A COMPREHENSIVE STUDY ON CRYPTOSPORIDIOSIS IN HIV/AIDS PATIENTS IN RAICHUR DISTRICT, KARNATAKA

A Dissertation Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

Under the Faculty of Medicine

BLDE (Deemed to be University),Vijayapura, Karnataka.

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December-2018

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ACKNOWLEDGEMENT

I am deeply indebted to Almighty God who has guided me through the whole period of my studies.

I express my deep sense of gratitude and heartfelt thanks to my guide Dr Annapurna G Sajjan, Professor, Dept. of Microbiology, Shri B M Patil medical college for her timely advices, suggestions and corrections that made this study fruitful.

My sincere thanks to my Co-guide Dr Kishore G Bhat, Director Professor & HOD, Department of Microbiology, Maratha Mandal's NGH Institute of Dental Sciences & Research Centre, Belgavi, for his technical and professional guidance during processing of my study samples.

My sincere acknowledgement goes to the entire administration and academic staff of BLDE Deemed to be University, Vijaypura, especially Department of Medical Microbiology who contributed so much to the building of my career.

I express my respect and sincere gratitude to Dr. M S Biradar, Honorable Vice Chancellor BLDE (Deemed to be University), Dr.J G Ambekar, Registrar, Vjayapur, Dr. SP Guggarigowdar, Principal, Dean. Faculty of Medicine. BLDE's Shri B M Patil Medical College, Vjayapur, Dr. Tejeshwini Vallabh Madam, Vice Principal & PHD Committee Chair Person, BLDE's Shri B M Patil Medical College, Vjayapur, Prof Dr. Kusal.K.Das sir Research member in BLDEU, Professor,Dept of Physiology. Dr.P.R.Shahapur , Professor and HOD, Department of Microbiology and Mr. Satish patil, Deputy Registrar BLDE (Deemed to be University),.

I am thankful to Dr Kavita patil Dean cum director, Dr B V Peerapur, Professor and HOD, Department of Microbiology, Dr Venkatesh Naik Associate professor, RIMS, Raichur for their whole hearted support.

I am thankful to Ph.D committee, BLDEU deemed to be University for their valuable suggestions and constant encouragement

I am very much thankful to staff and Heads of Medicine, Pediatrics and ART center RIMS, Raichur for helping me to complete this dissertation.

I also thank Mr. Manohar Pugaji, Microbiogist, Maratha Mandal’s NGH Institute of Dental Sciences & Research Centre, Belgavi for his skilled assistance and co-operation during my study.

I express my special appreciation to the statisticians, Dr Shannawaz and Mr.Ramesh whose guidance contributed significantly to the success. My special thanks are addressed to the RIMS, Raichur for its appreciable policy of promoting education at all levels. I thank Mrs. Vijay Lakshmi and sister Reena. HIV/AIDS counselor of voluntary counseling and testing center for helping in this study.

I thankful Mr.Narsimhalu and Mrs.Reshma, technicians for their assistance. I thankful to all non-teaching staff of microbiology in RIMS, Raichur and Shri B M Patil medical college, Vijaypura. My heartful thanks to my friend and English instructor Mrs Anupama Karaddi to her constant help for completion of the thesis.

I affectionately thanks to my fiancée Ravivarma, my children Master Yuvraj and Master Virat and my mother Sarala kumari, brothers and other family members for their support and constant encouragement during my study.

I am greatly indebted to the study subjects, for the co-operation in spite of pain and suffering with disease, without them this dissertation would not have been possible and lot of other people who have contributed of this work but names do not appear here.

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ABBREVATIONS

AIDS	-	Acquired immunodeficiency syndrome
HIV	-	Human immunodeficiency Virus
MZN	-	Modified Ziehl Neelsen staining
H_2SO_4	-	Sulphuric acid
IFT	-	Immunofluorescence staining technique
FITC	-	Fluorescein iso-thiocyanate
H_2O_2	-	Hydrogen peroxide
PBS	-	Phosphate buffer solution
IgG	-	Immunoglobulin G
IgM	-	Immunoglobulin M
ELISA	-	Enzyme-Linked Immuno Sorbent Assay
TMB	-	Tetramethylbenzidine
PCR	-	Polymerase chain reaction
TAE	-	Tris Acetate EDTA buffer.
EDTA	-	Ethylene diamine tetra acetic acid
DNA	-	Deoxy - ribo nucleic acid
CD_4	-	Cluster of Differentiation
Spp	-	Species
T cell	-	Thymus dependent lymphocyte
IFN	-	Interferon
BD FACS	-	Becton-Dickson facs
PH	-	Le potential Hydrogen
STD	-	Sexually transmitted diseases
ART	-	Antiretroviral therapy
USA	-	United States of America

Dedicated

To

My beloved mother, husband

and children

ABSTRACT

Cryptosporidium parvum is one of the most important enteric opportunistic parasitic infections in immunocompromised patients. *Cryptosporidium parvum* is a coccidian protozoal parasite that inhabits the brush border of enterocytes, damages the epithelial cells and causes diarrhoeal disease. It causes severe and prolonged diarrhoea in HIV sero-positive/AIDS patients with CD_4counts <200 cells/cumm. Early detection of cryptosporidium will enable the clinician in effective management of the disease. Various techniques based on different principles are available for the diagnosis of cryptosporidiosis. There is a need to evaluate these methods for optimal benefit of the patients. There is a high incidence of HIV infection/AIDS in Raichur District, Karnataka. Hence it is pertinent to study the incidence of the opportunistic parasitic diseases like cryptosporidial infection in these individuals. This cross sectional descriptive study was undertaken with the following aims and objectives.

Aims and Objectives

This study was aimed to determine the incidence of cryptosporidial infection in HIV sero- positive/AIDS patients both with diarrhoea as well as without diarrhoea, to evaluate various methods of detection of *C.parvum* and correlate the CD_4 counts with the incidence of cryptosporidiosis.

Materials and methods

Stool samples were collected from 110 HIV positive patients presenting with and without diarrhoea at RIMS, Raichur after obtaining informed consent. Each stool sample was divided into four parts and subjected to modified Ziehl Neelsen staining method, immunofluorescent microscopy, ELISA and PCR.

Results

Out of 110 cases studied, 65 patients presented with diarrhoea and the remaining 45 were without diarrhoea. The major group affected was 31- 40 years with mean age 34.4 years. Male preponderance was seen. Out of 110 patients, 80 (73%) patients had CD_4 count less than 200 cells/cumm. Maximum positivity was detected by ELISA i.e. 95.4% followed by Immunofluorescent Microscopy 92.6%, Modified ZN staining 77.3% and PCR 66.4%.In the present study 65 patients had diarrhoea as the predominant manifestation, followed by diarrhoea and weight loss in 63, weight loss and fever in 50 and fever in 45 patients.

Conclusion

Our study highlights the importance of routine examination of stool samples for cryptosporidium oocysts in all HIV sero-positive /AIDS patients, irrespective of gastrointestinal symptoms. ELISA was found to be the most reliable method for diagnosis.

INTRODUCTION

Cryptosporidiosis due to *Cryptosporidium parvum* is an important zoonotic disease distributed world-wide. The description of *Cryptosporidium parvum* was 1st reported in 1907 in the gastric crypts of laboratory mouse by Edward Tyzzer. This disease is now well documented inhuman beings, especially among immunocompromised individuals[1].

In 1981Michael Gottlieb and his colleagues at Los Angeles reported abroad range of opportunistic enteric parasites responsible for gastrointestinal infections like cryptosporidiosis, in patients with severe immunosuppression[2].*Cryptosporidium parvum* causes rigorous and protracted diarrhoea and is considered as one of the most important enteric opportunistic infection in AIDS[3]. *Cryptosporidium parvum* is a coccidian protozoal parasite that inhabits the brush border of enterocytes, damages the epithelial cells and causes diarrhoeal disease[3].

World health Organization (WHO) in 2004 considered cryptosporidiosis as the most ignored disease mainly in developing countries. This is attributed to the scarcity and non-availability of proper laboratory facilities. Cryptosporidiosis can cause severe mortality in immunocompromised (HIV sero positive/AIDS) and malnourished individuals mainly in countries which are underdeveloped[4].

Cryptosporidiosis occupies fifth position out of twenty four essential food borne parasitic infections. Globally, this parasite is recognized as a significant cause for diarrhoea[5].Morbidity due to cryptosporidiosis has all the time been a significant community health problem within the tropics, although the prevalence and severity might differ depending on the place and time [6]. HIV sero-positive/AIDS patients with CD4 (cluster of differentiation) cells above 200/cumm are tolerant but in patients with profound immuno-suppression, it leads to severe diarrhoea and weight loss. Antiviral

therapy helps to improve immunological condition in HIV/AIDS individuals who are suffering with cryptosporidiosis[7].

Cryptosporidiosis is more frequent among male homosexuals and children in HIV sero-positive/AIDS population. Route of transmission of this infection is commonly by oro-faecal route and also few occurrences are associated through water contamination[8].

Diarrhoealdisease is a recurrent problem in HIV infection. In developed countries frequency of diarrhoea is often 30-60% and around 90% in developing countries. Reports show that chronic diarrhoea in HIVsero-positive/AIDS patients due to cryptosporidiosis often leads to considerably shorter life span as compared to the patients who do not have cryptosporidiosis [9].

In developing countries, 80% of children and 90% adults with HIV infection suffer with diarrhoea, which may occur at any stage of the infection. If diarrhoea persists beyond one month it leads to loss of weight of around 10%,this condition is known as ‘AIDS –defining condition’. Chronic diarrhoea is an independent marker of poor prognosis in patients with the AIDS[10].

HIV sero-positive/AIDS individuals who do not manifest diarrhoea, still harbor cryptosporidium and act as carriers. Immunocompromised hosts show a broad range of infection from carrier state to cholera like illness. Self limited diarrhoea is generally noticed in normal population[11].

The immune condition is a key factor in determining the severity of cryptosporidiosis[12]. Eighty percent of AIDS patients with cryptosporidiosis have CD4 count below 200cells/cumm[13].Different studies have revealed various prevalence rates in varied geographical locations [14]. In India, prevalence rate of cryptosporidiosis is 80% [15].

Due to higher incidence of HIV sero-positivity /AIDS in our area, i.e., Raichur District of Karnataka, it is important to know the opportunistic parasitic diseases like cryptosporidiosis and its correlation with the immune status of the patient.

Early detection of cryptosporidium will enable the clinician in effective management of the disease. Various techniques based on different principles are available for the diagnosis of cryptosporidiosis. There is a need to evaluate these methods for optimal benefit of the patients.

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AIMS ANDOBJECTIVES

- To determine the incidence of cryptosporidiosis in HIV sero-positive/AIDS patients.
- To detect and compare cryptosporidiosis in HIV sero-positive/AIDS patients with diarrhoea and without diarrhoea.
- To evaluate various methods of detection of cryptosporidiosis.
- To correlate the CD4 count with the incidence of cryptosporidiosis.

2.1 HYPOTHESIS

Cryptosporidial infection may be linked with low CD4 counts and impaired immune system of the host and may precipitate fatal infection in HIV individuals.

REVIEW OF LITERATURE

3.1 Historical aspects

Clarke first identified cryptosporidium species in 1895 and described this species as “swarm spores lying upon the gastric epithelium of mice”[1].

Tyzzer was the first person to enlighten about cryptosporidiosis. He recognized the parasite on the gastric glands of experimental mouse. In 1907 he gave detailed explanations and illustrations about this parasite. Prior to the 1st report of human infection, cryptosporidium was described in different types of hosts and each was identified as a separate species. Accordingly, it was classified based on the host species[2].

In 1955, Slavin was the first person to report the cryptosporidium linked with mortality[1]. According to the structural characteristics of oocysts, nineteen different types of cryptosporidial species were found in birds, reptiles, fishes and in mammals in studies conducted between 1961-1986[1]. In 1971 validity of these results was questioned by Vetterling. Later, Tzipori et al., recommended and portrayed that cryptosporidium was only one genus on the basis of origin in broad acceptance of host[1].

In 1971, Panciera et al had done a research on the calves suffering with diarrhoea and identified the cryptosporidium in them. This study motivated the awareness in veterinary persons and lateron lead to much experimental research in animals. Tzipori, Angus and Current et al remarkably reviewed this study and recent articles have expanded the importance of the veterinary research in the field of human medicine[2].

In 1976 the 1st human cryptosporidial case was registered, later on a rare report was published that cryptosporidium can cause short term diarrhoea in immunosuppressed patients, especially the individuals with HIV/AIDS[1].

For a couple of years intermittently additional cases were reported mostly in immunocompromised patients who were suffering with immunological congenital diseases with immunodeficiency or were treated with immunosuppressive therapy (Bouzid et al)[3].

In 1982 cryptosporidialoutbreak had occurred in healthy individuals who were in close contact with infected calves and this created curiosity in medicine (Current et al. and CDC)[4,5]. Globally cryptosporidiosis in both immunodeficent and healthy individuals are associated with acute to chronic clinical manifestations (Ungar, Current)[4,6].

3.2 Taxonomy of cryptosporidium

Classification features:

- Phyla: Apicomplexa - An organ complex that appears as conical structures with the tapered end.
- Class: Sporozoasida - Both asexual and sexual reproduction is seen.
- Subclass: Coccidiasina - The life cycle usually includes “merogony, gametogony and sporogony”.
- Order: Eucoccidiorida - “Merogony or schizogony” is observed.
- Suborer: Eimeriorina - “Macrogaments and microgamonts” develop indepedndently with non-motile zygote.
- Family: Cryptosporidiidae - “Oocysts with 4 naked sporozoites”.
- Genus: Cryptosporidium-intracellular and extra cytoplasmic protozoan parasite.

- This classification was proposed in 1985 by Levine.

Cryptosporidium is a human intestinal spore-forming protozoan. Taxonomically termed 'Cryptosporidia'as a gut pathogen in humans.

3.3 Classification of cryptosporidium

Phyla	: 'Apicomplexa'
Group	: 'Sporozoa'
Sub group	: 'Coccidiae'
Order	: 'Eucoccidiorida'
Suborder	: 'Eimeriorina'
Family	: 'Cryptosporidiae'
Genus	: 'Cryptosporidium'
Species	: '*Cryptosporidium parvum*'.

Some researchers said that 13 species are applicable, in that C.parvum was the main parasiteaffecting mammalsin addition to human species (De Graaf*et al.*)[7].

3.4 Epidemiology and prevalence

*C. parvum*gained importance for the omnipresence, causes infection in many mammals and is highly contagious (Griffiths) [8]. In 1982, cryptosporidial cases were investigated in hundred countries and the impact of infection was maximum in developing countries (Ungar) [9].

Cryptosporidial infection represents as a major communal health problem. Cryptosporidiosis is recognized as 3rd or 4thinfectious disease in developing countries and infectivity levels are in extremes. Seasonal changes differ from one country to the other and incidence can reflect indirectly on events like rain fall and farming (Casemore, 1990)[10].

Children of Gambia suffered with cryptosporidiosis due to seasonal changes like high moisture and heavy rainfall (Adegbola *et al.,* 1994) [11].

Generally animals like calves and sheep are reservoirs for the transmission of the disease through contamination. Casemore described transmission of cryptosporidium by water/food contamination and other modes like nosocomial infection, sexual transmission or traveler's diarrhea [10].

Casemore(1990) stated that severity of the disease was more inimmunocompromised individuals and in children below 5 years of age. According to epidemiological information, the immunodeficent population is at increased risk of infection with cryptosporidiosis[10]. Drinking Water Health Advisory March 2001 conducted study on chemotherapy patients, HIV individuals, two extremes of age(children and old people) and reported high risk of infection in these patients[12, 13, 14].

In 1993, Cryptosporidium created much public attention after a major water borne outbreak in Milwaukee Wisconsin, within two weeks 4,00,000 people were infected, 5,000 identified with cryptosporidiosis and hundred fatalities noted[15].

Reports show that in developed countries 60% of HIV/AIDS individuals suffer with diarrhoea and more than 95% are affected in developing countries. In HIV/AIDS patients diarrhoea is a common clinical symptom[16].Treatment may not be effective and can cause mortality among these patients (Joachim A et al) [17].

3.5 Morphology

Cryptosporidium has six distinct forms of morphology and they include: oocyst, sporozoite, tropozoite, meront, microgamont and macrogamont[18].

Oocyst

These are 2 types:

1. **Unsporulated Oocyst**

It is spherical to oval, measures 4-6um in diameter and surrounded by a thin cyst wall[18].

2. **Sporulated Oocyst**

It is the infective stage of the parasite. It is spherical to oval and measures 4-6um in diameter and surrounded by a thick cyst wall. Each oocyst contains four slender sporozoites[18].The sporozoites are liberated in the intestine of the host and pass through the faeces and cause infection directly, can live for a long time in aquatic environment and has an exceptional ability to spread the infections anywhere because of its ubiquitous character (Tzipori, Griffiths and Mawdsley) [19,20].

Sporozoite

It is thin and crescent shaped measuring 1.5 -1.7um. It has a pointed anterior end and posteriorly contains a spherical nucleus.

Tropozoite

This is a transitional form of the parasite. It is spherical or elliptical and diameter is 2-2.5um. Each trophozoite consists of a large nucleus with or without a conspicuous nucleolus.

