

# 1. INTRODUCTION

## 1.1. INTRODUCTION

Salmonellosis refers to disease caused by any serotype of bacteria in the genus *Salmonella*, other than *Salmonella typhi* (Evans and Brachman, 1989).

Typhoid fever, an acute systemic infectious disease seen only in humans, is a classical example of enteric fever caused by *Salmonella enteric serovar typhi*

The classic presentation includes fever, malaise, diffuse abdominal pain, and diarrhea. Untreated, typhoid fever is a grueling illness that may progress to delirium, obtundation, intestinal hemorrhage, bowel perforation, and death within one month of onset. Survivors may be left with long-term or permanent neuropsychiatric complications (Evans and Brachman, 1989).

A blood bank is a cache or bank of blood or blood components, gathered as a result of blood donation or collection, stored and preserved for later use in blood transfusion. Blood transfusion is generally the process of receiving blood products into one's circulation intravenously (Evans and Brachman, 1989).

Transfusions are used for various medical conditions to replace lost components of the blood. Early transfusions used whole blood, but modern medical practice commonly uses only components of the blood, such as red blood cells, white blood cells, plasma, clotting factors, and platelets (Evans and Brachman, 1989).

On rare occasion, blood products are contaminated with bacteria. This can result in life-threatening infection, also known as transfusion-transmitted bacterial infection. The risk of severe bacterial infection is estimated in 2002, as about 1 in 50,000 platelet transfusions, and 1 in 500,000 red blood cell transfusion. Blood product contamination, while rare, is still more common than actual infection (Evans and Brachman, 1989).

The reason platelets are more often contaminated than other blood products is that they are stored at room temperature for short periods of time. Contamination is also more common with longer duration of storage, especially when exceeding 5 days and the sources of contaminants include the donor's blood, donor's skin, phlebotomist's skin, and from containers (Blajchman, 2002).

Two key issues have dominated discussion and research among blood transfusion scientists to date. One is the preservation of the viability of blood constituents in order to benefit patients receiving a transfusion. The other is the safety of blood and blood products for transfusion. Having succeeded in isolating the various immunological factors likely to cause adverse transfusion reactions, attention has shifted in recent years to excluding pathogens that are either blood-borne or transmitted via the donor or that are introduced during the process of preparing the blood and its products (Bove, 1990).

While efforts have been made to identify and curtail pathogens that pose a risk to the safety of the blood supply in developed countries (Hill, 2005)

It appears that there is little enthusiasm among stake holders for ensuring the safety of the blood supply from infectious agents in Sudan (Brown *et al.*, 2005).

Apart from some concern about HIV and HBV, which are blood-borne and can compromise the safety of the blood supply, policy-makers do not seem to be interested in screening for other blood-borne pathogens. Yet, no blood or blood product is safe until it is free from all agents that can create an untoward consequence after transfusion (Barr and Muir, 1990).

This is the reason why no effort should be spared in ensuring that all types of pathogens - viral or bacterial - are eliminated from the blood supply. Given that

salmonellosis is endemic in our region, there is a potential risk of *Salmonella* being transmitted via blood transfusion. Indeed, unscreened units of blood which harbour live *Salmonella* organisms or endotoxin could cause severe, possibly fatal, post-transfusion reactions (Corales and Schmitt, 2002)

Bacterial contamination of blood components is an infrequent complication of transfusion. However, if it does occur, the potential for fulminant sepsis in the recipient is associated with high mortality. It can result from contamination during venepuncture or if an asymptomatic donor is bacteraemic at the time of donation. Symptoms occur during or shortly after transfusion of the contaminated unit and include high fever, rigors, erythema and cardiovascular collapse (Kopko and Holland, 2001).

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RBCs are stored at 4°C. This makes contamination with Gram-negative bacteria such as *Yersinia enterocolitica* and *Pseudomonas* species more likely as they proliferate rapidly at this temperature. Gram-positive bacteria such as *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Bacillus* species proliferate more readily at room temperature and so are more commonly seen as platelet contaminants (Kopko and Holland, 2001).

## 1.2. RATIONALE

Salmonellosis is an important global public health problem causing substantial morbidity and thus also has significant economic impact. Typhoid fever occurs worldwide, primarily in developing nations whose sanitary conditions are poor. So the disease is endemic in Sudan. One of the different mode of *Salmonella* transmission is blood transfusion and transmission of *Salmonella* is highly risk especially for immune compromised patient, so, the study will detect the presences of *Salmonella* among healthy blood donors in central blood bank in Sudan in addition to, there are no screening tests currently available for detection of bacterial contamination.

## **1.3. OBJECTIVES**

### **1.3.1. General objective:**

To determine seroprevalence of Typhoid fever among Blood Donors in Central Blood Bank in Khartoum State in Sudan, during the period between September and December, 2014.

### **1.3.2. Specific objectives:**

1. To detect *Salmonella typhi* O and *Salmonella paratyphi* B on blood donors.

2. To determine the prevalence of anti-*Salmonella* antibodies by using

Widal test and Typhidot test (Immunochromatography test (ICT)) in the diagnosis of *Salmonella typhi*.

3. To determine the percentage of recent and previous typhoid infection by using Typhidot test (Immunechromatography test (ICT)).

4. To correlate between seropositivity and antigens distributions among studied group.

## **2. LITERATURE REVIEW**

### **2.1. History of *Salmonella*:**

At the beginning of the 19th century, typhoid was defined on the basis of clinical signs and symptoms and pathological (anatomical) changes. However, at this time, all sorts of enteric fevers were characterized as "typhoid"(Kenneth, 2012).

