SEROTYPES OF SALMONELLA ISOLATES FROM CHICKENS AND THEIR SUSCEPTABILITY TO LOCAL PLANT EXTRACTS

A Thesis Submitted in Partial Fulfillment
of Master Degree of Veterinary Science (M. V. Sc.)

By

NADA ABDELGALIL GAAFAR OSMAN
(B. V. Sc., University of Khartoum ٨٩٩١)

Supervisor
Dr. ISAM MOHAMED ALI EI JALII
(B. V. Sc., M. V. Sc., Ph. D)

Co-Supervisor
Dr. IMAD AHMED ALTAHIR
(B. V. Sc., M. V. Sc., Ph. D)

Department of Preventive Medicine and Public Health
Faculty of Veterinary Medicine, University of Khartoum

SEPTEMBER ١٤٣١٢٠١
PREFACE

The present study has been done at the Department of Preventive Medicine and Public Health, Faculty of Veterinary Medicine, University of Khartoum, and National Health laboratory of Sudan under supervision of Dr. Isam Mohammed Ali and Co-supervision of Dr. Imad Mohamed Altahir.
Dedication

This work is dedicated to

Nubians who are bleeding since ٤٦٩١ till now ,
soul of my father, soul of aunt Zahra, soul of Dr Garang ,
to my great mother, lovely sister, wonderful brother, Shawkat
recent Baankhi, Wardi, relatives,
friends and humanity

With deep love
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Subject</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preface</td>
<td>i</td>
</tr>
<tr>
<td>Dedication</td>
<td>ii</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>iii</td>
</tr>
<tr>
<td>List of Tables</td>
<td>vii</td>
</tr>
<tr>
<td>List of Figures</td>
<td>viii</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>x</td>
</tr>
<tr>
<td>Abstract</td>
<td>xii</td>
</tr>
<tr>
<td>Arabic abstract</td>
<td>xiii</td>
</tr>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Objectives</td>
<td>3</td>
</tr>
<tr>
<td>Chapter One: Literature Review</td>
<td></td>
</tr>
<tr>
<td>1.1 Salmonellosis</td>
<td>4</td>
</tr>
<tr>
<td>1.1.1 General Definition</td>
<td>4</td>
</tr>
<tr>
<td>1.1.2 Chicken Salmonellosis</td>
<td>4</td>
</tr>
<tr>
<td>1.1.2.1 Causative agent</td>
<td>4</td>
</tr>
<tr>
<td>1.1.2.2 Clinical signs</td>
<td>5</td>
</tr>
<tr>
<td>1.1.2.3 Pathogenicity</td>
<td>6</td>
</tr>
</tbody>
</table>
1.7.1 Cultural characteristics ......................................................... \***
1.7.2 Serotyping ........................................................................ \***
1.7.3 Taxonomy ........................................................................ \*0
1.7.4 Other avian species .......................................................... \*7
1.7.5 Antimicrobial activity ......................................................... \*9
1.7.6.1 Antimicrobial agents......................................................... \*9
1.7.6.2 Antibiotics susceptibility .................................................. \*9
1.7.6.3 Natural plants used as antimicrobial agent ....................... \*1

Chapter Two: Materials and Methods

1.8 Farms .................................................................................... \*4
1.8.2 Questionnaire ..................................................................... \*4
1.8.3 Samples ............................................................................. \*6
1.8.3.1 Collection ...................................................................... \*6
1.8.3.2 Culture media................................................................. \*6

Reagents.......................................................................................... \*9

1.8.3.3 Sterilization .................................................................... \*6
1.8.3.4 Culturing ......................................................................... \*6
1.8.3.5 Primary culture............................................................... \*6

1.8.5.1 Subculture ...................................................................... \*1
٦٫٣٫٢ Identification

Microscopic examination .............................................................٤٠١

٦٫٣٫٢ Microscopic examination ....................................................٤٠١

٢٫٦٫٣٫٢ Biochemical reactions

Motility test ..................................................................................٣٤١

٢٫٦٫٣٫٢ Motility test ........................................................................٣٤١

٢٫٦٫٣٫٢ Urease test ..........................................................................٣٤١

٢٫٦٫٣٫٢ Urease test ..........................................................................٣٤١

٣٫٢٫٦٫٣٫٢ Indole test ........................................................................٣٤٢

٣٫٦٫٣٫٢ Kilgler iron ager meaning..................................................٣٤٣

٣٫٦٫٣٫٢ Serological reaction ..........................................................٣٤٣

٣٫٥٫٢ Serotyping .............................................................................٣٤٤

٣٫٥٫٢ ((O)) typing ...........................................................................٣٤٤

٣٫٥٫٢ ((O)) typing ...........................................................................٣٤٤

٣٫٥٫٢ ((H)) typing ...........................................................................٣٤٤

٣٫٥٫٢ ((H)) typing ...........................................................................٣٤٤
Chapter Three : Result

1. Isolation .......................................................... 64

2. Microscopic examination and biochemical reactions........................................ 64

3. Serological reactions ................................................ 66

4. Serotyping of isolates .................................................. 66

5. Antimicrobial activity .................................................. 70

6. Antibiotics susceptibility testing ........................................ 70

7. Antimicrobial activity of some natural plants extracts...................................... 74

8. Questionnaire ................................................................ 77
Chapter four: Discussion

Discussion ........................................................................................................... \^*

Conclusions ........................................................................................................ \^6

Recommendations ............................................................................................... \^7

References .......................................................................................................... \^8
<table>
<thead>
<tr>
<th>Table</th>
<th>Table Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Epidemiological data for salmonellosis outbreak from different Farms</td>
<td>53</td>
</tr>
<tr>
<td>2</td>
<td>Distribution of lactose and non lactose fermenting isolates among Different farms</td>
<td>45</td>
</tr>
<tr>
<td>3</td>
<td>Distribution of salmonella isolates among different farms</td>
<td>55</td>
</tr>
<tr>
<td>4</td>
<td>Classification of salmonella isolates to serotype level</td>
<td>85</td>
</tr>
<tr>
<td>5</td>
<td>Five salmonella serotypes diameter of inhibition zones to four chosen antimicrobial agent</td>
<td>16</td>
</tr>
<tr>
<td>6</td>
<td>Antimicrobial activity of Acacia nilotica and Solenostemma Largel (Del) Hayne chloroformic and methanolic extracts in different concentrates against different serotypes of salmonella</td>
<td>56</td>
</tr>
<tr>
<td>7</td>
<td>Epidemiological data collected from different farms</td>
<td>97</td>
</tr>
<tr>
<td>Figure</td>
<td>Page</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>١. Poly (O) antisera subgroups typing</td>
<td>٥٤</td>
<td></td>
</tr>
<tr>
<td>٢. Poly (H) antisera subgroups typing</td>
<td>٧٤</td>
<td></td>
</tr>
<tr>
<td>٣. Soxhlets Used For Extractions</td>
<td>١٥</td>
<td></td>
</tr>
<tr>
<td>٤. (a&amp;b) poly (O) &amp; (H) subgroups serotyping</td>
<td>٩٥</td>
<td></td>
</tr>
<tr>
<td>٥. Effect of different antibiotics upon varied serotypes of Salmonella</td>
<td>٢٦</td>
<td></td>
</tr>
<tr>
<td>٦. Different serotypes reaction to different antibiotics</td>
<td>٣٦</td>
<td></td>
</tr>
<tr>
<td>٧. Antibacterial effect of Acacia nilotica extracts in concentration (١:١) upon S.gombe</td>
<td>٦٦</td>
<td></td>
</tr>
<tr>
<td>٨. Antibacterial effect of Acacia nilotica extracts in concentration (١:٢) upon S.gombe</td>
<td>٧٦</td>
<td></td>
</tr>
<tr>
<td>٩. Antibacterial effect of Acacia nilotica extracts in concentration (proportion) (١:٤) upon S.gombe</td>
<td>٨٦</td>
<td></td>
</tr>
<tr>
<td>١٠. Antibacterial effect of Solenostemma argel (Del) Hayne extracts in all concentration (١:١).</td>
<td>٩٦</td>
<td></td>
</tr>
<tr>
<td>١١. Effect of different concentration of Acaia nilotica chloroformic extract upon varied serotypes of Salmonella</td>
<td>٠٧</td>
<td></td>
</tr>
<tr>
<td>١٢. Different serotypes reactions to different concentration of Acaia nilotica chloroformic extract</td>
<td>١٧</td>
<td></td>
</tr>
</tbody>
</table>
\textbf{\textsuperscript{17}. Effect of different concentration of Acaia nilotica methanolic extract upon varied serotypes of Salmonella.}

\textbf{\textsuperscript{17}}

\textbf{\textsuperscript{15}. Different serotypes reactions to different concentrations of Acaia nilotica methanolic extract ....................}

\textbf{\textsuperscript{17}}

\textbf{\textsuperscript{16}. Different serotypes reactions to different concentration of Solenostoma laryel (Del) Hayne chloroformic extract.................................................. \textsuperscript{\textsuperscript{15}}}

\textbf{\textsuperscript{17}}

\textbf{\textsuperscript{18}. Effect of different concentrations of Solenostemma laryel (Del) Hayne chloroformic extract upon varied serotypes of salmonella ................. \textsuperscript{\textsuperscript{15}}}

\textbf{\textsuperscript{17}}

\textbf{\textsuperscript{17}. Effect of different concentration of Solenostemma laryel (Del) Hayne methanolic extract upon varied serotypes of Salmonella .................\textsuperscript{\textsuperscript{17}}}

\textbf{\textsuperscript{17}}

\textbf{\textsuperscript{18}. different salmonella serotypes reactions to different concentrations of Solenostemma laryel (Del) Hayne methanolic extract .................