Meront

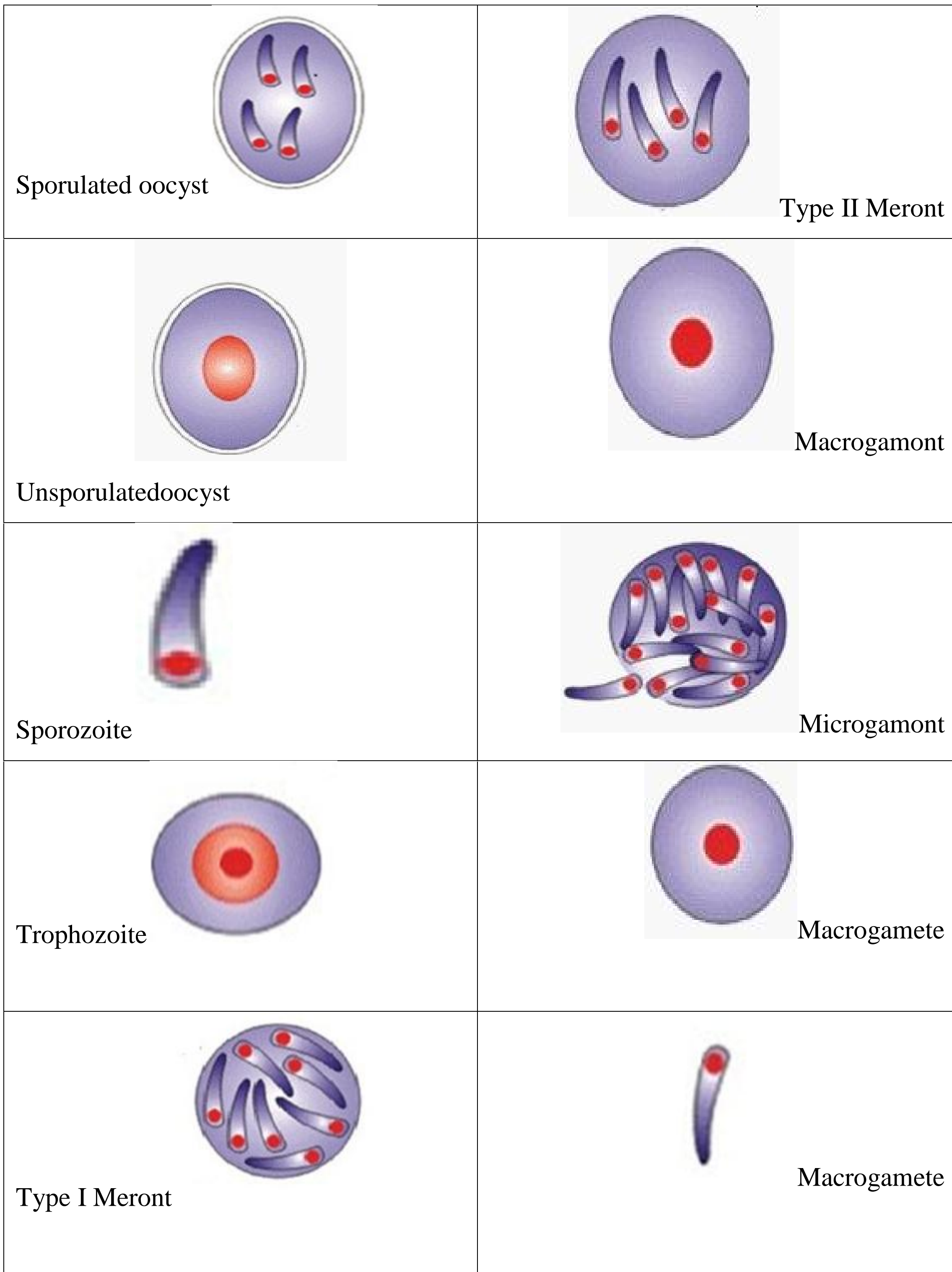
Meront are of two types- type I and type II. They are morphologically indistinguishable from one another. They are crescent shaped and measure about 1-5um in diameter.

Microgamonts

These are male sexual forms. They are wedge shaped, measuring 0.2 - 0.7um and are enclosed by a double-layered membrane.

Macrogamont

Macrogamonts are sexual forms of female. They are round, 3-5um in diameter and are covered by a double layered membrane[18].



Morphological forms of *C.parvum*(Fig. 1)

3.6 Mode of transmission

Cryptosporidial infection is mainly transmitted by contaminated food and water via oro-fecal route or animal to person contact or person to person contact[24]. Even one Oocyst can cause infection. Incubation period is 2 -14 days[15].

Food and water

In United States, 6outbreaks of cryptosporidiosis occurred due to consumption of contaminated drinking water[4]. Sources of waterborne contamination are generally by water surfaces, swimming pools and recreational pools. Other sources of contaminated water are untreated underground or well water[4, 5].

Another main source of transmission is contaminated food or infected person or an asymptomatic carrier with cryptosporidiosis. The children who consumed apple cider contaminated with animal stool in the fair got infected with cryptosporidium at Maine and this report was well documented[4]. Food sources include cool drinks, salads and the food which is not cooked properly. Oocysts do not survive in well cooked food [4].

Cryptosporidium parvum infection in pets is uncommon; however there is a specific relationship between animal and man. Studies conducted in the United States have shown 50% of calves shed cryptosporidial oocysts and infected dairy forms [4].

Person to person

Cryptosporidium parvum spread with high rate of infection mainly in baby-care centers. Care takers also can be infected easily by *C. parvum* and transmit infection to their families[20]. Hospitals also are main place of spread of cryptosporidiosis. There are many reports that infection spreads from patients to medical staff or patient-to-patient. Other people responsible for spread of infection include veterinary staff, livestock handlers and homosexuals (Casemore D.P et al) [21].

3.7 Life cycle

C.parvum completes its life cycle, sexual and asexual phases in one host (monoxenous life cycle).

Infective stage:

Infected host releases the thick-walled oocysts in feaces which are the infective stage of the parasite[22].

Mode of infection:

Man gets infected through ingestion of sporulated oocysts[23]. Excystation is triggered by different factors with reducing condition Co_2, temperature, pancreatic enzymes, bile salts and it occurs in small intestine (Robertson et al., 1993)[24]. Sporozoites are liberated through a split-shaped hole from one end of the oocyst (Fayeret al., 2000)[25].

In the parasitophorus vacuoles, sporozoites transform into trophozoites in the epithelial cells of the intestine. Trophozoite undergoes asexual multiplication (schizogony) in the mucosal cells and produce type I meront. Eight matured merozoites are liberated from the type I meront (merogony). These merozoites invade the neighboring epithelium and repeat schizogony which transform into type II meront and produce four mature merozoites (additional merogony)[26, 27].

Merozoites undergo sexual reproduction (gametogony) and produce female macrogamonts and male microgamonts[23].

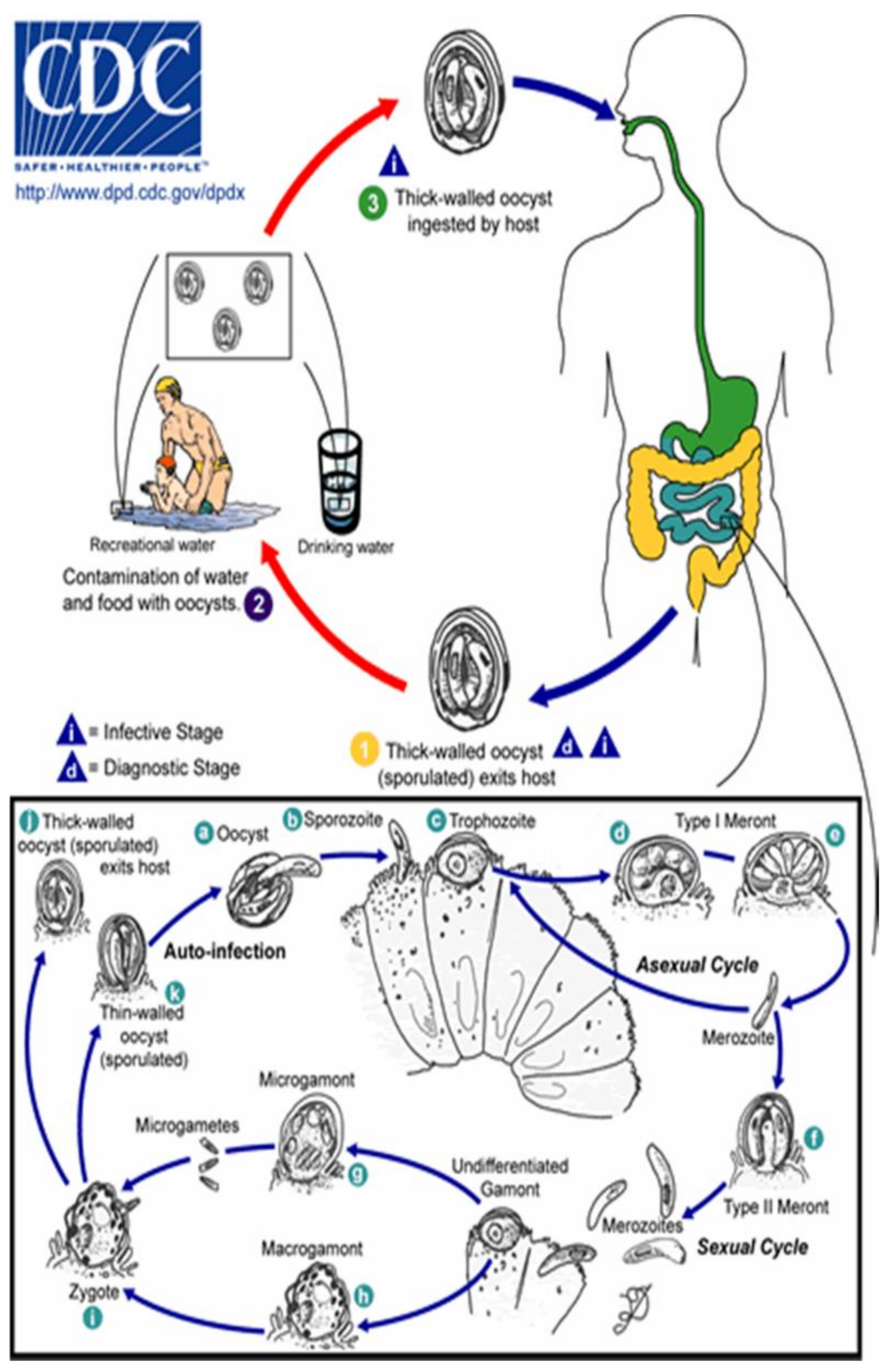
Microgamonts transform into microgametocytes and liberate sixteen mature microgamonts (male). Macrogamonts form macrogametocytes with single nucleus which mature into macrogametes (female).These gametes undergo fertilization, resulting in a diploid zygote which develops into oocyst[28].

These oocysts undergo additional asexual development (sporogony) to develop into sporulated oocyst with four sporozoites. Eighty percent of thick-walled (sporulated) oocysts are released into the faeces and transmit the infection from one person to other or to animals and 20% of thin walled oocysts infect the same host and start a new life cycle, this process is known as autoinfection. The oocysts which are fully mature on liberation infect immediately without further development[29].

Life cycle of the Cryptosporidium in humans involves auto-infective oocysts causing auto-infection. Release of merozoites or type I meront indicates that chronic infections can occur in host without effect of exogenous oocysts [30, 31].

Entire life cycle of the parasite is completed within 48 hours in many hosts and the infection can last for many months and is called prepatent period (Initial one to three weeks of cryptosporidial infection). Patent period (shedding of oocysts) lasts for many days to months and years which indicates the activity of infection[31].

Although many factors can affect the duration of infection, the most important one is the immunocompetency of the host and the cryptosporidium species (O'Donoghue) [23].



Life cycle of *Cryptosporidium parvum* (Fig. 2)

3.8 Virulence factors

Proteases (Excyststation)

Mechanism of cellular damage in cryptosporidium infection remains unknown. But many enzymes cause tissue damage directly, such as phospholipases, proteases and hemolysins.

Proteases play a significant role in the parasitic life cycle, like proteolysis, host-tissue attack and suppression of host immunity (Okhuysen, Nesterenko, Forney 1996)[32,33,34] .

It mediates the degradation of proteins, invades the host cells and decreases the immunity in host. The detection of functional proteases in sporozoite during excystation and prevention of infection by protease inhibitors suggest that proteases are very important in the initial stages of cryptosporidium infection. Distinct protease activities have been identified for cryptosporidium sporozoites: aminopeptidase, cysteine protease and serine protease activities which have been implicated in the excystation process[32].

Adherence factors

The first important step in infection is the adherence of glycoproteins and thrombospondins to epithelial cells of the host (Wanyiri and Ward)[35].

The circumsporozoite-like glycoprotein (1,300 kDa) is attached to the apical portion of sporozoites and merozoites (Riggs &Langer RC).Circum-sporozoite-like (CSL) glycoprotein is a soluble glycoprotein containing a ligand that helps to bind the receptors of intestinal epitheliumofhuman and bovine species[36, 37].And also a zoite ligand is responsible for binding and adhesion (Schaefer and Langer) [38, 39].

This mucin-like glycoprotein is present in micronemes and on the surface of invasive merozoites and sporozoites. It was recognized that glycoprotein 900delays

and mediates the invasion and motility. Some clinical trials have reported that specific antibodies present in the host inhibit thegp900and reduce cryptosporidial infection in vitro [40,41].

Locomotion

Gliding motility allows the sporozoites to migrate across the surface of host cells and activelyinvade them. This form of motility is conserved among different apicomplexan parasites. *C. parvum* sporozoites undergo circular and helical gliding movements, which are actinmyosin-tubulin dependent. For locomotion CPS-500, P23, TRAP-C1& CpMIC1 are responsible virulence factors[42].

Invasion & Membrane integrity

During gliding motility, sporozoites deposit trails of proteins, CpMuc, Cpa135, CpSUB Cp2 & secretory phospholipase which are involved in the attachment and invasion of host cells[43].

Membrane lysis & nutrient transport

Mainly 3 types of Heat shock proteins (HSPs) are detected based on molecular weight by electrophoresis (SDS-PAGE). They are HSP90, HSP70, and HSP65.

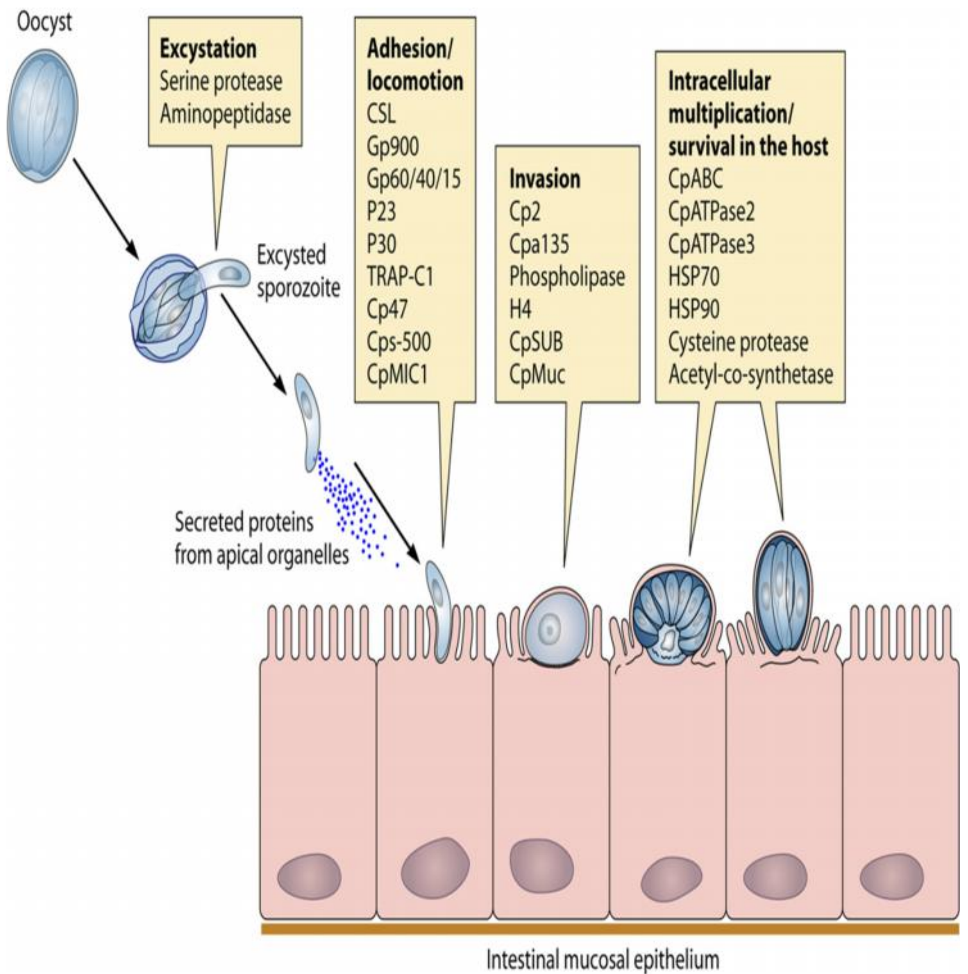
Only HSP90 and HSP70 are demonstrated in the cryptosporidium. HSP90 proteins increase the concentration of the HSPs synthesis and HSP70 increases dramatically in immunosuppressed patients or malnutrition patients (Woods, et al)[44]. The association between HSP expression level and cryptosporidium toxicity require further study. And also Hemolysin H4 & CpABC act as virulence factors for nutrient transport (Miller et al)[45].

Immune/cytokine modulation

Cysteine protease is also responsible for immune/cytokinemodulation[33,34].

Fatty acid metabolism

Acetyl co-synthetase is responsible for intracellular multiplication and fatty acid metabolism in the host[46,47].



Virulence factors(Fig.3)

3.9 Cell Biology

The basis for Cryptosporidium pathogenicity is poorly understood. Diarrhoea is the major symptom associated with cryptosporidiosis and could be due to:

a) Loss of absorptive epithelium (due to apoptosis) and villus atrophy resulting in malabsorption and release of inflammatory cell mediators which stimulate electrolyte secretion and lead to diarrhea[48].

b) Increased intestinal secretion may be partly due to enterotoxin-like activity as a result of endogenous secretory mediators like as prostaglandins which alter NaCl transport by stimulating the enteric nervous system (McCole and Fayer) [49,50].

c) Lean et al described that infectious cytokines are directly involved in the pathogenesis of diarrhoea [51].

d) TNF- stimulates chloride secretion in the intestine via a prostaglandin mediator (Clark and Sears, 1996). Interferon (IFN)- , confers resistance to the parasite, and also plays an important role in secretory diarrhea. Exogenous IFN- inhibits both of the Na/H ion exchanger and Na/K exchanger resulting in increase in intracellular Na concentration which leads to an increase in cell volume, which in turn has been implicated in the decreased expression of a number of transport and barrier proteins [52,53].

This leads to dysfunctional epithelium, manifesting into diarrhoea. Many virulence factors like 15/40/60 kDa glycoprotein (gp60), cryptosporidium oocyst wall protein, thrombospondin-related adhesive proteins, glycoprotein 900 (gp900), heat shock protein of 70 kDa (HSP70), 90 kDa (HSP90),proteases, phospholipases, haemolysin etc have been associated with cryptosporidium pathogenicity in humans and animals[53].