In 1880s, the typhoid bacillus was first observed by Eberth in spleen sections and mesenteric lymph nodes from a patient who died from typhoid. Robert Koch confirmed a related finding by Gaffky and succeeded in cultivating the bacterium in 1881. But due to the lack of differential characters, separation of the typhoid bacillus from other enteric bacteria was uncertain (Kenneth, 2012).

In 1896, it was demonstrated that the serum from an animal immunized with the typhoid bacillus agglutinated (clumped) the typhoid bacterial cells, and it was shown that the serum of patients afflicted with typhoid likewise agglutinated the typhoid bacillus. Serodiagnosis of typhoid was thus made possible by 1896 (Kenneth, 2012).

### **2.2 History of *Salmonella* research in Sudan:**

In Sudan, the prevalence of *Salmonella* serovars is not well documented, as salmonellae are not routinely isolated and identified. Only a few studies have been reported by few workers. (Horgan, 1947) made the first report on *Salmonella* infections in cattle. He investigated a food poisoning outbreak at Wad Madani town and isolated *Salmonella* serovar Dublin from faeces of two persons who fell sick after eating meat. Again the serovar Dublin was isolated from infected calves and from one of the apparently healthy animals (Soliman and Khan, 1959).

A survey to ascertain the incidence rate of *Salmonella* infection in animals was made in Khartoum (Khan, 1970). During the survey, 230 *Salmonella* cultures were recovered from different sources belonging to 63 serotypes. Subsequent *Salmonella* surveys which have been conducted at Khartoum and Malakal added 15 serovars to the list of Sudan. The serovars reported were: *S. amager*, *S. derby*, *S. kandle*, *S. reading*, *S. salford*, *S. adelaide*, *S. amersfoort*, *S. bertin*, *S. chester*, *S. mushmar-haemek*, *S. muenche*, *S. muensters*, *S. newport*, *S. pomona* and *S. poona* (Khan, 1970). In his attempt to assess the quality of fresh meats in Sudan, (Sariy Eldin, 1971) reported the occurrence of *Salmonella wein*, *S. dublin*, *S. havana*, *S. typhimurium*, *S. senegal* and *S. braenderup*. *S. dublin* was also isolated from sheep liver (Salih and Ibrahim, 1972). Fifty-eight *Salmonella* strains were isolated from slaughtered chicken in Khartoum North and Omdurman (Yagoub and Mohamed, 1987). The most common serotypes reported were: *S. mons*, *S. amek* and *S. uganda*. The incidence of *S. dublin* in the mesenteric lymph nodes and faeces of sick calves in Kuku dairy cooperative farm, Omdurman and El Obeid slaughter houses was also reported (Saliem, 1987).

Forty-five *Salmonella* isolates (not serotyped) were isolated from carcasses, liver, spleen, intestinal contents of chickens from a poultry farm in El Obeid .The isolation of *Salmonella enterica* subspecies *enterica* serotype San-Diego from three goats (3.84%) at Omdurman Central Abattoir was reported (El Tom *et al.*, 1999). Recently, *Salmonella* Umbadah plus 19 new serovars were reported from different sources at Khartoum (Hag Elsafi *et al.*, 2009).

## **2.3. Salmonellosis:**

### **2.3.1. Description:**

Salmonella infection (salmonellosis) is a type of gastroenteritis caused by Salmonella bacteria. Most *Salmonella* infections occur after eating contaminated food but also sometimes after contact with another person with the infection. There are about 2,500 different strains of *Salmonella*, many of which cause infection in both animals and humans. There are two strains of *Salmonella* that cause typhoid and paratyphoid fever respectively: (*Salmonella typhi* and *Salmonella paratyphi*) (Heymann, 2008).

### **2.3.2. Classification and Morphology of Salmonella:**

Kingdom: Bacteria

Phylum: Proteobacteria

Class: GammaProteobacteria

Order: Enterobacteriales

Family: Enterobacteriaceae

Genus: *Salmonella*

Species: e.g. *S. enterica*

Salmonellae are Gram-negative, flagellated, facultatively anaerobic bacilli possessing three major antigens: H or flagellar antigen; O or somatic antigen; and Vi antigen (possessed by only a few serovars) (Black *et al.*, 1960).

As with other Gram-negative bacilli, the cell envelope of salmonellae contains a complex lipopolysaccharide (LPS) structure that is liberated on lysis of the cell and, to some extent, during culture. The lipopolysaccharide moiety may function as an endotoxin, and may be important in determining virulence of the organisms. This macromolecular endotoxin complex consists of three components, an outer O-polysaccharide coat, a middle portion (the R core), and an inner lipid A coat.



Lipopolysaccharide structure is important for several reasons. First, the nature of the repeating sugar units in the outer O-polysaccharide chains is responsible for O antigen specificity; it may also help determine the virulence of the organism. Salmonellae lacking the complete sequence of O-sugar repeat units are called rough because of the rough appearance of the colonies; they are usually avirulent or less virulent than the smooth strains which possess a full complement of O-sugar repeat units. Second, antibodies directed against the R core (common enterobacterial antigen) may protect against infection by a wide variety of Gram-negative bacteria sharing a common core structure or may moderate their lethal effects. Third, the endotoxin component of the cell wall may play an important role in the pathogenesis of many clinical manifestations of Gram-negative infections. Endotoxins evoke fever, activate the serum complement, kinin, and clotting systems, depress myocardial function, and alter lymphocyte function. Circulating endotoxin may be responsible in part for many of the manifestations of septic shock that can occur in systemic infections (Chopra *et al.*, 1994).

