Sudan University Of Science And Technology(SUST)
College Of Post Graduate Studies

Frequency of *Shigella*, *Salmonella* species and Intestinal Parasites in a diarrheal diseases in Sinnar State

A dissertation submitted for partial fulfillment of the requirement of M.sc in medical laboratory science (medical microbiology)

by:Submitted

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DECLARATION

We hereby declare that this work is original research work; undertaken under the supervision of **Dr. Y. Fadal-Allah** and has not been presented elsewhere for award of a degree of certificate. All sources have been cited and appropriately acknowledged.

Name……………………………............ Date………………………….

Name……………………………............ Date………………………….

Dedication

To

The greatest love in my life ,thewoman whom I loved and give me the strength, well and support all the time………………..…dearest mother

To

Those whom always on my side………Brothers and Sisters

To

My supporters through my way…………Friends

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Abstract

Background: Diarrheal disease continues to be an important cause of morbidity and mortality among youngchildren in developing countries including Sudan. Globally, intestinal parasite, Shigella and Salmonella species remain major contributors to acute enteric infections. The study was aimed at determining the frequency ofintestinal parasite, Shigella and Salmonella species identified from diarrheic patients at Daweena Medical Centre, Abuhuggar Medical Centre, Abuhuggar Hospital and Senja model medical centre in Sinnar state**.**

Methods: This study based cross sectional study was conducted from November to May 2016. Parasitic andbacterial identification was done using standard parasitological and bacteriologicalprocedures.

Results: Out of the 103 stool samples, 48 (46.6%) samples were positive for intestinal parasite, Shigella and Salmonella species. Of these, 43 (41.7%), 2 (1.9%) and 5 (4.9%) samples were positive for intestinal parasites, Salmonella and Shigella species respectively. The dominant isolated parasite was Entamoeba histolytica with frequency of 11.7% followed by Hymenolepis nana (8.7%), then Giardia lamblia (7.8%). The least identified parasites were Ascaris lumbricoid and Taenia saginata about 1% for each one.

The majority 29 (58%) of enteropathogens were found in patients aged 6-15 years. Whereas, 17 (34%) in patients aged 16-25 , 2 (4%) observed in patients aged 26-35 and 1(2%) pathogen observed within patients aged >5 and <45. Double parasitic infections were observed in 4 patients (3.9%).

Conclusion: The presence of reasonably high amount of intestinal parasite and Salmonella and Shigella species, measures including health education, improvement of safe water supply and sanitation facilities
is crucial

مخلص الأطروحة

تظل امراض الاسهلات من المسببات الهامة للامراضية ومن اهم الاسباب لمعظم حالات الوفاة لاطفال الدول النامية ويعد السودان احد الدول النامية. عالميا, الطفيليات المعوية, انواع بكتيريا الشيقيلا, والإشريكيا القولونية المسببة للاسهالات و انواع بكتيريا السالمونيلا تعتبر المسبب الرئيسي للإصابات المعوية الحاده. هدفت هذة الدراسة لمعرفة تردد الممرضات المعوية لمصابي الاسهالات المترددين على كل من مركز داوينا الطبي, مركز أبوحجار الطبي, مستشفى أبوحجار و مركز صحي سنجه النموزجي بولاية سنار في الفترة من شهر فبراير الي شهر مايو سنة(2016) واعتمدت علي نظام التقاطع العرضي, كما تم التعرف التعرف على الطفليات المعوية و انواع البكتيريا الممرضة بواسطة الطريقه المثلى المتبعه داخل معامل البكتيريا و الطفيليات الطبية للتعرف علي مثل هذة الانواع من الممرضات.

تم جمع عدد 103 عينة من مصابي الاسهالات بالمنطقة المعنية وخلصت هذة الدراسة الي ان62/103 (60.2%) من المصابين بامراض الاسهالات حاملين للممرضات المعوية المسببة للاسهالات. وكانت هذة الممرضات موزعه كالاتي:43/103(41.7%) طفيليات معوية , 5/103 (4.9%) انواع بكتيريا الشيقيلا,2/103 (1.9%) انواع بكتيريا السالمونيلا و12/103 (11.7%) الاشريكيا القولونية نوع مصلي O157:H7.معظم الممرضات المعوية وجدت في مصابي الاسهالات الذين يتراوح اعمارهم 6-15 سنة بتردد 29/50 (58%), يليهم المصابين الذين يتراوح اعمارهم 16-25 سنة بتردد 17/50 (34%), ومن ثم المصابين الذين يتراوح اعمارهم 26-35 بتردد 2/50 (4%) واقل تردد للممرضات المعويه وجد فئتين من الصابين (المصابين الذين اعمارهم دون سن الخامسة والمصابين الذين يتراوح اعمار 36-45 سنة.وكان التردد الاعلي لبكتيريا الاشريكيا القولونية نوع مصلي O157:H7 بين تردد العزلات البكتيرية بتردد 12/53 (22.7%), تلية انواع بكتيريا الشيقيلا بتردد 5/53 (9.4%) و من ثم انواع بكتيريا السالمونيلا بنردد 2/53 (3.8%).

وكان التردد الأعلى لطفيل المتحولة الزحارية بين تردد الطفيليات بتردد 12/43 (27.9%), يليه طفيل المتحرشفة القزمة بتردد 9/43 (20.9%), ثم طفيل القيارديه اللمبيلية بتردد 8/43 (18.6%),ويليه طفيل المتحولة القولونية بتردد 6/43 (14.0%) ومن ثم طفيل السرمية الدودية بتردد 2/43 (4.7%). واقل تردد كان لكل من طفيل الشريطية الافريقية وطفيل الصَفر

الخراطينيَ بتردد 43/1 حوالي (2.3%) لكل منهما.وايضا شوهدت الازدواجية باصابة الطفيليات المعوية عند 43/4 (9.3%) من حاملي الطفيليات المعوية, وكانت كالأتي: 43/3 (7.0%) حاملي لطفيل المتحرشفة القزمة وطفيل القياردية اللمبياية و43/1 (2.3%) حاملي لطفيل المتحولة الزحارية وطفيل المتحولة القولونية

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CHAPTER ONE

Introdution, Rationale and Objectives

1.1. Introduction:

Diarrheal diseases in developed and developing countries are significant causes of morbidity and mortality and are caused by a variety of pathogens such as Bacterial, Parasitic pathogens, viral and others. Also caused by non biological substances like chemical toxins and …ate. Bacterial pathogens likeinfection with organisms of the *Salmonella* spp. is an important public health problem throughout the world. Non-typhoidal Salmonellosis is a common cause of food-borne infection, and typhoid continues to exact a considerable death toll in developing countries.The major causes of typhoid fever are *Salmonella enterica* serovar Typhi (*Salmonella typhi*) and also, to a lesser extent, strains of *Salmonella enteric* belonging to serovar paratyphi (*Salmonella paratyphi* A, B and C). In the UK, cases of typhoid tend to occur in travelers returning from locations where this disease is endemic or in people who have been in contact with patients who have become asymptomatic carriers of *Salmonella typhi* or *Salmonella paratyphi* (Chart *et al.*, 2007).

In Sudan isolation of *Salmonella* was reported by different investigators (El Hussein, 2010) isolated four *Salmonella* isolates from 119 fecal samples (3.4%) (Fath Elrahman, 2008) isolated only 7(15%) *Salmonella typhi* isolates from 47 blood specimens. (Ahmed, 2008) isolated only 2 (4.3%) *Salmonella paratyphi* A isolates from 46 blood specimens (Mahmood, 2008) isolated only 22 (46.8%) *Salmonella paratyphi* B isolates from 47 blood specimens (Mohammed, 2011).

Also diarrheal diseases caused by *Shigella* spp. Dysentery, was recognized by Hippocrates as a condition characterised by the frequent passage of stools containing blood and mucus. It was not known until 1875 when the cause of amoebic dysentery was discovered and later when Shiga isolated the organism that was later known as *Shigella dysenteriae* that the two dysenteric illnesses could be clearly differentiated. Dysentery has always played an important part in human history, influencing the course of military campaigns. Although the mortality rate among soldiers with bacillary dysentery was approximately 2.5%. At crucial times, many soldiers were infected and incapable of performing military duties. When people are herded together in goals or on ships, epidemic dysentery may sweep through the population with devastating effect. The first of the genus to be identified was *Shigella dysenteriae*, which for many years was known as Shiga's bacillus (Shiga, 1898). *Shigella flexneri* was originally described by Flexner in 1900 (Flexner, 1990). *Shigella sonnei* was first isolated in 1904, but it was not until 1915 that its pathogenic potential was recognized by Sonne (Sonne, 1915).

Parasites that causes diarrheal diseases wich expressed as parasitic intestinal infections and it's continue to be an important causes of morbidity and mortality in the developing world (Cheesbrough, 1987).

The most common parasites reported are protozoan infections mostly Giardiasis and Amebiasis. But also Helminthes can cause diarrheal diseases such as nematodes; Ascaris, Enterobius and cestodes like Taenia, Hymenolepis and other (Cheesbrough, 1987).

Rationale

Diarrheal diseases continues to be an important cause of morbidity and mortality among young children in developing countries including Sudan. Notably Sinnar state. So study like ours is needed specially in this area because few studies done in state rather than Khartoum. Studies form such this type of ours study it's not recently done in last few years in Sudan, more and more studies like form ours study is needed from states rather Khartoum and specially form like ours studies must be done every five years if not done annually to protect ours self from diarrheal diseases and it's causes.

1.2. Objectives:

1.2.1. General objective:

To identify frequency of *Salmonellae*, *Shigellae*, and Intestinal parasites in a diarrheal diseases.

1.2.2. Specific objectives:

a) To estimate incidence of *Salmonellae* in a diarrheal diseases.

b) To estimate incidence of *Shigellae* in a diarrheal diseases.

c) To estimate incidence of Intestinal parasites in a diarrheal diseases.

d) To determine the existence of statistically significant differences in the *Salmonellae*, *Shigellae*, Intestinal parasites in a diarrheal diseases.

CHABTER TWO

2. LITERATURE REVIEW

2.1. The genus *Salmonella:*

2.1.1. Historical background:

In the late 1800s, Dr. Theobald Smith, a researcher under Dr. Daniel E. Salmon in the USDA's Bureau of Animal Industry, was the first American to identify *Salmonella* as a separate strain or genus. Although Smith actually identified the bacteria, Salmon's name as administrator was listed first on the research paper, so the new bacterium was named for Salmon (FDA, 2008).

2.1.2. Definition:

Members of the genus *Salmonella* are fermentative facultative anaerobic, oxidase-negative Gram-negative rods. They are generally motile, non-lactose fermenting, urease negative, citrate utilizing and acetyl methyl carbinol negative (Gillespie *et al*., 2006). They are non-sporing and with the exception of *Salmonella typhi*, non-capsulate (Cheesbrough, 2006). Most strains of *Salmonella* are produce acid and gas during fermentation of glucose. They also produce H_2S from sulfur-containing amino acids (Cornelissen *et al*., 2013).