3. 10 Immunological Responses to *C.parvum*

Both ‘cell mediated immunity’ and ‘humoral immunity provide immune response to cryptosporidial infection.

Studies have showed that resistance to cryptosporidium of host increases with increased CD4 cells count in HIV sero-positive individuals compared to those with less CD4 cell counts. It is unclear whether the cryptosporidium infection affects the

helper cells, cytotoxic cells and cells mediated by antibody B (Akinbo, Anyimode, Adamu)[54,55,56].

3.11 Genetics

Nucleic acid analysis played a significant role in construction of the recent molecular, biochemical and taxonomic biology of the parasite. It is difficult to investigate the karyotype of cryptosporidium without chromosomal condensation during cell division. Somatic chromosomes can be identified by gel electrophoresis technique. This technique is used to measure the density of chromosomes and whole genome measures about 10 MB [57,58].

In 1988, Spano F distinguished the two genuses; *Cryptosporidium pavum* and *Cryptosporidium bailey* which are described with different banding patterns. Till date, 30 genes of *Cryptosporidium parvum* have been sequenced and characterized completely[59,60].

Studies have also been done on secondary bifunctional enzyme dihydrofolatereductase(DHFR) and thymidylate synthase(TS) separated from parental DNA by hybridization method [59].

Based on the data sequence two main findings are explained. Different DHFR positions containing new residues can cause antifolate resistance and point mutations that occur in other parasites[59].

Second finding provides the evidence that specialized gene sequences of DHFR-TS can be used to study the genotypic varieties in *Cryptosporidium parvum* strains[58].

An important benefit of genetics is the development of probes for the detection of cryptosporidium [61, 62]. Microsatellites are genetical equipments that are useful for

specific mutations because of the polymorphic nature and *C. parvum* types were differentiated by microsatellite analysis[59].

About 200AT-rich microsatellites are found in DNA of *Cryptosporidium parvum*. Composite mapped genes of *C. parvum* strains helps to recognize the gene of the parasite (Spano and Crisanti 2000)[59].

The main advantage of research on molecular genetics was to evaluate molecular markers on the categorization of cryptosporidium species[63].

Preliminary confirmation on phenotypic difference of *Cryptosporidium parvum* strains was associated with parasitic virulence, drugs investigation by iso-enzyme and western-blot methods[64.65].

3. 12 Mechanism of Pathogenesis

The pathogenic mechanisms by which cryptosporidium spp. causes diarrhoea and malabsorption are poorly understood. The initial host-parasite interactions of attachment, invasion and parasitophorous vacuole formation are complex processes that involve multiple parasite ligands and host receptors[66,67].

Invasive “zoite” stages of apicomplexans possess specialized secretory organelles (rhoptries, micronemes and dense granules) collectively known as the apical complex. The surface and/or apical complex proteins (such as CSL, GP900, p23/27, TRAP C1, GP15, CP15, CP60/15, cp47, gp40/45 and gp15/Cp17) are implicated in mediating these interactions[68].

In *C. parvum* infection, glucose-stimulated sodium absorption was inhibited and this paralleled the extent of villous and epithelial cell damage, increased mucosal prostaglandin production (e.g., PGE2 and PGI2), which can inhibit neutral NaCl absorption and result in secretary diarrhoea. The mechanisms of increased

prostaglandin production are not known. In addition, resident and recruited leukocytes in the mucosa have the potential to produce high levels of prostaglandins[69].

Alterations in the intestinal permeability also play a role in the *C. parvum* diarrhoea. In this context, increased levels of gamma interferon (IFN-) produced during the infection could increase the intestinal permeability and impair epithelial barrier function. Cytokines released, increase the intestinal secretions of sodium and chloride leading to inhibition of water absorption. Epithelial cell damage by parasitic invasion and multiplication leads to T cell-mediated atrophy/cell death. It produces up to 10-20 liters of watery stools per day[70].

3.13 Pathological Physiology

Pathological studies are limited on cryptosporidiosis. Blanshard et al (1992) identified cryptosporidium in 40% of intestinal biopsies and 52 % of rectal biopsies in individuals with AIDS-associated cryptosporidiosis[71].

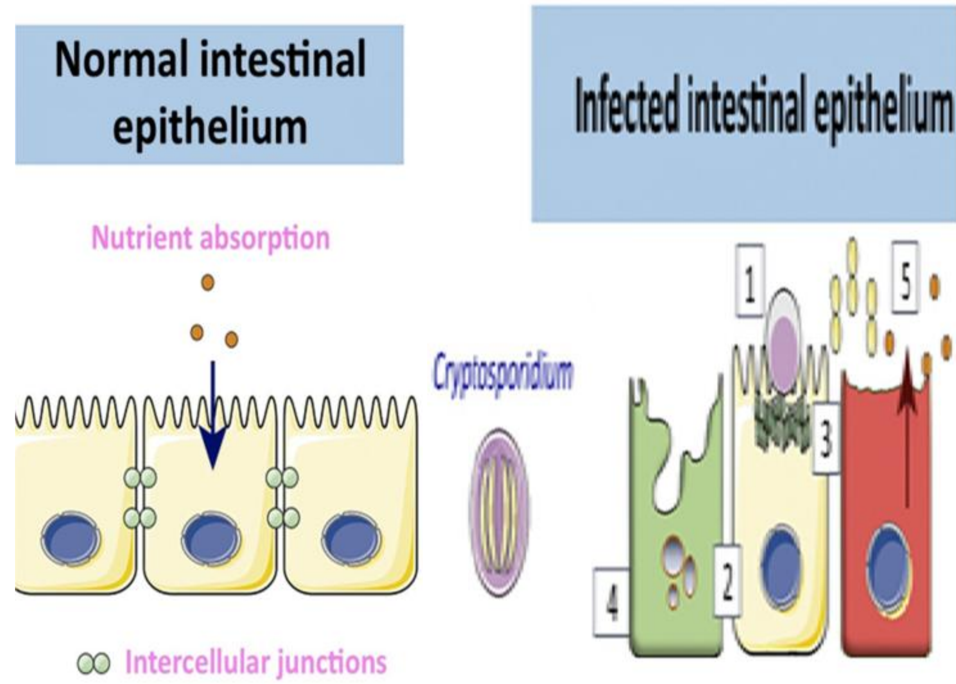
Clayton et al identified the disease in 2 types: Type one infection on the proximal end of small intestine and second type of infection on distal end of the small intestine. In patients with infection on proximal end, disease is very severe compared with second type of infection. Various anatomical parts of the small intestine and also ileo-cecal region were often colonized by the cryptosporidium[72].

3.14 Mucosal abnormalities

In 1976 Meisel explained cryptosporidial infection, in those with severe histopathological disorder, apart from AIDS patients[73].

One of studies in the United States described that the mucous membrane of the patient with cryptosporidial infection was related to more rigorous mucosal intestinal disease compared to those who do not have the pathogen. Study identified that

cryptosporidial infection increases in mitosis process of the cell division leading to villus atrophy (Kotler et al 1990)[74].



Mechanism of pathogenesis (Fig 4)

1. Interaction between Cryptosporidiosis and the apical surface of the epithelial surface results in the localized activation of signaling cascades in barrier disruption.
2. Polymerization of actins filaments.
3. The region of host-parasite interaction.
4. Cell death.
5. Complex interactions influence infectivity and disease outcome.

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3.15 Clinical manifestations

In immunocompetent persons exposure to *C. parvum* may result in asymptomatic carriage. Usually it causes self-limiting watery diarrhoea without blood and spontaneous remission on an average of 10 days. In immunodeficient persons, diarrhoea can be severe, protracted and can often be fatal[75].

Diarrhoea is the main clinical manifestation occurring with cryptosporidial infection in AIDS patients when the CD4 counts below 200cells/cumm[76,77,78]. It may cause acute diarrhoea or chronic diarrhoea lasting for more than a month[79,80]. Cryptosporidium also causes diarrhoea in patients with hemophilia common variable hypogammaglobuliemia[79].

Other manifestations in cryptosporidial infection include nausea, vomiting, weight loss, anorexia, cramping abdominal pain, low grade fever and chronic malabsorption. In immunosuppressed individuals infection in bile duct leads to jaundice and obstruction in biliary tract[80,81].

3.16 Laboratory diagnosis

Laboratory identification plays a significant role in the diagnosis of cryptosporidiosis. It is based on (a) Microscopic demonstration of the parasite (b) Culture methods (c) Immunological tests and (d) Molecular methods (DNA probes & PCR techniques)[82].

Collection of sample

Collection and transportation of the stool sample is important in the diagnosis of *C.parvum*. The stool samples are collected in a wide mouthed container preferably plastic one with tight lid to avoid spillage. Sample should not be contaminated with urine. No antiseptics should be used to wash the container. Every specimen should be labeled properly, with detailed clinical history included on the requisition forms and informed consent.[83].

Transport and preservation of sample

Once the specimen is collected, it should be transported to the laboratory immediately. In case of delay, the sample should be preserved to prevent the specimen from the deteriorating. The preservative are provided in 15 to 30ml plastic

or glass vials, with can be tightly sealed to prevent leakage. It is important that, correct proportions of faeces and fixatives are used and faecal material is mixed well with the preservative[84].

The commonly used preservatives are 5% or 10% formalin, polyvinyl alcohol, schaudinn's fixative and methiolate iodine formalin. Usually 1-2 gram of stool is mixed in 8-10ml of 10% formalin[85]. For routine collection and examination of faecal specimens, either 10% formalin or 5% formal-saline will adequately preserve cryptosporidium cysts[86].

Polyvinyl alcohol has been showed to be an excellent fixative for preservation of morphologic features of the parasite. In PVA and schaudinn's fixative, mercuric chloride is used as a base which is toxic and hazardous for human use and presents difficulties in disposal. Lynne.S.Garcia et al., in their study have tried copper sulphate as an alternative for mercuric chloride base in schaudinn's fixative and found that the recovery and morphology of the parasite was not good compared to mercuric chloride. In another study, zinc sulfate was used to compare it with the mercuric chloride. The study showed minimal differences in terms of organism identification[87].

Safety measures

In the stool samples oocysts remain infective for extended period. Stool specimen should be preserved in 10% buffered formalin or sodium acetate-acetic acid-formalin (SAF) to make oocyst nonviable. In addition, the usual safety measures for handling potentially infectious material should be adopted[88].

Processing of samples

Macroscopic Examination:

The specimen should be examined for consistency, colour, odour and presence of worms, mucous and blood[89].

Microscopic Examination

Direct microscopic examination of stool in saline, iodine and lacto-phenol cotton blue (LPCB).

Wet mounts

The wet mounts are used to detect motile trophozoites, larva, cysts, ova, pus cells, RBCs, bacteria, yeast cells, hyphal elements, fat globules and charcot layden crystals. In light microscopy, oocysts are seen as smooth, colorless, and spherical or slightly ovoid bodies, with size ranging from 3 to 8μm. However these are most often missed as they are unstained[90]

Lacto phenol cotton blue: They are done for the study of nuclear characteristics and glycogen mass[90].

Concentration techniques

Common methods used for the concentration are centrifugation, Sheather's sucrose flotation method, saturated salt flotation, and Allen and Ridley's formal-ether method. Symptomatic cases generally contain large number of oocysts than asymptomatic ones. Modified formal-ether method is commonly used[91].

Staining methods

Modified acid-fast staining method

In 1981, Henriksen and Pohlenz used acid-fast Ziehl–Neelsen (ZN) stain to identify the oocyst, which was modified by Casemore et al, with better results and subsequently, it became the widely used method for oocyst detection. The degree of the stain taken by the individual oocyst varies and can be confused with various structures such as feacal debris, yeast cells and bacterial spores which are also stained red but are comparatively smaller[92, 93].

Cryptosporidium oocysts are acid-fast and resist decolonization with 1% concentrated sulfuric acid. They appear pink-colored, round, about 5µm in diameter and contain 4 sporozoites within the oocyst. The background appears uniformly blue. Either hot or cold staining method can be used. The advantages are low cost, help in viewing more number of specimens and also they are permanent stains. The limitations include time-consumption, requires thorough expertise for interpretation [94].

Immunofluorescence microscopy technique

The rate of detection of cryptosporidium is increased using anti cryptosporidium specific fluorescent antibody staining over acid-fast staining. It is a rapid procedure and seems to be more sensitive than MZN method and is considered as a gold standard in many laboratories. This test is limited because of its expensive and availability of fluorescence microscope[95,96].

Electron microscopy

Electron microscope was used for the confirmation of infection in humans reported in the initial studies. However, its major disadvantage lies in the availability equipment [97].

Serological techniques

Antigen detection methods

Using antibodies labeled with fluorescent reporters

Cryptosporidium oocysts can be detected using monoclonal antibodies (mAbs) against oocyst wall antigen (C-mAbs). These mAbs recognize the epitopes on the surface of oocysts.

The commercially available mAbs are raised against the *C. parvum* but no antibody preparation is available for the specific epitopes on human pathogenic cryptosporidia. Therefore, different species and genotypes of genus cryptosporidium

which vary in the oocyst epitope expressionwill fluorescence less intensely. Hence negative samples should always be confirmed by either conventional methods or polymerase chain reaction (PCR) methods[97].

Using antibodies labeled with enzyme reporters

Antigen detection based on the antibodies labeled with enzyme is commercially available for enzyme immunoassay (EIA) or immunochromatographic (IC) methods. Coproantigen detection ELISA has the advantage of good specificity of 98%–100% and a large number of samples can be processed in a short time[97,98].

Antigen detection by immunochromatographic method(IC)

Immunochromatographic method (IC) assay is another popular assay because of its rapid results. Although the specificity is reported to be high (98%–100%), sensitivity is reported to be low. Agnamey et al. compared four commercial rapid immunochromatographic assays and found that the sensitivity and specificity depends on kits. Average sensitivity was 47.2%, 62.4%, 68.8%, and 70.6% for Crypto-Strip, RIDAQuick, Remel-Xpect, and ImmunoCard STAT, respectively. The specificity was 100% for Remel-Xpect, ImmunoCard STAT, Crypto-Strip, and 98% for RIDAQuick.[97].

Antibody detection methods

Demonstration of antibodies to cryptosporidium-specific antigens in serum, saliva or fecal samples is an indirect method for the diagnosis of infection or exposure. Demonstration of antibodies can merely reflect either recent infection or past exposure to the antigen which is not useful. Hence these tests are useful for sero-epidemiological surveys of the disease.

Serum antibodies are produced to the 27-kDa and 15/17-kDa antigens on the sporozoite surface. There are many formats for antibody detection using native

antigen or recombinant antigen of cryptosporidium. Initially, the ELISA was done using crude *C.parvum* extracts but now the availability of recombinant cryptosporidium antigens has increased its specificity with various recombinant proteins. Studies have reported that antibody to Cp23 correlates with past infection while that to Cp17 indicates recent infection. Specific anti-cryptosporidium IgG and IgM can be detected by ELISA[96]. The only advantage of ELISA is good specificity of 98%–100% and many samples can be tested in short time. Only disadvantage is the high cost of the kits and need for equipments (ELISA reader and plate washer) [98,99].

Histology

Diagnosis can be established by demonstrating cryptosporidium oocysts in intestinal mucosal biopsy. They appear as small, basophilic, spherical structures measuring 3-5µm, arranged in rows or clusters. PCR and immuno-histochemistry may be performed on paraffin-embedded tissue sections. Presently, it is rarely used such as the procedure is invasive and all the regions of intestine are not infected by the parasite. Also, sampling error can occur. Moreover, it is costly, time-consuming and not useful for routine diagnosis[100].

Molecular methods

Polymerase chain reaction (PCR) is a very sensitive method for the detection of cryptosporidium in both clinical and environmental samples. It helps in genotyping and sub typing of cryptosporidium.

Polymerase chain reaction

Earlier conventional and immunological methods were used in the diagnosis of cryptosporidiosis. However these methods are time-consuming, labor intensive, need skilled personnel and are prone to false-positive and negative results.

Molecular methods have changed the diagnostic modalities with the advent of PCR. This method is more sensitive with the detection range from 1 to10 oocysts and has the major advantage of speciation[101].

Restriction fragment length polymorphism (RFLP)

Restriction fragment length polymorphism helps to analyze the PCR products after amplification of genomic DNA. Genotyping, sub-typing, and species identification can be done. However, it is a time-consuming procedure[102].

Real-time polymerase chain reaction

This assay is sensitive, specific; reproducible and also improves turnaround time. It is a real-time technique that detects the DNA using hybridization probes. Expensive equipment is the only limitation of this method [103].

Multiplex real-time polymerase chain reaction

The main disadvantage in PCR-based assays is the difficulty in DNA extraction from the fecal sample and possibility of contamination. However, real-time PCR by fluorescence recognition probes were designed which reduce the possibility of contamination, work period and cost of reagents. Multiplex assay combines different targets into one assay. The only drawback is that technical expertise is needed[104, 105].

Culture methods

In vitro study using human epithelial cell culture, it has been demonstrated that intestinal epithelial cells undergo moderate level of apoptosis by *C. parvum*[106].

3.17 Treatment

There is no confirmed parasitic treatment for cryptosporidial infection although an attempt to treat the infection with numerous parasitic and microbial drugs is made. Initial information, recommended that spiramycin may be efficient, where

1gram 3-4 times daily was tried. In the controlled trial of 54 individuals with HIV sero-positive/ AIDS and cryptosporidiosis, showed no effective signs. Since the serum levels were low, the AIDS clinical trial group was tested with intravenous spiramycin but it was also ineffective. Paramycin an amino glycoside antibiotic has shown activity against cryptosporidium at concentration achievable in the intestinal lumen and has shown clinical improvement in AIDS associated cryptosporidiosis. Paramycin was tried for treatment with the cure rate of 30-70% using dosage of two 250mg tablets 3 or 4 times daily 2 weeks.[107,108].

3.18 Prevention and Control

Preventing the exposure of infection in individuals by safety sanitary measures to avoid soil or water contamination, drinking pure water and improved personal hygiene can help to prevent the infection[109].

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MATERIALS AND METHODS

4.1 Type of Study: The present study undertaken is a cross sectional descriptive study.

4.2 Source of Data:

The study group consisted of inpatients and out patients of Medical, Pediatrics, Skin and STD, ART Centre and other departments of Raichur Institute of Medical Sciences, Raichur.

