University Of Benghazi
Faculty Of Science
Department Of Zoology

Incidence Of Intestinal Parasites Among HIV- Infected Patients In Benghazi , Libya .

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Zoology.

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Benghazi , Libya

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أطروحة مقدمة كجزء من متطلبات الأجازة العليا (الماجستير) في العلوم

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بنغـــــازي – ليـبيـــا

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بسم الله الرحمن الرحيم

﴿وما أوتيتم من العلم إلا قليلاً﴾

صدق الله العظيم

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INTRODUCTION

INTRODUCTION

Human immunodeficiency virus (HIV) infection, is a worldwide infection and a serious public health problem in the present . A high rate of infection is found in many regions of the world . Gastrointestinal infections are very common in patients with HIV infection or AIDS (Surasiengsunk .,1998).

Current estimates showed that at least more than one-quarter of the world's population is chronically infected with intestinal parasites, which may play a significant role in morbidity due to intestinal infection and most of these infected people live in developing countries. However, intestinal parasites once considered to be controllable in developed countries still remain a major cause of morbidity and mortality worldwide (Fincham, and Adams 2003 and UN AIDS ,2007).

Parasitic infections of the gastrointestinal tract are a major cause of morbidity in developing countries and are increasingly important in certain populations from the developed countries, particularly in AIDS patients . Several species of intestinal parasites have been associated with diarrhoea which occurs with HIV infection. (Arora and Arora, 2009).

Approximately 40 million people are HIV infected, out of which 90% of infections in sub-Saharan Africa , Asia and South America. Co-infection between HIV and parasitic infections are common diarrhoea is a common clinical presentation of these infections. Reports indicate that diarrhoea occurs in 30-60 per cent of AIDS

patients in developed countries and in about 90 per cent of AIDS patients undeveloping countries (Framm and Soave ,1997) .

In particular, opportunistic protozoans play a major role as etiologic agents of chronic diarrhea in HIV-infected patients. Chronic diarrhea in such patients can profoundly compromise the absorptive function of the small intestine, and cause significant mortality (Escobedo and Nunez, 1999).Diarrhea is one of the most common AIDS-related illnesses causing a significant morbidity and mortality in HIV infected patients () , and is a common complication of HIV positive patients inducing weight loss and cachexia. (Mukhopadya *etal*.,1999 and Siddiqui *et al*.,2007) .

The objectives of this study to :-

A- Determine the incidence of intestinal parasites in HIV infected patients who were admitted or treated in the National Hospitals in Benghazi ,Libya.

B- Study the relationship between incidence and sex age .

LITERATURE REVIEW

2. LITERATURE REVIEW

2.1.AIDS:

The AIDS and HIV so-called epidemic is currently one of the most critical medical, social and governance issues facing the world. AIDS has been recognized worldwide since1989. There are two main types of HIV: type 1 (HIV-1) is the most common, while, HIV type-2 (HIV-2) found predominantly in Africa (Biggar, 1986 and Bradbeer,1986). While HIV-1 prevalence is increasing in many areas, the prevalence of HIV-2 has remained fairly stable, and the clinical course of HIV-2 disease is slower than that of HIV-1. double infection with HIV-1 and HIV-2 is possible, although it has been suggested that HIV-2 infection may confer some protection against HIV-1 acquisition(Berkelman *et al .,* 1989). Although mother-to-child transmission of HIV-2 has been documented, this occurs less frequently than with HIV-1 (Clavel *et al .,* 1986).

2.2. Human immunodeficiency virus (HIV-1) infection in Libya :

The first cases of HIV infection occurred in the 1980 , among hemophiliacs as a result of Factor VIII imported from Europe. Before 1998, the large majority of infections were reported among foreigners. In 1998, a severe HIV/AIDS infection occurred at Benghazi children's hospital due to contaminated blood products/ parenteral exposure . A total of 453 persons, mostly children, were infected and 53 have died.

The Libyan health system and the society as a whole faced major difficulties to react in an effective way to the infection, leaving a large number of families in distress (Giasuddin ,1991) .

2.3. Intestinal parasitic infections in Libya :

Like in many other developing countries, intestinal parasites are widely distributed in Libya largely due to the low level of environmental and personal hygiene, contamination of food and drinking water that results from improper disposal of human excreta (WHO, 1981and WHO, 1987). In addition, lack of awareness of simple health promotion practices is also a contributing factor(WHO, 1981and WHO, 1987) . In Libya the first report was published by Dar and Friend **,**1979 on the intestinal parasites in Benghazi school children . Later, El-Boulaqi *et al* ., (1980) from Libya studied the prevalence of intestinal parasites in primary school children in Benghazi **.** Several other studies on intestinal parasites in Libya (Al-Tawaty *et al* .,1998 ; El –Buni and Khan , 1998 ; Bugharara *et al* .,1999 ; Raof ,2002 ; Ibrahium , 2003 ; Burshan ,2004 ; El-Sanousi , 2006; El-Sanousi , 2007 Karor ,2007 ; Sadaga and Kassem ,2007) .

But So far no study on intestinal parasites among HIV seropositve patients was done in Libya .

2.4. Intestinal parasitic infections in HIV/AIDS patients:

Intestinal parasitic infections are the commonest and the major causes of morbidity and mortality in HIV positive patients, worldwide (Gupta *et al*.,2008) . These organisms usually cause a self limiting illness in immunocompetent individuals, but in the case of immune compromised

patients, they can cause life threatening, profuse watery diarrhoea (Meisel *et al .,* 1976).

The public health importance of intestinal parasites as a major concern in most developing countries has been pronounced with the co-occurrence of malnutrition and HIV/AIDS. With HIV/AIDS pandemic, many intestinal parasites, previously considered to be sporadic or zoonotic infections, have become opportunistic parasites causing uncontrollable and life- threatening diarrhoea (Wittner *et al*., 1993).

As compared to developed countries, the prevalence of opportunistic intestinal parasites is expected to be higher in developing countries among HIV infected population. This is also reflected by the prevalence of opportunistic intestinal parasites in a given geographical locality among the general population (Lindo *et al,* 1998 and Cimerman *et al.*, 1999). HIV infection has been shown to predispose the patient to intracellular opportunistic intestinal parasites (Wittner *etal*.,1993; and Goodgame, 1996). This does not seem to be the case with extracellular intestinal parasites.

Some studies have indicated that compared to the general population, there is relatively lower prevalence of non-opportunistic extracellular intestinal parasites in HIV/AIDS patients (Gomze Morales *etal.,* 1995 and Lindo *etal*, 1998). Although differences in exposure may not be ruled out, it is suggested that HIV- induced entropathy may not create conducive environment for the establishment of extracellular intestinal parasites (Wittner *et al*. 1993). Others have also argued that mucosa dwelling parasites may benefit from HIV-induced pathological changes and the reduced immune response due to HIV infection, which creates suitable

environment for opportunistic intestinal parasites in HIV/AIDS patients (Goodgame, 1996).

Most clinical manifestations of HIV/AIDS patients results either from the reactivation of pre-existing latent pathogens, as the individuals become immunosuppressed or is caused by exposure to locally predominant pathogens. Consequently, clinical presentations of AIDS and the pathogens responsible in different geographical areas reflect the differing prevalence of opportunistic intestinal parasitic infections in a given community (Colebunders *et al*,, 1988 and Lindo *etal*, 1998).

Several studies shown that intestinal parasitic infection are amongst the most widespread of all human immunodeficiency virus (HIV) (Tarimo *et al* ., 1996 ; Escobedo and Nunez , 1999 ; Wiwanitkit ,2001 ; Mohandas *et al* ., 2002 ; Guk *et al* ., 2005 ; Moges *et al*., 2006 ; Oguntibeju, 2006 ; Saksirisampant *et al*., 2009 ;Al-Megrin , 2010; Akinbo *et al*., 2010 ;Dehkordy *etal.(*2010) , Etok *et al.*, 2010 ; Getaneh *et al.,* 2010 ; Abaver *et al* . , 2011 ;Akinbo *et al*., 2011;Alemu *et al.*, 2011 ; Amatya *et al* ., 2011 ; Asma *etal* ., 2011 ;Deorukhkar *et al* ., 2011; Ochigbo *et al*., 2011 and Stensvold *et al.* ,2011).

2.5. Diarrhoea and HIV/AIDS:

Diarrhoea is a common clinical manifestation of HIV infections both in the developing (90%) and the developed (30-50%) countries (Colebunders *etal*., 1988). Its cause could be parasitic (commonly opportunistic intestinal parasites) (Germani, *etal*., 1998; Kelly, 1998). Chronic diarrhoea lasting for more than one month is one of the major complaints of AIDS patients

occurring in about 40% of cases and it is one of the WHO-stagingcriteria for AIDS (WHO, 1993).

It has been shown that at least 40-80% of AIDS patients report diarrhoeal episodes during their illness (Kelly, 1998). About 50% of chronic diarrhoea in AIDS patients may be explained by enteric infections with one or more species of pathogenic organisms, commonly opportunistic ones (Bartlett *et al*, 1992 and Prasad *et al.,* 2000). Gut architectural alteration secondary to local HIV infection, (usually referred to as HIV entropathy) a condition characterizing chronic diarrhoea in AIDS patients in whom no identifiable aetiological agent has been found for the diarrhoea (Kotler *etal*.,1984 and Bartlett *et al*, 1992). Patients with this syndrome have malabsorption, and small bowel histology revealing villous blunting and chronic inflammation (Kotler *etal.,* 1984 and Tadesse and Kassu, 2005).

There is another view that enteropathy syndrome may not represent a direct effect of HIV on gut mucosa, but rather could be due to opportunistic enteric pathogens that are difficult to detect and therefore as yet undiagnosed (Colebunders *et al*., 1988). It is also held that HIV related entropathy is not only the cause of unexplained diarrhoea, but may also create favourable environment for the invasion of intracellular opportunistic intestinal parasites (Chintu *etal*., 1995 and Germani *etal.,* 1998 and Boral *et al*., 2004).

Types of intestinal parasites in HIV/AIDS patients:

HIV/AIDS pandemic has brought about a great change in intestinal parasite fauna. As the spectrum of immunodeficiency progresses, HIV

infected individuals become susceptible to a variety of opportunistic parasite infections that occur with greater frequency and severity. Almost 80% of AIDS patients die from AIDS -related infections including intestinal parasites rather than HIV infection itself (Kelly, 1998 and Adamu and Petros , 2009). Several intestinal parasites previously considered non-pathogenic or with transient pathogenic potential in immunocompetent individuals are opportunistically becoming aggressive and causing debilitating illness in HIV/AIDS patients. Most of these infections are caused by organisms that do not normally affect immunocompetent individuals (Kaplan *et al*., 1996). The principal pathogenic intestinal parasites commonly reported as opportunistic and that cause chronic diarrhoea in HIV/AIDS patients are *Cryptosporidium parvum, Isospora belli*, *Cyclospora cayetanensis* and intestinal microsporidia (*Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*). These infections usually occur late in the course of HIV infection (Chintu *et al*.,1995; Tarimo *etal*., 1996 ; Brandonisio *et al.*, 1999 ; Cimerman *etal*., 1999; Sarfati *et al.,* 2006 ;Stark *et al*., 2007 and Aminuan and Yakubu, 2008).

a) *Cryptosporidium parvum*:

Cryptosporidium species are very small intestinal protozoa. They are dwelling in the stomach or in the small intestine of mammals, birds and reptiles. This apicomplexa parasite infects humans and animals, globally. Up to now eight valid *Cryptosporidium* species have been reported to be capable of infecting humans (Rimhanen-Finne, *et al*., 2009). *Cryptosporidium* has been initially reported in a mouse in 1912 by Tyzzer, but its impact on animal health has not been recognized until the early 1970, when association with diarrhea in calves mainly in the first 30 days

of age has been observed (Panciera *et al*., 1971). It can cause gastrointestinal illness in a wide variety of mammals, including humans, cattle, sheep, goats, pigs and horses worldwide (Fayer *et al*., 1997). The routes of transmission of this parasite can be person to person through direct or indirect contact, animal to animal, animal to human, waterborne through drinking water or recreational water, food borne and/or possibly air borne (Fayer *et al*., 2000 and Musa *etal*., 2011).

It is an intestinal pathogen having a zoonotic nature and responsible for clinical disease in mammalian species commonly infecting human beings. *C. parvum* has been reported in about 80 different animal species including cattle, pigs, horse, sheep and goats (Dubey, *e t al*., 1990). The first case of human infection with *C. parvum* was reported in 1976 (Mosier and Oberst, 2000 and Getaneh *et al* ., 2010). Since then, it is an increasingly recognised agent of intestinal infection as a common cause of severe diarrhoea in immunocompetent and immunocompromised humans and domestic animals (Hunter and Nicholes, 2002 and Dehkordy *et al.*, 2010). The oocyst measures 4-6m. in diameter. The major clinical symptoms are watery diarrhoea, malabsorption and wasting syndromes The severity of the disease depends on the immune status of the individuals (Martins and Guerrant, 1995; Botero *et al*., 2003 ; Ghimire *etal* 2004 and Gatei *etal.*,2008).

The nature of diarrhoea is usually secretory or malabsorpative, voluminous, intractable, watery and often cholera-like (Clark and Sears, 1996). Mucus may be associated with diarrhoea, but blood or leukocytes are rarely reported in AIDS patients with cryptosporidiosis. About 2 to 7 liters per day (sometimes more) of diarrhoea have been reported (Manabe, *etal*., 1998). Severe weight loss has been reported due to debilitating

diarrhoea (Mengesha, 1994). Abdominal pain with cramps, low-grade fever, vomiting, nausea and anorexia accompanies the diarrhoea (Clark and Sears, 1996 and Lindo *etal*., 1998). The diarrhoea can be bright yellowish-green in colour, offensive and may contain mucus (Martins and Guerrant, 1995). It has been estimated that in AIDS patients with diarrhoea, the association of *C. parvum* ranges from 10 to 30% in the developed countries and 30 to 50% in the developing world (Petersen, 1993). *C. parvum* has also been implicated in the etiology of extraintestinal cryptosporidiosis causing acalculous cholecystitis, sclerosing cholangistis and pancreatitis in HIV-infected patients (Le *et al*., 2008 and Lopez-velez, *etal*., 1995). In general *C. parvum* is the most common opportunistic protozoan parasite that is at present well established and documented as a worldwide cause of diarrhoea in immunosuppressed individuals in general and HIV/AIDS patients in particular. It is now an AIDS -defining illness as it is involved with chronic diarrhoea (longer than one month) and the most common cause of enteric disease in HIV/AIDS patients (WHO, 1993).

Cryptosporidium parvum is also an increasingly recognized agent of intestinal infections in immunocompetent humans (Kuhls *et al* ., 1994). Human infections in healthy individuals, are manifested with symptoms that are self-limited and usually last less than a month. In HIV/AIDS patients and immunocompromised individuals, diarrhoea can persist for months (or even for life) and eventually may become life threatening (Clark and Sears, 1996). Transmission of *C. parvum* occurs by ingestion of infective oocysts through mainly faecal-oral route of contamination, by human-to-human, animal-to-human or environmentally such as in water-borne outbreaks (Griffiths, 1998).

Nosocomial transmission (i.e. in hospital room mates) has also been described. An outbreak of cryptosporidiosis in a hospital in Copenhagen

had affected some 18 HIV-positive patients. The source of the outbreak was identified as ice from an ice machine in the ward, contaminated by an incontinent psychotic patient with cryptosporidiosis who was using his hands to pick out ice for cold drinks (Bruce *et al.,* 2000). In different parts of the world, *C. parvum* has reached the public health domain when it became widely recognised as the most serious and difficult to control cause of water-borne diarrhoea. This was confirmed by the major outbreak in Milwaukee, Wisconsin in1993 (Griffiths,1998).

b) *Blastocystis hominis*:

Diarrhoeagenic intestinal parasite that were not recognized as such up to the recent past are emerging and increasing these days. The problem has become more serious with onset of HIV/AIDS pandemic. Among these the status of *B. hominis* as a cause of diarrhoea is a controversial and not well-documented one (Zierdt, 1988). Although *B. hominis* is often the most frequently reported from stool samples, its epidemiology is not clearly understood. The reason behind this could be lack of appropriate information about the epidemiology of the parasite, conflicting and paradoxical ideas on its classification and pathogenicity. Based on ultrastuctural and structural evidences, *B. hominis* has now been classified under protozoa (Stenzel and Boreham, 1996).

