea and to co-relate their presence with CD4+ T-cell counts.

AIMS

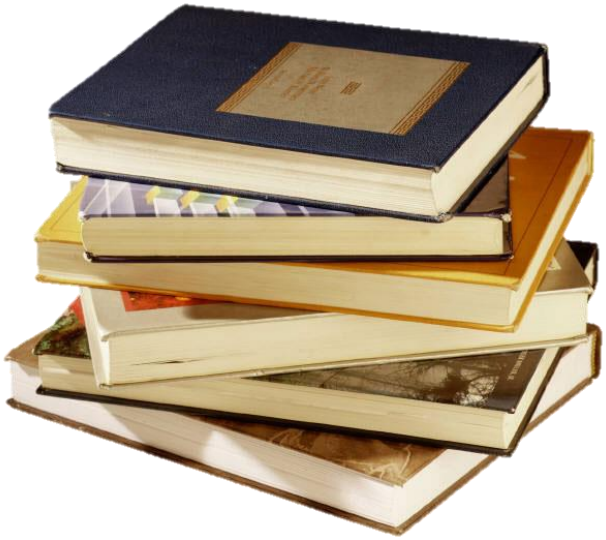
&

OBJECTIVES

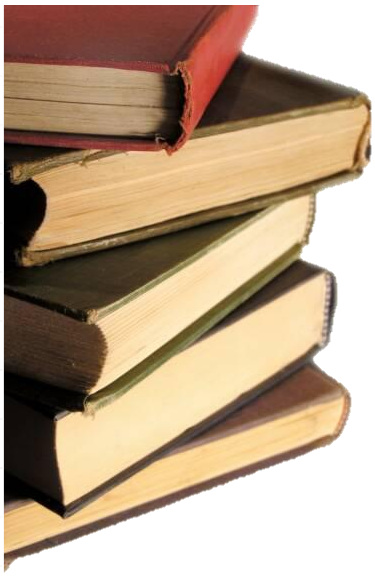
AIMS AND OBJECTIVES

1. To identify parasites in stool samples of HIV seropositive patients with diarrhoea.

2. To correlate the opportunistic intestinal parasitic diarrhoea in HIV seropositive patients with CD4+ T-cells counts.



REVIEW OF LITERATURE



REVIEW OF LITERATURE

HUMAN IMMUNODEFICIENCY VIRUS (HIV)

The pandemic of HIV infection, the cause of AIDS, is clearly the defining medical and public health issue of our generation and ranks among the greatest infectious disease scourges in history. Since the world first became aware of new immunodeficiency disease in gay men in US (1981) the disease has spread in successive waves in various regions around the globe.[13]

HISTORY AND EPIDEMIOLOGY

1984 - Retrovirus isolated by Montagnier group at Pasteur Institute in France, shown to be the cause of AIDS by Robert Gallo laboratory. CD4 identified as an HIV receptor. [14]

1988 - First World AIDS Day held on December 1.

1991 - Red ribbon becomes an international symbol of AIDS awareness

2000 - 34.3 million people worldwide believed to be HIV-positive. [15]

2009 - In November, UNAIDS published its annual Epidemic Update stating significant decline in new HIV infections (by 17% since 2001), due to the increased availability of HIV drugs, deaths had declined by 10% over the past five years. [16]

2010 - In July, WHO released its revised editions of the ART guidelines. The previous version of the guidelines (2006) recommended treatment initiation at a CD4 count of 200 cells/mm3, whereas the updated guidelines recommended treatment initiation at ≤350 cells/mm3 (or those with WHO clinical stage 3 or 4 if CD4 testing was unavailable).[17] The recommendation was based on evidence that showed starting treatment earlier slowed disease progression and reduced the risk of HIV transmission. The new guidelines would significantly increase the global number of people in need of antiretroviral treatment.

TABLE 1 : Global Epidemiology of HIV/AIDS [18]

	Estimate	Range
People living with HIV/AIDS in 2010	34 million	31.6-35.2 million
Proportion of adults living with	50	47-53
Children living with HIV/AIDS in 2010	3.4 million	3.0-3.8 million
People newly infected with HIV in 2010	2.7 million	2.4-2.9 million
Children newly infected with HIV in 2010	390,000	340,000-450,000
AIDS deaths in 2010	1.8 million	1.6-1.9 million

INDIA & HIV

According to annual report 2011 – 2012, released by National AIDS Control Organization (NACO), the National adult HIV prevalence in India is approximately 0.31% which corresponds to an estimate 2.39 million people living with HIV in the country.[3]

The HIV/AIDS epidemic came to India in 1986 when the first case of HIV was detected in Chennai. Since then, the number of infected people has grown substantially.[19] Most of the initial cases had occurred through heterosexual sex; but at the end of the 1980s, a rapid spread of HIV was observed among injecting drug users in Manipur, Mizoram and Nagaland. [20]

1992 -NACP I launched to slow down the spread of HIV infection

1999 -NACP II begins, focussing on behaviour change, increased decentralization and NGO involvement.

2007 - 2012 - NACP III was implemented to halt and reverse the HIV/AIDS epidemic in India.[19]

KARNATAKA AND HIV [21]

Karnataka is one of four large states in South India with a relatively advanced HIV epidemic, with the adult HIV prevalence in some districts exceeding 1% (HIV Sentinel Surveillance). Northern Karnataka is more severely affected by HIV than southern Karnataka, in part because of the large concentration of commercial sex

networks in northern Karnataka. In addition, in northern Karnataka, the HIV epidemic tends to be more advanced in rural than in urban areas, unlike many other areas in India. The continuing high HIV prevalence in core and bridging populations indicates an epidemic that is largely driven by local sexual networks involving clients of FSWs, FSWs and MSM-T (men who have sex with men and transgender)

ETIOLOGIC AGENT

The etiologic agent of AIDS is HIV, which belongs to the family of human retroviruses (Retroviridae) and the subfamily of lentiviruses. Nononcogenic lentiviruses cause disease in other animal species, including sheep, horses, goats, cattle, cats, and monkeys. The four recognized human retroviruses belong to two distinct groups: the human T lymphotropic viruses (HTLV)-I and HTLV-II, which are transforming retroviruses; and the human immunodeficiency viruses, HIV-1 and HIV-2, which cause cytopathic effects either directly or indirectly.[22] HIV-1 is further divided into three groups: 'major' group, M; 'outlier' group, O; and 'new' group, N. Group M has several subtypes or clades (subtypes A to K). [23] HIV-1 is the more common type associated with AIDS in the United States, Europe, and Central Africa, whereas HIV-2, first identified in 1986, causes a similar disease principally in West Africa.[24]

Both HIV-1 and HIV-2 are zoonotic infections. The Pan troglodytestroglodytes species of chimpanzees has been established as the natural reservoir of HIV-1 and the most likely source of original human infection. HIV-2 is more closely related phylogenetically to the simian immunodeficiency virus (SIV) found in sooty mangabeys than it is to HIV-1. [22]HIV is a highly mutable virus. The great variability of HIV is believed to be due to the error prone nature of reverse transcription.[25]

STRUCTURE OF HIV

HIV is a spherical enveloped virus, about 90 – 120 nm in size. The nucleocapside has an outer icosahedral shell and an inner cone shaped core, enclosing the ribonucleoproteins. The genome is diploid, composed of two identical single stranded, positive sense RNA copies.[25] The RNA genome is coated with the Nucleocapsid protein, and the RNA–protein complexes are enclosed in a capsid composed of multiple subunits .[26]

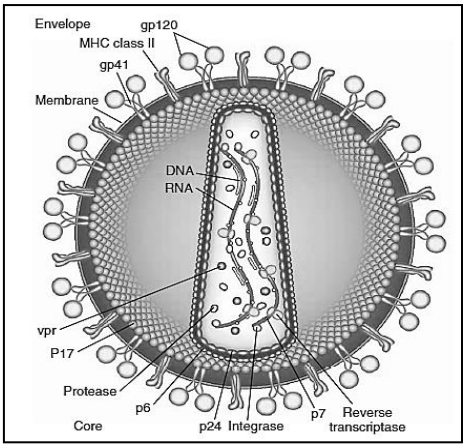


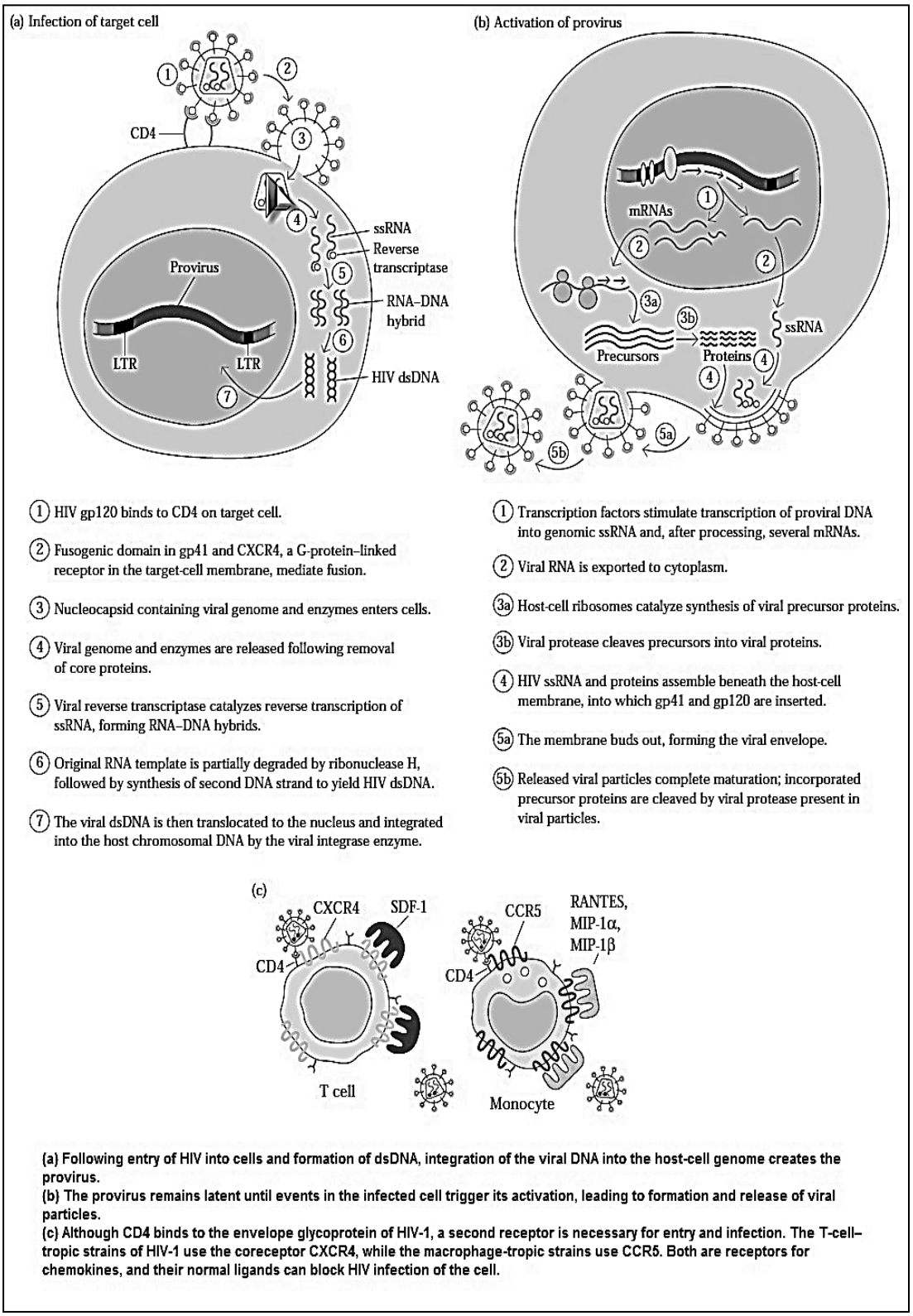
FIGURE 1: HIV structure[27]

TABLE 2: Genes and Gene Products of HIV-1 AND HIV-2[27]

Gene	*Proteins*	*Size (kDa)*	*Function/Properties*
gag	p17		Matrix protein; interacts with gp41
	p24		Core protein
	p6		Core protein; binds to Vpr
	p7		Nucleocapsid; binds to RNA
	p1		
	p2		
pol	Protease	10	Proteolytic cleavage of Gag and Pol
	Reverse transcriptase	66, 51	Polymerase and RNase H activity (p66 only)
	Integrase	32	Integration into chromosome
env	gp120		Envelope; viral entry into cell
	gp41		Transmembrane protein; cell fusion
vif	Virion infectivity protein	23	Efficient cell-free transmission
vpr	Viral protein R	18	Enhances viral replication in primary cells, virion-associated protein; G_2/M phase arrest; nuclear localization
tat	*Trans*-activator of transcription	14	Major viral *trans*-activator, immune suppression
rev	Regulator of expression of virion protein	19	Enhances expression of unspliced and singly spliced RNAs
*vpu**	Viral protein U	15-16	Enhances virion release from cells; downregulates CD4 and MHC class I surface expression
nef	Negative regulatory factor	27	Inhibits or enhances viral replication depending on strain and cell type
			Downregulates CD4; MHC class I
			Antiapoptosis
vpx†	Virion protein x	25	Packaged into the virion

*HIV-1 only.
†HIV-2 only.
MHC, major histocompatibility complex; RNase H, ribonuclease H.

REPLICATION [28]



(a) Following entry of HIV into cells and formation of dsDNA, integration of the viral DNA into the host-cell genome creates the provirus.
(b) The provirus remains latent until events in the infected cell trigger its activation, leading to formation and release of viral particles.
(c) Although CD4 binds to the envelope glycoprotein of HIV-1, a second receptor is necessary for entry and infection. The T-cell–tropic strains of HIV-1 use the coreceptor CXCR4, while the macrophage-tropic strains use CCR5. Both are receptors for chemokines, and their normal ligands can block HIV infection of the cell.

FIGURE 2: Overview of HIV infection of target cells and activation of provirus.

NATURAL HISTORY OF HIV

PRIMARY HIV INFECTION [29]

Primary infection usually presents as an acute febrile illness 2–4 weeks post exposure, often with lymphadenopathy, pharyngitis, maculopapular rash, orogenital ulcers and meningoencephalitis. Profound transient lymphopaenia (including low CD4) can develop, and opportunistic infections may occur, but these infections should not be confused with clinical staging events developing in established HIV infection. Primary HIV infection can be identified by recent appearance of HIV antibody or by identifying viral products (HIV-RNA or HIV-DNA and/or ultrasensitive HIV *p*24 antigen) with negative (or weakly reactive) HIV antibody.

IMMUNE STATUS IN ADULTS

The normal absolute CD4 count in adolescents and adults ranges from 500 to 1500 cells per mm^3 of blood. In general, the CD4 (%CD4+ or absolute count) progressively decreases as HIV disease advances. As in children, individual counts may vary within an individual adult or adolescent and assessing the CD4 count over time is more useful.[30, 31, 32] The CD4 count usually increases in response to effective combination antiretroviral therapy, although this may take many months.[33]

The production of virus by CD4+ T-cells maintains of a steady state of viral load and T-cell number. A dynamic relationship exists between the number of CD4 cells and the amount of virus produced. As virus is produced, new CD4 cells are infected, and these infected cells have a half-life of 1.5 days. In progression to full AIDS, the viral load increases and the CD4 T-cell count decreases before onset of opportunistic infections. If the viral load is decreased by anti-retroviral treatment, the CD4+ T-cell number increases almost immediately. [28]

The proposed immunological classification outlines four bands of HIV related immunodeficiency – no significant immunodeficiency, mild immunodeficiency, advanced immunodeficiency and severe immunodeficiency. The likelihood of disease progression to AIDS or death without ART increases with increasing immunodeficiency (decreasing CD4),[34] opportunistic infections and other HIV related conditions are increasingly likely with CD4 counts below 200 per mm^3. Response to ART is affected by the immune stage at which it is started, people commencing ART with advanced immunodeficiency (CD4 >200–350 per mm^3) appear to have better virological outcomes than those who commence with more severe immunodeficiency. Adults starting ART with CD4 <50 per mm^3 have a much greater risk of death. Adults who commence ART with only mild immunodeficiency do not appear to obtain any additional benefits. Revised antiretroviral therapy recommendations reflect this. Pregnancy does affect the CD4 count although the significance of these changes is not clearly understood, and for practical purposes the immunological classification remains the same.[29]

TABLE 3 : WHO immunological classification for HIV infection.[29]

HIV-associated immunodeficiency	Age-related CD4 values			
	<11 months (%CD4+)	12–35 months (%CD4+)	36–59 months (%CD4+)	>5 years (absolute number per mm^3 or %CD4+)
None or not significant	>35	>30	>25	> 500
Mild	30–35	25–30	20–25	350–499
Advanced	25–29	20–24	15–19	200–349
Severe	<25	<20	<15	<200 *or* <15%

TABLE 4: Correlation between CD4 count & HIV associated diseases [35]

>500 cells/ mm^3 • Acute primary infection • Persistent generalized lymphadenopathy • Recurrent vaginal candidiasis	**<200 cells/mm^3** • Pneumocystis carinii pneumonia • Cryptosporidiosis • Oesophageal candidiasis • HIV associated wasting • Mucocutaneous Herpes simplex • Microsporidiasis • Extra pulmonary TB • Peripheral neuropathy
<500 cells/mm^3 • Pulmonary TB • Herpes zoster • Extra-intestinal salmonellosis • HIV associated ITP • Lymphoid interstitial pneumonitis • Pneumococcal pneumonia • Oropharyngeal candidiasis • Kaposi's sarcoma • Cervical intraepithelial lesions • Interstitial pneumonitis	**<100 cells/mm^3** • Cerebral Toxoplasmosis • Progressive Multifocal leukoencephalopathy • Cryptococcal meningitis • HIV associated dementia **<50 cells/mm^3** • CMV retinitis • Disseminated mycobacterium avium intracellulare

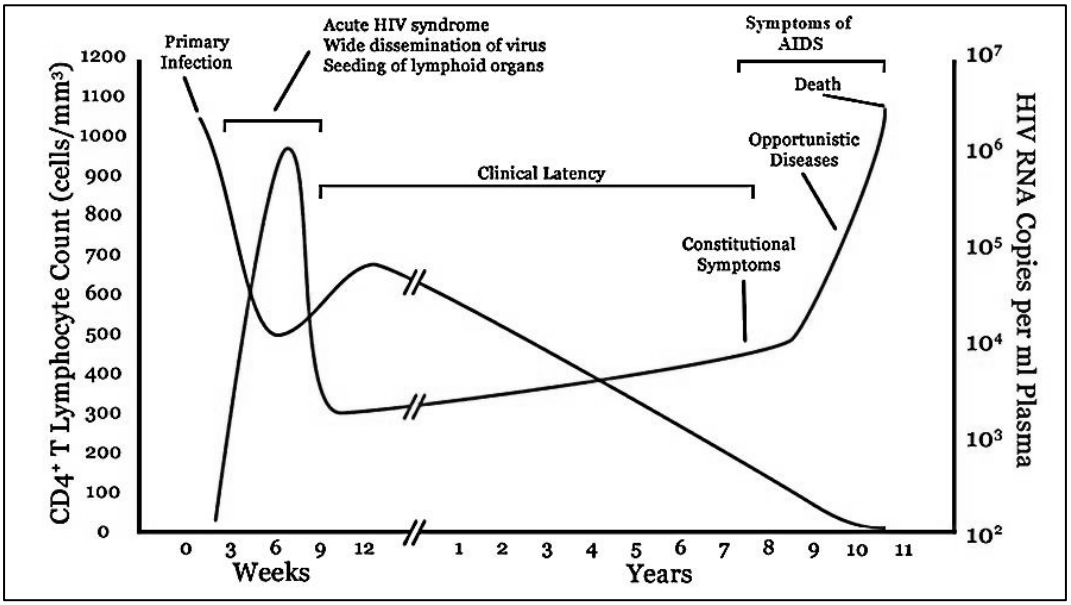


FIGURE 3: The natural history of HIV infection [36]

DIAGNOSIS OF HIV INFECTION

Detection of anti-HIV antibodies is the mainstay of testing for HIV and diagnosis of HIV. Tests to detect specific HIV antibodies can be classified into Screening tests (ELISA/EIA and Rapid) and Supplemental tests (ELISA/EIA and Rapid and Western Blot). Screening tests are performed to screen units of donated

blood and blood products and for surveillance. Supplemental tests are performed on serum sample reactive in screening test for the purpose of diagnosis of the individual. If a specimen is reactive in two different systems, it has to be tested again using one of the supplemental tests which may be a third ELISA/Rapid test or a Western Blot test (WB) as the case may be. Whichever commercial kit is selected, it should be ensured that it detects antibodies against HIV-1, HIV-2 and their subtypes. Strategies devised by UNAIDS and WHO which are based on clinical presentation of the individual and prevalence of HIV have to be followed.[37]

Laboratory Markers Associated with Progression of HIV Infection [38]

The laboratory markers available for assessing immune status of HIV infected patients can be classified into:

1. Viral Markers

I. Quantitative Viral Load – It can be estimated by assaying, Plasma HIV Viral RNA Load, Serum p24 Antigen or Serum p24 Antibody.

II. Assays for Qualitative Viral Change – For example Syncitium Inducing Strains, which are more virulent.

2. Surrogate Markers

I. **Virus specific markers** - Decline in, or absence of antibodies to various HIV antigens including p24, p17, gp120, gp41 and nef gene product have been used as surrogate markers in the past, but they are not very sensitive.

II. Non-specific markers

A. **Cellular Markers / Markers of Immune Function** – These include CD4 cell count, Percentage of CD4 cells, CD8 cell count, Multitest, delayed type hypersensitivity (DTH) skin test and Phenotypic markers of lymphocytic activation.

B. **Soluble Markers –** For example Neopterin and Beta 2- microglobulin (β2-m).

C. **Other Markers -** Other surrogate markers of immune function, such as soluble interleukin-2 receptor levels, serum IgA levels and serum cytokine levels have been studied. Other potentially promising immunologic prognostic markers include percentage of CD38, CD4 cells and CD4, CD29 "bright" memory cells.

CD4 T lymphocyte Count [39]

CD4, a surface glycoprotein on certain T cells, serves as a receptor for HIV, and cells expressing this protein usually decline in number with progressive HIV infection. The number of cells that express the CD4 antigen is therefore a usual guide to the pathological effects of HIV on the immune system. Studies have shown that subjects with low CD4 count are at risk of specific AIDS related illnesses such as various opportunistic infections. Several technologies for determining the absolute number of CD4 cell counts have been developed. These are:

Flow cytometric method – Immunofluorescence analysis by flow cytometry is the gold standard for CD4 T lymphocytes measurements and also the method of choice if a large through put of samples is required. To date, many single-platform flow cytometric technologies have been developed commercially, some of them are – FACS Count microbead based system, Modified flow cytometry, Guava Easy CD4 volumetric system, PartecCyFlow counter (volumetric system) and Point CARE system.

Non- flow cytometric methods – Manual methods - Microscope based, low cost, microbead separation of CD4 T lymphocytes from other blood cells, followed by standard manual cell counting techniques using a light microscope. Dynabeads CD4 T lymphocytes quantitation is another example.

HIV AND DIARRHOEA

Infections of the small and large intestine leading to diarrhoea, abdominal pain, and occasionally fever are among the most significant gastrointestinal problems in HIV-infected patients. They include infections with bacteria, protozoa, fungi and viruses. [6]

TABLE 5: Enteric pathogens in AIDS [40]

Viruses	Bacteria and mycobacteria	Parasites [41]	Fungi
Cytomegalovirus	Salmonella	Cryptosporidium	Histoplasma
Astrovirus	Shigella	Cyclospora	Candida albicans
Picornavirus	Campylobacter	Isospora belli	
Coronavirus	Clostridium difficile	Microsporidia	
Rotavirus	Treponemapallidum	Entamoeba histolytica	
Herpesvirus	Spirochaetes	Giardia lamblia	
Cytomegalovirus	Neisseria gonorrheae	Blastocystis hominis	
Adenovirus	Vibrio cholerae	Strongyloides stercoralis	
Small round virus	Pseudomonas	Ascaris lumbricoides	
HIV	Staphylococcus aureus	Hookworm	
	Mycobacterium avium-complex, MTB		

THE OPPORTUNISTIC ENTERIC PARASITES

PROTOZOA [27]

The phylum Protozoa is composed of morphologically simple eukaryotic organisms. Protozoa may be divided, for convenience, into four distinct groups based on method of locomotion: mastigophora (flagella), sarcodina (pseudopodia), apicomplexa (microtubule complex, commonly referred to as sporozoa), and ciliophora (ciliates). Cryptosporidium spp., Cyclospora, Isospora, Toxoplasma gondii& Giardia lambia, all have been noted to cause severe diseases in patients with acquired immunodeficiency syndrome.