4.3 Inclusion criteria:

All HIVsero-positive/AIDS patients with and without diarrhoea were included in the study.

4.4 Exclusion criteria: HIV sero-negative patientswere excluded.

4.5 Data Collection Procedure

Ethical consideration

Approval of Institutional Ethical committee of BLDE Deemed to be University, Vijayapur and Raichur Institute of Medical Sciences, Raichur was obtained. Before including in the study, informed consent was obtained from each participant.

A standard questionnaire was prepared to obtain demographic and clinical information of the patient including geographical area, level of education, occupational status, age, sex, socioeconomic status, duration of diarrhoea and CD_4 cell counts.

Specimen collection and Transport

Stool samples were collected from 110 HIV positive patients presenting with and without diarrhoea at RIMS, Raichur after obtaining informed consent. Stool samples were collected in a clean dry sterile plastic container, labeled with

information of the patient and sent for laboratory examination immediately along with requisition form with detailed clinical history.

4.6 Processing of samples

Macroscopic examination:

The stool samples were observed forcolour, odour, PH & consistency. The presence of blood or mucous, live worms or segments of the worms was also noted.

Each sample was divided into 4 portions.

- First portion wasused to prepare the smear and stained by Modified ZiehlNeelsen staining technique (MZN).
- Second portion was used to prepare the smear, fixed with ethanol and stored at room temperature for Immunofluorescent microscopy technique(IFT).
- Third portion was preserved in formalin at room temperature for processing by Enzyme-Linked ImmunoSorbent Assay (ELISA).
- Fourthportion was preserved in ethanol and stored at $-4^{0}C$ for further analysis by Polymerase chain reaction (PCR).

4.7 STUDY PLAN

INFORMED CONSENT

↓

HISTORY TAKING FROM PATIENT

↓

SAMPLE COLLECTION

↓ ↓ ↓ ↓

MZN IFT ELISA PCR

4.8ModifiedZiehl-Neelsenstaining (MZN) [1]

Requirements

Reagents

a) 1% cold carbolfuchsin

Composition

Basic fucshin 1g

Ethanol 20ml

Distilled water 100ml

Preparation

Dissolve basic fucshin in ethanol and add phenol in distilled water to it. Filter and store in amber colored stopper bottle.

b) 3% hydrochloric acid in 95% alcohol i.e. Ethanol

Composition

Concentrated hydrochloric acid 3ml

Absolute alcohol 97ml

Preparation

3ml of concentrated hydrochloric acid is added slowly to a bottle containing 97ml of absolute alcohol.

c) 1% Methylene blue

Composition

Methylene blue 1gm

Distilled water 100ml

Preparation

Dissolve 1gm of methylene blue in 100ml of distilled water.

Materials

Microscope, Glass slides and cedar wood oil.

Procedure

- After heat fixation of the prepared feacal smear, following staining procedure was employed.
- Flood the slide with carbolfuchsin and allow it to act for 10-15 minutes.
- Rinse with tap water.
- Decolorize with 3%-6% suphuric acid by rocking the slide until color stops leaking from smear.
- Rinse with tap water.
- Counter stain with 1% Methylene blue for 30seconds -1minute.
- Dry the smear and examine under the microscope with oil immersion objective.

Observation: Oocysts appear as pink-colored, round, about 5µm in diameter. The background appears uniformly blue.

4.9Immunofluorescent stainingtechnique (IFT) [2]

Requirements

Reagents

- Pre cooled ethanol
- Phosphate buffer solution
- Primary antibody: Goat IgGPolyclonal antibody- FITC antibody - Oocystspurified from bovine feaces(Unconjugated).
- Secondary antibody: Anti-Goat IgG (Whole molecule) - FITC antibody produced in rabbit affinity isolated antibody, buffered aqueous solution.
- 5% H_2O_2
- Glycerol

Materials and equipment

- Fluorescent microscope.
- Microscope slides.
- Jars

Procedure

- The stool sample is smeared on the glass slide and fixed with ethanol.
- The smear is placedin a jar fixed with pre-cooled ethanol for 5 minutes.
- Rinse the slide with PBS.
- Then block the slide with 5% H_2O_2 for 10 minutes.
- Briefly immerse the slide in a jar of PBS for 2minutes.
- Apply diluted primary antibody (1:500 dilution) for 40 minutes on the slide.
- Wash the slide with PBS for 2-3 minutes.
- Then apply diluted secondary antibody (1:400 dilution) for 30 minutes on the slide.
- Again immerse the slide in PBS for 2-3 minutes.

- Observe under fluorescent microscope.
- Preservation in 90% glycerol in PBS with cover slip

Observation: Bright apple green oocysts are round and measure 4 to 5 µm in diameter. Sporozoites aresometimes visible inside the oocysts

4.10 Enzyme-Linked ImmunoSorbent Assay (ELISA) [3]

Requirements

Reagents

- Test strips - Microwells containing anti-cryptosporidium antibodies
- Enzyme conjugate - anti-cryptosporidium antibodies conjugated to horseradish peroxidase with thimersal.
- Positive control - cryptosporidium positive antigen.
- Negative control - buffered protein solution.
- Chromogen - tetramethylbenzidine (TMB) and peroxide.
- Wash buffer - 25ml of concentrated buffer with detergent and thimersal.
- Dilution buffer - buffered protein solution with thimersal.
- Stop solution- 5% phosphoric acid.

Materials

a) Transfer pipette
b) Squeeze bottle for washing strips.
c) Graduated Cylinder
d) Reagent grade (D) water
e) Micropipettes
f) Applicator sticks
g) Simple dilution tubes

Equipment: ELISA plate reader capable of reading biochromatically at 450/620-650nm.

Procedure

- Break the required number of wells needed from the microtitre plate including two control wells (positive and negative) and place in the holder.
- Using the micropipette add 100ul of positive control and negative control into wells.
- Add 50ul of dilution buffer to each sample well (Don't add dilution buffer to control wells).
- Add 50ul of stool sample to each sample well and incubate for 60 minutes at room temperature (15-25^{0}C).
- Then wash and slap the wells to remove excess of buffer.
- Add 2 drops of enzyme conjugate to each well and incubate for 30 minutes at room temperature (15-25^0)
- Then wash and tap the washed plate upside down on an absorbent paper to remove excess of buffer.
- Add 2 drops of chromogen to every microwell and incubate for 10 minutes
- Add two drops of stop solution to each well and gently shake the wells.
- Read reaction results within 5 minutes visually or by ELISA plate reader.

Interpretation

Positive: Absorbance reading of 0.08 OD and above indicates the presence of cryptosporidium antigen.

Negative: Absorbance reading less than 0.08 OD indicates the absence of detectable levels of cryptosporidium antigen.

4.11 Polymerase Chain Reaction (PCR) [4]

Requirements

Reagents:

1. Ampliqon RED (PCR Mastermix):10µl
2. PCR Primers specific for Cryptosporidium parvum -

   SRY (forward) - 0.5 µl & SRY (reverse): total 1µl
3. DNA Template: 2µl
4. Molecular grade water: Added to make final volume to 20 µl.

Reagents used for Detection of amplified products (Agarose gel Electrophoresis)

Agarose

Ethidium bromide

Amplified product

Acetate EDTA buffer : 50times

Tris Base : 12.1gm

Glacial acetic acid : 2.850ml

0.5M EDTA : 5ml

Distilled water : Made upto 50 ml.

Stored at -20°C

Type ofPCR : Conventional type.

Primers: *C. parvum*F 5’- GGTACTGGATAGATAGTGGA-3’

C.parvum R 5’-TCGCACGCCCGGATTCTGTA-3.

These primers amplify a 680 base pair fragment specific to undefined region of *C.parvum.*

Instruments:Water bath, vortexer, centrifugemicropipettes, Eppendorf tubes, .laminar air flow, Thermal cycler, Electrophoresis apparatusand Gel documentation system.

Extraction of DNA[4, 5]

Stool sample is centrifuged at 12000rpm for 5 minutes at 4^0C

500ul lysis buffer is added

The sample is vortexed and centrifuged at 12000rpm for 5 minutes at 4^0C

Then incubated in water bath at 65^0C for 90min

After incubation 20ul of Proteinase K is added

Incubated in water bath at 65^0C for 90min

The sample is vortexed& centrifuged at 12000rpm for 5 minutes at 4^0C

Incubated at -20^0C for 5 minutes

500ul of phenol - chloroform - isoamyl alcohol is added

The suspension is vortexed& centrifuged for 5 minutes at 4^0C

The upper layer is transferred to new micro tubes (PCR tubes)

500ul of chloroform is added

The mixture is again vortexed& centrifuged for 5 minutes at 4^0C

The upper layer is carefully transferred to new micro tubes (PCR tubes)
& 500ul propane is added

The suspension is vortexed& centrifuged for 12000 rpm for 3 minutes at 4^0C

The upper layer is slowly discarded & 500ul of 70% ethanol is added to precipitate DNA

Again it is vortexed& centrifuged at 12000rpm for 3 minutes at 4^0C

Theupper layer is discarded slowly 50ul of buffer is added and stored at -20 ^{0}C, until theamplification.

Procedure[6]:

1. A premixture was prepared and aliquoted into each sample tube.
2. The sample was gently vortexed and spinned down.
3. The sample tube was placed in thermal cycler.

The PCR cycle was as follows

Initial denaturation - 94^0 C, 5min, followed by 35 cycles of

Denaturation - 95^0 C, half minute

Annealing - 56^0 C, half minute

Extension - 72^0 C, half minute

And last extension - 72^0 C for 5 min.

Samples were kept at 4^0 C following PCR.

Detection of amplified products (Agarose gel Electrophoresis)

- Amplified products were subjected to electrophoresis through 2% Agarose gel containing 1 x Tris Acetate EDTA buffer.
- 15 µl of each amplified product were loaded into each well. Electrophoresis was done at 25V for two hours.
- The gel was visualized under UV transilluminator after staining with ethidiumbromide
- The gel picture was captured and analyzed using Gel Documentation System.

Analysis:

- The target region from the DNA of each sample was amplified and will be separated on the agarose gel depending on their size.
- The selection of primer set decided the target region to be amplified and the size of that amplified product.
- The primer set which was selected amplified a 680 base pair fragment which is specific to *C.parvum.*
- The DNA ladder was run simultaneously with each gel to obtain the bands of known sizes and used in locating the band positions of test samples.

Interpretation: Band at position 680 base pair was considered as positive and non-specific bands or no bands were taken as negative.

4.12 CD4 cell count test

The flow cytometer (BD FACS count) was used to measure CD4 cell counts for patients and these reports were obtained from ART centre, RIMS,Raichur.

4.13 Statistical analysis

All characteristics were summarized descriptively. For continuous variables, the summary statistics of mean± standard deviation (SD) were used. Chi-square (^{2}) test was used for association between two categorical variables.

The formula for the chi-square statistic used in the chi square test is:

$$\chi_c^2 = \sum \frac{(O_i - E_i)^2}{E_i}$$

The subscript “c” is the degrees of freedom. “O” is observed value and E is expected value.

C= (number of rows-1)* (number of columns-1)

In cases of more than 30% cell frequency <5, Freeman-HaltonFisher exact test was employed to determine the significance of differences between groups for categorical data. The difference of the means of analysis variables between two independent groups was tested by unpaired t test.

The t statistic to test whether the means are different can be calculated as follows:

$$t = \frac{(\overline{x_1} - \overline{x_2}) - (\mu_1 - \mu_2)}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

where $\bar{x}_1$ = mean of sample 1

$\bar{x}_2$ = mean of sample 2

n_1 = number of subjects in sample 1

n_2 = number of subjects in sample 2

s_1^2 = variance of sample 1 = $\frac{\Sigma(x_1 - \bar{x}_1)^2}{n_1}$

s_2^2 = variance of sample 2 = $\frac{\Sigma(x_2 - \bar{x}_2)^2}{n_2}$

The difference of the means of analysis variables between two time points in same group was tested by paired t test.

Sensitivity- specificity was done to check relative efficiency.

sensitivity or true positive rate (TPR)

eqv. with hit rate, recall

$$TPR = TP/P = TP/(TP + FN)$$

specificity (SPC) or true negative rate

$$SPC = TN/N = TN/(FP + TN)$$

precision or positive predictive value (PPV)

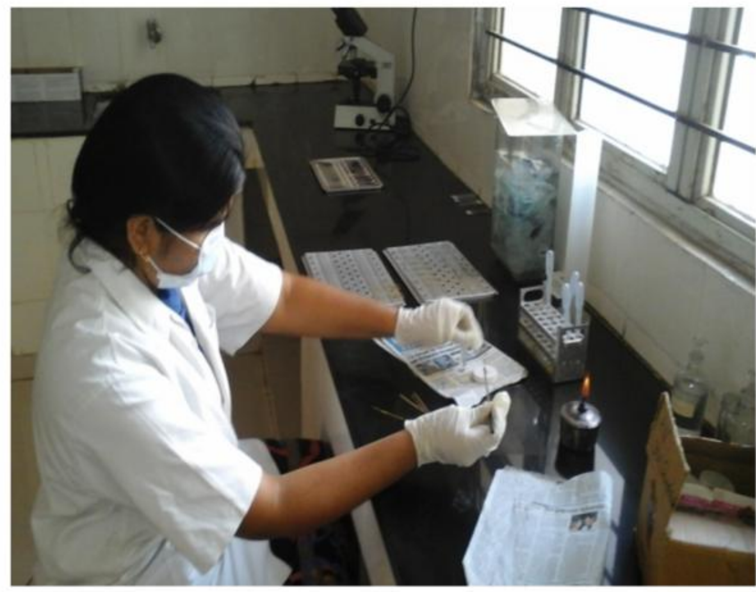
$$PPV = TP/(TP + FP)$$

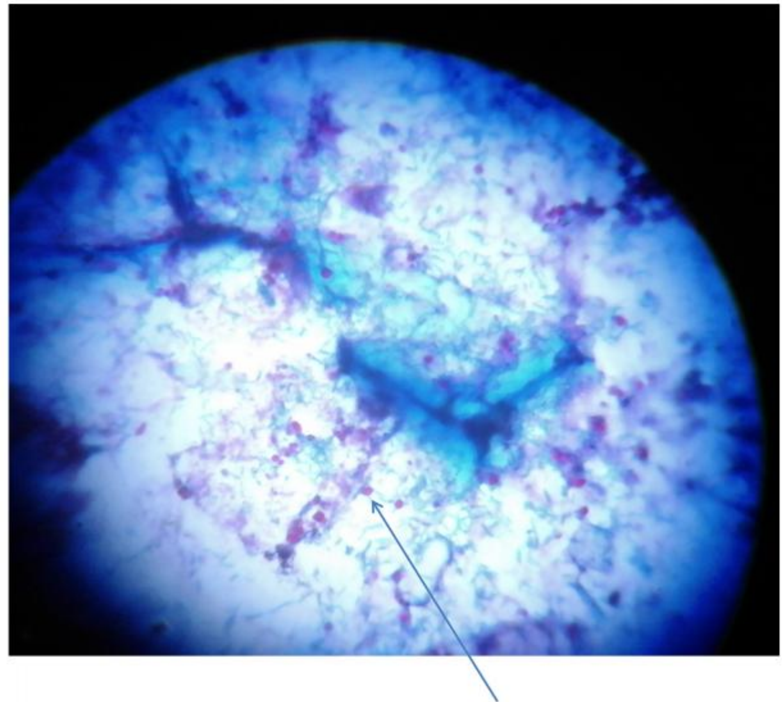
negative predictive value (NPV)

$$NPV = TN/(TN + FN)$$

If the p-value was < 0.05, then the results were considered to be statistically significant otherwise it was considered as not statistically significant. Data were analyzed using SPSS software v.23.0and Microsoft office 2007.

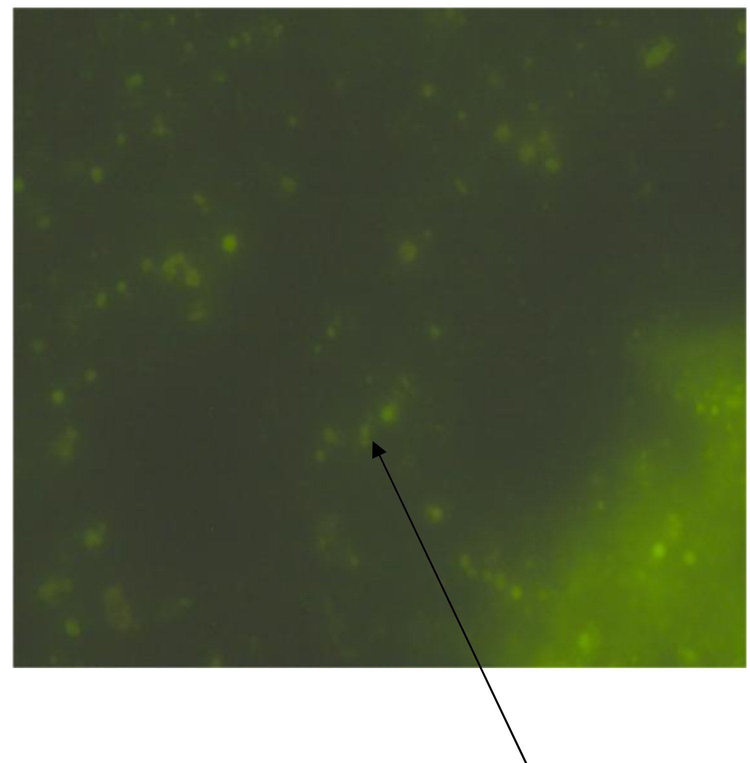
Modified ZiehlNeelsen staining





Oocysts of Cryptosporidium parvum

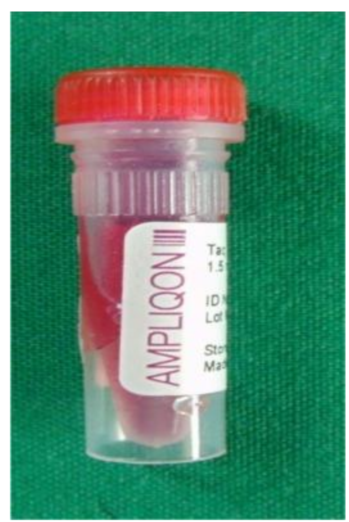
Immunofluorescent Microscopy



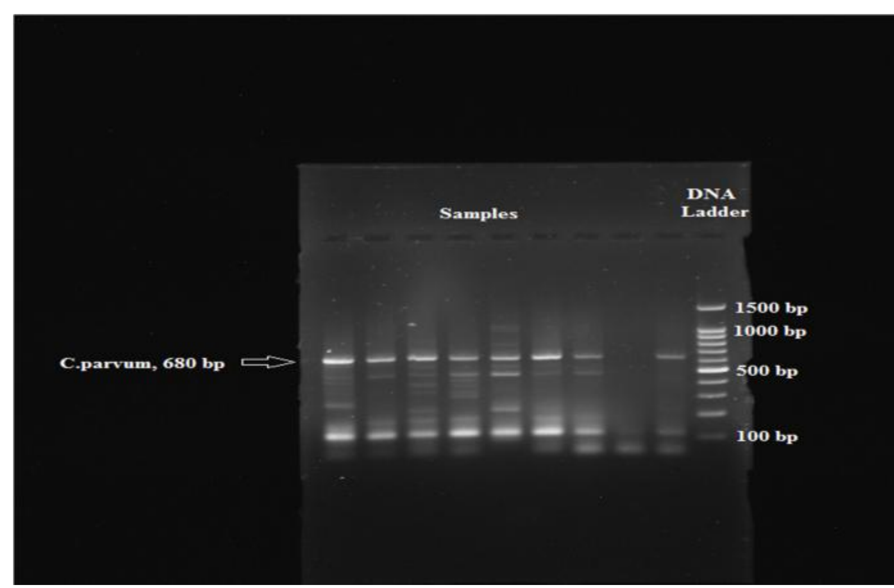
Oocysts of Cryptosporidium parvum

ELISA

Polymerase Chain Reaction (PCR)



Polymerase Chain Reaction (PCR)



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RESULTS

This study was conducted ona total of 110 stool specimens obtained from HIV infected patients admitted to RIMS Hospital, Raichur.