Some investigators have presented strong evidence for its pathogenicity .while others have considered it to be a commensal. Reports of asymtomatic and symptomatic *B. hominis* infections in humans are worldwide. *B. hominis* infections are predominantly reported from developing countries of tropical and subtropical regions. Travellers from

the developed countries might be affected with *B..hominis* infection when they travel to these regions of the world. (Shlim *et al*., 1995).

The infection rate has been reported to vary from 1.6% in industrialized countries to more than 50% in developing countries (Gericke, *et al*.,1997). Well recognized clinical signs due to *B. hominis* in symptomatic individuals, include abdominal discomfort or pain, diarrhoea, nausea, vomiting flatulence, gastroenteritis, colitis and other minor complaints (Stenzel and Boreham, 1996). However, whether or not *B. hominis* can act as an etiologic agent of enteritis has been debatable. At least two reasons for the controversy are evident. The organism, like other controversial agent of enteritis such as yeast (*Candid abacus*), can be found in individuals without evident pathogenic manifestation or abdominal discomfort. There is also a strong evidence to show that clinical manifestation of illness due to *B. hominis*, when large numbers of organisms mostly greater than five parasites per high power field (>5/HPF) are seen in the presence of symptoms and in the absence of other well recognised viral, bacterial or parasitic agents(Telalbasic *et al*.,1991).

Infection with *B.hominis* has gained attention in case of immunocompromised individuals and HIV/AIDS patients (Libre *etal*., 1989; and Escobedo and Nunez, 1999). The association of *B.hominis* with diarrhoea in immunosuppressed patients has been suggested in one study among Tanzanian children with chronic diarrhoea. Furthermore, molecular and immunological evidences have revealed that strain variation might be associated with pathogenic potentials (Clark, 1997 and Kaneda *et al*., 2002). It is generally accepted that *B. hominis* is transmitted by faecal-oral contamination, in a manner similar to other gastrointestinal protozoa

(Stenzel and Boreham ,1996 Leelayoova, *et al*., 2004). Other protozoan parasites such as *Cryptosporidium, Isospora, Cyclospora* and microsporidia, which were previously considered to be non-pathogenic or to have low pathogenicity, are also recognised as causes of human diseases, especially in immunosuppressed individuals and HIV/AIDS patients. Unlike the above mentioned opportunistic intestinal parasites, the possible association of *B. hominis* infection with HIV/AIDS is not well documented. Thus, during the investigation of the etiology of gastrointestinal diseases the relationship of *B. hominis* infection in case of HIV/AIDS patients and immunocompromised individuals has to be considered.

c) *Cyclospora cayetanensis*:

Another newly defined coccidian opportunistic intestinal parasite in humans is *C. cayetanensis*. Cyclosporiosis is characterized by mild to severe watery diarrhoea, nausea, anorexia and abdominal cramps. It has been described from HIV/AIDS patients with protracted diarrhoea (Sifuenes-Osonio, *etal*., 1995). In immunocompetent individuals diarrhoea appears to be prolonged but self-limited, lasting from just over one week to a mean duration of about three weeks. Although infections appear eventually to resolve spontaneously, in some cases, both in immunosuppressed and immunocompetent individuals. But in AIDS patient recrudescence of the symptoms is a major problem (Curry and smith, 1998) *Cyclospora* resembles *Cryptosporidium*, but the size varies ranging from 8-10 m with 2 sporocysts and having 2 sporozoites in each sporocyst, whereas *Cryptosporidium* measures 4-6m in size and has 4 naked sporozoites (Curry and smith, 1998).

d) *Isospora belli*:

Isospora belli is another well-defined coccidian opportunistic intestinal parasite in HIV/AIDS patients and in some areas it is the cause of gastroenteritis (Lumb and hardiman, 1991). It mostly causes watery diarrhoea and weight loss. Diarrhoea produced by *I. belli* infection in AIDS patients is often secretory-like, without blood and leads to dehydration; low grade fever, eosinophlia, abdominal pain, vomiting and malaise are some of the symptoms reported (Bartlett *et al*, 1992; Lindsay *et al*., 1997and Lainson and Da silva,1999).

Isospora belli infections are essentially cosmopolitant in distribution but are more common in tropical and subtropical regions, especially Haiti, Mexico, Brazil, El Salvador, tropical Africa, the Middle East and South East Asia. In developed countries immigrants are suspected as introducers of the disease (Sorvillo *et al*.,1995 and Curry and smith, 1998). Chronic infections are developed in some patients and oocysts are excreted for a long duration, several months to years (Lindsay *etal* 1997). This information has important implication in the era of HIV/AIDS pandemic. Most oocyts of *Isospora* are excreted unsporulated and undergo a developmental period sporulation outside the host and become infectious. Sporulated oocyst of *I. belli* are characterized by having two sporocysts and each sporocyst in turn containing four sporozoites. The oocysts of *Isospora belli* in humans measure 20-33 µm by 10-19 µm. They are elongated and ellipsoidal (Curry and smith, 1998). Transmission in humans occurs via faecal-oral route, mainly by ingestion of infectious oocysts from contaminated food and/or water. Diagnosis depends on microscopic identification of oocysts in the stool. Like *Cryptosporidium*,

the oocysts of *Isospora* are acid fast during staining, but differ from oocyst of *Cryptosporidium* by their size and shape.. In HIV/AIDS patients recurrence of infections are commonly reported (Verdier *et al*., 2000) .

e. Three genera of amoeba may inhabit the intestinal tract of humans: *Entamoeba, Iodamoeba and Endolimax.* Members of these genera considered non-pathogenic include *Entamoeba hartmani, Entamoeba gingivalis, Entamoeba coli, Endolimax nana*, and *Iodamoeba butschlii* (Mahon and Manuselis 2000 ; Raccurt *et al*., 2006)

Entamoeba histolytica: This parasite was named for its remarkable ability to lyse human tissues. A requirement to amoebic invasion is the parasite's ability to colonize and penetrate colonic mucins overlying the intestinal epithelium (Chen *et al*., 2007).

Entamoeba histolytica infection is one of the most common parasitic infections worldwide, infecting about 50 million people, often in developing countries, resulting in 40,000 to 100,000 deaths per annum It has long been known that although about 500 million people each year have amoebiasis, only about 10% experience symptomatic disease (WHO, 1997; Ayeh-Kumi *et al.*, 2001).

It is probable that 90% of the infections previously ascribed to *E. histolytica* are actually *E. dispar*, while only the remaining 10% are *E. histolytica* infections (Zaki and Clark, 2001). Mora *et al.,* (2008) found that the *E. histolytica/E. dispar* prevalence rates according to the direct, Ritchie and trichromic staining methods were 20.09%, 13.79% and 12.15%, respectively; while prevalence rates according to PCR for *E. histolytica* and

E. dispar were 6.31% and 4.44%, respectively, in a study they conducted in Venezuela. Direct stool examination from 134 individuals with diarrhoea detected *E. histolytica/E. dispar* in eight (6%) whiles analysis by PCR showed *E. dispar* in ten (7.5%) and *E. histolytica* in two cases (1.5%) in a related study conducted in Nicaragua (Leiva *et al.*, 2006). In northern Ghana, (Verweij *et al*., (2003) showed a high prevalence (39.8%) of *E. histolytica*/*dispar* complex by microscopy but 82.8% of *Entamoeba dispar* and only one case of *Entamoeba histolytica* by PCR. *Entamoeba histolyticais one* of the most common intestinal protozoan parasites infecting humans worldwide. They are known to be the most important diarrhoea-causing protozoa (Marshall *et al.*, 1997 and Tsai *et al*., 2006). It is estimated that 10% of the world's population is infected with *E. histolytica* with the highest prevalence in developing countries (Fontanet *etal*., 2000;Vandenberg *et al.*, 2006).

f. Intestinal Microsporidia :

Other important intestinal parasites in HIV/AIDS patient are the microsporidia. They are ubiquitous, obligate intracellular spore forming protozoan parasites increasingly detected as opportunistic pathogens in HIV/AIDS patients. Since the onset of HIV/AIDS pandemic, a number of parasitic microsporidian pathogens of humans have been recognised. The frequency with which they are encountered and reported in clinical practice and the intensity of infections with opportunistic microsporidian parasites in AIDS patients have tremendously increased (Goodgame *et al*, 1996).

Up to date, at least, thirteen species of microsporidia have been reported to infect animals (Didier *et al*., 1998). Among these are six known genera that have been associated with human disease, namely *Enterocytozoon,* and *Encephalitozoon* (Weber *et al*., 1994). Infection in humans can occur in different tissues including the small intestine, kidney, cornea and liver with various clinical manifestations. Before 1985, however reports of clinical disease related to intestinal microsporidial infection in humans were rare. Since 1985, when *Enterocytozoon bieneusi* was identified as an aetiological agent of diarrhoea (Desportes *etal*., 1985), clinical syndromes associated with intestinal microsporidiosis in AIDS patients were frequently reported (Kotler and Orenstein, 1998). Diarrhoea and malabsorption are the most common clinical syndromes associated with intestinal microsporidial infections in AIDS patients (Weber *et al*., 1994)

Infection by *Enterocytozoon bieneusi* and/or *Encephalitozoon intestinalis* have been identified as main causes for watery diarrhoea and wasting syndrome in AIDS patients (Asmuth *et al*.,1994). In Libya, so far, there is no information concerning intestinal microsporidial infection in HIV/AIDS patients.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3. 1. Study area:

Benghazi is the second largest city in Libya it is situated on the north of grate Libyan desert and on the southern coast of Mediterranean sea. (Fig.1) It was founded in fourth century B.C. occupying an area of approximately 43, 535 km^2. Benghazi has Mediterranean climate with moderate winter and hot summer. The monthly average of temperature (19.5 C ^{o} to 34.4C ^{o}) , Rainfalls in millimeters (0.0 to 38.3). Relative humidity 50 to 70% . (according to Benina meteorological station, 2010) .

3.2.1. Study design and patients: -

Across- sectional study was carried out on patients admitted or treated to the HIV wards at Benghazi hospitals (HIV Center , Isolation center , central medical laboratory in Al- Jamhouria hospital and 7 October hospital) during the period from February to December 2010, Four hundred fifty patients, their ages ranged between 5 to 40 years with or without diarrhea , were asked to participate in this study, out of the total number three hundred AIDS confirmed patients (HIV seropositive) (153 females and 147 males) , and one hundred fifty were HIV seronegative (the seronegative patients were used as control groups (51 females and 99 males) .

Figure (1) : Map of Benghazi city - showing place of the study .

3.2 .2 Questionnaires:

A questionnaire was distributed to patients and out patients admitted to HIV wards , The questionnaire was prepared to collect socio-demographic and clinical data from each patient including age, gender , nationality , HIV positive or negative were recorded for each participant . (Index)

3.2.3. Collection and preparation of sera:

Serum sample of each patient was separated from whole blood (plain or EDTA) , 5 cc tube . were obtained from enrolled . Either fresh serum or plasma can be used for this assay. Blood was collected by venipuncture should be allowed to clot naturally and completely the serum / plasma was separated from the clot as early as possible to avoid hemolysis of the RBC, care should be taken to ensure that the serum samples are clear and not contaminated by microorganisms . Any visible particulate matters in the sample should be removed by centrifugation at 3000 RPM for at least 20 minutes at room temperature, blood (serum plasma) then transferred into a plain tube with caps until required for immunoglobulin analysis. Samples were stored at 2-8 C^{o} . Non required samples for assaying within 3 days should be stored frozen (- 20 C^{o} or below) . The patients serum samples were used for HIV testing . HIV serostatus of the patients was determined by using commercially available ELISA using National AIDS Control Organization (NACO,2007) of HIV antibodies. In order to confirm the infection and non-infection with HIV.

3.3.2.1 Enzyme linked immunosorbent assay (ELISA) for qualitative detection of antibodies to Human Immunodeficiency Virus (HIV) :

HIV 1+2 antibodies enzyme immunoassay test kit was purchased from PRECHEK Bio , Inc (215 N , State college Bivd .k, Anaheim CA92806 , USA .WWW. Prechekbio . com) (Plate 1a) . The material provided with the kit are microwell plate wells(12 × 8 well strips per plate) Each well coated with HIV1+2 antigens , HRP(Horseradish peroxidase) – conjugate reagent , negative and positive control , Wash buffer , chromogen solution A chromogen solution B , stop reaction (Diluted sulfuric acid solution 2.0 M H2SO4). The kit stored at 2-8 ^{o} C (Plate 1b)

The test was done according to the following manufacturer procedure (in brief)

1- Add 100 µ l of positive control, negative control and specimens into their respective wells.

2- Incubating (1): cover the plate with the plate cover and incubate for 30 minutes at 37 ^{o} C

3- Washing (1): at the end of the incubation remove and discard the plate cover. Wash each well 5 times with diluted wash buffer. Each time, allow the micro wells to soak for 30-60 seconds . (Plate 2) .

4- Adding HRP – conjugate .Add 100 µ l into each.

5- Incubating (2): cover the plate with the plate cover and incubate for 30 minutes at 37 ^{o} C.

6-Washing (2) After end of the incubation remove and discard the plate

cover . Wash each well 5 times with diluted wash buffer . Each time , allow the microwells to soak for 30-60 seconds. (Plate 2) .

7-Dispense 50 µ l of chromogen A and 50 µ l of chromogen B into each well and incubate for 15 minutes at 37 ^{o} C.

8-Add 50 µ l stop reaction solution into each well and mix gently for 30 second.

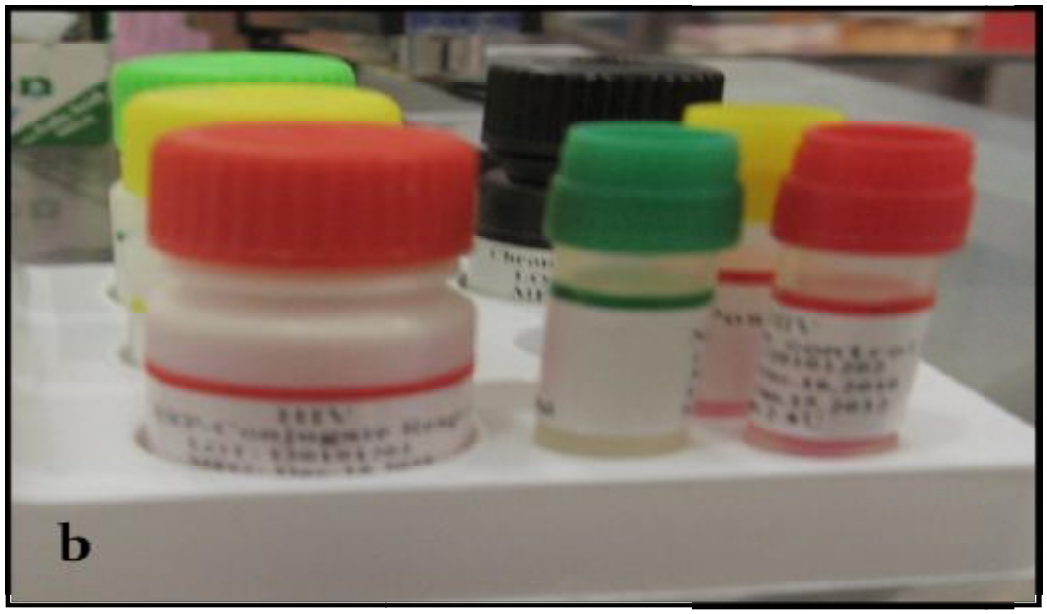
9-Read the absorbance at 450 nm within 5 minutes with microwell reader. (Plate 3) .

HIV 1+2 Ab index was calculated by dividing the optical density of sample by optical density of cut-off calibrator.

HIV 1+2 Ab index = **cut-off value (c.o.) = *NC+0.1**

Interpretation of the results.

- **Positive result ≥0.500.**
- **0.300 to 0.499 are indeterminate and need to be retested.**
- **Negative ≤0.300.**



Plates 1 : (a and b): Kit Enzyme linked immunosorbent assay (ELISA) (HIV) .