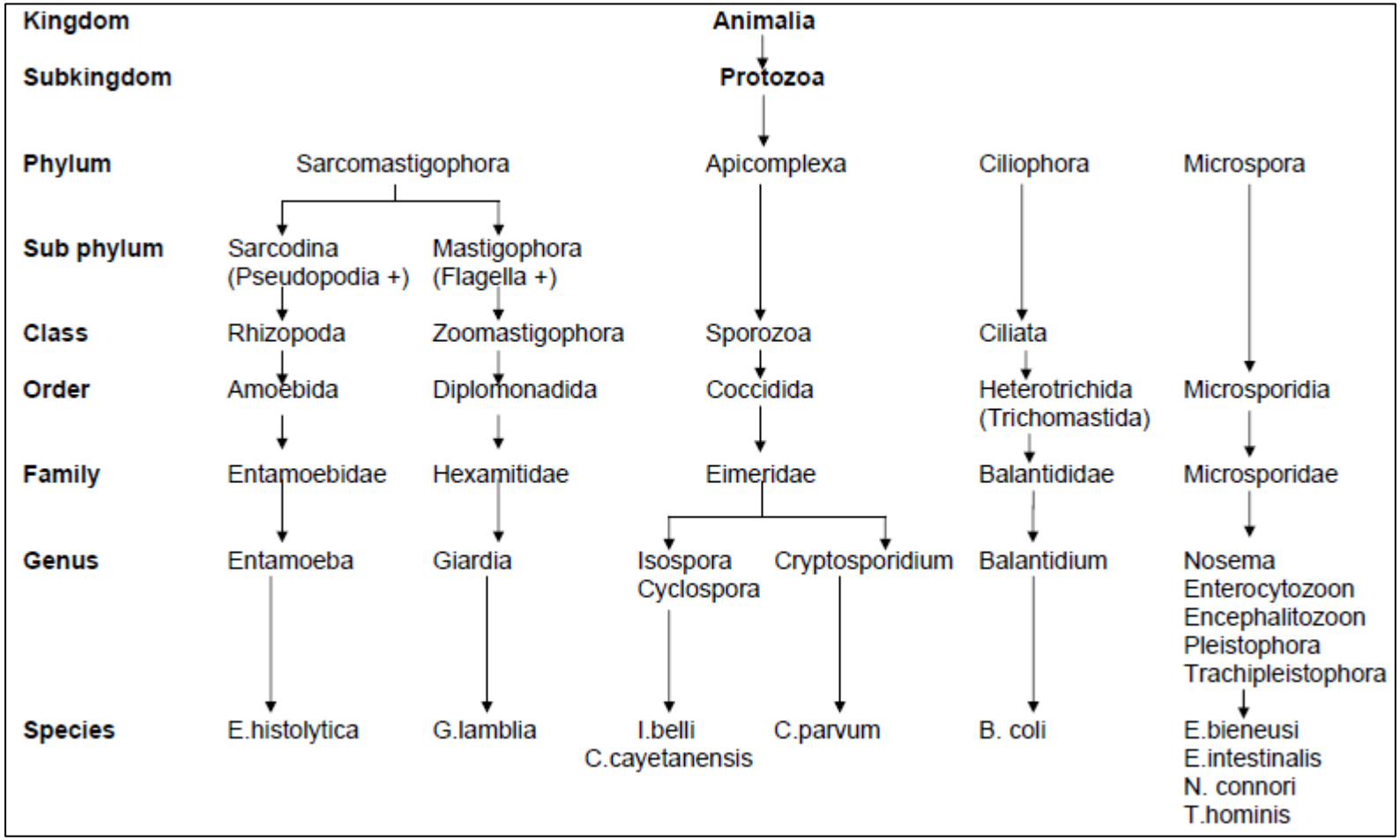


FIGURE 4: Taxonomy of intestinal parasites [42, 43, 41]

COCCIDIAN PARASITES [11]

Opportunistic infections constitute a major health problem in patients infected with HIV. Among these, intestinal parasitic infections are the commonest and are a major cause of morbidity and mortality in HIV positive individuals worldwide. The coccidian parasites (Cryptosporidium spp., Isospora belli, Cyclospora spp. and Microsporidium spp.) are foremost among the enteric parasites in these patients.

CRYPTOSPORIDIUM

HISTORY

Clarke in 1895, may have been the first to observe a species of Cryptosporidium which he described as "swarm spores lying upon the gastric epithelium of mice." In retrospect, these small organisms were probably the motile merozoites of C. muris, the type species named and described approximately 12 years later by the well-known American parasitologist, E. E. Tyzzer.[44]

The advent of the AIDS epidemic and the recognition of water-borne outbreaks of cryptosporidiosis (particularly the 1993 Milwaukee epidemic affecting more than 400 000 individuals) led to the inclusion of Cryptosporidium as an emerging infectious pathogen.[45]

TAXONOMIC AND MORPHOLOGIC FEATURES

The genus Cryptosporidium consists of a group of protozoan parasites within the protist subphylum Apicomplexa, class Sporozoa and subclass Coccidida. There are 10 recognized Cryptosporidium species based on host specificity, morphology, and molecular biology studies. Besides humans, the parasite can infect many different species of animals (eg, mammals, birds, reptiles) and is pathogenic to immunocompetent and immunocompromised hosts .[46] Two species mainly infect humans: Cryptosporidium hominis (previously Cryptosporidium parvum genotype 1),

which infects only humans, and C. parvum (previously C. parvum genotype 2), which infects humans and animals. Cryptosporidium canisinfects dogs and humans. Additional subspecies of Cryptosporidium parvum have been identified in stools of AIDS patients. [47]

Cryptosporidium parvum oocyst average width is 4.5 µm and average length is 5 µm.The 4 sporozoites are naked within the oocyst (i.e., not inside sporocysts). Oocysts are excreted in higher numbers, fully sporulated and infectious when excreted (sporozoites can be visualized when oocysts are excreted).[48]

EPIDEMIOLOGY [45,47]

Cryptosporidium has a wide geographic distribution, though infection is more prevalent in regions of the world with poor sanitary conditions. Infection is more common during warm rainy months. The reported prevalence of infection varies widely and is influenced by geographic region, age, immune status, local outbreaks and the range of sensitivities and specificities offered by different diagnostic modalities. In moist environments, Cryptosporidium oocysts may remain infectious for 6 months. The infectious dose can be as low as 10 oocysts, though considerable variability exists among isolates and a much higher infectious dose is often required in previously exposed seropositive individuals.

Transmission of Cryptosporidium:

- Water-borne - Epidemic and endemic disease
- Person-to-person - Endemic disease
- Foodborne
- Animals

Oocysts have been detected in apparently pure surface water sources, though protected spring water sources are less likely to be contaminated. Untreated or raw

waste water is substantially contaminated with oocysts. Moreover municipal wastewater treatment centers, runoff from animal agriculture and various wildlife populations all contribute to a remarkable release of oocysts into the aquatic environment. Oocysts are highly resistant to chlorination and can bypass certain filtration methods. Accordingly, sources of treated potable water can contain significant numbers of oocysts. Oocysts can survive for a period of time in seawater, and indeed shellfish in coastal areas have been found to be contaminated with infectious oocysts. Most cases among immunocompetent hosts have been associated with waterborne outbreaks and have involved either contaminated drinking water or recreational water sources such as swimming pools and lakes. Disease is also well-described in returning travelers, persons with animal contact (e.g., farmers) and amongst daycare personnel working with young children. Direct person-to-person transmission via the fecal-oral route is also common in a number of settings including during sexual activity. Health care workers should be cognizant of the potential for nosocomial transmission. Foodborne transmission, though relatively infrequent, has been reported from a number of sources including inadequately pasteurized beverages and raw fruits and vegetables.

Cryptosporidium parvum is a frequently identified parasite in HIV infected individuals with diarrhoea in India and other parts of the world. [49]

LIFE CYCLE

The life cycle can be divided into six major developmental events: excystation, the release of infective sporozoites; merogony, the asexual multiplication within host cells; gametogony, the formation of micro- and macrogametes; fertilization, the union of micro- and macrogametes; oocyst wall formation, to produce an environmentally resistant stage that transmits infection from one host to

another; and sporogony, the formation of infective sporozoites within the oocyst wall. Each intracellular stage of C. parvum resides within a parasitophorous vacuole confined to the microvillous region of the host cell, whereas comparable stages of Eimeria or Isospora species occupy parasitophorous vacuoles deep (perinuclear) within the host cells. Oocysts of C. parvum undergo sporogony while they are within the host cells and are infective when released in the faeces, whereas oocysts of Eimeria or Isospora species do not sporulate until they are passed from the host and exposed to oxygen and temperatures below 37°C.

Studies with experimentally infected mice have also shown that approximately 20% of the oocysts of C. parvum within host enterocytes do not form a thick, two-layered, environmentally resistant oocyst wall. The four sporozoites of this autoinfective stage are surrounded only by a single unit membrane. Soon after being released from a host cell, the membrane surrounding the four sporozoites ruptures and these invasive forms penetrate into the microvillous region of other enterocytes and reinitiate the life cycle. Approximately 80% of the oocysts of C. parvum found in enterocytes develop thick, environmentally resistant oocyst walls and are passed in the faeces. Thick walled oocysts are the life cycle forms that transmit the infection from one host to another. The presence of auto infective, thin-walled oocysts & type I meronts that can recycle are believed to be the life cycle features of C. parvum responsible for the development of severe infections in hosts exposed to only a small number of thick-walled oocysts and for persistent, life-threatening disease in immune deficient persons who are not exposed repeatedly to these environmentally resistant forms. [44]

The life cycle of Cryptosporidium species is completed within the small intestine and colon of the host, with the developing stages associated with luminal

surface of the intestinal epithelial cells, where it remains intracellular but extracytoplasmic. Ingestion in a new host is followed by the ingestion of as few as 10 oocysts. This is followed by the release of motile sporozoites in the intestine that invade the epithelial cells. The invasive process involves Gal/GalNac epitopes of sporozoites surface glycoproteins and recruitment of the host actin cytoskeleton to form a parasitophorous vacuole.

A feeder organelle at the site of attachment is believed to function as a portal to allow nutrients from the host cell to the parasite. Asexual (merogony) and sexual reproduction of the parasite occurs within the extracytoplasmic vacuole, resulting in merozoites that infect adjacent epithelial cells and the production of sporulated thin-walled and thick-walled oocysts. Thin- walled oocysts can excyst endogenously resulting in autoinfection, which helps to explain the mechanism of persistent infections in patients with AIDS. The thick-walled environmentally hardy oocysts, shed in the fecal material of the infected host are immediately infectious, hence can be transmitted from person to person.

The alterations in the intestinal structure and physiology that lead to the pathogenesis of Cryptosporidiosis include rapid loss of microvillous border, shortening and fusion of the villi, lengthening of the crypts resulting in malabsorption due to loss of membrane bound digestive enzymes, decreased absorption, reduced glucose-NaCl absorption and increased Chloride anion secretion. Proinflammatory cytokines specifically INF-α and TNF-γalso contribute to the pathogenesis of cryptosporidiosis by increasing the production of prostaglandins, neural peptides and reactive nitrogen intermediates, disruption of the epithelial border leading to a leaky and dysfunctional epithelium and alteration of solute transport leading to osmotic diarrhoea .[50]

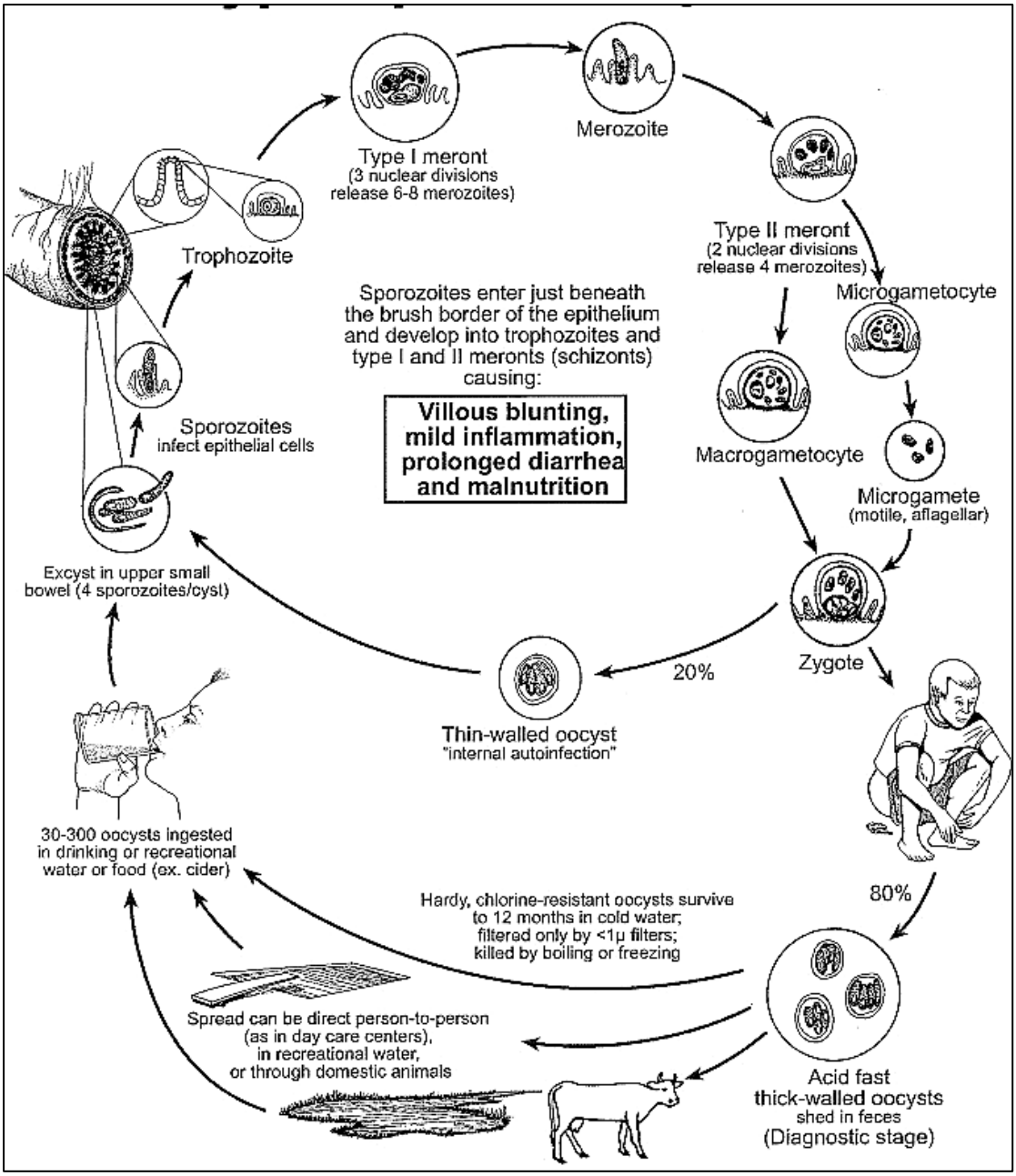


FIGURE 5: Lifecycle Of Cryptosporidium [51]

CLINICAL MANIFESTATION

Cryptosporidiosis has been described in patients with a broad range of immunodeficiencies, including HIV/AIDS, In such patients, cryptosporidiosis is usually indolent in onset and manifestations are similar to those seen in normal hosts, but the diarrhoea is more severe. Fluid losses of up to 25 L/day have been described. Patients with biliary cryptosporidiosis present with typical manifestations of

cholecystitis and cholangitis. Unless the immunologic defect is reversed, the disease usually persists for the duration of the patient's life. Weight loss is often prominent. The prognosis depends on the nature of the underlying immunologic abnormality. [52]

Patients clinically present with profuse watery diarrhoea in association with cramping abdominal pain, anorexia, nausea and vomiting. In the patient with HIV, the course of cryptosporidiosis often correlates with the immune status of the individual. Patients with CD4 counts above 200cells/μL are likely to have a clinical course similar to immunocompetent hosts. Patients with AIDS and progressively declining CD4 counts are more likely to present with foul smelling bulky stools in the context of chronic diarrhea and weight loss. Severely immunocompromised individuals with CD4 counts less than 50 cells/μl develop a more fulminant cholera-like disease with watery and voluminous diarrhea. Biliary and respiratory tract disease is more likely to manifest in severely immunocompromised persons with CD4 counts less than 50 cells/μL. Biliary tract involvement may result in biliary strictures, papillary stenosis, pancreatitis, acalculous cholecystitis, or sclerosing cholangitis. These may manifest with right upper quadrant pain, nausea, vomiting and low grade fever.[47]

Cryptosporidia infection is most devastating in patients with advanced HIV disease because of its ability to cause severe dehydration, electrolyte imbalance and wasting. Most patients have chronic illness with many exacerbations and remissions. Extra-intestinal infections involving the liver, biliary tract and lung have also been reported.

Antiretroviral therapy (ART) greatly influences the outcome of cryptosporidiosis both indirectly by immune restitution and increase in CD4 counts and by the direct effect of protease inhibitors on oocysts shedding. However, despite the use of highly active antiretroviral therapy (HAART), HIV infected patients can

still present with coccidian diarrhoea, possibly due to non-compliance with medications, viral resistance to drugs or decreased bioavailability of drugs.[53]

DIAGNOSIS

The diagnosis of cryptosporidiosis is established by the recovery and identification of Cryptosporidium oocysts in a recently passed or preserved diarrheal stool. Oocysts excretion is most intense during the first week of illness, tapers during the second week, and generally stops with the cessation of diarrhoea. [52]

Henricksen introduced the use of acid-fast stain to differentiate yeast cells from oocysts to avoid false-negative diagnosis, since yeast cells resembled oocysts in size and morphology.[54]The diagnosis of cryptosporidiosis rests on the identification of the 4-5 □m spherical oocysts (or oocyst components) in stool.[53]

In a study conducted in New York Sheather's sugar cover-slip floatation was more effective than formalin-ether sedimentation for the concentration of oocysts.[54]
In wet mount, they appear pink tinged, spherical, translucent bodies. Under phase contrast microscopy, they appear as bright and birefringent, containing up to four sporozoites and dark granules. Oocysts stain with modified acid fast stains. Uneven staining due to variable uptake by oocyst wall may be seen. Some may appear as ghost cells that do not take up the stain. They can also be stained by Methanamine silver, Giemsa, Auramine - rhodamine and PAS. [55, 53]

Polyclonal and monoclonal conjugates with Fluorescein Isothiocyanate can be used as an immunofluorescent technique. ELISA for antibodies to C. parvum coproantigen (CCAg) from stool is available. Flowcytometric method can be used for quantitation of cysts.[55] Polymerase chain reaction targeting COWP-190 or small subunit RNA have been developed.[53]

TABLE 6: Diagnostic options for Cryptosporidium detection [53]

Technique	Advantages	Disadvantages
Acid-fast stain (Ziehl-Neelsen, Kinyoun)	Low cost	Low sensitivity and specificity
Auramine-rhodamine	Rapid screening	Low sensitivity and specificity; high cost (equipment)
EIA	Sensitive (32.9 ng of Cryptosporidium protein [Meridian Diagnostics, Inc., Cincinnati,Ohio]); minimal training required	High cost
IFA	Sensitive (100 oocysts/ml [Meridian Diagnostics, Inc., Cincinnati, Ohio])	High cost
PCR	Sensitive (1 oocyst) (150); permits genotyping	Non-standardized methods; high cost; specialized training and equipment required

TREATMENT

In the immunocompetent patient, the disease is self-limited and attempts at specific antiparasitic therapy are not warranted; rehydration may be required in small children. In the immunocompromised host, the severity and chronicity of the diarrhea warrants therapeutic intervention. Unfortunately, there is no uniformly effective anticryptosporidial agent available at this time. The only uniformly successful approach has been the reversal of underlying immunologic abnormalities. [26]

One of the most biologically intriguing and clinically frustrating features of Cryptosporidiosis is its resistance to antimicrobial drugs. There is no curative therapy despite in vivo and in vitro testing of hundreds of compounds. One possible explanation is that, Cryptosporidium establishes a compartment within the host cell, which is morphologically different from the setting used by the related species. This unique parasitophorous vacuole may somehow shelter the parasite from antimicrobial drugs.[53]

In immunocompromised hosts, particularly AIDS patients with CD4 cell counts below 200/mm^3, cryptosporidiosis can be life-threatening and must be treated aggressively. Initially, the nutritional, hydration, and electrolyte status of the patient should be assessed and corrected with intravenous hydration, if necessary.

Antimotility agents, such as opiates and somatostatin analogues, may also be used. In people with AIDS, the ideal treatment involves partial restoration of immune function with HAART. Several case reports have demonstrated the resolution of diarrhoea coincident with rise in CD4 count upon HAART therapy.[53]

Paramomycin has been most widely used and has consistently displayed at least partial activity in clinical trials. In a study conducted Kadappu et al., Azithromycin was found to be a safe and effective antiprotozoan against Cryptosporidiosis in HIV positive patients. Combination of Paramomycin and Azithromycin has also been proposed. [56, 53]

Nitazoxanide (NTZ) is the latest drug to be widely tested. It is a nitrothiazole benzamide with broad spectrum activity. Study of NTZ in 15 Mexican AIDS patients with Cryptosporidiosis found parasite clearance in nearly 100% of patients. [57, 53]

CYCLOSPORA

HISTORY [58]

Cyclosporan organisms were first noted in the intestines of moles in 1870 by Eimer. Schneider created the genus Cyclospora in 1881. In 1902, Schaudinn reported the first life-cycle study of Cyclospora. Cyclospora species have been subsequently been found in snakes, insectivores, and rodents.

Between 1986 and 1993, there are nine reports linking diarrhoeal illness in more than 200 immunocompetent and immunocompromised children and adults due to an unidentified acid fast organism resembling a “large cryptosporidium”. On the basis of electron microscopic studies in 1990 that revealed photosynthesizing organelles within the organism, similar to those of blue-green algae, Long et al. suggested that it was a cyanobacterium similar to Chlorella species.

The organism has been called by several names like a coccidian-like body,

cyanobacterium-like body (CLB), a blue-green alga, a Cryptosporidium muris-like cyst, a fungal spore, and a species of Blastocystis.

In 1993, Orgeta et al. succeeded in inducing Cyclospora to sporulate and showed that, when mature it has two sporocysts, each containing two sporozoites. Thus he proved that the organism is the oocyst stage of Coccidian parasite Cyclospora.

The first report of human Cyclospora infection, which had largely gone unnoticed until 1993 came from Papua New Guinea in 1979, before the popularization of acid-fast stained stool smears and the advent of molecular Phylogenetic analysis.

TAXONOMIC AND MORPHOLOGICAL FEATURES

Cyclospora cayetanensis is a protozoan parasite : subphylum Apicomplexa, subclass Coccidiasina, order Eucoccidiorida, family Eimeriidae.[59] In 1994, Ortega et al. christened the organism Cyclospora cayetanensis, deriving the species name cayetanensis from the name of the Peruvian university (Universidad Peruana Cayetano Heredia) where their principal studies had been conducted. [48]

Cyclospora resembles Isospora in that oocysts are excreted unsporulated and require a period of time outside the host for maturation to occur. In the laboratory, sporulation occurs after 5 – 11 day incubation in either distilled water or in 2.5% potassium dichromate at temperatures between 25°C and 32°C.

Cyclospora oocysts are spherical and are 8 – 10μm in diameter. When observed by means of light microscopy, they appear as non-refractile spheres that contain a cluster of refractile membrane bound globules. Electron microscopic studies reveal an outer fibrillar coat that is 63nm thick and a cell wall that is 50nm thick. Within each oocyst there are two sporocysts, each ~ 4μm in diameter with a cell wall

that is 62 nm thick. Two sporozoites are contained within each sporocyst; these sporozoites are 1.2μm wide and 9.0μm long and contain a membrane - bound nucleus and micronemes characteristic of Apicomplexans.[58]

EPIDEMIOLOGY [58]

Cyclospora is widely distributed throughout the world; it has been identified in both residents and travelers from various regions including America; the Caribbean islands; Eastern Europe; India; South Africa; and Southeast Asia. Persons of all ages have been infected. Most of the current knowledge of the epidemiology of Cyclospora species is derived primarily from Nepal, Haiti, and Peru because the parasite appears to be endemic in these countries. In Katmandu, Nepal, workers have documented an annual surge of Cyclosporiasis that coincides with the rainy season (between May and October)**.**

In 1995, excess numbers of cases of Cyclospora infection was documented in New York and Florida. Although eating unwashed berries was implicated as a risk factor, it could not be proven. Data on outbreaks in Chicago and Nepal provide compelling evidence that Cyclospora is acquired through contaminated water. Transmission via contaminated food, lettuce, undercooked meat, and raw beef has been suggested but not proven. Direct animal-to-human or person-person transmission of Cyclospora has not been documented**.**

Cyclospora oocysts in freshly excreted stool are not infectious. Thus, direct person-to-person transmission through fecal exposure is unlikely. Oocysts become infectious (i.e., sporulate) in the environment, days to weeks after excretion. The rate of sporulation, which usually takes at least 1 week under laboratory conditions, is influenced by environmental factors. For example, storage of oocysts at 4°C or 37°C (vs. at 22°C–32°C) slows sporulation .Preliminary data suggest that oocysts do not

sporulate after exposure to -20°C for 24 h or to 60°C for 1 hr. To maintain transmission, Cyclospora must survive in the environment long enough both to sporulate and to be ingested thereafter by a susceptible host. A moist environment is probably more conducive to survival than a dry one. Methods to assess viability (beyond sporulation and excystation) are not yet available. No documented outbreaks have been associated with cooked or commercially frozen food.[48]

LIFE CYCLE [27]

Unsporulated oocysts are excreted in the stool of infected individuals. Oocysts are quite resistant and can survive under diverse environmental conditions including freezing, 2% formalin, 2% potassium dichromate, and chlorination.