\textbf{\textsuperscript{17}}
ACKNOWLEDGMENTS

First of all thanks for Allah , who gave me power and patient to complete this work . I am indebted to Dr Mohammed Elamin Hamid , Dr Isam Mohammed Ali , Dr Imad Eltahir . I would like to express my sincere appreciation and deep gratitude to them, for friendly and enthusiastic guidance, encouragement, support and patience.

My deep appreciation is due to Dr Najeeb, Khalil Abbass , Nuha Ahmed , Amina , Hassan , Ibraheem and Zeinb  in Microbiology Laboratory and all entire staff of National Health Laboratory .

My thanks extended to Dr. Awatif and Dr. Ashraf Nabeel all entire staff of Medicinal and Oramatic Plant Centre.

Sincere thanks to Adil Mahguob , Tahani all entire staff of Department of Preventive Medicine and Public Health, Fucalty of Vetrinary Medicine, University of Khartuom .

A very special thank and sincere gratefulness should be expressed to my mother Haram , sister Leina (Nahid), brother Mohammed, uncel Shawket and my friends Eshraga,Amel,Fatima , Rawda Babikir , Rawda Mohamed, Halla, Lwahiz, Mohammed, Mawadah, Tahani, Eihab, Nagla, Hamaad, Abeer, Yosra and all friends and relatives.
My deep thanks to all sudanese and all teachers from the primary school to the university and to any one who contribute to teach me even a letter.

Deep love to my son Mohamed and daughters Maiy, Muraam and Zahra.
ABSTRACT

Chicken salmonellosis is considered as threatening factor to poultry industry. On the other hand, human food-borne infection due to *Salmonella* from poultry products is of significant value. No much data regarding the main serotypes encountered in chicken and human infection in Sudan is available, hence this study was designed to determine the main serotypes of *Salmonella* prevalent in some open system laying farms in Khartoum State and to evaluate drug sensitivity and effect of some natural plant extracts on isolated *Salmonellae*.

One hundred and ten faecal, manure(dry faeces) and drinking water samples were collected for bacteriological examinations from four laying farms to detect the presence of *Salmonella*, two of the farms from which the samples were taken are known to harbour the infection, while birds in the other two farms were showing no clinical symptoms of salmonellosis. From the total isolates, eighty three non lactose fermenting isolates were recovered and thirty were proved to be *Salmonella*. Identification of *Salmonella* isolates to the genus level was based on morphological, cultural characteristic and biochemical reactions and confirmation was based on serological test.
Salmonella isolates, twelve have been serotyped. Five serotypes have been identified. They were Salmonella gombe, S. bonariensis, S. potsdam and two unclassified salmonella serotypes. Classification to the serotype level was based on serotyping method using specific antisera subgroup (O) and (H).

The five isolated serotypes were tested for in vitro antibiotic sensitivity using disk diffusion test. Four antibiotics namely, gentamycin, tetracycline, neomycin and colistin were used. Generally the serotypes showed different responses to different antibiotics. S. bonariesis and S. gombe were sensitive to gentamycin, the two unclassified serotypes (N5) (N19) were found to be sensitive to neomycin while S. potsdam was less sensitive to all antibiotics.

In vitro testing using natural plants extracts was carried out for the same serotypes. Acacia nilotica (Cissus quandrugularis) locally named (Garad) and Solensomma largel (laryl) (Del) Hayne locally named (Hargel) were used. Garad extracts in different concentrations were found to be highly effective against all serotypes of salmonella. The methanol extract was found to be more effective compared with chloroform extract. Hargel extracts were found to be of low activity against salmonella serotypes in different concentrations.

The epidemiological investigations showed that all farms with good hygiene management have low infection rate with Salmonella, on the other hand, in farms with poor hygiene, high rate of infection occurred.
It could be concluded that salmonellosis was endemic in the examined farms and affect the productivity. New serotypes were isolated, gentamycin and extracts of Garad specially the methanol extracts are highly effective against Salmonella and could be used for the treatment of the disease.
(2) العقل العالي و الفعالية الفعالة لا يمكن للكليانة أن تؤدي إلى النجاح إذا لم يتعلم الكليانة. 
(2) العقل العالي و الفعالية الفعالة لا يمكن للكليانة أن تؤدي إلى النجاح إذا لم يتعلم الكليانة. 

بالتالي، يمكن أن تكون النتائج الايجابية بناءً على الوعي والتعليم الفعالة. 
بالتالي، يمكن أن تكون النتائج الايجابية بناءً على الوعي والتعليم الفعالة. 

(2) العقل العالي و الفعالية الفعالة لا يمكن للكليانة أن تؤدي إلى النجاح إذا لم يتعلم الكليانة. 
(2) العقل العالي و الفعالية الفعالة لا يمكن للكليانة أن تؤدي إلى النجاح إذا لم يتعلم الكليانة. 

بالتالي، يمكن أن تكون النتائج الايجابية بناءً على الوعي والتعليم الفعالة. 
بالتالي، يمكن أن تكون النتائج الايجابية بناءً على الوعي والتعليم الفعالة.
INTRODUCTION

Salmonellosis is a disease caused by any organism of the *salmonella* group (Coma, ٣٢٢٢). The disease infects wide range of animal species as well as human. Poultry as general affected by the disease and a large range of acute and chronic diseases caused by any one or more members of the Genus *Salmonella*. Chicken salmonellosis is a composite term including diseases caused by *S. pullorum* (pullorum disease), *S. gallinarum* (fowl typhoid), *S. Arizona* (arizonosis) and other *salmonella* infection which are often referred to as salmonellosis (Gordon, ٢٩٩١).

The poultry industry has undergone considerable development in the Sudan in recent years. Chicken salmonellosis is one of the important problems that accompany this industry. Salmonellosis affect production in many ways like decreased in egg production, young chicks death and stunting growth of the broilers as well as stress which is often considered as the common catalyst in outbreaks of *salmonella* in both layer and broiler.

Food borne infection constitutes a major health problem in many countries. Many pathogens are encountered in these infections but *Salmonella* has significant contribution. It is worthy to mention that more than ٣٢٢ serotypes have been isolated in infected persons and animals of which
*S.typhimrium*, *S.enteritidis*, *S.infant*, *S.cholerasuis* and *S. heideberg* are most common. (Coma, Ꜯ・・・).

Recently many *Salmonella* species have been isolated from chicken. (Clanek *et al.*, Ꜯ١٩٩١, Imad, Ꜯ٣٠٠٢). Full serotyping of *Salmonella* isolates is very important for epidemiological purposes regarding the infection in specific area. The knowledge of the serotypes prevalent in the area will greatly help in the designing a suitable program for controlling the disease in chicken. Serotyping requires the use of polyvalent and monovalent O and H antisera and is usually carried out in a specialist public health laboratory (Monica, Ꜯ٣٠٠٣). Serotyping is a critical component of a public health response to the global challenge of salmonellosis. Support for serotyping as part of national Salmonella surveillance, and for rapid international communication of the results will help target future prevention strategies (WHO, Ꜯ٥٩٩١).

In many developing countries, the availability and use of antibiotics are poorly controlled, which results in a high rate of resistance, particularly to the older antibiotics. The extensive use of antibiotics, not only in human but also in chickens for disease prevention or as growth promoting feed additives, has led to a serious increase in and spread of multiple antibiotic-resistant bacteria (Moellering, Ꜯ٨٩٩١).

Natural plants extracts were traditionally used for various medicinal purpose worldwide. *Cissus quadrangularis* (Garad) have been used in India
and Srilanka as antimicrobial agent. Various parts of the plants are selected especially roots, young shoots and stem (Gupta, 1991). Fruitful results were obtained. In Sudan, Garda and Hargel are widely found. Traditionally they were used in the treatment of many infections and strong believe of their usefulness, as medicine is common.

OBJECTIVES

The main objectives of the present study were to:

\(1\) - Determine the serotypes of salmonella prevalent in studied laying farms

\(2\) - Examine antibacterial activity of some antibiotics and natural plants extracts on isolated serotypes.