Plate (2): Microplate washer SAGA

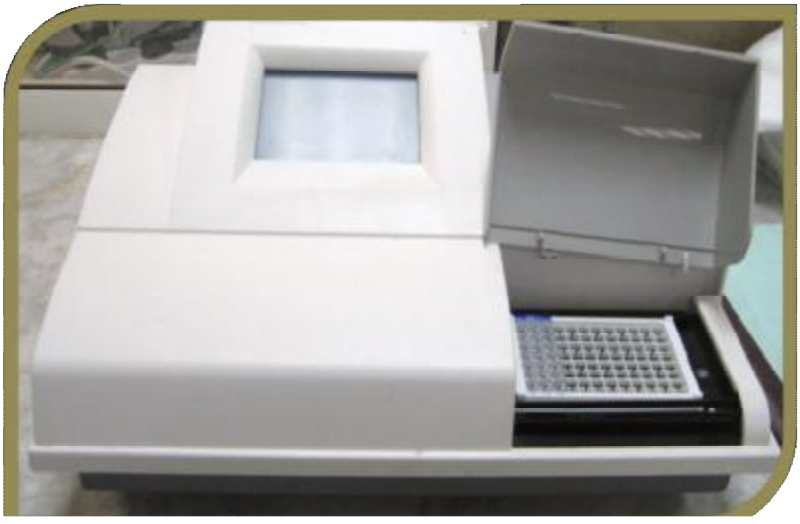


Plate (3): Microplate reader

3.3. 3. Collection of stool samples :

Four hundred and fifty stool samples were collected from patients and outpatients from Benghazi hospitals (HIV center , Isolation center and central medical laboratory in Jamhouria and 7 October hospitals)

A single fecal sample from each patient was collected in a dry, clean, leak-proof plastic container. Half of each sample was stored in 10% formalin to prevent bacterial action (Mohandas *et al.,* 2002). All samples were concentrated directly or stored in refrigerator at 4ºC for further use. Each sample was labeled with the patient's name and place of collection.

3.3.3.1. Macroscopic examination:

Stool specimens were examined by naked eye for appearance color, consistency and presence of blood mucous and adult or larvae helminthes (Chessbrough,2004).

3.3.3.2. Microscopic parasitological examination

3.3.3.2. Direct microscopy method (wet mount):

I. Direct Normal saline solution (0.85% NaCl solution):-

To confirm the presence of motile intestinal parasites and trophozoites under light microscope (40X magnification).

Procedure :-

One drop of normal saline mixing with small amount of faeces until mixture become a uniform suspension under 22x22 mm cover slip .the entire cover slip area was examined using low power (10x) then high power (40x) and (100x) objectives .

II. Direct wet mount Iodine preparation . (Markell *et al*, 1999 and Nobale, 1994): -

Procedure:

One drop of gram iodine was put in the middle of the slide, About 2 mg of stool sample was taken from the faecal sample and then mixed with the drop of iodine, then covered with cover glass, At first the examination started by using 10 x eye-piece to have a general view of the slide, then more comprehensive examination, was done using 40x objective. The procedure was repeated when indicated each field examined by the 40x objective to check for the presence intestinal parasites .

Lugol's iodine :

was used to stain protozoan cysts and trophozoite of intestinal parasites.

3.3.3.2. Formalin - ethyl acetate sedimentation technique: (Markell *et al*.,1986) :-

This technique was performed as recommended by the Centre of Disease Control and Prevention (2003). Each sample was concentrated by formalin - ethyl acetate sedimentation method prior to microscopic examination. The concentration procedures maximize the recovery of oocysts by separating parasites from fecal debris to increasing the chance of detecting protozoan parasite oocysts.

Procedure :

1- The specimen was mixed well .

2- 5 ml of the fecal suspension were strained through wetted cheese cloth-type gauze placed over a disposable paper funnel into a 15 ml centrifuge tube .

3- Saline (0.85%) was added through the debris on the gauze to bring the volume in the centrifuge tube to 15ml .

4- Sample was centrifuged at 500 x g for 10 minutes .

5- Supernatant was decanted, then 10 ml of 10% formalin was added to the sediment and mixed thoroughly with wooden applicator stick .

6- 4ml of ethyl acetate were added; the tube was stoppered, and shook vigorously in an inverted position for 30 seconds .

7- Each sample was centrifuged again at 500 x g for 10 minutes .

8- The plug of floating debris was removed from the top of the tube by ringing the sides with an applicator stick. The top part of supernatant was decanted .

9- A cotton-tipped applicator was used to remove debris from the sides of the centrifuge tube .

10- Five drops of 10% formalin were added to resuspend the concentrated specimen. After samples were concentrated, fecal materials were analyzed microscopically for intestinal parasites at 4x and then on 40x or 100x with oil .

3.3.3.2.3. Modified Zeihl-Neelsen Staining Method:

Modified Ziehl-Neelsen technique (acid-fast stain) used for detection of oocysts of opportunistic coccidial intestinal parasite- *Cryptosporidium* spp, *Isospora belli* and *Cyclospora cayetanensis* (Current and Garcia ,1990) .

Procedure:-

Thin smears were prepared directly from fresh faecal samples as well as from sediment of concentrated stool and allowed to air dry , Fixed with absolute methanol for a few seconds, then air –dried and stained by Zeihl-Neelsen acid-fast stain the same procedures were used for smears prepared after concentration. Smears were prepared from the concentrated stool samples and were stained as described by Adegbola *et al*.(1994) with some modifications. In this technique, the slides were stained with carbol-fuchsine for 30 minutes and then washed with tap water. The slides were decolorized in 1% HCl acid-alcohol for 1 minute and counterstain with 0.25% malachite green (or methylene blue) .for 30
sec. Rinse in tap water, the stained smears were microscopically examined using 100x magnification .(Endeshaw *et al* ., 2004) .

Plate (4) : Modified Zeihl-Neelsen (acid-fast stain)

Plate (5) : Staining Zeihl-Neelsen technique .

3.3.3. Statistical analysis:

Statistical analyses were carried out to determine the incidence and significance of the data. The logistic regression (Dowdy *et al.,* 2004) used to find the relationships between the parasitic infection age, sex and nationality of patients . incidence was calculated as the percentage of infected HIV" seropositive" and participates with intestinal parasites.

Chi-squar X^2 was employed to find out the significance or non-significance of the relationships between age, sex, diarrhea , and the presence or absence of the parasites.

The accepted level of significance $P < 0.05$ was considered significant during the using the test. All analysis were computed in windows environment of statistical of program (SPSS) version 17.0 .

RESULTS

4. RESULTS

4.1. Incidence :

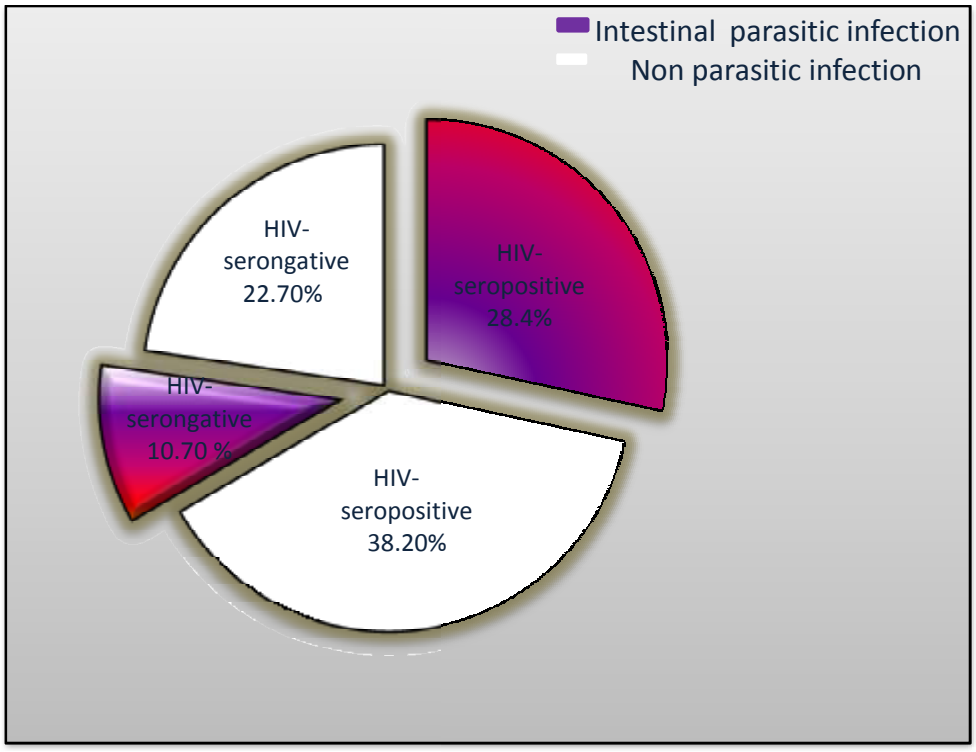
The present study was carried out on four hundred fifty patients comprising three hundred HIV –seropositive patients and one hundred fifty were HIV- seronegative . The results revealed that the overall incidence of infection with intestinal parasites in HIV- seropositive was (28.4%) higher than HIV- seronegative group (10.7%) (Fig.2) . The results showed that one hundred and twenty eight (42.7%) out of HIV-seropositive patients and forty eight (32.0%) out of HIV- seronegative with a total one hundred seventy six (39.1 %) of both groups out of the total examined were infected with intestinal parasites (Table 1 and Fig. 3)

The results showed that there was a significant difference between parasitic infection in HIV- seropositive (P=0.001) and between parasitic infection in HIV- seronegative , (P=0.000) .

Among HIV- seropositive patients , the results revealed that seven species of intestinal parasites were detected , these parasites were *Blastocystis hominis* , *Entamoeba histolytica / Entamoeba dispar* , *Giardia lamblia , Entamoeba coli, Endolimax nana* , *Cryptosporidium parvum* and *Enterobius vermicularis* (plates 6-14)

The most common intestinal protozoan parasite detected were *B. hominis* with highest incidence at 18.7% (56) followed by *E. histolytica E . dispar* 10.3% (31) , *G. lamblia* 8% (24) , *E . coli* 5.3 % (16) , *E. nana* 4% (12) *and C. parvum* 3.3%(10), and only one helminth parasite *E. vermicularis* was detected at low incidence rate 1% (3) (Table 2 and Fig. 4) .

Figure (2) : Overall incidence rate of intestinal parasitic infection among examined patients (n=450)



χ^2 = 13.1 ; P < 0.05 ; df = 3 ; P-value = 0.004*

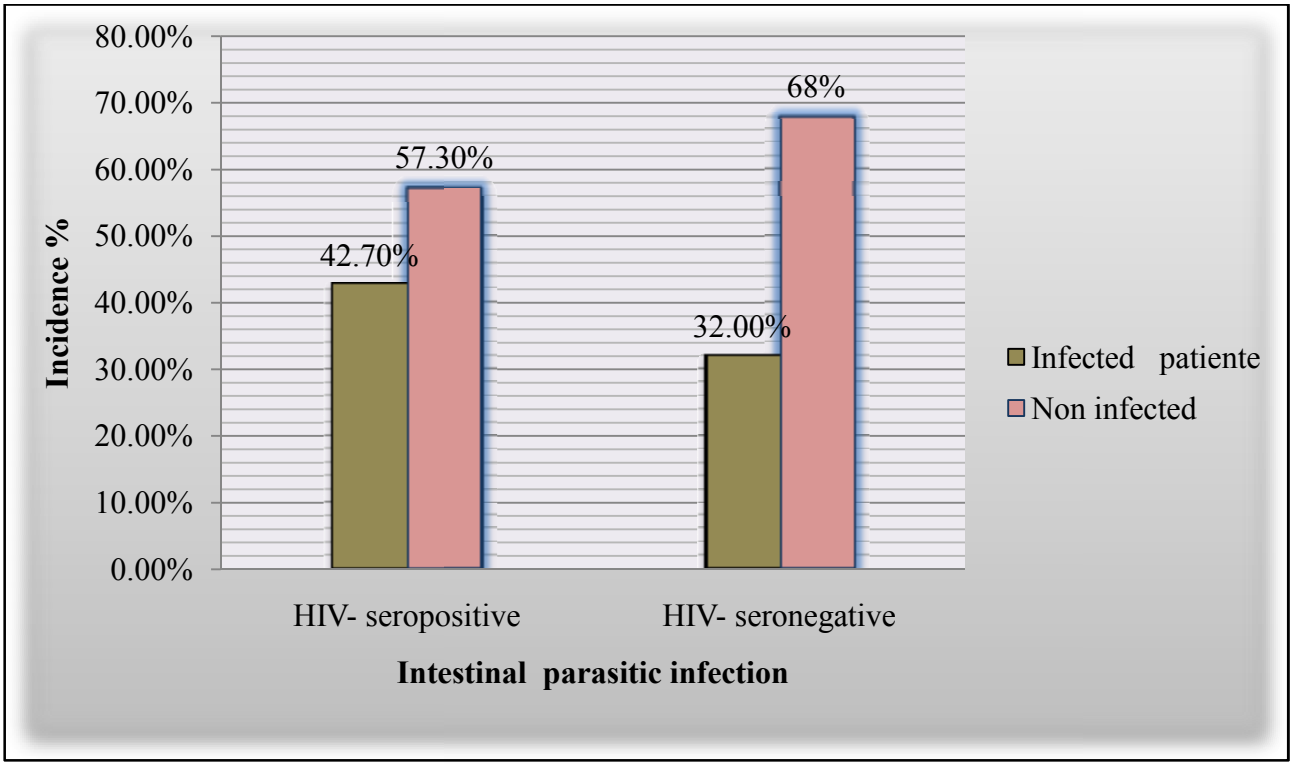
Table (1) : Overall incidence rate of intestinal parasitic infection among examined patients (N=450) :

Parasitic infection	HIV seropositive (N=300)	HIV seronegative (N=150)	Total (N=450)
Infected	128 (42.7%)	48 (32.0 %)	176(39.1%)
Non infected	172 (57.3%)	102 (68%)	274(60.9%)
Total	300 (100%)	150 (100%)	450(100%)

HIV-serpositive (n=300) χ^2 = 6.453 ; df = 1 ; P-value = 0.011*

HIV-seronegative (n=150) χ^2 = 150 ; df = 1 ; P-value =0.000*

Figure (3) : Overall incidence rate of intestinal parasitic infection in examined patients (N=450) :



There were a significant differences in incidence among *B . hominis* (P=0.00), *Entamoeba histolytica E. dispar* (P=0.00), *G. lamblia* (P=0.00), *E . nana* (P=0.00), *C. parvum* (P=0.02) , *E. coli* (P=0.00) and *E . vermicularis* (P=0.136) .

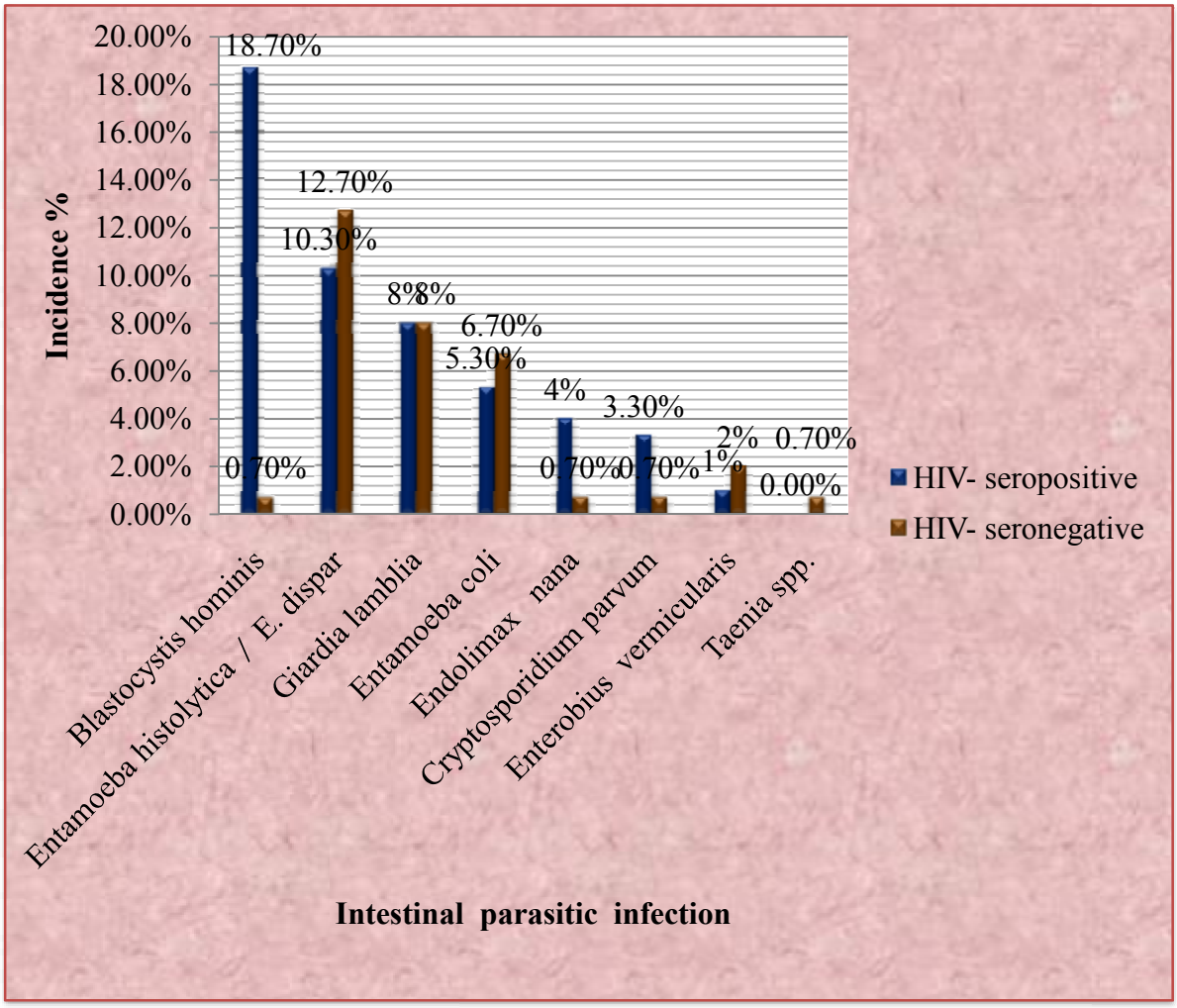
Among examined HIV- seronegative group , the results revealed that eight species of intestinal parasites were detected , these parasites were *B . hominis* , *E. histolytica / E. dispar* , *G . lamblia , E .coli* , *E. nana* , *C. parvum* , *E . vermicularis* and *Taenia* sp .