2.1.3. Classification:

The genus *Salmonella* is the most complex genus in the family Enterobacteriaceae, with more than 2400 serotypes currently listed in the Kauffman-White scheme. Because multiple nomenclature systems are used, classification of the organism can be confusing. Previously, *salmonellae* were classified as having three distinct species: *Salmonella choleraesuis*, *Salmonella typhi*, and *Salmonella enteritidis*. Most of the described serotypes belonged to the species *Salmonella enteritidis*. *Salmonellae* were

further grouped (A, B, C, etc.) based on somatic O antigen and further divided into serotypes (1, 2, 3, etc.) based on flagellar H antigens (Guerrant *et al*., 2004). All *Salmonella* and *Arizona* isolates are now considered to be a single species i.e. *Salmonella enterica* (Guerrant *et al*., 2004). DNA hybridization studies have now shown that all pathogenic salmonellae belong to a single species, *Salmonella enterica* which is subdivided into 7 subspecies (subsp.) (Cheesbrough, 2006). They separated into seven distinct subgroups: I, II, IIIa, IIIb, and IV through VI. Subgroup I contains most of the *Salmonella* serotypes responsible for human disease. Based on this new schema, the complete name for all isolates is designated as, for example, *Salmonella* enterica subspecies *enterica* serotype *Typhi*. However, due to wide clinical use and familiarity, it is still appropriate to address them as, for example, *Salmonella typhi*, as long as it is understood that these organisms are a serotype of the species *Salmonella enterica* and not a distinct species (Guerrant *et al*., 2004).

2.1.4. Antigenic structures:

The *salmonellae* carry a complex antigenic structure. The antigens which have been detected on these organisms include somatic (O) antigen, flagellar (H) antigen, surface antigens (such as Vi), fimbrial (F) antigen, M and N antigens as well as antigens present on rough strains and designated as R antigen (Bhatia and Ichhpujani, 2008).

2.1.4.1. Surface Antigens:

The surface antigens which are located on the surface of the *salmonellae* and at times render the bacteria unagglutinable by antibodies against O antigen. These include Vi (virulence) antigen is a surface polysaccharide which is formed by most of the strains of *Salmonella typhi* and some strains of *Salmonella paratyphi* C, *Salmonella dublin* as well as *Citrobacter. freundii*;

it is heat labile and destroyed at 100°C within 60 minutes. M and N antigens are responsible for mucoid character and F antigen is carried on fimbriae, as well as R antigen responsible for smooth to rough variation of *Salmonella* (Bhatia and Ichhpujani, 2008).

2.1.4.2. O Antigens:

These are heat stable polysaccharide antigens that are part of lipopolysaccharide of the cell wall. These antigens are resistant to boiling up to 2 hours and 30 minutes and alcohol-stable, withstanding treatment with 96% ethanol at 37°C for 4 hours. These also remain unaffected in suspensions having 0.2% formaldehyde. Till date 67 somatic (O) antigens have been described and with their help *salmonellae* have been divided into 46 O serogroups (Bhatia and Ichhpujani, 2008).

2.1.4.3. H Antigens:

These are flagellar antigens. They are heat and alcohol labile but are well preserved in 0.04 to 0.2% formaldehyde. The production of these antigens can be biphasic in some, but not all strains. In Phase 1 (also called as specific phase) a large number of flagellar antigens have been found in this phase (more than 80). A different set of H antigens expresses when the bacterium is in phase 2 (also called as group phase). This phase is non-specific phase because numerous *Salmonellae* share the same antigens when these are in phase 2 (Bhatia and Ichhpujani, 2008).

2.1.5. Habitat:

The genus *Salmonellae* includes a large number of pathogens of human beings as well as other mammals which are antigenically related to one another. Currently 2324 serotypes of*Salmonella* are known. With the exception of a handful of these (such as *Salmonella typhi* and

Salmonellaparatyphi) all are primary pathogens of animals with capability to infect human beings also (Bhatia and Ichhpujani, 2008).

2.1.6. Transmission:

Most infections result from the ingestion of contaminated food products and, in children, from direct fecal oral spread. The incidence of disease is greatest in children younger than 5 years and adults older than 60 years who are infected during the summer and autumn months, when contaminated foods are consumed at outdoor social gatherings. The most common sources of human infections are poultry, eggs, dairy products, and foods prepared on contaminated work surfaces (e.g., cutting boards where uncooked poultry was prepared) (Murray *et al.*, 2013). Salmonellosis also is transmitted by direct contact with animals, by non-animal foods, by water, and occasionally, by human contact (Murray *et al.*, 2007).It is generally believed that 1% to 4% of patients with acute typhoid fever become carriers, but this rate is a function of the age and health of the patient. The carrier rate is higher in women and increases with increasing age and prevalence of gallbladder disease. Fecal carriers usually outnumber urinary carriers by 10 to 1, but in areas endemic for *Schistosoma haematobium,* urinary carriers are often more common. The carrier rate within communities varies considerably (Guerrant *et al.*, 2004).Food can be infected directly by water used to wash it or prepare it, by carriers, by vomits and dust, and probably by flies. In many cases, the initial concentration of organisms is too low to cause human disease, but under optimal environmental conditions the organisms can multiply in food. In August 2000, the Ohio Department of Health reported sexual transmission of *Salmonella typhi* in a cluster of nine cases (seven culture-confirmed) in men in Ohio with no recent travel abroad,

all but one of whom reported having had sexual contact with one asymptomatic male *Salmonella typhi* carrier (Guerrant *et al.*, 2004).

2.1.7. Pathogenesis:

Broadly speaking the disease may present in three ways-enteric fever, septicemia and enteritis (Bhatia and Ichhpujani, 2008). The primary mode of transmission of the typhoid bacillus is the fecal-oral route through contaminated food and water. The incubation period is usually 14 days but may range between 5 to 20 days. The organisms on entering the gastrointestinal tract attach to and penetrate the epithelial lining of the small intestine. Following penetration the bacteria are phagocytosed by macrophages but are unfortunately not destroyed. These organisms multiply within the macrophages. The macrophages carry these to the reticuloendothelial system. These events occur during the first week of infection and may be accompanied by fever, malaise, lethargy and aches and pains. During the second week extended bacteraemia is present and the foci of infection may occur in various tissues; often the gallbladder becomes infected. Bacteria may be shed from the gallbladder back into the intestinal lumen. During this time ulcerative lesions of Peyer's patches may develop and the patient is often severely ill with a constant fever as high as 40°C, abdominal tenderness, diarrhoea or constipation, and vomiting. By the third week the patient may be exhausted but still be febrile. Death may occur in 10% of untreated patients. After recovery, multiplication of these bacteria in the gallbladder has been seen in 3% of the patients. These persons may become chronic carriers and serve as source of future outbreaks (Bhatia and Ichhpujani, 2008). Studies done in human volunteers using the Quailes strain of *Salmonella typhi* showed that, in healthy previously unvaccinated male adults, ingestion of 10^5 organisms led to clinical disease in 25% of

volunteers (median infective dose ID_{25}), ingestion of 10^7 organisms caused disease in 50% (ID_{50}), and 10^9 organisms caused disease in 95% (ID_{95}). As the number of organisms increased, the attack rate increased, the incubation period decreased, but the clinical syndrome was unchanged. Nothing is known about the relationship between differences in strains of *Salmonella typhi* and infectivity except that strains that do not have Vi antigen are less infective and less virulent. A gastric pH of less than 1.5 will kill most of the organisms, and those patients who chronically ingest antacids, have had a gastrectomy, or have achlorhydria due to aging or other reasons require lower numbers of organisms to produce clinical disease (Guerrant *et al*., 2004).Relapse: apparent recovery can be followed by relapse in 5–10% of untreated cases. Relapse is usually shorter and of milder character than the initial illness, but can be severe and may be fatal. Severe intestinal haemorrhage and intestinal perforation are serious complications that can occur at any stage of the illness (Greenwood *et al.,* 2012).

2.1.8. Laboratory diagnosis:

Laboratory diagnosis of typhoid fever depends upon following three parameters: Isolation of causative agent, detection of microbial antigen and titration of antibody against causative agent (Bhatia and Ichhpujani, 2008).

2.1.8.1. Culture methods:

Blood culture has the promise of diagnosis in the first week and is very specific, but its sensitivity is poor due to various factors. The most important factor is the very few numbers of bacteria needed to cause severe infection, which can be as low as 10/ml. Hence, positive culture yields are very low and elude definitive diagnosis. Other limiting factors, beside the bacteriostatic effect of antibiotics (already administered before the culture sample is taken), may be the nature of culture medium employed, the time of

blood collection, the host's immune response system, and the intracellular characteristics of *Salmonella typhi* (Haque *et al.,* 1999).Isolation of the causative agent by culture has remained the gold standard for diagnosis of typhoid fever. However, it is well recognized that facilities for blood culture are not readily available everywhere. Moreover, it is time consuming, expensive and the number of cultures showing positive results is also small. In the present study, out of 62 clinically diagnosed cases of typhoid fever, only 7 (11.29%) were found to be culture positive for *Salmonella typhi*. The reported figure from other studies varies from 6.92% to 26.7%. Two investigators from Bangladesh reported isolation rates of *Salmonella typhi* as 16.67% and 26.7%. In contrast, others, reported isolation rates of only 8.4% and 6.92%. The relative low sensitivity of the blood culture in diagnosing typhoid fever is due to widespread use of antibiotics and the difficulties in obtaining adequate volume of blood for culture (Jindal *et al.,* 2014).

Bone marrow culturing has a higher sensitivity than blood culturing, but is a more invasive procedure (Mitra *et al.,* 2010). Faeces; Organisms can usually be isolated from 40–50% of patients during the second week of infection and from about 80% of patients during the third week. Faecal culture is useful for detecting *Salmonella typhi* carriers (Cheesbrough, 2006).Urine; Organisms can usually be isolated from about 25% of patients after the second week of infection especially from those with urinary schistosomiasis. The bacteria are not excreted continuously and therefore several specimens may need to be cultured before the organisms are isolated (Cheesbrough, 2006).Deoxycholate citrate agar (DCA) is highly selective; the growth of coliforms and Gram-positive organisms is strongly inhibited due to sodium citrate and sodium deoxycholate in the medium. Xylose lysine deoxycholate (XLD) agar is a popular medium, and as it is less inhibitory than DCA.

Wilson and Blair medium is a more reliable modification of the original bismuth sulphite agar. It is highly inhibitory to other species and is particularly useful for the isolation of *S. typhi* from heavily contaminated specimens. Selection depends on the presence of brilliant green, sodium sulphite and bismuth ammonium citrate. *Salmonella typhi* has a characteristic silvery sheen with an adjacent brown-black zone in the agar (Gillespie *et al*., 2006).Because the media used for culture are highly selective, some pathogens that should grow may be inhibited. The more media inoculated, the more likely a pathogen will be isolated**.** Use of enrichment broth or the use of both XLD and HEK will detect more pathogens, but the cost-benefit may not make the expense and time practical (Garcia and Isenberg, 2007).