\(3\) - Evaluate the effect of hygiene on salmonellosis in these farms.
CHAPTER ONE
LITREATURE REVIEW

١.١. Salmonellosis

١.١.١. General Definition

Salmonellosis is a term used to describe infection with any organism of the salmonella group (Coma, ٨٠٠٠). Avian Salmonellosis is an inclusive term designating a large group of acute or chronic disease of fowl caused by any one or more members of the Genus Salmonella which is a member of the large family Enterbacteriaceae (Clanek et al., ٦٩٩١).

١.١.٢. Chicken Salmonellosis

Is a composite term including diseases caused by S. pullorum (pullorum disease), S. gallinarum (fowl typhoid), S. arizona (arizonosis) and other salmonella infections which are often referred to as salmonellosis (Gordon, ٦٨٩١).

١.١.٢.١. Causative Agent

Salmonellosis is caused by any member of Genus Salmonella. S. pullorum produce salmonellosis in most animal and birds (Ewards et al, ٦٩٤٨). Fowl typhoid was reported among turkey, fowl, peafowl, duckling (Pomeroy, ٦٧٧٧). Fox (٦٧٧٧) isolated S. gallinarum from parrots, swans, sparrows, ringdoves and ostriches. Paratyphoid was reported in a variety of

### Clinical Signs

Most adult birds affected with *Salmonella pullorum* are carriers and seldom show clinical signs although in some cases egg production, fertility and hatchability are adversely affected. Mortality rate of salmonelosis (pullorum disease) in chicken may vary from nothing to 100% losses. And in fowl typhoid mortality rate vary from 10-50% or more (Gordon, 1984) (Hall, 1994).

### Pathogenicity

Salmonellosis constitutes a major threat to both human and animal health and to welfare (Imad, 1993). *Salmonella enteritidis* is a common pathogen of all species of mammals and fowls (Shoko, 1994). *Salmonella* of various serotypes are commonly found in the digestive tract of animals, illness
are usually associated with ingesting food contaminated with salmonella (Morris et al., 1999). In chicken, infection of salmonella is life long. The lighter breeds, particularly leghorn, have had fewer reactors with infected flocks than found in heavy breeds (Snoeyebos, 1985). It has been postulated recently that the genus Salmonella virulence evolved acquisition of Salmonella pathogenicity island (SPI 1) by plasmid or phage-mediated horizontal gene transfer SPI 1 was likely obtained by lineage ancestral to all Salmonella serotypes (Ochman and Groisman, 1993). SPI1 encodes virulence factors mechanisms used by salmonella serotypes during the intestinal phase of infection, including invasion of intestinal epithelial cells (Galan and Curtiss, 1989; Jones et al., 1994), induction of neutrophils recruitment (McCormik et al., 1995; Galyov et al., 1997), and secretion of intestinal fluid (Galyov et al., 1997). Serotypes belonging to S.enterica possess a second pathogenicity island, designated SPI 2, that is not present in S.bongori serotypes (Ochman and Groisman, 1993, Hensel et al., 1993). In S.enterica subspecies 1, the SPV (Salmonella plasmid virulence) operon, is found in only a few of serotypes, including S.gallinarum, S.enteritidis, S.choleraesuis S. Pullorum, S. abortusovis, S. paratyphi and S.Dublin (Popoff et al., 1984, Mahon and lax, 1989, Baumber et al., 1990 Guling et al., 1993). Salmoella plasmid virulence (SPV) have a role during infection of the host. The SPV operon is required for systemic phase of the disease caused by Salmonella Serotypes. (Guling and
Epidemiological evidence provide support for the idea that the SPV operon is also important for gthogensis of extra-intestinal infection associated with nontyphoidal \textit{Salmonella} serotypes in human (Fierer \textit{et al.}, 1997). Pathogens that lack host specificity, such as \textit{S. typhimurium} and \textit{S. enteritidis}, tend to be more frequently associated with disease in young animals than in adult, suggesting that they are not optimally adapted to cope with a fully nature immune system. Host specific serotypes tend to be more virulent as illustrated by the fact that they cause higher mortality rates (Baumler \textit{et al.}, 1996; Poppoff and LeMinor, 1997).

\textbf{\textit{\textbf{\textbf{1.1.1.4. Transmission}}}}

The primary role of infected hatching eggs in transmitting infection was recognized early in the course of investigation as a third of the eggs laid by infected hens contain \textit{Salmonella}. Chiefly as a result of contamination of the ovum following ovulation, transmission of infection during hatching from infected to non infected chicks can result in extensive dissemination (Snoeyenobs, 1984; Clanek \textit{et al.}, 1991). Egg transmission plays a dominant role in spread of infection of \textit{Salmonella} (Hinshaw \textit{et al.}, 1977). \textit{S. enteritidis} can be transmitted through the complete life cycle of flies and the infection may continue as long as 4 weeks within flies and \textit{Salmonella} may be recovered from flies up to 7 miles from point of origin (Greenberg, 1988). Paratyphoid
isolations were reported from houses flies (Kaye et al., 1981). Transmission also occur with in flock as a result of cannibalism of infected birds, egg eating and entry of salmonella through wounds. Eating contaminated egg has been demonstrated experimentally since S. pullorum has rarely been demonstrated in feed. In contrast to frequent contamination by S. paratyphoid, also the infected birds (reactor and carriers) could be considered as the most important mean of permeation and spread. Chicken buyers, visitors who travel from house to another and from farm to another may carry infection. Wild birds, animals, and flies may be important mechanical spreaders, especially if they have been feeding on carcasses of dead birds or offal from packing plants or hatcheries (Clanek et al., 1991). Broilers will infect the packing station and environment at slaughtering. Man must be recognized as a possible source of infection to poultry, which may involve poultry a pendants and human waste products (Mc Neil, 1984).

1.1.2.4. Diagnosis

A definitive diagnosis of pullorum disease requires isolation and identification of S. pullorum. Flock history and signs are of limited value in arriving at a diagnosis because of the similarity to a number of other disease. Lesions, particularly in severely affected chicks and poults, may be highly suggestive and used as a basis for a tentative diagnosis. Positive serologic findings have major value in detecting infection in a control program but
should not be considered adequate for a definitive diagnosis. Delay of \( \text{3-10} \) or more days in appearance of agglutinating antibodies following infection often result in mortality before antibody development ( Calnek et al., 1991 ).

*Salmonella* is generally identified as being a non-lactose fermenting (NLFs) Gram negative rod. To diminish the risk of obtaining false negative result, a non-selective enrichments and plating on two selective media is preformed. The non selective medium usually used is buffered peptone water. The selective enrichments used tetrathionate broth (Miller- Kauffman) and rappoport vassiliadis soy peptone (RVS). For subculturing xylose lysine desoxycholate (XLD) a gar and Brilliant Green agar (BGA) are used.