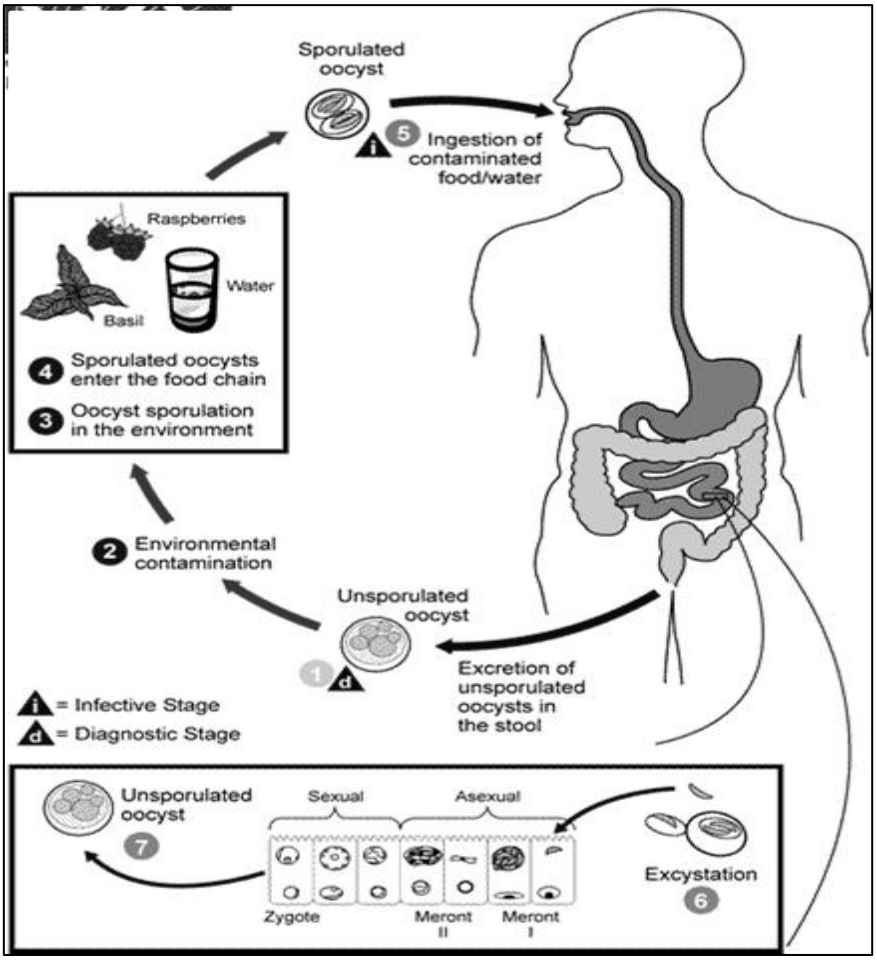


FIGURE 6: Life cycle of Cyclospora[60]

Sporulation is required for infectivity and requires at least 7 days of maturation outside of the human host; experimentally, in moderate temperatures, sporulation occurs within 7 to 13 days.

Each sporulated oocyst contains two sporocysts that each holds two sporozoites. After ingestion of sporulated oocysts, excystation occurs in the proximal small bowel. Sporozoites penetrate the epithelial cells of the small intestine, where both asexual and sexual reproduction takes place. Although the asexual life cycle can continue endogenously within the intestinal epithelium, sexual reproduction leads to the development of zygotes. Zygotes mature into oocysts within the intestinal epithelium, which in turn are released in the stool after causing rupture of the host cells.

CLINICAL MANIFESTATIONS

The incubation period of Cyclospora infection ranges from 2 to 11 days. Although Cyclosporiasis is said to be clinically indistinguishable from Cryptosporidiosis and Isosporiasis, diarrhoea may not be the presenting or predominant symptom for patients who have Cyclospora infection. [58]

Clinical manifestations of Cyclosporiasis include watery diarrhoea that occurs in relapsing, cyclical pattern, sometimes alternating with constipation. Important associated symptoms include profound fatigue, "indigestion or heartburn: - like symptoms, nausea, abdominal cramps, anorexia, weight loss, and vomiting. A flu-like prodrome with accompanying myalgia's and arthralgia may precede the onset of diarrhoea. Although the disease is self-limited, it may be prolonged and last for weeks; progressive fatigue, anorexia, and weight loss may overshadow the resenting diarrhoeal symptoms. [58]Usually not life-threatening but not trivial either. Complications can include malabsorption, Reiter's syndrome, and possibly Guillain-

Barre syndrome.Cessation of symptoms and excretion of oocysts typically occur within a few days to 1 or 2 weeks of each other.[48]

Small bowel infection with Cyclospora results in an inflammatory process with villous fusion and atrophy, which potentially reduces the surface area and leads to decreased absorption. Malabsorption of D-xylose and increased excretion of fecal fat occurs. Among the immunocompromised, clinical illness is prolonged and severe and is associated with high rate of recurrence that can be attenuated with long term suppressive therapy. There is also evidence of biliary tract infection with Cyclospora in AIDS patients.[48] With the use of electron microscopy it has been shown that the organisms were present inside vacuoles within the cytoplasm of epithelial cells in jejunal biopsy specimens. The pathological changes seen in patients with Cyclospora infection are reminiscent of tropical sprue, and the possibility that the parasite may trigger the latter condition has been raised. [58]

DIAGNOSIS [27,58]

The diagnosis on Cyclospora infection is based on microscopic detection of oocysts in fecal specimens. Examination of wet mounts of fresh, unpreserved stool by means of bright - field microscopy reveals non-refractile spheres that are 8 - 10μm in diameter and contain numerous refractile globules enclosed within membranes. The oocysts are acid - fast and thus can be seen using one of the many acid - fast staining techniques, including the modified Ziehl - Nielsen stain and Kinyoun acid - fast stain. Cyclospora cysts have a variable appearance (unstained, pink, or dark red) on acid - fast staining. Despite their distinct characteristics, Cyclospora oocysts may be confused with Cryptosporidium oocysts unless their diameter is measured with a micrometer. Additional stains including auramine, safranin and lactophenol cotton blue can also be used.

Cyclospora oocysts are autofluorescent and appear as neon blue circles when examined with an ultraviolet fluorescence microscope fitted with a 365 nm excitation filter; this property appears to wane over time. Whether persons infected with Cyclospora shed oocysts intermittently, as do those with Isospora infection, is also unknown. It is important to underscore that the rudimentary nature of our diagnostic techniques may in part contribute to under recognition of Cyclospora infection.

Species-specific real-time polymerase chain reaction assays have been developed that are capable of detecting low concentrations of oocysts in stool. Although polymerase chain reaction may be more sensitive than conventional diagnostic methods, it is not widely available and requires additional validation in clinical settings. Flow cytometry has been proposed as an alternate method of diagnosis. Antibodies to Cyclospora can be detected, but serologic tests are not commercially available.

In addition to being found in faeces, Cyclospora has also been detected in jejunal aspirates and biopsy specimens by means of light microscopy and electron microscopy.

TREATMENT [27,58]

Trimethoprim - sulfamethoxazole (TMP - SMZ) is the drug of choice for treating Cyclospora infection. HIV patients infected with Cyclospora were successfully treated with TMP – SMZ (160/800mg) four times daily for 10 days, followed by secondary prophylaxis with TMP - SMZ (160/800mg) three times weekly. Patients who cannot tolerate TMP-SMX may be treated with ciprofloxacin 500mg twice daily for 7 day.

ISOSPORA BELL

HISTORY

Isospora belli was first discovered by Virchow in 1860 and was named by Wenyon in 1923.[41] The name 'belli' was given because several cases of infection with this parasite were seen among troops stationed at the Middle East during the first world war (Bellium meaning war).[43]

TAXONOMIC AND MORPHOLOGICAL FEATURES

Isospora belli are host-specific coccidian protozoan parasites of the phylum Apicomplexa , Class Sporozoea , subclass coccidian , order Eucoccidiida , suborder Eimeriina and genus Isospora. [42] Each oocyst is elongated and measures 20 to 33 μm in length and 10 to 19 μm in breath. The oocyst is surrounded by a cyst wall having two layers. Immature oocyst, passed in human faeces, contains two sporoblasts which mature in to sporocysts (in 48 hours at room temperature). Each sporocyst contains four sporozoites which are crescent shaped.[42, 43]

EPIDEMIOLOGY [43], [61]

I. belli is common in tropical and subtropical environments. Incidence is higher in Central and South America, Africa and South – East Asia. Sporadic outbreaks have occurred in mental institutions and in day-care centers in the United States. The infection is common in immunosuppressed patients, particularly those with AIDS living in tropical areas. Transmission probably occurs by the fecal-oral route by contaminated food and water. Infection by oral-anal sexual contact is also reported in USA. Chronic diarrhea is the major clinical manifestation.

LIFE CYCLE:

Oocysts are the infective form of parasite. Mode of infection is by ingestion of food and drink contaminated with faeces containing mature cysts. The parasites

invade the epithelium of the proximal jejunum and distal duodenum and remain intracellular. Inside the cytoplasm of the enterocyst the parasite undergoes asexual multiplication (merogony) to produce trophozoites. Some of these trophozoites undergo sexual cycle (sporogony or gametogony) and produce unsporulating oocysts, each containing a single sporoblast. These unsporulating oocysts are excreted in the faeces and further development takes place outside the host.

These oocysts sporulate and mature into sporulating oocysts with two sporocysts each containing a single sporoblast, these unsporulating oocysts are excreted in the faeces and further development takes place outside the host. These oocysts sporulate and mature into sporulating oocysts with two sporocysts each containing four sporozoites. These mature oocysts are infective form of parasites. [62]

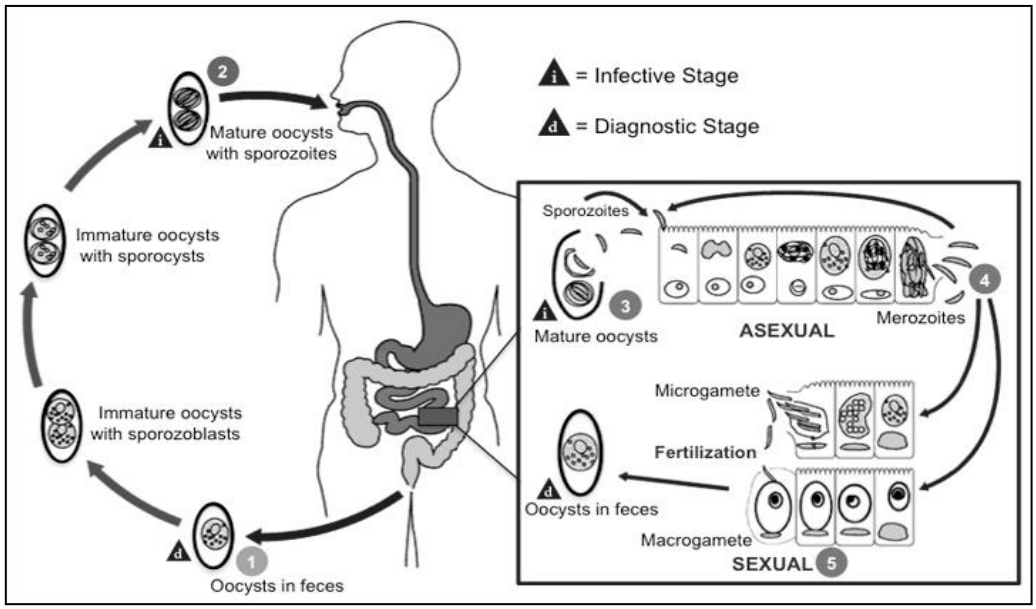


FIGURE 7: Lifecycle of Isospora belli[60]

CLINICAL FEATURES

Oocyst invades proximal small intestinal enterocytes resulting in severe prolonged diarrhoea in immunocompromised patients. Symptoms occur within 1 week after infection with oocysts and include profuse, non-bloody watery diarrhoea with abdominal cramping, indistinguishable from that caused by Microsporidia and

Cryptosporidium parvum. Vomiting, headache, fever and malaise may also be present and dehydration follows when diarrhoea is severe.

A notable feature of I. belli infection is its propensity to stimulate an eosinophilic response in the lamina propria, with concomitant Charcot– Leyden crystals detectable in stool samples. In addition, mild to moderate systemic eosinophilia in the absence of leukocytosis is commonly reported. [45]Although the pathogenesis of diarrhea is not known, it is believed to be due to mechanisms similar to Cryptosporidiosis. In AIDS patients extra – intestinal infection can occur, though it is rare. Necropsy occasionally reveals infection of mesenteric lymph nodes, liver, and spleen. Biliary diseases have also been reported.[41]

DIAGNOSIS [41]

Diagnosis can be established by the demonstration of characteristic I. belli oocyst in the faeces by examination of unstained or iodine stained direct smear preparation and by zinc sulphate as well as formalin – ether concentration methods. Oocyst can also be detected in faecal smears following acid – fast staining or staining with auramine – rhodamine. With acid fast stain oocyst appear red in colour.

Unstained oocysts are auto fluorescent, appearing violet under ultraviolet light and green under green or blue – violet light. Various life cycle stages can be detected with in the epithelial cells of intestinal mucosa obtained by biopsy. A highly sensitive and specific method for diagnosis has employed PCR with primers for small subunit rRNA sequences of I. belli.

TREATMENT [43]

The drug of choice is trimethoprim – sulphamethoxzole, pyrimethamine – sulfadiazine is also an effective combination. In immunocompromised patients treatment of underlying cause should be done simultaneously.

MICROSPORA

HISTORY [63]

Microsporidia are obligate intracellular protozoan parasites infecting a broad range of vertebrates and invertebrates. In 1857 these parasites were first recognized as pathogens in silkworms, and long before they were described as human pathogens they were recognized as a cause of disease in many nonhuman hosts. The first human case of microsporidial infection was reported in 1959 and only ten well-documented human infections with microsporidia were described until 1985.

The first microsporidium found in an AIDS patient was Enterocytozoon bieneusi, an entirely new genus and species causing chronic diarrhoea. [45]

TAXONOMY & MORPHOLOGY [42, 64, 41]

Microspodia belongs to the phylum microspore (Sprague 1977) class Microsporea Order Microsporida (Balbiani 1987). To date more than 1200 species belonging to 143 genra have been described infecting a wide range of vertebrates and invertebrate hosts. There are 7 genera Enterocytozoon, Encephalitozoon, Nosema, Pleistophora, Thelohanea, Trachipleistophora& Vittaforme, which are known to cause human disease Microsporidiosis particularly in AIDS patients. Most cases (90%) of intestinal microsporidiosis are caused by Enterocytozoon bieneusi and the rest are caused by E. intestinalis. E. hellem is associated with the infection of cornea and conjunctiva causing kerato-conjunctivitis. E. intestinalis infects the intestine, gall bladder, liver and kidney.

All developmental stages are formed in direct contact with the host cell cytoplasm, and no sporophorous vesicles or pansporoblastic membranes are present . The parasite has no diplokarya in any stage of development and contains elongated nuclei during early development (unique to this genus). The proliferative and

sporogonial forms are rounded multinucleate plasmodia measuring up to 6 □m in diameter and limited by a unit membrane. Other organelles unique to this genus are electron-lucent inclusions which are present throughout the life cycle and electron-dense discs which are formed during sporogony and represent precursors to the polar tube and anchoring discs. Sporoblasts develop from large plasmodial sporonts that are divided by invagination of the plasmalemma. The oval spores display typical microsporidial ultrastructure and measure 0.7 to 0.98 by 1.08 to 1.64 □m. The polar tubule has five to seven coils that appear in two rows when seen in cross sections by transmission electron microscopy. [65]

EPIDEMIOLOGY [66]

The interest in microsporidia has grown during the last 20 years with the recognition of new species that cause opportunistic infections in AIDS patients. Transmission of infection to other individuals is possible by faecal-oral or urine-oral, inhalation of contaminated aerosols or by ingestion of infected food or water. The most frequent cause of human microsporidium infection is Enterocytozoon bieneusi and Encephalitozoon hellem, Encephalitozoon cuniculi, Encephalitozoon intestinalis, and Nosema corneum. Enterocytozoon bieneusi is estimated to be one of the most important intestinal pathogens in severely immunodeficient HIV-infected patients. Microsporidia are an important cause of disease in HIV infected patients and are now increasingly recognized as pathogens in non-HIV infected patients with or without immunosuppression.

LIFE CYCLE

The life cycle of microsporidia includes three distinct phases: first, the infective phase, i.e., the spore stage, made up of spore release into the environment, spore transmission, stimulation of the spore necessary to trigger the extrusion of the

polar tubule, and inoculation of infective spore content (termed sporoplasm) into a host cell; second, the proliferative vegetative phase, termed merogony (schizogony), during which the parasites multiply intracellularly; and third, the intracellular sporogony, during which infective spores are formed.

In human microsporidial infection, the life cycle of the organisms is completed within the human host, and there is no evidence of an intermediate host or a vector transmitting developmental stages of microsporidia.

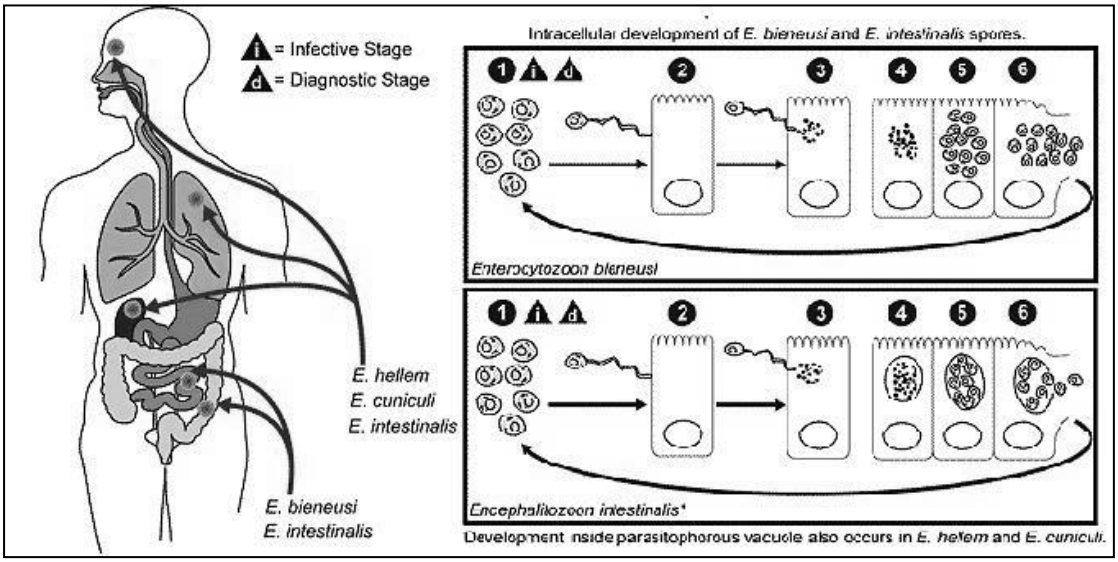


FIGURE 8: Lifecycle of Microspora [60]

CLINICAL FEATURES

The spectrum of clinically manifest microsporidial infection includes intestinal, ocular, muscular, and systemic disease. The most prevalent microsporidian-associated disease in HIV-infected patients is chronic diarrhea with wasting syndrome, but disseminated infection is increasingly recognized.

Microsporidiosis is most common in patients with severe cellular immunodeficiency, i.e., when CD4 cell counts drop below 50 to 100/µl. When microsporidiosis is diagnosed, the majority of the patients have had other opportunistic infections. The main symptoms are chronic non bloody diarrhea without

fever, anorexia, weight loss of about 2 kg/month, and bloating. Some patients experience intermittent diarrhea, and a few excrete microsporidial spores without having diarrhea. The stool is watery or soft, and the number of bowel movements is usually about three to seven per day, rarely, up to 20 per day.

DIAGNOSIS [65]

Diagnosis of microsporidial infection is dependent on morphological demonstration of the organisms themselves by light or electron microscopic examination. Serological tests to detect antibodies to Encephalitozoon spp. have been developed, but available results suggest that these tests may not be feasible for diagnosis of human microsporidiosis, particularly in patients with immunodeficiency.

Encephalitozoon sp. and Nosema sp. have been isolated by using cell culture systems, but these tests are fastidious and costly, and the most common human species, Enterocytozoon bieneusi, has not been continuously propagated. Molecular probes are being developed but are currently not available outside of research laboratories.

Detection of microsporidial parasites has often been based on electron microscopic examination of tissue specimens because of the organism's small size; staining properties that sometimes hamper visualization of the spores and developing stages, using routine staining techniques; and the unfamiliarity of pathologists and parasitologists with the histopathologic appearance of this infection.

Serologic assays (including carbon immunoassay, indirect immunofluorescence test, enzyme-linked immunosorbent assay [ELISA], and Western blot immunodetection) have been useful in detecting antibodies to Encephalitozoon cuniculi in several species of animals, but reliable serologic tests for diagnosis of human microsporidiosis are lacking.

None of the procedures that are routinely used to concentrate ova and parasites results in concentration of microsporidial spores in stool specimens. The formalin - ethyl acetate concentration procedure or different flotation methods produce significant removal of fecal debris, and smears prepared from these concentrates might appear easier to read by light microscopic examination compared with smears from unconcentrated specimens. Such concentration techniques, however, lead to a substantial loss of microsporidial spores and false -negative results.

The "gold standard" for diagnosis of Microsporidiosis is transmission electron microscopy of jejunal biopsies. The Chromotrope - based staining technique for light microscopic examination of stool specimens includes steps similar to those used in the trichrome staining procedure of Wheatley. The spores have a specific appearance when stained with this technique. The spore wall stains bright pinkish - red; some spores appear transparent, and others show a distinct pinkish – red - stained, belt like stripe that girds the spores diagonally or equatorially. Most background debris in stool specimens counterstains faint green. Some other fecal elements, such as yeasts and some bacteria, may also stain reddish, but they are distinguished from microsporidial spores by their size, shape, and staining pattern.

Both Calcofluor white and the Modified Trichrome Staining methods are equally sensitive, specific, and robust in the laboratory diagnosis of intestinal microsporidiosis, and the decision to use either one may be based on technical criteria such as the availability of a fluorescence microscope.

The calcofluor white staining method is considered to be as sensitive as Chromotrope based staining. It has the advantages of speed and ease of performance. For laboratories that stock calcofluor white and have an epifluorescence microscope fitted with the appropriate filters, calcofluor white method may be an appropriate

screening test for microsporidial spores. A potential problem with the calcofluor white stain is the lack of specificity resulting from the widespread occurrence of cellulose and chitin - containing objects in feces. A high degree of sensitivity and specificity appears achievable when including a second staining procedure, such as Chromotrope - based stain, on those specimens positive by calcofluor white.

Giemsa staining results in a light – blue staining of microsporidia, and sometimes a characteristic darkly stained nucleus may be visualized. The very small, blue - stained microsporidial spores, however, are difficult to identify in smears of stool specimens and to differentiate from other fecal elements, which all are also blue stained.

Fluorescein - tagged polyclonal antibodies have been used by some investigators for histologic and cytologic detection of microsporidia in human specimens and to visualize different microsporidial developmental stages in cell cultures.

TREATMENT

Microsporidiosis has been generally refractory to medical therapy. Albendazole has been used often with either poor success or only a temporary response. Fumagillin has also been studied as an antiprotozoal drug in humans, but it is currently not licensed for human use Sinefungin and albendazole, which have been reported to inhibit growth of various protozoal parasites in vitro, and fumagillin have been found to reduce the number of microsporidia and to cause growth deformities of Encephalitozoon spp. propagated in cell cultures. Different substances, among them the calcium channel blocker nifedipine and the antifungal agent itraconazole, have been shown to inhibit Encephalitozoon hellem spore germination in an in vitro system.

ENTAMOEBA HISTOLYTICA

HISTORY

Entamoeba histolytica was first described by Lambl in 1859 and Losch in 1875 established its pathogenic nature in the stool of a Russian suffering from dysentery. He proved it to be a pathogen by producing intestinal lesions in a dog by infecting it with a dysenteric stool.[43]

Kartulis in 1886 conclusively demonstrated the amoeba as a causative agent of amoebic dysentery and amoebic liver abscess and showed Entamoeba histolytica (E.histolytica) trophozoites in hepatic capillaries. Schaudinn described trophozoites of pathogenic and non-pathogenic intestinal amoebae. The etiological role of E. histolytica in causing amoebic dysentery was conclusively shown by Walker and Sellards in 1913 by conducting studies on human volunteers. [43, 67]

E. histolytica is the third leading parasitic cause of death in the developing countries. It remains as an important cause of diarrhoea in homosexual men suffering from AIDS in the developed countries. [68]

TAXONOMY AND MORPHOLOGY

Genus Entamoeba belongs to the Phylum Sarcomastigophora, Subphylum Sarcodina, Superclass Rhizopoda, Class Lobosea, Subclass Gymnamoebia, and Order Amoebida. [42] The taxonomy of E. histolytica has changed significantly in the last decade and it has recently been reclassified into two species which are morphologically identical but genetically distinct: E. histolytica (Schaudinn, 1903), an invasive disease-causing parasite, and E. dispar (Brumpt, 1925), a non-invasive parasite. This separation was initially proposed in 1925 by Brumpt, who found that only one of the species caused disease in kittens or human volunteers, and named the non-pathogenic species E. dispar. [67]

Entamoeba histolytica appears in **three stages**:

- **The trophozoite** has hyaline ectoplasm (pseudopodium) granular endoplasm. Also it has red blood corpuscles and tissue debris. It changes its shape constantly and size is 20-30μm. The nucleus is spherical, 4-6μm in diameter. It has a karyosome, small dot like, central in position and surrounded by a clear halo. The nuclear membrane is lined by fine chromatin granules and the space between karyosome and nuclear membrane is traversed by a fine thread of lenin network.
- **Precystic stage**: Smaller, 10-20μm in diameter, round or ovoid with blunt pseudopodium. The endoplasm is free of red blood cells and other ingested food particles.
- **Cystic stage**: the parasite has cyst wall and mature cyst is a quadrinucleate spherical body.

Transmission from man to man is affected through its encysted stage and infection occurs through ingestion of these cysts. Faecal contamination of drinking water, vegetables and food are primary causes. [42]

EPIDEMIOLOGY

Amoebiasis is responsible for approximately 100,000 deaths per year, mainly in Central America, Africa and India. Worldwide amoebiasis is the third most cause of death due to parasitic infection after malaria and schistosomiasis.

Amoebiasis infections are endemic in most temperate climates in developing world. In industrialized countries, amoebiasis occurs in sexually active homosexual men, immigrants, tourists who travel to areas of endemic infection and HIV positive individuals.

Epidemiological studies have shown that low socioeconomic status and unsanitary

conditions are significant independent risk factors for infection. In addition, people living in developing countries have a higher risk and earlier age of infection than those in developed regions.[69]

LIFE CYCLE [67]

The E. histolytica life-cycle is relatively simple and consists of an infective cyst and an invasive trophozoite form. The quadrinucleate cyst is the infectious form of the parasite, is resistant to chlorination, gastric acidity and desiccation, and can survive in a moist environment for several weeks. After ingestion excystation of the cyst occurs in the intestine where the cyst undergoes nuclear and cytoplasmic division to form eight trophozoites. The trophozoites can then colonize and/or invade the large bowel. Cysts are never found within invaded tissues.

