**Sudan University of Science Technology**

## College of Graduate Studies

# Bacteriological and Clinical Study on Diarrheic Sheep Salmonellosis in Khartoum State, Sudan

# دراسة بكتيرية سريرية لمرض السالمونيلا في الضان المصابة بالإسهالات في ولاية الخرطوم، السودان

Thesis Submitted to the College of Graduate Studies in the Fulfillment of the Requirement of Master Degree in Veterinary, Pathology, Microbiology and Parasitology (Microbiology)

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## Dedication

I dedicate this work

To my mother

To the soul of my Father

And to

My sisters for supportingme to complete this work.

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## Acknowledgment

Firstly, Praise to Almighty Allah for giving are the strength and stamina to finish this work. With a great touch of pleasure and gratitude, I would like to express thanks to my supervisorProfessor **Siham Elias Suliman** for her grateful help.

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Finally my sincere gratitude to whom gave me their help to complete this study.

## Abstract

The aim of this Study was to estimate main clinical parameters of sheep suspected to have salmonellosis and investigation presence of Salmonella causing the disease and isolation and identification of bacteria causing the disease. A total of 53 sheep with abdominal pain and fluid diarrhea were examined clinically for pulse rate, respiration and body temperature in February to December 2020 in Omdurman locality, Khartoum State. Fecal samples were collected from these animals for bacteriological examination. MacConkey and salmonellashigella agar media were used for culturing and Gram's stain was used for examining the morphology of bacteria. Biochemical testswere applied for identification of the bacteria. The results, revealed that the pulse rale was (110.00±1.01 to 99.10±1.00), respiration (38.10±2.10 to 35.10±1.10) and body temperature (40.21±1.00 to 39.10±0.20) were increase the culture of the fecal samples in MacConkey agar medium showed that 19 (35.8%) the colonies were small, round, slightly elevated glister of yellow color that did not ferment of lactose and glistening colonies, while in salmonella shigella agar medium revealed black colonies. The results of Gram's stain showed gram-negative rod-shaped bacteria in culture of fecal sample. Biochemical tests showed that all the isolates were positive to catalase test and negative to oxidase, indole and urease test. In conclusion, the disease is prevalent in the study area in sheep which leading to economic loss due to death of the animals, costs of laboratory diagnosis, treatment of clinical cases, cleaning and disinfection and also control and prevention costs.

## المستخلص

هدفت هذه الدراسة لتقدير المعالم السريرية الرئيسية في الضأن القابلة بالإصابة بداء الساموئيلا. وكذلك عزل ومعرفة البكتريا المسببة لهذا المرض. كان العدد الكلي للضأن 53 التي يظهر عليا ألم بطني وإسهال مائي. لفحصها سريرياً وذلك لقياس معدل النبض، التنفس ودرجة حرارة الجسم من الفترة من فبراير إلى ديسمبر 2020م في محلية أم درمان، ولاية الخرطوم. تم جمع عينات البراز من هذه الحيوانات للفحص البكتيري. أوساط الغراء التي استعملت في نمو المستعمرات البكتيرية هي مكوي وسالمونيلا شيغلا. وذلك بصبغة غرام لوصف أشكال البكتريا. ولمعرفة نوع البكتيريا تم عمل الإختبارات الكيميائية الأحيائية. أوضحت النتائج إرتفاع معدل النبض ( 110.00±1.01, 99.10±1.00). والتنفس (1.10 35.10 2.10, 38.10). ودرجة الحرارة (40.21±1.00,39.10±0.20). وعند زراعة هذه العينات في وسط غراء مكونكي ظهرت مستعمرات صغيرة، مستديرة، مرتفعة قليلاً، صفرا اللون، ولامعة، غير مخمرة لسكر اللاكتوز. بينما في وسط غراء سالمونيلا شيغلا ظهرت ظهرت مستعمرات سوداء. نتائج صبغة غرام أوضحت بكتريا عصوية الشكل سالبة لصبغة غرام. الإختبارات الكيميائية الأحيائية أظهرت أن البكتريا المعزولة موجبة لإختبار كاتاليز وسالبة لإختبار أكسيدز، إندول واليوريا. وفي الختام أن هذا المرض شائع في منطقة الدراسة ولم يؤدي إلى خسارة إقتصادية في الضان نسبة لنفوق الحيوانات، تكلفة في التشخيص المعملي، علاج الحيوانات المصابة، النظافة، المطهرات، وكذلك تكلفة التحكم والوقاية.

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## Introduction

Sheep Salmonellosis is a gastrointestinal disease that causing very acute scouring and death. This disease may occur in different symptoms, septicemia, acute enteritis and chronic enteritis depending on types of the strains and their severty [Radostits*etal*., 2000]. Serovars associated with the disease in sheep are serovar*abortous*, dublin and *Typhimurium*.*Serovardublin*causing both abortion and enteritis. Generally, the disease characterized by meiritis, anorexia and loss of wool. But, in the lambs the organisms developing diarrhea high mortality rate. Serovar *typhimurium* usually associated with enteritis [Uzzau*etal*., 2001]. It is generally accepted intervention of some precipitating factor such as transport, intercurrent disease, anesthesia and surgery, dosing with antimicrobials or anthelmintics, acute deprivation of food or parturition is usually necessary to cause the disease [Radostits*etal*., 2007].

The disease is known as important food and water – borne disease that infect humans and animals leading to significant morbidity and mortality [Laconcha*etal.*, 1998; Poppe*etal.*, 1998, Akkina*etal.*, 1999]. In human the disease causing and intestinal inflammation characterized by diarrhea, fever, vomiting and abdominal cramp 12 – 72 hours after infection [Santos, *etal*. 2001].