The most common intestinal protozoan parasites detected were *E. histolytica E . dispar* , with the highest incidences at 12.7% (19) followed by *G. lamblia* 8% (12), *E . coli* 6.7% (10) *B. hominis* 0.7% (1), *C . parvum* 0.7% (1), *E. nana* 0.7% (1), all were protozoan parasites and two helminth parasites , *E. vermicularis* and *Taenia* sp . were detecte at low incidences rates 2% (3) and 0.7% (1), respectively (Table 2 and Fig. 4) . There were a significant differences in incidences among *E. histolytica / E. dispar* (P=0.000), *G. lamblia* (P=0.000), *C. parvum* (P=0.00) *E . coli* (P=0.001) and no significant differences among *B. hominis* (P=.144), *E. vermicularis* (P=0.08) , *E. nana* (P=0.144), and *Taenia* sp. (P=0.144).

Table(2) : Incidence of intestinal parasitic infection among examined HIV - seropositive and HIV – seronegative patients :

Type of parasites	HIV- serostatus (%)		Total (%) (n=450)
	HIV- seropositive (n=300)	HIV- seronegative (n=150)	
Blastocystis hominis	**18.7%(56)**	**0.7% (1)**	**12.7% (57)**
Entamoeba histolytica E. dispar	**10.3% (31)**	**12.7% (19)**	**11.1% (50)**
Giardia lamblia	**8 % (24)**	**8 % (12)**	**8% (36)**
Entamoeba coli	**5.3% (16)**	**6.7% (10)**	**5.8% (26)**
Endolimax nana	**4 % (12)**	**0.7% (1)**	**2.9% (13)**
Cryptosporidium parvum	**3.3% (10)**	**0.7% (1)**	**2.4% (11)**
Enterobius vermicularis	**1% (3)**	**2 % (3)**	**1.3% (6)**
***Taenia* sp.**	**0.0% (0)**	**0.7% (1)**	**0.2% (1)**

Figure(4) : Incidence of intestinal parasitic infection among examined HIV - seropositive (n=300) and HIV – seronegative patients (150)



4.2. Incidence and sex:

Although the results revealed that the incidence rate was higher in females 70 (45.8%) than in males 58 (39.5%) in HIV- seropositive patients . No significant difference (P=0.195) was found between males and females HIV-seropositive patients . On the other hand the incidence rate of intestinal parasites in HIV – seronegative was higher in females 19 (37.3%) than in males 29 (29.3%) .There was no significant difference was detected between females and males (P = 0 .322) . The relationship between incidence of intestinal parasitic infection in HIV-seropositive and HIV- seronegative and sex is presented in Table (3) and Fig.(5) .

Seven species of intestinal parasites were noted in both females and males .The most common parasite that identified in both sexes was *B.hominis* . at overall incidence 10.7% (32 / 300) in females and 8% (24 /300) in males .No significant difference in overall incidence was detected between females and males (P=.308) . *E. histolytica* [redacted] *E. dispar* was higher in 6.33% (19 / 300) than that of females 4%(12 [redacted] 300) There was no statistically significant difference between overall incidence in each of males and females (P=.148) .

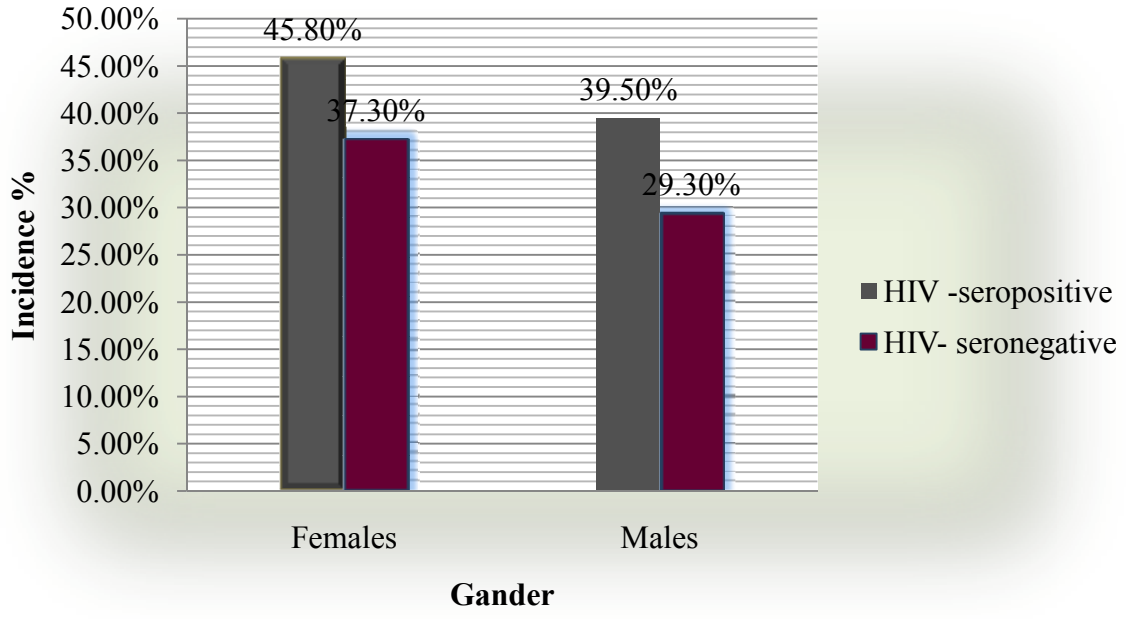
Table (3) : Relationship between incidence of intestinal parasitic infection and sex among HIV- seropositive and HIV- seronegative

Gander	HIV – seropositive (n=300)		HIV seronegative (n=150)	
	No.examined	Infected	No.examined	Infected
Females	153	70(45.8%)	51	19 (37.3%)
Males	147	58(39.5%)	99	29(29.3%)
Total	300	128(42.7%)	150	48(32%)

HIV-seropositive (n=300) χ^2 = 4.706 ; df = 3 ; P-value =.195

HIV-seronegative (n=150) χ^2 = .981 ; df = 1 ; P-value =0 .322

Figure (5) : Relationship between incidence of intestinal parasitic infection and sex among HIV- seropositive and HIV-seronegative



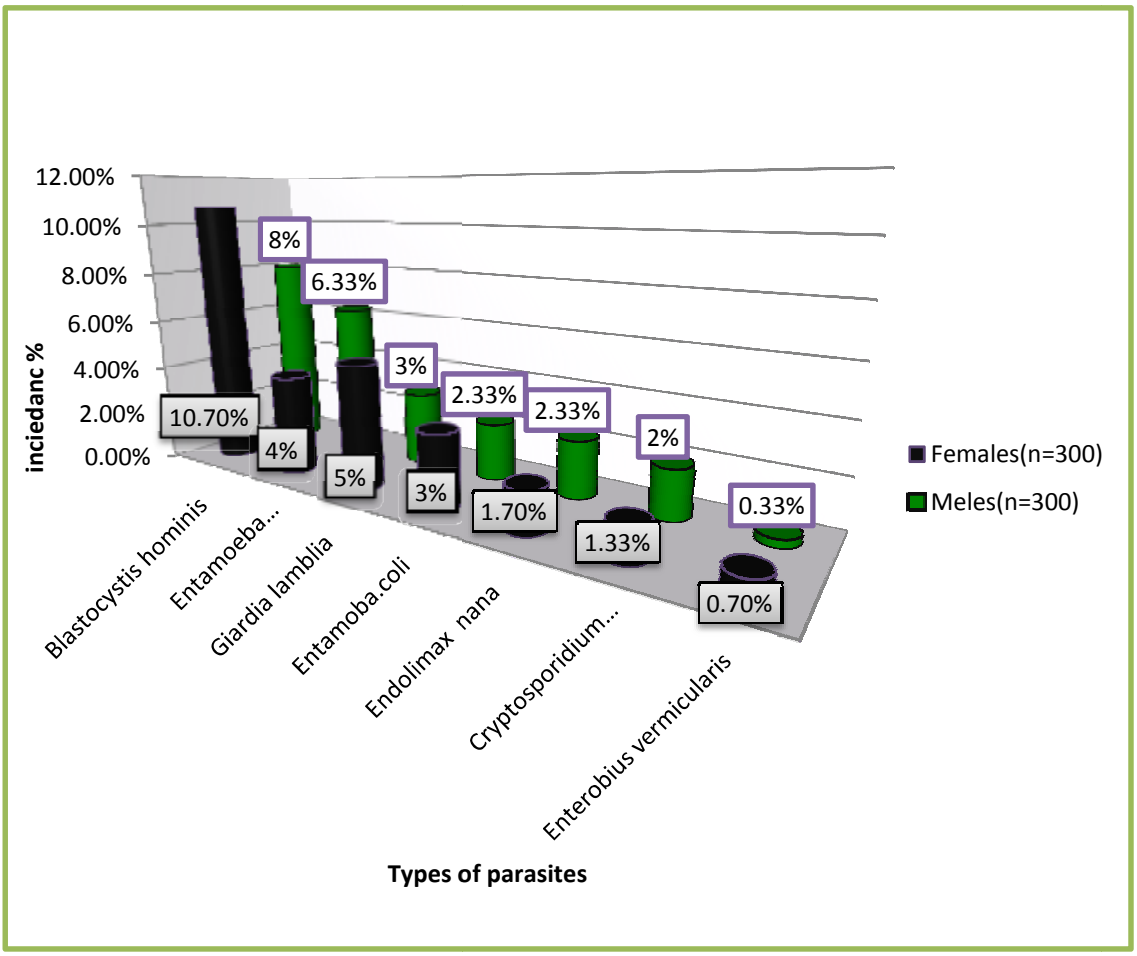
.*G. lamblia* was detected in 5 % of females out of total (300) examined HIV- seropositive, which was higher than that of males 3% . Overall incidence of this parasite was significantly different between females and males (P=0.240) .

The results revealed that the overall incidence of *E.coli* was 3% (9 300) in females and 2.33% (7 300) among males . The results revealed that there were significant difference between the overall incidence of this parasite in both sexes (P=.666) . *E.nana* was noted in 7 (2.33%) males and 5(1.7%) females out of the total 300 specimens examined . There was no significant differences between overall incidence of this parasite in both sexes (P=0.02) . *C.parvium* showed on overall incidence that was higher in males 2% (6 out 300) and 1.33 % (4 300) among femles . There was no significant difference between overall incidence of this species in both sexes (P=0.479) . *E. vermicularis* is the only helminth parasites detected among HIV-seropositive patients at overall incidence 0.7% in females comparing with males 0.33% . There was no significant difference between overall incidence of this species in both sexes (P=.585) (Table 4 and Fig .6) .

Table (4) : Incidence of intestinal parasitic species and sex among HIV-seropositive patients (n=300) :

Types of parasites	**NO. Examinned(%)**		**Total(n=300)**
	Females(n=300)	Males(n=300)	
Blastocystis hominis	32(10.7%)	24(8.0%)	56(18.7%)
Entamoeba histolytica E . dispar	12(4.0%)	19(66.3%)	31(10.3%)
Giardia lamblia	15(5.0%)	9 (3.0%)	24(8%)
Entamoeba coli	9(3.0%)	7(2.33%)	16(5.3%)
Endolimax nana	5(1.7%)	7(2.33%)	12(10%)
Cryptospordium Parvum	4(1.33%)	6(2.0%)	10(3.3%)
Enterobius vermicularis	2(0.7%)	1(0.33%)	3(1%)

Figure (6) : Incidence of intestinal parasitic species and sex among HIV-seropositive patients (n=300).



4.3. Incidence and age :

The incidence of intestinal parasitic infections in different age groups (years) of HIV- seropositive patients is presented in Table (5) and Fig. (7) . The results revealed that the highest incidence rate (50%)was observed in age group of 30-39 years followed by age group 10-19 and age group 20-29 years old at incidence rates 44.4% and 35.9 % respectively the low incidence rate was recorded among age group 0-9 years old at 28.6% . Age had a significant influence on the incidence of intestinal parasitic infection in HIV- seropositive patients (P= 0.004).

The highest incidence of intestinal parasitic infection among different age groups of HIV-seronegative was detected among age groups 0-9 years old at 46.7% followed by age groups 20-29 , 10-19 , > 40 and 30-39 years old at incidence rates 35.4 %, 29.8% , 27.8 %, 22.8 respectively. Age had no significant influence on the incidence of intestinal parasites in HIV seronegative (P= 0.581)(Table 5 Fig.7).

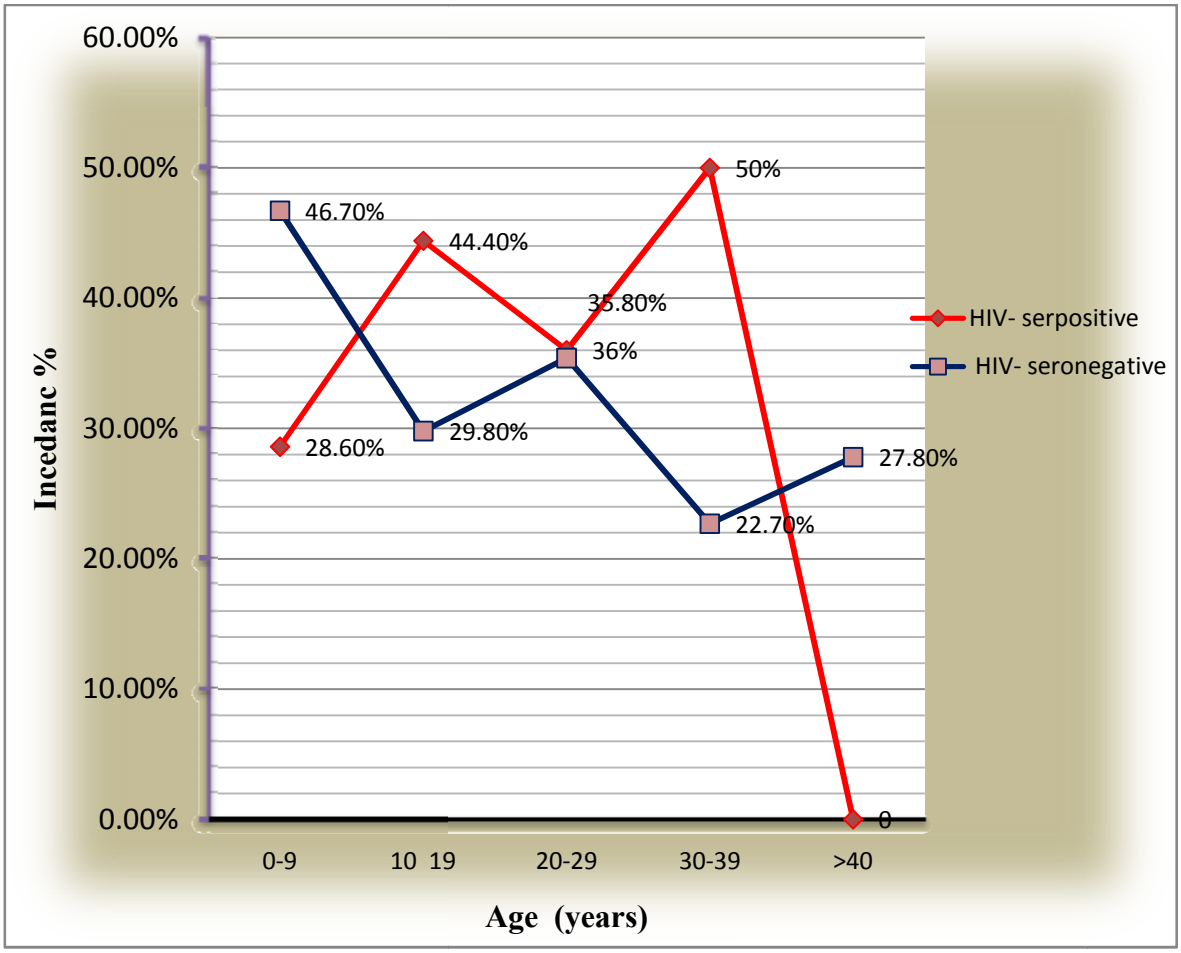
Table (5): Incidence of intestinal parasitic infection in each age group among HIV- seropositive and HIV- seronegative :

Age groups (years)	HIV-seropositve		HIV-sernegative	
	Examined (n=300)	infected (%)(n=128)	Examined (n=150)	Infected (%) (n=48)
0-9	7	2 (28.6%)	15	7(46.7%)
10-19	234	104 (44.4%)	47	14 (29.8%)
20-29	53	19(35.8%)	48	17(35.4%)
30-39	6	3(50 %)	22	5(22.7%)
>40	0	0	18	5(27.8%)
Total	300	128(42.7%)	150	48(32%)

HIV-seropositive (n=300) $\chi 2 = 24.7$; df = 9 ; P-value = 0.004

HIV-seronegative (n=150) $\chi 2 = 2.86$; df = 4 ; P-value = 0.581

Figure (7): Incidence of intestinal parasitic infection in each age group among HIV- seropositive and HIV- seronegative ..