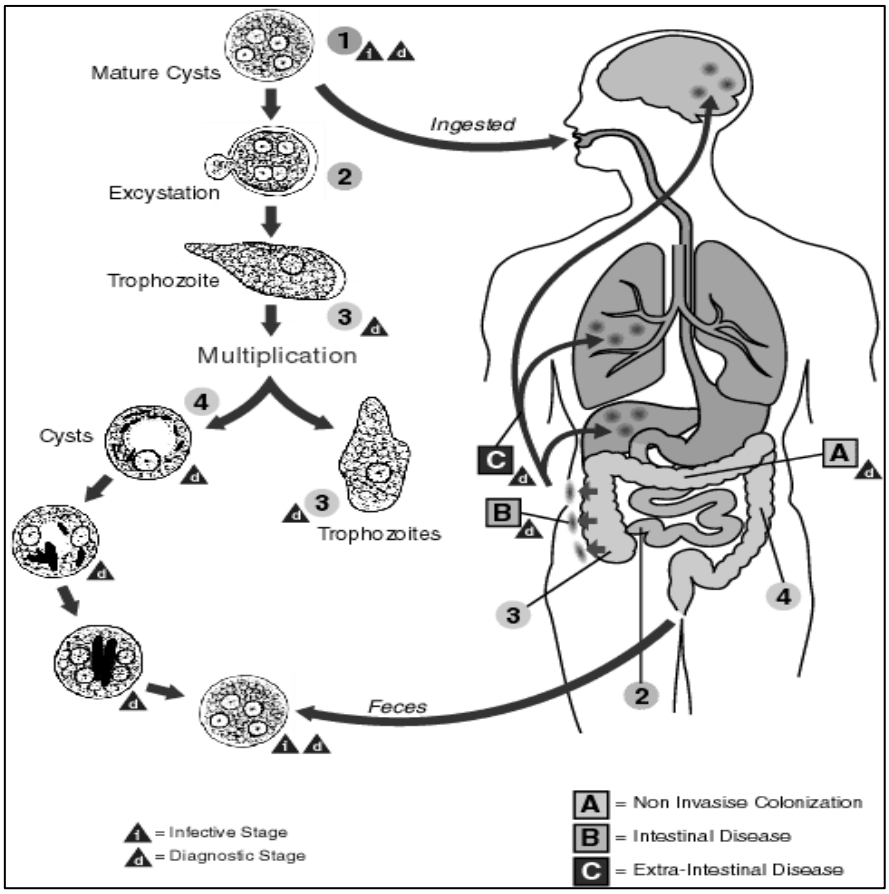


FIGURE 9: 1: Life cycle of E. histolytica [70]

Invasion of the colonic intestinal epithelium by trophozoites leads to the formation of the classically described flask-shaped ulcers. Hepatic abscesses occur due to migration of the parasite via the portal vein. All infections with E. histolytica are not alike, and whether infection results in colonization or invasion may be influenced by the E. histolytica strain and its interaction with bacterial flora, host genetic susceptibility and factors such as malnutrition, sex, age and immunocompetence.

CLINICAL FEATURES

Incubation period is variable, from 4 days to a year or longer. Asymptomatic infection is the most frequent. Symptomatic infection may be uncomplicated intestinal infections, with symptoms of diarrhoea, cramps and flatulence. Amoebic dysentery patients presents with fever, profuse diarrhoea that contains blood and mucous, and severe abdominal pain. Compared to bacillary dysentery, it has usually insidious onset and abdominal tenderness is less marked and localised.

Amoebic colitis (chronic amoebic dysentery) is characterised by intermittent diarrhoea over a long period of time often misdiagnosed as ulcerative colitis or irritable bowel syndrome.

Amoeboma is a localised tumour - like lesion which usually results from chronic ulceration. Histologically, it consists of granulation tissue.

Extra intestinal amoebiasis, the liver is primarily involved. Systemic signs of infection - fever, leucocytosis - eosinophilia, rigors are present. Pain over the liver with hepatomegaly and elevation of the diaphragm is usually observed. [43]

DIAGNOSIS

Demonstration of trophozoites or cysts is usually necessary for diagnosis of E. histolytica. Examination of stool samples is the most effective means of diagnosis

of gut infection. Stool specimens can be examined either unstained or stained with Lugol's or D'Antoni's iodine. Iodine stains make the nucleus perfectly visible. The appearance of chromatoid bodies is the same as in wet mount preparations. Although several other stains, including Giemsa, methylene blue, Chorazole black E, Wright's, and iodine-trichrome, may be used successfully, Wheatley's trichrome staining or one of the modified iron hematoxylin stains for permanent smears has been suggested for routine use in diagnosis of E. histolytica/E. dispar. Lighter infections of cyst passers may be detected with concentration techniques, such as zinc sulfate flotation. [69]

Nested PCR and monoclonal antibody methods are now available for distinguishing between E. dispar and E. histolytica in fresh and preserved stool samples, including those with mixed infections. These methods are based on the observation that the two amoeba species differ at specific sites in their SSU-rDNA. [46]

TREATMENT [46]

Metronidazole (a 5-nitroimidazole derivative) has become the preferred drug in treatment of amoebiasis. It is low in toxicity and is effective against both extraintestinal and colonic infections, as well as cysts. Despite immunosuppression from HIV infection and the complicated disease course of invasive amoebiasis, clinical responses to metronidazole therapy were favourable in terms of rapid defervescence and a low attributable mortality rate.[68]

GIARDIA LAMBIA

HISTORY

Giardia lamblia was first discovered by Leeuwenhock in 1681 in his own stool specimen, but was not described until 1859 by Lambl. [55] The organism is named Giard after professor Giard of Paris and Lamblia after professor Lambl of Prague who made a detailed study of the parasite. [43]

Giardia holds a special place in the affection of all protozoologists because the parasite that causes Giardiasis, Giardia lamblia (also known or G. Intestinalis or G. duodenalis) was the first parasitic protozoan ever to be seen.

The first good illustrations of Giardia are those of Vilem Lambl in 1859 but the parasite received very little attention until the Second World War. The returning troops with diarrhoea were found to have Giardia parasites in their faeces, the cysts of which caused similar disease in laboratory animals.

In 1921 the distinguished British protozoologist, Clifford Dobell, suggested that Giardia could be a serious pathogen. This fact became widely recognized, when the detailed studies of Robert Rendorff produced unambiguous evidence linking the parasite with the disease. [55]

TAXONOMY & MORPHOLOGY

It belongs to the phylum Sarcomastigophora, subphylum Mastigophora, class Zoomastigophorea, the order Diplomonadida and the family Hexamitidae.

Giardia lamblia exists in two forms, trophozoite and cyst.

- **Trophozoite** appears like a longitudinally split pear. The dorsal surface is convex and the ventral surface is concave with a sucking disk. The size is 14µm X 7µm. The anterior end is broad and rounded and posterior end tapers to a sharp point. It is bilaterally symmetrical and all the organs of the body are paired.
- **Cyst** is oval in shape measuring about 12µm X 7µm in size. The axostyles lie more or less diagonally forming a sort of dividing line within the cyst wall. There are four nuclei, which may remain clustered at one end or lie in pairs of opposite poles. The remains of flagella and the margins of the sucking disc may be seen inside the cytoplasm. [42]

EPIDEMIOLOGY

Giardiasis is one of the most common intestinal parasitic infections in human and is distributed worldwide in developed and developing countries. Geographical distribution varies considerably worldwide with wide range of prevalence. There are differences in the numbers of infections, not only between countries but within geographic regions. Infections seem to be more common in children than in adults. There has also been an increase in the prevalence of Giardiasis in the male homosexual population. Susceptibility to infection with

Giardia is influenced by sex, age, environmental conditions, socioeconomic conditions, occupation, nutritional status, gastric acidity and overall host immune status. Giardiasis is one of the most common cause of traveller's diarrhoea and has been reported from all parts of the world. [55] In developed regions of the world, Giardia is usually transmitted via contaminated water. However, its ubiquitous distribution makes person-to-person transmission frequent in settings of poor fecal–oral hygiene, and food transmission is increasingly recognized. [45]

LIFE CYCLE [47]

This one-celled flagellated protozoan has a simple life cycle. Cysts are the transmission stage and are excreted in the faeces of infected individuals into the environment where they can survive for weeks. When ingested, exposure to the low pH of the stomach and pancreatic enzymes induces excystation, with two trophozoites developing from each cyst. Trophozoites attach to epithelial cells of the upper intestine, primarily the jejunum but also the duodenum, where they grow and divide.[47]

CLINICAL FEATURES[47]

The majority of symptomatic people have acute self-limited diarrhoea, lasting 7 to 15 days while a small proportion of symptomatic patients develop chronic

diarrhoea accompanied by signs of malabsorption and significant weight loss. Symptoms usually begin 7 to 14 days after cyst ingestion but can begin as late as 4 weeks later. The onset is usually acute with diarrhoea accompanied by diffuse abdominal cramping and discomfort, bloating, flatulence and fatigue. Nausea can be present but vomiting is rare. Fever, when present, is low-grade and seen early in the course of infection. Initially stools are usually profuse and watery but later in the course of the disease can become greasy and malodorous.

DIAGNOSIS [47, 43, 42]

The traditional method of diagnosis is examination of freshly passed stool for Giardia trophozoites and cysts, the former are found in a diarrhogenic stool or after a purgative. Saline wet mount, stains such as iodine, Giemsa, or trichome, aid in microscopic identification.

Recently, new assays have been developed based on detection of Giardia antigens. The direct fluorescent antibody test (DFA), uses a Giardia-specific antibody conjugated to a fluorophore to stain stool specimens. On a single stool specimen the sensitivity is between 96 - 100%. Other antigen-detection tests detect soluble Giardia-specific proteins in the stool. There are two different types of soluble-antigen-detection tests, the enzyme immunoassays (EIA) and immunochromatographic dipsticks (ICT) which work on the same overall principal, i.e., immobilization of antibodies specific for Giardia proteins on a plate (EIA) or a piece of chromatography paper (ICT) that then capture Giardia proteins in the stool. ICTs are relatively new and have a lower sensitivity than EIAs (80% versus 94 to 98%).

Giardia trophozoites may be recovered from aspirates of duodenum and jejunum by Enterotest. Antigiardia antibody detection in serum is not useful in diagnosis of disease. DNA - techniques (DNA probes for Giardia) are available now.

TREATMENT [47, 42]

The derivatives of imidazole are the most commonly used drug to treat Giardia infection. In adults, 250 mg thrice daily for 5-7 days has been shown to be effective in 80 to 95% of cases. Other like trimidazole (2 gms once). Furazolidone (100 mg 4 times daily for 7 - 10 days) have been found to be effective for Giardiasis.

STRONGYLOIDES STERCORALIS

Strongyloides stercoralis might be described as 'the military worm'. It was first described by a military physician in soldiers returning from war. Bavay (1876) first described Strongyloides stercoralis in French soldiers returning from Indochina (now Vietnam), who were suffering intractable diarrhoea. [45] Stiles and Hassal in 1902 worked out the life cycle and pathogenesis of the parasite. [43]

Strongyloides stercoralis belongs to phylum Nemathelminth, class Nematode, superfamily Rhabditoidea and genus Strongyloides.[42]

It is worldwide in distribution more common in tropics and subtropics of Africa, South America and Asia including India. It is an important opportunistic pathogen in immunocompromised host (HIV/ AIDS). [41]

Most infected immunocompetent individuals acquire immunity after primary infection against a reinfection. Though both humoral and cell mediated response occurs, but cell mediated immunity (CMI) plays a vital role in controlling infection. When CMI is depressed as in immunosuppressed (e.g. HIV/AIDS), hyper infection occurs, and disseminated or generalised Strongyloidiasis develops.[43]

Strongyloides stercoralis exists in both a free living form in the soil and as an intestinal parasite. The parasitic females are 2.2 mm in length, semi-transparent and colourless and lie embedded within the mucosal epithelium of the proximal small intestine where they deposit their eggs. A single female worm will produce up to 50

eggs per day. There is no parasitic male and reproduction is by parthenogenesis. [47]Rhabditiformlarva hatch out and are passed in faeces and develop in soil or metamorphose in the lumen of intestine to Filariform larvae and cause autoinfection.[41]

The clinical manifestations of uncomplicated strongyloidiasis are cutaneous, pulmonary and gastrointestinal. Following penetration of the skin, there may be a localized, erythematous, papular, pruritic eruption. Migrating larvae may produce a serpiginous urticarial rash - larva currens that can progress as fast as 10 cm/hr. This is frequently seen on the buttocks, perineum and thighs and may represent autoinfection. Adult worm may cause diarrhoea with blood and mucous. Abdominal pain due to adult worm is common. [43] In immunocompromised patients autoinfection may lead to a chronic strongyloidiasis. Such individuals are at risk of hyperinfection syndrome characterised by massive larvae invasion of the lungs with respiratory distress, or any other organs including central nervous system, heart and liver. This is a life threatening condition and untreated disseminated disease is fatal. [43]

The diagnosis of strongyloidiasis can be established by identification of the rhabditiform larvae in saline or iodine wet mount of stool. Very low number of larvae produced on a daily basis by an adult worm. Furthermore, release of larvae is intermittent. Multiple microscopic examinations using large volumes of stool concentrated by formol - ether or Baermann's method are often necessary. Frequently, the only clue to the diagnosis is the presence of unexplained eosinophilia. [42]

A variety of methods have been developed to increase the likelihood of finding larvae in a stool specimen. One method employs an agar culture on which a stool sample is placed on nutrient agar and then incubated for several days. As the motile larvae crawl over the agar, they carry bacteria with them leaving visible tracks. In one large study, this technique was found to be 96% sensitive in determining the

presence of strongyloides. Serologic tests have been developed that can aid in the diagnosis of strongyloidiasis. An enzyme-linked immunoassy is available from the Centre for Disease Control (Atlanta) which has a sensitivity of 95%. Specificity is less as a result of cross-reactivity with other helminthic infections. An immediate hypersensitivity type of skin test has been developed which has a sensitivity of 82-100%, but has significant cross-reactivity with filarial infections. [45]

The goal of treatment of strongyloidiasis is eradication of the organism. The drug of choice for treatment of strongyloidiasis is ivermectin given orally at a dose of 200µg/kg once daily for 2 days. Thiabendazole is equally effective at a dose of 25 mg/kg twice daily for 3 days, but has a much greater incidence of side effects including nausea, vomiting, foul taste and foul smelling urine. Albendazole has also been used, but is less effective than either thiabendazole or ivermectin, and requires 7 days of treatment. [43]

BLASTOCYSTIS HOMINIS

Blastocystis hominis is a parasite whose taxonomic status is unclear. The life cycle includes vacuolar, amoeboid, precystic, and cyst stages. Ameboid stages divide by binary fission and phagocytize bacteria. Two kinds of cysts are formed: thin walled and thick walled. The former evidently contain schizonts and are possibly autoinfective, whereas the latter are likely the means of external transmission. Several species of Blastocystis have been described from ducks, geese, camels, and even koalas. Blastocystis hominis has been implicated in various intestinal disorders, including traveler's diarrhea and irritable bowel syndrome, but a clear link between infection and disease has yet to be established. [46]

Blastocystis was first definitively described as a distinct organism by Alexeieff in 1911. Brumpt (1912), believing that different species of Blastocystis

were present in different hosts, proposed the name B. hominis for the organism from humans. The classification of Blastocystis remains controversial, it has been described as a yeast, a fungus, an alga, the cyst of other organisms (including Trichomonas spp.) and as a morphological form of Dietamoebafragilis. Ultrastructural and physiological studies, first performed by Zierdt et al. (1967), have demonstrated that Blastocystis has protozoan characteristics.[45]

A number of morphological forms of Blastocystis have been reported from culture and from faecal material. The forms most commonly reported in faeces include the vacuolar, granular, multivacuolar and cyst forms. An amoeboid form has been reported only rarely, and there are a number of conflicting reports on its morphology. An avacuolar form is thought to be present in the intestine of humans.[45]

A number of life-cycles have been proposed for Blastocystis, but none have been verified in vitro or in vivo. The elucidation of several new forms of Blastocystis (particularly the cyst form) has invalidated most of the earlier life-cycles. This includes the life-cycle proposed by Zierdt (1973). [45]

Infections with the organism are worldwide and appear in both immunocompetent and immunodeficient individuals. Symptoms attributed to gastrointestinal B. hominis infection are generally non-specific and include diarrhoea, abdominal pain and cramps or discomfort, nausea, flatulence and fever. Other signs and symptoms include rectal bleeding, faecal leukocytes, eosinophilia, hepatomegaly and splenomegaly, cutaneous rashes and itching. [45]

The cyst form is the more recognizable and more recent discovery that has helped in the advancement of understanding the way the infection is transmitted. A permanent stained smear using a stain such as the trichrome stain is most preferred for determining the presence of this organism. Iodine smears, used to identify B. hominis

organisms, are preliminary procedures for a number of other parasites. As compared to the other three forms, the cyst is generally smaller in size with a thicker cyst wall of several layers. It lacks a large central vacuole and has a few nuclei, although there are small multiple vacuoles and food storage deposits that may be seen. The cyst form is the most resistant form of this parasite and is able to survive in environmental conditions that may not be conductive to life for other organisms due to temperature extremes and drying out because of its thick cyst wall. [71]

Treatment for Blastocystis is initiated only when the organism is detected in significant numbers. The agent, metronidazole, that is effective against a variety of parasitic pathogens and some bacteria is the predominant drug used for treatment. Iodoquinol is also sometimes used for treatment when necessary. [71]

IMPACT OF HELTHMINTHIC INFECTION ON HIV/AIDS

Helminth infection in HIV-infected children may impact how the host responds to infectious diseases and immunizations, indirectly through the pathway of malnutrition, as well as directly through immunologic mechanisms. Beyond nutritional deficits, helminths also induce immunosuppressive responses, creating an ideal environment for chronic helminth infection, and inhibiting the host's ability to control other diseases such as HIV.[72] Clinical studies suggest that deworming HIV-infected individuals may delay HIV progression, as measured by CD4 count and HIV viral load .[73]

The World Health Organization (WHO) recommends annual or bi-annual school-based deworming as a cost-effective strategy to diminish the consequences of chronic helminth infection. Deworming could also be considered part of the nutritional care package for HIV-infected children, to reduce the consequences of malnutrition and anemia in HIV. Incorporating deworming into routine HIV care and

treatment is an ideal way to improve the nutritional health of HIV-infected children, and may provide additional benefits. [73]

Albendazole treatment of individuals with human immunodeficiency virus type 1 (HIV-1) and Ascaris lumbricoides coinfection has led to significantly improved CD4+ cell counts and a trend for lower plasma HIV-1 RNA levels Treatment of A. lumbricoides co-infection may delay HIV-1 disease progression by reducing helminth-induced, IL-10–mediated immunosuppression.[72]

Ramakrishnan K. et al stated that in HIV/AIDS patients with diarrhea, Entamoeba histolytica infection was noted in 17.5%, while Cryptosporidium parvum was observed in 28.7%. The prevalence of these infestations was significantly higher than in the HIV negative group. Study suggests that enteric infections in HIV infected patients are different from those in HIV uninfected persons in south India. This may be due to differences in immunological profile of susceptibility.[74]

Prasad K. N. et al studied emerging parasites in HIV-related diarrhea. He concluded that, emerging parasites were significantly higher compared to conventional pathogens. Isospora belli (27%) was the most frequently-encountered pathogen in our patients, followed by Cryptosporidium (11%) and Blastocystis hominis (8%) as single pathogens.[49]

Lekha T. et al studied 366 HIV positive patients with diarrhea, Cryptosporidium (39.8%) was most commonly found in the HIV positive patients followed by Microsporidia spp. (26.7%). There were (25.1%) cases of mixed infections. The maximum parasitic isolation was in group of patients who had CD4 cell counts below 200cells/μL. The isolation rates decreased with increase in the CD4 cell counts.[75]

Joshi M. et al examined a total of 110 stool specimens from the 94 AIDS

patients. Parasitological studies revealed a high positivity of 56.4%. The most frequently detected protozoan was Isospora belli in 16 patients (17%) followed by E. histolytica in 14 (14.9%). The most common helminthic parasite detected was Strongyloides stercoralis in 5 patients (5.3%).[76]

Kulkarni S. V. et all studied 137 patients. Enteric parasites were detected in 48 (35%) stool samples, of which 30 (62.5%) were opportunistic and 18 (37.5%) were non opportunistic. Opportunistic parasites were Cryptosporidium parvum (16), Isospora belli (11), Microsporidia (2) and Cyclospora (1). The proportion of opportunistic pathogens in patients with CD4 count <200 cells/µl was significantly higher.[4]

Satheesh S. Kumar et al studied a total of 150 HIV positive patients. The most predominant parasite was Isospora belli (18%), followed by Cryptosporidium (14%), Microsporidium and Cyclospora.[77]

MATERIAL & METHODS

MATERIAL AND METHODS

TYPE OF STUDY - A descriptive cross sectional study design was used.

SOURCE OF DATA -

A total of 110 patients of both sexes irrespective of age groups who were HIV seropositive with diarrhea attending BLDEA's Sri B.M. Patil Medical College Hospital & Research Centre, Bijapur from DEC 2011 to MAY 2013 were included in the study after the approval of institutional ethical committee and with the consent of the subjects.

INCLUSION CRITERIA -

Both sexes irrespective of age groups who were HIV seropositive (HIV I or HIV II or both) with diarrhea.

EXCLUSION CRITERIA -

1. Cases who were HIV seronegative.
2. Cases HIV seropositive without diarrhea.
3. Patients who had received Anti-parasitic medications, anti-diarrheal agents in the previous 2 weeks.

METHOD OF COLLECTION OF SAMPLE:

The study was done on stool samples obtained from 110 HIV positive patients, who tested positive according to NACO guidelines. Strategy IIB & III were used for diagnosis of HIV infection. All patients were tested positive for HIV using three separate kits .When a serum sample tested reactive once by a system of ELISA / Rapid (E/R) test, the test was repeated immediately by a different system in order to confirm the diagnosis. The sample was then taken up for supplemental tests to confirm the diagnosis. Supplemental tests like E/R, Western Blot (WB), Indirect fluorescent antibody test (IFA), Radioimmunoprecipitation test (RIPA) etc.

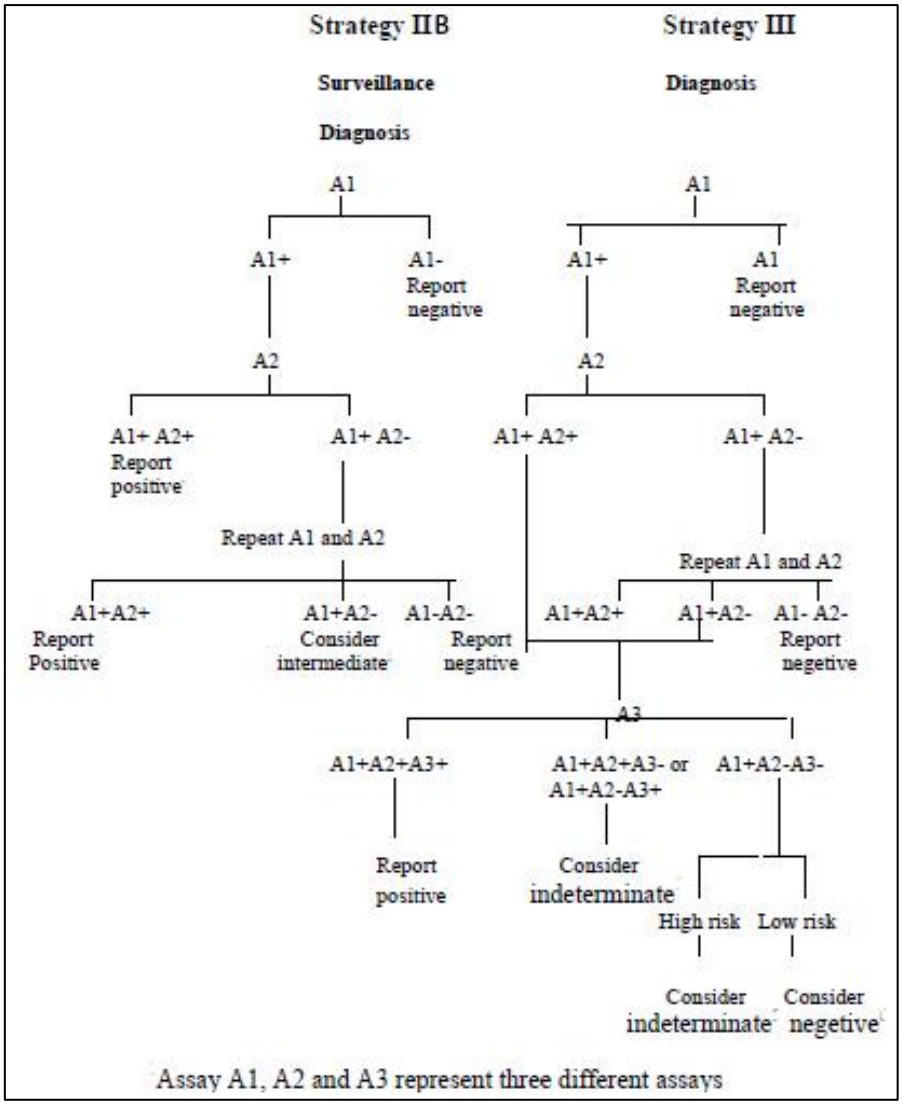


FIGURE 10: Strategies for HIV diagnosis as per NACO guidelines

Diarrhoea was defined as 3 or more semi-liquid or liquid stools in twenty four hours. Diarrhoea associated with HIV infection may be acute or chronic. Acute diarrhoea is defined as diarrhoea of <14 days duration and chronic diarrhoea is defined as diarrhoea lasting for >14 days.

The stool samples were collected in sterile leak proof plastic containers with a wide mouth and a tight - fitting lid without any preservative. The patients were asked to collect their stool sample preferably in the morning. They were instructed to avoid contamination of the stool specimen with urine or water. A disposable plastic spoon was kept inside each container for their convenience while collecting the sample.