Subsequently it is confirmed with biochemical tests and serological test and serotyping whether the colonies resembling *Salmonella* on XLD and BGA are *Salmonella*. It is recommended to use the TSI (triple sugar iron) agar, urea a gar (Christensen) L-lysine decarboxylase, B-galactosidase (ONPG), Voges Proskauer and indole test in this order in addition; the *Salmonella* colonies were serotyped and classified on subspecies level. The biochemical confirmation of *Salmonella* and serotyping may be performed at the same time. Molecular analysis was used for characterization of some strains of *salmonella* and they were showed the \( 16S \) rDNA sequency pattern, and belonged to one group. The \( 16S \) rDNA genes proved to be unsuitable for epidemiological typing of *salmonella* strains below the species level. RAPD-PCR techniques
were used to detect the mechanism of some *salmonella* strains resistance to chloramphincol, tetracycline ampicilin and cephalixin and in diagnosis of *Salmonellae* (Imad, ٣٠٠٢). Both flagellin genes of *S. typhimurium* and cloned overlapping fragments of these genes in an expression vector were isolated. Monoclonal antibodies (MAbs) to both *S. typhimurium* flagellins were produced and used to select two recombinant flagellin fragments of ٦٨ and ٢٠١ amino acids, specific to the H:i and H:٢٠ antigens, respectively. These peptides appeared to be highly suitable antigens in an ELISA detecting *S. typhimurium*-specific antibodies. More than ٤ different serotypes of *salmonella* were found, and some serotypes probably mobilized from widespread animal-rearing activities were detected only during storm events. These serotypes may be good indicators of specific contamination sources. Furthermore, the RS-HP method based on the PCR amplification of the intergenic intergeneric spacer region between the ٦٨S and ٣٢S rRNA genes can produce amplicon profiles allowing the discrimination of species at both serotype and intra serotype levels. This method represents a powerful tool that could be used for rapid typing of *Salmonella* isolates (Baudart et al., ٠٠٠٢). The usefulness of selected PCR-protocols for the detection of *Salmonella* in ٣٦١ samples of animal origin (٣٦ raw minced meat, ٣٦ raw chicken meat, ٣ raw sausages, and ٣٥ egg samples, as well as ٣٠ poultry faecal, and caecal swabs samples) and DNA-fingerprinting typing was shown. To establish an
accurate PCR-procedure for *Salmonella* detection, the following parameters were evaluated: two pre-PCR concentration procedures, centrifugation and immunomagnetic separation (IMS) using Dynabeads anti-*Salmonella*; the specificity and sensitivity of \( \cdot \) sets of primers; and different conditions of the amplification reaction. In light of the results obtained from the use of PCR-based procedures alone or in combination with conventional methods, the following findings could be underlined: First, IMS was more efficient than centrifugation in the recovery of *Salmonella*. Second, the selected IMS/PCR-detection protocol was less time-consuming than the IMS/culture procedure, and a good concordance between them was found when the Kappa coefficient was calculated (\( \cdot \cdot \cdot \)). Third, PCR-ribotyping technique showed a very low discrimination power, being able to differentiate only three profiles. Fourth, RAPD technique using specific primers supports previous works in which it was proposed as a simple and useful tool for discriminating isolates between and within serotypes. Fifth, the efficiency, rapidity, and flexibility of the PCR-protocols applied were high, and they can be performed using two PCR-programs and the same basic equipment. (A.del et al., \( \cdot \cdot \cdot \)). The PCR primers selected can lead to inaccurate results. False-positive results arise from mispriming of nucleic acid sequences that are similar to target DNA, particularly when samples contain DNA from ingested material, fecal flora, and/or mammalian cells (Nuovo, \( \cdot \cdot \cdot \)). For instance, there is over \( \cdot \cdot \cdot \)
homology between the genomes of *Salmonella* and *Escherichia coli* (Slayers, 1994), and this can cause considerable mispriming and lead to false-positive amplicons. Misprimed amplicons similar in length to the amplicon of interest are also difficult to distinguish on agarose gels but can be identified by Southern hybridization with internal nucleotide probes (Nuovo, 1996; Stone, 1995; Zingg, 1994).

1.1.4.1. Epidemiology

Although the chicken appears to be the natural host of *S. pullorum*, the turkey has also proved to be an important host. In these hosts, infection is usually life long. Infection in other species have usually been minor and little long –term significance (Calnek *et al.*, 1991).Morbidity of fowl typhoid may vary and also mortality may range from 10 to 50% or more and that the seasonal incidence of the disease coincides with the period of peak of egg production (Hall *et al.*, 1949). Survival of *Salmonella* on soil for 2-30 days in summer and 12-18 days in winter and the organism also survive from 10-105 days on dirty wood work inside poultry houses at temperature of 2°C to 37°C and humidity’s of 30-70% and outdoors the organism survived for 2-34 days at 30 to 31°C (khashimov, 1984). Mortality from paratyphoid infections of poultry is more frequent during the first two weeks after hatching and the highest occur between the sixth and tenth days and that the infection rarely cause mortality in birds more than one month old. Also mortality among young
birds under natural conditions usually vary from 10 to 40% and mortality rates of 50% or more were also reported in severe outbreaks (William, 1974).

V Treatment

Reasonably effective prophylactic and therapeutic drugs were first discovered among the sulfonamides since that time other compounds including nitrofurans and several antibiotic have been found effective reducing mortality from the disease, sulphonmides in particular frequently suppress growth and may interfere with feed and water intake and egg production (Severens, 1945). In the USA and Canada many efforts made to eradicate the disease. In the USA the food and drug, admistration has approved use of sulpha quionoxaline and nitrofrans (Furazolidone) (Anonymous, 1988). Also treatment was done in experimental animal (albino mice), the antimicrobial drugs used were ciprofloxacin, ampicillin, sulphamethoxazole+ trimethoprin and cefalexine and there were different levels of response. Cephaletin made complete clearance of cure of mice while ciprofoxcin succeed to eliminate Salmonella from stool of mice. Bactrim (Sulfamethoxazol + trimethoprim) Produce clearance of Salmonella from stool of some groups of mice and failed in other groups. Ampicillin was effective in all groups of mice (Imad, 2002). Lactic acid and chlorine solution was used to inhibit growth of staphylococcus aureus and Salmonella derby (Ockerman et al., 2004). In addition to control measures such as vaccination and the use of competitive exclusion flora, the
supplementation of feed with acidic compounds has also been proposed to combat Salmonella in chicks (Van, et al., 2002). Currently, short-chain fatty acids (SCFA; formic, acetic, propionic, and butyric acid) are commonly used in the poultry industry for this purpose. SCFA decrease fecal shedding and the levels of colonization of the ceca and internal organs of chickens by Salmonella (Hanton and linton, 1981; Thompson and Hinton, 1997).

Control

In simplest sense it may be stated that only requirement to establish breeding flocks free of Salmonella pullorum and to hatch and rear their progeny under circumstances that preclude. Direct or indirect contact with infected chicken or turkey (Calnek et al., 1991). It has commonly accepted methods of management to prevent introduction of infectious disease agent are generally applicable to prevent introduction of S. pullorum. The fact that egg transmission plays a dominant role in spread of infection makes it mandatory that only eggs from flocks known to be free of pullorum disease be introduced into hatcheries (Hinshaw et al., 1971). Fumigation of incubators and hatchers with formaldehyde was originally developed to decrease spread of disease and destroy residual infection during cleaning between hatches. It has been established that chicken and turkey flocks can be developed and maintained free of pullorum disease by adhering to well defined management procedures. There should be no mixing of pullorum free stock at any time with other
poultry or confides birds not known to be free of disease (Calnek et al., 1991). Rodent control is essential so mice can be a significant amplifier of Salmonella enteridits infection in layer. During final preparation of eggs and egg-containing dishes in our homes, we can protect our selves by following simple food safety rules, including proper refrigeration and through cooking (Morris, 1999). Use of effective drugs in control and treatment of outbreak and recognition of the importance of environment as a source of reinfection.

Chicks and poults should be placed in an environment that can be cleaned and sanitized to eliminate any residential Salmonella from previous flocks. Chick and poults should receive palletized crumbled feed to minimize the introduction of S. gallinarum and other Salmonella. Free flying birds are commonly found to be carrier of Salmonella. Poultry houses should be bird proof. Insect control is important particularly, against flies, poultry mites, and less meal worm. These pests may provide a means of survival of Salmonella and other avian pathogens in environment. Another animals e.g. dogs and cats may be carrier of Salmonella, these should kept away from the poultry. Potable drinking water must be used or chlorinated water should be provided. In some areas, surface water is collected in open ponds for use as drinking water for lives stock and poultry. Human may be mechanical carrier of the organism on foot, water and clothing as well as poultry equipment processing trucks and poultry crates. Every precautions should be made to prevent introduction of
Salmonella by these means. Proper disposal of dead birds is essential (Clanek et al., 1991). There are vaccines available for salmonellosis in a limited number of countries. But there may be better alternatives for their prevention or control (David, 1981). Control of Salmonellosis can begin only after strict standard of sanitation if applied to the production of animal feed ingredients (Moran, 1977).

1.1.4.5. Hygiene and Salmonellosis

The recent increase in the number of outbreaks of food poisoning due to S. enteritidis in man was epidemiologically analysed and it was considered that contaminated eggs or egg product were the major source of this infection (Shoko, 1991). Poultry meat remains a major vehicle for human Salmonella infection. Epidemiologic data indicated that the current increase in outbreaks of Salmonella enteritidis arises from an important new source which is intact shell eggs (Grace, 1991). When paratyphoid infection becomes established in the brooder, it is rapidly transmitted by inhalation or direct consumption of faecal material or contaminated feed and water by young chicks. Repeated passage of the organism results in an infection of increased severity. The organism may directly be transmitted to young birds from older fowls that are a symptomatic chronic intestinal carriers (William, 1978). Poultry feed may be a common and very important source of paratyphoid in the epidemiology of salmonellosis in the poultry and man (pomeroy et al, 1965). Incorporation of
dried egg powder unfit for human consumption into poultry feed has been responsible for the introduction of some new *Salmonella* types in Britain (Wilson, 1991). Feed ingredients may become contaminated with *Salmonella* on the farm, storage bins, box cars and feed mills (Pomeroy, 1972). *Salmonella* did not find in freshly cooked poultry feed emphasized the importance of recontamination of the cooked product (Watkins et al, 1989). Small numbers of *Salmonella* present in contaminated feed may multiply in mashes under favorable moisture condition and temperature (Gordan, 1991). Six *salmonella* serotypes were isolated from cotton seed and soybean oil meal used in preparation of poultry feed (Grumble and flower, 1981). It was found that both treated and untreated chicken bone meal contaminated with *Salmonella* (Smith and Tucker, 1982). Man may be recognized as a possible source of infection to poultry which may involve poultry a pendants and human waste products (Mc Neil, 1941). On the other hand, poultry may also serves as a source of salmonellosis for other animals on the farm (Ladehoff, 1999). Rats and Mices are frequently carry intestinal *salmonella* particularty *S.typhimurim and S.enteristidis* and their litter is encountered in poultry feed, such rodents should be suspected as a possible source of the infection (Schnurrenberger et al, 1988). *S. gallinarum* was isolated from tick *Argas persicas* and bug *Cimex lectularuis* and louse (Gyurove, 1973).