2.1.8.2. Serological tests:

2.1.8.2.1. Widal test:

The Widal test uses O and H antigens from the *Salmonella typhi* and *Salmonella paratyphi* in a simple bacterial agglutination test to aid the diagnosis of enteric fever. Patients infected with *Salmonella typhi* produce serum antibodies to the O and H antigens of this pathogen, and the detection of these specific antibodies forms the basis of this test, the standardized protocol for which was established in 1950. Although simple to perform, the Widal test is difficult to interpret, requiring a detailed knowledge of the patient's medical, travel and vaccination history. Acute and convalescent serum should be obtained and a positive diagnosis made on the basis of a fourfold rise or fall in *Salmonella typhi* O and H antibodies. Certain patients may have high H antibody titers due to previous infection or vaccination. The O antibody concentrations fall about 6 months after previous exposure to typhoid (Gillespie *et al*., 2006).Widal test is not a candidate for early

detection of the disease because specific antibodies take at least one week to reach detectable levels. Beside these shortcomings, its value is further diminished by its non-specificity. It is particularly unreliable with single titers (Haque *et al.,* 1999). In endemic areas the Widal test produces many false positive and false negative test results. False positive results occur because *Salmonella typhi* shares O and H antigens with other *Salmonella* serovars and cross-reactions also occur with other enterobacteriacae (Cheesbrough, 2006). Cross-reacting antibodies from previous exposure to other salmonellae may confuse the results of serodiagnosis (Greenwood *et al.,* 2012).Widal test is quite sensitive but has become highly nonspecific. In a study in Peru, O and H antibodies were found to be raised in 30% and 75%, respectively, of the normal population. In another study in Pakistan, Widal test was positive in 28% of the general population. Another shortcoming of the Widal test is that it becomes positive only in the second week of illness, so its value for early detection of the disease is limited (Haque *et al.,* 1999).

2.1.8.3. Molecular methods:

2.1.8.3.1. Labeled probes:

Although detection of *Salmonella typhi* by using labeled probes is 99% specific, its sensitivity is poor because it cannot detect less than 500 bacteria/mL (Haque *et al.,* 1999).

2.1.8.3.2. Polymerase Chain Reaction:

Due to the need for extensive infrastructure and specialized skills, the PCR facility cannot be made available everywhere, especially in developing countries. Another factor to be considered is the relatively high cost, which is almost twice that of blood culture (Haque *et al*., 1999).

2.1.9. Prevention:

Cook poultry, ground beef, and eggs thoroughly. Do not eat or drink foods containing raw eggs, or raw (unpasteurized) milk. If you are served undercooked meat, poultry or eggs in a restaurant, don't hesitate to send it back to the kitchen for further cooking. Wash hands, kitchen work surfaces, and utensils with soap and water immediately after they have been in contact with raw meat or poultry. Be particularly careful with foods prepared for infants, the elderly, and the immunocompromised. Wash hands with soap after handling reptiles, birds, or baby chicks, and after contact with pet feces. Avoid direct or even indirect contact between reptiles (turtles, iguanas, other lizards, snakes) and infants or immunocompromised persons. Don't work with raw poultry or meat, and an infant (e.g., feed, change diaper) at the same time. Mother's milk is the safest food for young infants. Breastfeeding prevents Salmonellosis and many other health problems (CDC, 2010).

2.1.10. Treatment:

Management of hospitalized patients requires proper use of antibiotics, good nursing care, adequate nutrition, careful attention to fluid and electrolyte balance, prompt diagnosis and treatment of intestinal perforation, intestinal bleeding, and other complications and the use of high-dose corticosteroids in severely ill patients. Medications that are appropriate for the treatment of typhoid fever include ampicillin, amoxicillin, cefotaxime, ceftriaxone, chloramphenicol, trimethoprim-sulfamethoxazole, or a fluoroquinolone. Of these, fluoroquinolones have been found to result in the shortest fever-clearance times. Azithromycin has also recently been shown to be effective. Oral therapy is indicated for uncomplicated disease and parenteral therapy is required for severe illness. Choice of a specific antibiotic is based on the expected or known susceptibility of the organism. Caution must be applied

when interpreting results of susceptibility testing. Although *Salmonella typhi* is often susceptible in vitro to drugs including cephalexin, aminoglycosides, furazolidone, and second-generation cephalosporins, use of these

2.2. Shigella:

2.2.1. Description of Organism:

Shigellae are small Gram-negative rods, non- or late lactose fermenters, fermenting sugars without gas production. Although non-motile using conventional tests, recent studies have shown the presence of flagellar genes and expression of a motile phenotype under certain conditions (Giron, 1995). *Shigellae* do not survive as well as *salmonella* in clinical specimens and so should be plated onto isolation medium as quickly as possible. Some selective media, deoxycholate citrate agar (DCA) or Wilson and blair designed for the isolation of *Salmonella*, can be too inhibitory for *Shigella*; thus, it is useful to use a non-inhibitory medium such as MacConkey, *Salmonella–Shigella* (SS) or xylose lysine–deoxycholate (XLD) in parallel.

2.2.2. Classification:

The genus *Shigella* is part of the enterobacteriacae, which have been classified together on the basis of the disease they cause. The *shigellae* have been shown to be closely related to *Escherichia coli* by DNA hybridization, isoenzyme analysis and the presence of Shiga-like toxin (Brenner *et al*., 1969; Crosa *et al*., 1973; Goullet, 1980).Recently, the full genome of *Shigella flexneri 2a* was sequenced and comparisons with *Escherichia coli* K12 showed that *Shigella* were phylogenetically indistinguishable from *Escherichia coli* (Jin *et al*., 2002; Wei *et al*., 2003). It has been suggested that *Shigella* spp. should be reclassified and new nomenclature introduced to clarify the relationship between these two species (Lan and Reeves, 2002).

They are most closely related to the EIEC, which also are capable of producing a dysenteric illness. The genus *Shigella* contains four species: *Shigela dysenteriae*, *Shigella flexneri*, *Shigella boydii* and *Shigella sonnei*. The species are differentiated on the basis of simple biochemical tests and serology of their LPSs: *Shigella dysenteriae* are non-mannitol fermenter and their *O*-polysaccharide LPS is unrelated antigenically to the other *shigellas*. *Shigella flexneri* and *Shigella boydii* ferment mannitol, but the latter are antigenically distinct from *Shigella. flexneri*. All *Shigella flexneri* O-specific polysaccharides of the LPS antigens have a common rhamnose-containing tetrasaccharide (Robbins, Chu and Schneerson, 1992).Included among the *Shigella boydii* are variants, some of which are capable of producing gas from glucose. *Shigella sonnei* differs from the other members of the genus in that it is a late lactose fermenter, which can be detected with ONPG, unlike other members of the genus which are non-lactase fermenting. The O-side chain is composed of a repeating disaccharide containing an unusual monosaccharide altruronic acid (Robbins, Chu and Schneerson, 1992). This antigen is identical to that of *Plesiomonas shigelloides* 017 which make up most human isolates and is the source of the cross-reactions between these two species. The *O*-polysaccharide of the LPS is used to divide the species into serotypes as follows: *Shigella dysenteriae* into 12 serotypes, *Shigella flexneri* into 13, *Shigella boydii* into 18 and a single serotype of *Shigella sonnei*(Robbins, Chu and Schneerson, 1992).

2.2.3. Pathogenesis:

Shigella spp. are largely limited to mucosal infection of the distal ileum and colon; intestinal perforation, although rare, has been reported (Azad, Islam and Butler, 1986); toxic megacolon may occur in up to 3% of patients in developing countries (Bennish, 1991).Bacteriaemia is the most important

lethal complication (Struelens *et al*., 1990); meningitis and pneumonia due to general spread are rare (Bennish, 1991). Seizures are common especially with *Shigella. dysenteriae* infection, and these resemble febrile seizures, although they occur in children over 5 years old (Rawashdeh, Ababneh and Shurman, 1994;Thapa *et al*., 1995).To establish an infection, *Shigella* must invade the enteric epithelium via the basolateral epithelial surface. The organisms gain access to the basolateral side via M-cells, the specialized antigen presenting cells of the lymphoid follicles (Wassef, Keren and Mailloux, 1989; Perdomo *et al*., 1994). Subsequent killing of macrophages in the submucosa leads to the release of cytokines (Zychlinsky, Prevost and Sansonetti, 1992; Zychlinsky *et al*., 1994), which attracts many PMNLs. A path for *Shigella* invasion is opened via the tight junctions which are broken down by the PMNLs (Perdomo, Gounon and Sansonetti, 1994).Recent research has shown that *Shigella flexneri* can also invade the colonic epithelial layer by manipulation of the epithelial cell tight junction proteins in an M-cell/PMN independent manner (Sakaguchi *et al*., 2002). Once inside the submucosa, *Shigella flexneri* makes contact with the basolateral membrane of the epithelial cells (Tran Van Nhieu *et al*.,1999).*Shigellae* have a virulence plasmid that encodes two loci required for invasion; the *ipa*locus and the *mxi-spa* locus (Jennison and Verma, 2004). *Shigella dysenteriae* type 1 produces a potent exotoxin (Stx) which enhances local vascular damage. It is structurally closely related to the Shiga-like toxins of *Escherichia coli*. It produces fluid accumulation in the rabbit ileal loop model but does not appear to be necessary for intracellular killing of mucosal epithelial cells. Systemic distribution of the toxin results in microangiopathic renal damage with subsequent development of the hemolytic uremic syndrome (HUS) (Obrig *et al*.,1988).

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2.2.3.1.Epidemiology:

It is estimated that there are 164.7 million cases of bacillary dysentery annually, of which 163.2 million are in developed countries and 1.5 million in industrialized countries (Kotloff *et al*., 1999). Approximately 1.1 million people die from shigellosis each year – 61% of these are children under 5 years old (Kotloff *et al*., 1999). Infection is spread by the faecal–oral route with great ease, as the infective dose is low (102–103 cfu). *Shigella* spp; are obligate human pathogens and do not infect other hosts, although experimental infection can be achieved in primates. Most cases of bacillary dysentery spread from person to person, and this may occur rapidly, especially in closed communities and when individuals are brought together in large numbers and sanitary arrangements are inadequate. Dysentery is a disease of poverty, and the incidence of infection can be correlated with poor housing and sanitary facilities and when large populations are suddenly displaced through war or natural disaster. Epidemics of disease may be both water and food borne and can be associated with faecally contaminated wells. Shigellosis is a disease of children under 5 years old in developing countries, reaching a peak at between 18 and 23 months (Henry, 1991). Disease is more severe in children who are malnourished (Bennish,1991). In one study, all of the fatal cases were severely malnourished (Thapa *et al*., 1995). Patients suffered convulsions, bacteriaemia, renal failure, intestinal perforation and toxic megacolon (Rawashdeh, Ababneh and Shurman, 1994; Thapa *et al*., 1995). *Shigella dysentery* has a significant effect on childhood mortality and on growth in excess of dysenteric illnesses of other etiologies (Bennish, 1991; Henry, 1991). In developing countries, *Shigella flexneri* and *S. dysenteriae* are most frequently isolated, whereas *Shigella sonnei* and *Shigella flexneri* predominate in developed countries (Kotloff *et al*., 1999).

Hemolytic uremic syndrome is associated with infection with *Shigella dysenteriae*. Between 2000 and 2004, the annual number of cases per 100 000 population per year in the United Kingdom varied between 1.6 and 1.1 (data from the Health Protection Agency, Communicable Disease Surveillance Centre, London). A study of infectious intestinal disease among cases in the community in England, carried out between August 1993 and January 1996, showed that *Shigella* spp. was isolated in 0.8% of faecal samples from cases and none of the controls (Kotloff *et al*., 1999). However, in many industrialized countries the scale of bacillary dysentery is not clearly established as many cases do not come to medical attention or are neither investigated bacteriologically nor notified to public health authorities. Infection is more common in children than in adults. *Shigella* is an important cause of traveler's diarrhea, especially in individuals returned from developing countries (Vila *et al*., 1994). Data from the Health Protection Agency on *Shigella* isolates received between April and June 2004 showed that 24% of all reports of *Shigella* infection recorded recent travel abroad, 63% of which specified travel to the Indian subcontinent, 19 of those were due to *Shigella sonnei* (Health Protection Agency, 2004).