### **2.3.3. Antigenic Structures:**

As with all *Enterobacteriaceae*, the genus *Salmonella* has three kinds of major antigens with diagnostic or identifying applications: somatic, surface, and flagellar.

#### **2.3.3.1. Somatic (O) or cell wall antigens:**

Somatic antigens are heat stable and alcohol resistant. Cross-absorption studies individualize a large number of antigenic factors, 67 of which are used for serological identification. O factors labeled with the same number are closely related, although not always antigenically identical (Kenneth, 2012).

#### **2.3.3.2. Surface (Envelope) antigens:**

Surface antigens, commonly observed in other genera of enteric bacteria (e.g., *Escherichia coli* and *Klebsiella*), may be found in some *Salmonella* serovars. Surface antigens in *Salmonella* may mask O antigens, and the bacteria will not be agglutinated with O antisera. One specific surface antigen is well known: the Vi antigen. The Vi antigen occurs in only three *Salmonella* serovars (out of about 2,200): Typhi, Paratyphi C, and Dublin. Strains of these three serovars may or may not have the Vi antigen (Kenneth, 2012).

#### **2.3.3.3. Flagellar (H) antigens:**

Flagellar antigens are heat-labile proteins. Mixing *Salmonella* cells with flagella-specific antisera give a characteristic pattern of agglutination (bacteria are loosely attached to each other by their flagella and can be dissociated by shaking). Also, anti-flagellar antibodies can immobilize bacteria with corresponding H antigens.

A few *Salmonella enteric* serovars (e.g., Enteritidis, Typhi) produce flagella which always have the same antigenic specificity. Such an H antigen is then called monophasic. Most *Salmonella* serovars, however, can alternatively produce flagella with two different H antigenic specificities (Kenneth, 2012).

#### **2.3.4. Mode of transmission of *Salmonella*:**

*Salmonella* bacteria are mainly spread to humans via poorly cooked food made from infected animals' e.g. meat, poultry, eggs and their by-products. Spread by 'cross-contamination' can occur when the bacteria contaminate ready-to-eat food, eg. when food that will not be cooked further is cut with a contaminated knife. *Salmonella* can spread from person to person if hands are not washed properly,

particularly after going to the toilet or changing nappies. It can also spread from animals to humans (Heymann, 2008).

### **2.3.5. The disease in human:**

#### **2.3.5.1. Pathogenesis:**

Salmonellosis includes several syndromes (gastroenteritis, enteric fevers, septicemia, focal infections, and an asymptomatic carrier state). Particular serovars show a strong propensity to produce a particular syndrome (*S typhi*, *S paratyphi-A*, and *S schottmuelieri* produce enteric fever; *S choleraesuis* produces septicemia or focal infections; *S typhimurium* and *S enteritidis* produce gastroenteritis); however, on occasion, any serotype can produce any of the syndromes. In general, more serious infections occur in infants, in adults over the age of 50, and in subjects with debilitating illnesses (Finlay *et al.*, 1989).

Most non-typhoidal salmonellae enter the body when contaminated food is ingested. Person-to-person spread of salmonellae also occurs. To be fully pathogenic, salmonellae must possess a variety of attributes called virulence factors. These include (1) the ability to invade cells, (2) a complete lipopolysaccharide coat, (3) the ability to replicate intracellularly, and (4) possibly the elaboration of toxin(s). After ingestion, the organisms colonize the ileum and colon, invade the intestinal epithelium, and proliferate within the epithelium and lymphoid follicles (Finlay *et al.*, 1989). The mechanism by which salmonellae invade the epithelium is partially understood and involves an initial binding to specific receptors on the epithelial cell surface followed by invasion. Invasion occurs by the organism inducing the enterocyte membrane to undergo “ruffling” and thereby to stimulate pinocytosis of the organisms). Invasion is dependent on rearrangement of the cell cytoskeleton and probably involves increases in cellular inositol phosphate and calcium. Attachment and invasion are under distinct genetic

control and involve multiple genes in both chromosomes and plasmids ( Finlay *et al.*, 1992).

#### **2.3.5.2. Symptoms:**

*Salmonella* infections can have a broad range of illness, from no symptoms to severe illness. The most common clinical presentation is acute gastroenteritis. Symptoms include diarrhea and abdominal cramps, often accompanied by fever of 100°F to 102°F (38°C to 39°C) (American Academy of Pediatrics, 2006; Miller and Pegues, 2005).

Other symptoms may include bloody diarrhea, vomiting, headache and body aches. Symptoms usually develop 6-72 hours after exposure to the bacteria, but sometimes up to 2 weeks. Symptoms typically last between four and seven days but can sometimes last much longer. *S. typhi* and *paratyphi* generally cause a bacteremic illness—*Salmonella* found in the blood—of long duration. This illness is called enteric, typhoid, or paratyphoid fever (Miller and Pegues, 2005). Symptoms start gradually, and include fever, headache, malaise, lethargy, and abdominal pain. In children, it can present as a non-specific fever. The incubation period for *S. typhi* is usually 8 to 14 days, but it can range from 3 to 60 days. For *S. paratyphi* infections, the incubation period is similar to that of non-typhoidal *Salmonella*, 1 to 10 days (Miller and Pegues, 2005; Behraves, 2008).