Antibiotics and supportive care are indicated for animals with systemic disease, but early treatment is essential for septicemia salmonellosis trimethoprim – sulfadoxine combination is recommended and given parenterally dailyuntil recovery [Radostits*etal.*, 2007]. The treatment by appropriate antibiotics in human and the drugs fluoroquinolones and ceftriaxone are resistant to some population, but azithromycin is better

intreating the disease [Onwuezobe*etal.*, 2012].

## Objectives

1) To examine sheep with signs of salmonellosis clinically in Khartoum State.
2) To isolate and identify the organisms causing the disease.

# Chapter One
# Literature Review

# Chapter One

# Literature Review

## 1. Salmonellosis

### 1.1. Etiology

The genus*Salmonella* consists of S.*enterica*, *S.bongoria*, *S.typhimurium* and *S.dublin*in sheep and goats [Radostits*etal*., 2007]. These bacteria are Gram-negative, rods, motile, aerobic and facultative anaerobic.The *Salmonella*is belonging to the *Enterobacteriaceae* family [Barrow and Feltham, 2003; D'Aoust and Maurer, 2007], but failed to ferment lactose, sucrose, salicin and urea, reduce nitrite and most are phototropic (Tindall *et al.*, 2005). The species of *Salmonella* multiply optimally at a temperature between 35ºC-37ºC, pH about 6.5-7.5, and water activity between 0.94-0.94. They are chemo-organotrophic organisms (Scott, 2010). Theyare also able to multiply in the environment with low level or no oxygen (Diagnostic Services of Manitoba, 2009). These bacteria are sensitive to heat and will not survive at temperature above 70ºC; so, it is sensitive to pasteurization, but resist to drying, even for years (Acha and Szyfres, 2001). Also, these species ferment glucose to form gases.

Strains of *Salmonella* are classified intoserovarson the basis of extensive diversity of lipopoly saccharide(LPS) or O antigens and flagella protein or H antigen in accordance with the white – Kauffmann - Le minor scheme, currently more than 2600 serovars that are recognized (Issenfuth – Jeanjean*et al.*,2014). Commonly, serovars that cause infections in humans and animals belong to subspecies *enterica*.

## 1.2. Epidemiology

Salmonellosis can affect all species of domestic animals including sheep and goats and also human causing zoonosis complex. These species are different according to geographic areas which are different on climate, population density, land use, farming practices, food harvesting and processing technologies and consumer habits. The disease is most prevalent in areas of intensive animals or husbandry when the animals are stressed (Sewell andBrocklesby, 1990; Radostits*et al.,* 1994).

Generally, the occurrence of *Salmonella* in sheep and goats in variouscountries varies from 1 to 51.5% (D'Aoust, 1989). The mortality rate in lambs reached 50% in newZealand(Hemmingen*et al.,* 1982). But in the infected flocks up to 20% of lambs or kids could be died during the first ten days of life (Leondidis*et al.*, 1984). Also, abortion is wildly occurred in sheep and goats naturally infected bysalomellosis(Linklater, 1983; Leondidis*et al.*,1984; Verma *et al.*,1998). The organisms may survive for long periods inthe environment as carrier state that provides the major source of infection for animals and human. Thus, persistence of infection in animals and environment is important epidemiological features of salmonellosis (Baird – Parker, 1990; Clarke and Gylse, 1993, Radostits*et al.*, 1994)

Farm animals are infected through various sources and these are feeding stuffs, birds, bedding, flies, rodents, sewage, soil and water. Also during transportation cross contamination may occur to the animals as results of excretion of *Salmonella* in the feces. In the slaughtering process, meat contaminated from carrier animals and this is very important source of *Salmonella* in human food chain. Carcass contaminated due to poorly

disinfected knives and other slaughtering equipment and poor hygiene among plant personnel on carcass contamination(Semltzer*et al.*,1980; Semltzer and Thomas, 1981;D'Aoust, 1989; Baird – Parker, 1990). The transmission of *Salmonella* in sheep and goats is most commonly by the fecal oral route. Fleece laden in fecal material has been identified as an important source of carcass contamination and rendered products and incorporation in finishing feed ensure a continuous recycling of infection within the animal food chain (Kimberling, 1988; D'Auoust, 1989; Smith and Sherman, 1994). The infection of newborn by the disease depending on the size of challenge dose of the immunological status of the animal itself that dependent on the colostrum intake in neonates, previous exposure to the infection andexposure to stressor(Radostis*et al.*, 1994).

### 1.3. Pathogenesis

Salmonellae need to colonize in the distal small intestine or colon to cause the disease. The growth of these bacteria are inhibited by volatile organicacids produced by the indigenous normal anaerobic flora blocks access to attachment site required by these species of bacteria. The organisms after ingestion by the animals, they are rapidly growing and multiply in the rumen and pass into small intestine. The symptoms of the infection are marked enteritis due to release of endotoxin from disintegrated organisms. Diarrhea as aresult from the inflammationsbut also the organism may also elaborates enterotoxins that haveright secretion of epithelial cells leading to accelerated fluid and electrolyte loss from the gut (Kimberling, 1988, Smith and Sherman, 1994). Enteric salmonellosis is involving an increase mucosal cell cyclic AMP content and prostaglandin concentration as well as and the inflammatory response tothe invading bacteria (Radostits*et al.*, 2007). This bacteriain small

intestine penetrate mucous membranes,invaded of the payers' patches, mesenteric lymph nodes and finally enter the blood which reach all organs. The organism may colonize in the liver, gall bladder, spleen and mesenteric lymph nodes. The actionbacteria may reduce the peristalsis and increase colonization in the intestine (Quinn *et al.*, 1994; Bhatia and Khhpujani, 1994).