Blastocystis hominis showed that the highest incidence rate among age group 0-9 years 2 (28.6%) followed by 20-29 years 11(21%) and 10-19 years , 42(18%) age group and 30-39 years was 1(17%) . *E. histolytica* *E. dispar* showed the highest incidence among age group 0-9 years at 1 (14.3%) followed by 10-19 years was 26 (11%) and the low incidence rate was detected among age group 20-29 years at 4(7.5%) *E. histolytica* *E. dispar* infection was not detected among age group 30-39 years, *G. lamblia* showed that the highest incidence rate among age group 30-39 years at 1(17%) followed by 10-19 years at 20(8.5%) and the low incidence rate among age group 20-29 years at 3 (7 %) *G.lamblia* infection was not detected among age group 0-9 at 0(0.00%) . *E. coli* showed the highest incidence among age group 30-39 years 3(50%) followed by 10-19 years was 13 (5.5 %) *E.coli* infection was not detected among age 0-9, 20-29 at 0(0.00%) . *E. nana* showed the highest incidence among age group 0-9 years 1(14.3%) followed by 10-19 years was 10(4.2%) and low incidence rate was among age group 20-29 years year at 1(2%)in. *E.nana* infection was not detected among age group 30-39 years at 0(0.00%) . *C. parvum* showed among age group 10-19 years 10(4.2%) only . *E. vermicularis* showed the highest incidence 1 (17 %) among age group 30-39 years followed by age group 20-29 years at 1 (17 %) and 10-19 years 1 (0.4 %) and not detected among age group 0-9 years (Table 6) .

Table (6): Incidence of intestinal parasitic species in each age group among HIV patients .

Age / Parasite	0-9 (n=7)	10-19 (n=234)	20-29 (n=53)	30-39 (n=6)	Total(n=300)
Blastocystis hominis	**2(28.6%)**	**42(18%)**	**11(21%)**	**1(17%)**	**56(18.7%)**
Entamoeba histolytica E. dispar	**1(14.3%)**	**26(11%)**	**4(7.5%)**	**-**	**31(10.3%)**
Giardia lamblia	**0(0.0%)**	**20(8.5%)**	**3(7%)**	**1(17%)**	**24(8.0%)**
Entamoeba coli	**-**	**13(5.5%)**	**-**	**3(50%)**	**16 (5.3%)**
Endolimax nana	**1(14.3%)**	**10(4.2%)**	**1(2%)**	**-**	**12(3.9%)**
Cryptosporidium parvum	**-**	**10(4.2%)**	**-**	**-**	**10(3.3%)**
Enterobius vermicularis	**-**	**1(0.4%)**	**1(2%)**	**1(17%)**	**3(0.9%)**

On the other hand , incidence of intestinal parasitic infection among different age groups (years) in HIV - seronegative , the result showed that *B.hominis* was detected only among age group 0-9 years at incidence rate 6.7% and not detected in other age groups . *E. histolytica* *E. dispar* showed the highest incidence among age group 0-9 years was 3(20%) followed by > 40 years was 3(16.7%) and 20-29 years was 7(14.6%), 30-39 was 3(13.6%) and the low incidence rate was detected among age group10-19 years 3(6.4%) . *G. lamblia* showed the highest incidence among age group 20-29 years was 8 (16.7%) followed by 30-39 years 2 (9.1 %) , age group 0-9 years 1(6.7%) and 10-19 years 1 (2.1 %) the parasite was not detected among age group 40 years old . *E. coli* showed the highest incidence among age group 0-9 years 2 (13.3 %) followed by 10-19 years 4(8.5%) and 20-29 years was 4(8.33%) .No infection was detected among age group 30-39 years and >40 years old . *E. nana* showed the highest incidence among age group >40 years was 1 (5.5 %) and the non infected among age group from 0-9 years to 30-30 years old .*C. parvum* showed among age group 0-9 years 1(6.7 %)and and the non infected among age group from 10-19 years to >40 years old . *E. vermicularis* showed the highest incidence among age group 10-19 years was 2(4.25 %) followed by >40 years was 1(5.5%) and the non infected among age group 0-9,20-29,30-39 *Taenia spp* showed only among age group 30-39 years was1(4.5%) . (Table 7)

Table (7): Incidence of intestinal parasitic species in each age group of HIV-seronegative (n=150).

Parasite	Age group (years)					
	0-9 (n=15)	10-19 (n=47)	20-29 (n=48)	30-39 (n=22)	>40 (n=18)	Total(n=150)
Giardia lamblia	1(6.7%)	1(2.1%)	8(16.7%)	2(9.1%)	0.00%	12(8%)
Entamoeba histolytica E. dispar	3(20%)	3(6.4%)	7(14.6%)	3(13.6%)	3(16.7%)	19(12.7%)
Blastocystis hominis	1(6.7%)	0.00%	0.00%	0.00%	0.00%	1(0.76%)
Endolimax nana	0.00%	0.00%	0.00%	0.00%	1(5.5%)	1(0.76%)
Cryptosporidium parvum	1(6.7%)	0.00%	0.00%	0.00%	0.00%	1(0.76%)
Entamoeba coli	2(13.3%)	4(8.5%)	4(8.33%)	0.00%	0.00%	10(6.76%)
Enterobius vermicularis	0.00%	2(4.25%)	0.00%	0.00%	1(5.5%)	3(2%)
Taenia spp.	0.00%	0.00%	0.00%	1(4.5%)	0.00%	1(0.76%)
Total	8(5.33%)	10(6.7%)	19(12.7%)	6(4%)	5(3.33%)	48(32%)

Control (n=150) $\chi 2 = 43.4$; $P < 0.05$; $df = 32$; P-value = 0.082

4.4.Incidence and diarrhea :

Incidence rate of intestinal parasites in HIV- seropositive with and without diarrhea were 51 (75 %) with diarrhea and the rest 77 (33.2%) patients without diarrhea .The results revealed that those HIV-seropositive patients with diarrhea was higher than those without diarrhea. There was a significant difference was detected between incidence of parasitic infection and diarrhea . (P= 0 .000).

Infection rate with intestinal parasites in HIV -seronegative was found 6 (60 %) had diarrhea and the rest 42(30 %) patients without diarrhea . There was a significant difference was detected between incidence and diarrhea . (P=0.056) among HIV –seronegative . (Table 8 and Fig.8).

B.hominis had the highest incidence (35.3%) among HIV-seropositive patients with diarrhea followed by *E. histolytica* *E. dispar* (26.4%) , *E.coli* (11.8%) , *C .parvum* (10.2%) , *G. lamblia* (8.8%) , *E. nana* (4.4%) and *E. vermicularis* (1.4%) .

B.hominis had the highest incidence (13.7%) among HIV- seropositive patients without diarrhea followed by *G. lamblia* (7.7%) , *E. histolytica* *E. dispar* (5.6 %) whereas *E. nana*(3.9%) , *E.*coli (3.4%) , *C.parvum* (1.3%) and *E. vermicularis* (0.086 %) had the lowest infection rates .

B.hominis and *E.coli* had the highest incidence (20.0 %) among seronegative patients with diarrhea followed by *G. lamblia* (10.%) and *C. parvum* (10.0 %), and whereas *E. histolytica* *E. dispar* , *E. nana* , *E. vermicularis* and *Taenia* sp non seen infection rates .

B.hominis had the highest incidence (12.1%) among seronegative patients without diarrhea followed by *E. histolytica* *E. dispar* (8.6%) , *E. coli* (5.7%), *E. vermicularis* (2.1 %) , *E. nana* and *Taenia* sp (0.71%) in both ,while no infection with *G. lamblia* and *C.parvum* was detected (Table . 9 and Fig .9(a,b) .

Table (8): Relationship between intestinal parasitic infection and diarrhea among HIV - seropositive and HIV seronegative patients

Diarrhea	Total examined (n=300)	With pararsitic infection (n=128)	Total examined (n=150)	With pararsitic infection (n=48)
With diarrhea	**68**	**51 (75 %)**	**10**	**6(60%)**
Without diarrhea	**232**	**77 (33.2%)**	**140**	**42 (30%)**

HIV- seropositve (n=300) χ^2 = 47.8 ; df = 3 ; P-value =0.000*

HIV- seronegative (n=150) χ^2 = 3.860 ; df = 1 ; P-value =0.049

Figure (8): Relationship between intestinal parasitic infection and diarrhea among HIV - seropositive patients and HIV - seronegative patients .

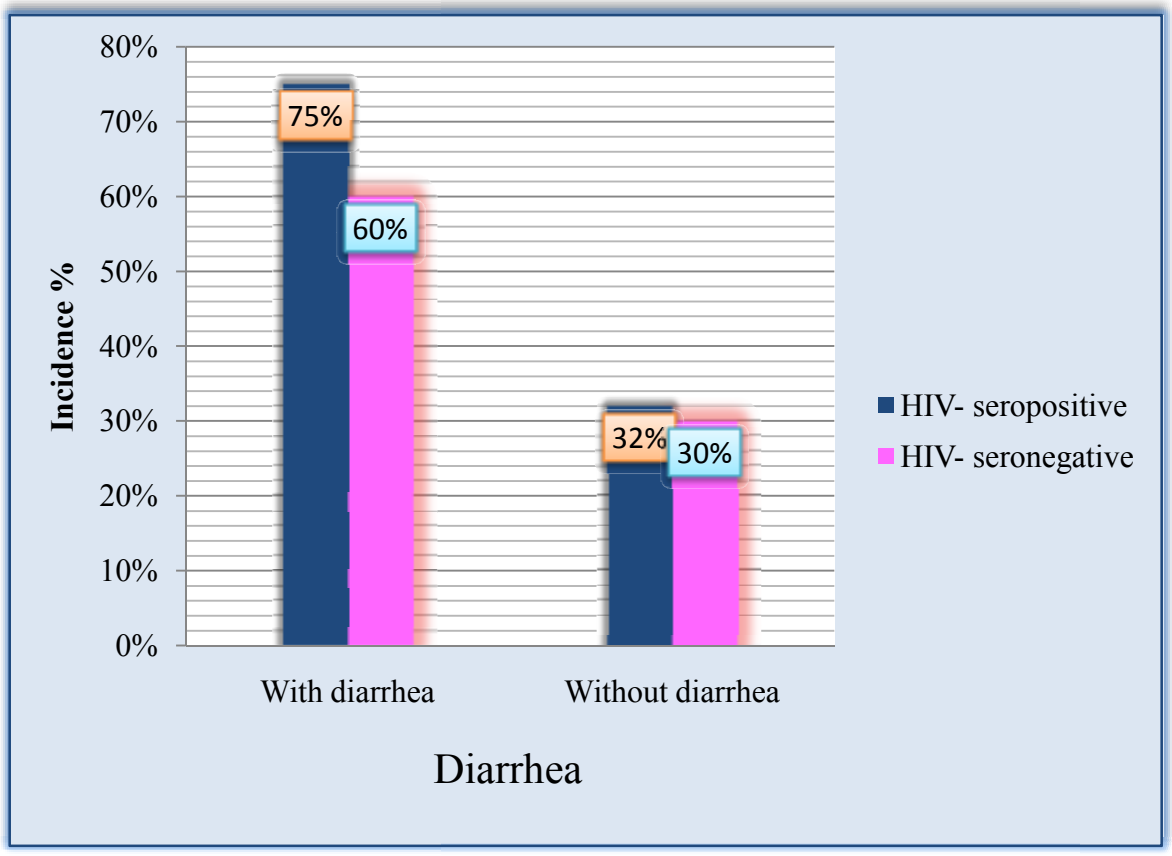
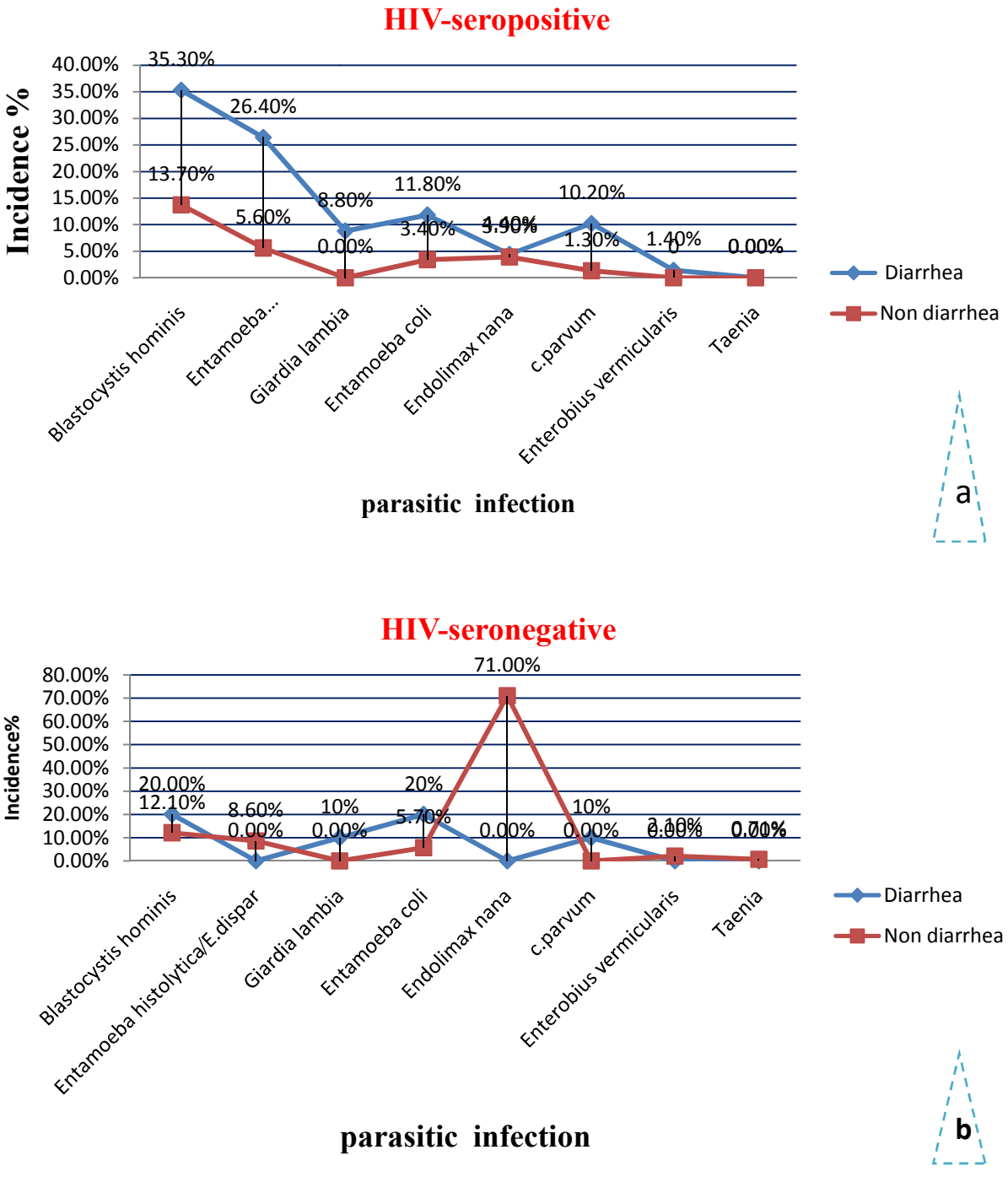


Table (9) : Incidance of intestinal parasitic species infection in HIV – seropsitive and HIV – seronegative patients with and without diarrhea .