Patients were instructed to drop the spoon along with the sample inside the container and close the lid tight. The containers were labelled properly and transported to the laboratory without delay. The specimens were processed within 1-3 hours of collection. Patient details pertaining to age and sex, duration of HIV infection, symptoms at presentation, extra-intestinal opportunistic infections and CD4 counts were obtained.

EXAMINATION OF STOOL SAMPLE

MACROSCOPIC EXAMINATION: [78, 42]

The specimens were examined by naked eye for:

- Colour
- Consistency
- Presence of blood, mucus
- Adult worms or segments of worms

MICROSCOPIC EXAMINATION: [64, 78, 43]

Direct microscopic examination of faeces in saline, iodine and lactophenol cotton blue (LPCB) suspension was done to detect trophozoites, ova, cysts, larvae and oocysts.

i. **Saline preparation**:

- One drop of 0.85% NaCl was placed on a clean glass slide. A small amount of representative fecal specimen was picked up on the end of an applicator stick and emulsified thoroughly.
- A 22 X 22 mm clean coverslip was carefully placed over the suspension avoiding air bubbles.
- This was used for identifying various forms of intestinal parasites (motile trophozoites, ova, cysts, oocysts and larvae).

ii. **Iodine preparation**: the preparation was made in which a drop of Lugol's iodine was added to the drop of saline emulsion of stool sample and then a coverslip was placed on the preparation.

iii. **Lactophenol Cotton Blue Mount**: This was done similar to the above wet mounts using Lacto-phenol Cotton Blue solution.

These preparations were examined under the low power and high power objectives (10X and 40X) of the light microscope. The wet mounts were screened by examining the slides starting from one end of the coverslip. The doubtful and detailed structures were examined under the high power objective.

The saline mount was examined for the motile trophozoites, larva, cysts, ova, pus cells, RBCs, bacteria, yeast cells, hyphal elements, fat globules and Charcot layden crystals. The iodine mount and LPCB was examined particularly for the study of nuclear characteristics and glycogen mass.

Calibration of Microscope with an Ocular Micrometer was done. Measurements are made with a micrometer disk that is placed in the ocular of the microscope; the disk is usually calibrated as a line divided into 50 units. Depending on the objective magnification used, the divisions in the disk represent different measurements. The ocular disk division must be compared with a known calibrated scale, usually a stage micrometer with a scale of 0.1- and 0.01- mm divisions.

Permanent Staining Methods: Used for detecting coccidian parasites in the faeces specimens. A small portion of the sample was placed at the center of a clean slide and a thin smear (one should be able to see through the wet material before it dries) was prepared by gently rolling an applicator stick to spread it. Smear was air dried and heat fixed.[78]

Modified Kinyoun's Acid-Fast Staining Method (Cold method).[78]

- Heat fixed faecal smear was covered with a strip of filter paper. The slide was flooded with Kinyoun's carbolfuchsin and allowed to stain for 5 min.
- The stain was tipped off and filter paper removed. The slide wasrinsed briefly (3 to 5 sec.) with 50% ethanol and then thoroughlywith water.
- 1% Sulfuric acid was used to decolorize for 2 min or until no morecolour ran from the slide. Slide was then rinsed in water anddrained.
- Counterstaining was done with methylene blue or malachite green for 1 min.
- Slide wasrinsed with water and air dried.
- Then the slide was examined with low and high objective.To see the detailedmorphology, oil immersion objective was used(total magnification, 100X).

Interpretation: Cryptosporidium species oocysts and Cyclospora species oocyst stained bright pink and appeared asspherules on a pale green or blue background.The degree and proportion of staining varies with individual oocysts.The internal structures take up the stain to varying degrees. Someappeared amorphous, while others contained the characteristic cresentricform of the sporozoites.Isospora belli oocysts stain pink and appear as large elongatedovoid bodies, tapered at the end and containing either a granular zygote ortwo sporoblasts.

CONCENTRATION TECHNIQUES

Concentration techniques like Formol ether sedimentation and Sheather's sugar floatation technique was employed for all specimens for the concentration of the parasitic ova and cysts.

Sheather's sugar floatation technique :[79, 45]

Principle: The specific gravity of helminth ova and larvae and protozoan cysts are in

the range of 1.050 – 1.150. The floatation principle utilizes liquid medium denser than the parasites so that they rise to the surface and can be skimmed from the surface film.

Procedure:

- 1gm of faeces was transferred with an applicator stick to 3ml of sucrose solution in a test tube and was mixed thoroughly.
- With gentle stirring, sufficient sucrose solution was added to form a positive meniscus at the top of the test tube.
- Gently a coverslip was placed on to the surface of the positive meniscus. After 20 min the coverslip was lifted vertically together with its hanging drop and place on to a clean microscope slide.
- Examined for the presence of parasite, using 10X objective lens. Identification of any definitive morphological features under the 40X objective was done.

Formol ether concentration: [45, 43, 42, 62]

Principle: The faecal material is dissolved in solutions of density below that of the ova/cysts. In this case the ova/cysts are concentrated at the bottom.

Procedure: Add 10 ml of 10% formalin to approximately 1 g of faeces and stir using an applicator stick, to get a cloudy suspension.

- This suspension is strained through the gauze filter into a centrifuge tube until the 7ml mark is reached.
- 3 ml of ether is added and mixed for one minute.
- It is centrifuged for 2000 rpm for 2 minutes and then allowed to settle.
- Loosen the fatty plug (debris) with an applicator stick, and pour away the supernatant by quickly inverting the tube.
- Replace the tube on its rack and allow the fluid on the sides of the tube to drain down to the sediment.

- After concentration techniques were employed, the saline, iodine and LPCB preparations were done and examined under the microscope with low and high power objective. Smears were prepared to identify the parasites by staining techniques like Modified Kinyoun's Acid-Fast Stain and examined under the microscope with oil immersion objective.

ESTIMATION OF CD4 COUNT [39, 37]

Sample: CD4 count was estimated form 5 ml of K_2EDTA blood collected from each patient and was processed the same day. The CD4 count was done by automated flow cytometry analyser FACS calibur (Beckton Dickinson).

Principle of Flow Cytometry: Signals are generated by cells or particles in suspension passingthrough a light (usually LASER) source in the flow cell, in a single file(aligned by Sheath Fluidic System) and are analysed electronically in a flowcytometer. The parameters measurable include forward scatter (an indicator ofcell size), side scatter (an indicator of granularity of the cell) and signals frommultiple fluorescent dyes (e.g. FITC, phycoerythrin) tagged to cell surfacephenotypic marker-specific antibodies.

BD FACS Calibur is a flow cytometer which is capable of measuring the scatter and the fluorescence parameter. It can detect the scatter parameter namely the forward and the side scatter which gives information about the size and granularity of the cell. The BD FACS Calibur can detect up to 3 fluorescence parameters. It can measure both absolute CD4 + T-lymphocyte count as well as % CD4 count.

Antibody panels - BD TriTEST CD3 fluorescein isothiocyanate (FITC)/CD4 phycoerythrin (PE)/CD45 peridinin chlorophyll protein (PerCP) was used, which is a three-color direct immunofluorescence reagent to identify and determine the percentages and absolute counts of mature human T lymphocytes (CD3) and

helper/inducer (CD3+CD4+) T-lymphocyte subsets in erythrocyte-lysed whole blood. When used with TruCOUNT Tubes, absolute counts of these populations can be enumerated from a single tube.

Procedure

- Required numbers of BD TruCOUNT Tubes were taken. 20µl of Tritest antibody reagent is added to each tube, by placing it on the side wall of the tube, to avoid disturbing the bead at the bottom.
- 50µl of well mixed whole blood collected in K2EDTA is added to each tube in a similar way and vortexed.Incubated in dark at room temperature for 15 minutes.
- 450µl of 1x Lysing solution was added and vortexed. This cause lysis of RBCs and fixation of cells on the beads.
- Incubated in dark at room temperature for 15 minutes.
- Reading was obtained on the BD FACS Calibur.

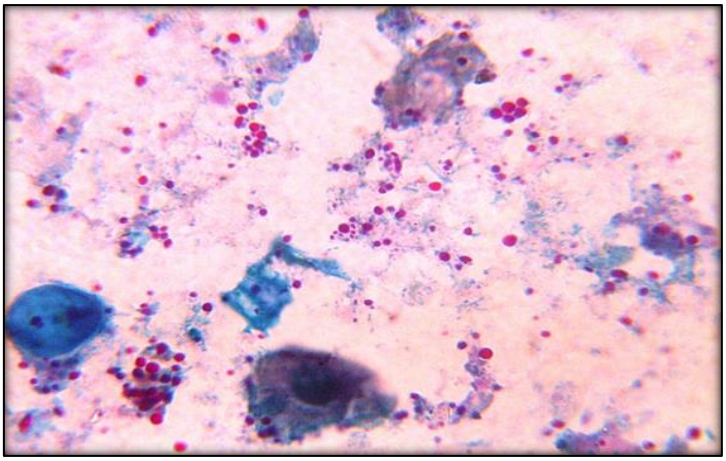


FIGURE 11 :Cryptosporidium parvum (MKAFS)

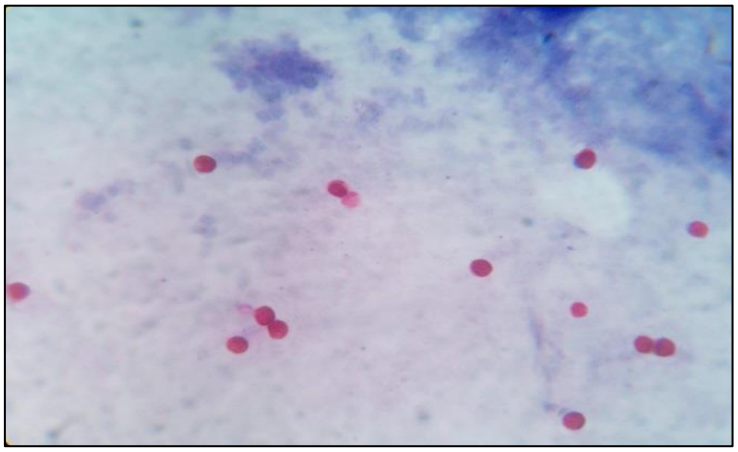


FIGURE 12 :Cyclospora cayetanensis (MKAFS)

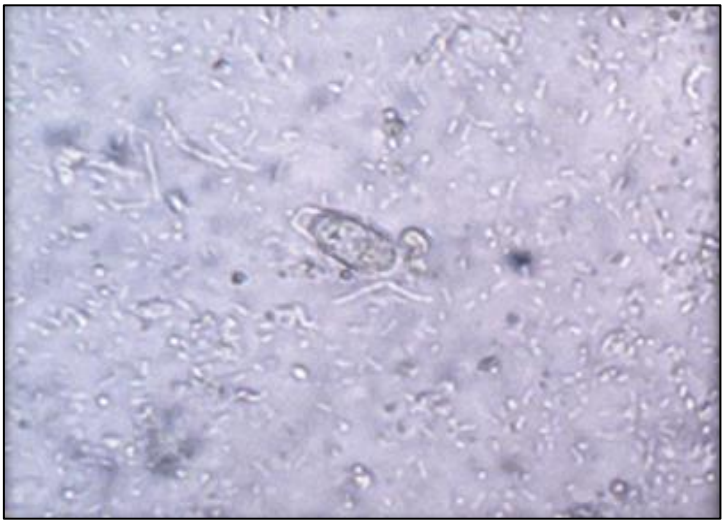


FIGURE 13: Isospora belli (saline wet mount)

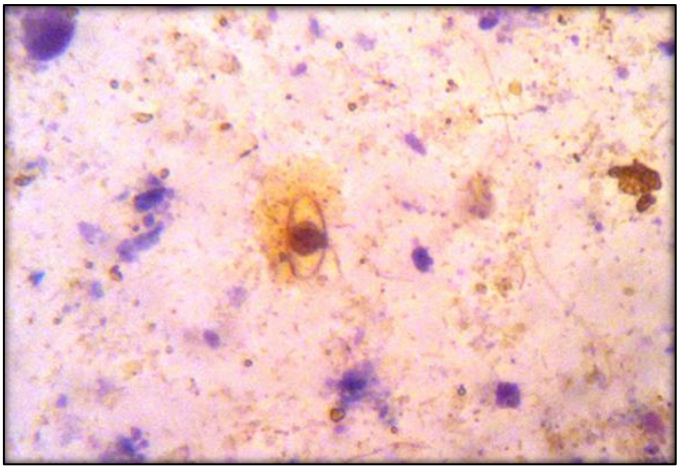


FIGURE 14: Isospora belli (iodine wet mount)

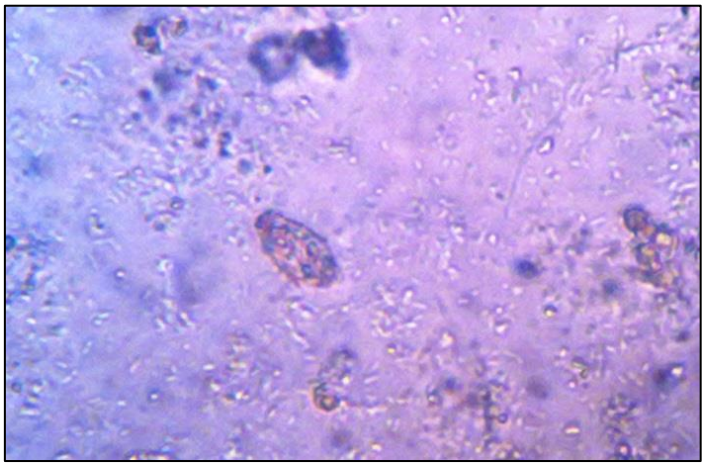


FIGURE 15: Isospora belli (LPCB)

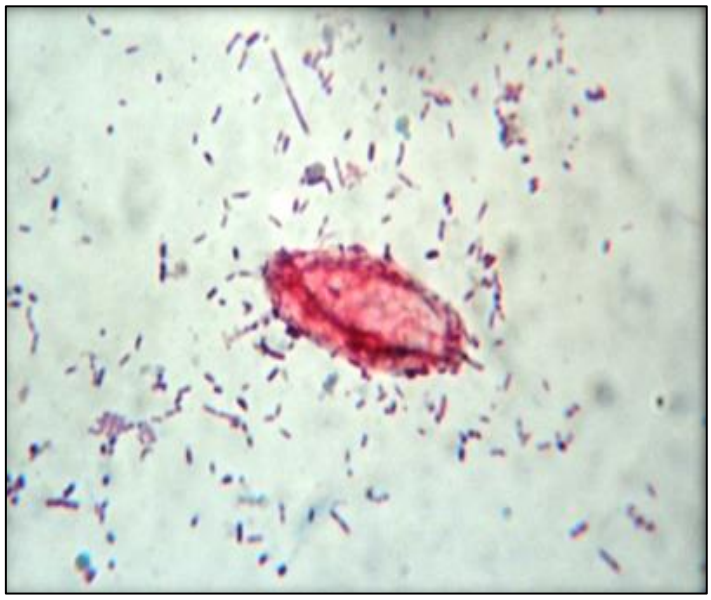


FIGURE 16: Isospora belli (MKAFS)

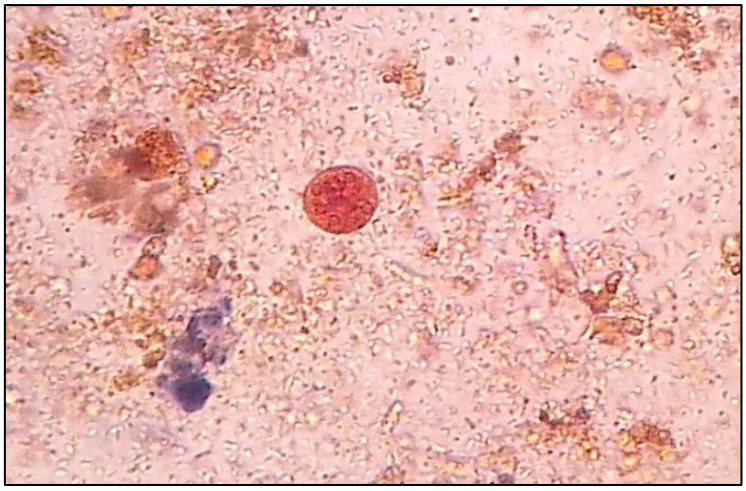


FIGURE 17: Entamoeba coli (Saline wet mount)

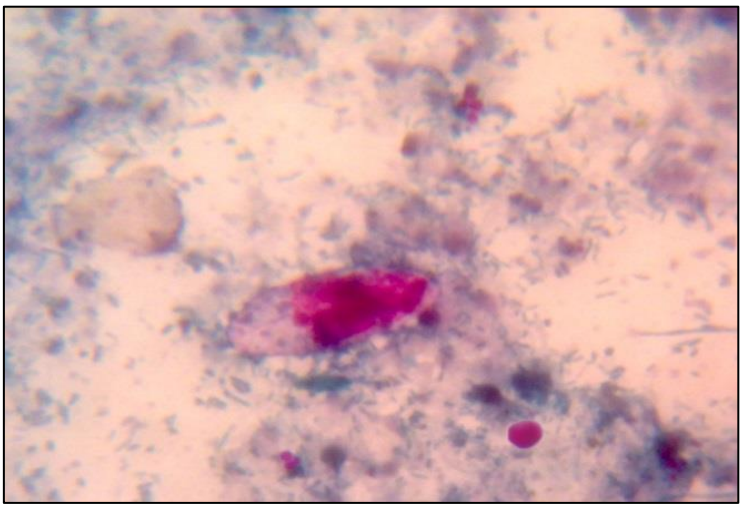


FIGURE 18: Mixed infection (MKAFS)

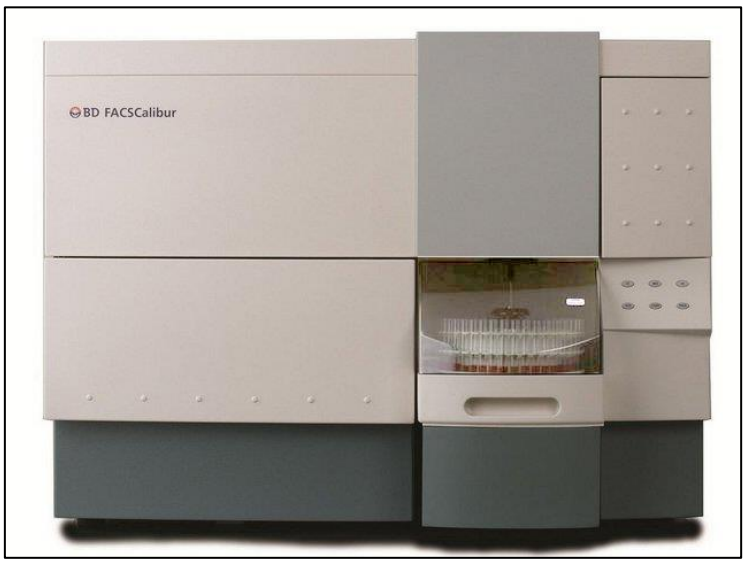


FIGURE 19:BD FACS Calibur for CD4 estimation.

RESULTS

RESULTS

A total of 110 HIV/AIDS seropositive individuals with diarrhoea were enrolled in this study from 01.12.2011 to 31.05.2013, attending BLDEA's Sri B.M. Patil Medical College Hospital & Research Centre, Bijapur.

TABLE 7: Sex distribution in study group.

Sex	Study Group	Percentage
Male	61	55.45 %
Female	49	44.55 %

Out of 110 cases of HIV/AIDS studied male predominance was observed with number of male 61 (55.45%) followed by female population of 49 (44.55%). Male to Female Ratio is 1.2:1.

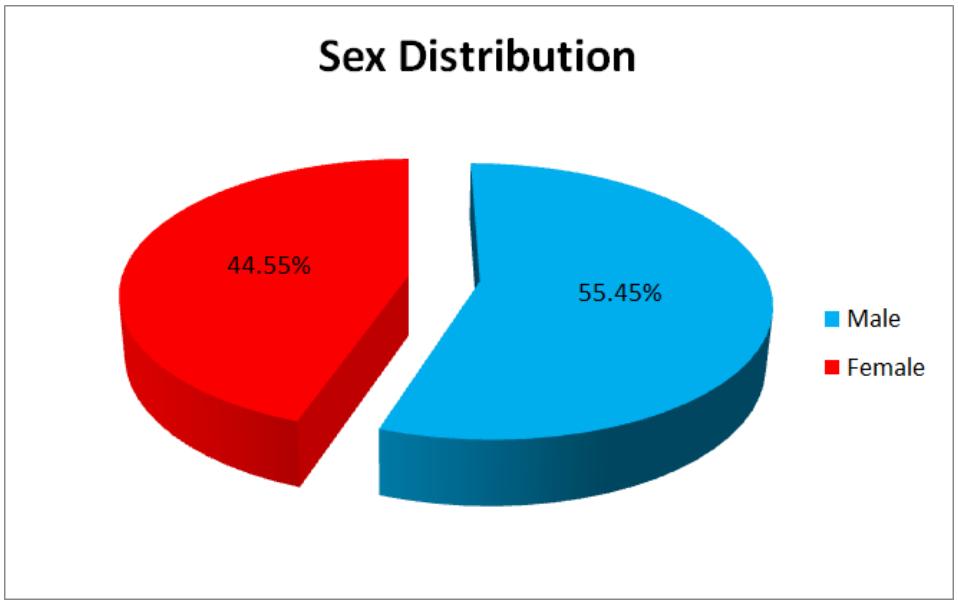


FIGURE 20: Sex distribution of study group

TABLE 8: Age wise distribution of study group

Age (years)	No. of cases	Percentage
≤ 10	3	2.72 %
11 - 22	3	2.72 %
21 - 30	19	17.28 %
31 - 40	48	43.64 %
41 - 50	27	24.55 %
≥ 51	10	9.09 %
Total	110	100

Table No. 8 depicts maximum number of 48 cases (43.64%) were in the age group of 31-40 years and least number of cases 3 each were seen in age groups <10 years & 11 – 20 years respectively.

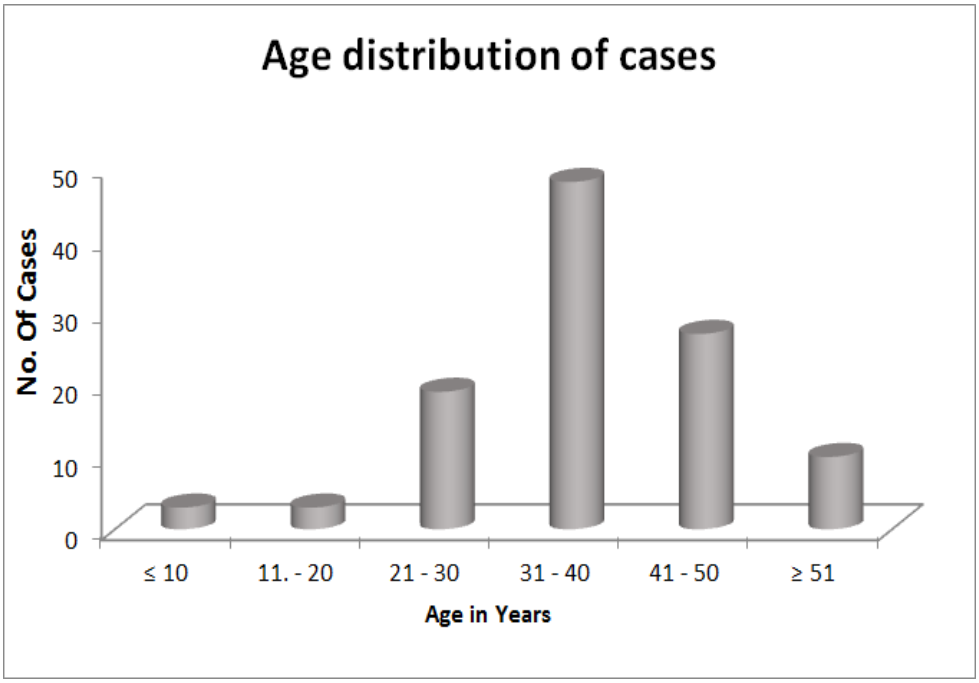


FIGURE 21: Age distribution of study group

TABLE 9: Age and Sex Distribution Among Study Group

Age (years)	Male		Female		Total	
	Number	Percentage	Number	Percentage	Number	Percentage
≤ 10	3	2.72 %	0	0 %	3	2.72 %
11 - 20	1	0.90 %	2	1.82 %	3	2.72 %
21 - 30	6	5.46 %	13	11.82 %	19	17.28 %
31 - 40	27	24.55 %	21	19.09 %	48	43.64 %
41 - 50	18	16.37 %	9	8.18 %	27	24.55 %
≥ 51	6	5.45 %	4	3.64 %	10	9.09 %
Total	61	55.45 %	49	44.55 %	110	100 %

Table No.9 indicates the highest prevalence of males in 31 – 40 years age group i.e. 27 cases (24.55%) followed by 41-50 years of age group with 18 cases (16.37%). Of the females studied 31-40 years of age group showed highest prevalence with 21 cases (19.09 %) followed by age group 21 – 30 having 13 cases (11.82%). Overall age group 31 – 40 years accounted for a maximum number of cases 48 (43.63%).