When the organism entered the bloodstream, a febrile reaction occurs and acute phase of the disease develops 24 – 48 hours. The strains that produce septicemia can be destructed by the host and multiplication within macrophages of liver and spleen as well as intravascularly. This destruction within the blood stream is prevented by D-repeat units of the lipopoly saccharide. It is known that they mask determinants on bacterial cell surface that would normally bind complement and activate it by means of the alternative pathway. As the result, chemotaxis will reduce, opsonisation and phagocytosis. These bacteria are survived within the phagocyte in no immune animals. Iron binding proteins of the animal is removed by siderphores that secreted by these invaded Salmonellae(Quinn *et al.*, 1994; Radostits*et al.*, 2007). Death from the disease usually occurs due to shock, septicemia, endotoxemia dehydration and acidosis (Kimbrling, 1988; Smith and Sherman, 1994).

## 1.4. Diagnosis

### 1.4.1. Clinical Finding

In sheep the only recognized form is acute enteritis. However, the early stage of an outbreak in some cases of septicemic form which characterized by profound depression, dullness, prostration, high fever (40.5 – 4 ℃, 105 – 107

°F) and death within 24 – 48 hours. Generally, the main signs of the disease of sheep are watery diarrhea, septicemia, meritis, abortion and high fever (40 – 41 ℃, 104 – 106 °F).

Dysentery with tenesmus in some cases, also the feces hare putrid smell and contain mucus, fibrous cells. These is complete anorexia, sometimes increased thirst, theheart and respiratory rates are rapid and mucous membranes are congested (Radositis*et al.,* 2007).

### 1.4.2. Laboratory diagnosis

#### 1.4.2.1. Bacteriology

The organism can be cultured from fecal samples, bulk tank milk, milk and environmental sites.Warnick*et al.,* 2003; Schott*et al.,* 2001 reported that the samples can inoculated in selenite broth for 24 – 48 hours followed by culture on Brilliant Green Agar. Generally the organism required 48 hours for the growth and biochemical tests for confirmation(Radosititts*et al.,* 2007). But the major medium today is MacConkey agar which included lactose and neutral red as indicator (Barrow and Feltham, 2003). Also an antigen – capture ELISA with enrichment culture for detection of salmonellae from fecal samples is applied as rapid than routine culture method.

#### 1.4.2.2. Indirect tests

These tests including hematology for the changes in leucocyte counts and clinical chemistry of electrolytes. A leukopenia and neutropenia occur when total and differential white cell counts measured. Also, sodium and potassium changes due to dehydration of the action of the bacteria (Blood*et al.,* 1989;Radostits*et al.,* 2007).

#### 1.4.2.3. Serological Procedures

Several serological tests have been developed in recent years for the rapid detection of salmonellae using antigensin tissue specimens and fecal samples (Carter *et al.,* 1995).

Species– specific agglutinins are used as diagnoticaids. These organisms do not appear in the serum until about 2 weeks after infection. The agglutination tests including fluorescent antibody test, passive hemoagglutination test, radiometric assay and latex agglutination procedures(Blood*et al.,* 1989; Carter *et al.,* 1995).

Masala *et al.,* (2007) and Martin – Atance*et al.,* (2012) reported that serum agglutination test (SAT) for detection of 1 gm, but these antibodies are not found in some animals after 2-3 months. Also they described other serological tests and these are additional agglutination assays, immunofluorescence, complement fixation test and gel immune diffussion test.

Meat juice enzyme – linked immunosorbont assay – Danish mix-ELIAS (DME) is a combination of lipopolysaccharide extraction of *Salmonella*O antigen. The DME was designed for surveillance and is recommended for monitoring herd and detection of high levels of *Salmonella* infection (Bensin*et al.,* 2002).

Polymerase chain reaction (PCR) is highly specific and sensitive test for detection of salmonellosis. Direct detection is done by amplification of part of OMPC after extraction of DNA from feces. PCR assay has been used to detect *Salmonella* DNA Persisting in the environment with field after electrophoresis. Assay needs enrichment of fecal specimens followed by genomic DNA extraversion for detection salmonellosis. (Schott *et al.,* 2001).

### 1.4.2.4 Serogroups and Serotypes

*Salmonella spp*. are classified into different serogroups and part into different serotypes. According to the Kanffmann-White Scheme. Under this serotype Scheme, numbers and letters are assigned to the different O (somatic) and H (Flagellar) antigen typing the O antigen determined the serogroup and typing H antigen. Defines the serotype. This scheme recognizes 46 D serogroup and 114 H antigens. In various combination and characterized 2523 serotypes(Popoff *et al.,* 2003).

The most. common serogroups (ATOE) and commonly designated by letters (Fitzgerald*et al.,* 2003). At least three antibody-antigen reaction are required to identify *Salmonella* serotype and the less common serovars often requires further tests for correcting the characterization (Kim *et al.,* 2006). For detect capsular or virulence (VI) antigen is required to screening of *Salmonella* serotype especially *Salmonella*serotype typhi and *Salmonella* serotype para-typhi possess Vi antigen that make the strains non agglutination antisera. These cultures agglutination in Vi antigen by boiling the culture for ten minutes. The specific O antigen is confirmed by slide agglutination with factor antigen. H antigen is usually determined by tube agglutination test. Determined by the (O antigen the organism should be motile), and second phase H antigen only is usually sufficient for the identification of typhoid fever and paratyphoid fever organisms (WHO, 2003).

The major surface structure is O antigen which is part of the lipopolysaccharide (LPS). The LPS consists of lipid A and core oligosaccharide domain in addition of O-specificpolysaccharide chain (D antigen). D antigen is polymer with repeating units of three to six sugars in

salmonella serogroup A to E. the variation in O antigen structure is represented by the different types of sugar present (WYK and Reere S., 1989; Luk*et al.*, 1993; Fitzgerald *et al.*, 2003).

### 1.4.2.5 Molecular Technique

The method currently used for detecting *Salmonella* environmental samples spp require two days to produce the result at half limited sensivity. The result produced in 18-24 hours. Premiers and propose specific to the gene INVS-group D and *Salmonellaenterica*serovarsenteritidePCR will not report any false result. The receiver operating characteristics (ROC) analysis documents excellent agreement between the result from culture and PCR method (Kasturi and Orgon, 2009).