Parastic infection	HIV- seropositive No . positive (%)			HIV seronegative No . positive (%)		
	Diarrhea (n=68)	Non-diarrhea (n=232)	Total (n=300)	Diarrhea (n=10)	Non-diarrhea (n=140)	Total (n=150)
Blastocystis hominis	24(35.3%)	32(13.7%)	56(18.7%)	2 (20.0%)	17(12.1%)	19(10.7%)
Entamoeba histolytica E. dispar	18 (26.4%)	13 (5.6%)	31(10.3%)	0 (0.0%)	12(8.6%)	12(8%)
Giardia lamblia	6 (8.8%)	18 (7.7%)	24(8%)	1(10.0%)	0(0.0%)	1(.7%)
Entamoeba coli	8(11.8%)	8(3.4%)	16(5.3%)	2 (20.0%)	8(5.7%)	10(6.7%)
Endolimax nana	3(4.4%)	9 (3.9%)	12(3.9%)	0 (0.0%)	1 (.71%)	1(.7%)
Cryptosporidium parvum	7(10.2%)	3 (1.3%)	10(3.3)	1(10%)	0(0.0%)	1(.7%)
Enterobius vermicularis	1(1.4%)	2(.086%)	3(.9%)	0(0.0%)	3(2.1%)	3(2%)
Taenia sp.	0 (0.0%)	0 (0.0%)	**0 (0.0%)**	0 (0.0%)	1 (.71%)	1(.7%)

Figure: 9 (a.b): Incidance of intestinal parasitic species infection in HIV – seropsitive and HIV – seronegative patients with and without diarrhea



4.5.Incidence of single and mixed infection :

In the present study the distribution of single and mixed incidence of intestinal parasites among HIV seropositive patients was One hundred and six (82.8%) had a single intestinal parasitic infection (Infected with one species of parasite) and twenty two (17.2%) had mixed infection (Infected with more than one species of parasites) . The results showed that there was a high significant difference between incidence and types of parasitic infection (P = 0.000) (Table 10 and Fig 10) .

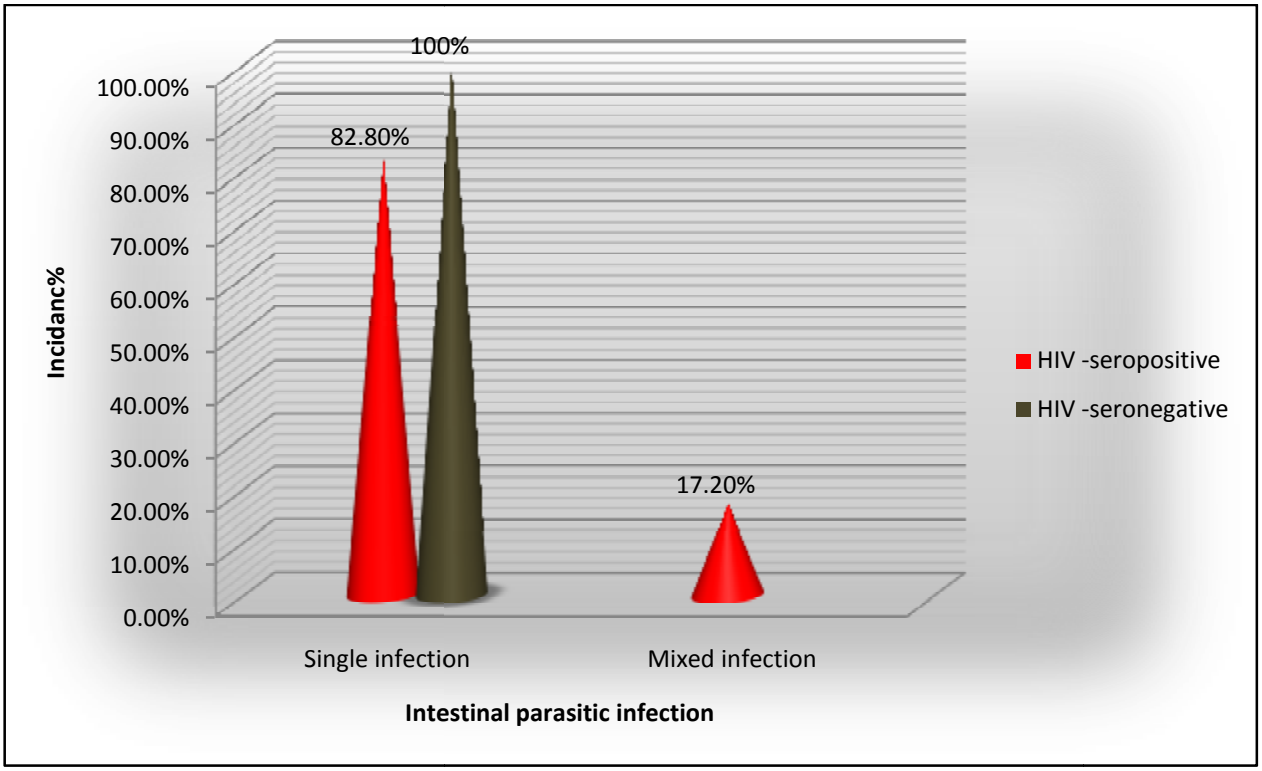
While the distribution of single and mixed incidence of intestinal parasites among HIV- seronegative patients showed in forty eight (100%) had a single of intestinal parasites and there was not mixed infection detected in HIV- seronegative patients .

Table (10): Single and mixed infections of intestinal parasites among HIV- seropositive and HIV-seronegative

Type of infection	HIV- seropositive (%) (n=128)	HIV -seronegative(%) (n=48)
Single infection	**106 (82.8%)**	**48(100%)**
Mixed infection	**22(17.2%)**	**-**
Total	**128 (100%)**	**48(100%)**

χ^2 = 113.0; P < 0.05 ; df =2 ; P-value = 0.000 *

Fig (10): Single and mixed infections of intestinal parasites among HIV-seropositive and HIV- seronegative .



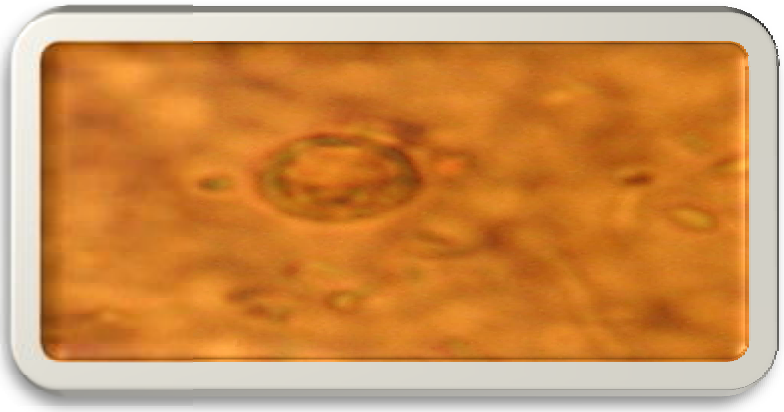
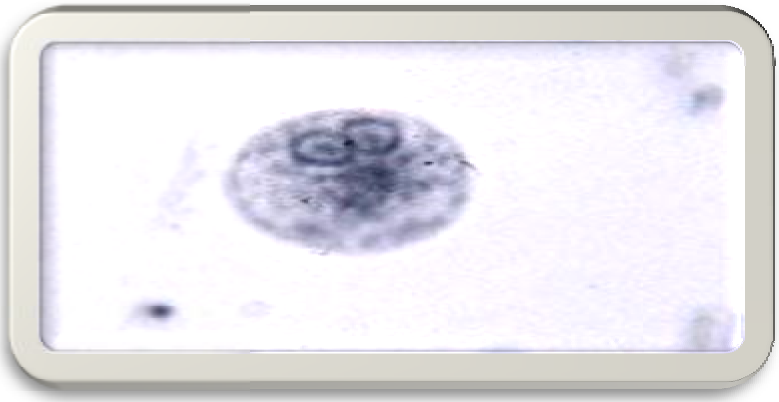


Plate (6): *B. hominis* vacuolar form (v : vacuole. w: wall , n: nuclei). (x 100)



Plate(7): *E. histolytica* *E.dispar* cyst (c: cyst wall , n: nuclei). (x 100)

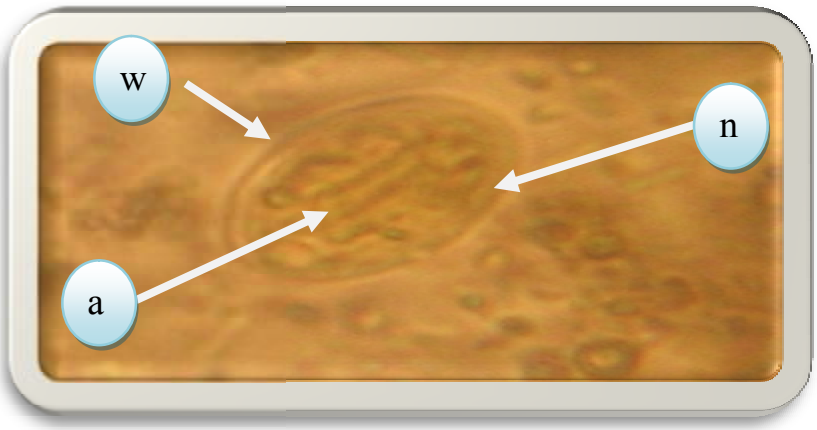
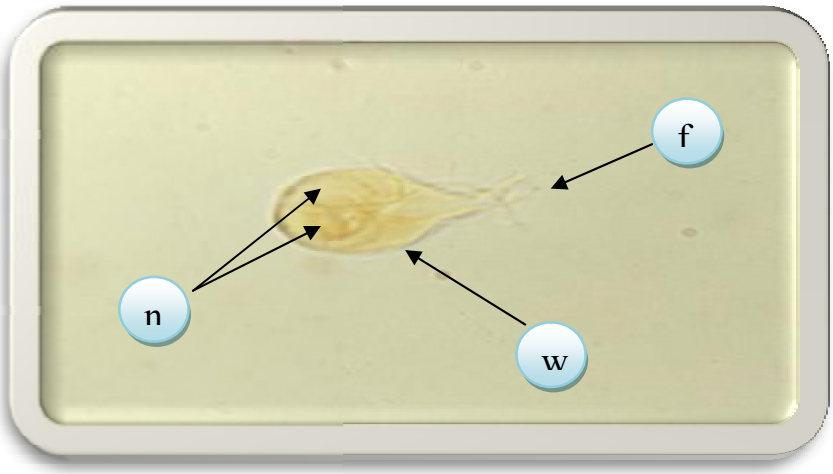
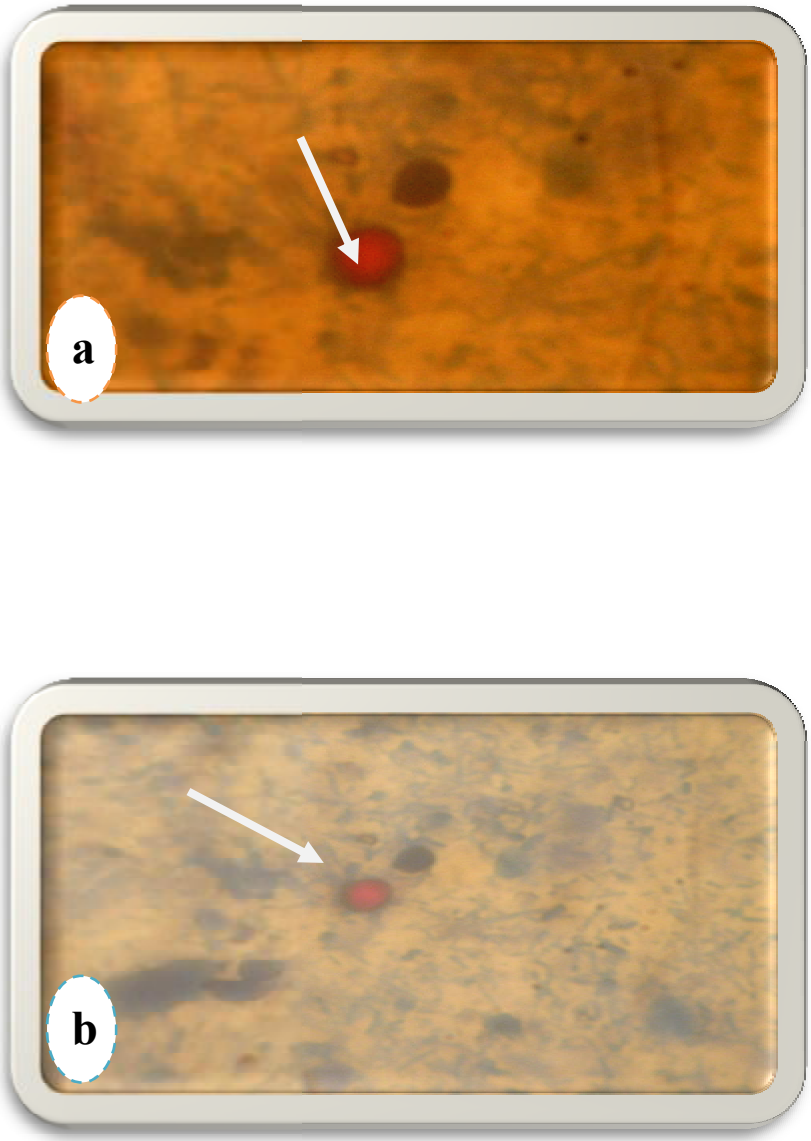


Plate (8): *G. lamblia* cyst (w: wall , n: nuclei, a: axonemes). (x 100)



Plate(9): *G. lamblia* trophozoite (w: wall , n: nuclei, f: flagella). (x 100)



Plates (10 a and b): *Cryptosporidium parvum* (x 100)

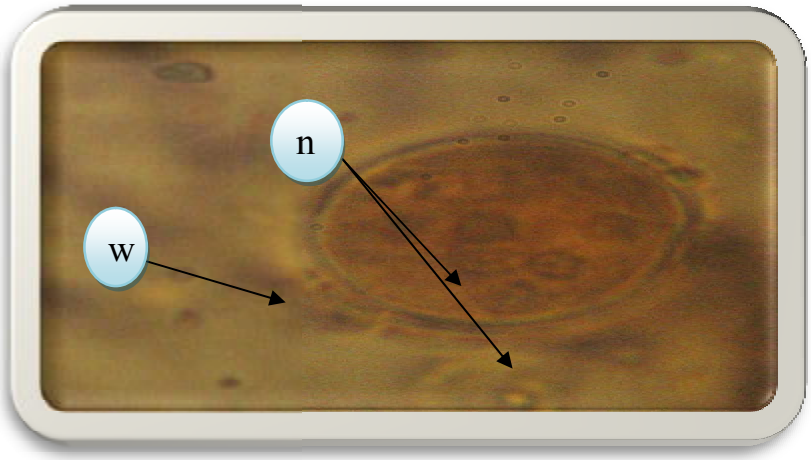


Plate (11): *Entamoeba coli* cyst (w: wall ,n: nuclei). (x100)