5.1 Study groups

Out of 110 cases studied 65 patients presented with diarrhoea and the remaining 45 were without diarrhoea.

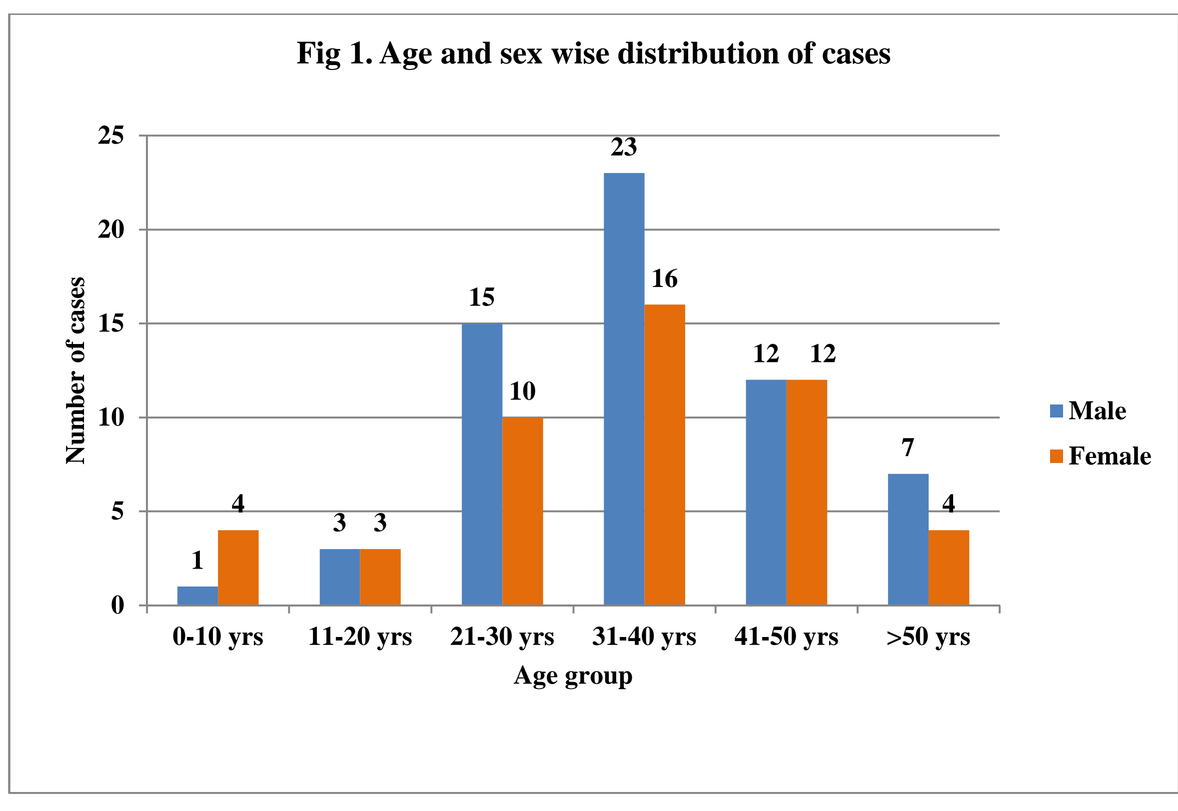
Table 1.Study groups

Study group	Description	No. of individuals
I	HIV seropositive /AIDS patients with diarrhoea	65 (59%)
II	HIV seropositive /AIDS patients without diarrhoea	45 (41%)
Total	-	110 (100%)

Diarrhoeal vs. Non diarrhoeal is 2 = 3, P- value is <0.05 SIG.

5.2Age and sex wise distribution of study individuals

The distribution of the patients in different age groups ranged from 01-60 years.Maximum numberof cases was in the age group of 31-40 years. Least number of cases was found in the age groups 0-10 years & 11-20 years. Mean age of cases in the study is 34.4 years.Amongst the 110 HIVsero-positive /AIDS cases studied, male predominance was observed i.e. 61(55%) and female population wasof 49 (45%).Male to Female ratio was 10:6.The commonest age group affected amongst males was 31-40 yearsand lowest was 0-10years. Amongst the females maximum cases belonged to 31-40 years age group and the lowest group affected was 11-20 years(^{2} =3.1 & P =0.692).



5.3CD4 counts of the 110 study subjects

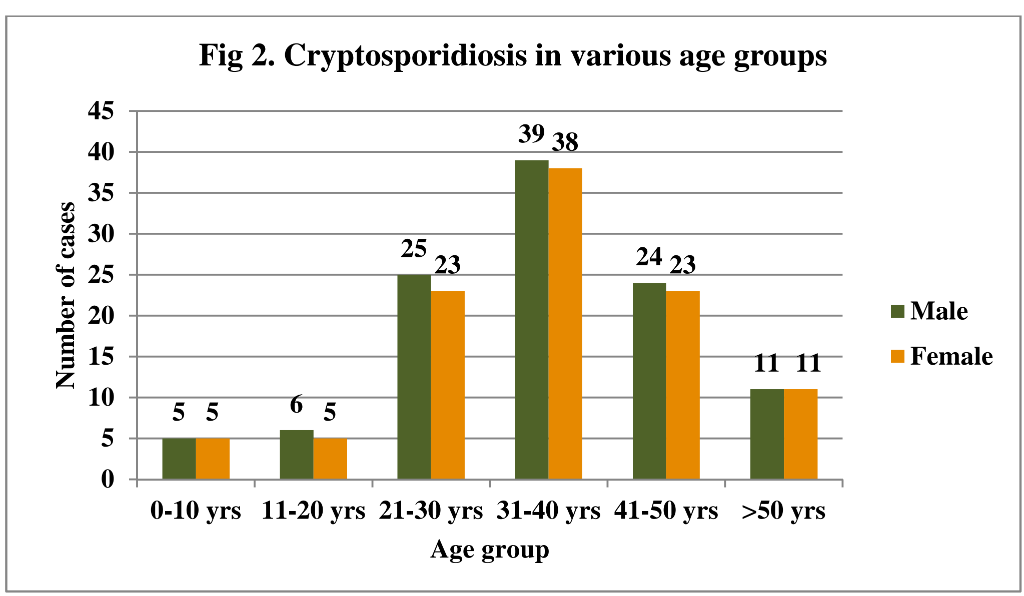
Out of 110 patients, 80 patients (73%) had CD4 count less than 200cells/cumm, 29patients (26%) had CD4 count less than 500 cells/cummand in one patient CD4 count was above 500 cells/cumm.

Table 2.CD4 counts of the 110 study subjects

CD4 counts (cells/ mm^3)	No. of cases(%)
0 – 200	80 (73%)
201 – 500	29 (26%)
>500	01 (1%)
Total	110 (100%)

5.4Cryptosporidiosis in various age groups

The age group with the highest incidence of infection was 0-10 years and above 50years. However, the difference in the incidence of infection in relation to age was not statistically significant (^{2} =5.1 & P =0.395).



5.5Correlation of CD4 count, diarrhoea and cryptosporidial positivity

Maximumcases of cryptosporidiosis had CD4 counts of less than 200 cells/cumm i.e.73%.

CD4 count versus cryptosporidium positivity is significantly correlated inversely with -0.231(P value 0.015 SIG) correlation coefficient.

CD4 count versus diarrhoea is significantly correlated inversely with -0.313(P value 0.001 SIG) correlation coefficient.

Diarrhoea versus cryptosporidium positivity is correlated directly with 0.207 (P value 0.03 SIG) correlation coefficient.

Table 3.Correlation of CD4 Count, Diarrhoea and Cryptosporidial positivity

CD4 counts(cells/cumm)	Diarrhoea	Cryptosporidial Positivity
0 – 200	54	80
201 – 500	10	25
>500	00	00
Total	64	105

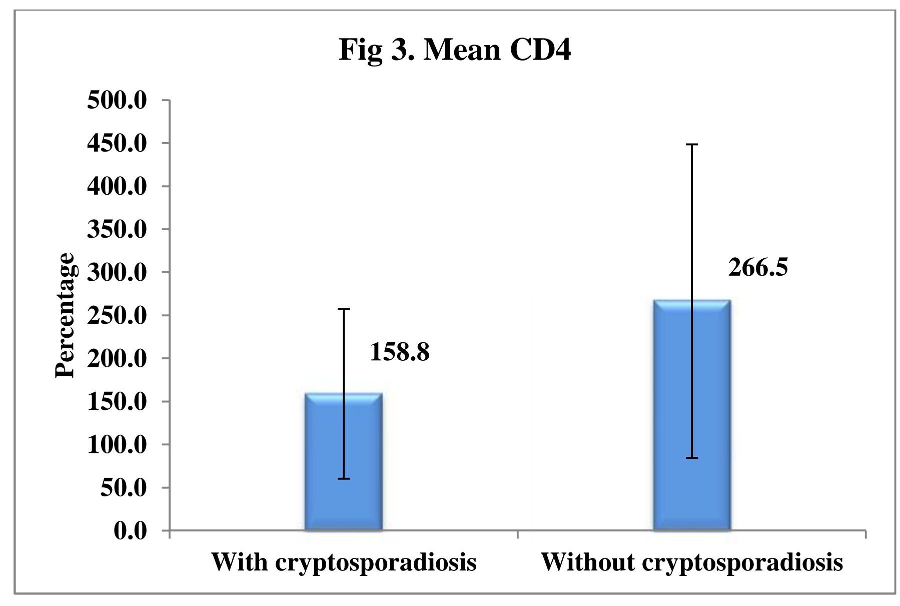
5.6Relationship between CD4 cell count and cryptosporidiosis in HIV sero-positive/AIDS patients

Majority of cryptosporidium positive cases had CD4 cells less than 200 cells/cummand mean value is 158.8with SD 98.4.

Maximum cryptosporidium negative cases were found to have CD4 cells more than 200 cells/cumm and mean value 266.5 with SD 182.0. P value is 0.015 (sig).

Table 4. Relationship between CD4 Cell Count and Cryptosporidiosis

CD4	With cryptosporidiosis		Without cryptosporidiosis		p value
	Mean	SD	Mean	SD	
	158.8	78.4	266.5	82.0	0.015 (sig)



5.7 Detection and comparison of cryptosporidiosis in HIV sero-positive /AIDS patients with diarrhoea and without diarrhea

Results of Modified Ziehl Neelsen staining:

Out of 85 positive cases, 61 cases were with diarrhoea and 24 cases without diarrhoea. P value is <0.001 (sig).

Table 5.Modified Ziehl Neelsen staining

MZN	Diarrhoea				P value
	Positive		Negative		
	N	%	N	%	
Positive	61	93.8	24	53.3	<0.001 (sig)
Negative	4	6.2	21	46.7	
Total	65	100.0	45	100.0	

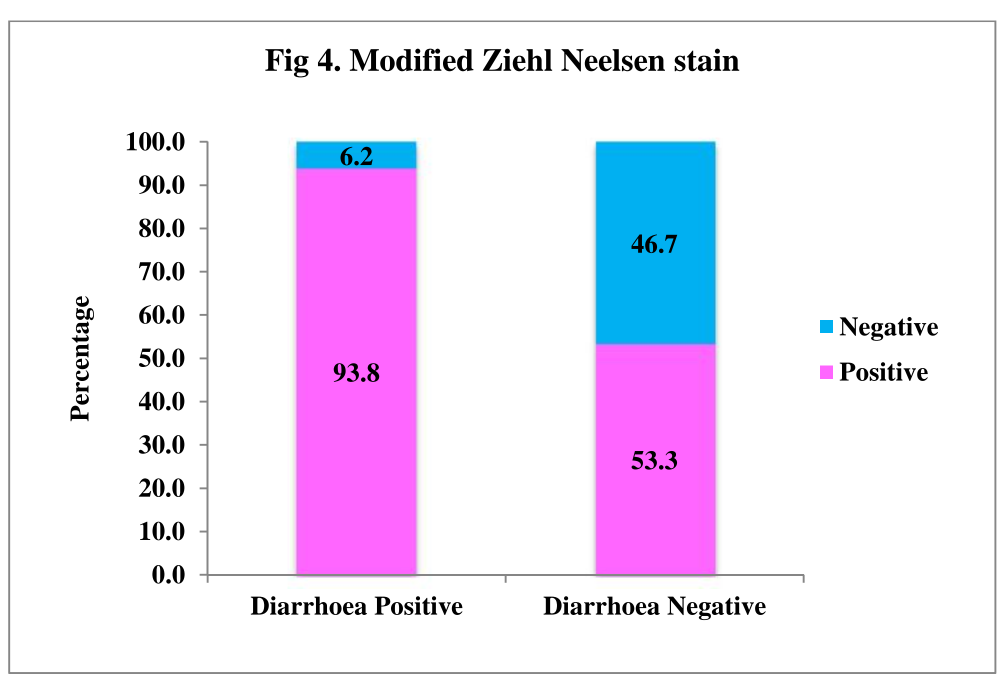
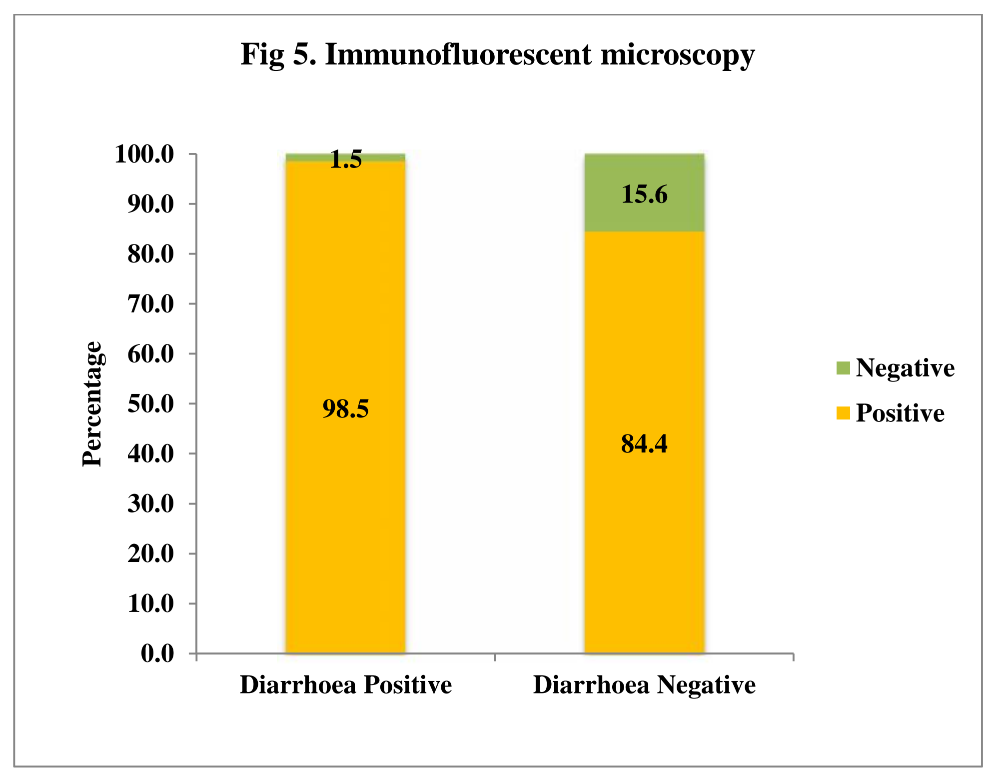


Fig 4. Modified Ziehl Neelsen stain

Results of IFT Method: Among 102 positive cases,64 cases with diarrhoea and 38 cases without diarrhoea. P value is 0.005(sig).