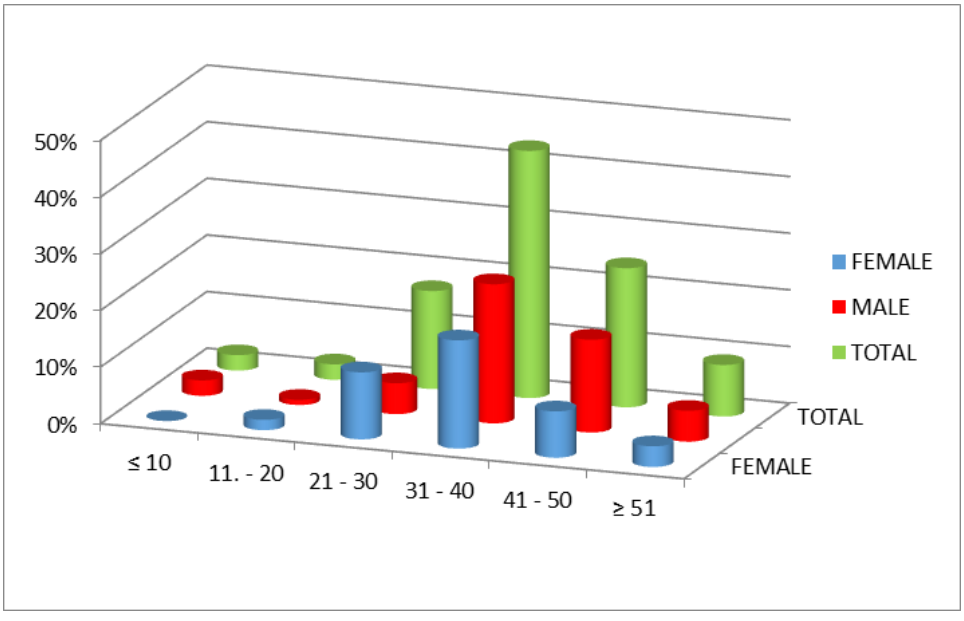


FIGURE 22: Age and Sex Distribution Among in Study Group

TABLE 10: Distribution of type of diarrhoea

Diarrhoea	No. of Negative cases	No. of Positive cases	Total Cases n = 110
Acute	52	11 (17.5%)	63(57%)
Chronic	9	38 (80.85%)	47(43%)
Total (n)	61	49	110(100%)

Out of 110 cases studied 63 patients (57%) were with acute diarrhoea and the remaining 47 cases (43%) were chronic diarrhoea patients.

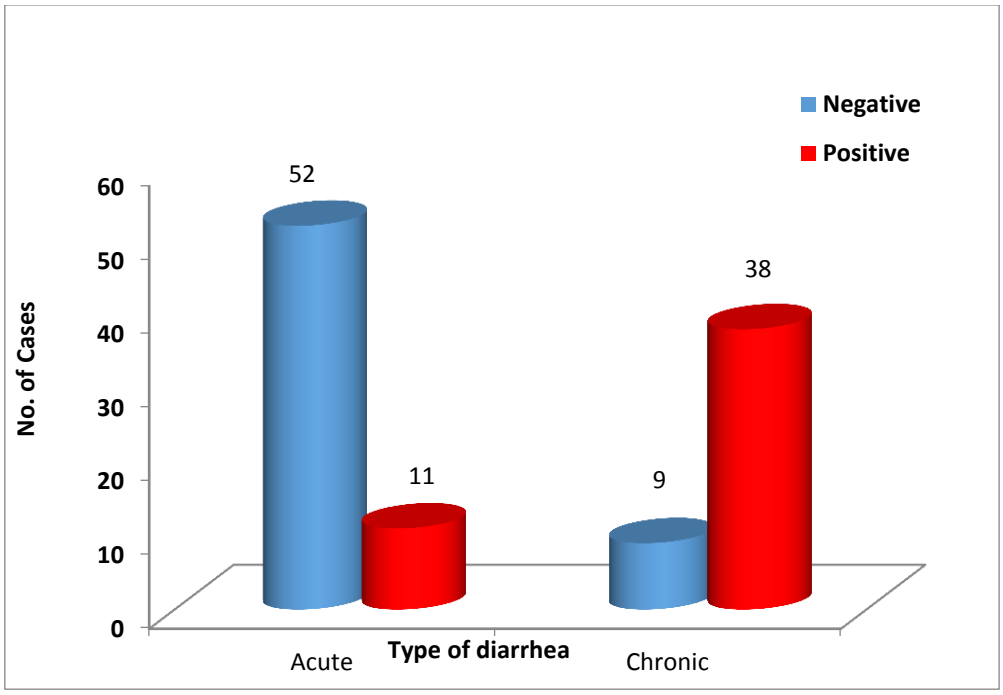


FIGURE 23: Distribution of type of diarrhoea

TABLE 11: Type of diarrhea and CD4 count Correlation

Diarrhea	No. of Patients	Mean CD4 Count	95% Cl. interval		P Value
			Minimum	Maximum	
Acute	63	300.4	257.6	343.3	0.036
Chronic	47	157.5	120.4	194.6	0.0005

TABLE 12: Symptoms along with Diarrhoea

Symptoms	No. of cases	Percentage
Weight loss	106	96.36 %
Pain Abdomen	83	75.45 %
Fever	48	43.63 %
Weight Loss +Pain Abdomen	83	75.45 %
Weight Loss + Fever	48	43.63 %
Pain Abdomen + Fever	41	37.27 %

In the present study 106 patients (96.36%) had weight loss as the predominant clinical manifestation along with diarrhoea followed by pain abdomen and combined weight loss & pain abdomen accounting for 83 cases (75.45%) each.

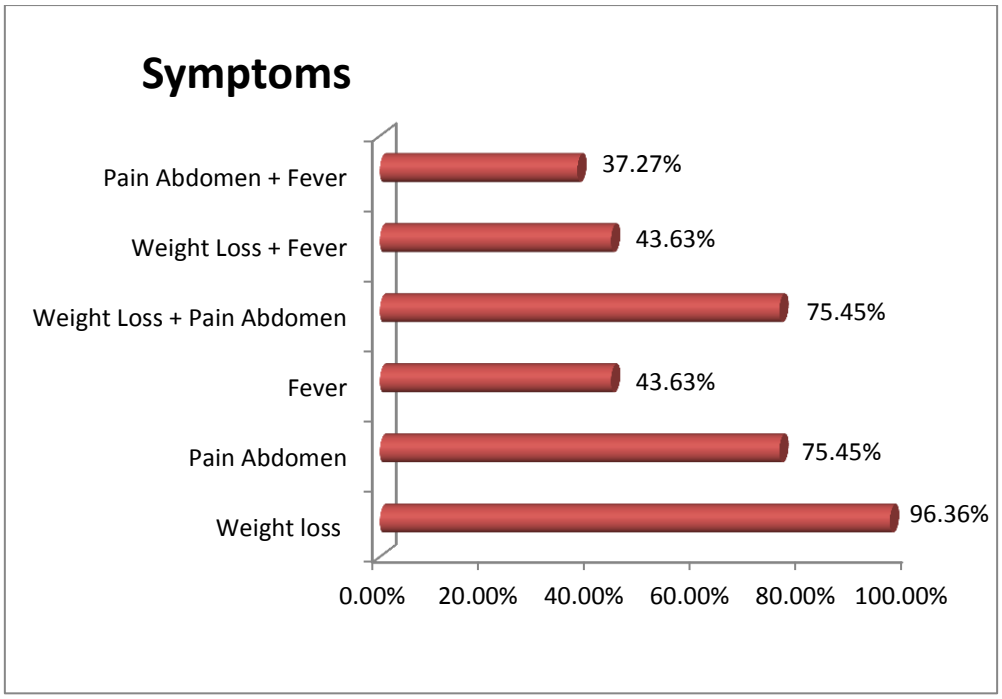


FIGURE 24: Symptoms along with Diarrhoea

Pulmonary Tuberculosis was the commonest clinical diagnosis seen in study group (45 cases - 40.90%), of which 24 cases (53.3 %) were positive for Coccidian parasites, followed by the cases of anaemia (26 cases - 23.6%) out of which 14 (53.8%) showed coccidian parasite

TABLE 13: Consistency of stool correlated with presence of parasites

Consistency of stool	No. of Samples n = 110	No. of positive samples	Percentage
Formed	11(10%)	3	27.27 %
Semi - formed	73 (66.4%)	39	53.42 %
Watery	26 (23.6%)	7	26.92 %

Among 110 HIV/AIDS patients with diarrhea studied majority (66.4%) were having Semi-formed stool followed by 23.6% watery and only 10% had formed stool. Detection of parasites was maximum (53.42 %) in semi-formed stool samples.

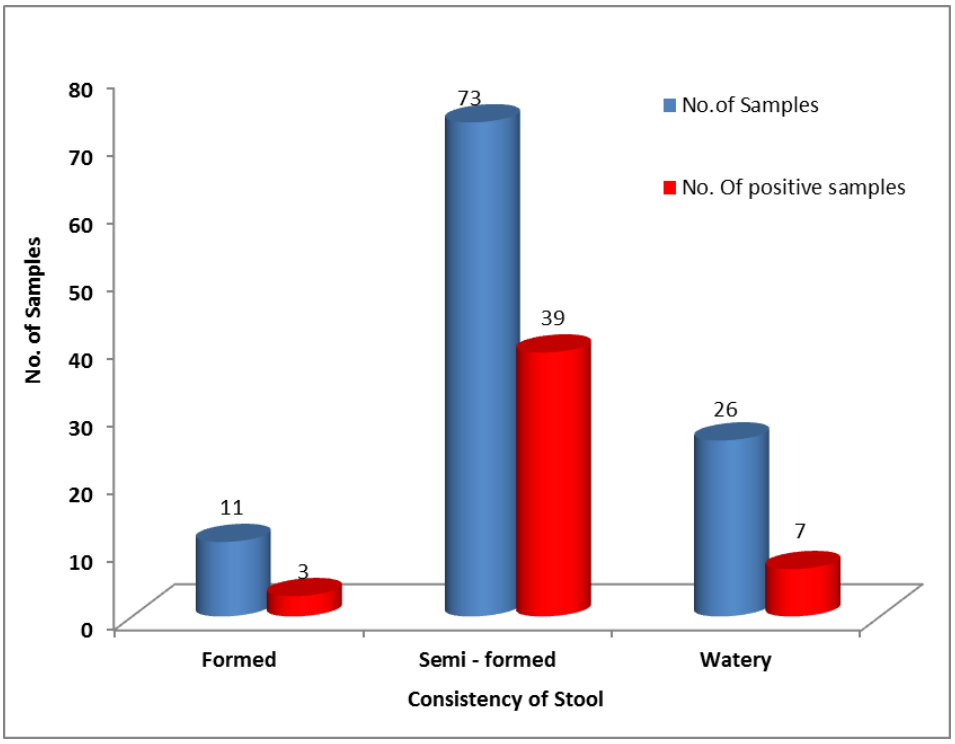


FIGURE 25: Consistency of stool correlated with presence of parasites

TABLE 14: Different parasites identified

Parasites identified	No. of Positive Samples (n=110)	Percentage
Cryptosporidium parvum	36	32.73 %
Cyclospora cayetanensis.	3	2.72 %
Mixed Infection	10	9.09 %
Total Positive Samples	49	44.54 %

Out of total 110 samples, 49 (44.54%) were positive for parasites and 61 were negative for parasites. Maximum among these were Cryptosporidium parvum positive 36 (32.73%) followed by Mixed infection 10 (9.09 %).

TABLE 15: Pattern of Mixed infection in different age groups

Pattern of Mixed Infection	No. of Positive Samples (n=110)	Percentage
Cr. + Cy.	8	7.27 %
Cy. + I	1	0.9 %
Cr. + Cy. + I	1	0.9 %
Total Positive Samples	10	9.09 %

Mixed infection of Cryptosporidium parvum with Cyclospora was seen in maximum samples 8 (7.27%) followed by 1 case each of Cyclospora with Isospora belli and Cryptosporidium parvum with Cyclospora and Isospora belli.

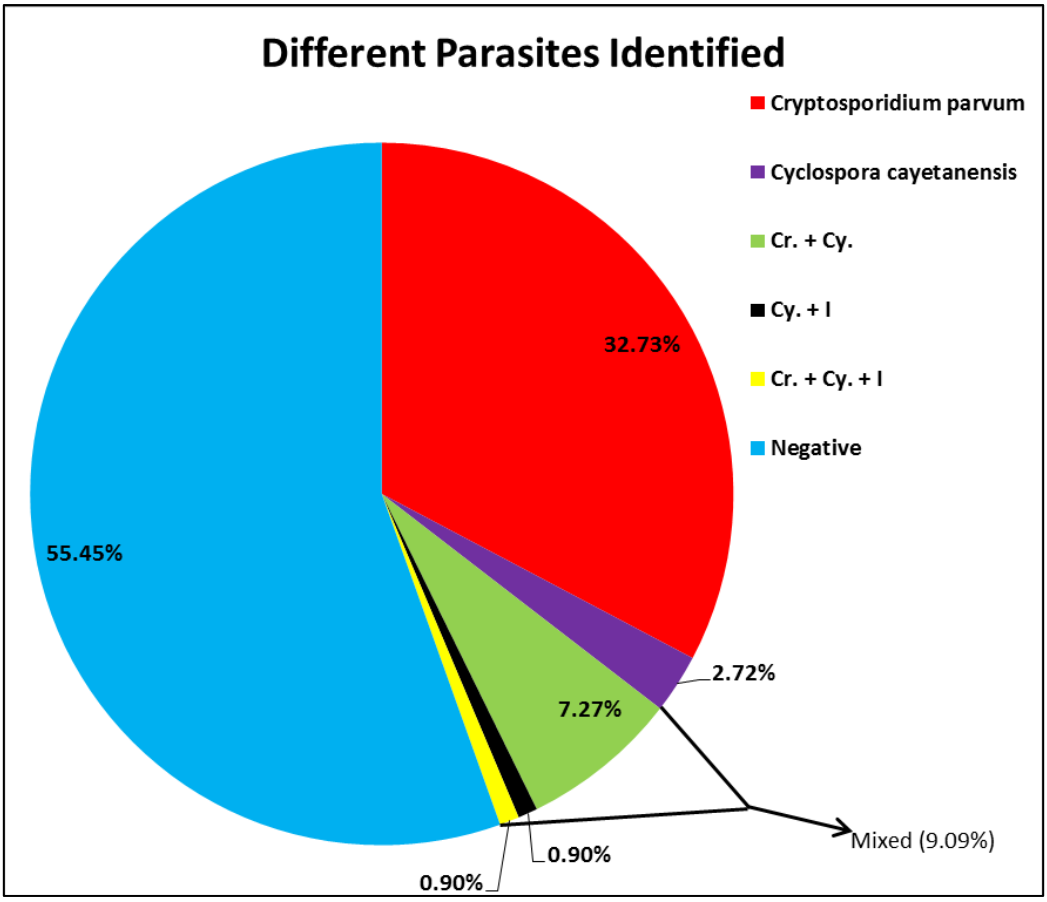


FIGURE 26: Different parasites identified in stool samples

A case of 28 years old female having watery diarrhea since 7 days with history of weight loss, pain in abdomen and CD4 count of 544 cells/ mm3. Stool examination revealed Entamoeba coli cysts with no evidence of other parasites.

TABLE 16: Parasites identified by different methods of examination

Method of identification	Cryptosporidium parvum	Cyclospora cayetanensis	Isospora belli
SWM	-	5	2
IWM	-	5	2
LPCB	-	5	2
MKAFS	45	13	2

MKAFS showed maximum identification o f parasites i.e. Cryptosporidium parvum followed by Cyclospora cayetanensis & Isospora belli. Whereas other methods showed maximum of Cyclospora cayetanensis followed by Isospora belli.

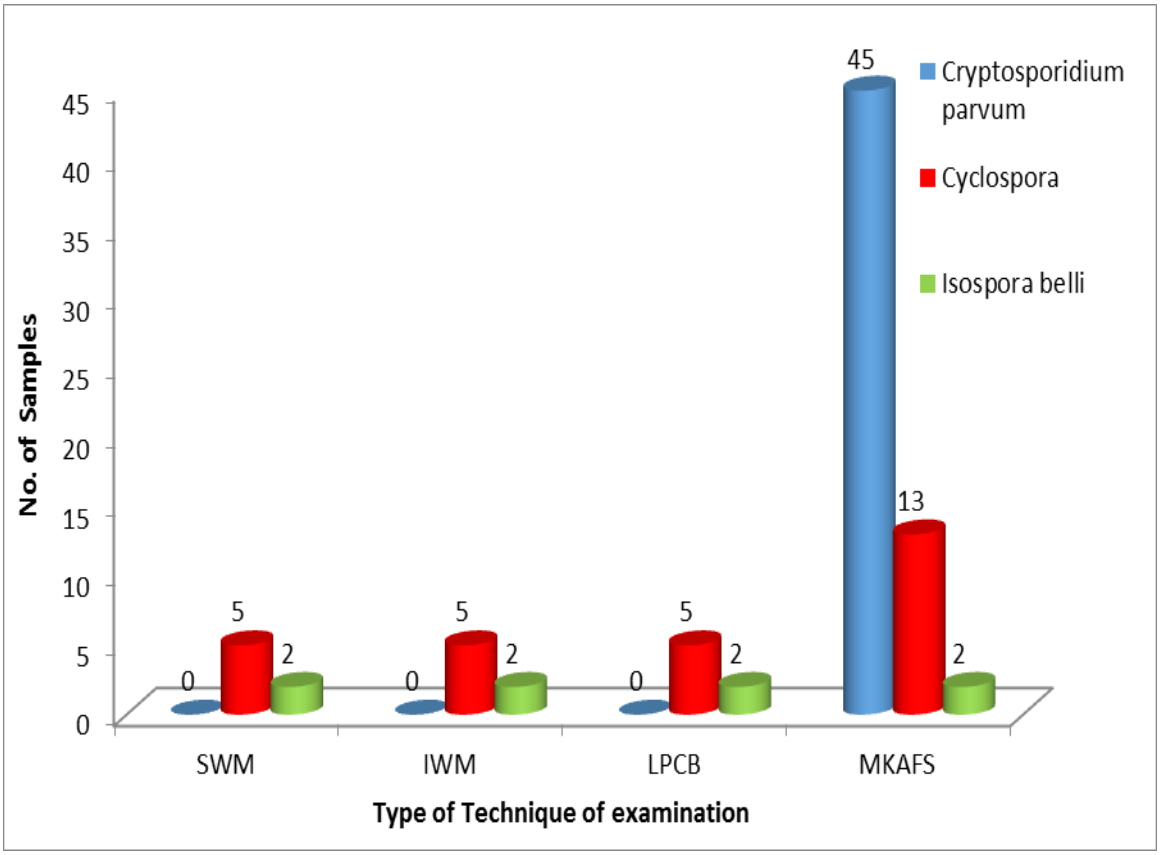


FIGURE 27: Parasites identified by different methods of examination.

TABLE 17: Correlation of CD4 counts and no. of cases positive for parasites.

Parasites		CD4 counts (Cells/mm^3)			
		< 100	100 - 200	200- 500	>500
Cryptosporidium parvum		18	15	3	0
Cyclospora cayetanensis		0	3	0	0
Mixed infection	Cr. + Cy.	5	3	0	0
	Cy. + I	0	1	0	0
	Cr. + Cy. + I	1	0	0	0
Total		24	22	3	0

Based on CD4 counts Cryptosporidium parvum was the commonest parasite seen in 75% of patients with counts <100 cells/mm3 and 68.19% in patients with counts 100 – 200.Whereas none of the cases of coccidian parasitic diarrhoea were observed in patients with CD counts >500.

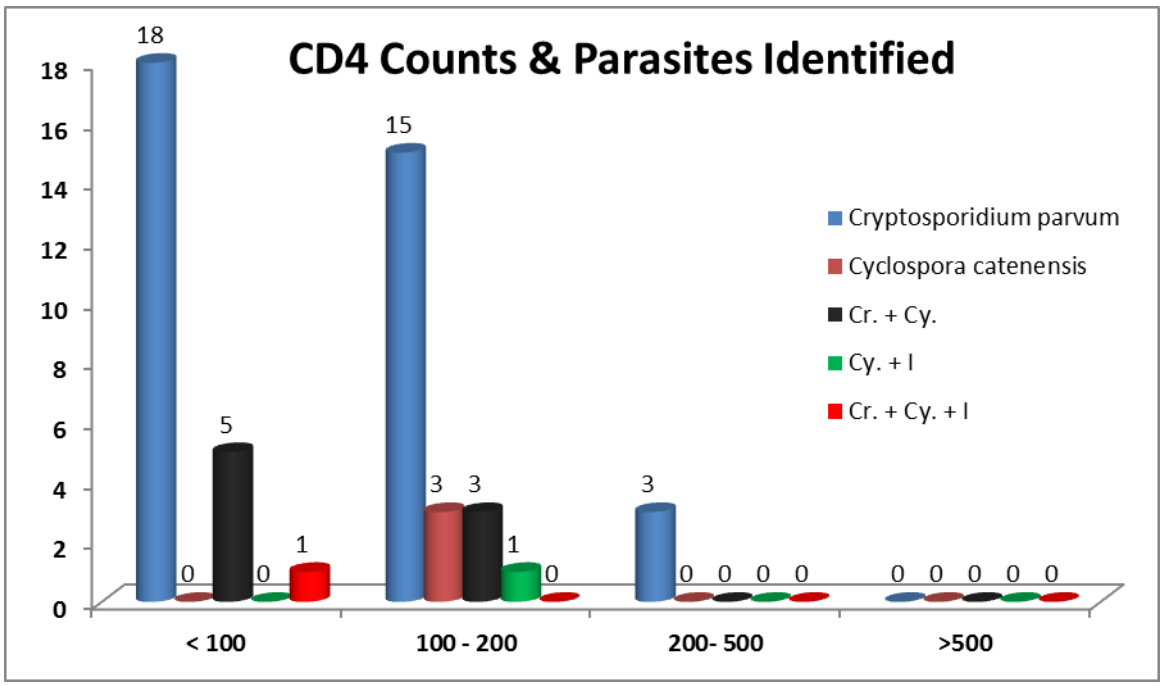


FIGURE 28: CD4 counts and no. of cases positive for parasites

TABLE 18: HAART association with parasite isolation

HAART	Positive for parasites	Negative for parasites	Total (%)
On treatment	43(44.8%)	53(55.2%)	96(87.3%)
Not on treatment	6(42.9%)	8(57.1%)	14(12.7%)
Total	49	61	110

In the present study, 87.3% (96 cases) were on HAART treatment, amongst these 44.8% (43 cases) were positive for enteric parasite. Secondly, 6 cases (42.9%) were detected with parasites who were not on HAART treatment.

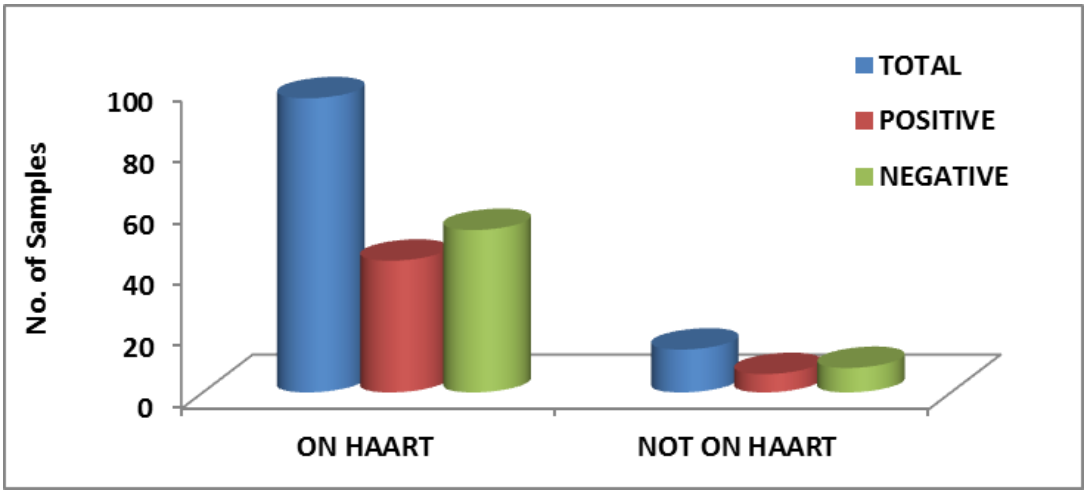


FIGURE 29: HAART association with parasite isolation

DISCUSSION

DISCUSSION

Parasitic infections are among the most widespread of all chronic human infections worldwide. India has the distinction of having the largest number of people living with human immunodeficiency virus (HIV) in the world. Despite the widespread HIV awareness programmes going on at present, a large number of patients either go undiagnosed or present late with multiple infections.

With the emergence of AIDS, parasitic diarrhea has gained significance, as it is one of the important causes of morbidity and mortality. The line of treatment being different for diverse parasites necessitates a definitive diagnosis and study of the etiological agents causing diarrhea, especially when it can be fatal in this vulnerable group of individuals.

Diarrhea is among the most frequent clinical symptoms in HIV-infected patients. It occurs in approximately 50% of patients in North America and in up to 90–100% of patients living in developing countries, resulting in significant morbidity, mortality and reduced quality of life. There are multiple aetiologies for AIDS related diarrhea, among which infectious agents, including bacteria, parasites, mycobacteria and viruses, are predominant. This susceptibility to microbial infections can be related to the course of progression of HIV disease. As the CD4 count decreases, patients become susceptible to an increasing number of opportunistic parasitic infections, especially when the CD4 count decreases < 200 cells/mm^3.

A review of literature shows varying incidence of Cryptosporidium, Isospora & Cyclospora in stool samples from different parts of world. This variation appears to stem from the selection of cases, the number of cases, method employed, seasonal variation and geographical variations. Literature also reveals that there is overall higher incidence of Cryptosporidium reported from developing countries compared to

developed countries.

Present study was done as data on the spectrum of parasites responsible for diarrhoea in HIV-seropositive patients was lacking in our area necessitating the need to identify enteric parasites in HIV-seropositive patients and correlate it with CD4 count. Moreover early detection of causative parasites played a significant role in implementing timely and correct treatment. This was an additional motivational force for the present study.