1.1.1.1. Zoonotic Importance
Over 400 serotypes have been described within the *salmonella* group. Of these, more than 200 have been isolated in infected persons and animals of which *S. typhimrium*, *S. enteritidis*, *S. infant*, *S. cholerasuis* and *S. heideberg* are most common. *Salmonellosis* is the most common zoonotic disease in developed countries (Coma, 2002).

*Salmonella* infection in animals represent a threat to human health, particularly if infected animals used as food products (Leibisch and Schwarz, 1991). Food borne salmonellosis constitutes a major health problem in many countries, moreover the costs associated with salmonellosis could be considerable (Person and Jendreg, 2002). The recent increase in the number of outbreaks of food poisoning are due to *S. enteritidis*. Human food poisoning cases were analysed, and it was considered that contaminated eggs or eggs products were the major source of this infection (Shoko, 2002). A significant proportion of human *Salmonella enteritidis* outbreaks in recent years have been traced to the consumption of contaminated eggs produced by infected laying flocks (Richard, 2002). *Salmonella spp* are important zoonotic agent as a result of food borne transmission (Mead *et al.*, 1999, Winokur *et al.*, 2002). Large outbreaks have been associated with ingestion of poultry, meat, milk and other dairy products (Bean *et al.*, 1997). Typhoid fever is transmitted by water and food in endemic areas and in particular by carriers handing food provided to the children from 3-14 years of age (Ivanoff, 2002). Human illness are
usually associated with ingesting food contaminated with *salmonella*, although transmission also may occur from person-to-person through the fecal-oral route, when personal hygiene is poor and by the animal-to-man route (Morriss *et al.*, 1999). According to the statistical report of the Ministry of Health, Khartoum State, typhoid infection found to be 132,107 and 91 for the years 1999, 2000 and 2001 respectively. The enteric fever and diarrhea found to be 2108, 1477, and 2892 for the year 1999-2000, and 2001 respectively calculated from the hospital cases (Imad, 2003).

*Salmonellae* are responsible for 16 million annual cases of typhoid fever; 1.7 billion cases of gastroenteritis resulted in more than 5 million annual death (Groisman and Ochman, 2000). *Salmonella enterica* serotype *enteritidis* remains the most common *salmonella* serotype isolated from human in UK, with 10296 cases (Liebana *et al.*, 2001). Typhoid fever was reported in developing counties like South East Asia, India, Jordan, Bangladesh and Nigeria (Ivanoff, 2002).

11.1.11. Economic Importance

11.1.11.1. Poultry Economic Importance
In Sudan, commercial poultry farming concentrated around the capital Khartoum and in Gezira Area. In some other parts of the country, there are governmental units, which provide chicks and feed to the citizens. The common practice all over the country is the back yard system of production where the families raise up a small flock of one or more birds. The products are usually consumed by the family and sometimes sold as additional income (Hayat, 1987). The poultry population was estimated as 5.32 million birds with an annual output of 0.352 tons of eggs and 0.241 tons of meat according to the six year development plan 1987/88-1992/93. On national level, this amount to a per capita consumption of 3.4 eggs per year and 0.9 kg of meat (Hayat, 1987). No reliable data are available to show poultry production. This is evident by the records of Ministry of Animal Resources (1990, 1991) which showed that poultry population to be 3.54 million, while the Food and Agriculture Organization (FAO) gives the total population to be 3.83 million. Recent estimate by Arab Organization for Agriculture Development (AOAD) 1997 stated the population in modern sector to be 3,434,000 broiler and 3,330,000 layers (Suad, 1998).

1112,1114. Salmonellosis Economic Importance
Salmonellosis constitutes a major threat to both human and animal health and to welfare. Salmonellosis cause a decrease in egg production (Imad, ٣٠٠٢). Stress is often the common catalyst in outbreaks of salmonella in both layer and broiler (Morries, ٩٩٩١). The food borne salmonellosis constitutes major problem. Moreover, the costs associated with salmonellosis are considerable. There are thus strong arguments for preventive efforts. Ambitious, often government-sponsored, programmes aimed at preventing and controlling salmonellosis for instance poultry production represent one alternative to lower salmonellosis related illness and economic costs (Perssan, ٢٩٩١). Mortality rate of chicken salmonellosis (pullorum disease) varies from no losses to ١٠٠٪ in serious outbreak and in fowl typhoid from ١٠٠-٥٪ or more (Hall, ٤٤٩١). Some diseases of poultry are significance because of their zoonotic nature (Gordon, ٢٨٩١).

١١١١١١١١١١١. Chickens Salmonellosis in Sudan

In the Sudan, the first record of isolation of Salmonella from chickens is of S.gallinarum recovered from two outbreaks of fowl typhoid (Annual report, Sudan Veterinary Service, ١٩٩٣). Tow strains of the organism were again isolated from fowl, in Malakal Town (Soliman and Khan, ٩٥٩١). ٢٩٠ chickens, ٨ swans, one shoebill and one demoiselle orane were examined by Khan,(٩٥٩١). No Salmonella was isolated from chickens or swan but S.Dublin and S.typhimurium were recovered from the shoebill and demoiselle orane.
respectively. Khan in 1991 investigated about apparently healthy fowl were from farms around Khartoum, diseased chicken, samples of Poultry droppings, eggs, other avian species including pigeon, cattle, egret, kites, sparrow, clurabirds, sand grousse, sparrow hawk, ducks, ostrich, white cockato, red macaw parrot, white peacock chakour and vultures were examined. Khan Isolated *S.chicago, S.london, S.omdurman S. gaminara, S.newport and S. uganda from fowls. S.typhimurium from Pigeons and S.east bourne, S.reading and S. typhimurium from wild bird (Khan, 1991). On the other hand, poultry were investigated from Khartoum Province, samples from the Sudanese- Kwaiti Company from poultry lohmann imported from German and from bird markets. (1998) *Salmonella* serotype were Isolated included *Salmonella infantis, S. Kalama, S.emek S.mons, S.wien, S.heidelberg, S. Livingstone, S. derby, S.cerro, S. Uganda , S.ruiru , S.alachua, S. Vogan , S.meleagridis, S. : L , V: , S. : Z Z - subspII,S. : Z Z Subsp I, monophas.var subsp 'S. Montevideo, S.stockholm S.amersfoort, S.give and S.rough from R:r: 1,7 sub. Sp' (Ygoup, 1987). samples collected from poultry were examined then *S.amersfoort , S. Eneritidis S. heidel* and other *salmonella* serotypes were isolated( Imad, 2003).

1.7. Genus Salmonellae
\textbf{1.4.1. Morphologhy}

Salmonella is a Gram- negative bacteria. Its shape is long and slender with rounded ends occurring singly but rarely with chain of more than two bacilli. (Monica, ٠٠٠٢). Salmonella is non acid fast, non sporing, non capsulated and about ٦-٧٠ mm in length and ١٠-٢٠ mm in diameter and mostly occur singly and occasionally in pairs. They tend to stain at the poles than the centre (Pomeroy, ٢٧٩١).

\textbf{1.4.2. Cultural Characteristic}

Salmonellae are facultative. They grow between ٥١-٥٤°C with optimum temperature of ٧٣°C. A selective medium is required to isolate salmonella from specimens. In Blood agar, Salmonellae produce grey – white ٦-٧ mm diameter colonies similar to those of many other enterobacteriaceae. Some strains have mucoid colony appearance. In xylose lysine deoxycholate medium (XLD) Salmonellae produce hydrogen sulphide(H₂S) forming pink red colonies ٦-٨ mm in diameter with black centers. Most strains of S. paratyphi A does not produce H₂S. In Deoxycholate citrate agar (DCA) and MacConkey agar, salmonellae produce non lactose fermenting pale colored colonies while on deoxycholate citrate medium (DCA) which indicated by black centers colonies (Monica, ٠٠٠٢).