### 1.4.3. Necropsy Findings

In animals died peracutly there may be no gross lesions, but extensive submucosal and subserosal petechial hemorrhages are usually occur. In acute enteritis the changes are associated with septicemic form and damage is mainly in the large and small intestines. The inflammation ischaracterized by mucoenteritis with submucosalpetechiation to diffuse hemorrhagic enteritis. Similar lesions found in abomasum.

In cases that have survived for long time, there is superficial necrosis and fibrin exudation which develop to extensive diphthericpseudo membrane and fibrin casts (Radostits*et al.*, 2007).

The mesenteric lymphnodes are enlarged, edematous and hemorrhagic. The wall of gallblader may be ticked and lines is enlarged and moist with

accumulation of fast (Kimberling *et al.*, 1988; Radostits*et al.*, 2007).

The chronic form is manifested by discrete areas of necrosis of the wall of the cecum and colon. The wall is thickened and covered by yellow-grey necroticmateial of overlying a red granular mucosal surface. In some cases there is ulceration in cecum around ileocecal valve. The mesenteric lymph nodes and spleen are swollen. In all species there is chronic pneumonia, polyarthritis and osteomyelitis (Radostits*et al.*, 2007).

### 1.5. Treatment

Tetracycline's,chloramphenicol, ampicillin, neomycin, penicillin, amoxicillin, apramycin and sulfonamides have been used. But there are multi-drug resistant strain(Carter *et al.*, 1995).Ceftiofur in neonates at dose rate 5 mg/kg BW intramuscularly per 24 hours is effective in experimental salmonellosis. Also the synergism of trimethoprim and sulfadoxine orally or parentally are effective. Thetherapy sulfadimidine and framycin are widly recommended in the treatment of salmonellosis. Nitro furazonc is commonly used as drug choice of treatment the disease(Adams, 2001; Radostitis*et al.*, 2007).

Fluorquinolone is effective as antimicrobial spectrum in wide variety of animal (Brown, 1996). Sulphounides and neomycin also used for treating salmonellosis.

## 1.6. Prevention and Control

For controlling of salmonellosis there are national and international policies are setup with the objective of reducing of the disease in animals in order to reduce outbreaks of human.

The reduction can begin from the farms to the food processor and to retail outlets, together with comprehensive educational programs for the consumers and food handers(D'Auost, 1989).

The principle of controlling of salmonellosis in the farms that by limitation of introduction of spreading of the disease within the hard purchased animals should be free from the disease that by preventing the farms from carrier animals. Carrier animals should be identified and either culled or isolated and treated. Random mixing of the animals should be avoided and the movements around the farms should be restricted to limit the infection (Aiello and Mays, 1998). Also ensuring that feeding and drinking water are free from the organims depending on the integrity of the source. Fecd and water supplies must be protected from fecal contamination, cleanliness of contaminated buildings and contaminated materials must be disposed. Prophylaxis by antibiotics of food and water should be considered. All workers should be aware of the hazards of working with infected animals and the importance of personal hygiene (Aiello and Mays, 1998; Radostits*et al.*, 2007).

Salmonellosis is zoonotic disease causing hazard analysis critical control growth or contamination with*Salmonella* is a pre-requisite for the effective and economic control of human and animal salmonellosis.

Antemortem and post – morten inspection are important for removing of diseased animals from food chain. But, majority of animals carrying the disease in their digestive tract do not show any signs of infection (Baird – Parker, 1990).

For increasing of immunization of the animals vaccines are applied. The animal resistance to disease relies initially on the production of inflammatory cytokines leading to infiltration of inflammatory cells in the tissues for cleaning of the organism be T and B cells – dependent and the establishment of long – lasting acquired immunity to reinfection (Radostits*et al.,* 2007).

A vaccine containing live attenuated to some species is used (*S.typhimurium*, *S.dublin*, *S.choleraesuis*). The antogenousbacterin which must be precipitated on aluminum hydroxide and given 2 injections per 2 weeks apart is given good immunity (Carter *et al.*, 1995; Radostits*et al.*, 2007).

# Chapter Two
# Martials and Methods

# Chapter Two

# Martials and Method

## 2.1. Study Area

Khartoum is the capital of Sudan and located in the semidesert zone in between latitude 15.08 degree and 16.39 degree North and longitude 31.36 and 34.25 degree East. It is divided into 3 major localities and these are Khartoum, Khartoum north [Bahry] and Omdurman. The topography is flat, expected for some scattered mountains. It is hot and dry with rains in summer and cool and dry winter. The annual rainfall ranges from 75 to 160 mm, falling mainly in July and August. Generally the average temperature is 21.6 ℃, the maximum temperature in summer exceed to 40 ℃, while the minimum during April and reaches 9.3 ℃/day. The dry period extends for 8 – 10 months. Livestock production systems or milk, meat and poultry production are operational within and around Khartoum city. In addition, some animal units [45 million] pass throw the state for export and trade purpose [El-Siddig*et al.*, 2006].

## 2.2. Clinical Examination of Sheep Suspected to Salmonellosis

A total of 53 sheep with abdominal pain, severe fluid diarrhea were examined clinically for estimation of pulse rate, respiratory rate and body temperature perminute [Kelly, 1984] from February to December 2020 in Omdurman Locality, Khartoum State.

## 2.3. Collection of Fecal Samples

Fecal samples [53 samples] were collected from sheep in sterile containers and transported in ice box to Microbiology Laboratory in College of Veterinary Medicine, Sudan University of Science for bacteriological examination.

## 2.4. Bacteriology

### 2.4.1. Method of Sterilization

#### 2.4.1.1. Dry Heat

##### 2.4.1.1.1. Hot Air Oven

This method was used for sterilization of clean glass containers which were wrapped in Foil, Temperature of 160℃ was applied for one hour [Barrow and Feltham, 1993].