TABLE 19: Sex Distribution: Comparison studies

STUDIES	MALE (%)	FEMALE (%)
Mohandas et al 2002 [80]	0.64	0.36
Hailemariam et al 2004 [81]	0.538	0.462
Beena et al 2009 [82]	0.58	0.42
Vyas et al 2012 [12]	0.692	0.308
Raytekar et al 2012 [83]	0.559	0.4401
Present Study	0.5545	0.4455

There were 55.45 % of males in comparison to 44.55 % of females with male to female ratio being 1.2:1. This male preponderance in the present study also coincides with the findings of other authors, Mohandas et al 2002 [80] (64% males, 36% females); Hailemariam et al 2004 [81] (53.8% males, 46.2% female), & Beena et al 2009 [82] (58% male & 42% female), Raytekar et al 2012 [83](55.9%Males, 44.01 % Females). Predominance of male cases may be due to their migration to the metropolitan cities in search of work. Staying away from the families for longer periods leading to chances of exposure.

TABLE 20: Age Distribution of Study Group : Comparison studies

STUDIES	AGE GROUP	PERCENTAGE
Hailemariam et al 2004 [81]	25- 44 Years	61.5 %
Beena et al 2009 [82]	21 - 40 Years	78 %
Deorukhkar et al 2011 [84]	21 - 40 Years	66.27 %
Jha et al 2012 [85]	26 - 35 Years	64.94 %
Present Study	21 - 40 Years	60.92 %
	31- 50 Years	68.19 %

In the present study, a total of 110 HIV seropositive patients were studied. Majority of the patients (68.19 %) belonged to the age group of 31- 50 years. Mean age in our study is 37.78 years ± SD 11.13. Comparable age related data has been reported by other authors Hailemariam et al 2004 [81] (61.5%); Beena et al 2009 [82] (78%) and Deorukhkar et al 2011 [84] (66.27 %) in their studies carried out on HIV positive patients. This reflects the increased positivity of HIV/AIDS in the sexually active age group as per the demographic distribution of the disease. Similarly majority of the patients (64.94 %) belonged to the age group of 26 - 35 years with the mean age of 32.36 years was seen in study by Jha et al. [85]

TABLE 21: Age& Sex Distribution: Comparison studies

Studies	Age (years)	Male		Female		Total	
		Number	%	Number	%	Number	%
Hailemariam et al 2004 [81]	**15 - 24**	7	8.90%	6	7.70%	-	-
	25 - 44	27	34.60%	21	26.90%	-	-
	>45	5	6.40%	2	2.60%	-	-
Beena et al 2009 [82]	**21 - 30**	10	20%	6	12%	16	32%
	31 - 40	12	24%	11	22%	23	46%
Raytekar et al 2012 [83]	**21 - 30**	8	6.30%	14	11.02%	22	17.32%
	31 - 40	19	14.96%	20	15.74%	39	30.70%
	41 - 50	14	11.02%	7	5.51%	21	16.53%
Present Study	**21 - 30**	**6**	**5.46%**	**13**	**11.82%**	**19**	**17.28%**
	31 - 40	**27**	**24.55%**	**21**	**19.09%**	**48**	**43.64%**
	41 - 50	**18**	**16.37%**	**9**	**8.18%**	**27**	**24.55%**

In present study there were 55.45 % of males (Mean age - 38.623 ± SD - 11.360, two tailed P Value < 0.0001 - extremely significant) in comparison to 44.55

% of females (Mean age - 36.735 ± SD - 10.864, two tailed P Value < 0.0001 - extremely significant). In our study there is highest prevalence of males in 31 – 40 years age group i.e. 27 cases (24.55%) followed by 41-50 years of age group with 18 cases (16.37%). Of the females studied 31-40 years of age group showed highest prevalence with 21 cases (19.09 %) followed by age group 21 – 30 having 13 cases (11.82%). Overall age group 31 – 40 years accounted for a maximum number of cases i.e. 48 (43.63%).

Comparable data was presented in study by Beena et al 2009 [82] where highest prevalence of males were in age group 31 – 40 i.e. 12 (24%) , followed by 21 – 30 age group i.e. 10 (20%). Of females highest number was in the age group 31- 40 i.e. 11 (22%) followed by age group 21 – 30 years having 6 cases (12%). Overall age group 31 – 40 years accounted for a maximum number of cases 23 (46%). The figures in this study were very much similar to that of our study. Whereas in other study by Raytekar et al 2012 [83]where highest prevalence of males were in age group 31 – 40 i.e. 19 (14.96%) , followed by 41 – 50 age group i.e. 14 (11.02%). Of females highest number was in the age group 31- 40 i.e. 20 (15.74%) followed by age group 21 – 30 years having 14 cases (11.2%). Overall age group 31 – 40 years accounted for a maximum number of cases 39 (30.70%).

TABLE 22: Distribution of type of diarrhoea: Comparison studies

Studies	Acute Diarrhea (+ ve %)	Chronic Diarrhea
Dwivedi et al 2007 [7]	12/23 (52.2%)	24/27 (88.9%)
Tuli et al 2008 [75]	78.50%	50.70%
Vyas et al 2012 [12]	34/100 (34%)	128/166 (77.1%)
Present Study	11/63(17.5%)	38/47 (81%)

In the present study, 47(43%) of the HIV positive patients had a history of chronic diarrhoea and 63(57%) had acute diarrhoea. The percentage of parasitic

identification in our study was 80.9% (38) in chronic cases and 17.5% (11) in acute cases. Thus, there is a statistically significant difference (P<0.0001 by Fisher's exact test) in the detection rate of the parasite with respect to type of diarrhoea.

The present study revealed higher parasitic isolation in chronic diarrhoea cases (81%) as compared to acute diarrhea (17.5%) cases. Similar data was presented in studies by Dwivedi et al 2007 [7] and Vyas et al 2012 [12] where percentages of parasite isolation were higher in chronic diarrhea patients compared to acute diarrhea . While in study by Tuli et al 2008 [75] percentage of parasite isolation was more in acute diarrhea patients than chronic diarrhea. The higher parasitic isolation in chronic diarrhoea cases as compared to acute diarrhea cases in our study may be perhaps due to majority of parasites detected were of the coccidian group which are more frequently encountered in chronic diarrhoea cases.

TYPE OF DIARRHEA AND CD4 COUNT CORRELATION

Diarrhea is a common symptom in HIV-infected subjects. The frequency of diarrhea has been associated with the degree of immunosuppression, with a reported prevalence of 6% in patients with less than 249 CD4 cells/mm^3 and 3.2% in those who had CD4 count >700 cells/mm^3. [86]

In present study 63 cases were of Acute diarrhea having mean CD4 count 300.4 (95% CI Minimum - 257.6 & Maximum - 343.3) and P value 0.036, where as in 47 cases of chronic diarrhea mean CD4 count was 157.5 (95% CI Minimum - 120.4 & Maximum - 194.6) and P value 0.0005 highly significant. Similar finding was reported by Attili et al 2006 [87] in acute diarrhea cases mean CD4 count was 314.9 and in chronic diarrhea cases mean CD4 was 195.5. The higher level of immunosuppression in the chronic cases predisposes them to infection by opportunistic enteric parasites. Also these coccidian parasites are not controlled by

empirical anti-diarrhoeal drugs which are received by most of the chronic diarrhoea cases.

SIGN & SYMPTOMS

In the present study in addition to diarrhoea 106 patients (96.36%) had weight loss as the predominant clinical manifestation followed by pain abdomen in 83 (75.45%) patients and fever in 48 (43.63%). Whereas in study done in North India by Jha et al 2012 [85]- pain abdomen was reported in 61.69% and fever accounted for 19.48% of cases. A Thai study by Manatsathit et al 1996 [88] - weight loss was found in all cases studied whereas pain in abdomen was associated in 54% of the patients. This varied symptomatology of the patients may be perhaps due to association with other opportunistic diseases which are commonly associated with such patients.

HIV-TB co-infection is a serious problem worldwide, but especially of concern in India where background rates of TB are the highest in the world. Prevalence of HIV among patients with radiologic or bacteriologic confirmation of TB in India ranges from 2.8 to 9.4 per cent. In India, the most common opportunistic infection among people with HIV infection is pulmonary TB. [89]

In our study pulmonary tuberculosis was the commonest clinical diagnosis seen among the study group i.e. 45 cases (40.90%). This is in well accordance with Kumarasamy et al 2003[1] where pulmonary tuberculosis (49.3%) was most common disease associated. Also in study by Joshi et al 2002 [76] pulmonary tuberculosis was associated with most (40.4%) of the study group.

Just as HIV infection can contribute to the severity of TB, there is increasing evidence that TB can affect HIV disease progression. Patients with active TB were found to have higher HIV plasma viral loads (PVLs) than asymptomatic patients with HIV and those with Opportunistic infections other than TB. Pro-inflammatory

cytokine production, particularly TNF-α, by tuberculous granulomas is thought to contribute to HIV viral replication. [89]

CONSISTENCY OF STOOL

The physical characteristics of a fresh fecal specimen may aid in determining what types of organisms may be present. Fecal specimens are described as formed (solid) or unformed (semi-formed, or watery).

In our study parasites were identified in 83.34% of unformed (Semi-solid + watery) stool samples and 27.27% of formed stool samples. Thus probability of finding a parasite from unformed stools is approximately three times compared to formed stool samples in this study. Whereas study by Tuli et al 2008 [75] where probability of finding a pathogen from watery and semi formed stools was approximately four times greater as compared to formed stools. Moreover, Saksirisampant et al 2009 [90] reported that the patients with stool of unusual (semi-formed, or watery) consistency were 4.9 fold more likely to have opportunistic parasitic infection with parasitic protozoa. This can be attributed to greater shedding, more inflammatory response and greater virulence of the pathogens causing watery diarrhea. [75] High index of suspicion of opportunistic parasitic infection in semi-formed or watery stool should be observed which will also be helpful for choosing appropriate diagnostic methods.

PARASITES IDENTIFIED

Various studies from India and other countries have reported a high prevalence of intestinal parasites ranging from 15% to 75%. From our study, the overall identification of enteric parasitosis is 44.54%. Nearly similar percentage of detection was seen in Saksirisanpant et al 2009 [90] where overall positive samples were 45.6%. Whereas Prasad et al[49] in 2000 from Lucknow, north India detected enteric

pathogens in 73% of study group. In a study from Madurai, South India by Ramakrishnan et al 2007 [74] parasiteswere detected in 38.7% of cases. Akinbo et al 2010 [91], from Nigeria, reported 15.3% samples as positive for parasites.

TABLE 23: Parasites identified: Comparison studies

Studies	Parasites Identified		
	Cr.	Cy.	Mixed
Mohandas et al 2002 [80]	10.8%	3.3%	-
Tuli et al 2008 [75]	39.8%	24.0 %	25.1%
Gupta et al 2008 [11]	29.2%	4.2%	-
Basak et al 2010 [92]	28.4 %	2 %	2 %
Sucilathangam et al 2011 [93]	36 %	3 %	9 %
Jha et al 2012 [85]	60.42%	2.9%	-
Vyas et al 2012 [12]	25.18%	8.65%	-
Saksirisanpant et al 2009 [90]	34.4%	1.1%	-
Present Study	**36 (32.73 %)**	**3 (2.72 %)**	**10 (9.09 %)**

In our study the maximum detected parasites i.e. 36 (32.73 %) were Cryptosporidium parvum, followed by Cyclospora cayetanensis 3 (2.72 %) and mixed infection 10 (9.09 %). Nearly similar percentage of detection was seen in Saksirisanpant et al 2009 [90] where 34.4% were Cryptosporidium and 1.1% were Cyclospora positive. Jha et al 2012 [85] were having Cyclospora positivity similar to that of our study i.e. 2.9% but Cryptosporidium (60.42%) was much higher compared to our studies. Several studies from India and other parts of the world also have reported Cryptosporidium parvum as the predominant pathogen. [80,75,85,94] Whereas in various studies from different geographical areas other parasitic pathogens like E. histolytica[87,83], Giardia intestinalis [84], helminths [81], Isospora belli [11,77], microsporidium [95] were found in predominance.

In our study a case of 28 years old female having watery diarrhea since 7 days with history of weight loss, pain in abdomen and CD4 count of 544 cells/ mm^3, her stool examination revealed Entamoeba coli cysts with no evidence of other parasites. Entamoeba coli was also seen in studies by Raytekar et al 2010 [83] and Nkenfou et al

2013 [94] but due to uncertainty of its pathogenesis they were not reported.

This discrepancy in the findings may be attributed to geographical variation, differences in population characteristics such as socio-economic status and access to potable water and key climatic variables, particularly humidity and temperatures which always had a relationship to waterborne diseases like cryptosporidiosis[75].

Different screening, staining methods and molecular methods for detection of parasites were used by several studies leading to variation in percentage and pattern of parasites identified. Samples with low numbers of oocysts may get overlooked by conventional acid fast staining techniques. [7] In Tuli et al[75] and Anuradha De et al [96] Microsporidia was also reported along with other Coccidian parasites. Lack of identification of Microsporidia in our study could be because of inability to use stains specific for it due to cost constrains. Further study is needed utilizing these stains along with molecular diagnostic methods.

In addition in this study we noticed a low level of Isospora belli and Cyclospora. As many of the study population might be under Trimethoprim - Sulphamethoxazole prophylaxis. Treatment with trimethoprim Sulphamethoxazole for other infections in AIDS is said to confer some protection against Isospora belli and Cyclospora [77]

Helminths and trophozoites/oocysts of Entamoeba, Giardia in majority of studies conducted previously were reported whereas in our study no isolation of helminths and trophozoites/oocysts of pathogenic protozoa were observed. The reason could not be revealed as most of the included patients belonged to low socioeconomic group and were illiterate leading to improper history of medication. Tuli et al in 2008[75] and Vyas et al in 2012 [12] reasoned that most of the patients with diarrhoea were on empirical anti-helminthic/anti-diarrhoeal treatment. Moreover, in our country

these drugs are freely available across the counter in the drug store even without prescription.[75]

PATTERN OF MIXED INFECTION IN DIFFERENT AGE GROUPS

In our study mixed infection was 9.09% (10 Samples) out of which Cryptosporidium parvum with Cyclospora was seen in maximum samples 8 (7.27%) followed by 1 case each of Cyclospora with Isospora belli and Cryptosporidium parvum with Cyclospora and Isospora belli. In a case report by Chakrabarti et al 2004 [96] mixed infection of Cryptosporidium parvum with Cyclospora and Isospora belli was seen, while Basak et al 2010 [92] 2% of such cases were reported. In a study by Sucilathangam et al 2011[93] mixed infection of Cryptosporidium parvum with Cyclospora was seen in 3 % of samples. Venkatesh et al 2012 [97] also reported co-infection of Cyclospora and Isospora belli in 1 patient.

A large proportion of patients in the study population had diarrhoea for which no etiological agents could be attributed. Variety of unknown/unidentified infections or HAART related toxicities or to a lesser extent malignancies could have contributed to the etiology of these cases. [87] Direct mucosal HIV infection and bacterial overgrowth, probably related to gastric hypoacidity may play a role. In addition to microbes, other factors such as medication, immune deregulation and autonomic dysfunction play a substantial role in diarrhoea of HIV positive patients. [98]

PARASITES IDENTIFIED BY DIFFERENT METHODS OF EXAMINATION

In this study, Saline, Iodine and Lactophenol cotton blue wet mounts were used for ova and cyst identification. Modified Kinyoun's acid-fast staining was done for coccidian parasites. Sensitivity of all the three wet mounts was similar in detection of Isospora. Oocysts were better visualized in iodine and LPCB mounts compared to saline mounts. In general, structures were more discernible with iodine and LPCB

mounts compared to saline mounts. While saline and iodine mounts got dried fast, LPCB mounts could be examined even after a few hours.

While wet mounts could detect 5 (only 38.5%) Cyclospora oocysts, MKAF staining could identify 13 (100%) whereas both methods were equally effective for identification of Isospora oocysts. Moreover all wet mounts were futile in detecting oocysts of Cryptosporidium. Only MKAF staining could detect Cryptosporidium spp. Thus in our study Modified Kinyoun's Acid Fast (MKAF) stain was observed to be the most useful microscopic method to detect opportunistic intestinal coccidian parasites. MKAF was useful to detect C. parvum, I. belli and Cyclospora which is in accordance with Sehgal et al [99] and Garcia et al [100]. LPCB was found to be useful to detect and identify I. belli and Cyclospora, which is similar with the study of Parija et al [101]& Venkatesh et al [97].

Observations made in the present study suggest that the routine use of combination of wet mounts with MKAF staining to be effective in detecting a variety of enteric parasites, in stool specimen of HIV patients. As many HIV-infected asymptomatic patients are known to harbour enteric parasites, it is better to screen all HIV patients periodically for them, especially when CD4 counts are low.

CD4 COUNT CORRELATION

Extent of deterioration of immunity, as measured by CD4 count could predict the presence of coccidian parasites with or without multiple infections. In our study almost all the positive sample patients with a CD4 count which was < 500 cells/µl, were found to have opportunistic coccidian parasitic infections (44.5%). Maximum patients which were positive for parasites had CD4 counts <100 i.e. 24 (50%), followed by CD4 counts 100 - 200 i.e. 22 (44.9%), whereas only 3 (6.1%) have CD4 counts 200 - 500. No parasites were isolated in patients with CD4 counts > 500

cell/mm^3.

In a study by Tuli et al 2008 [75] the maximum parasites isolated was in the group of patients with CD4 cells counts < 200 cells/mm^3. The isolation rate decreased with increase in CD4 count. In study by Vyas et al 2012[12] 72.8% (118/162) patients testing positive for parasites have CD4 T-cell counts <200 cells/mm^3, while 27.1% (44/162) of patients have CD4 T-cell counts in the range between 200-500 cells/mm^3 and more than 500 cells/mm^3, cumulatively. In study by Sucilathangam et al 2011 [93] all the patients (57) with a CD4 count less than 500 cells/mm^3, were found to have opportunistic coccidian parasitic infections (55.3%). In study by Basak et al 2010 [92] correlation of CD4 count with percentage of parasite isolation was [CD4 <100 - (34.4%), 100 -200 (41.3%), 200-500 (17.2%)] similar to that of our study. Raytekar et al 2012 [83] study shows intestinal parasites more commonly in patients with CD4 counts 200-499 cells/mm^3. Overall the rate of isolation of pathogens causing diarrhea in HIV was higher in HIV seropositive individuals with CD4 counts less than 200 cells/μL as compared to HIV seropositive individuals with CD4 counts of 200-500 cells/μL or > 500 cells/μL.

In Present study based on CD4 counts Cryptosporidium parvum was the commonest parasite seen in 50% of patients with counts <100 cells/mm^3 and 41.4 % in patients with counts 100-200 cells/mm^3. Patients with CD4 counts 200-500 cells/mm^3 showed 8.3% Cryptosporidium parvum and no parasite was seen in patients with CD4 counts >500 cells/mm^3. In study by Sucilathangam et al 2011[93] Cryptosporidium parvum (43%) was the predominant pathogen in patients with CD4 T-cell counts which was < 200 cells/mm^3. Raytekar et al [83] from Loni (Maharashtra) , Kulkarni et al [4] from Pune and Vyas et al 2012[12] stated that Cryptosporidium parvum was mainly associated with CD4 counts < 200 cells/mm^3. The reason for this may be

that the immunocompromised patients were either more susceptible to acquire particular parasites and/or were unable to get them cleared once the infection was established.

In our study Cyclospora was identified only in patients with CD4 count 100 - 200 i.e. 3 (2.72%) and mixed infection was seen in 10 (9.09%) patients of which majority 6 (60%) were in patients with CD4 counts <100 and remaining 4 (40%) in patients with CD4 counts 100-200. In study by Sucilathangam et al 2011[93] all identification of Cyclospora and mixed infection was seen in patients with CD4 counts < 200 cells/mm^3. In study by Vyas et al 2012 majority of Cyclospora identified i.e. 16.9% was seen in patients with CD4 count <200 cells/mm^3. In study by Gupta et al 2008 [11] mixed infection were seen in mean CD4 T cell count of 105.3 cells/mm^3.

We have found that the extent of deterioration of immunity, as measured by the CD4 count, could predict the status of diarrhea, i.e., acute or chronic and the presence of coccidian parasites. It has been observed that patients with Cryptosporidium, Cyclospora, or multiple infections who presented with chronic diarrhea had a lower CD4 count. These findings are consistent with other studies. It is relevant to mention here that CD4 T lymphocytes are necessary for the resolution of both acute and chronic Cryptosporidiosis.

The results of experimental infection studies with Cryptosporidiumin mice and calves have shown that immunity is dependent on the number of CD4 T cells. CD4 T cells help in increasing the intraepithelial lymphocyte population and generating gamma interferon. Interleukin-12 may play a role, possibly through its ability to induce gamma interferon production. Antigen-driven interleukin 12 production in macrophages requires interaction between CD40 on antigen presenting cells and CD40 ligands on CD4 T-lymphocytes.[7]

HAART ASSOCIATION WITH PARASITE ISOLATION

The widespread use of highly active antiretroviral therapy (HAART) has resulted in a significant decrease in the incidence of opportunistic enteric pathogens as a consequence of immune recovery. Nonetheless, patients with advanced HIV-1 disease who were recently diagnosed or have poor response to HAART can still suffer from opportunistic infections with pathogens such as Cryptosporidium, Microsporidia, Isospora belli, Cyclospora cayetanensis, Mycobacteriumavium complex, and cytomegalovirus, among others. [86]

In study by Aseefa et al 2013 [102] from Northeast Ethiopia the overall prevalence of intestinal parasites in pre-ART and on-ART was 39% and 17.6%, respectively with significant decrease of intestinal parasite in the ART era ($p < 0.001$). Another study by Akinbo et al [91] from Nigeria showed a low prevalence (5.3%) of intestinal parasitic infections among patients on HAART therapy.

In the present study the group of HIV seropositive patients studied were mostly having the CD4 count or was referred for the same to VCTC centre, Bijapur. Nearly 87.3% (96 cases) of our study group was on HAART treatment. Patients on HAART and positive for enteric parasite were 44.8% (43 cases) whereas on HAART with no enteric parasite detected were 55.2% i.e. 53 cases. Out of 14 (12.7%) patients not on HAART parasites were identified in 6 cases (42.9%) and not identified in 8 cases (57.1%). By Fischer's exact test P value is 1.0000, which is not significant. It has been reported that HIV-infected patients on HAART can still present with coccidian diarrhoea, possibly due to non-compliance with medications, viral resistance to drugs or decreased bioavailability of drugs. There is need to evaluate this further on higher sample size as a prospective/cohort study on the duration of HAART and its effect on enteric opportunistic infection

SUMMARY

SUMMARY

The study was a cross sectional one and was carried out from December 2011 to May 2013 at the Department of Microbiology of BLDEU'S Shri B M Patil medical College, Bijapur. Stool samples from hundred and ten HIV seropositive patients complaining of diarrhoea attending OPD and inpatients at various wards of our hospital regardless of their age and sex were included. Identification of Enteric parasites was carried out using Saline, Iodine, LPCB wet mounts, Modified Kinyoun's acid-fast staining followed by microscopic examination. CD4 cell counts done of HIV infected individuals were used as indicators of immune status to analyse the results obtained.

Following observations were made in our study:

- In the present study, majority of the study population belonged to the age group of 31 – 50 years (68.19%).
- There was a male preponderance accounting for 55.45%, with Male to female ratio 1.2:1.
- Out of 47 patients presenting with chronic diarrhoea, 80.9 % were positive for enteric parasitosis, whereas 63 had acute diarrhoea with parasites identified in 17.5 %.
- Chronic diarrhoea had higher percentage of positivity due to higher level of immunosuppression.
- Parasites were identified in 83.34% of unformed stool samples and 27.27% of formed stool samples. Probability of finding parasites in unformed stool samples were high compared to formed stool.
- 110 samples from HIV seropositive patients with diarrhoea were studied and parasites were identified from 49 samples i.e. 44.54% positivity.

- Cryptosporidium was identified in maximum number of positive cases (32.73%) followed by mixed infection (9.09%) and Cyclospora infection (2.72%).
- Modified Kinyoun's acid-fast staining could identify maximum number of Isospora, Cyclospora and Cryptosporidium. Cryptosporidium were seen only by this method.
- Maximum patients which were positive for parasites had CD4 counts <100 i.e. 24 (50%), followed by CD4 counts 100 - 200 i.e 22 (44.9%), whereas only 3 (6.1%) had CD4 counts 200 - 500. No pathogenic parasites were isolated in patients with CD4 counts > 500 cell/mm^3.
- 87.3% of our study group was on HAART and in maximum (55.2%) cases on treatment no parasite was identified. However further studies correlating HAART and enteric parasitosis is required.

CONCLUSION

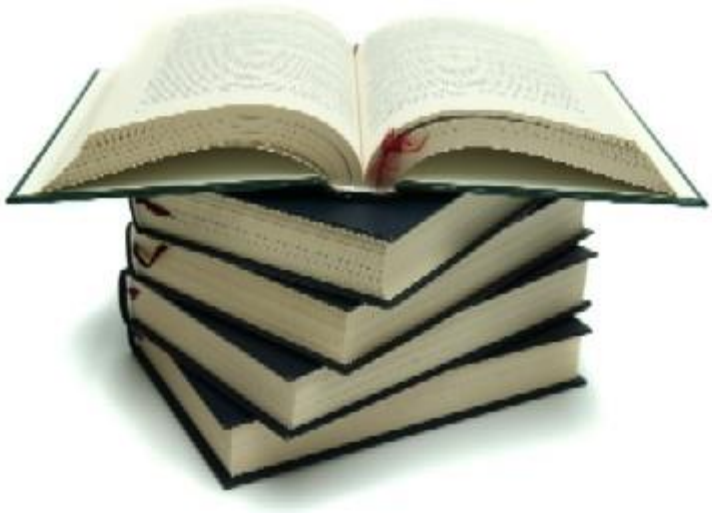
CONCLUSION

Individuals with HIV/AIDS, because of their compromised immune status are at a higher risk of infections. Opportunistic enteric parasites affect the small intestine and produce overwhelming results with grave prognosis. As parasites cause prolonged, life-threatening diarrhoea in AIDS patients, identification of these opportunistic parasites at the earliest will enable the clinician to give effective treatment and save the patient from increasing mortality.