\textbf{1.4.3. Serotyping}
Based on their O and H antigen composition, more than \( \gamma \gamma \) \( \text{salmonella} \) serotype are described in the Kauffmann-white scheme, accordingly, \( \text{Salmonella} \) is placed in groups by the antigens (A,B,C) and subdivided by their H(phase 1 and 2) antigens. Polyclonal antisera containing antibodies to major groups can be used to isolate and identify biochemically suspected \( \text{salmonella} \). Full serotyping (for epidemiological purposes) requires the use of polyvalent and monovalent O and H antisera and is usually carried out in a specialised public health laboratory (Monica, \( \gamma \gamma \)). Most \( \text{Salmonella} \) serotypes alternatively express either phase-1 or phase-2 flagellar antigens, encoded by the \( \text{fliC} \) and \( \text{fljB} \) genes, respectively. Variable internal regions of the \( \text{fliC} \) genes encoding the H:i, H:r, H:l,v, H:e,h, H:z(\( \gamma \)), H:b, and H:d antigens have been sequenced; and the specific sites for each antigen in selected \( \text{Salmonella} \) serotypes have been determined. These results, together with flagellar G-complex variable internal sequences obtained by the food borne and diarrheal diseases branch at the centers for disease control and prevention in Atlanta, GA, have been used to design a multiplex PCR to identify the G-complex antigens as well as the H:i, H:r, H:l,v, H:e,h, Hz(\( \gamma \)), H:b, and H:d first-phase antigens. These antigens are part of the most common \( \text{Salmonella} \) serotypes possessing first-phase flagellar antigens. \( \text{Salmonella enterica} \) serotype enteritidis is identified by adding a specific primer pair. This multiplex PCR includes \( \gamma \) primers. A total of \( \gamma \gamma \) \( \text{Salmonella} \) strains
associated with \(^\text{27}\) different serotypes were tested. Each strain generated one first-phase-specific antigen fragment ranging from \(^\text{001}\) to \(^\text{005}\) bp; \(Salmonella\) serotype enteritidis, however, generated two amplicons of \(^\text{005}\) bp that corresponded to the G complex and a \(^\text{334}\)-bp serotype-specific amplicon, respectively. Twenty-three strains representing \(^\text{91}\) serotypes with flagellar genes did not generate any fragments. The method is quick, specific, and reproducible and is independent of the phase expressed by the bacteria when they are tested. (Herrera \textit{et al.}, \(^\text{4002}\)). The PCR primers selected can lead to inaccurate results. False-positive results arise from mispriming of nucleic acid sequences that are similar to target DNA, particularly when samples contain DNA from ingested material, fecal flora, and/or mammalian cells (Nuovo, \(^\text{6991}\)). For instance, there is over \(^\text{90.7}\) homology between the genomes of \(Salmonella\) and \(Escherichia coli\) (Slayers, \(^\text{4991}\)), and this can cause considerable mispriming and lead to false-positive amplicons. Misprimed amplicons similar in length to the amplicon of interest are also difficult to distinguish on agarose gels but can be identified by Southern hybridization with internal nucleotide probes (Nuovo, \(^\text{1996}\), Stone, \(^\text{1995}\), Zingg, \(^\text{1994}\)).

\(^\text{1,4,4\; Taxonomy}\)

The family Enterobacteriaceae is subdivided into five primary groups, group \(^1\) contains the Genus \(Salmonella\) plus four other genera (Bergey’s Manual of Microbiology, \(^\text{1974}\)). The Genus \(Salmonella\), named for late
eminent USA Veterinarian Daniel E. Salmon (Snoeytenbos, 1989).

Historically, species names were arbitrarily given to serovar for convenient reasons in medical practice. Some serovars' names denoted syndrome (s.typhi) or relationship (s.paratyphi A,B,C). Other names were correlated with syndrome and host specificity which was right in some cases (S.abortus-ovis, S.abortus-equus) or wrong in other cases (S.typhimurium, S.cholerae-suis). To avoid possible source of confusion, names indicating geographical origin of first strain of new serovar (S.London, S.panama, S.tel.el-kebir) were used. It was decided that compound names would be hereafter condensed in simple names (S.typhimurium, S.cholerae-suis, S.tel.el-kebir) these names, wrongly considered as species names. Names were maintained only for subspecies enterica serovars, which account for more than 99.9% of isolated Salmonella strains. In the clinical practice, the subspecies names does not need to be indicated as only serovars of subspecies enterica bear a name: Typhimurium, London or Montevideo are serovars of subspecies enterica. The name Salmonella ser. Typhimurium or Salmonella Typhimurium may be used for routine practice. Serovars of other subspecies S. enterica and those of S.bongri are designated only by their antigenic formula (Kauffman-White Scheme, 1981).

The Genus Salmonella consists of two species:-
(1) *S. enterica*, which is divided into six subspecies: *S. enterica* subsp *mohanenterica*, *S. enterica* subsp *salamae*, *S. enterica* subsp *arizonae*, *S. enterica* subsp *indica*, *S. enterica* subsp *diarizoae*, *S. enterica* subsp *houtenae*. (2) *S. bongori* (formerly called *S. enterica* subsp. *bongori*) These species and subspecies can be distinguished on basis of differential characters e.g. *S. enterica* subspecies included the following serotypes: *S. typhimurium*, *S. enteritidis*, *S. choleaeuis*, *S. gallinarum*, *S. pullorum*, *S. abortusovis*, *S. paratyphi* C and *S. Dublin* (Popoff *et al.*, 1981; Woodward *et al.*, 1981; Poppe *et al.*, 1989; Baumler *et al.*, 1991; Mohan and lax, 1993; Roudiar *et al.*, 1991).

### 1.4.2 *Salmonella* in Other Avian Species

*Salmonella* is a facultative intracellular pathogen, typically colonizing reptiles, birds, and mammals, with some serovars *typhi*, *Dublin* and *galliarum* infect human, cattle and bird respectively (Falkow, 1991). *Salmonella pullorum* like most of the recognized *salmonella* tend to produce symptoms of salmonellosis in most animals and avian species. It was isolated from turkey, pheasant, canaries, parrots, calves, swine, dogs, foxes, minks, cats, Chinchilla and man (Edward *et al.*, 1984). Also *S. pullorum* reported from ducks (Chute and Gershman, 1987) and from bull finch (Hudson and Beaudette, 1983) and from guinea pigs and rabbits (Rettger, Hull and Sturges, 1971) and from rats (Sato, 1977). Fowl typhoid was reported among turkey
S. gallinarum isolated from parrots, swans, sparrows, ringdoves, ringdoves and ostriches (Truches, 1943). Paratyphoid organisms were reported in a variety of avian species other than the chicken. Turkeys were the first report (Paff, 1941). Paratyphoid infection reported in pigeon, particularly in squabs (Moore, 1890). The serotypes isolated from turkeys include S. typhimurium (Cherrington, Gilddow, and Moore, 1947) S. newington (Hinshaw and Mcneil, 1947) and S. infantis (Gordon and Tucker, 1957). Isolation of Salmonellae reported from tropical psittacine birds including parrots, parrotlets, parakeets and conures. Many other workers reported paratyphoid infections in birds and isolated a etiological organisms. The species of birds investigated included different types of gulls, parakeets, Guinea fowls, different types of fiches, cordon bleu, cutthroat, sparrows, a peafowl, Japanese robins, a secretary bird, a diamond dove, a sugar bird, callistes, cookato, hoatzin, horn bill, starlings, rusty black birds, a rook, a red throat driver, a mallard and a mute swan (William, 1977). The first report of a paratyphoid outbreak in chicken (Mazza, 1899). Paratyphoid outbreak reported in chicks due to S. thompson and typhimurium (Wilson, 1944). The occurrence of S. anatum, S. bareilly, S. california, S. london, S. montevideo and S. thompson was reported in chicks (Gordon and Buxton, 1947).
Antimicrobial Activity

Antimicrobial Agents

May be defined as those chemical substances interfere with the growth and activity of microorganisms generally. The term denotes inhibition of microbial growth (biostatic) and/or microbial destruction (biocidal). Such as antibacterial or antifungal are frequently employed to refer to activity agent against specific group of microorganism (Pelczar et al., 1979). The advent of synthetic methods has, however resulted in a modification of the definition, and antibiotic may be used to substance, produced by a microorganism (natural) or to similar substance, produced wholly (synthetic) or particularly by chemical synthesis (semi synthesis) which low concentration inhibits the growth of other microorganism (Pratt, 1979). A characteristic property of antibiotics is bacteriostatic and/or bactericidal action on microbes. Bacteriostatic agent inhibits the growth of bacteria without killing it, while bactericidal agent kills bacteria (Pelczar et al, 1979).