#### **2.3.5.3. Complications:**

In approximately 5% of non-typhoidal infections, patients develop bacteremia (Miller and Pegues, 2005; Jones, 2008). In a small proportion of those cases, the bacteria can cause a focal infection, where it becomes localized in a tissue and causes an abscess, arthritis, endocarditis, or other severe illness. Infants, the elderly, and immune-compromised persons are at greater risk for bacteremia or invasive disease (Miller and Pegues, 2005; Buzby and Roberts, 2009). Additionally, infection caused by antimicrobial-resistant non-typhoidal *Salmonella*

serotypes appears to be more likely to cause bloodstream infections (Varma *et al.*, 2005; Buzby and Roberts, 2009).

Overall, approximately 20% of cases each year require hospitalization, 5% of cases have an invasive infection, and one-half of 1% die. Infections in infants and in people 65 years of age or older are much more likely to require hospitalization or result in death (Jones *et al.*, 2008). There is some evidence that *Salmonella* infections increase the risk of developing digestive disorders, including irritable bowel syndrome (Mearin, 2005; Cremon, 2014).

Although most persons that become ill with diarrhea caused by *Salmonella* recover without any further problems, a small number of persons develop a complication often referred to as Reactive Arthritis (Townes and John, 2010). Symptoms of reactive arthritis include inflammation (swelling, redness, heat, and pain) of the joints, the genitourinary tract (reproductive and urinary organs), or the eyes.

#### **2.3.5.4. Group at risk:**

*Salmonella* bacteremia is being identified with increasing frequency in persons infected with the human immunodeficiency virus. Salmonellosis may occur in patients with an established diagnosis of acquired immunodeficiency syndrome (AIDS), or it may be the first manifestation of this disorder. In patients with AIDS, salmonellosis is characterized by recurrent bacteremia despite treatment and a relative paucity of gastrointestinal manifestations. *Salmonella* bacteremia is being identified with increasing frequency in persons infected with the human immunodeficiency virus. Salmonellosis may occur in patients with an established diagnosis of acquired immunodeficiency syndrome (AIDS), or it may be the first manifestation of this disorder. In patients with AIDS, salmonellosis is characterized by recurrent bacteremia despite treatment and a relative paucity of gastrointestinal manifestations (Steven and Charles, 1987).

### **2.3.6. Laboratory diagnosis:**

#### **2.3.6.1. Isolation and Identification of *Salmonella*:**

A number of plating media have been devised for the isolation of *Salmonella*. Some media are differential and nonselective, i.e., they contain lactose with a pH indicator, but do not contain any inhibitor for non salmonellae (e.g., bromocresol purple lactose agar). Other media are differential and slightly selective, i.e., in addition to lactose and a pH indicator, they contain an inhibitor for nonenterics (e.g., MacConkey agar and eosin-methylene blue agar).

The most commonly used media selective for *Salmonella* are SS agar, bismuth sulfite agar, Hektoen enteric (HE) medium, brilliant green agar and xylose-lisine-deoxycholate (XLD) agar. All these media contain both selective and differential ingredients and they are commercially available.

Most strains grow on nutrient agar as smooth colonies, 2-4 mm in diameter. Most strains are prototrophs, not requiring any growth factors. However, auxotrophic strains do occur, especially in host-adapted serovars such as *typhi* and *paratyphi* A.

#### **2.3.6.2. Metabolism and Biochemical properties:**

Motile, Gram-negative bacteria, lactose negative; Products acid and gas from glucose, mannitol, maltose, and sorbitol; no Acid from adonitol, sucrose, salicin, lactose, Ortho-Nitrophenyl- $\beta$ -galactoside (ONPG) test negative (lactose negative) indole test negative ,methyl red test positive ,Voges-Proskauer test negative, citrate positive (growth on Simmon's citrate agar), lysine decarboxylase positive, urease negative ,ornithine decarboxylase (positive) , H<sub>2</sub>S produced from thiosulfate ,do not grow with potassium cyanide (KCN ),phenylalanine and tryptophan deaminase negative ,gelatin hydrolysis negative.

### **2.3.6.3. Serological identification tests of *salmonella*:**

#### **2.3.6.3.1. Widal test:**

The Widal test is one method that may be used to help make a presumptive diagnosis of enteric fever, also known as typhoid fever. Although the test is no longer commonly performed in the United States or other developed countries, it is still in use in many developing countries where enteric fever is endemic and limited resources require the use of rapid, affordable testing alternatives (Keddy, 2011).

This test demonstrates the presence of somatic (O) and flagellar (H) agglutinins to *Salmonella typhi* in the patient's serum using suspensions of O and H antigens. Antigens of *S. paratyphi* A and *S. paratyphi* B are included in most commercial kits. The recommended method of performing the Widal test is by the tube agglutination technique where serial two-fold dilutions of the subject's serum from 1:20 to 1:1280 are tested. There are many controversies regarding the Widal test especially involving the quality of the antigens used and interpretation of the result, particularly in endemic areas. There has been no consensus on the diagnostic titre for a single Widal test. The O agglutinins are first to appear and H agglutinins appear later and last for a longer time than O agglutinins. Traditionally, a positive Widal test is based on a fourfold rise in O agglutinins in repeated tests or a titre of > 1:160 (in endemic areas like Sudan) in a single test. From the sensitivity and specificity data, it is clear that some culture proven cases of typhoid fever may be associated with a negative Widal test particularly if done early in the course of illness, so that the predictive value of negative Widal test is limited. Culture isolation of *Salmonella typhi* from blood and bone marrow is the standard diagnostic test to confirm typhoid fever (Olopoenia and King, (2000).