##### 2.4.1.1.2 Red Heat

This method was used for sterilization of wire loops and holding over flame as near and vertical as possible until it become red hot [Barrow and Feltham, 1993].

##### 2.4.1.1.3. Moist Heat – Autoclave

This method was used for sterilization of culture media, solutions, plastic wares such as rubber stoppers which could not withstand the dry heat. The holding temperature 115℃ - 121℃ under 10 -15 pound pressure for 15 to 20 minutes [Barrow and Feltham, 1993].

### 2.4.2. Culture Media

#### 2.4.2.1. Solid Media

The media used were prepared according to Oxiod[2006].

##### 2.4.2.1.1. MacConkey's Agar Medium

The medium was prepared by according to manufacture addition of 53 grams agar powder to litre of distilled water and brought to boiling until dissolved completely the PH was adjusted to 7.4, thensterilized by autoclaving at 121℃ for 15 minutes. Then it was aseptically distributed in sterilized petri–dishes as 15ml and left to solidify.

##### 2.4.2.1.2. Simmons Citrate Agar

Thirty two grams of medium powder were dissolved in boiling distilled water 1 liter. Then dispensed in sterile test tubes and sterilized by autoclaving at 121ºC for 15 minutes. After removing from the autoclave, the test tubes were set in slope position until cooling.

##### 2.4.2.1.3. Urea agar Base Medium

Amount of 2.4 grams of urea agar base powder was suspended in 95 ml of distilled water and dissolved by boiling, then sterilized by autoclaving at 121 ℃ for 15 minutes and cooled to 50℃ and then 5 ml of sterile urea solution were aseptically added and mixed well, poured in 3 volumes into bijou bottles, and then put in the slope position until cooling.

#### 2.4.2.1.4 Salmonella Shigella Agar Medium

Boiling of 5.5 grams of dehydrated medium in 100 ml distilled water until dissolving and then autoclaving at 121 ℃ for 15 minutes. The medium was distributed in petri dishes [15ml].

#### 2.4.2.1.5 Semi Solid Medium

The motility medium was described by Cruickshank *etal*.,(1975) and 0.2% of nutrient broth was distributed in sterile test tubes containing craigie tubes and then the medium was autoclaved at 121ºCfor 15 minutes and pound/squareinch.

### 2.4.3. Reagents

#### 2.4.3.1. Hydrogen Peroxide

Hydrogen Peroxide produced by British Drug House [B.D.H] was diluted to 3% aqueous solution for catalase test.

#### 2.4.3.2. Oxidase Test Reagent

Tetra – methyl – pheynlene – diaminedihydrochoride was prepared as 1% aqueous solution. Filter paper 50×50 millimeter size were impregnated in the reagent before and dried at 50℃ .

### 2.4.3.3. Kovac's Reagent for Indole

The aldehyde p-dimethyamino – benz aldehyde 50 gram was dissolved in alcohol (75ml) and warmed water at (50 - 55℃). Then cooled of the mixture and addition of hydrochloric acid was performed (25ml).

### 2.4.4Culture of the Samples in Special Medium:

All samples were cultured in MacConkey and *Salmonella shigella* agar media by using wire loop under the flame.

### 2.4.5. Microscopic Examination

### 2.4.5.1. Preparation of Smear

Culture part of typical isolated colony was put in drop of sterile normal saline and spreaded on clean microscopic slide. The smears were allowed to dry in air, then fixed by gentle flaming and placed in rack. All stained by Gram stain.

### 2.4.5.2. Gram Stain

Gram Stain was done according to Barrow and Feltham (1993), as follows:

- Crystal violet solution was added to afixed smear for one minute.
- The slide was washed by water.
- Then lugol's iodine was added for 1 minute.
- Then the slide was washed by water.
- Decolorization of the slide by acetone for no time.
- The slide was washed by water.

- Diluted carbol fuchsine was added for 1 minute and the slide washed by water.
- Then the slide was dried by air and examined microscopically under oil immersion lens.

### 2.4.6. Biochemical Tests

#### 2.4.6.1. Primary Biochemical Tests

All biochemical tests were used as described by Barrow and Fetlham (2003).

##### 2.4.6.1.1. Catalase Test

A drop of 3% aqueous solution of hydrogen peroxide was placed an clean slide, then a colony of the tested samples on nutrient ager was picked and mixed with the hydrogen peroxide. A positive reaction was indicated by production of gas bubbles.

##### 2.4.6.1.2. Oxidase Test

The test was performed by placing the oxidase reagent – soaked dried filter paper strip on a clean slide and small amount of fresh culture was smeared on the strip. Apositive reaction gives deep purple color within seconds.

##### 2.4.6.1.3 Oxidation Fermentation Tests [OF]

Two tubes of Hugh and Leifson's medium were inoculated with the tested culture. One of them was covered with layer of sterile paraffin oil to adepth of 1-2cm and the 2 tubes were incubated at 37℃ and examined daily.

Then oxidative bacteriagive yellow color in open tube only, while the fermentive bacteria give yellow color in both tubes.

#### 2.4.6.1.4. Motility Test

The test of motility medium was stabbed by the inoculum stabbed straight with a wire loop to a depth about 15mm. The culture was incubated at 37 ℃ for 24 hours. Motility of bacteria indicate by the migration of the organism through craigie tube and the medium is become turbid, no growth seen in non motile bacteria.

#### 2.4.6.1.5. Sugar Test - Fermentation of Sugars

Sugars were weighted [0.5 gram] and dissolved with trypticase sodium chloride [0.5gram] and phenol red [0.0189mg] in 100ml distilled water and transferred into conical flasks. Addition of 0.5% to 1% of desired carbohydrate into all flasks and dispensed into test tubes. Insertion of inverted Durham tubes into all tubes. Sterilization at 115℃ for 15 minutes. Inoculation of the broth with bacterial culture and incubation of the tubes at 18 – 24 hours at 37 ℃. Yellow color means acid production, while yellow color and presence of small bubbles in the inverted Durham tubes means acid and gas production.