The present study shows that Cryptosporidium is the predominant enteric protozoan parasite in HIV seropositive patients with diarrhoea. The isolation rate was comparatively higher in patients with chronic diarrhoea than in cases of acute diarrhoea.

HIV-infected individuals with lower immunity, as indicated by CD4 counts, suffered more with enteric opportunistic infection. Parasites were common in lower immune status. Cryptosporidiumwas detected in a wide range of immune status, but highest rate of infection was seen with CD4 counts < 200 cells/mm^3. Mixed infection of Cryptosporidium, Cyclospora, Isospora were also reported in patients of low immune status with chronic diarrhoea.

In the present study, simple techniques like wet mounts and Modified Kinyoun's acid fast staining could successfully identify a variety of enteric parasites in HIV patients. These methods should be employed as part of routine testing for the detection of intestinal protozoa in HIV seropositive patients. This will guide the clinicians to start an early and appropriate treatment and thus improve the quality of life of such patients. Further studies on effect of HAART on opportunistic enteric parasites need to be done.



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ANNEXURE

ANNEXURES I

SOLLUTION'S AND STAINS

Reagents and stains used for processing stool samples

1. Normal saline (0.85% saline)

Composition

- Sodium Chloride 0.85 gms
- Distilled water 100 ml

Preparation:

Dissolve 0.85 gms of sodium chloride in 100 ml of distilled water.It is sterilized by autoclaving at 121°C for 15 minutes. When cool, it is stored at 4°C.

2. Lugol's iodine

Composition

- Iodine crystals (powdered) : 5 gm
- Potassium iodide : 10 g
- Distilled water : 100 ml

Preparation:

Potassium iodide and iodine crystals are dissolved in distilled water. The potassium iodide solution is saturated with iodine, with some excess crystals left in the bottom of the bottle. This stock solution is stored in a brown bottle at room temperature and in the dark. The shelf life is 24 months. The stock solution remains good as long as an excess of iodine crystals remains in the bottom of the bottle. The working solution is prepared by diluting a portion 1:5 with distilled water for routine use. The working solution is placed into o brown dropper bottle. The working solution should have a strong tea colour and is discarded when the colour lightens (usually within 10 to 14 days).

3. Modified Kinyoun's Acid Fast stain :

A. Concentrated carbolfuchsin

Composition:

- Solution A:
    - Basic fuchsin ; 4 g
    - 95% ethanol : 20 ml
- Solution B:
    - Phenol crystals : 8 g
    - Distilled water : 95 ml

Preparation:

Mix solutions A and B together. Filter and store in amber coloured stoppered bottle at room temperature. This mixture is stable for 1 year.

B. 1% Sulphuric acid

Composition:

- Concentrated sulphuric acid : 1 ml
- Distilled water : 99 ml

Preparation:

Add 1 ml of concentrated sulphuric acid slowly to 99 ml of distilled water. Store this at room temperature. This mixture is stable for 1 year.

C. Methylene blue :

Composition:

- Methylene blue : 0.3 g
- 95% ethanol : 100 ml

Preparation:

Dissolve 0.3 g of methylene blue in 100 ml of 95% ethanol. Store this at room temperature.

D. **Malachite green**

Composition:

- Malachite green : 400 mg
- Distilled water : 100 ml

Preparation:

Dissolve 400 mg of malachite green in 100 ml of distilled water.

Saturated sugar solution for Sheather's sugar floatation technique

Composition:

- Sucrose : 500 g
- Phenol : 6.5 g
- Distilled water : 320 ml

Preparation:

Mix the sugar in distilled water and add phenol.

Solutions required for Formol ether sedimentation technique:

A. 10% Formol saline

Composition:

- Formalin : 10 ml
- Normal saline : 90 ml

Preparation:

Add 10 ml of formalin (40% v/v solution of formaldehyde in water) to 90 ml of 0.85% of sodium chloride solution.

B. 3 ml of concentrated ethyl acetate solution

ANNEXURES II

PROFORMA

1. NAME : CASE NO:

2. AGE/SEX : IP NO:

3. OCCUPATION : DOA:

DOD:

LAB NO:

4. RESIDENCE :

5. CHIEF COMPLAINTS :

6. PAST HISTORY :

7. CLINICAL EXAMINATION:

A. General Physical Examination

Pallor : Icterus : Pulse : Temperature:

BP:

Level of dehydration: Mild / Moderate / Severe

B. Systemic Examination

CVS :

RS :

PA :

CNS :

C. Local examination :

8. Investigations : Hb %

Complete blood count

Urine analysis

CSF analysis

CD4 count

9. **Stool Examination :**

A) Gross

Consistency : Watery / Semi-formed / Formed

Colour :

Odour :

pH :

Presence of mucus and blood:

Presence of Worms, segments of Tapeworm:

B)Microbiological study :

I. Direct microscopic examination :

a) Saline wet mount :

b) Iodine wet mount :

c) Lacto-phenol Cotton blue :

II. Modified Kinyoun's Acid Fast Staining :

III. Formol Ether sedimentation technique :

IV. Sheather's sugar floatation technique :

ANNEXURE III

MASTER CHART

Sl. No.	IP/OP No.	AGE (years)	SEX	OCCUPATION	CD4 COUNT	Clinical Manifestation				Past History			HIV/AIDS	ART	Clinical Diagnosis	STOOL EXAMINATION									REPORT
						Diarrhoea (Days)	Wt. Loss	Pain Abdomen	Fever	H/o Blood Transfusion	H/O TB	Personal H/O Exposure				Consistency of Stool	SWM		IWM		LPCB		MKAFS		
																	BCT	ACT	BCT	ACT	BCT	ACT	BCT	ACT	
1	24170	26	F	Housewife	124	15	+	+	+	+	–	NO	P	+	ANEMIA	SF	–	–	–	–	–	–	**Cr**	**Cr**	**Cr**
2	23868	60	F	Housewife	92	15	+	+	–	–	+	NO	P	+	P.T.B.	W	–	–	–	–	–	–	**Cr**	**Cr**	**Cr**
3	24348	22	F	Housewife	91	21	+	+	–	–	–	NO	P	+	Ch. D	SF	–	–	–	–	–	–	**Cr**	**Cr**	**Cr**
4	24647	28	F	Housewife	440	4	+	–	+	–	–	NO	P	+	A.G.E.	SF	–	–	–	–	–	–	–	–	**NIL**
5	24616	43	M	Business	96	45	+	–	–	–	+	NO	P	+	P.T.B.	W	–	–	–	–	–	–	**Cr**	**Cr**	**Cr**
6	23704	48	F	Housewife	206	14	+	+	–	–	–	NO	P	+	Pleomorphic Adenoma	SF	–	–	–	–	–	–	–	–	**NIL**
7	24658	37	F	Housewife	311	5	+	+	+	–	–	NO	P	+	Lt. Forearm Cellulitis	SF	–	–	–	–	–	–	–	–	**NIL**
8	5872	50	F	Housewife	504	20	+	–	+	–	–	NO	P	+	P.U.O	SF	–	–	–	–	–	–	–	–	**NIL**
9	26986	38	F	Housewife	92	45	+	+	–	–	–	NO	P	+	Ch. D	SF	**Cy + I**	**Cy + I**	**Cy + I**	**Cy + I**	**Cy + I**	**Cy + I**	**Cr + Cy + I**	**Cr + Cy + I**	**Cr + Cy + I**
10	4739	45	M	Farmer	203	4	+	+	+	+	–	NO	P	+	P.T.B.	SF	–	–	–	–	–	–	–	–	**NIL**
11	6704	35	F	Housewife	213	15	+	–	+	–	+	NO	P	+	P.T.B.	W	–	–	–	–	–	–	**Cr**	**Cr**	**Cr**
12	7685	37	F	Housewife	190	3	+	+	+	–	+	NO	P	+	P.T.B.	SF	–	–	–	–	–	–	**Cr**	**Cr**	**Cr**
13	7845	46	F	Business	137	2	+	+	+	–	+	NO	P	–	A.G.E. + P.T.B	W	–	–	–	–	–	–	**Cr**	**Cr**	**Cr**

14	6384	40	M	Farmer	593	5	–	+	–	–	–	NO	P	–	A.G.E	SF	–	–	–	–	–	–	–	–	**NIL**
15	6389	30	M	Carpenter	247	10	+	+	+	+	–	NO	P	+	A.G.E. + ANEMIA	SF	–	–	–	–	–	–	–	–	**NIL**
16	7811	45	M	Wireman	414	4	+	+	+	+	–	NO	P	+	ANEMIA	F	–	–	–	–	–	–	–	–	**NIL**
17	7625	60	F	Housewife	149	4	+	+	+	–	–	NO	P	+	A.G.E. + Oral Candidiasis	SF	–	–	–	–	–	–	–	–	**NIL**
18	7343	60	F	Housewife	305	4	+	+	+	–	+	NO	P	+	P.T.B.	F	–	–	–	–	–	–	–	–	**NIL**
19	11231	55	M	Artist	259	2	+	–	+	–	–	NO	P	+	Lt. Leg Cellulitis	SF	–	–	–	–	–	–	–	–	**NIL**
20	26525	22	M	Student	494	10	+	+	+	+	–	NO	P	+	ANEMIA	SF	–	–	–	–	–	–	–	–	**NIL**
21	12607	61	M	Business	43	15	+	–	–	+	–	NO	P	+	ANEMIA	SF	–	–	–	–	–	–	**Cr**	**Cr**	**Cr**
22	15171	37	M	Business	254	4	+	–	–	–	+	NO	P	+	P.T.B.	SF	–	–	–	–	–	–	–	–	**NIL**
23	14668	39	M	Driver	169	3	+	+	+	–	+	NO	P	+	P.T.B.	SF	–	–	–	–	–	–	–	–	**NIL**
24	14651	60	M	Farmer	53	7	+	+	–	–	+	NO	P	+	P.T.B.	W	–	–	–	–	–	–	**Cr + Cy**	**Cr + Cy**	**Cr + Cy**
25	12583	41	M	Driver	74	60	+	–	–	+	–	YES	P	–	ANEMIA	SF	–	–	–	–	–	–	**Cr**	**Cr**	**Cr**
26	12633	33	M	Farmer	53	15	+	–	+	+	–	NO	P	+	ANEMIA	SF	–	–	–	–	–	–	**Cr + Cy**	**Cr + Cy**	**Cr + Cy**
27	15639	40	M	Clerk	320	3	+	+	+	+	+	NO	P	+	P.T.B.	F	–	–	–	–	–	–	–	–	**NIL**
28	15613	38	M	Driver	169	30	+	–	–	–	+	NO	P	+	P.T.B.	SF	–	–	–	–	–	–	**Cr + Cy**	**Cr + Cy**	**Cr + Cy**
29	15659	50	M	Farmer	112	21	+	–	+	+	–	NO	P	–	HIV	SF	–	–	–	–	–	–	**Cr**	**Cr**	**Cr**
30	4015	38	F	Housewife	95	14	+	+	–	–	+	NO	P	+	ANEMIA + P.T.B.	W	–	–	–	–	–	–	**Cr**	**Cr**	**Cr**
31	5995	27	F	Housewife	138	4	+	+	–	–	–	NO	P	+	A.G.E	W	–	–	–	–	–	–	–	–	**NIL**
32	4235	42	M	Business	134	5	+	+	+	–	–	NO	P	+	A.G.E	SF	–	–	–	–	–	–	–	–	**NIL**
33	4171	22	F	Housewife	16	21	+	+	–	–	–	NO	P	+	AIDS	SF	–	–	–	–	–	–	**Cr**	**Cr**	**Cr**
34	3025	35	M	Driver	184	4	+	+	–	–	–	NO	P	+	A.G.E.	W	–	–	–	–	–	–	–	–	**NIL**
35	2617	32	M	Business	33	21	+	–	–	–	+	NO	P	+	ANEMIA + P.T.B.	SF	–	–	–	–	–	–	**Cr**	**Cr**	**Cr**
36	2266	55	M	Cobbler	195	3	+	+	+	+	+	NO	P	+	A.G.E. + P.T.B	W	–	–	–	–	–	–	–	–	**NIL**
37	1426	40	M	Farmer	95	21	+	+	–	–	+	NO	P	+	P.T.B.	SF	–	–	–	–	–	–	**Cr**	**Cr**	**Cr**

38	2618	35	M	Driver	85	4	+	+	+	–	–	NO	P	+	A.G.E.	F	–	–	–	–	–	–	–	–	**NIL**
39	1309	30	F	Housewife	17	30	+	+	–	–	+	NO	P	+	ANEMIA + P.T.B.	SF	**Cy**	**Cy**	**Cy**	**Cy**	**Cy**	**Cy**	**Cr + Cy**	**Cr + Cy**	**Cr + Cy**
40	26627	50	M	Farmer	109	2	+	+	+	+	–	NO	P	+	A.G.E.	SF	–	–	–	–	–	–	–	–	**NIL**
41	26504	41	M	Coolie	90	7	+	+	–	–	–	NO	P	+	AIDS	SF	–	–	–	–	–	–	**Cr**	**Cr**	**Cr**
42	26310	40	M	Business	149	4	+	–	+	+	–	NO	P	+	P.T.B.	SF	–	–	–	–	–	–	–	–	**NIL**
43	422	30	M	Mechanic	38	7	+	+	–	–	+	NO	P	+	P.T.B.	SF	–	–	–	–	–	–	**Cr**	**Cr**	**Cr**
44	26987	45	F	Housewife	324	7	+	+	–	–	–	NO	P	+	A.G.E.	W	–	–	–	–	–	–	–	–	**NIL**
45	21813	45	M	Business	271	3	+	+	–	–	–	NO	P	+	ANEMIA	SF	–	–	–	–	–	–	–	–	**NIL**
46	25702	50	F	Housewife	163	60	+	+	–	–	+	YES	P	+	P.T.B.	F	–	–	–	–	–	–	**Cr**	**Cr**	**Cr**
47	227716	28	F	Housewife	303	7	+	+	+	+	–	NO	P	–	ANEMIA	SF	–	–	–	–	–	–	–	–	**NIL**
48	227717	30	F	Housewife	464	4	–	+	+	–	–	NO	P	+	A.G.E.	SF	–	–	–	–	–	–	–	–	**NIL**
49	227715	28	M	Business	595	7	–	+	–	–	–	YES	P	–	AIDS	SF	–	–	–	–	–	–	–	–	**NIL**
50	18472	9	M	Student	163	14	+	+	–	–	–	NO	P	+	P.E.M	SF	–	–	–	–	–	–	**Cr**	**Cr**	**Cr**
51	18749	40	F	Housewife	257	7	+	+	–	–	+	NO	P	+	P.T.B.	SF	–	–	–	–	–	–	–	–	**NIL**
52	18755	35	F	Housewife	101	27	+	–	–	–	–	NO	P	+	ANEMIA	SF	–	–	–	–	–	–	**Cr + Cy**	**Cr + Cy**	**Cr + Cy**
53	18946	12	F	Student	71	7	+	–	–	+	–	NO	P	–	P.E.M	SF	–	–	–	–	–	–	**Cr**	**Cr**	**Cr**
54	15333	42	M	Labour	58	14	+	+	–	–	+	NO	P	+	P.T.B.	SF	–	–	–	–	–	–	**Cr + Cy**	**Cr + Cy**	**Cr + Cy**
55	14668	38	M	Electrician	218	5	+	+	–	+	–	NO	P	+	ANEMIA	SF	–	–	–	–	–	–	–	–	**NIL**
56	18073	39	M	Business	119	14	+	+	–	–	+	NO	P	+	P.T.B.	SF	–	–	–	–	–	–	**Cr**	**Cr**	**Cr**
57	18055	50	M	Farmer	294	7	+	+	+	–	–	NO	P	+	C.C.F	W	–	–	–	–	–	–	–	–	**NIL**
58	18072	32	F	Housewife	180	45	+	–	–	–	–	NO	P	+	AIDS	SF	**I**	**I**	**I**	**I**	**I**	**I**	**Cy + I**	**Cy + I**	**Cy + I**
59	18786	36	M	Farmer	247	45	+	–	–	+	+	NO	P	+	P.T.B. + Anemia	F	–	–	–	–	–	–	–	–	**NIL**
60	19320	48	M	Carpenter	207	60	+	–	–	–	+	NO	P	+	P.T.B.	SF	–	–	–	–	–	–	**Cr**	**Cr**	**Cr**
61	381	36	M	Driver	436	8	+	+	+	+	–	NO	P	+	ANEMIA	W	–	–	–	–	–	–	–	–	**NIL**
62	30895	32	F	Clerk	493	4	+	+	+	–	–	NO	P	–	A.G.E.	W	–	–	–	–	–	–	–	–	**NIL**
63	1469	6	M	Student	34	30	+	–	–	+	–	NO	P	+	C.C.F + Anemia	SF	–	–	–	–	–	–	**Cr**	**Cr**	**Cr**
64	1660	44	M	Farmer	184	15	+	–	–	–	+	NO	P	+	P.T.B.	SF	–	–	–	–	–	–	–	–	**NIL**

65	1654	50	F	Housewife	52	20	+	+	—	+	—	NO	P	+	ANEMIA	SF	—	—	—	—	—	—	**Cr**	**Cr**	**Cr**
66	30832	40	M	Driver	206	30	+	—	—	—	+	NO	P	+	P.T.B.	SF	—	—	—	—	—	—	—	—	**NIL**
67	1715	44	M	Business	436	4	+	+	+	—	+	NO	P	+	P.T.B.	W	—	—	—	—	—	—	—	—	**NIL**
68	1880	28	M	Business	540	5	+	+	+	—	—	NO	P	+	A.G.E.	W	—	—	—	—	—	—	—	—	**NIL**
69	30424	45	M	Farmer	182	8	+	—	—	+	+	NO	P	+	P.T.B. + Anemia	F	—	—	—	—	—	—	—	—	**NIL**
70	3543	45	F	Farmer	185	5	+	+	+	+	+	NO	P	+	P.T.B. + Anemia	SF	—	—	—	—	—	—	—	—	**NIL**
71	3893	12	M	Student	116	14	+	+	+	—	—	NO	P	+	A.G.E.	SF	**Cy**	**Cy**	**Cy**	**Cy**	**Cy**	**Cy**	**Cy**	**Cy**	**Cy**
72	3989	52	M	Farmer	88	60	+	—	—	—	+	NO	P	+	Ch. D + P.T.B.	SF	—	—	—	—	—	—	**Cr**	**Cr**	**Cr**
73	6026	55	F	Housewife	108	30	+	+	—	+	—	NO	P	+	ANEMIA	SF	—	—	—	—	—	—	**Cy**	**Cy**	**Cy**
74	8664	38	M	Business	580	2	—	+	+	—	—	NO	P	+	A.G.E.	W	—	—	—	—	—	—	—	—	**NIL**
75	9026	40	M	Business	481	14	+	+	—	—	+	NO	P	+	P.T.B.	F	—	—	—	—	—	—	—	—	**NIL**
76	12483	35	M	Driver	116	30	+	+	—	—	—	NO	P	—	Ch. D	SF	—	—	—	—	—	—	**Cr**	**Cr**	**Cr**
77	12478	42	M	Electrician	510	4	+	+	+	—	—	NO	P	+	A.G.E.	SF	—	—	—	—	—	—	—	—	**NIL**
78	18054	40	M	Clerk	110	15	+	+	+	—	—	NO	P	+	Ch. D	SF	**Cy**	**Cy**	**Cy**	**Cy**	**Cy**	**Cy**	**Cy**	**Cy**	**Cy**
79	301	3	M	—	369	4	+	+	+	—	—	NO	P	+	P.E.M	F	—	—	—	—	—	—	—	—	**NIL**
80	401	35	F	Housewife	587	2	+	+	+	—	—	NO	P	—	A.G.E.	SF	—	—	—	—	—	—	—	—	**NIL**
81	408	33	M	Driver	606	15	+	+	—	—	+	NO	P	+	P.T.B.	SF	—	—	—	—	—	—	—	—	**NIL**
82	409	45	F	Housewife	196	30	+	+	—	+	—	NO	P	+	ANEMIA + Ch. D.	SF	—	—	—	—	—	—	**Cr**	**Cr**	**Cr**
83	411	28	F	Housewife	544	7	+	+	—	—	+	NO	P	—	P.T.B.	W	E. coli	E. coli	—	—	—	—	—	—	**NIL**
84	420	31	F	Clerk	659	2	+	+	+	—	—	NO	P	+	A.G.E.	W	—	—	—	—	—	—	—	—	**NIL**
85	441	30	F	Farmer	169	15	+	+	—	+	—	NO	P	+	ANEMIA	SF	—	—	—	—	—	—	**Cr**	**Cr**	**Cr**
86	482	50	M	Farmer	147	30	+	+	—	—	—	NO	P	—	Ch. D	W	—	—	—	—	—	—	**Cr**	**Cr**	**Cr**
87	504	36	F	Housewife	344	7	+	+	+	—	—	NO	P	+	A.G.E.	W	—	—	—	—	—	—	—	—	**NIL**
88	518	35	M	Farmer	40	45	+	+	—	+	+	NO	P	+	P.T.B. + Anemia	SF	**Cy**	**Cy**	**Cy**	**Cy**	**Cy**	**Cy**	**Cr + Cy**	**Cr + Cy**	**Cr + Cy**
89	527	45	M	Farmer	74	20	+	+	—	—	+	NO	P	+	P.T.B.	SF	—	—	—	—	—	—	**Cr**	**Cr**	**Cr**

90	587	12	F	Student	198	15	+	+	+	–	–	NO	P	+	P.E.M + Ch.D	SF	–	–	–	–	–	–	**Cr**	**Cr**	**Cr**
91	540	26	F	Housewife	149	90	+	+	–	–	–	NO	P	+	Ch. D	SF	–	–	–	–	–	–	**Cr + Cy**	**Cr + Cy**	**Cr + Cy**
92	541	35	F	Housewife	302	2	+	+	+	–	–	NO	P	+	A.G.E.	W	–	–	–	–	–	–	–	–	**NIL**
93	633	39	M	Driver	173	45	+	–	–	–	+	NO	P	+	P.T.B.	F	–	–	–	–	–	–	**Cr**	**Cr**	**Cr**
94	666	33	M	Business	373	15	+	–	–	–	+	NO	P	+	P.T.B.	SF	–	–	–	–	–	–	–	–	**NIL**
95	681	39	M	Business	257	30	+	+	–	+	+	NO	P	+	P.T.B. + Anemia	SF	–	–	–	–	–	–	**Cr**	**Cr**	**Cr**
96	692	35	F	Housewife	134	45	+	+	–	–	–	NO	P	+	Ch. D	F	–	–	–	–	–	–	**Cr**	**Cr**	**Cr**
97	693	26	F	Housewife	371	4	+	+	+	–	–	NO	P	+	A.G.E.	W	–	–	–	–	–	–	–	–	**NIL**
98	705	40	F	Housewife	512	2	+	+	+	–	–	NO	P	–	A.G.E.	W	–	–	–	–	–	–	–	–	**NIL**
99	708	47	F	Housewife	210	6	+	+	–	+	–	NO	P	+	ANEMIA	SF	–	–	–	–	–	–	–	–	**NIL**
100	1080	35	F	Housewife	84	15	+	+	+	–	+	NO	P	+	P.T.B.	SF	–	–	–	–	–	–	**Cr**	**Cr**	**Cr**
101	1140	38	F	Housewife	37	40	+	+	–	–	+	NO	P	+	P.T.B.	SF	–	–	–	–	–	–	**Cr**	**Cr**	**Cr**
102	1180	40	F	Housewife	225	4	+	+	+	+	–	NO	P	+	ANEMIA	W	–	–	–	–	–	–	–	–	**NIL**
103	1211	40	M	Mechanic	192	2	+	+	+	–	+	NO	P	+	P.T.B.	W	–	–	–	–	–	–	–	–	**NIL**
104	1340	40	F	Housewife	460	10	+	–	–	–	+	NO	P	+	P.T.B.	SF	–	–	–	–	–	–	–	–	**NIL**
105	1850	38	M	Farmer	483	20	+	+	–	–	–	NO	P	+	Ch. D	SF	–	–	–	–	–	–	–	–	**NIL**
106	1888	38	F	Farmer	210	24	+	+	–	–	–	NO	P	+	Ch. D	SF	–	–	–	–	–	–	–	–	**NIL**
107	1906	30	F	Housewife	540	2	+	+	+	–	–	NO	P	+	A.G.E.	SF	–	–	–	–	–	–	–	–	**NIL**
108	2202	35	F	Housewife	336	45	+	–	–	–	+	NO	P	+	P.T.B.	SF	–	–	–	–	–	–	–	–	**NIL**
109	2407	30	M	Business	555	5	+	+	+	–	–	NO	P	–	A.G.E.	W	–	–	–	–	–	–	–	–	**NIL**
110	4448	55	M	Business	117	30	+	+	–	–	+	NO	P	+	P.T.B.	SF	–	–	–	–	–	–	**Cr**	**Cr**	**Cr**

ANNEXURE IV

KEY TO MASTER CHART

A.G.E. : Acute Gastroenteritis

ACT : After concentration Technique

AIDS : Acquired Immunodeficiency Syndrome

BCT : Before concentration Technique

C. C. F. : Congestive Cardiac Failure

Ch. D. : Chronic Diarrhoea

Cr : Cryptosporidium parvum

Cy : Cyclospora cayetanensis

E. coli : Entamoeba coli

F : Formed

H/O TB : History of Tuberculosis

HIV : Human Immunodeficiency Virus

I : Isospora belli

IP/OP No. : In Patient / Out Patient Number

IWM : Iodine Wet Mount

LPCB : Lacto Phenol Cotton Blue

Lt. : Left

MKAFS : Modified Kinyoun's Acid Fast Stain

P. E. M : Protein Energy Malnutrition

P. T. B. : Pulmonary Tuberculosis

SF : Semi formed

Sl. No. : Serial Number

SWM : Saline Wet Mount

W : Watery

Wt. Loss : Weight Loss