### **2.3.7. Antibiotic Susceptibility:**

During the last decade, antibiotic resistance and multiresistance of *Salmonella* spp. have increased a great deal. The cause appears to be the increased and indiscriminate use of antibiotics in the treatment of humans and animals and the addition of growth-promoting antibiotics to the food of breeding animals. Plasmid-borne antibiotic resistance is very frequent among *Salmonella* strains involved in pediatric epidemics (e.g., *Typhimurium*, *Panama*, *Wien*, *Infantis*). Resistance to ampicillin, streptomycin, kanamycin, chloramphenicol, tetracycline, and sulfonamides is commonly observed. Colistin resistance has not yet been observed (American Academy of Pediatrics, 2006).

Until 1972, Typhi strains had remained susceptible to antibiotics, including chloramphenicol (the antibiotic most commonly used against typhoid) but in 1972, a widespread epidemic in Mexico was caused by a chloramphenicol-resistant strain of *S. Typhi*. Other chloramphenicol-resistant strains have since been isolated in India, Thailand, and Vietnam. Possible importation or appearance of chloramphenicol-resistance strains in the United States is a real threat. *Salmonella* strains should be systematically checked for antibiotic resistance to aid in the choice of an efficient drug when needed and to detect any change in antibiotic susceptibility of strains (either from animal or human source). Indiscriminate distribution and use of antibiotics should be discouraged (AAP, 2006).

### **2.3.8. Epidemiology:**

Contaminated food is the major mode of transmission for non-typhoidal salmonellae because salmonellosis is a zoonosis and has an enormous animal reservoir. The most common animal reservoirs are chickens, turkeys, pigs, and



cows; dozens of other domestic and wild animals also harbor these organisms. Because of the ability of salmonellae to survive in meats and animal products that are not thoroughly cooked, animal products are the main vehicle of transmission. The magnitude of the problem is demonstrated by the following recent yields of salmonellae: 41% of turkeys examined in California, 50% of chickens cultured in Massachusetts, and 21% of commercial frozen egg whites examined in Spokane, WA ( Mishu *et al.*, 1994).

In typhoid fever and non-typhoidal salmonellosis, two other factors have epidemiologic significance. First, an asymptomatic human carrier state exists for the agents of either form of the disease. Approximately 3% of persons infected with *S typhi* and 0.1% of those infected with non-typhoidal salmonellae become chronic carriers. The carrier state may last from many weeks to years. Thus, human as well as animal reservoirs exist. Interestingly, children rarely become chronic typhoid carriers. Second, use of antibiotics in animal feeds and indiscriminant use of antibiotics in humans increase antibiotic resistance in salmonellae by promoting transfer of R factors.

Salmonellosis is a major public health problem because of its large and varied animal reservoir, the existence of human and animal carrier states, and the lack of a concerted nationwide program to control salmonellae (Mishu *et al.*, 1994).

### **2.3.9. Treatment:**

General salmonellosis treatment measures include replacing fluid loss by oral and intravenous routes, and controlling pain, nausea, and vomiting. Specific therapy consists of antibiotic administration. Typhoid fever and enteric fevers should be treated with antibiotics. Antibiotic therapy of non-typhoidal salmonellosis should

be reserved for the septicemic, enteric fever, and focal infection syndromes. Antibiotics are not recommended for uncomplicated *Salmonella* gastroenteritis because they do not shorten the illness and they significantly prolong the fecal excretion of the organisms and increase the number of antibiotic-resistant strains (Stephen *et al.*, 1985).

### **2.3.9. Control:**

Salmonellae are difficult to eradicate from the environment. However, because the major reservoir for human infection is poultry and livestock, reducing the number of salmonellae harbored in these animals would significantly reduce human exposure. In Denmark, for example, all animal feeds are treated to kill salmonellae before distribution, resulting in a marked reduction in salmonellosis. Other helpful measures include changing animal slaughtering practices to reduce cross-contamination of animal carcasses; protecting processed foods from contamination; providing training in hygienic practices for all food-handling personnel in slaughterhouses, food processing plants, and restaurants; cooking and refrigerating foods adequately in food processing plants, restaurants, and homes; and expanding of governmental enteric disease surveillance programs (Mishu *et al.*, 1994).

Recently, The U.S. Department of Agriculture has approved the radiation of poultry to reduce contamination by pathogenic bacteria, e.g. *Salmonella* and *Campylobacter*. Unfortunately, radiation pasteurization has not yet been widely accepted in the U.S. Adoption and implementation of this technology would greatly reduce the magnitude of the *Salmonella* problem (Mishu *et al.*, 1994).

Vaccines are available for typhoid fever and are partially effective, especially in children. No vaccines are available for non-typhoidal salmonellosis. Continued research in this area and increased understanding of the mechanisms of immunity to enteric infections are of great importance (Mishu *et al.*, 1994).

#### **2.3.10. Vaccination against Typhoid Fever:**

Vaccines are available for typhoid fever and are partially effective, especially in children. No vaccines are available for non-typhoidal salmonellosis. Continued research in this area and increased understanding of the mechanisms of immunity to enteric infections are of great importance (Mishu *et al.*, 1994).

Three types of typhoid vaccines are currently available for use in the United States: (1) an oral live-attenuated vaccine; (2) a parenteral heat-phenol-inactivated vaccine; (3) a newly licensed capsular polysaccharide vaccine for parenteral use.