2.2.3.2. Clinical Features:

Dysentery is an infection, not a toxemia, and symptoms result from changes in the bowel wall, caused by multiplication of the organisms after ingestion. The incubation period appears to be 2–3 days on average. Clinical features vary markedly among the different types of *shigellae*, sonnei dysentery being the mildest. Diarrhoea is frequently the only symptom in sonnei dysentery and consists of several loose stools in the first 24 h. This acute stage passes off rapidly, and in most cases, by the second day, the condition largely subsides. In some, the infection can be a mild and trivial affair; for

others the symptoms are more severe and vomiting can lead to dehydration, especially among the young. Fever is usually absent, but sometimes, at the onset, there is a sharp rise in temperature and general signs and symptoms may suggest meningeal involvement. Isolation of *Shigella* from blood and CSF specimens is rare (Struelens *et al*., 1990).*Shigella* septicemia is most common in infants and in people with malnutrition or in the immunocompromised (Huskins *et al*., 1994).The highest rates of *Shigella* bacteriaemia. *Shigella dysenteriae*, *Shigella flexneri* and *Shigella boydii* were isolated from blood cultures more frequently than *Shigella sonnei*, raising the possibility that these serotypes may be more invasive (Gupta *et al*., 2004).Abdominal pain is not usually a prominent symptom, although the illness may mimic appendicitis or even intussusception in babies. In some severe cases, the onset may be sudden, with vomiting, headache, rigors, severe colic and exhausting diarrhea. This may lead to dehydration, tetany and meningeal signs. While the illness caused by *Shigella flexneri* and *Shigella dysenteriae* may be no worse than sonnei dysentery, in most cases the patient is more acutely ill with constitutional upset. Diarrhea is severe and persistent. Abdominal pain and tenderness are frequent features, and the patient is toxic and febrile. The pulse is rapid and weak, and the patient becomes feeble, the skin punched, the tongue coated and urine scanty. The patient suffers from great thirst and cramps in the limbs; confusion or delirium may ensue. Generally, symptoms gradually subside over a period of 10–14 days, but relapses do occur, with a flare-up of the dysenteric diarrhea and rapid death. Fulminant choleraic or gangrenous forms are usually due to *Shigella dysenteriae*. Bacteriaemia, pneumonia, meningitis, seizures and hemolytic uremic syndrome HUS are recognized complications. During the acute stage of the disease, *Shigella* organisms are excreted in large numbers

in the faeces, but during recovery the numbers fall, although the organism may remain in the faeces for several weeks after the symptoms have subsided.

2.2.4. Laboratory diagnosis :

The organisms are usually present in large numbers in the intestinal mucus or the faeces in the early stages. Freshly passed stools should be examined, although rectal swabs showing marked faecal staining may be used. If the specimen includes blood and mucus, these should be plated directly. When faeces are kept alkaline, *shigellae* may survive for days, but in acid stools they die in a few hours. If there is

likely to be much of a delay, it may be useful to collect faeces into buffered 30% glycerol saline solution. Nothing is to be gained by culturing urine or blood, since these are invariably negative. Numerous waterborne epidemics are on record, and in several instances, shigellas have been isolated from water itself. Microscopic examination of the mucus in the early stages of acute bacillary dysentery shows a marked predominance of polymorphonuclear cells and red cells. Faecal material is plated out directly onto deoxycholate citrate agar (DCA), *Salmonella–Shigella* (SS) agar or xylose–lysine–deoxycholate (XLD) medium. *Shigellae* appear as small colorless or slightly pink colonies on DCA and as a pink or red colonies with, in some cases, a pink or yellow periphery on XLD. A few strains grow poorly on inhibitory media, and it is advisable to use MacConkey agar and to examine any non-lactose-fermenting colonies after overnight incubation. A preliminary identification can often be made by slide agglutination with specific antisera from the growth on the primary plate. In any event, full biochemical and serological tests must be performed on subcultures that have been checked for purity. The biochemical identification of *Shigella* is

complicated by the similarity of some strains of other genera, in particular strains of *Hafnia*, *Providencia*, *Aeromonas* and atypical *Escherichia coli*; non-lactose-fermenting or anaerogenic strains of *Escherichia coli* are a common problem. The PCR can be used to identify *Shigellae* either from a cultured isolate or directly from faeces. The *ipa*H gene is a good target for PCR as it is a multiple-copy element found on the large invasion plasmid (pINV) and the chromosome (Islam *et al*., 1998; Dutta *et al*., 2001). This PCR test also detects EIEC, as this group of diarrhoeagenic *Escherichia coli* also harbours pINV. Molecular typing techniques, such as pulsed-field gel electrophoresis (PFGE), can be used to investigate outbreaks of *Shigella* infection and to trace the source of infection (Chen *et al*., 2003).

2.2.5.Serotyping of *Shigella* spp:

Serotyping is subtyping method based on the immuno-reactivity of various antigens. Microorganismsproduce a variety of antigens: structural components of the cells (cell wall constituents, capsules orenvelopes, flagellae, fimbriae); secretion products of the cells (toxins, extracellular enzymes) or antigenscontained in the interior of the cells. Chemically the antigens used for such purposes are of two mainkinds: proteins and carbohydrates (including mixtures of both components).The *Shigellae* are by definition non-motile, as such, only the somatic (O) antigens are utilized for thedetermination of serotype. Flagellar (H) antigens are not expressed. The O antigen consists of repeatunits of oligosaccharide, and is part of the lipopolysaccharide (LPS) of the outer membrane of Gramnegativebacteria and contributes to the main antigenic variability on the cell surface.On the basis of O antigen structure and biochemical profile, *Shigella* spp can be classified into four species: *Shigella dysenteriae*, *Shigella flexneri*, *Shigella*

boydii and *Shigella sonnei*. Each species (with the exception of *Shigella sonnei*) can be further divided into multiple serotypes).

Group A or *Shigella dysenteriae*: includes 15 serotypes (*Shigella dysenteriae* 1 to the 15).

Group B or *Shigella flexneri*: includes 8 serotypes (*Shigella flexneri* 1 to the 6, *Shigella flexneri* X and *Shigella flexneri* Y) that can be divided into subserotypes according to its antigenic group factors(designated 3,4 – 6 and 7,8). Therefore with all the possible different antigenic combinations, theserotypes 1 to 5 of *S. flexneri* are subdivided into 11 subserotypes.

Group C or *Shigella boydii*: includes 20 serotypes (*Shigella boydii* 1 to the 20).

Group D or *Shigella sonnei*: contains only one serotype that may occur in two forms, form I(smooth) and form II (rough) (WHO, 2010).

2.2.6. Antimicrobial Therapy:

During the last decade, quinolones such as norfloxacin, ciprofloxacin, ofloxacin and fleroxacin have emerged as drugs of choice for the treatment of various bacterial enteric infections, including shigellosis (Bhattacharya and Sur, 2003). Controlled trials have shown that quinolones in varying regimens, from a single dose (Salam *et al*., 1994).To 5 days of treatment, significantly reduce the intensity and severity of traveler's diarrhoea as well as shigellosis (Wistrom and Norrby, 1995). Quinolones resistance is presently uncommon among *shigellae*, but it is inevitable that resistance will develop from increased usage of these agents. Antimotility drugs such as diphenoxylate (Lomotil) are not recommended in the treatment of diarrhoea, although loperamide, a synthetic antidiarrhoeal agent, has been shown to decrease the number of unformed stools and shortens the duration of

diarrhoea caused by *Shigella* in adults treated with ciprofloxacin (Bhattacharya and Sur, 2003).

2.3. Intestinal parasites:

Parasitic infections, caused by intestinal helminthes and protozoan parasites, are among the most prevalent infections in humans in developing countries (Savioli and Albonico, 2004).

2.3.1. Intestinal protozoa:

In developed countries, protozoan parasites more commonly cause gastrointestinal infections compared to helminthes (Savioli and Albonico, 2004). The most common intestinal protozoan parasites are: *Giardia lamblia*, *Entamoeba histolytica*, *Cyclosporacayetanenensis*, and *Cryptosporidium spp*. The diseases caused by these intestinal protozoan parasites are known as giardiasis, amoebiasis, cyclosporiasis, and cryptosporidiosis respectively, and they are associated with diarrhea (Davis *et al*, 2002). *G. lamblia* is the most prevalent parasitic cause of diarrhea in the developed world, and this infection is also very common in developing countries. Amoebiasis is the third leading cause of death from parasitic diseases worldwide, with its greatest impact on the people of developing countries. The World Health Organization (WHO) estimates that approximately 50 million people worldwide suffer from invasive amoebic infection each year, resulting in 40-100 thousand deaths annually (WHO, 1997). Cryptosporidiosis is becoming most prevalent in both developed and developing countries among patients with AIDS and among children aged less than five years. Several outbreaks of diarrhoeal disease caused by *C. cayetanensis* have been reported during the last decade (Herwaldt, 2000).

2.3.1.1. *Entamoeba histolytica:*

Entamoeba histolytica is an aerobic parasitic protozoan belonging to the genus *Entamoeba* and an etiology agent of amoebasis. *Entamoeba histolytica* is pathogenic in the caecum and colon of human being. The term *'histolytica* literally means "tissue dissolving'' referring to the carnivorous habit of the organism. *Entamoeba histolytica* is the most unique among the Amoebas because of its ability to hydrolyse host tissue. It can become a highly virulent and invasive organism causing diarrhea. Acute infection of amoebasis may be presented with other infection apart from bloody diarrhea such as ulceration of the colonic mucosa, abdominal pain and a palpable mass in corresponding areas of the abdomen. Amoebasis may give rise to amoebic liver abscess and intestinal pathologies (Aribodor *et al*, 2012).

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 year. It has long been known that although about 500 million people each year have amoebiasis, only about 10% experience symptomatic disease (WHO, 1997). There are only two stages to the life cycle of *Entamoeba histolytica*, the infectious cyst stage and the multiplying, disease-causing trophozoite stage. In the majority of cases, infection results from the ingestion of fecally-contaminated water or food that contains *Entamoeba histolytica* cysts. Much less often the cyst or the trophozoite forms can be transmitted as a result of oral or oral/anal sexual practices (Abhay *et al*, 2009).Trophozoites vary remarkably in size from 10 to 50 μm in diameter and when they are alive they may be actively motile (Mahon and Manuselis, 2000). The amoebae are anaerobic organisms which do not have mitochondria. The finely granular endoplasm contains the

nucleus and food vacuoles, which in turn may contain bacteria or red blood cells. The parasite is sheathed by a clear outer ectoplasm. The nucleus has a distinctive central karyosome and a rim of finely beaded chromatin lining the nuclear membrane (Sodeman and William, 1990). The cyst is a spherical structure, 10 to 20 μm in diameter, with a thin transparent wall (Sodeman and William, 1990). Fully mature cysts contain four nuclei with the characteristic amoebic morphology. Rod like structures (chromatoidal bars) are present variably, but are more common in immature cysts. Inclusions in the form of glycogen masses also may be present (Beaver *et al*, 1984).Approximately, 90% of infected individuals are asymptomatically colonized, amoebic dysentery usually occurs gradually, with symptoms (such as abdominal pain and tenderness, painful sudden bowel evacuation (tenesmus) and diarrhoea followed by weight loss(Manson and Bell, 1987).