Antibiotics Susceptibility

Resistance of some salmonella serotypes isolated in Sudan to erythromycin, penicillin, cephaloridine and ampicillin was reported, with some swear moderately sensitive. However, a wide range of variation was reported in response to streptomycin and tetracycline (Yagoub et al., 1986). Some of salmonella serotypes were highly resistance to tetracycline, furazolidone,
cephalixin, colistin and ampicillin respectively, while they were sensitive to ciprofloxacin and gentamycin and resistance to sulfamethoxazole + trimethoprim (Imad, 2002). Widespread use of antibiotics in recent years has revolutionized the therapy and has substantially reduced mortality from infectious disease. Misuse of antibiotics, irrational selection, aberrant dosage, possible poor patient compliance and easy over the counter availability of prescription drugs has unfortunately helped in the emergence of multiple-drug resistant (MDR) strains of salmonella. MDR salmonella isolates were found to be quite susceptible to a question, alcoholic extract of fruit of C.fistula alone as well as in combination with amoxicillin. Minimal inhibition concentration (MIC) of C.fistiula when tested alone was found to be in the range of 0.93-1.21 mg/ml, however, the combination of amoxicillin and fruit extract of C.fistula, MIC of C.fistula lowered to 0.93-0.65 mg/ml and there was four-fold decrease in MIC of amoxicillin for MDR salmonella typhii. MIC alone was 1.06-2.72 mg/ml and in combination 0.39-1.02 mg/ml (Shahana, et al., 2002).

Progressive rise in drug line drugs, chloromphenicol, co-trimoxazole and ampicillin/amoxicillin has led to excessive use of floroquinolones as agents of choice for the treatment of enteric fever. MIC of hundred blood isolates of salmonella typhi and paratyphi was measured by agar dilution method, and the result showed 17% of isolates were resistant to nalidixic acid (MICs>24 mg/ml) and to oflexicon (MIC>0.12 mg/ml)(Ahmed et al, 2004).
Natural Plants Used as Antimicrobial Agents

The roots, young shoots and stem of *cissus quandranqularis* (Garad) have been used for various medicinal purpose in India and Sirlanka, A new unsymmetrical tetracycline tritepenoid, \(\gamma\)-oxoonoce-\(\alpha\)-ene-\(\beta\) beta, \(\alpha\) alpha-aerial was isolated from the aerial parts of this plant. The structure was elucidated on the basis of spectral and chemical evidence (Gupta, 1991). Leaf extract of some plants (Adathoda Vasica- Vitex nequndo, Azdirachta India, Sansevieriasp. *Cissus quandranqualris* (quadranqhl) and Aloe sp.) as a% aqueous extracts sprayed on the P. iatus population were reduced in all treatment, compared to untreated control, at 5 and 10 days after treatment but. L nod, flora and Aloe sp. recorded the lowest populations at \(\cdot\) mites per leaf (Palaniswamy-s : Ragini-Jc 2002).

The alcoholic extracts of 23 plants, selected on the basis of literature references and use in folklore, were studied for their activity against 4 pathogenic bacteria and 5 fungi. Only eight of these plants were included *Solenostemma argel* and markedly antifungal. There was a correlation between the antimicrobial activity and the content of physiologically active principles (Ross *et al.*, 1984). Aerial parts of *Solenostemma – argel* were successively extracted with Methanol /water in different proportions (4 fractions) Phytochemical and chromatographic screening as quantitative determination of flavonoid and saponin content were carried out for each fraction. The antimicrobial activity of the 4 fractions against 8 bacteria (*staphylococcus aureus*, *Micrococcus*, *streptococcus*, *Bacillus anthraces*, *E.coli*, Klebsiella
Pneumoniae, pseudomonas aeruginosa and proteus vulgaris) observed and Fungi (Fusarium, Aspergillus parasiticus, A.glaucus, pencillium, chrysosporium, condida albicans, Mucor and Rhodotorula) were studied. The greatest effect was for streptococcus spp, and moderate action against E.coli, B.anthracis, S.aureus, Klebsiella pneumoniae and proteus vulgaris fraction no. I showed antifungal activity against A.niger and Mucor while fraction ^ showed activity against A.niger, A.candidus chrysosporium, Candida albicans, Candida spp and Rhodotorula. Fraction ^ had an effect on A.niger, Candida spp and Rhodotorula fraction ^ was highly effective against A.parasiticus and A.candidus. The ^ fractions showed different degrees of antifungal activity against the examined ^ fungal species (El-Hady et al., ^). Aerial parts of Solenostemma argel were successively extracted with chloroform/ methanol and in different proportion (^ fractions). Phytochemical and chromatographic screening as well as quantitative determination of the flavonoid and saponin content was carried out for each fraction. The antimicrobial activity of the ^ fraction against ^ bacteria: (Stamphylococcus aureus, Micrococcus, Streptococcus spp, Bacillus anthraces, E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and proteus Vulgaris) and ^ fungi (Fusaurium, penicillium, Sapergillias parasiticus, A. Flavus A.niger A.Candidus A.glaucus, Chrysosporium crypytococcus neoformans, Candida spp . C. albicans, Mucor and Rodotorula were studied. The greatest effect was observed for streptococcus spp and moderate action against E.coli B.anthracis, S.aueus, Klebsiella pneumoniae and proteus vulgaris there was no effect on
Micrococcus and Pseudomonas while a weak fungicidal activity was observed (El- Hady et al., ٤٩٩١). Four compounds isolated from the steam of the desert shrub S. argel which is used in local medicine were screened only one (from the saponifiable fraction) showed any activity against both Gram- positive and Gram- negative bacteria (Tharib et al., ٤٩٩١).
CHAPER TWO
MATERIALS AND METHODS

١٫١. Farms

The studied farms were open system laying farms. They were located in Khartoum State specifically in Alkalakla, Aldekhenat, Elfetiah and Tabatelhassanab. The chickens in all farms were of ٨-٢١ months old and their breed was lohmann. The farms were divided into two groups, each one included two farms. The first group chickens were suspected for having salmonella infection with the following signs, dullness with whitish to greenish diarrhea, decrease in egg production, in postmortem there was congested and misshaped ova. The second group chicken was randomly surveyed and, they have no clear signs except decrease in egg production.

١٫٢. Questionnaire

A questionnaire was designated to collect information on methods of husbandry, medications used, mortality rate and outbreak of Salmonellosis occurred in these farms

Epidemiological data collected during visit illustrated in Table (١)
٣.٢. Samples

٣.٢.١. Collection

Faecal, manure and water samples were collected from each farm. The sample were ١١١ faecal samples, ٧ manure and ٤ water. They were collected during the period from June ١٠٠٢ to March ٣٠٠٢. All samples were cultured to isolate *Salmonellae*.

٣.٢.٢. Culture Media

٣.٢.٢.١. Selenite Broth

It was composed of bacteriological peptone (Oxoid L٤٤٤) Lactose , Sodium phosphate . The medium was prepared by suspension of ٩١ g of bisodium Selenite (oxoid L١٢١) powder in one liter of distilled water to dissolve mixed well and filled out into containers to depth of ٠ cm then, sterilized in boiling water bath or in free flowing steam for ٠١ minutes without autoclaving .

٣.٢.٢.٢. MacConkey Agar

This medium contain peptone ,lactose , bile salts ,neutral red ,agar . The medium was prepared by dissolving suspension of ٤٧ g of powder in one liter of distilled water then boiled to dissolve completely and sterilized by autoclaving at ٤٠٣٠٣ ٤ for ١٥ minute before dispensing into sterile Petri dishes .
٣.٣.٣. Xylose Lysine Desoxycholate (XLD)

This medium composed of lysine hydrochloride, xylems, lactose, sucrose, sodium desoxycholate, sodium chloride, sodium thiosulphate, ferric ammonium citrate, phenol red and agar. The medium was prepared by suspension of ٣٥٠ g of powder in one liter of distilled water and heated with frequent agitation until the medium boiled and transferred to water bath at ٠.٦٥٠ then dispensing into sterile Petri dishes.

٣.٣.٤. Nutrient Agar (Oxoid)

It was composed of lab-lemco powder, yeast extract, peptone, sodium chloride, agar. The medium was prepared by suspension of ٨٢٠ g of powder in one liter of distilled water then boiled to dissolve completely and sterilized by autoclaving at ١٢١٠ for ٥١٠ minute before dispensing into sterile Petri dishes.

٣.٣.٥. Motility Media

It was contain meat extract, peptone, agar, gelatin, sodium chloride. The medium was prepared by suspension of ٢٠١٠ g of powder in one liter of distilled water then boiled to dissolve completely and sterilized by autoclaving at ١١١٠٠ for ٥١٠ minutes before dispensing into sterile Petri dishes.

٣.٣.٦. Christensen’s Medium (Urea Medium)

The base medium contains peptone, sodium phosphate, dextrose, potassium dihydrogen phosphate, phenol red and agar. The medium was prepared according to manufacture’s instructions by dissolving ٤٠٠٤ g of urea’s agar (Oxoid) in ٥٩٠ ml distilled water by boiling. Sterilized and the PH was adjusted to ٥٠٠ then cooled to ٠.٦٥٠. Five ml of sterile ٤٠٠٤ urea
solution (Oxoid SR) were added aseptically. The mixture was distributed in 1 ml amounts into McCartney bottles and allowed to solidify in slope position.