##### **2.3.10.1. Live oral vaccines:**

Although oral killed vaccines are without efficacy, vaccines using living avirulent bacteria have shown promise. The Live Oral Typhoid Vaccine should not be given to children younger than 6 years of age. It is given in four doses, 2 days apart, as needed for protection. The last dose should be given at least 1 week before travel to allow the vaccine time to work. A booster dose is needed every 5 years for people who remain at risk (Ryan *et al.*, 1989)

##### **2.3.10.2. Parenteral heat-phenol-inactivated vaccine:**

It has been widely used for many years. In field trials involving a primary series of two doses of heat-phenol- inactivated typhoid vaccine,

Since the inactivated vaccines contain the O antigen (endotoxin), local and general reactions occur. Vi antigen extracted following the methodology used for the meningococcal vaccine seems to avoid reactions to endotoxin.

The inactivated Typhoid Vaccine should not be given to children younger than 2 years of age. One dose provides protection. It should be given at least 2 weeks before travel to allow the vaccine time to work. A booster dose is needed every 2 years for people who remain at risk (AAP, 2003).

#### **2.3.19.3. Vi capsular polysaccharide (ViCPS):**

It is composed of purified Vi ("virulence") antigen, the capsular polysaccharide elaborated by *S.Typhi* isolated from blood cultures. In recent studies, one 25-ug injection of purified ViCPS produced seroconversion (i.e., at least a fourfold rise in antibody titers) in 93% of healthy U.S. adults. Two field trials in disease-endemic areas have demonstrated the efficacy of ViCPS in preventing typhoid fever. In one trial in Nepal, in which vaccine recipients were observed for 20 months, one dose of ViCPS among persons 5-44 years of age resulted in 74% fewer cases of typhoid fever. ViCPS has not been tested among children less than 1 year of age (Fraser *et al.*, 2007)

### **3. MATERIALS AND METHODS**

#### **3.1. Study type and design:**

Cross sectional descriptive study was carried out.

#### **3.2. Study area:**

This study was conducting in Central Blood Bank in Sudan.

#### **3.3. Study population:**

Study populations were healthy blood donors.

#### **3.4. Study period:**

This study was carried out from September to December, 2014.

#### **3.5. Ethical consideration:**

The consent was taken from donor after been informed by the nature of the study .

#### **3.6. Data collection:**

After explaining the purpose of the study , data were collected from volunteers by records ,the data include age ,sex and history of previous blood donation . Blood sample was collected in sterile plain container.

#### **3.7.2. Laboratory work:**

##### **3.7.2.1. Sample collection:**

Aliquots of five ml of whole venous blood were collected using sterile disposable syringes. And left to clot for one hour .The collected specimens were transported to

the laboratory, centrifuged for 3000 r.p.m for five minutes and serum was separated and stored at -20°C until tested.

### **3.7.2.2. Widal test (Standard Agglutination Test (SAT) :**

All the samples were subjected to the tube agglutination test to find out exact titer of antibodies, the Widal test was done as following:

Series of serum dilutions were made for each antigen to be tested, including tubes with 0.5 ml saline for control of each antigen to be used.

Perfectly clean and dry test tubes were made and prepared dilutions beginning with 1:10 and doubling through 1:320.

0.1 ml of serum were added to 0.9 ml of physiological saline and then diluted serially by mixing 0.5 ml diluted serum with 0.5 ml saline and discarding 0.5 ml from the last tube. From specimen submitted to detect possible rise in titer, series of 10 dilutions were prepared, ending with 1:320. The prepared tubes were incubated at 37°C for 24 hours, after incubation period observe the agglutination reaction within the tubes (Cheesbrough, 2006).

Each serial dilution had been interpreted as the follow: 1/20, 1/40, 1/80, 1/160 and 1/320; negative, insignificant, doubtful, significant and significant, respectively

To quality control the positive and negative control sera were run in parallel with each performed batch. Duplicates of each tested serum were used to assure that the antigens used in the test were sensitive as well as specific (Cheesbrough, 2000).

### **3.7.2.2. Immunochromatography test (ICT):**

This test was proceeded to confirm presencesn of *Salmonella* among blood donors .Commercial typhidot Ict was used as the flowing:

After determination of titer of the widal test , serum was added to square well, up to area A(control line), 3 drops of buffer were added to oval wells then pulled the clear plastic tab and one drop of buffer was added to square well .Finally waited for 15 minutes and read the result which were shown by colour bands when there is IgM in serum against coated antigen. Pink purplish coloured lines confirm a positive test result. It was compared with positive control lines.

### **3.8. Data analysis:**

The Statistical Package for Social Sciences (SPSS-s) is used for statistical analysis.

## RESULTS

One hundred blood donors were enrolled in this study, males were 81(81%) and females were 19 (19%); table 1.

As shown in fig.2 the reactive sera for Widal test of total sample were 33 (33%) samples and non-reactive sera for Widal test were 67 (67%) of total sample.

The titration result of Typhoid fever among blood donors for *Salmonella typhi* OAg were (significant, doubtful, insignificant and negative) as (19(19%), 11(11%), 3(3%), 67(67%)) respectively as shown in table.2.