Table 6.IFT Method

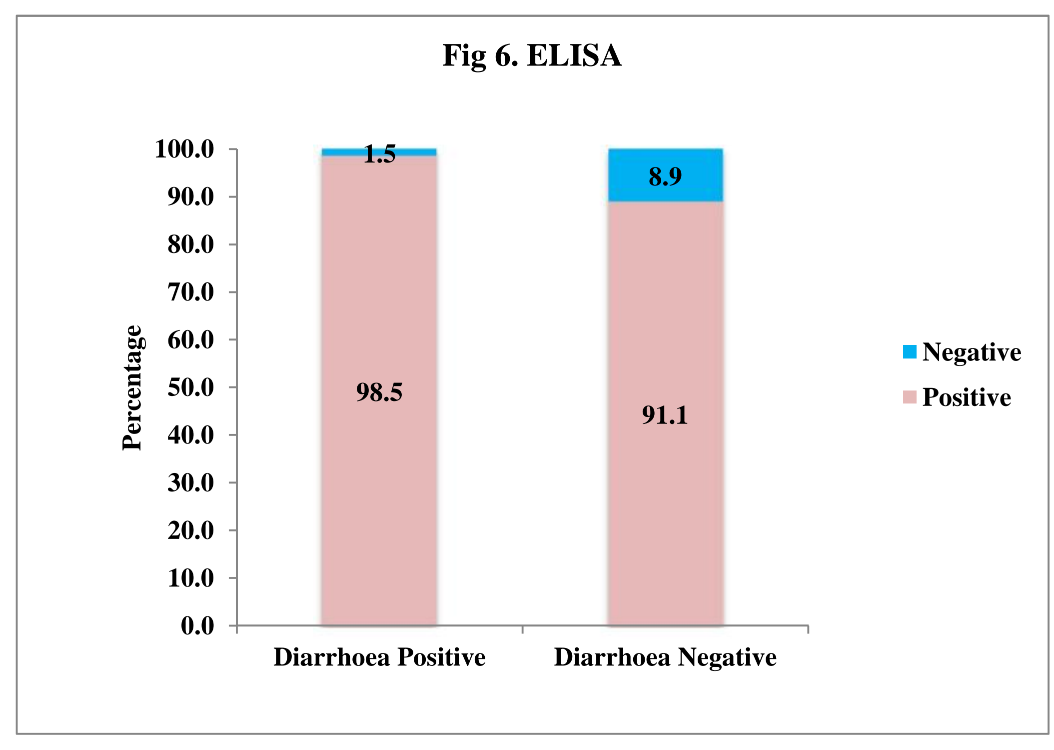
IFT	Diarrhoea				P value
	Positive		Negative		
	N	%	N	%	
Positive	64	98.5	38	84.4	0.005(sig)
Negative	1	1.5	7	15.6	
Total	65	100.0	45	100.0	



Results of ELISA Method: Of 105 positive cases, 64 cases were with diarrhoea and 41 cases were without diarrhoea. P value is0.03 (sig).

Table 7.ELISA Method

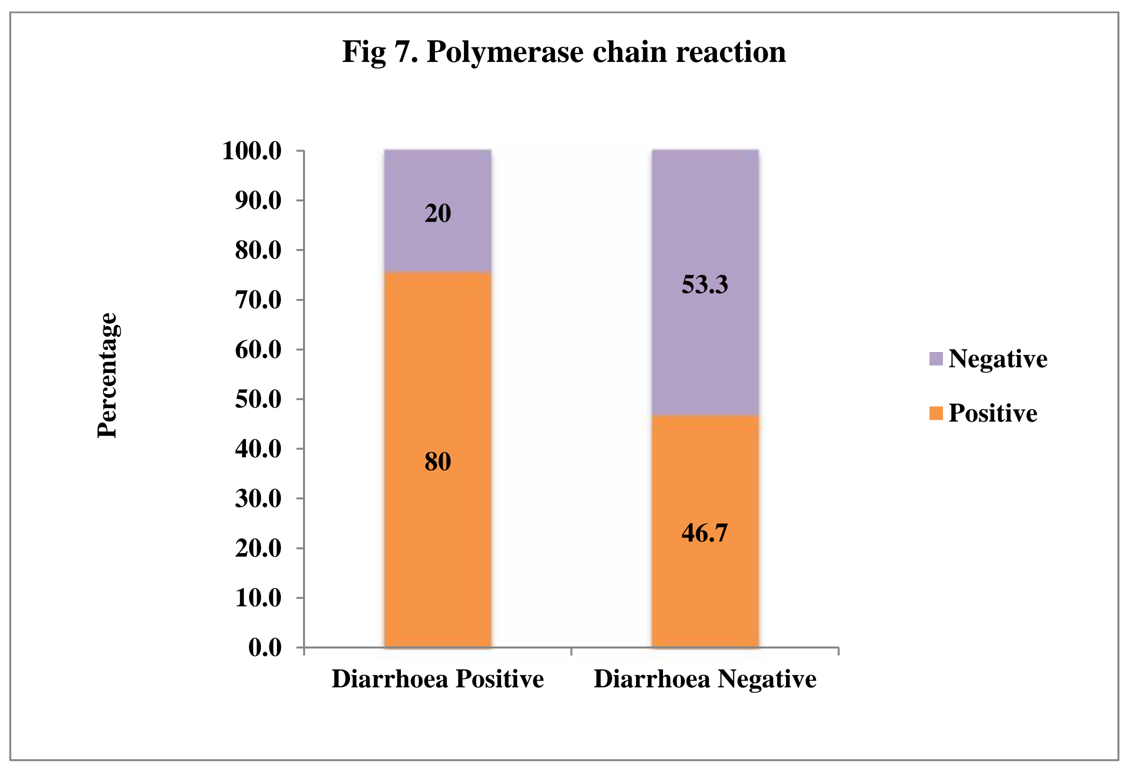
ELISA	Diarrhoea				P value
	Positive		Negative		
	N	%	N	%	
Positive	64	98.5	41	91.1	0.03(sig)
Negative	01	1.5	04	8.9	
Total	65	100.0	45	100.0	



Results of PCR Method: Out of 73 positive cases, 52 cases were with diarrhoea and 21 cases without diarrhoea. P value is 0.002 (sig).

Table 8.PCR Method

PCR	Diarrhoea				P value
	Positive		Negative		
	N	%	N	%	
Positive	52	80	21	46.7	0.002(sig)
Negative	13	20	24	53.3	
Total	65	100.0	45	100.0	



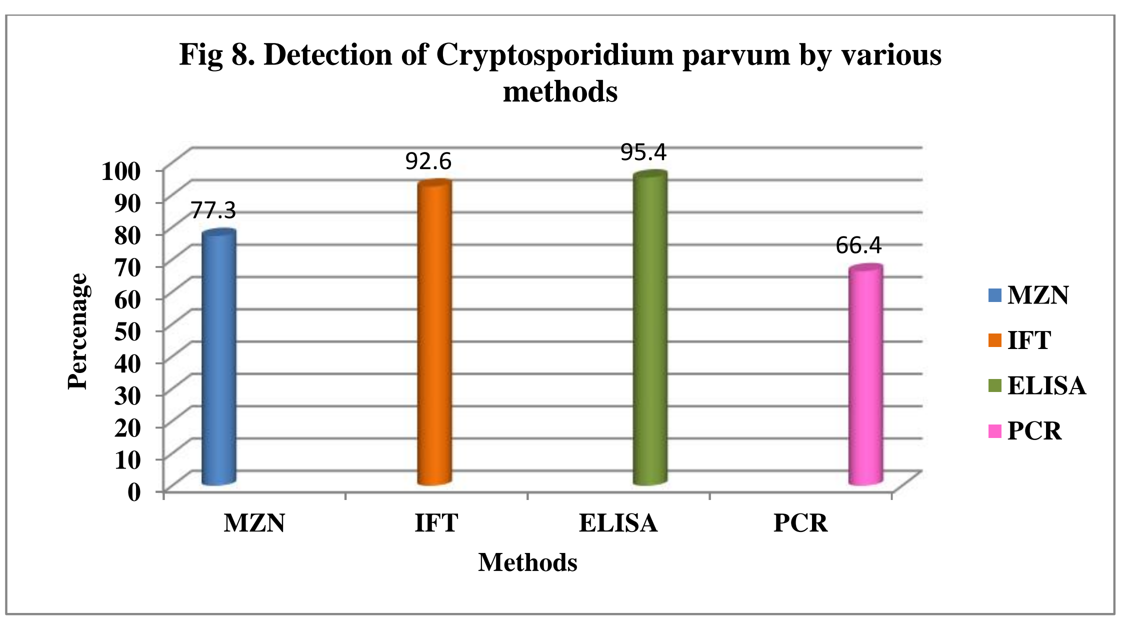
5.8Detection of *Cryptosporidium parvum* by various methods

Maximum positivity was detected by ELISA i.e. 95.4% followed by Immunofluorescent microscopy 92.6%, Modified ZN microscopy 77.3% and PCR 66.4%. ELISA was found to be the most reliable method with sensitivity and specificityof 98% and 100%.

P<0.005 is considered to be significant, since all the tests have a P value of <0.005.Hence it is considered to be highly significant.

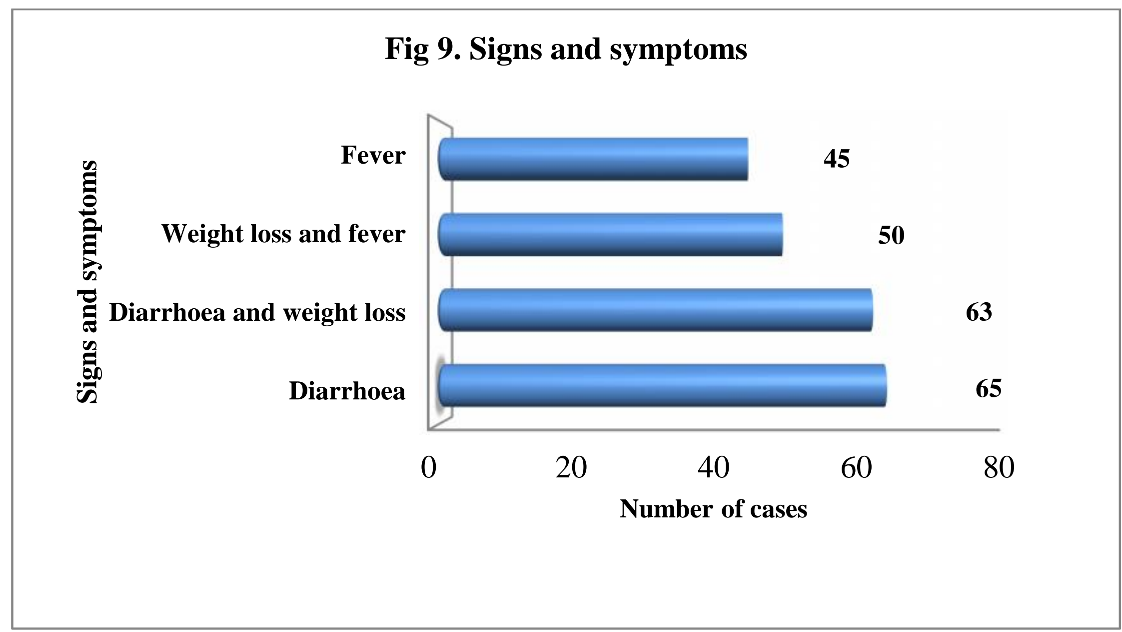
Table 9.Detection of *Cryptosporidium parvum* by various methods

Method	Total cases	Positive	%	Sensitivity	Specificity
Modified ZN Microscopy	110	85	77.3	94%	100%
Immunofluorescent Microscopy	110	102	92.6	96.2%	100%
ELISA	110	105	95.4	98%	100%
PCR	110	73	66.4	83%	100%



5.9Signs and Symptoms

In the present study 65 patients (59%) had diarrhoea as the predominant manifestation, followed by diarrhoea and weight loss in 63 (57%), weight loss and fever in 50 (45.5%) and fever alone in 45(41%) patients.



5.10 Socioeconomic and demographic data of participants

Amongst 110HIVsero positive/AIDS patients enrolled in this study, majority was from rural areas and labourers were the most affected group.

Table.10

Characteristics	N=110	Percentage(%)
Residence		
Rural	92	86.6
Urban	18	16.4
Patients Classification		
Outpatients	45	40.9
Inpatients	75	68.1
Occupation		
Professional	10	9
Sellers	12	11
Labourers	60	54.5
Children	O6	5.5
House wives	22	20

DISCUSSION

6.1Incidence

The present study depicts a high incidence of cryptosporidiosis i.e. 95.4% amongst HIV sero-positive / AIDS cases in Raichur District of Karnataka. One of the studies by A. Singh from Manipal incidence of cryptosporidiosis was 42.9% quite lower than our study and another study by Anand from Manipur it was 94.4% which is equal to my study[1,2]. Globally the incidence rate of cryptosporidiosis is 60.3%[3].

6.2 Age and sex distribution

In the present study, amongst 110 HIV sero-positive / AIDS cases, 61(55%) were males and 49 (45%) were females. Male predominance was observed (Male to Female ratio was 10:6). These findings are in accordance with the reports of Darji et al (2013) at Gujarat (males-61% and females-39%), Paudyal et al (2013) at Kathmandu (males-66% and females-34%)and A.Singh et al (2003) at Manipal (males-97% and females-3%)[4, 5, 1].Males are more prone to develop HIV infection as compared to females due to increased mobility, work-related issues and similar findings have been reported in other studies conducted by S.V. Kulkarin et al and S. Gupta etal[6, 7].

Our study shows that majority of cases were in the age group between 31- 40 years (mean age is 34.4 years) in that 23 (37.7%) were males and 16 (32.6%) were females. Similar findings are reported by Kashyapet al and Takerkhani H et al[8,9]. Statistically, there was no significant association between gender and the immunocompromised state (^{2} =3.1 & P =0.692).

6.3 Cryptosporidiosis in various age groups

Cryptosporidiosis can be acquired at any time during the course of HIV/AIDS infection. In the present study, highest incidence of cryptosporidiosis was observed in the age group of 0-10 years and above 50 years followed by 11-20, 21-30 & 31-40 years. Similar findings were observed in the studies conducted by Yunusa et al in Nigeria, Salyer et al in Uganda, Certad et al in Venezuela and Erhabor O et al in Niger Delta. There was no association between cryptosporidial infection and age. (^{2} =5.1 & P =0.395)[10,11,12,13].

6.4 Correlation between CD4 counts and cryptosporidiosis of the study subjects

In this study, majority of the subjects (73%) with cryptosporidiosis had CD4 counts less than 200 cells/cumm. This finding is in accordance with the studies of Erhabor O et al (2011) and Aminu M. et al (2014)[13, 14]. The study indicates a strong association between cryptosporidiosis and low CD4 cell counts i.e. less than 200 cells/cumm. This result was found to be statistically significant (P value > 0.015 and Mean value 158.8 + SD 98.4).

In a study by Tuli et al (2008) maximum cryptosporidium infectivity was in the group with CD4 cell counts <200 cells/cumm. The rate of infection decreased with increase of CD4 count[15]. In the study by Vyas et al 2012, 72.8% patients tested positive for cryptosporidium had CD4cell counts <200 cells/cumm[16]. In studies by Sucilathangam et al 2011 and Basak et al 2010 cryptosporidial infection was associated with CD4 countless than 500 cells/cumm[17,18].

Similar to our study, Raytekar et al 2012 study shows cryptosporidiosis to be more commonly associated with CD4 count <200 cells/cumm. Overall infection rate of the cryptosporidium causing diarrhoea was elevated in HIV sero positive/AIDS

individuals with CD4 counts less than 200 cells/cumm as compared to those with CD4 counts below 500 cells/cumm[19].

Seventy three percent of patients with CD4 counts <200cells/cumm showed cryptosporidiosis in our study. Kulkarni et al at Pune stated that cryptosporidial infection is primarily related to CD4 counts <200 cells/cumm and the cause of infection in immunocompromised individuals might be because they are more susceptible or incapable to get rid of the infection[20].

Experimental studies of infection in mice and calves with cryptosporidium showed that immunity is mainly dependent on CD4 counts. CD4 T cells increases the number of epithelial lymphocytes and help to produce -interferon[21].

In this study there is a positive association between cryptosporidiosis and CD4 count <200 cells/cumm (P value less than 0.005).HIV infection increases the opportunity of cryptosporidial infection as it destroys the cell mediated immune system which is provided by the CD4 lymphocytic cells[22].

6.5 Correlation of CD4 count, diarrhoea and cryptosporidial positivity

Out of 65 diarrhoea positive cases, 55 showedCD4 counts below 200 cells/cumm and 10 cases showed CD4 counts above 200cells/cumm. P value is 0.03(significant). CD4 count versus cryptosporidium positivity is significantly correlated inversely with -0.231 (P value 0.015 SIG) correlation coefficient.CD4 count versus diarrhoea is significantly correlated inversely with -0.313 (P value 0.001 significant) correlation coefficient. Diarrhoea versus cryptosporidium positivity is correlated directly with 0.207 (P value 0.03 significant) correlation coefficient. The results were in accordance with the studies of Taherkhaniet al (2007) and S.M. Darji et al (2013)[3, 9].

An increase of CD4 count enhances immune-system and simultaneously the rate of infection decreases. Elevated mortality rates with cryptosporidiosis was reported by Sauda FC et al (1993) and Brandoniso O et al (1993)[23, 24].

Cryptosporidial infection and diarrhoea were common in HIV infected patients with low CD4 cell counts. Rate of diarrhoea was found to be inversely proportion in HIV patients with CD4 counts <200 cells/cumm. According to M. Darji et al and Newman RD et al *C. parvum* is an important cause of persistent diarrhoea in patients with AIDS and it can cause severe symptoms in immunocompromised patients[25] (Baqai R et a) [3, 25, 26].

In our study higher incidence of cryptosporidial infection was observed among patients with diarrhoea (98.5%). A study conducted in Ethiopia by Girma et al 2014 found a strong association between the incidence of cryptosporidial infection as well as duration of diarrhoea[27]. Ninety four percent of HIV sero-positive patients had diarrhoea with cryptosporidial infection in Tzipori S et al study [28].

Our results showed similar relationship between HIV seropositivity, CD4 counts and cryptosporidiosis. In the present study 80 cryptosporidiosis positive patients had CD4 count less than 200cells/cumm and 64 patients had diarrhoea .This is statistically significant and favorable with previous studies by Erhabor O et al 2011and Dwivedi KK et al (2007)[11, 29].

These lymphocytes when significantly decrease less than 200 cells/cumm patients are prone to get opportunistic infection and invariably have more chance of acquisition of *C. parvum* infection[30].

6.6 Cryptosporidiosis with diarrhoea and without diarrhoea

In the present study, cryptosporidiosis cases with diarrhoea were 98.5% and without diarrhoea were 91.1% .The presence of cryptosporidium in HIV positive

cases with and without diarrhoea indicates an existing high risk of infection by this parasite as found in Brazil study (^{2} = 3, P- value is <0.03 SIG)).In India asymptomatic carriage rate is 94.4% [31, 32, 33].