### 2.4.6.2. Secondary Biochemical Tests

#### 2.4.6.2.1. Citrate Test

This was incubated as single streak over the surface of a slope of Simmons citrate medium and examined daily for up to 7 days for the growth of bacterial and the color changed. Positive reaction is blue color and streak of

tested bacterial was citrate utilized and original green color citrate not utilized [Barrow and Feltham, 2003].

#### 2.4.6.2.2. Urease Test

The tested organism streaked on to aslope of urea agar medium and incubated at 37℃ for 2 days. Pink color indicated positive reaction [Barrow and Feltham, 2003].

#### 2.4.6.2.3. Indole Test

Peptone water was inoculated with tested organism and incubated at 37℃for 48 hours. One ml of the KOVAC reagent was administered by the side of the tube. When a pink ring appeared in the reagent layer within a minute, the test was considered positive.

#### 2.4.6.2.4. Kligler Iron agar

The medium contained bacto – beef extract [3 gms], bacto – yeast extract [3gms], bacto – peptone [15gms], protease peptone [5gms], glucose[10gms],Ferrow sulfate [0.2gms], sodium chloride [5gms], sodium thiosulfate [0.3gms], agar [12gms], phenol red [0.024gms] and distilled water [1.000 ml]. The ingredients were dissolved in boiling distilled water. The medium dispensing in test tubes and sterilized in the autoclave for 15 minutes at 15 pounds pressure [120℃] positive test was red for species that ferment lactose or dextrose [Barrow and Fetham, 1993].

## 2.5. Statistical analysis

The data were analyzed using SPSS software [Statistical Package for theSocial Sciences, Version 16.0].Clinical data was analyzed by using one way ANOVA and the statistical significance wasset at$P \leq 0.05$.

# Chapter Three
# Results

# Chapter Three

# Results

## 3.1 Survey

The suspected sheep for salmonellosis [53animals] showed fever, watery diarrhea, abdominal pain and respiratory distress, while 14 of them were recumbent of as shown in Table 1.These animals were examined clinically for pulse rate, respiratory rate and body temperature. There were increased in these parameters.

**Table (3.1)Estimation of clinical Parameters of infected sheep [N=19] and non-infected sheep [N=34] for salmonellosis in Omdurman Locality, Khartoum State**

| No. | Parameters | Infected sheep | Non-infected sheep |
|---|---|---|---|
| 1 | Pulse rate/min | 110.00 ± 1.01 | 99.10 ± 1.00 |
| 2 | Respiratory rate/min | 38.10 ± 2.10 | 35.10 ± 1.10 |
| 3 | Body Temperature/ ℃ | 40.21 ± 1.00 | 39.10 ± 0.20 |

**Table (3.2)Percentage of Sheep Salmonellosis [N=19] in Omdurman Locality, Khartoum State**

| No. | Variable | Frequency | Non-infected sheep |
|---|---|---|---|
| 1 | Positive | 19 | 35.8 |
| 2 | Negative | 34 | 64.2 |
| 3 | **Total** | **53** | **100** |

### 3.2 Growth on Solid Media

#### 3.2.1 MacConkey's Agar Medium

Collected samples were cultured 19 (35.8%) of them were showed growing colonies. These colonies were small, round, slightly elevated, glistening yellow in color and no fermented of lactose (Fig. 1).