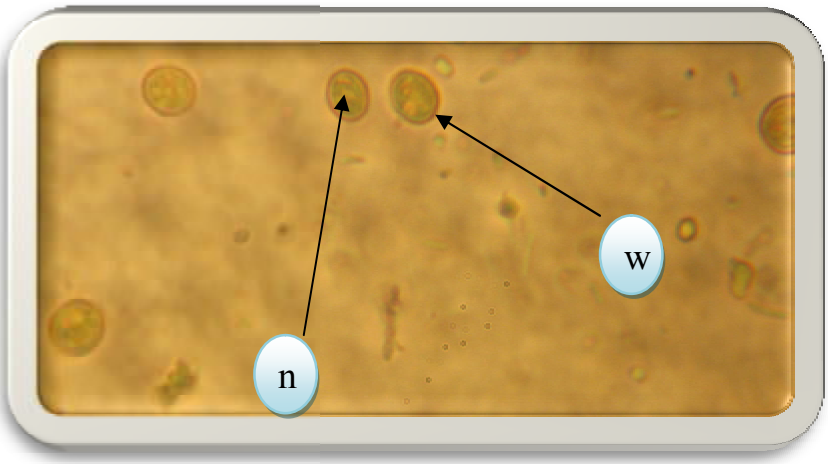


Plate (12) : *Endolimax nana* (w: wall , n: nuclei). (x 100)

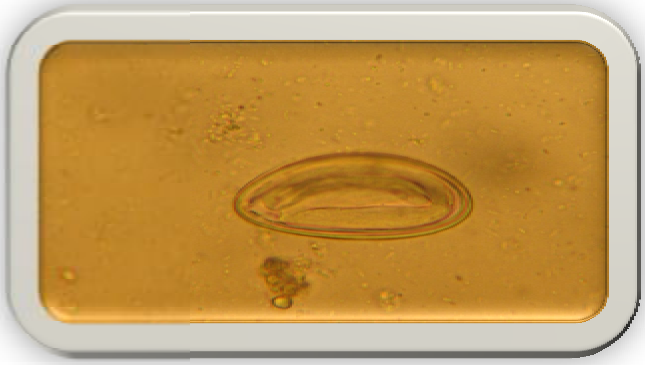


Plate (13) : *Enterobius vermicularis* egg(x100)

Plate (14) : ***Taenia* sp** egg (x100)

DISCUSSION

5. DISCUSSION

To our knowledge, this is the first study to be carried out on the incidence of intestinal parasites in HIV – seropositive patients in Benghazi , Libya . The results obtained in this study can provide important information for future understanding of intestinal parasitic infection in HIV – seropositive patients. . This study was undertaken in HIV infected patients who were admitted or treated in an ambulatory way in the National Hospital Centers of Benghazi which is the Libyan reference centers for HIV management to determine the incidence of intestinal parasites among HIV positive and negative individuals . An informed consent was obtained for each patient prior to his/her inclusion in the study.

Intestinal parasitic infections are the commonest and the major causes of morbidity and mortality in HIV positive individuals , worldwide (Gupta *etal*.,2008). These parasites usually cause a self limiting illness in immunocompetent individuals, but in the case of immune compromised patients, they can cause life threatening, profuse watery diarrhoea (Meisel *et al*., 1976).

5.1. Incidence :

In the present study intestinal parasites was detected in both HIV/ AIDS patients and HIV uninfected controls . This finding is in agreement with findings in previous reports from different countries of the world (Greten *et al* ., 1993 ; Méndez *et al* .,1994; Tarimo *et al* . ,1996; Lindo *et al*. 1998 ; Tsegaye *et al* . , 1999 ; Fontanet *et al* . ,2000 ; Wiwanitkit , 2001 ; Mohandas *et al* . ,2002 ; Botero *et al*. ,2003 ; Ghimire *et al*., 2004; Lim *et al* .,2005; Moges *et al*. ,2006 ; Stark *et al* .,2007; Gatei *et al .,* 2008; Assefa *et al*.,2009; Akinbo *et al*.,2010; Akinbo *et al*. ,2011 and Alemu *et al.* , 2011) . The detection of intestinal parasites in both HIV patients and controls could be a reflection of the poor environmental sanitation and personal hygienic practices, which emphasize the need for interventional measures at the community level to reduce the risk factors of acquiring intestinal parasites in immunocompromised patients

The incidence of intestinal parasitic infection detected in the present study was 28.4 % among HIV/AIDS patients .This is a higher than in the HIV uninfected control (10.7%) , but this difference was statistically significant . The high incidence of parasitic infection among HIV/AIDS patients than HIV uninfected controls in the present study is in agreement with findings in previous reports from other countries of the world (Hailemariam *et al*., 2004; Ramakrishman *et al*.,2007; Gupta *et al*., 2008; Mariam *et al* ., 2008; Akinbo *et al* ., 2010 ; Berenji *et al* ., 2010 ; Abaver *et al* ., 2011 and Alemu *et al* ., 2011) .

The incidence of parasitic infection among HIV/AIDS patients in the present study (42.7 %) was lower than those results reported in other studies in the world, 47% in Turky (Boral *et al* ., 2004); 52.6 % in Ethiopia (Hailemariam *et al* ., 2004) ; 56.6% in Ethiopia (Getachew *et al*.,2004) ; 51.3% in Thailand (Pinlaor *et al* ., 2005); 67%in Ethiopia (Moges *et al* ., 2006); 50% in Thailand (Wiwanitkit, 2001) ; 80.3% in Bahir Dar city ,North west Ethiopia (Alemu *et al* ., 2011) . On the other hand ,the incidence of parasitic infection among HIV/AIDS patients was higher than those reported in other studies in the world ; 27.9 % in South Italy (Brandonisio *et al* ., 1999) ; 26% in Thailand (Termmathurapj *et al* .,2000) ; 11.4% in Iran (Meamar *et al*.,2007) ; 28.3% in New Delhi, India (Gupta *et al*.,2008) 35% in India (Kulkarni *et al* ., 2009); 8.7% (Etok *et al*.,2010) ; 22.7% in Abuja – Nigeria (Abaver *et al*., 2011); 33% in Denmark (Stensvold *et al* ., 2011) and 33.8 % in Lagos (Sanyaolu *et al*., 2011) .

The examination of fecal specimens of HIV seropositive and HIV seronegative patients revealed that eight species of intestinal parasites were detected of which six protozoan parasites and two helminthes . This findings more or less similar to other previous studies in the world among HIV – seropositive and HIV –seronegative individuals , (Lindo *et al* .,1998; Termmathurapoj *et al* ., 2000 ; Wiwanitkit , 2001; Guk *et al* . , 2005; Moges *et al*., 2006; Ramakrishnan *et al* ., 2007; Gatei *et al* ., 2008 and Daryani *et al* ., 2009) .

The present study showed that intestinal protozoan parasites were more prevalent than helminthic infection , this in agreement with

previously reported results (Lindo *et al* ., 1998 ; Pinlaor *et al* ., 2005 ; Gatei *et al* ., 2008 ; Ramakrishnan *et al* ., 2007 ; Gupta *et al* ., 2008 Daryani *et al* ., 2009 ; Nasiri *et al* ., 2009 ; Kulkarni *et al* ., 2009 ; Al-Megrin , 2010 and Asma *et al* ., 2011) .

On the other hand this findings disagree with previous results from other part of world , where intestinal helminthic parasites were more prevalent than protozoa (Tarimo *et al* ., 1996 ; Fisseha *et al* ., 1999 ; Fontanct *et al* ., 2000 ; Kassu *et al* ., 2003 ; Okodua *et al* ., 2003 ; Hailemariam *et al* ., 2004; Moges *et al* ., 2006 ; Meamar *et al* ., 2007 ; Assefa *et al* ., 2009) .

Blastocystis hominis was more prevalent in HIV AIDS patients (18.7%) than controls (0.7%) , as has been reported in other studies (WHO 1986 ; Greten *et al* ., 1993 ; Lindo *et al* ., 1998 ; Hailemariam *et al* ., 2004 ; Udeh *et al* ., 2008) . This results disagree with other studies were no *B* . *hominis* infection was reported (Escobedo and Nunez 1999; Kumar *et al* ., 2002; Kassu *et al* ., 2003; Okodua *et al* ., 2003; Kaminsky *et al* ., 2004; Guk *et al* ., 2005) . *B.hominis* was more common parasite in the present study this in consistant with other findings , 71.4% (Kurniawan *et al* ., 2009) ; 61 % (Meamar *et al* ., 2007) This indicates that the organism might be a possible pathogenic agents among immunocompromised patients . Was the most highly encountered parasite in this study at incidence rate 18.7% . This is consistent with the high HIV seropositve infected patients and HIV seronegative patients were the rate 2.08 % . Lindo , *et al* . (1998) reported that the incidence rate was 1.9 % in HIV seropositive , while in HIV seronegative not detected in any infection with *B. hominis*

(Brandonisio *et al.,* 1999) , 6.5% (Termmathurapoj *et al.,* 2000) , 3.3 % (Mohandsa *et al.,* 2002) , 14.1% (Hailemariam *et al* ., 2004) 6.1 % (Meamar *et al.,* 2007) . *B. hominis* is a ubiquitous protozoan parasite found in the intestinal tracts of humans evidence accumulated over the last decade suggests association of *B. hominis* with gastrointestinal disorders involving diarrhea , abdominal pain , constipation , nausea and fatigue (Puthia *et al.,* 2008) .This parasites is thought to be conducted through travel since it has been reported in westerners traveling to the tropics (Sheehan *et al.,* 1986 , Shlim *et al.,* 1995 and Tsegaye ,1999).

On the other hand , Low incidence rates of *B. hominis* were reported previously , 3.9 % (Mariam *et al* ., 2008 ; 1.9 % (Lindo , *et al* ., 1998) in HIV seropositive while in HIV seronegative not detected in any infection with *B. hominis* (Brandonisio *et al.,* 1999) , 6.5% (Termmathurapoj *et al.,* 2000) , 3.3 % (Mohandas *et al.,* 2002) , 14.1% (Hailemariam *et al* ., 2004) 6.1 % (Meamar *et al.,* 2007)

The incidence rate of *E. histolytica / E. dispar* in the present study was 10.3 % in HIV seropositve patients was more or less similar when compared to the results reported among HIV seronegative controls (12.7 %) . However incidence rate of *E. histolytica / E. dispar* among HIV seropositive patients in the present study was in agreement with those results reported in the world , 17.5 % in India (Ramakrishnan *et al*., 2007), 24.6 % in Ethiopia (Fontanet *et al*., 2000) . 27% in Ethiopia (Adamu and Petros 2009). But lower than that results reported in Ethiopia (Kassu *et al*., 2003) at 31%.

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The incidence rate of *E. histolytica* *E. dispar* in the present study was relatively high as compared to the results reported among in HIV seropositve patients from world , as 7 % in Indian (Kulkarni *et al*., 2009) , 5.8 % in Honduras (Lindo *et al*., 1998) , 1.7 % in Northern India . (Mohandas *et al*., 2002) , 5.7 % in Nigeria (Okodua *et al*., 2003) , 3.6 % in North west Ethiopia (Moges *et al*., 2006) and 6.7 % in Malaysian (Oguntbeju 2006)

Cryptosporidium parvum is an enteric coccidian parasite that has become a cause of concern as an important agent of diarrhea. Cryptosporidiosis in immunocompetent host is usually mild , self limiting and recover within a few weeks , the infection may have a severe ,

The incidence rate of *C. parvum* in the present study was 3.3 % in HIV seropositve patients and 0.7 % among HIV seronegative . this results agree with other previous studies in the world among HIV seropositive and HIV seronegatve was at 14% in Zambia (Chintu *et al .,* 1995) , 11.9% in Koura (Angel and Fidel 1998) , 7.7 % in Honduras (Lindo *et al .,* 1998) , 8.6% in Thailand (Termmathurapoj *et al .,* 2000) , in Indian 10.8 % (Mohandas , *et al .,* 2002) , 10.5 % in Korea (Guk *et al .,* 2005) , 9.4% Iran (Daryani *et al .,* 2009) 12% in India (Kulkarni *et al .,* 2009) , 8.1 % in Saudi Arabia (AL-Megrin ,2010) . High prevalence of *cryptosporidium* was reported from other previous studies at 21.5 % in South Italy (Brandonisio *et al* ., 1999) , 33.3 % in Delhi India (Dwivedi *et al* ., 2007) ; 28.7 % in South India (Ramakrishnan *et al* ., 2007) 30 % in Abuja , Nigeria (Udeh *et al* ., 2008) , 43 % in India (Gupta *et al* ., 2008) and 36 % in Tanzania (

Zali *et al* ., 2004 . The present findings was higher than results reported previously , 5.7 % in Nigeria (Okodua *et al* ., 2003) ,0.9 % in Iran (Meamar *et al* ., 2007), 4.9% in Jakarta , Indonesia (Kurniawan *et al* ., 2009).

Cryptosporidium parvum is one of ubiquitous intestinal protozoan parasite , with worldwide distribution and it causes a significant enteric disease in immunocopromised hosts such as HIV patients . (Kaushik *et al* ., 2009) , Transmission of this parasite in mainly through fecal-oral route , as well as through drinking contaminated water ,person to person spread and contact infected animals (Getaneh *et al* ., 2010)

In immunocompetent individuals, *Cryptosporidium* usually causes a self-limiting diarrhea, whereas in immunodeficient patients it may cause a severe, chronic and progressive gastroenteritis. Because no effective therapy is available for cryptosporidiosis, prolonged diarrhea may lead to dehydration, wasting and frequently death (Dionisio , 2002), (Hunter and Nichols, 2002), A number of studies have determined prevalence of cryptosporidiosis among HIV-positive patients and showed results differing quite markedly from one another and ranging from 0 to 100% . (Getaneh *et al* ., 2010).

The incidence rate of *G. lamblia* in the present study was 8% in HIV seropositve patients and 8 % among HIV seronegative . The present finding higher than previous results in other countries , 1.9% in Indonesia (Kumiawan *et al* ., 2009) , 3.8 % in Ethiopia (Hailmeriam *et al* ., 2004) , 3.8% in Iran (Nasiri *et al*., 2009) , 4.2 % in India (Gupta *et al*., 2008) , 5.7 % in Abuja (Yvonne *et al*., 2011) , 6 % in Cuba

(Escobedo and Nunez ,1999) and 1.9 % in Jakarta , Indonesia (Kurniawan *et al* ., 2009) .

Giardia lambelia , compared to intestinal coccidians is not considered an opportunistic agent and less frequently observed to cause severe illness in HIV/AIDS patients . (Ferrreria ,2000) . In the present study , *G. lamblia* was the third most prevalent parasite detected . *G. lamblia* is a common parasite in HIV-seropostive homosexual males. The transmission of *G. lamblia* may be direct by ingestion of faeces or indirect through drinking of contaminated water or ingesting of contaminated food (Neva and Brown , 1994) *G. lamblia* may cause acute or chronic diarrhea , steatorrhoea , malabsorption of fat and weight loss (Benenson , 1990).

Other intestinal protozoan parasites detected in the present study among HIV seropositive and HIV seropositive patient were *E. coli* 5.3 % and among HIV sronegative *E. coli* 6.7% *E. nana* 4 % among HIV seropositive and *E. nana* 0.7 % among HIV seronegative . the incidence of *E. coli* 5.3 % among HIV seropositive which is those to that recorded in Ethiopia (12.6%) (Assefa *et al* ., 2009) . However the prevalence of *E. coli* (12.5 %) was relatively high when compared to previous reports 0.8% in Northern India (Mohandas *et al* ., 2002) ,1.1 % Thailand (Termmathurapoj *et al* ., 2000) . 1.9 % in Ethiopia (Assefa *et al* .,2009), 1.6% in North of Iran (Daryani *et al.,* 2009) 2.9%

(among HIV seropositive in Australia (Stark, *et al* .,2007) ; 3.7% in Saudia Arabia (AL-Megrin 2010) ; 4.7% in Nigeria (Udeh , *et al* .,2008) and 7.7 % in Honduras (Lindo , *et al* ., 1998) , 3.9% in

Iran (Zali *et al* ., 2004) . But it was lower than previously recorded in North West Ethiopia (17%) (Taedesse and Kassu , 2005) ,in South India (20%) (Ramakrishnan *et al* ., 2007) .

The incidence rate of *E. nana* in the present study was 4% in HIV seropositve patient was relatively high when compared to previous studies intestinal of parasites in HIV patients . (Termmathurapoj *et al* .,2000 ; Udeh *et al* .,2008 ; AL-Megrin 2010; and Kulkarni *et al* .,2009) . While in Jamaica (Barrett *et al* .,2008) .