2.3.1.2.Entamoeba coli:

Entamoeba coli is a nonpathogenic protozoan with a widehuman distribution. The presence of this organism in a patient'sstool is a useful indication of fecal-oral exposure. The life cycleof *Entamoeba coli* is identical to that of *Entamoeba histolytica*, and the two organismscan be found concurrently in up to 10% to 30% ofpatients in endemic areas. However, *Entamoeba coli* is nonpathogenicand requires no specific treatment. The cysts and trophozoitesof *Entamoeba coli* can be distinguished from those of the pathogenic *Entamoeba histolytica* on the basis of nuclear morphology and cyst size.Whereas *Entamoeba histolytica* cysts usually have fewer than five nucleiand are 10 to 15 μm in diameter, the cysts of *Entamoeba coli* are greaterthan 15 μm and often have five to eight nuclei(Guerrant *et al*., 2004).

2.3.1.3. *Giardia lamblia:*

Giardialamblia, also called *Giardia intestinalis* and *Giardia duodenalis*, is one of the most common intestinal parasites in the world, occurring in both industrialized and developing countries with an estimated 2.8 million new cases annually. First observed by Anton Van Leuwenhoek in 1681 in a sample of his own diarrheal stool, *Giardia* was initially thought to be commensal and has only been recognized as a pathogen since the mid 1900s (Abhay *et al*, 2009). *Giardia lamblia* is the most commonly isolated intestinal parasite throughout the world. Prevalence rates of 20-40% are reported in developing countries, especially in children (Fraser, 1994).*Giardia* transmission occurs by the fecal-oral route, either directly, via person to person contact or indirectly, via contamination of surface water or food. The salient features of *Giardia* cysts that influence disease transmission include their stability in the environment, their immediate infectivity upon leaving the host and the small number of cysts required to cause infection (Abhay *et al*, 2009). *Giardia lamblia*has a simple life cycle consisting of two stages: trophozoite and cyst, cysts are the transmission stage and are excreted in the feces of infected individuals into the environment where they can survive for weeks (Abhay *et al*, 2009). Trophozoite of *Giardia lamblia* has two nuclei and each nucleus contains a prominent karyosome, giving the parasite its characteristic face like appearance. In addition, it has four pairs of flagella, an axostyle (a microtubule containing organelle to which the flagella attach), a ventral disk and two median bodies, organelles whose function is not known. Cysts, which are slightly smaller than trophozoites, have a carbohydrate rich cell wall which likely protects them from the environment and two to four nuclei

(Abhay *et al*, 2009).*Giardia* infection may be asymptomatic or it may cause disease ranging from self limiting diarrhoea to a severe chronic syndrome (Meyer, 1990). The length of the incubation period, usually 1 to 3 weeks, depends at least partly on the number of cysts ingested. Normal human hosts with giardiasis may have any or all of the following signs and symptoms: loose, foul smelling stools, steatorrhea (fatty diarrhea), malaise, abdominal cramps, excessive flatulence, fatigue and weight loss or a coeliac disease like syndrome (Washington *et al*, 2006). Although most cases are seen in hosts with some concurrent condition, such as an immune deficiency, protein calorie malnutrition, or bacterial overgrowth of the small intestine, some cases of severe giardiasis occur in apparently normal hosts. Different strains of *Giardia lamblia* possibly vary in virulence (Smith and Wolfe, 1980).A small percentage of symptomatic individuals will have chronic infection, lasting months or longer, Chronic giardiasis is frequently accompanied by weight loss, which can be significant, and malabsorption of fats, vitamins A and B12, disaccharides, especially lactose, and protein are observed, with malabsorption of fats and lactose being most common (Abhay *et al*, 2009).

2.3.1.4. Diagnosis of intestinal protozoa:

Intestinal protozoa are diagnosed by identifying cysts or trophozoites in fecal specimens or histologically by visualizing cysts in biopsy specimens or secretions of intestinal mucosa (Sheehan *et al*, 1979). The conventional direct wet mount preparation for microscopy to identify motile trophozoites and a formalin ethyl acetate concentration step to identify cysts (Qvarnstrom *et al*, 2005), and when appropriately conducted with or without iodine stain, the conventional wet mount establishes the diagnosis of *Giardia lamblia* in

up to 70-85% of cases after two stool examinations (Sodeman and William, 1990). The sensitivity of the acid fast stain for oocysts of *Cryptosporidium* in the direct examination of stools is approximately 30% after one sample examination (Sodeman and William, 1990).

2.3.2. Intestinal helminthes:

Intestinal worm infections are widely prevalent in tropical and subtropical countries and occur where there is poverty and poor sanitation. Soil transmitted helminthes (STH) infections form the most important group of intestinal worms affecting two billion people worldwide and the main species are *Ascaris lumbricoides* (roundworms), *Trichuris trichiura* (whip worms) and *Necator americanus, Ancylostoma duodenale* (hookworms)(Sodeman and William, 1990).Recent estimates suggest that *A. lumbricoides* can infect over a billion, *Trichuris trichiura* 795 million, and hookworms 740 million people (de Silva *et al*, 2003). Other species of intestinal helminthes are not widely prevalent. Intestinal helminthes rarely cause death. Instead, the burden of disease is related to less mortality than to the chronic and insidious effects on health and nutritional status of the host (Stephenson *et al*, 2000). In addition to their health effects, intestinal helminthes infections also impair physical and mental growth of children, thwart educational achievement, and hinder economic development (Drake *et al*, 2000).

2.3.2.1. *Taenia species:*

Taenia spp are long, segmented, parasitic tapeworms (family: Taeniidae, subclass: Cestoda). These parasites have an indirect life cycle, cycling between a definitive and an intermediate host (Beaver *et al*, 1984).Taenia

infections are estimated to affect 100 million people worldwide, with major endemic areas located primarily in the developing countries of South America, Africa, India, China and Southeast Asia. Taenia infections are less common in North America; however, neurocysticercosis has been recognized as an important health problem in California. Although this disease is mainly seen in migrant workers from Latin American, it has also been reported in US residents who have not traveled to endemic countries (Abhay *et al*, 2009). *Taeniaspecies* are hermaphrodite and are very long enough to measure in metres (*Taenia solium* measure 3-5 meters while that of *Taenia saginata* measures 5- 10 meters). The body is divided into head, neck and a long segmented body (strobilla). The head size is about 1 mm in diameter. Head of both species bears four suckers. The head of *Taenia solium* has a rostellum armed with hooklets. In contrast, the head of *Taenia saginata* does not have the armed rostellum instead a depression. Each segment contains independent male and female sex organs. The terminal mature segments measuring 15-25 x 5-7 mm keep on detaching from the body and are passed in the feces. The eggs of*Taenia spp* are golden brown in color, measure 30-40 micron in diameter and are indistinguishable morphologically. The onchosphere bears three pairs of hooklets (Rai *et al*, 1996). Humans become infected after eating uncooked or under cooked beef or pork containing cysticerci. After ingestion, the cysticerci attach to the intestinal mucosa and develop into adult worms (Tadesse *et al*, 2008). Taeniasis is an infection with the adult tapeworm which usually remains confined to the small intestine. Most often, such infection results in minor gastrointestinal irritation and is frequently accompanied by nausea, diarrhea, constipation, hunger pains, chronic indigestion and passage of proglottids in the faeces. Although these symptoms are usually milder when the infection

is caused by *Taenia solium*, the risk of developing cysticercosis remains high (Abhay *et al*, 2009).

2.3.2.2*Hymenolepis nana:*

Hymenolepis nana (dwarf tapeworm) is a common human parasite and the smallest tapeworm known to infect humans. The lifecycle of *Hymenolepis nana* does not require an intermediate host, complete development occurring within a single host (Tadesse *et al*, 2008). *Hymenolepis nana* is widely distributed in countries with warm climates including those of Africa, South America, Mediterranean region, and SouthEast Asia, the infection is more frequently seen in children although adults are also infected (Tadesse *et al*, 2008).Adult worm measures 1-3 cm in length. It is made up of head (scolex), neck and segmented body. The head carries four suckers and a rostellum armed with one row of hooks. The segments of the body are divided into mature and gravid segments. In the mature segment, there are three testes in the middle. The eggs, which is immediately infective when passed by the patient, is rounded, about 40 microns in diameter. It contains a six hooked oncosphere within a rigid membrane (the embryophore). This embryophore has two polar thickening or knobs from which project 4 to 8 long, thin filaments called polar filaments (Assafa *et al*, 2004).Infection in man takes place by ingestion of eggs through contaminated foods or drinks. Autoinfection (the onchosphere hatched while the eggs being inside the intestine penetrate the villi and develop into cysticercoid larva that later develop into adult worm) also occurs (Rai *et al*, 1996).Light infections produce no symptoms. In fairly heavy infections, children may show lack of appetite, abdominal pain and diarrhea (Assafa *et al*, 2004).

2.3.2.3 *Ascaris lumbricoides:*

Round worm (*Ascaris lumbricoides*) is the largest of the human intestinal parasites. It lives and matures in the ileum and sometimes jejunum of the small intestine. Roundworm is often regarded as a parasite of children, but people of all ages may be infected (Obeng, 1997).Ascariasis, a soil transmitted infection, is the most common human helminthes infection. Current estimates indicate that more than 1.4 billion people are infected worldwide. In the United States, there are an estimated 4 million people infected, primarily in the southeastern states and among immigrants (Abhay *et al*, 2009).Important factors associated with an increased prevalence of disease include socio-economic status, defecation practices and cultural differences relating to personal and food hygiene as well as housing and sewage systems. Most infections are subclinical; more severe complications occur in children who tend to suffer from the highest worm burdens (Abhay *et al*, 2009).Round worm is long, cylindrical and tapers toward both ends. Female worms are longer than the males. (Obeng, 1997). At the anterior end, there are three prominent lips with dentigerous ridge. Posterior end of male is curved ventral. The tail is bluntly pointed. The spicules in male genital organ are simple and measure 2-3 mm in length. In female, vulva is present at about one third of the body length from the anterior end (Assafa *et al*, 2004). Since the sexes are separate, it requires infection with both male and female worms to produce fertile eggs in the host. It has been reported that, generally, infected persons harbor more females than male worms with an estimated ratio of 10 female worms to 3 male worms. There is always the possibility that a host may be infected only by female or by male worms. In such cases, the female worms produce the unfertilized eggs, which are incapable of developing further. Each female worm lays about 200,000 eggs per day, for as long as she is fertilized and in the intestine. Adult worms in

the human host live for less than 10 months, with maximum life spans of up to 1.5 years. Each has a protective durable shell. The eggs are discharged into the lumen of the intestine and leave the host with the feces into the environment (Obeng, 1997). Infection in man takes place by ingestion of embryonated eggs through contaminated food or drinks (unfertilized eggs are non infectious) (Assafa *et al*, 2004).The adult worm normally feeds on partly digested food from the intestine in humans. It has been reported that the host (having about 26 worms) may lose 10 per cent of his/her total daily intake of protein (Obeng, 1997).Although most individuals infected with *Ascaris lumbricoides* are essentiallyasymptomatic, the burden of symptomatic infection is relatively high as a result of the high prevalence of infection on a worldwide basis. Symptomatic disease is usually related to either the larval migration stage or manifests as pulmonary disease, or to the intestinal stage of the adult worm (Abhay *et al*, 2009). The early symptoms of round worm infection are a pneumonitis with cough and sometimes blood stained sputum (which may contain larvae), dyspenia, wheezing, persistent non productive cough, substernal pain, fever and diarrhea; These symptoms begin 5 to 6 days after infection, usually last 10 to 12 days and are caused by the round worm larvae migrating and developing inside the human body. A heavy presence of adult worms in the small intestine may cause digestive disorders, nausea, abdominal pain, vomiting, restlessness and disturbed sleep (Obeng, 1997). The large size of the adult worms also presents problems, especially if the worms physically block the gastrointestinal tract. *Ascaris* seems to be especially sensitive to anesthetics, and numerous cases have been documented where patients in surgical recovery rooms have had worms migrate from the small intestine, through the stomach, and out the patient's nose or mouth (Assafa *et al*, 2004).