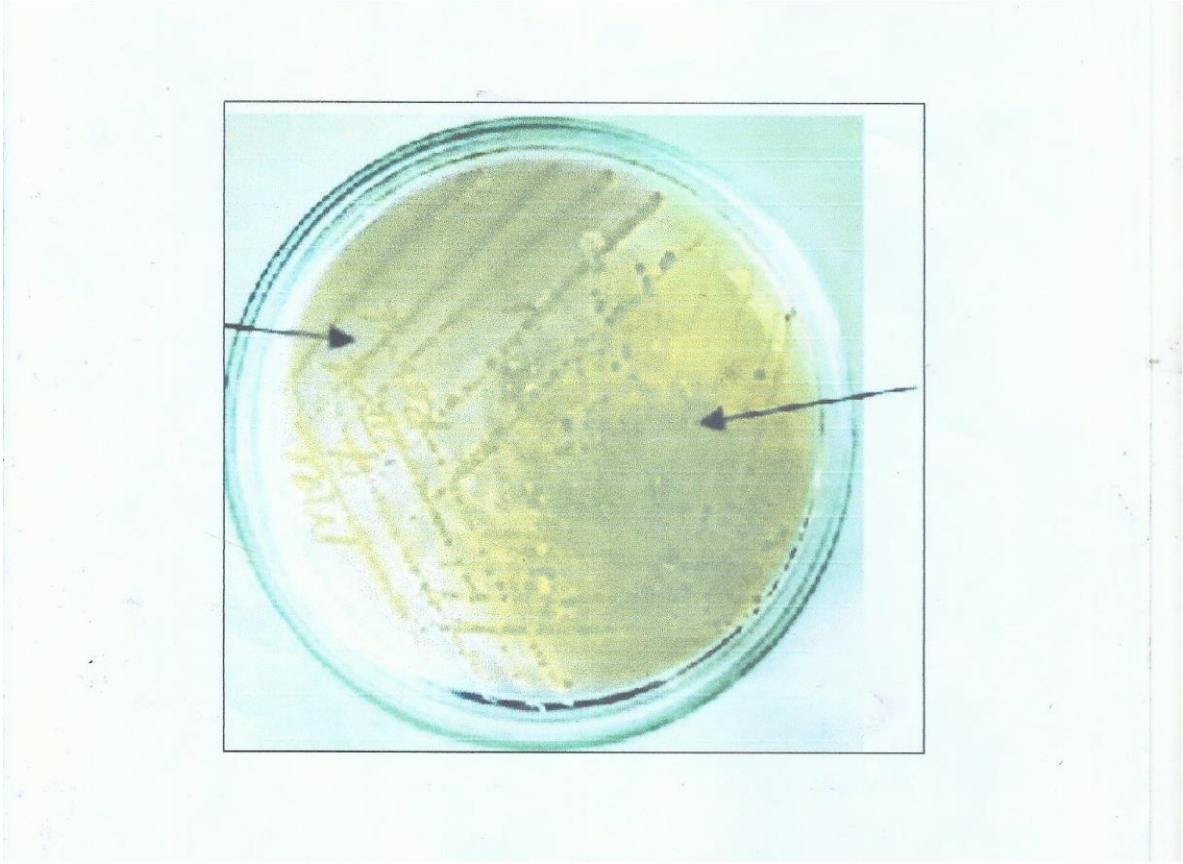

**Fig. (1) Yellow Color - Non-Fermentation of Lactose in MacConkey Agar Medium**

#### 3.2.2 Salmonella shigella Agar Medium

The samples (19 samples) on *Salmonella shigella* agar medium showed black colonies (Fig. 2).

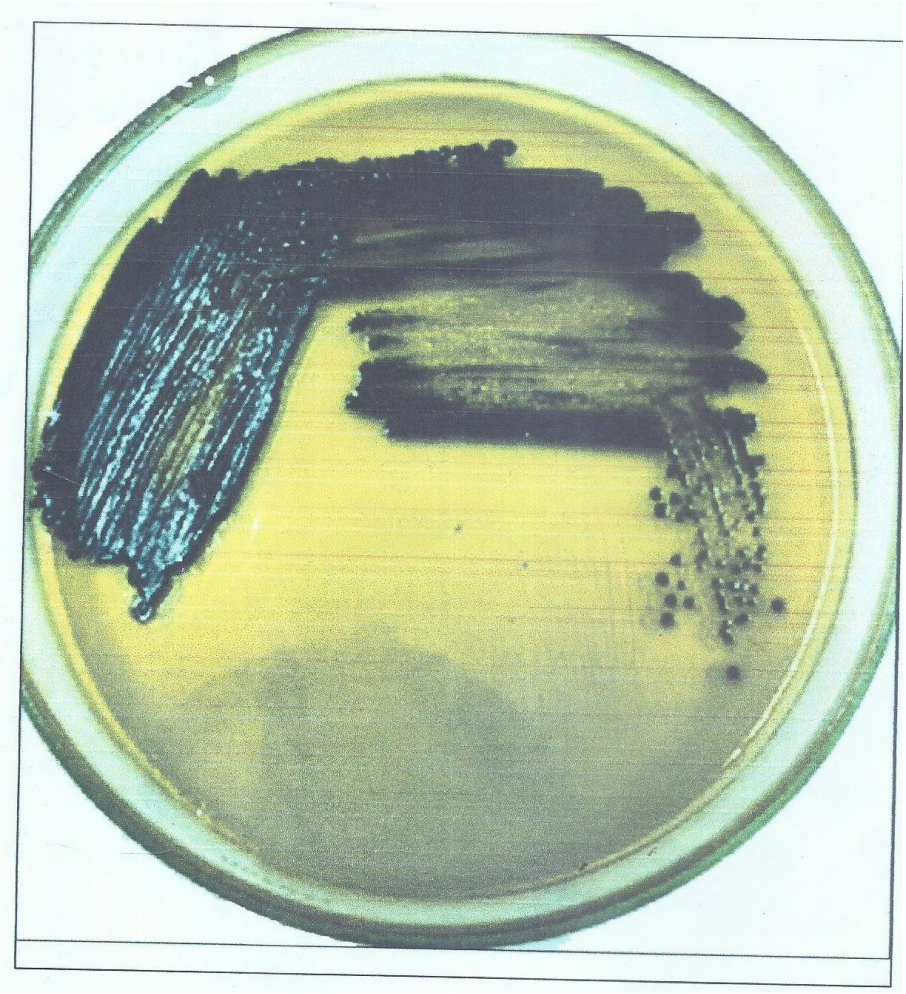

**Fig. (2): Black colonies of Fermentation of SimmonsCitrate in *Salmonella shigella* Agar Medium**

### 3.3 Microscopic Examination

Microscopic examination of the isolated revealed gram negative-rod table (3.2).