6.7 Detection of cryptosporidiosis by various methods

Detection of cryptosporidium oocysts: Microscopic technique

There is an increasing demand for diagnostic testing of *cryptosporidium parvum*, with a priority being placed on obtaining diagnostic results in an efficient and timely manner.

Cryptosporidiosis is diagnosed by microscopic techniques by demonstrating cryptosporidium oocysts in stool samples. Oocysts size ranges from 4.5- 5 microns and shape is spherical or slightly oval[34].

In the present study, positivity of cryptosporidial infection by Modified Ziehl-Neelsen staining was 77.3% which is closer to the results of two studies i.e Darji's et al at Gujarat (74%), Ibrahim R Aly Shalash et al at Egypt (72.2%) and results of two other studies were lower than our study i.e Amadi et al at Zambia (50%) and Blanco et al at Guniea (56.6%). Soumendra Nath Maity et al from Hyderabad found highest incidence (100%) amongst all studies[35,36,37,38].

Demonstration of characteristic oocysts with Modified Ziehl Neelsen stain is the common method to detect cryptosporidiosis. However this method is laborious and less sensitive (Bialek et al 2002)[40].Oocysts of cryptosporidium are very small and can easily be missed in faecal samples as artifacts. They can also be easily confused with other oocysts. This staining method does not distinguish oocysts of cryptosporidial species. Also the red and pink stains taken by stool debris add to the confusion. It is a disadvantage as compared to other methods. The advantage of this method is that it is inexpensive and adoptable method, for this reason all

underdeveloped and developing countries still depend on this method (Connelly et al)[40].

Modfiied Zeihl Neelsen staining	
Different (years)	**Percentage (%)**
Darji et al-Gujarat(2013)	74
Ibrahim R AlyShalash et al Egypt (2016)	72.3
Our study	77.3

Detection of cryptosporidium oocysts: Immunofluorescent Microscopic Technique (IFT)

To detect cryptosporidiosis by IFT, phenol auramine method is commonly used to stain the oocysts[35,40]. Cryptosporidial positivity by immunofluorescent microscopy was 92.7% which varies with the findings of two studies 66.67% by Salah H Elsafi at Damman (2008) and 27% by M. Srisuphanut at Thailand, which is quite lower than our study (2005)[41,42].

Immunofluorescent microscopic technique and auramine staining procedures are very sensitive and accurate methods for identification of cryptosporidium in stool specimens. Direct and indirect immunofluorescent techniques are highly expensive than routine microscopic methods, however oocysts are easily recognized. Additionally, many studies considered IFT as gold standard technique to detect oocysts[43,44]. Immunofluorescent test helps to examine more number of samples, moderately labor intensive and short turnaround time [45-50].

Immunofluorescent test	
Different studies (years)	**Percentage (%)**
M. Srisuphanut et al Thailand (2005)	66.6
Shalah et al at Damman (2008)	27
Present study	92.7

Detection of cryptosporidium antigen: Enzyme-linked Immuno Sorbent Assay (ELISA)

Amongst 110 HIV sero-positive/AIDS cases, 105 were positive for cryptosporidium antigen by ELISA (95.4%). The sensitivity and specificity of the test were 98% and 100%. Detection of specific antigen of cryptosporidium by using monoclonal antibodies in sandwich ELISA is highly specific and sensitive when compared with other methods. A study conducted by Fabiana Rangel Marques et al has reported a sensitivity of 100% and specificity of 96% by immunoassay[51].

Three other studies showed incidences of cryptosporidial infection of98.1% by Ibrahim R Aly Shalash at Egypt (2016), 74.3% by U U Nwodo at Nigeria (2014) and 86% by Redlinger et al (2002) Mexican/US border by ELISA[36,52& 53].

Enzyme linked Immunosorbent Assay is an ideal technique due to its high sensitivity and specificity. It is simple, rapid and easily standardized in recognizing cryptosporidial antigen in feacal specimens[54]. Hence ELISA is an excellent screening method that helps to conduct large scale epidemiological studies mainly when screening huge samples in short time (Casemore et al and Xiao et al)[55.56,57].

Research carried out by Jayalakshmi et al found ELISA method was useful in routine diagnosis[58]. According to the study conducted by Barua (2008) modified ZN technique was the efficient procedure for routine screening of stool samples to detect cryptosporidiosis compared to ELISA[59].

But in our study ELISA was found to be more efficacious in the detection of cryptosporidium antigen. Out of 110 samples, 105 were found positive by ELISA and 85 samples positive with modified acid fast staining method.

ELISA	
Different studies(years)	**Percentage (%)**
U U Nwodo Nigeria(2014)	74.3
Ibrahim R Aly Shalash et al Egypt (2016)	98.1
Our study	95.4

Identification of cryptosporidium genome by Molecular Technique

Polymerase chain reaction is the most sensitive method for the identification of cryptosporidium[60, 61,62].

In the present study PCR showed an incidence of cryptosporidial infection of 66.4%.Resultsof the studies conducted by UU Nwodo at Nigeria was 65.7% and in another study by Reza Ghafari's at Iran was 70. 8% which is quite higher than our study[19, 63].

Determining the exact incidence of cryptosporidiosis in one region is important for epidemiological surveys. It is a highly sensitive, specific and consistent technique for the detection ofcryptosporidium[64].

Type of PCR used in our study was conventional type. Conventional PCR detects the presence or absence of DNA, but not the RNA. It cannot quantitate the

amount of DNA and it cannot differentiate between viable and non-viable microbe. It is a highly sensitive method for detection of cryptosporidium, but its sensitivity in feacal matter could be significantly reduced by the presence of inhibitors in PCR like complex polysaccharides.PCR is a costly method than others, consumes time, require expertise and costly equipment[39].

Of the 110 samples, 73 (66.4%) samples showed a gene of the predictable range (680bp) demonstrating the occurrence of *Cryptosporidium parvum.*

Detection of Cryptosporidium genome by PCR cannot explain the infectivity state but only shows the presence of cryptosporidium in the sample[66,67]. Despite differences in PCR and ELISA methods, statistical significance was not noticed using both the methods.

Polymerase chain reaction(PCR)	
Different studies (years)	**Percentage (%)**
U U Nwodo, Nigeria (2014)	65.7
Reza Ghafari, Iran (2018)	70.8
Present study	66.4

6.7 Evaluation of various methods of detection of *cryptosporidium parvum*

Evaluation of various methods shows different percentages in identification of cryptosporidial incidence in the study i.e 95.4% by sad-ELISA, 92.6% by IFT, 77.3% by Modified ZN staining and 66.4% by PCR. The other two studies conducted on sad-ELISA by Beauty E. Omoruyi et al and Ibrahim R Aly Shalash et al showed 74.3%and 98.1% respectively[36, 53].Salah H. Elsafi et al and M. Srisuphanut et al studies depicted incidences of 27% and 66.6% by IFT respectively[42, 43]. Modified ZN

staining showed positivity of 74% & 72.3% by Darji et al and Ibrahim R Aly Shalash et al respectively[1,36]. Positivity of PCR was 65.7% and 70.8% by U U Nwodo et al and Reza Ghafari et al respectively [52, 64].

The sensitivities of various techniques were: PCR-83%, MZN-94%, IFT-96.2% and sad-ELISA-98%. ELISA showed the highest sensitivity of 98% and was found to be the most reliable method with sensitivity and specificity of 98% and 100%.

Difference within sensitivity and specificity are explainable because the ELISA identifies the cryptosporidial antigen, in the similar way IFT detects the stained oocysts of cryptosporidium, equally in Modified ZN staining oocysts observed and similarly PCR technique picks up genome of the infective cryptosporidium.

6.8 Socioeconomic and demographic data of participants

In our study, majority of cases were from rural areas and considerably affected groups was laborers and same was observed in Yunusa et al study[67] and Nyamwange study[68]. In our study 86.6% subjects were rural residents and 16.4% urban residents. The main cause of cryptosporidiosis in rural areas may be water contamination and lack of individual hygiene and poor access to health facilities. The best prophylactic measure would be to improve general sanitation, water supply and to educate HIV infected individuals about the disease[35]

Previous studies documented that intestinal parasitic infections often occur in HIV sero positive /AIDS patients. Kumar et al study shows the information regarding variations in incidence of *C.parvum* in HIV infected patients in different geographic areas. It was documented that *C.parvum* was the common parasite, mainly found in southern part of India[69]. In present study the incidence of *C. parvum* was 95.4%.

6.9 Signs and symptoms

In Ayyaggari et al study, all the patients showed more than one symptom. Various symptoms presented by these patients were weight loss 45, fever 25and 13 diarrhoea[70]. Another study by Jane et al also reported patients with weight loss 36, fever 23 and with diarrhoea 41[71].

In our study, 65 patients had diarrhoea as the predominant manifestation, followed by diarrhoea and weight loss in 63, weight loss and fever in 50 and fever in 45.

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SUMMARY AND CONCLUSION

7.1 Summary

- The incidence of cryptosporidiosis amongst the HIV sero-positive/AIDS patients was 95.4%, in Raichur District of Karnataka.
- Major group affected was 31-40 years with mean age 34.4 years andmale preponderance was seen.
- Seventy three percent of the cases had CD4 count of <200 cells/cumm.
- Cryptosporidial positivity rate was 98.4% amongst the patients who presented with diarrhea and 91.1% in non-diarrhoeal cases.
- Maximum positivity was detected by ELISA i.e. 95.4% followed by Immuno-fluorescent microscopy (92.6%), modified Ziehl Neelsen staining (77.3%) and PCR (66.4%).
- ELISA was found to be the most reliable method with sensitivity and specificity of 98% and 100%.
- Amongst the study group, 65 patients had diarrhoea as the predominant manifestation, followed by diarrhoea and weight loss in 63 patients, weight loss and fever in 50 and fever alone in 45 patients.
- Majority of the study individuals were from rural areas and labourers were the most affected group.

7.2 Conclusion

- This study enhanced the awareness of cryptosporidiosis among HIV sero-positive / AIDS patients in Raichur District of Karnataka.
- Our study highlights the importance of routine screening for cryptosporidiosis in all HIV sero-positive/AIDS patients, irrespective of gastrointestinal symptoms.
- ELISA can be considered the most reliable method for the detection of cryptosporidial oocysts in fecal specimens.

7.3 Limitations of Study

The study is confined to HIV sero-positive /AIDS patients with limited sample size, studies with larger sample size could throw better light on the exact prevalence in this region.

7. 4 Future Prospective

Culture may be useful for drug evaluation studies.To isolate pure parasite material for future vaccine development and passive immunotherapy.Animal models are needed to know the mechanism of pathogenesis of cryptosporidiosis.

MASTER CHART

Sl.No	IP/OP No	Age	Sex	Religion	Occupation	Lab No	Clinical Manifestation				Past history		HIV/AIDS	Clinical diagnosis	Stool examination			
							Diarrhoea	Weight loss	Pain Abdomen	Fever	H/O Blood transfusion	H/O TB			MZN	ELISA	PCR	IFT
1	10984	33	M	H	C	1	YES	YES	YES	YES	NO	YES	+	PTB, PUO	+	+	+	+
2	1353	40	M	H	F	2	NO	YES	NO	YES	NO	YES	+	PTB, PUO	+	-	+	+
3	1515	45	M	H	C	3	YES	YES	NO	YES	NO	YES	+	PTB, PUO	+	-	+	+
4	12286	35	F	H	C	4	YES	YES	YES	YES	NO	YES	+	PTB, PUO	+	+	+	+
5	33768	30	M	H	B	5	YES	YES	NO	YES	NO	YES	+	PTB, PUO	+	+	+	+
6	33776	50	M	H	F	6	YES	YES	YES	YES	NO	YES	+	PTB, PUO	+	+	+	+
7	37015	20	F	H	C	7	NO	NO	NO	YES	NO	NO	+	PUO	+	+	+	+
8	37013	45	M	H	C	8	NO	NO	NO	YES	NO	NO	+	PUO	-	+	-	-
9	2291	7	F	H	Chi	9	YES	YES	YES	YES	YES	NO	+	PUO	+	+	-	+
10	52564	35	M	H	B	10	YES	YES	YES	YES	NO	NO	+	PUO	+	+	+	+
11	2274	1	F	H	Chi	11	YES	YES	NO	YES	NO	YES	+	PTB, PUO	+	+	+	+
12	2242	5	M	H	Chi	12	YES	YES	NO	YES	YES	NO	+	PUO	+	+	+	+
13	15973	3	F	H	Chi	13	YES	YES	YES	NO	NO	NO	+	PUO	+	+	+	+

14	8651	48	M	H	C	14	YES	YES	NO	NO	NO	NO	+	PUO	+	+	+	+
15	9652	30	F	M	C	15	NO	YES	YES	NO	NO	NO	+	PUO	-	-	+	+
16	227621	48	F	M	C	16	YES	YES	NO	YES	NO	YES	+	PTB, PUO	-	+	+	+
17	1970	40	M	H	C	17	YES	YES	YES	NO	NO	YES	+	PTB, PUO	+	+	+	+
18	246597	40	M	H	C	18	NO	NO	NO	NO	NO	YES	+	PTB, PUO	+	+	+	+
19	20818	26	F	H	HW	19	NO	NO	NO	NO	NO	NO	+	PUO	-	+	+	+
20	26525	54	M	M	C	20	NO	NO	NO	YES	YES	YES	+	PTB, PUO	+	+	+	+
21	360036	30	F	H	HW	21	NO	NO	NO	YES	YES	YES	+	PTB, PUO	-	-	-	-
22	13848	35	M	H	C	22	YES	YES	YES	YES	NO	NO	+	PUO	+	+	+	+
23	14275	27	F	M	HW	23	YES	YES	YES	NO	NO	NO	+	PUO	+	+	+	+
24	14202	56	M	H	C	24	YES	YES	YES	NO	NO	YES	+	PTB, PUO	+	+	+	+
25	14200	30	M	H	C	25	YES	YES	YES	YES	NO	YES	+	PTB, PUO	+	+	+	+
26	14249	35	F	H	HW	26	YES	YES	YES	YES	NO	YES	+	PUO	+	+	+	+
27	94292	35	F	H	HW	27	YES	YES	YES	YES	NO	NO	+	PUO	+	+	+	+
28	94301	60	F	H	HW	28	YES	YES	YES	NO	NO	NO	+	PUO	+	+	+	+
29	2917	47	M	H	C	29	NO	NO	NO	YES	NO	NO	+	PUO	+	+	+	+
30	5619	45	F	M	C	30	YES	YES	YES	NO	NO	YES	+	PTB, PUO	+	+	-	+
31	2374	32	F	H	B	31	NO	YES	YES	YES	NO	NO	+	PUO	+	+	+	+
32	5272	40	F	H	C	32	YES	YES	YES	YES	NO	YES	+	PTB, PUO	+	+	+	+
33	91821	33	F	H	HW	33	YES	YES	NO	YES	NO	YES	+	PTB, PUO	+	+	+	+
34	91825	3	F	H	Chi	34	YES	YES	YES	YES	NO	YES	+	PTB, PUO	+	+	+	+
35	61255	35	M	H	B	35	NO	NO	NO	YES	NO	YES	+	PTB, PUO	+	+	+	+
36	2562	43	F	H	C	36	NO	YES	YES	YES	NO	YES	+	PTB, PUO	+	+	-	+
37	95915	22	M	M	C	37	YES	YES	YES	YES	NO	YES	+	PTB, PUO	+	+	+	+
38	94038	55	F	H	HW	38	YES	YES	YES	YES	NO	NO	+	PUO	+	+	+	+
39	6157	40	M	C	C	39	YES	YES	YES	YES	NO	NO	+	PUO	-	+	+	+

40	15830	40	F	C	C	40	YES	YES	YES	YES	NO	NO	+	PUO	+	+	_	+
41	15385	40	M	H	C	41	NO	NO	NO	YES	NO	YES	+	PTB, PUO	+	+	_	_
42	16313	28	F	H	HW	42	YES	YES	YES	NO	YES	YES	+	PTB, PUO	+	+	_	+
43	16672	45	F	C	C	43	NO	YES	YES	YES	YES	YES	+	PTB, PUO	+	+	_	+
44	16921	35	F	H	C	44	YES	YES	YES	YES	NO	YES	+	PTB, PUO	+	+	_	+
45	16287	35	F	H	H	45	NO	NO	NO	YES	YES	YES	+	PTB, PUO	_	+	_	_
46	17962	45	F	H	HW	46	NO	YES	NO	YES	NO	YES	+	PTB, PUO	_	+	_	+
47	17721	40	F	H	C	47	NO	YES	NO	YES	NO	NO	+	PUO	_	+	+	+
48	17990	46	F	H	C	48	Y>7	YES	YES	YES	YES	NO	+	PUO	+	+	_	+
49	18236	45	F	H	C	49	NO	YES	NO	NO	NO	NO	+	PUO	_	+	_	_
50	18477	58	F	M	C	50	NO	NO	YES	NO	NO	NO	+	PUO	_	+	_	+
51	1785	40	M	H	C	51	Y>8	NO	YES	YES	NO	YES	+	PTB, PUO	_	+	_	+
52	18646	56	M	H	C	52	NO	YES	NO	YES	NO	YES	+	PTB, PUO	_	+	_	+
53	18539	30	M	H	C	53	NO	YES	NO	YES	NO	NO	+	PUO	+	+	_	+
54	5743	27	F	H	HW	54	NO	YES	NO	YES	NO	YES	+	PUO, PTB	+	+	_	+
55	27157	18	M	H	S	55	YES	YES	YES	NO	YES	NO	+	PUO	+	+	_	+
56	3887	17	M	H	S	56	NO	NO	YES	YES	YES	YES	+	PUO, PTB	_	+	_	+
57	3202	24	M	H	C	57	YES	YES	YES	YES	YES	YES	+	PUO, PTB	+	+	_	_
58	4613	35	F	H	HW	58	NO	YES	YES	NO	NO	NO	+	PUO, PTB	+	+	+	+
59	4871	36	M	C	C	59	NO	YES	YES	YES	YES	YES	+	PUO, PTB	+	+	_	+
60	4787	45	F	H	HW	60	YES	YES	YES	YES	NO	NO	+	PUO	_	+	_	+
61	5083	47	M	C	C	61	NO	YES	NO	YES	YES	YES	+	PUO, PTB	+	+	_	+
62	5470	20	F	M	C	62	YES	YES	YES	NO	YES	YES	+	PUO, PTB	+	+	+	+
63	5675	26	F	H	C	63	YES	YES	YES	YES	YES	YES	+	PUO, PTB	+	+	+	+
64	5745	30	F	H	HW	64	YES	YES	YES	YES	NO	YES	+	PUO,PTB	+	+	+	+
65	4025	37	M	H	C	65	NO	YES	NO	NO	NO	NO	+	PUO	_	+	+	+