2.3.2.4 *Enterobius vermicularis:*

Enterobius vermicularis commonly referred to as pin worm, has the largest geographical distribution of any helminth. Discovered by Linnaeus in 1758, it wasoriginally named *Oxyuris vermicularis* and the disease was referred to as oxyuriasisfor many years. It is believed to be the oldest parasite described and was recentlydiscovered in ancient Egyptian mummified human remains as well as in DNAsamples from ancient human coprolite remains from North and South America (Abhay *et al*, 2009). *Enterobius* is one of the most prevalent nematodes in the United States and in Western Europe. At one time, in the United States there are an estimated 42 million infected individuals. It is found worldwide in both temperate and tropical areas. Prevalence is highest among the 5-10 year-old age group and infection is uncommon in children less than two years old. Enterobiasis has been reported in every socioeconomic level; however spread is much more likely within families of infected individuals, or in institutions such as child care centers, orphanages, hospitals and mental institutions. Humans are the only natural host for the parasite (Abhay *et al*, 2009).*Enterobius vermicularis* is a spindle shaped parasite of man and attaches to the mucosa of the lower ileum, ceacum and ascending colon. Pinworm eggs are infective shortly after being excreted. After ingestion, the eggs hatch in the upper intestine and liberate larvae which migrate to the region of the ileum. Copulation (mating) of the worms takes place in the lower small intestine, and then the females migrate to the ceacum or lower bowel and pass through the anus where upon contact with the air they shower their sticky eggs on the perianal skin (Tadesse *et al*, 2008). The eggs of *Enterobius vermicularis* naturally transparent and colorless, measure 50-60 x 20-30 mm in size, and are ovoid

and asymmetrical, one side being more convex than the other. They embryonate in 6 h and can remain viable for 20 days in the environment (Burkhart and Burkhart, 2005).Infection is facilitated by factors including overcrowding, wearing soiled clothing, lack of adequate bathing and poor hand hygiene, especially among young school aged children. Infestation follows ingestion of eggs which usually reach the mouth on soiled hands or contaminated food. Transmission occurs via direct anus to mouth spread from an infected person or via air borne eggs that are in the environment such as contaminated clothing or bed linen (Abhay *et al*, 2009). The majority of enterobiasis cases are asymptomatic; however the most common symptom is perianal or perineal pruritus. This varies from mild itching to acute pain. Symptoms tend to be most troublesome at night and as a result, infected individuals often report sleep disturbances, restlessness and insomnia. The most common complication of infection is secondary bacterial infection of excoriated skin. Folliculitis has been seen in adults with enterobiasis. Gravid female worms can migrate from the anus into the female genital tract. Vaginal infections can lead to vulvitis, serous discharge and pelvic pain (Abhay *et al*, 2009).

2.3.2.4. Diagnosis of intestinal helminthes:

Although clinical signs may evoke the suspicion of helminthiasis, diagnosis is still dependent upon the isolation and identification of helminthes from the feces. Adult worms or their segments can also be demonstrated macroscopically when the adult worm is spontaneously passed in stool or vomitus; administration of an antihelminthic drug may result in expulsion of the worm. The definitive methods usually involve microscopic detection of helminth eggs from fecal preparations via smears or after concentration.

Microscopy, however, requires trained experts, has low sensitivity for detection of light and moderate infections, and may result in misdiagnosis leading to delayed or inadequate treatment (Verweij *et al*, 2007). Numerous flotation and concentration methods are available, such as the Kato-Katz, formol ethyl acetate sedimentation and zinc sulphate flotation technique techniques (Martin and Beaver, 1968). Commercial antibody detection tests are available for some STH infections (Verweij *et al*, 2007).Colonoscopy is useful for the detection of whipworms in the rectum (Cooper, 1999).The most successful diagnostic method for *Enterobius* is the "Scotch tape" or "cellophane tape" method; this is best done immediately after arising in the morning before the individual defecates or bathes (Abhay *et al*, 2009).

CHAPTER THREE

3.MATERIALS AND METHODS

3.1. Material:

3.1.1. Instruments:

Table 3.1. Instruments:

- Refrigerator	Coldair	Sudan
-Autoclave	Express Equipment	England
-Water bath	Memert	Germany
-Microscope	Olympus optical	China
-Incubator	Tone Picanardi	Italy
-Sensitive balance	OHAUS	UK
- Hot air oven	Memert	Germany
- Dry cabinet	GARANTITO-Cheshi Engineering	UK

3.1.2 . Media:

Table 3.1.2. Media:

-XLD	Hi media laboratories	India
- Nutrient Agar	Hi media laboratories	India
-Muller Hinton Agar	Hi media laboratories	India
- Peptone water medium	Oxiod L.T.D	UK
- Urea medium	Mast diagnostic	UK
- Koser's citrate medium	Mast diagnostic	UK
-KIA	Oxiod L.T.D	UK

3.1.3. Reagent and stains:

Table 3.1.3. Reagent and stains:

Kovac's reagent	B.D.H.chemical	Japan
Normal saline	Industry Ltd	Saudi Arabia
Crystal violet	Sod-fine-CHEM-Ltd	China
Lougol's iodine	Sod-fine-CHEM-Ltd	China
70% Alcohol	Sod-fine-CHEM-Ltd	China
Saffranine	Sod-fine-CHEM-Ltd	China
wooden sticks		India
oil immersion		India
Filter paper		England
McFarland Standard	Kimball Ltd	Sudan
Formalin	Kimball Ltd	Sudan
Ether	Kimball Ltd	Sudan
Shigella spp antisera	B.D.H.chemical	Japan

3.1.4. Glasswares:

Table 3.1.4. Glasswares:

Slides	Bomix	China
Petridishes	Pyrex	USA
Test tubes	Pyrex	USA
Bottles	Pyrex	USA
Cover glass	Bomix	China

3.1.5. Other materials:

Table 3.1.5. Other materials

Sterile containers	Lebanon
Benzene burner	Italy
Pair of forceps	England
Wire loops	India
Cotton-wool swab	England
Racks	Sudan
Sieve	Sudan

3.2. Methods:

3.2.1. Study design:

3.2.1.1. Type of study:

This is a cross-sectional study.

3.2.1.2. Study area:

This study was conducted in Sinnar State. These were Daweena Medical Centre, Abuhuggar Medical Centre, Abuhuggar Hospital and Sinja model Medical Centre.

3.2.1.3. Study duration:

During the period from February to May 2016 the study was done.

3.2.1.4. Sample size:

Hundred and three stool samples were collected**.**

3.2.1.5. Inclusion criteria:

patients suffering by diarrheal diseases.

3.2.1.6 Exclusion criteria:

patients with other illness.

3.3. Collection of stool specimens:

The stool specimens were collected, from patients diagnosed clinically to have diarrheal diseases in a clean, dry and leak proof containers and transported to the laboratory as soon as possible. The stool specimens were investigated by three techniques, culture in suitable medium ,wet preparation and concentration technique.

3.3.1. Culture:

Small portion (3-5mg) of stool specimen was inoculated in Selenite F broth (Appendix 1) and incubated at 37°C for 18- 24 hours. At the end of incubation period the resultant growth was sub cultured in XLD agar

(Appendix 2) using a wire loop of suspension and incubated for 18- 24 hours. Suspected colonies were picked up and streaked on slope of nutrient agar (Appendix 3), incubated overnight at 37˚C then stored for further investigation.

3.3.2. Identification:

3.3.2.1. Gram smear:

Dried smear was fixed, covered fixed smear with crystal violet (Appendix 4) stain for 30–60 seconds, rapidly washed off the stain with clean water (When the tap water is not clean, use filtered water or clean boiled rainwater), all the water were tipped off, and the smear cover with Lugol's iodine (Appendix 5) for 30–60 seconds, wash off the iodine with clean was water, then decolorized rapidly (few seconds) with acetone–alcohol (Appendix 6), then washed immediately with clean water (Acetone–alcohol is highly flammable, therefore use it well away from an open flame), the smear was covered with safranin (Appendix 7) stain for 2 minutes, washed off the stain with clean water, the back of the slide clean was wiped, and place it in a draining rack for the smear to air-dry, smear was examined microscopically, first with the 40 objective to check the staining and to see the distribution of material, and then with the oil immersion objective to report the bacteria and cells (Cheesbrough, 2006).

3.3.2.2. Biochemical tests:

3.3.2.2.1. Oxidase test:

A piece of filter paper was Placed in a clean petridish and 2 or 3 drops were added of freshly prepared oxidase reagent (Appendix8), using a piece of stick or glass rod (not an oxidized wire loop), a colony of the test organism was removed and smear it on the filter paper and looked for the development of a blue-purple color within a few seconds. Result blue-purple color

positive oxidase test (within 10 seconds) and negative oxidase test no blue-purple color (within 10 seconds). Note: Ignore any blue-purple color that develops after 10 seconds (Cheesbrough, 2006).

3.3.2.2.2. Sugars fermentation, H_2S and gas production:

This is a multi tests carried out in Kligler Iron Agar (KIA) medium. They performed by inoculating KIA medium. A straight wire was used to inoculate KIA medium (Appendix 9), first the butt was stabbed and then the slope was streaked in a zig-zag pattern, after inoculation (make sure the tube tops are left loose). KIA reactions are based on the fermentation of lactose and glucose (dextrose) and the production of hydrogen sulphide. A yellow butt (acid production) and red-pink slope indicate the fermentation of glucose only, the slope is pink-red due to a reversion of the acid reaction under aerobic conditions, cracks and bubbles in the medium indicate gas production from glucose fermentation A yellow slope and a yellow butt indicate the fermentation of lactose and possibly glucose, a red-pink slope and butt indicate no fermentation of glucose or lactose and blackening along the stab line or throughout the medium indicates hydrogen sulphide (H_2S) production (Cheesbrough, 2006).

3.3.2.2.3. Indole test:

The tested organism was inoculated in a bijou bottle containing 3 ml of sterile tryptone water (Appendix 10), then incubated at 35–37°C for up to 48 h, indole was tested by adding 0.5 ml of Kovac's reagent (Appendix 11). Then Shaked gently and examined for a red color in the surface layer within 10 minutes.Red surface layer indicate positive indole test and no red surface layer indicate negative indole test (Cheesbrough, 2006).