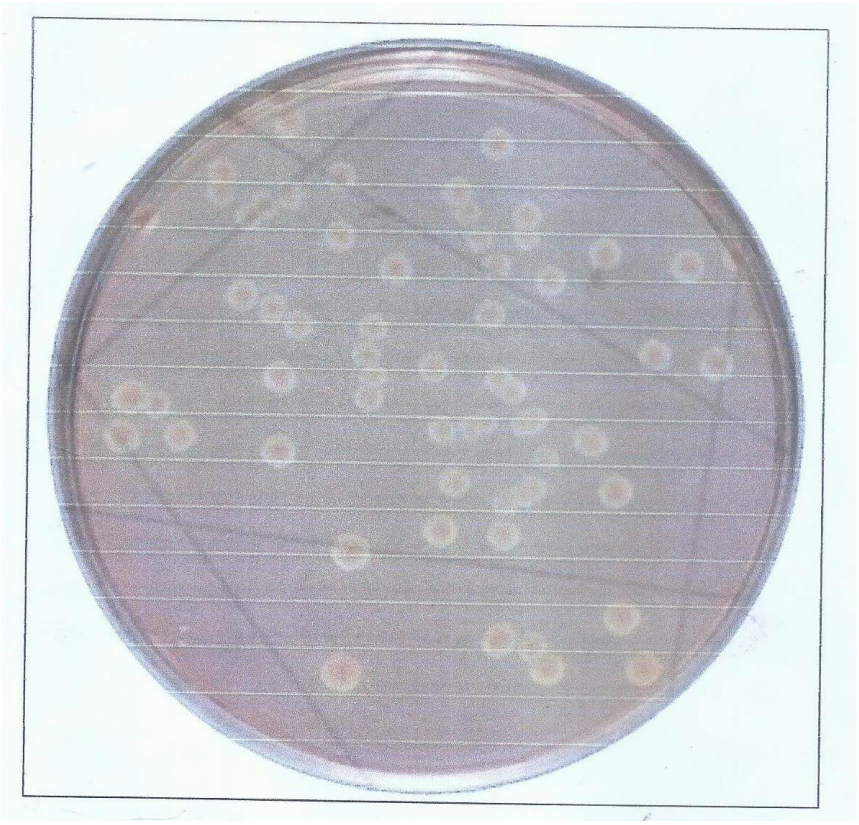

**Fig. (3): No Fermentation of Lactose in MacConkey Agar Medium**

## 3.4 Biochemical Characteristics of Isolates

### 3.4.1 Oxidase Test

All isolate were oxidase negative (table 3.4).

### 3.4.2 Indole Test

The isolated were negative.

### 3.4.3 Urea Test

Urea test showed negative reaction to isolates.

### 3.4.4 Kligler iron Test

The test was positive to isolates.

### 3.4.5 Hydrogen sulfide Test

The isolates showed black colonies in hydrogen sulfide test.

### 3.4.6 Citrate Test

The isolates also showed black colonies in citrate test.

The results of primary biochemical tests revealed that these samples were positive to catalase, oxidation fermentation test, motility test and suger fermentation test. But these samples were negative to oxidation test. Whereas, secondary biochemical test of these samples were negative to indole and urease [Table 3].

The clinical and bacteriological description showed that these organisms were *Salmnella*species.

**Table (3.3) Primary Biochemical Tests for Salmonella spp in Omdurman Locality, Khartoum State**

| No. | Test subtract | Results | | Salmonella spp reaction |
|---|---|---|---|---|
| | | **Positive** | **Negative** | |
| 1. | Catalase | Air bubbles | No air bubbles | + |
| 2. | Oxidase | Color change - Yellow color | No change in color | + |
| 3. | Motility | Motile | No motility | + |
| 4. | Sugar fermentation | Color change - Pink color | No change in color | + |
| 5. | Oxidase | Color change - Purple color | No change in color | - |

**Table (3.4)Some biochemical reactions of Salmonella Species in Infected Sheep [N=19] in Omdurman Locality, Khartoum State**

| No. | Test / Substrate | Results | | *Salmonella* |
|---|---|---|---|---|
| | | **Positive** | **Negative** | **Spp.Reaction** |
| 1 | **Oxidase** | Color change [purple] | No color change | - |
| 2 | **Indole** | Color change [pink] | No color change | - |
| 3 | **Urease** | Color change [pink] | No change in color | - |
| 4 | **Kligler iron tests** | Color change [pink] | No change in color | + |
| 5 | **Hydrogen Sulfide** | Black color | No change | + |
| 6 | **Citrate** | Black color | No change | + |

# Chapter Four

# Discussion, Conclusion and Recommendations

# Chapter Four

## Discussion, Conclusion and Recommendations

### 4.1 Discussion:

In this study, the symptom of infected sheep with salmonellosis in infected sheep (fever, water diarrhea, abdominal pain and respiratory distress)are in agreement with findings of Radositis*etal*.,(2007) who stated that the form of enteritis characterized by fever, severe fluid diarrhea and tenesmus. The increasing of pulse and respiratory rates and body temperature (Table3.1) due to severity of the disease (Blood *et al.*, 1989). For diagnosis of the definitive etiologic of *Salmonella* species in sheep bacteriological techniques are important (Table 3.2). The description of this species is necessary for the disease evaluation (Merchant and Packer, 1967). Cultue of the organisms and application of biochemical tests (Table 3.3) are ways for detection of the disease (Barrow and Feltham, 2003).

Many studies are applied these techniques for identification of *Salmonella* species in other animals and poultry (Kabour*et al.*, 2012; Mohamed – Noor *et al.*, 2012; Moutz*et al.*, 2012; Abdalla *et al.*, 2013; Ahmed *et al.*, 2013; Shuaib *et al.*, 2015; Omer *et al.*, 2016; Suliman *et al.*, 2016; Hessien*et al.*, 2018; Abdurrahman*et al.*, 2019; Salih *et al.*, 2019).

In Sheep *Salmonella* species canact as potential carrier of *Salmonella*because these species are facultative intracellular that can survive in the phagolysosome of macrophages and can avoid the bactericidal effect of antibody complement (Radostits*et al.*, 2007). The infection may persist in

lymph nodes or tonsils with no salmonella in the feces. Then the animals may begin shedding the organism or ever become a clinical case under the stress (Fraser and Mays, 1986).

Salmonellosis as zoonotic implication has assumed increasing importance recently because of occurrence of human salmonellosis with animal salmonellosis (Sanchez *et al.,* 2002). The transmission of the disease to human can occur via food chain through carcass contamination with animal feces at slaughter and during processing or through food or food handlers. Also, human infection by *Salmonella* species may occur through contaminated water, pest and exotic animals. Controlling of these routes of transmission are an effective way for prevention of the disease (El Hussien*et al.,* 2010).

# Conclusion and Recommendation

## 4.2 Conclusion

From the results the infected sheep by salmonellosis was 35.8% in the study area due to common spreading among herd and this indicates that the disease is serious problem. The diagnosis for detection of salmonella species by bacteriological techniques are the way of importance.

## 4.3 Recommendations

- Infected animals should be isolated from healthy animals and treatment of diseased animals is essential for control and prevention.
- Hazard analysis critical control point [HACCP] system must be applied in human food to control zoonosis for reducing contamination and also human public health sanitation.

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