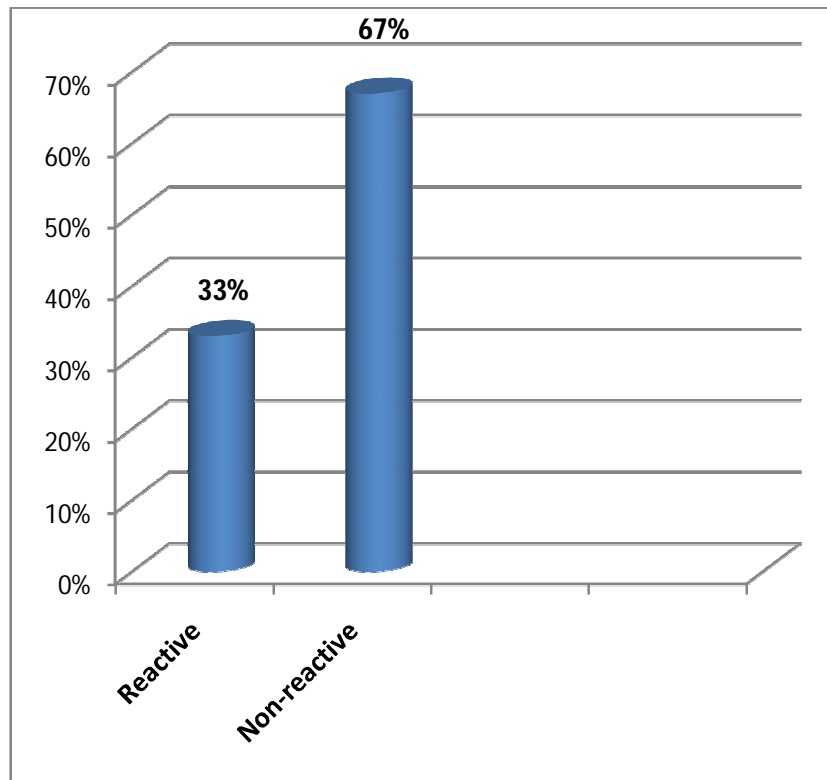
The titration result of Typhoid fever among blood donors for *Salmonella Paratyphi B* Ag were (significant, doubtful, insignificant and negative) as 11(11%), 12 (12%), 4 (4%) 73 (73%) respectively as shown in table 3.

This study was conducted Immunochromatography test (ICT) and the result is shows in fig.2 as: positive sera were 46(46%) and negative sera were 54 (54%). All positive reactions in Immunochromatography test (ICT) were positive for IgM antibody and there is no reaction for IgG antibody.

Fig.3 showed the positive and negative sera for immunochromatography test (ICT) among blood donors according to their age group.

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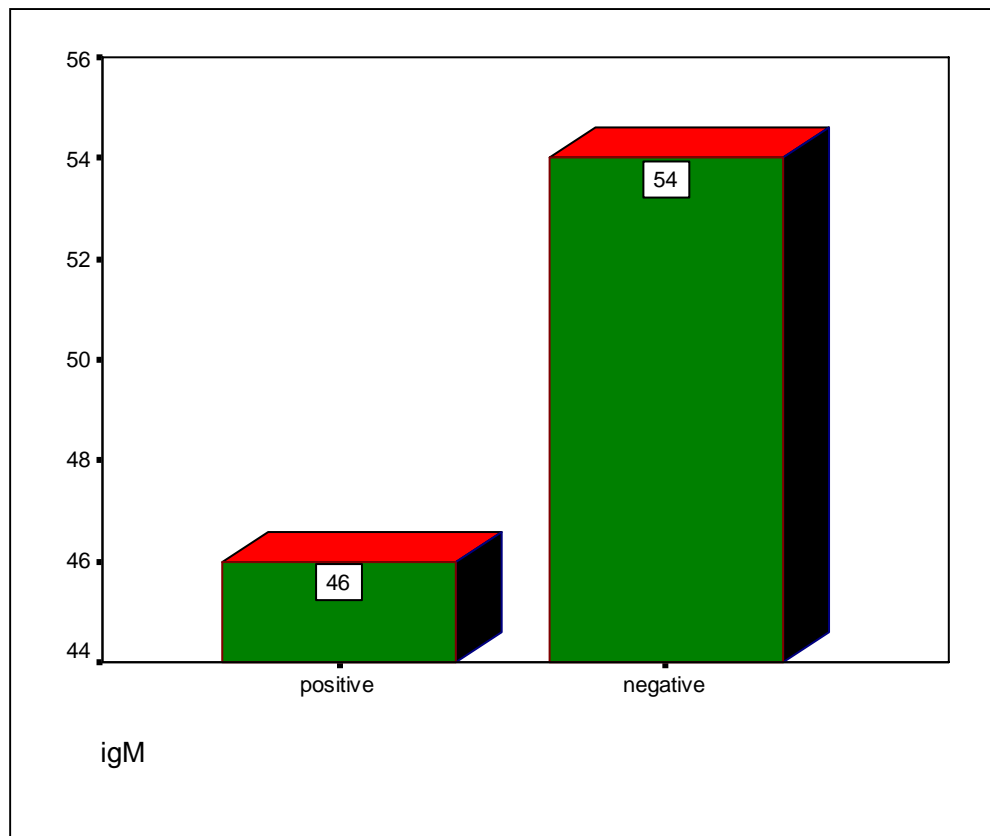
**Fig.1 Reactive and non-reactive sera of Widal test.**