3.3.2.2.4. Urease test:

A dense 'milky' suspension of tested organism was prepared in 0.25 ml physiological saline in a small tube, urease tablet was added (Appendix 12) then the tube was closed and incubated at 35–37°C (preferably in a water bath for a quicker result) for up to 4 hours or overnight (*Proteus* and *M.morganii* organism give a positive reaction within 4 hours). Red/purple color indicate a positive urease test while yellow/orange color indicate a negative urease test (Cheesbrough, 2006).

3.3.2.2.5. Citrate Utilization Test:

A dense bacterial suspension was prepared of the tested organism in 0.25 ml sterile physiological saline in small tube, citrate tablet was added (Appendix 13) and the tube was stoppered and incubated overnight at 35–37°C. Positive citrate test is indicated by red color while yellow-orange color indicate a negative citrate test (Cheesbrough, 2006).

3.3.2.2.6. Serological identification of *Shigella* spp:

Conventional serotyping of *Shigella* spp. is based on an antigen / antibody reaction. The isolate (antigen) is mixed with antisera of known specificities; the pattern of reactivity observed determines subgroup and serotype. It is important to note that antisera producers typically will recommend a method for preparing a bacterial antigen suspension. The method for preparing the bacterial antigen suspension. Draw a circle approximately 2cm in diameter on to a clean glass slide. Add 20ul of physiological solution to center of the circle. Collect a small amount of bacteria (1uL loop) from the overnight isolate and suspend the bacteria in the saline. Then add the antisera. Draw a circle approximately 2cm in diameter on to a clean glass slide. Place 20 uL of antigen suspension and 20 uL of physiological solution into the circle and mix thoroughly with the microbiological loop or stick to prepare control

negative. Gently tilt the slide back and forth for 1 minute. Observe for agglutination under indirect light. If no agglutination is observed proceed to subgroup / serotype determination. If agglutination is observed; the isolate is rough, refer to "Limitations of Procedure" and do not attempt serotyping.Serologic identification of *Shigella* begins with the use of polyvalent antisera. Polyvalent antisera is used to identify the species (i.e. *Shigella dysenteriae*, *Shigella boydii*, *Shigella flexneri*, or *Shigella sonnei*). If agglutination is observed with polyvalent antisera, the isolate is then tested with the individual monospecific serum found in the polyvalent antisera. Isolates should be initially tested with polyvalent antisera that covers the species / serotypes which are most common in the region. - If agglutination is observed with one of the polyvalent antisera, the serotype of the isolate is determined by testing the isolate with monovalent antisera specific to the serotypes / sub-factors found in the polyvalent sera (WHO, 2010).

3.3.3. wet preparation:

Wet preparation was made by mixing small portion of stool taken with an applicator wooden stick with a drop of normal saline on slide and covered with cover slip and examined systematically under microscope using 10X and the high magnification 40X for observation of more details(Cheesbrough, 1987).

3.3.4. Formal ether concentration technique:

Approximately, one gram of feces was collected from different parts of the specimen and emulsified in 5 ml of formal saline in glass beaker. Further 5 ml from same solution was added and mixed. The resulting suspension was strained through the sieve. The filtered sample was poured back into a

centrifuge tube and then equal volume of ether was added. The tube was mixed for one minute and then centrifuged for 5 minutes at 2000 rpm. All upper 3 layers were discarded and the sediment was transferred into slide wich was covered with cover slip and examined under microscope using 10X and 40X magnifications(Cheesbrough, 1987).

2.3.5. Data analysis:

All information and data was entered in computer for analysis using Statistical Package for Social Science (SPSS) version 16 for windows.

CHAPTER FOUR

4. Results:

Stool specimens of 103 patients were collected and examined for the presence of intestinal parasites and cultured for *Salmonella* and *Shigella* species. Out of the total 103 study participants 51 (49.51%) were males and 146 (50.49%) were females. The age of the studied patients ranges from 2years of age to 45 years with mean age of 15.03 year. The majority 67 (65%) of the study subjects were between 7-17 years of age.

Out of the 103 stool samples, 48 (46.6%) samples were positive for intestinal parasite, Shigella and Salmonella species. Of these, 43 (41.7%), 2 (1.9%) and 5 (4.9%) samples were positive for intestinal parasite, Shigella and Salmonella species respectively. The dominant isolated parasite was *Entamoebahistolytica* with frequency of 11.7% followed by *Hymenolepisnana* (8.7%), then *Giardialamblia* (7.8%). The least identified parasites were *Ascarislumbricoid* and *Taeniasaginata* about 1% for each one (Table 5).The distribution of enteropathogens according to the different age groups is listed in figure 1. The majority 29 (58%) of enteropathogens were found in patients aged 6-15 years. Whereas, 17 (34%) in patients aged 16-25 , 2 (4%)observed in patients aged 26-35 and 1(2%) pathogen observed within patients aged >5 and <45. Double parasitic infections were observed in 4 patients (Table 5). The commonest double infections were *Hymenolepisnana + Giardialamblia* 3 (2.9%) and *Entamoebahistolytica + Entamoebacoli* 1(1%).

4.1. Shigella spp serotyping results:

After *Shigella* spp serotyping found that; three isolates are *Shigelladysentery* serotype A and two isolates are *Shigellasonnei* not found of others spp of *Shigella*

Table 1. Distribution of stool specimens according to area of collection:

area of collection	Number	%
Sinja model medical centre	29	28.1
Abuhogar hospital	21	20.4
Daweena medical centre	35	34
Abuhogar medical centre	18	17.5
Total	103	100

Table 2. Distribution of stool specimens according to gender:

Gender	Number	%
Male	51	49.51
Female	52	50.49
Total	103	100

Table 3. Frequency and percentage of infections:

Infetion	Frequency	Percent
Yes	50	48.5
No	53	51.5

Table 3. Frequency and percentage of infections:

Infetion	Frequency	Percent
Yes	50	48.5
No	53	51.5
Total	103	100.0

Table 4.Frequency and percentage of bacterial isolates:

Isolated bacteria	Frequency	Percent
Shigella spp	5	4.9
Salmonella spp	2	1.9
Escherichiacoli	33	32.0
Proteusmirabilis	7	6.8
Proteusvulgaris	2	1.9
Pseudomonasaeruginosa	4	3.9
no growth	50	48.5
Total	103	100.0

Table 5. Frequency and percentage of intestinal parasites founded:

Founded parasite	Frequency	Percent
Entamoeba histolytica	12	11.7
Giardia lamblia	8	7.8
Hymenolepis nana	9	8.7
Entamoeba coli	6	5.8
Enterobius vermicularis	2	1.9
Taenia saginata	1	1.0
Ascaris lumbercoides	1	1.0
Co infection by *Giardialamblia&Hymenolepisnana*	3	2.9
Co. *Entamoebacoli&* *Entamoebahistolytica*	1	1.0
not seen	60	58.3
Total	103	100.0

Figure 1. Distribution of enteropathogens according to the different age groups
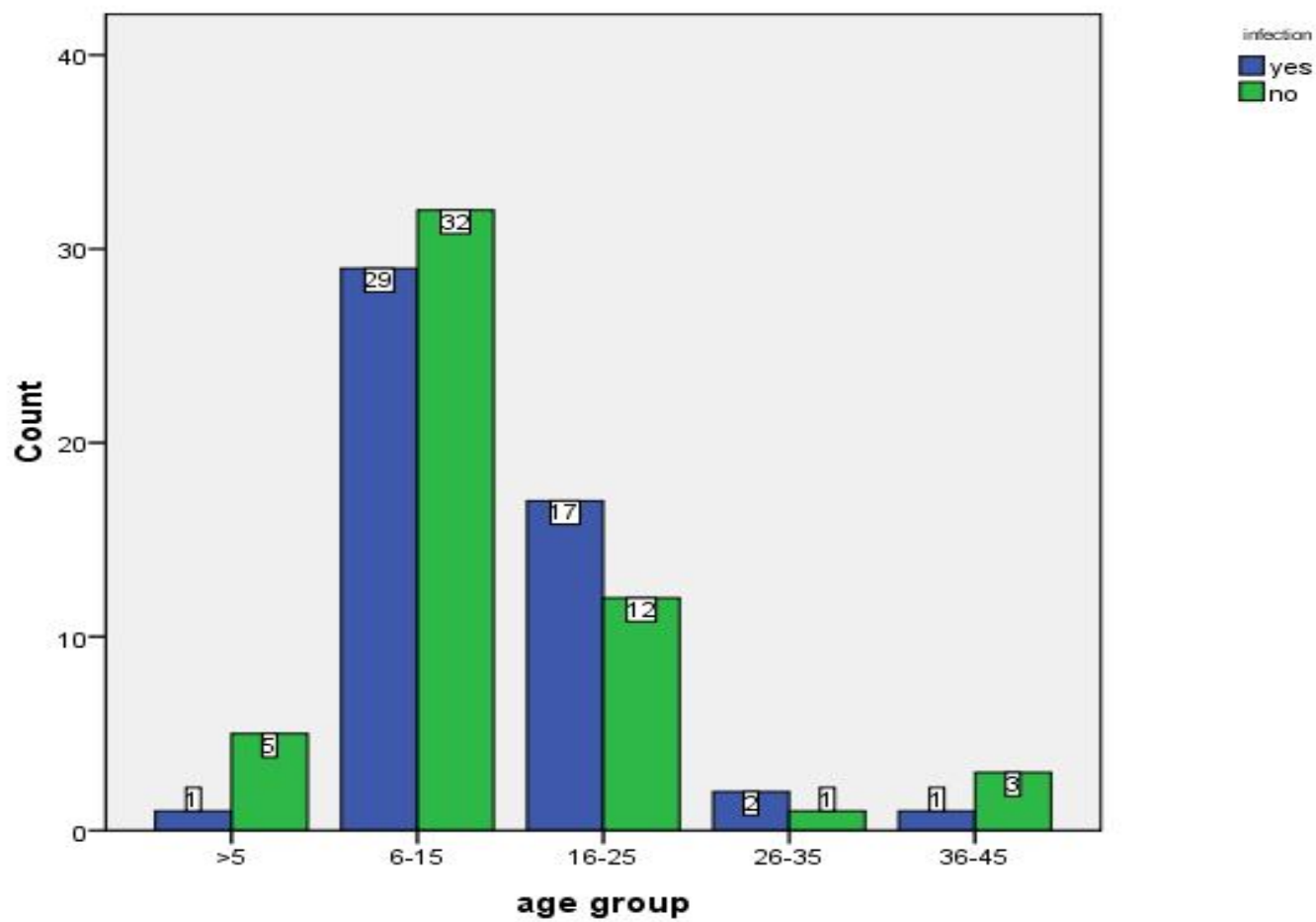
CHAPTER FIVE

5. Discussion:

The study results indicated, of the total 103 study participants, 43 (41.7%) of the symptomatic patients were infected with one or more intestinal parasites. It is comparable with study done by Unasho in southern Ethiopia, where 170 (41.9%) children were found to have single and double intestinal parasitic infections (Unasho, 2013) and also to study done by Beyene and Tasew in southwest Ethiopia where 107 (41.1%) children were positive for at least one intestinal parasite (Beyene and Tasew, 2014). but higher than study conducted in Gondar where the observed prevalence of intestinal parasites was 34.2% (Endris et al 2010), and the study conducted in Gamo area where 342 (39.9%) study subjects were found positive for at least one intestinal parasite (Wegayehu et al., 2013). Our study prevalence result was lower compared with reports of other similar studies, 72.9% in Gondar, Azezo (Wegayehu et al., 2013), 83% in Jimma (Ali et al 1999). and 83.8% in South East of Lake Langano (Legesse and Erko, 2004). These variations in prevalence might be due to differences in climatic conditions, environmental sanitation, economic and educational status of parents and study subjects, and previous control efforts.