Only one helminth *E. vermicularis* was detected among HIV-seropositive patients but it was absent among HIV seronegative patient but other helminthes reported among HIV seronegative *Taenia* sp.(2%) this species of parasites absence among HIV seropositve patient . (Fontanet *et al* ., 2000) in Ethiopia reported 1.3% , other studies in Ethiopia reported 3.6% (Moges *et al* ., 2006) .

5.1.1 Incidence and sex :

The overall incidence of intestinal parasites in HIV-seropositive patients and HIV seronegative patients among females and males and reported in the present study was 45.8 % and 39.5 % respectively while in HIV seronegative reported 37.3 % in females and 29.3 % in males . In both . No significant differences was exist between incidence and sex . This results in agreement with the results reported previously Lindo *et al* ., 1998 **;** Kumar *etal .,* 2002 **;** Kaminsky *et al.,* 2004 **;** Moges *etal .,* 2006; Daryani *etal.,* 2009 and Babatunde *etal.* 2010) . The detection of intestinal parasites among females and males immunocompromised patients indicating that both genders are

equally susceptible to infection . This agrees with the findings of Zali *et al* .,(2004) and Al – Megrin (2010).

The present study shown that infection in females was higher than males among HIV- seropositve group . But these results are in contrast with previous findings (Savioli *et al* ., 1992 and Etok *et al* ., 2010) which reported that there were more HIV –positive agreed with those in which females were HIV – seropositive than males. On the other hand males was higher than females among HIV seronegative group this may due to females have fewer restrictions than males whose leisure house are strictly controlled (Akogun and Badaki , 1998 ; Agha Rodina and Teodorescu , 2002 and Suwansaksri *etal .,* 2005) .

5.1.2 Incidence and age :

The age distribution of intestinal parasites among HIV –positive patients in the present study suggests that the infection tends to be common in 30-39 year old (50%) . This result agrees with those reported previously (Moges *et al* ., 2006 ; Simpore *et al* ., 2009 and Nasiri *et al* ., 2009) .However , no significant difference were observed between different age groups , the same results was reported previously (Al-Megrin , 2010) .

The present study showed all age groups were infected . This suggests HIV seronegative patients of all ages are susceptible to parasitic infection. The minimum incidence of parasites was discernible in old age group ,this may due to the develop of immunity to infection . The absence of the difference in the incidence between age groups was reported previously (Raccurt *etal.,* 2006 ;

Al-Megrin 2010 ; Hailemariam *etal.,* 2004 ; Oguntibeju 2006) . On the other hand similar results in age and incidence of intestinal parasites in HIV seropositive infected patients and HIV seronegative patients was found statistically significant (Ghimire *etal*., 2004 ; Lim *etal*., 2005 ; Okodua *etal*., 2003) .

5.1.3 Incidence and diarrhea :

Diarrhoea is a common complication of infection with HIV, leading to weight loss and cachexia. It occurs in almost 90% of the HIV patients (Kumar *et al .,* 2002) . The present study revealed that the infection rate with intestinal parasites in HIV seropositive was found to be 42.7 % ; of which diarrheal and non diarrheal cases accounted for 75% and 33.2 % respectively . The persons without diarrhea higher than those with diarrhea this rate of infection similar to previous studies in Honduras (Lindo *et al* ., 1998) .The same results were reported previously (Mohandas *et al* ., 2002 ; Brink *et al* ., 2000; Dwivedi , *et al* ., 2007 ; Zali *et al* ., 2004 ; and Deorukhkar *et al* ., 2011) .

5.1 .4 Incidence of single and mixed infection :

The present study revealed that 82.8% and 17.2% of infection were single and mixed infection respectively among HIV seropositive patients . Single and mixed infection has been reported by various workers (Guk *et al* ., 2005 ; Raccurt *et al* ., 2006; Moges *et al* ., 2006; Ramakrishnan *et al* ., 2007 , Tadess and Kassu , 2005 Assefa *et al* ., 2009 ; Kurniawan *et al* ., 2009 and Al- Megrin , 2010) . Multiple parasitic infections were more common in HIV/ AIDS patients while its not

detected in HIV - seronegative controls .This finding was reported previously (Hailemariam *et al* ., 2004).This strongly indicates the facilitated establishinent of parasites in immuno compromised patients .

SUMMARY

SUMMARY

This study was conducted on four hundred and fifty HIV seropostive and HIV seronegatve patients, fecal samples were taken from three Hospitals (HIV center , 7th October hospital ,Isolation center in Al jamhoria hospital and central laboratory) in Benghazi city. The collected samples were examined for intestinal parasitic infection by :

(1) Direct smear examination using normal saline and iodine soluation
(2) Sedimention method (Formalin –Ethyl Acetate method) to detect all cysts an trophozoites of protozoa and adult , eggs and larvae of helminthes .
(3) A modified version of the Ziehl – Neelsen technique was used to detect the coccidian parasites .

The blood samples were screened for the presence of HIV antibody using ELISA method for those particptate in this study .

Parasitic infection was identified in one hundred and twenty eight (70 females and 58males) giving an overall incidence rate at 42.7% in HIV seropositive while the parasitic infection was identified in forty eight (19 females and 29 males) giving an overall incidence of (32%) HIV-seronegative .

The result revealed that seven species of intestinal parasites were detected among HIV – seropositive patients , these parasites were *B. hominis* *E. histolytica* *E. dispar* , *E . coli* , *E. nana* , *G. lamblia* ,*C. parvum* and *E. vermicularis*.

The most common protozoan parasites in HIV seropositive was *B. hominis* with the highest incidence at (18.7%) . followed by *E. histolytica* *E. dispar* (10.3%) , *G. lamblia* (8%) , *E. coli* (5.3%) , *E . nana* (4%) , *C. parvum* (3.3%) and *E . vermicularis* (1%) The results showed that a significant differences were detected between incidence and each parasites .(p=0.000) . A significant differences were in overall incidence were statistically significant for each parasite (P=0.000) . No such difference were noted in *E . vermicularis* (P=0.136) . ,the most common protozoan parasites . Among HIV seronegative was *E. histolytica* *E. dispar* with the highest incidence at (12.7%) followed by *G. lamblia* (8%) , *E. coli* (6.7 %) ,*C. parvum* (0.7%), *B . hominis* (0.7%), *E . nana* (0.7%) . Among helminthes intestinal parasites was *E. vermicularis* (2%) and *Tenea* sp (0.7%) . The apparent differences in overall incidence were statistically significant for each of the following parasites *E. histolytica* *E. dispar* *G. lamblia , C. parvum* , *E. coli* (P=0.000) . No such differences were noted in *E . vermicularis* (P=0.08) *B . hominis* , *Tenea* sp and *E . nana* (P=.144)

Both sexes were infected with intestinal parasites in HIV seropositive the overall incidence was 45.8% in females and 39.5% in males . *B . hominis* had the highest incidence in both sexes with 21% in females and 16.3% in males followed by *E. histolytica* *E. dispar* at 9% in females and 3% in males , *G. lamblia* 10 % in females and 6.1% in males , *E. coli* 6% in females and 5% in males , *E . nana* 3.3% in females and 5% in males , *C. parvum* 3% in females and 4.1% in males , *E . vermicularis* 1.3% in females and 0.7% in males . No significant differences was exist between overall incidence and sexes (P = 0.195)

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While in HIV- seronegative both sexes were infected with intestinal parasite at overall incidence 37.3% in females and 29.3% in males . No significant difference was exist between overall incidence and sexes(P = 0 .322) .

Age had no effect on the incidence of intestinal parasites in HIV-seropositive (P= 0.570). The results showed that all age groups were infected with intestinal parasitic infection , the highest incidence rate with intestinal parasites was showed among age group 30-39 at 50% followed by 10-19 (44.4%) , 20-29 (35.8%) and 0-9 (28.6%) *B.hominis* showed high incidence rate in all age groups followed by *E. histolytica E. dispar , G. lamblia , E. coli, , C. parvum* and *E. nana* low incidence was detected in *E. vermicularis*

Age had no significant influence on the incidence of intestinal parasites HIV seronegative (P = 0.082) .The intestinal parasitic infection showed that the high incidence rate was detected among age groups 0-9(46.7%) , followed by 20-29(35.4%) , 10-19 (29.8%) ,> 40(27.8%) and 30-39 (22.7%) . *E. histolytica E. dispar* showed high incidence in all age groups followed by *, G. lamblia , E. coli , E. vermicularis* and low incidence was detected in *B.hominis , C. parvum , E. nana , Taenia* sp.

The results showed that the incidence rate of intestinal parasites in HIV – seropositive with and without diarrhea were 75% with diarrhea and 33.2% without diarrhea The results revealed that those HIV-seropositive patients with diarrhea was higher than those without diarrhea. There was a significant difference between incidence of parasitic infection and diarrhea . (P= 0 .000)

Infection rate with intestinal parasites in HIV -seronegative was found at 6 (60 %) had diarrhea and the rest 42(30 %) without diarrhea . There was a significant difference between incidence rate and diarrhea . (P=0.056) .

Single and mixed infections were detected at 82.8% and 17.2% receptivity in HIV seropositive . There was a high significant difference between single and mixed infection with parasitic infection in HIV – seropositive patients (P = 0.000) .

While the incidence of single infection of intestinal parasites among HIV seronegative patients showed in forty eight (100%) had single .No mixed infection was detected .

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الخلاصة باللغة العربية

الخلاصـــــــــــة

في عام 1998، حدثت عدوى حادة ب فيروس نقص المناعه الايدز(HIV/ AIDS) في مستشفى الأطفال في مدينة بنغازي.حيث أصيب حوالى 453 شخص، معظمهم أطفال وتوفى منهم 53. يعتبر هؤلاء المرضى عرضة للأمراضِ الانتهازية المُخْتَلِفةِ مثل الطفيليات المعويةِ.

أجريت هذه الدراسة لتحديد معدل حدوث الاصابة بالطفيليات المعوية فى مرضى الايدز فى مدينة بنغازي نظرا لتأثيرها على الحالة الصحية العامة ,شملت الدراسة 150 حالة غير مصابة بمرض الايدز لمجموعة سيطرة .

تم فحص ما مجموعة 450 عينة براز اشخاص مصابين وغير مصابين بمرض الايدز ذلك فى الفترة من فبراير الى ديسمبر 2010 ف . حيث شملت 300 حالة مصابة بمرض الايدز و150 حالة غير مصابة بمرض الايدز . لتحديد معدل انتشار الطفيليات المعوية .

اعتمدت الطرق المستخدمة فى هذه الدراسة للكشف عن تواجد الطفيليات المعوية فى عينات البراز بين الاشخاص المصابين و غير مصابين بفيرس الايدز الاتى :

أ – الفحص المباشر لمسحة من البراز باستخدام المحلول الملحى الفسيولوجى ومحلول الايودين

ب- استخدام تقنية الترسيب (Sedimentation method (Formalin –Ethyl Acetate

ج- استخدام صبغة A modified version of the Ziehl – Neelsen لكشف عن طفيليات الكوكسيديا (coccidian)

د- استخدمت طريقة الفحص المناعى ال ELISA لتأكيد من وجود فيروس الايدز فى المرضى المصابين وعدم وجوده فى المرضى غير مصابين .

اظهرت النتائج ان معدل انتشار الطفيليات المعوية فى المرضى المصابين بالايدز كانت 128 حاله بنسبة % 42.7 من الاشخاص المفحوصين .

دلت نتائج فحص عينات من المرضى المصابين بالايدز عن وجود سبعة أنواع من الطفيليات المعوية وهى :

B. hominis , *E. histolytica* /*E. dispar* , *E . coli* , *E. nana* , *G. lamblia* ,*C. parvum* and *E. vermicularis* .

اظهرت النتائج ان معدل انتشار الطفيليات المعوية فى الاشخاص غير مصابين بمرض الايدر كانت 48 بنسبة % 32 من الاشخاص المفحوصين عن وجود ثمان انواع من الطفيليات المعوية وهى :

B. hominis , *E. histolytica* /*E. dispar* , *E . coli* , *E. nana* , *G. lamblia* ,*C. parvum* , *E. vermicularis* and *Taenia sp* .

سجل طفيلي *B. hominis* أعلي معدل انتشار (18.7%) مقارنة الطفيليات المعوية الأخرى يليه الطفيليات *E. histolytica /E. dispar* بنسبة (10.3%)

G. lamblia (8%)*C. parvum* (3.3%)*E. vermicularis* (1% ، وقد كانت *E. coli*(5.3 %) *E.nana* (4%) .و كان هناك اختلافات معنوية بين المعدل العام للطفيليات وكل طفيلي (P=0.000) *E. vermicularis* (P=0.136)

كما سجل طفيلي *E. histolytica* / *E. dispar* (12.7%) أعلى معدل انتشار بين الاشخاص غير مصابين بالايدز مقارنة مع الطفيليات المعوية الاخرى يليه الطفيليات

G. lamblia (8%) ، *E. coli* (6.7%) ، *E. . vermicularis*(6.25%) ، *B. hominis* , *C. parvum* , *Taenia* *E.nana* فكان معدل انتشار العدوى فيها منخفظ (0.7%) . اظهرت الدراسة وجود اختلافات معنوية بين المعدل العام للاصابة بللطفيليات وكل طفيلي (P=0.000) عدا *B. homini* , *E.nana* , *Tenea* sp, *E. . vermicularis* (P=.144) (P=0.08) .

اظهرت النتائج ان معدل حدوث الطفيليات المعوية فى الجنسين كانت (45.8%) في الإناث و 39.5% في الذكور في عينات البراز للمرضي المصابين بالايدز . دلت النتائج على عدم وجود اختلافات معنوية بين الاصابة بالطفيليات المعوية والجنسين في مرضي الايدز بين الجنسين (P = 0. 195) .

اما معدل انتشار الطفيليات المعوية بين الاشخاص غير مصابين بالايدز فقد كان في الجنسين كانت 37.3%في الاناث و 29.3 % فى الذكور ولايوجد اختلافات معنوية بين الاصابة بالطفيليات المعوية بين الجنسين (P = 0 .322)

دلت النتائج المتحصل عليها ان اعلى معدل اصابة بين الفئات العمرية كانت فى الفئة العمرية (30-39) سنة وكان بنسبة 50% للمرضى المصابين بالايدز وسجل طفيل ال *B.hominis* اعلى نسبة اصابة بنسبة 28.6% فى الفئة العمرية (0-9) سنوات

اما الحالات غير مصابة بمرض الايدز فقد سجلت اعلى اصابة فى الفئة العمرية (9-10) سنوات بنسبة 46.7% يليها الفئات العمرية (10-19) بنسبة % 44.4 و(29-20)بنسبة 35.8%. و سجل طفيل *E. histolytica /E. dispar* *اعلى معدل اصابة* بنسبة 20% فى الفئة العمرية (0-9) سنوات .

حيث سجلت معدلات اصابة منخفضة فى كل الفئات العمرية هناك عدم وجود فروق معنوية فى نسبة انتشار الطفيليات المعوية بين المجموعات العمرية المختلفة (P = 0.570)
بينما في الاشخاص الغير مصابين بمرض الايدر فقد كان انتشار الاطوار الخضرية او المتكيسة للطفيليات المعوية فلم يكون هناك فروق معنوية في نسبة انتشار هذة الطفيليات والفئات معدل انتشار عالى بين جميع الفئات العمرية (p=
0.082)

الفئات العمرية اما باقي الطفيليات المعوية فقد سجلات معدلات إصابة منخفضة فى باقى الفئات العمرية .

اظهرت النتائج ان نسبة الاصابة بطفيليات المعوية فى الحالات المصابة بلاسهال اعلى 75% من الحالات غير مصابة بلاسهال % 33.2 بين مرض الايدز . ودلت النتائج على وجود فروق معنوية بين المجموعتين (P=0.00)كما سجلت ايضا نسبة عالية فى الحالات غير مصابة بالايدز فكان نسبة الطفيليات حالات الاسهال 60% اما الحالات غير المصابة

بالاسهال فكانت بنسبة 30% حيث سجل فرق معنوى (P=0.049)

اظهرت النتائج ان (% 82.8) من بين الحالات المفحوصة للمرضى المصابين بالايدز كانت مصابة بالطفيليات المعوية فى حالة اصابة مفردة (اصابة بطفيلى واحد) و(17.2%) من الحالات مصابين بأكثر من طفيلى . وكانت هناك احتلافات معنوية بين الاصابة الفردية والمختلطة (P=0.000) .

ولم تسجل اى اصلبة مختلطة فى الاشخاص الغير مصابين بمرص الايدز .