66	5710	47	M	H	C	66	NO	YES	NO	YES	NO	YES	+	PUO, PTB	+	+	_	+
67	6011	55	M	H	C	67	YES	YES	YES	YES	YES	YES	+	PUO, PTB	+	+	+	+
68	6454	35	M	C	P	68	NO	YES	NO	YES	NO	YES	+	PUO, PTB	_	+	+	+
69	6060	65	M	H	C	69	YES	YES	YES	NO	NO	NO	+	PUO	+	+	+	+
70	6457	35	F	H	HW	70	YES	YES	NO	YES	YES	YES	+	PUO, PTB	+	+	+	+
71	6140	30	M	H	C	71	YES	YES	YES	YES	NO	YES	+	PUO, PTB	+	+	+	+
72	12013	45	F	H	HW	72	YES	YES	YES	YES	YES	YES	+	PUO, PTB	+	+	+	+
73	6579	30	F	H	HW	73	YES	YES	YES	YES	YES	YES	+	PUO, PTB	+	+	_	+
74	48594	26	M	H	C	74	YES	YES	YES	YES	NO	YES	+	PUO, PTB	+	+	+	+
75	6379	50	F	H	HW	75	YES	YES	YES	YES	YES	YES	+	PUO, PTB	+	+	+	+
76	7584	22	M	M	C	76	NO	YES	NO	YES	NO	YES	+	PUO, PTB	_	_	+	+
77	7560	50	M	H	C	77	YES	YES	NO	NO	NO	NO	+	PUO	+	+	+	+
78	7774	46	M	H	C	78	NO	YES	NO	YES	NO	NO	+	PUO	+	+	_	+
79	7820	50	F	H	HW	79	YES	NO	YES	YES	YES	YES	+	PUO, PTB	+	+	+	+
80	7850	30	M	H	P	80	YES	YES	YES	YES	NO	YES	+	PUO, PTB	+	+	+	+
81	7857	43	M	H	C	81	YES	YES	YES	YES	NO	YES	+	PUO, PTB	+	+	_	+
82	7973	26	M	H	P	82	YES	YES	YES	YES	NO	NO	+	PUO	+	+	_	+
83	8039	37	M	H	P	83	NO	YES	NO	YES	NO	YES	+	PUO, PTB	_	+	_	_
84	41816	50	M	H	C	84	NO	NO	NO	NO	NO	YES	+	PUO, PTB	+	+	+	+
85	8293	40	M	H	C	85	YES	YES	YES	NO	NO	YES	+	PUO, PTB	+	+	+	+
86	9187	33	F	H	C	86	NO	YES	NO	NO	NO	YES	+	PUO,PTB	_	+	_	+
87	9098	60	M	M	C	87	YES	YES	YES	YES	NO	YES	+	PUO,PTB	+	+	_	+
88	8626	35	M	H	C	88	NO	YES	NO	YES	NO	YES	+	PUO,PTB	+	+	_	+
89	8680	35	F	H	C	89	YES	YES	YES	YES	NO	NO	+	PUO	+	+	+	+
90	9291	35	F	H	HW	90	NO	YES	YES	YES	NO	NO	+	PUO	+	+	+	+
91	9260	33	M	H	C	91	YES	YES	NO	YES	NO	NO	+	PUO	+	+	+	+

92	9787	40	F	H	HW	92	YES	YES	YES	YES	NO	YES	+	PUO,PTB	+	+	+	+
93	9753	30	M	H	C	93	NO	YES	NO	YES	NO	NO	+	PUO	-	+	+	+
94	10068	35	M	H	P	94	YES	YES	YES	NO	NO	NO	+	PUO	+	+	+	+
95	10508	27	M	H	P	95	YES	YES	YES	YES	NO	NO	+	PUO	+	+	-	+
96	10889	42	F	H	HW	96	YES	YES	YES	YES	NO	NO	+	PUO	+	+	+	+
97	10672	30	M	M	P	97	NO	YES	YES	YES	NO	YES	+	PUO,PTB	-	+	+	+
98	11245	60	M	H	C	98	YES	YES	YES	NO	YES	YES	+	PUO,PTB	+	+	+	+
99	11282	18	M	H	S	99	YES	YES	YES	NO	NO	YES	+	PUO,PTB	+	+	+	+
100	11375	35	M	H	C	100	NO	YES	YES	YES	NO	NO	+	PUO	+	+	-	+
101	11519	60	F	H	C	101	NO	YES	NO	YES	YES	YES	+	PUO,PTB	-	+	+	+
102	11530	35	F	H	HW	102	NO	YES	YES	YES	NO	YES	+	PUO,PTB	+	+	-	+
103	11651	28	M	H	C	103	YES	YES	YES	YES	NO	YES	+	PUO,PTB	+	+	+	+
104	11795	35	M	H	C	104	YES	YES	NO	YES	NO	YES	+	PUO,PTB	+	+	+	+
105	11669	40	M	H	C	105	NO	YES	NO	YES	NO	YES	+	PUO,PTB	-	+	+	+
106	12010	45	M	H	P	106	NO	NO	NO	YES	NO	NO	+	PUO	+	+	+	+
107	15425	27	F	H	HW	107	YES	YES	YES	NO	YES	YES	+	PUO,PTB	+	+	+	+
108	15640	19	F	H	HW	108	NO	YES	YES	YES	NO	YES	+	PUO,PTB	-	-	-	-
109	16582	28	F	C	HW	109	NO	YES	NO	YES	NO	YES	+	PUO,PTB	+	+	-	+
110	16076	40	M	H	C	110	YES	YES	YES	YES	NO	YES	+	PUO,PTB	+	+	-	+

Abbreviations of Master chart

HIV-Human deficiency virus

TB-Tuberculosis

PUO-Pyrexia of unknown origin

MZN-Modified Ziehl Neelsen staining

IFT- Immunoflourescent test

ELISA-Enzyme-linked Immune Sorbent Assay

PCR- Polymerase chain reaction

F-Female

M-Male

HW-House wife

D-Driver

C-Coolie

P-Professional

S-Student

chi-children

B.L.D.E. UNIVERSITY

(Declared vide notification No. F.9-37/2007-U.3 (A) Dated. 29-2-2008 of the MHRD, Government of India under Section 3 of the UGC Act,1956)

The Constituent College

SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

IEC Ref No,91/2014-15

July 02, 2014.

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this University met on **26th June 2014** *at* **11 AM** *to scrutinize the Synopsis / Research projects of Postgraduate student / Undergraduate student / Faculty members of this University / college from ethical clearance point of view. After scrutiny the following original / corrected & revised version synopsis of the Thesis / Research project has been accorded Ethical Clearance.*

Title A Comprehensive study on Cryptosporidiosis in HIV/AIDS patients in Raichur District Karnataka.

Name of Ph.D. / P. G. / U. G. Student / Faculty member. Mrs.Sandhya. P. Department of Microbiology.

Name of Guide :Dr.Prashant.Parandekar Professor Department of Microbiology.

Dr. Sharada Metgud
Chairperson, I.E.C
BLDE University,
BIJAPUR – 586 103

Dr.G.V.Kulkarni
Secretary, I.E.C
BLDE University,
BIJAPUR – 586 103.

Member Secretary,
Institutional Ethical Committee,
BLDE University, BIJAPUR.

Following documents were placed before Ethical Committee for Scrutinization.

- Copy of Synopsis / Research project
- Copy of informed consent form
- Any other relevant documents.

Smt. Bangaramma Sajjan Campus, Sholapur Road, Bijapur – 586103, Karnataka, India.
University: Phone: +918352-262770, Fax: +918352-263303, Website: www.bldeuniversity.org, E-mail: office@bldeuniversity.org
College: Phone: +918352-262770, Fax: +918352-263019, Website: www.bldea.org, E-mail: bmpmc1@yahoo.co.in

Government of Karnataka

Raichur Institute of Medical Sciences, Raichur.

(Autonomous Institution) Phone / Fax-08532 238488/89

Institutional Ethics Committee Approval Letter

Date: 06-01-2014

To
Mrs. Sandhya. P
Tutor
Dept of Microbiology,
RIMS, Raichur.

Ref : Protocol titled **"A COMPREHENSIVE STUDY ON CRYPTOSPORIDIOSIS IN HIV/AIDS PATIENTS IN RAICHUR DISTRICT OF KARNATAKA"**
with Ref No : RIMS/IEC/ /2013-14/ 09 Dated : 12-12-2013

Sub: Institutional Ethics Committee approval for the study.

Dear Mrs. Sandhya. P

We have received from you, following study related documents vide letter dated 12-12-2013
A) Application Form B) Proforma for Registration of Synopsis for Dissertation C) Information Sheet
D) Informed Consent Sheet
The above mentioned documents were examined by Dr. Shivanadn K G Assoc. Prof. and IEC Member, and suggested for Approval. After consideration, the committee other members also have decided to approve the aforementioned study proposal.

The members who Approved the study proposal were:

1) Dr. Vasant R Chavan, Prof. & Head, Dept. of Pharmacology, RIMS, Raichur. - Chairman
2) Dr. S.D. Mandolkar, Prof. & Head, Dept of Gen. Surgery, RIMS, Raichur. - Member
3) Dr. Shivanand K.G, Assoc. Prof., Dept of Physiology, RIMS, Raichur. - Member
4) Dr. Din Prakash Ranjan, Assoc. Professor, Dept. of P&SM RIMS, Raichur. - Member
5) Mr. V. Satish, Advocate, Raichur - External Member
6) Dr. Kavita Patil , Prof. & Head, Dept. of Ophthalmology, RIMS, Raichur. - Member Secretary

It is understood that the study will be conducted under the guidance of **Dr. B.V. Peerapur,** Prof. Head, Dept. of Microbiology, RIMS, Raichur.

You are required to submit the progress report to the IEC every six months and at the completion of the project.

Yours sincerely,

Dr. Vasant R Chavan,
I/C, Chairman
Institutional Ethics Committee
Raichur Institution of Medical Sciences, Raichur.

Dr. Kavita Patil
Member Secretary,
Institutional Ethics Committee
Raichur Institution of Medical Sciences, Raichur.

\

INFORMED CONSENT FORM

Study Title: - "A COMPREHENSIVE STUDY ON CRYPTOSPORIDIOSIS IN HIV/AIDS PATIENTS IN RAICHUR DISTRICT OF KARNATAKA"

Study Number: -…………………………………………………………………………

Subject's full Name: - ……………………………………………………………………

Date of Birth / Age …………………………………………………………………………

Address ……………………………………………………………………………………

……………………………………………………………………………………………….

1. I confirm that I have read the information in this form (or if has been read to me). I was free to ask any questions and they have been answered.
2. I have read and under stood this consent form and information provided to me.
3. I have been explained above the nature of the study.
4. I have been explained about duration of participation with number of participants.
5. I have been explained about procedures to be followed and about investigations, if any to be performed. I have been explained that I don't have to pay or bear the cost of procedure/investigations.
6. My rights and responsibilities have been explained to me by the investigators.
7. I have been adequately explained risks and discomforts associated with my participation in the study.
8. I have been explained about benefits of my participation in the study to myself, community and to medical profession.
9. If despite following the instructions I am physically harmed because of any substances or any procedures as stipulated in the study plan my treatment will be carried out free of cost at investigational site and the sponsor will bear all

the expenses, If they are not covered by insurance agency or by Government program or any third party. I have had my questions, answered to my satisfaction

10. I have been explained about available alternative treatments.
11. I understand that my participation in the study is voluntary and I am free to withdraw at any time, without giving any reason and without my medical care or legal rights being affected.
12. I hereby give permission to the investigators to release information obtained from me as result of participation in the study to the sponsors, representatives of sponsors, regulatory authorities, Government agencies & ethics committee. I understand that they may inspect my original records.However, I understand that my identity will not be revealed in any information released to third parties or published.
13. I agree not to restrict the use of any data or results that arise from the study provided such a use is only for scientific purpose (S).
14. I am exercising my free power of choice, hereby give my consent to be included as participant in the present study.
15. I agree to co-operate with investigator and I will inform him/her immediately. If I suffer unusual symptoms.
16. I am aware that if I have any questions during the study, I should contact at one of the address listed.

By signing this consent form I attest that this document has been clearly explained to me and understood by me.

Signature (or Thumb impression) of the Subject/Legally Acceptable

representative:___________

Signatory's Name______________ Date_______________

Signature of the Investigator________ Date_______________

Study Investigator's Name __________

Signature of the Witness ___________ Date_______________

Name of the Witness ____________________

PROFORMA FOR COLLECTION OF SAMPLE

Name: Age:

Sex : Occupation:

Address: DOA:

IP/OP No: Lab No.:

HISTORY OF PRESENT ILLNESS:

I. Presenting Complaints

- Diarrhoea Frequency Duration
- Weight Loss <10% >10%
- Pain AbdomenYes/No
- Dehydration mild/ moderate/ severe
- Fever
- Loss of Appetite
- Other Symptoms

II. Past History

H/O Blood transfusion

H/O Tuberculosis

Drug Addiction

STOOL EXAMINATION:

<u>MACROSCOPY</u>

- Consistency
- Colour
- Odour
- pH
- Presence of blood / mucus
- Presence of segments of worms

MICROSCOPY

MODIFIED KINYOUN'S ACID FAST STAINING:

IMMUNOFLOURESENT MICROSCOPY

ELISA FOR CRYPTOSPORIDIUM ANTIGEN:

PCR FOR CRYPTOSPORIDIUM:

CD4 COUNT:

BLDE (DEEMED TO BE UNIVERSITY)

Annexure -I

PLAGARISM VERIFICATION CERTIFICATE

1. **Name of the Student: Mrs Sandhya Papabathini Reg No:12PHD008**
2. **Title of the Thesis: "A COMPREHENSIVE STUDY ON CRYPTOSPORIDIOSIS IN HIV/AIDS PATIENTS IN RAICHUR DISTRICT, KARNATAKA".**
3. **Department: Microbiology**
4. **Name of the Guide & Designation: Dr. Annapurna G Sajjan, MD, Professor**
5. **Name of the Co Guide & Designation: Dr. Kishore G bhat, MD, Professor &HOD**

The above thesis was verified for similarity detection. The report is as follows:

Software used: TURNITIN **Date:** 7/12/18

Similarity Index (%) : Four Percent (4%) **Total word Count:** 16752

The report is attached for the review by the Student and Guide.

The plagiarism report of the above thesis has been reviewed by the undersigned.

The similarity index is below accepted norms.

The similarity index is above accepted norms, because of following reasons:

...The thesis may be considered for submission to the University. The software report is attached.

Signature of the Guide	Signature of the Co-Guide	Signature of the Student
Name & Designation	Name & Designation	

Professor
Department of Microbiology
B.L.D.E. University's
Shri B.M. Patil Medical College
BIJAPUR-586 103.

Verified by (Signature)

Name & Designation

Shri B.M. Patil Medical College,
Bijapur.

111

PAPER PRESENTATIONS

Oral presentation in National conference

Topic: Detection of cryptosporidium parvum by modified acid fast stain and ELISA in HIV/AIDS patients in Raichur district, Karnataka

National conference of Parasitology (PARACON-17) 04/02/2017 & Sree Balaji medical college& Hospital, Bharath university, Chennai

Poster presentation in State conference

Topic: Detection of cryptosporidium parvum in HIV/AIDS patients in correlation with CD4 count Raichur district, Karnataka; Microcon KC-17,18/02/2017 & VIMS, Bellary.

BHARATH UNIVERSITY
SREE BALAJI MEDICAL COLLEGE & HOSPITAL
Chennai - 44.

Certificate

This is to certify that ~~Dr.~~ Mrs. SANDHYA PAPABATHINI

has presented (Oral ✓ / Poster Presentation)

at **PARACON - 2017** National Conference on

Parasitology organised by

Department of Microbiology,

Sree Balaji Medical College and Hospital,

Chennai on 04th Feb. 2017

ACCREDITION

The TN Dr. M.G.R. Medical University has Awarded 20 Credit Points for Participating in this National Conference and workshop in category-III.

Dr. D.R.Gunasekaran	**Dr.P.Saikumar**	**Dr. Chitralekha Saikumar**
Dean, SBMCH.	Vice Principal, SBMCH	HOD, Dept. of Microbiology. SBMCH.
Conference Chairman	Conference Vice Chairman	Organising Secretory

MICROCON KC 2017

INDIAN ASSOCIATION OF MEDICAL MICROBIOLOGISTS (KC)
DEPARTMENT OF MICROBIOLOGY,
Vijayanagar Institute of Medical Sciences, Ballari

Certificate

This is to certify that

Mrs. SANDHYA PAPABATHINI

bearing Reg. No. - registered with - , Address **BMPMC, Vijayapura** has participated as Delegate/ ~~Chairperson~~/ Presented a Scientific Paper (~~Oral~~/Poster) in the **XXI Annual Conference of IAMM (KC)** conducted at VIMS, Ballari on 18th February 2017.

Karnataka Medical Council has granted 2 credit hours vide letter KMC/CME/929/2016 dated 03.12.2016

Zonal Chairman	Dr. Surekha Y A	Dr. Krishna S
Karnataka Medical Council	Organising Secretary	Organising Chairman
CME Accreditation Committee	Department of Microbiology	Department of Microbiology
	VIMS, Ballari	VIMS, Ballari

PUBLICATIONS

Sandhya.P et al., A Study on Cryptosporidium Parvum In HIV/AIDS Sero-Positive Patients in Raichur International Journal of Recent Scientific Research Vol. 8, Issue, 10, pp. 21244-21246, October, 2017 DOI:http://dx.doi.org/10.24327/ijrsr.2017.0810.1036(Pub med).

Sandhya.Pet al.2017, Comparative Study of Cryptosporidiosis By Modified Acid Fast Stain And Elisa Among HIV/AIDS Sero-Positive Patients. Int J Recent Sci Res. 8(12), pp. 22132-22136. DOI: http://dx.doi.org/10.24327/ijrsr.2017.0812.1207 (Pub med).