Among helminthiasis, H. nana 9 (8.7%) was the most prevalent parasite and followed by Enterobius vermicularis 2 (1.9%) and T. saginata Ascaris lumbercoides , whereas amoebiasis was the leading infection among protozoan infections followed by giardiasis.

Though the rate of prevalence is different the dominancy of H. nana is in line with the study conducted in Gondar (Endris et al 2010). But different with study result done by Abayne in south Ethiopia (Unasho, 2013), study done by Beyene and Tasew in southwest Ethiopia and with previous studies done in Assedabo (Jimma, Ethiopia), where Ascaris lumbricoides was the dominant parasite (56.4%) (Ali et al 1999). The difference could be mainly difference in climatic condition.

In this study multiple parasitic infections were seen in 4 patients (3.8%) from the total 103 study subjects and 9.3% among patients who were infected with parasites and this result is lower than the study conducted in Gondar, where multiple infections (polyparasitism) occurred in 14 individuals or 4.6% of the total examined subjects and 13.5% of those who had intestinal parasites (Gelaw et al., 2013). The difference could be due to geographical location or variation in study subject or sample size. Comparison with previous study results conducted in different parts of Ethiopia is difficult since the parasite prevalence varies with agro-ecozone, altitude and other environmental factors which are not studied here.

Shigellosis is primary a pediatric disease, with more than half of all infections occurring in patients between five years 13 years of age as observed in previous Ethiopian study (Mache, 2001). The isolation of Shigella species (4.9%) in this study is comparable to (5%) reported by Mache 2001 (Mache, 2001) and lower than (20.1%) from the different study subjects and area. Also the study was conducted in different age groups, our

prevalence rate of 4.9% lowers than that a report by Ashenafi, 1983 (9%) (Ashenafi, 1998) and 11.7% isolation rate reported by Asrat et al., 1999 at Tikur Anbessa, Ethio-Swedish children's hospital (Asrat et al., 1999). The low isolation of Shigella in this study compared to the previous study could be due to differ in environmental conditions.

Epidemiological investigation of salmonellosis in developing countries like Sudan is difficult because of the very limited scope of the studies and lack of coordinated surveillance systems. The overall prevalence of Salmonella in this study was 1.9%. This is lower than the studies conducted in Ethiopia at different times, 4.5% in Addis Ababa (Ashenafi and Gedebou, 1985), 6.4% in Addis Ababa (Mache 2002), 4.5% in Addis Ababa (Ashenafi, 1998), reported in Jimma (15%) (Mache 1997).

5.2. Conclusion:

This study indicated that intestinal parasite and some enteric bacteria such as *Salmonella* and *Shigella* species are responsible for the majority cases of diarrheal diseases. Infection with non-typhoidal *Salmonella* commonly produces self-limited diarrhoea. Therefore, measures including health education, improvement of safe water supply, sanitation facilities and continuous monitoring of microbiological and diseases surveillance is crucia

5.3. Recommendations:

1. Increase the sample size to validate the results of this study.

2. Use a more selective media such as Hektoen Enteric Agar for isolation of *Salmonella spp.*

3. improvement of safe water supply, sanitation facilities and continuous monitoring of microbiological and diseases surveillance.

4. More studies needed to be done in this area to validate the results of this study.

5. Select other endemic areas for collection of stool specimens.

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APPENDICES

Preparation of reagents and culture media

Appendix (1): Selenite Broth (Selenite F Broth)(HiMedia Laboratories Pvt. Ltd. Mumbai, India)

Ingredients	g/L
Part A	
Casein enzymichydrolysate	5.000
Lactose	4.000
Sodium phosphate	10.000
Part B	
Sodium hydrogen selenite	4.000

Preparation

Suspend 4.0 grams of Part B in 1000 ml distilled water. Add 19.0 grams of Part A. Mix well. Warm to dissolve the medium completely. Distribute in sterile test tubes. Sterilize in a boiling water bath or free flowing steam for 10 minutes. Don't autoclave. Excessive heating is detrimental.

Appendix (2): Xylose-Lysine Deoxycholate Agar (XLD Agar) (HiMedia Laboratories Pvt. Ltd. Mumbai, India)

Ingredients	g/L
Yeast extract	3.00
L-Lysine	5.00
Lactose	7.50
Sucrose	7.50

Xylose	3.50
Sodium chloride	5.00
Sodium thiosulphae	6.80
Sodium deoxycholate	2.50
Ferric ammonium citrate	0.80
Phenol red	0.08
Agar	15.00

Final pH (at 25°C) 7.4 ± 0.2

Preparation

Suspend 56.68 grams in 1000 ml distilled water. Heat with frequent agitation until the medium is boils. Don't autoclave or over heat. Transfer immediately to a water bath at 50°C: After cooling pour into sterile Petri plates.

Appendix (3): Nutrient Agar (HiMedia Laboratories Pvt. Ltd. Mumbai, India)

Ingredients	g/L
Peptic digest of animal tissue	5.00
Sodium chloride	5.00
Beef extract	1.50
Yeast extract	1.50
Agar	15.00

Final pH (at 25°C)

Preparation

Suspend 28 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well before pouring.

Appendix (4): Crystal violet (HiMedia Laboratories Pvt. Ltd. Mumbai, India)

Ingredients	g/L
Crystal violet	20 g
Ammonium oxalate	9 g
Ethanol or methanol, absolute	95 ml

Preparation

Weigh the crystal violet on a piece of clean paper (pre weighed), transfer to a brown bottle pre marked to hold 1 liter, add the absolute ethanol or methanol (technical grade is suitable) and mix until the dye is completely dissolved, weight the ammonium oxalate and dissolve in about 200 ml of distilled water, add to the stain, make up to the 1 liter mark with distilled water, and mix well (Caution: Ammonium oxalate is a toxic chemical, therefore handle it with care), label the bottle, and store it at room temperature. The stain is stable for several months.

Appendix (5): Lugol's iodine (HiMedia Laboratories Pvt. Ltd. Mumbai, India)

Ingredients	g/L
Potassium iodide	20 g
Iodine	10 g

Preparation

Weigh the potassium iodide, and transfer to a brown bottle pre marked to hold 1 liter, add about a quarter of the volume of water, and mix until the potassium iodide is completely dissolved, weight the iodine, and add to the potassiumiodide solution. Mix until the iodine is dissolved (Caution: Iodine is injurious to health ifinhaled or allowed to come in contact with theeyes, therefore handle it with care in a wellventilated room and make up to the 1

liter mark with distilled water, and mix well. Label the bottle, andmark it Toxic. Store it in a dark place at room temperature. Renew the solution if its colorfades.

Appendix (6): Acetone-alcohol decolorizer (HiMedia Laboratories Pvt. Ltd. Mumbai, India)

Ingredients	g/L
Acetone	500 ml
Ethanol or methanol, absolute	475 ml

Preparation

Mix the distilled water with the absolute ethanol (ethyl alcohol) or methanol (methyl alcohol), transfer the solution to a screw-cap bottle of 1 liter capacity, technical grade is adequate, measure the acetone, and add immediately to the alcohol solution mix well (Caution: Acetone is a highly flammable chemical that vaporizes rapidly, therefore use it well away from an open flame) and label the bottle, and mark it Highly Flammable. Store in a safe place at room temperature. The reagent is stable indefinitely.

Appendix (7): Safranin (HiMedia Laboratories Pvt. Ltd. Mumbai, India)

Ingredients	g/L
Safranin O	0.50
Ethyl alcohol, 95%	100.00

Appendix (8): Oxidase reagent (HiMedia Laboratories Pvt. Ltd. Mumbai, India)

Ingredients	g/L
Tetramethyl-p-phenylenediamine dihydrochloride	0.1 g

Preparation

Dissolve the chemical in the water. The reagent is not stable. It is therefore best prepared immediately before use.

Appendix (9): Kligler Iron Agar (HiMedia Laboratories Pvt. Ltd. Mumbai, India)

Ingredients	g/L
Peptic digest of animal tissue	15.00
Beef extract	3.00
Yeast extract	3.00
Protease peptone	5.00
Lactose	
10.00	
Dextrose	1.00
Ferrous sulphate0.20	
Sodium chloride	5.00
Sodium trisulphate	0.30
Phenol red	0.024
Agar	15.00

Final pH (at 25℃) 7.4 ± 0.2.

Preparation

Suspend 57.52 grams in 1000 ml distilled water. Heat to boil to dissolve the medium completely. Mix well and distribute into tubes. Sterilize by autoclaving at 15 Ibs pressure (121℃) for 15 minutes. Cool the tubes tis etas slopes with 1 inch butts.

Appendix (10): Peptone Water (HiMedia Laboratories Pvt. Ltd. Mumbai, India)

Ingredients	g/L
Peptic digest of animal tissue	10.00

Sodium chloride	5.00

Final pH (at 25°C) 7.4 ± 0.2.

Preparation

Suspend 15.0 grams in 100 ml distilled water. Mix well and dispense into tubes with or without inverted Durham's tubes and sterilize by autoclaving at 15Ibs pressure (121 °C) for 15 minutes.

Appendix (11): Kovac's Reagent (HiMedia Laboratories Pvt. Ltd. Mumbai, India)

Ingredients	g/L
p-dimethyl aminobenzaldehyde	10 g
isoamyl alcohol	150 ml
concentrated hydrochloric acid	50 ml

Preparation

Kovac's reagent is prepared by dissolving 10 gm of p-dimethyl aminobenzaldehyde in 150 ml of isoamyl alcohol and then slowly adding 50 ml of concentrated hydrochloric acid.

Appendix (12): Urea Agar (Christensen) (HiMedia Laboratories Pvt. Ltd. Mumbai, India)

Ingredients	g/L
Peptic digest of animal tissue	1.00
Dextrose	1.00
Sodium chloride	5.00
Dipotassiumphpsphate	1.20
Monopotasiumphosphate	0.80
Phenol red	0.012

Agar   15.00

Final pH (at 25°C) 7.4 ± 0.2.

Preparation

Suspend 24 grams in 950 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 Ibs pressure (121 °C) for 15 minutes. Cool to 50°C and aseptically add 50 ml of sterile 40% urea solution and mix well. Dispense into sterile tubes and allow to set on slanting position. Don't over heat or reheat the medium as urea decomposes very easily.

Appendix (13): Simmons Citrate Agar (HiMedia Laboratories Pvt. Ltd. Mumbai, India)

Ingredients	g/L
Magnesium sulphate	0.20
Ammonium dihydrogen phosphate	1.00
Dipotassiumphpsphate	1.00
Sodium citrate	2.00
Sodium chloride	5.00
Bromothymol blue	0.08
Agar	15.00

Final pH (at 25°C) 7.4 ± 0.2.

Preparation

Suspend 24.28grams in 100 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense as desired in tubes or flasks. sterilize by autoclaving at 15 Ibs pressure (121 °C) for 15 minutes.