**Table 2. The Widal test results for *Salmonella typhi* OAg**

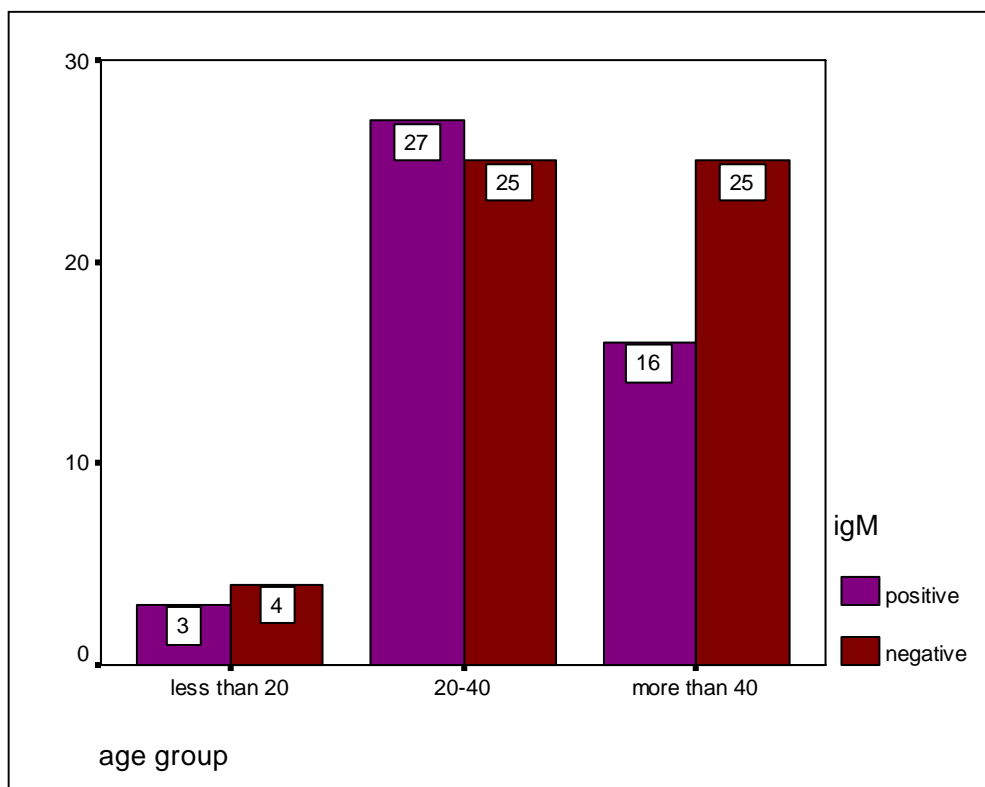
<b>Result</b>	<b>Frequency</b>	<b>Percent%</b>
Significant	19	19%
Doutfull	11	11%
Insignificant	3	3%
Negative	67	67%
<b>Total</b>	100	100%

**Table.3: The Widal test result for *Slmonella* Para typhi B Ag**

<b>Result</b>	<b>Frequency</b>	<b>Percent%</b>
Significant	11	11%
Doutfull	12	12%
Insignificant	4	4%
Negative	73	73%
<b>Total</b>	100	100%



**Fig.2: Positive and negative sera for immunochromatography test (ICT)**



**Fig. 3. The positive and negative sera for Immunochromatography test (ICT) among blood donors according to their age group**

## 5. DISCUSSION

### 5.1. Discussion

This study was aimed at assessing the prevalence of *Salmonella* among blood donors in Central Blood Bank in Khartoum state in Sudan.

The 33% of the total donor population found to be Widal-positive in this study which is higher than that reported by Nsutebu *et al.*, 2002 in Yaounde, Cameroon, who found that 10% of blood donors were showed positive result. Whereas our finding was lower than that reported by Teddy *et al.*, 2010, in Nigeria, it was 53%. This variation in prevalence rate of typhoid fever may attribute to the level of hygiene in these countries.

Typhidot is a new, inexpensive, and reliable serodiagnostic test recently available commercially and studied in many endemic areas with reports of higher sensitivity and specificity in the present study, 46% of cases were positive by typhidot test (ICT). Other studies have shown higher percentage of positivity 79% (Sherwal , 2004), 78% (Narayanappa, 2008), 70% (Bhutta and Mansurali, 1999), 56% (Membrebe and Chua, 1999), otherwise , previous study that reported low percentage of typhidot 9% (Jesudason and Sivakumar, 2006).

In present study all positive results of typhidot test (ICT) were positive for IgM antibody that indicates for suffering of these patients from recent *Salmonella* infection, so blood transfusion of those patients will be very dangerous especially for immunocompromised patients.

In this study typhidot positive results were exhibited higher percentage in age group 20-40 years (27%), followed by more than 40 year (16%), and less than 20 years (3%) which is quite comparable with the study of (Balakrishna, 2010); It showed 33% of patients belong to the age group 11-20 years. 24% were in the age group of 21-30 years. 15.5% were in the age group of 31-40 year.

Actually this research is the first one was conducted in the central blood bank in Khartoum state in Sudan.

## **5.2. CONCLOUTION**

Prevalence of disease among blood donors is a potential and dangerous source to whom directly received the blood transfusions.

### 5.3. RECOMONDATIONS

- 1- Screening of the blood of donors to detect the presence of *Salmonella* before donation is very crucial.
- 2- It is more gainful and easy to do screening test by typhidot test which gives reliable, sensitive, specific and rapid results.
- 3- *Salmonella's* blood bags must be avoided from donation especially immunocopromized patients or patients who's receiving immunosuppressive treatment.
- 4- Need attention of health institutions of the importance of *Salmonella* transfusion through blood and take the necessary actions to curb its prevalence through the blood.
- 5- Imperative need of specific researches on the prevalence of *Salmonella* in blood banks in Sudan.



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