\textbf{\textit{\textnumero, \textnumero, \textnumero, \textnumero. Peptone Water}}

It was composed of peptone and NaCl. The medium was prepared by dissolving 10 g of peptone water and 9 g of sodium chloride in one liter of distilled water. The pH was adjusted to \( \text{pH} = 2.7 - 4.7 \). This medium was used in indole test.

\textbf{\textit{\textnumero, \textnumero, \textnumero, \textnumero. Kilgler Iron Agar}}

It was composed of meat extract, yeast extract, peptone mixture, sodium chloride, dextrose, ferric, sodium, phenol red and agar. The medium prepared by suspension of 6.55 g of powder in one liter of distilled water then boiled and dissolved completely and sterilized by autoclaving at 121°C for 15 minutes.

\textbf{\textit{\textnumero, \textnumero, \textnumero, \textnumero. Swarm Media}}

It was composed of bacto beef extract, bacto peptone water, bacto agar, nitrium desoxycholate and water. The medium prepared by suspension of 18 g of powder in one liter of distilled water boiled and dissolved completely and sterilized by autoclaving at 121°C for 15 minutes.

\textbf{\textit{\textnumero, \textnumero, \textnumero, \textnumero. Nutrient Broth (Oxoid Cm)}}

This medium was composed of lab-lemco, peptone water; sodium chloride and beef extract. It was prepared by dissolving 17 g of powder in one liter of distilled water and sterilized by autoclaving at 121°C for 15 minutes.
١٫٣٫٣٫٢. Reagants

١٫٣٫٣٫١. Gram Stain Reagants

١٫٣٫٣٫١٫١. Crystal Violet

It was composed of two solutions. Solution (A) consisted of ten ml of crystal violet and ٠٠١ ml of ethanol (٥٩٪). Solution (B) was composed of ammonium oxalate ١٪. The reagent was prepared by mixing ٠٢ ml of solution (A) and ٠٨ ml of solution (B).

١٫٣٫٣٫١٫٢. Gram Iodine (Mordant)

The reagent was composed of iodine crystal, potassium iodine, and distilled water. It was prepared by grounding ١ g of iodine crystal and ٢ g of potassium iodine together in a mortar and ٠٠٢ ml of distilled water added slowly.

١٫٣٫٣٫١٫٣. Decolourizer

It was composed of ethanol (٥٩٪).

١٫٣٫٣٫١٫٤. Concentrated Cabrol Fuchsin (Counter stain)

It was composed of one gram of basic fuchsin, ١ ml of ethanol (٥٩٪) and ٥ g of phenol.

١٫٣٫٣٫١٫٥. Dilute Cabrol Fuchsin (Counter stain)

It was prepared by mixing ١ ml of concentrated carbol fuchsin with ٥ ml of distilled water.

١٫٣٫٣٫٢. Kovac's Reagent
It was composed of five grams of P-dimethyl-amino benzaldehye, 5 ml iso-amyl alcohol and 5 ml concentrated hydrochloric acid. The aldehyde was dissolved in the alcohol by gentle heating in water bath at 65°C. The mixture was cooled and this was followed by addition of the acid. The reagent was stored at 4°C in a dark bottle. It was used for detection of indole production.

4.3.4. Sterilization

4.3.4.1. Sterilization of Equipments

Glassware such as tubes, pipettes, soxhlet apparatus, flasks, Petri dishes and mortars were sterilized in the hot air oven at 161°C for an hour. Others like Bijou and universal bottles were sterilized in the autoclave at 10 lb for 10 minutes at (121°C). Instrument such as forceps, scissors were sterilized in the hot air oven at 161°C for an hour or by flaming after dipping in 7% of alcohol while used (Merchant and Packer, 1971).

4.3.4.2. Sterilization of Culture Media

Culture media were sterilized in the autoclave at 10 lb at 121°C for 10 minutes (Monica, 2000).

4.3.5. Culturing

4.3.5.1 Primary Culture

Primary culture for all samples was done into selenite broth. Water sample was taken by disposable pipette and put into Eperndrov tube then centrifuged at 8000 rounds for 5 minutes and the sediment was cultured into selenite broth while 1 gram of faecal and manure samples were cultured directly
into selenite broth. Then all cultured samples were incubated at $\sim 73°C$ for $\sim 42$ hours. The selenite broth colour change from pale yellow to red, indicating the bacterial growth.

**Subculture**

After $\sim 42$ hours, subculture were made from selenite broth onto MacConkey agar and incubated at $73 – 34°C$ for $\sim 42$ hours. Then, non lactose fermenting colonies (suspected *salmonella*) picked up from each plate and plated onto Xylose Lysine Desoxycholate (XLD) and incubated at $\sim 34°C$ for $\sim 42$ hours. Lactose fermenting colonies were discarded. From (XLD) non-lactose fermenting colonies with black centers producing hydrogen sulphide (H$_2$S) picked up. Then sub cultured onto nutrient agar and incubated at $\sim 34°C$ for $\sim 42$ hours.

**Identification**

**Microscopic Examination**

Smears were made directly from the pure cultures onto nutrient agar by emulsifying of each colony on a drop of sterile normal saline placed on a clean dry glass slide. The smears were allowed to dry on air then fixed by passing three times over the flame. Then the slide was flooded with $\sim 2$ crystal violet for $\sim 1$ minute. The stain was poured off and the slide was washed with distilled water. Then, it was flooded with lugol’s iodine for $\sim 1$ minute and poured off to be decolourized with absolute ethanol for $\sim 51$ seconds and rinsed with water. After that, slide was stained with dilute cabrol fuchsin for $\sim 51$ seconds, washed with distilled water and blotted to dry. A drop of oil immersion was put over
the smear and examined under light microscope. The Gram-negative bacteria stained red/pink (Barrow and Feltham, 1991).

Biochemical Reactions

Pure non-lactose fermenting colonies suspected salmonella, which showed Gram negative rods were examined biochemically according to Monica (2000).

Motility Test

Tubes containing motility medium (xylose lysine deoxycholate) agar were stab- inoculated with pure colony, to depth of about 5 mm then incubated at 37°C for 48 hours. Growth of pink –red colonies with black center at inoculated area, indicated xylose fermentation with acid and H2S production what meaning positive reaction and organism was motile.

Urease Test

A loop full of organism from pure culture inoculated heavily in small tubes containing 5 ml of sterile Christenings modified urea agar slope and incubated at 37°C for 48 hours. No change in color indicating that organism is negative.

Indole Test

From pure culture, organism was inoculated in tube containing 5 ml of sterile peptone water and incubated at 37°C for 48 hours. Then 5 ml of Kovacs reagent added and skated gently. There was no ring fermentation in upper layer of the mixture so organism was negative to indole.
٤٫٢٫٦٫٣٫٢.

**Kilgler Iron Agar Meaning (KIA)**

Straight wire full of organism used to inoculate slope KIA medium, firstly stabbed the butt and then, streaking the slope in zigzag pattern, then the tube tops left loose. The slope became red-pink color with blackening along the stab line or throughout the medium. Red Pink color indicated glucose fermentation due to version of acid reaction under aerobes conditions and blacken indicated hydrogen sulphide (H₂S) production indicating to positive reaction.

٣٫٦٫٣٫٢.

**Serological Reactions**

All pure non lactose fermenting colonies that showed Gram-negative bacilli and identified as *salmonella* biochemically were examined serologically on basis of agglutination test which detect the presence or absence of surface antigen (O) and flagella (H) antigen of *salmonella* (Kauffman-white scheme). The test procedure was done as following:

A loop full of normal saline was placed on a glass slide. From pure nutrient agar culture, separated colony (antigen) was removed by a sterile loop and mixed into the saline drop on the slide, the same step was repeated with a negative control. After ensuring opaque suspension in both drops, two drops of poly (O) antisera was added and mixed by sterile loop on the suspension and left up to one minute. Agglutination occurred on suspected drop and the negative control was homogenous. All isolates gave agglutination to poly (O) antisera were examined again with poly (H) antisera using the same procedure.
Serotyping of the Isolates

All isolates proved to be *salmonella* according to biochemical and serological reactions were then serotyped to subgroup level.

Poly (O) Subgroups Typing

Poly (O) subgroups of antisera used for serotyping were namely OMA, OMB, O:6, O:7, O:8, O:41, O:02. The subgroups typing was done using agglutination test as described in Poly (O) subgroups. Figure 1 illustrated poly (O) subgroup typing.
Poly “O” antisera

OMA  OMA  OMB  OMB

O: ٦  O: ٧  O: ٨  O: ٩٠  O: ٩٠

Figure ١: Poly (O) Subgroups Typing
\textbf{٢،٣،٤،٥. Poly (H) Subgroups Typing}

Poly (H) sub grouping was done as follows:

The isolates were sub cultured into swarm media by inoculating one spot of \textit{salmonella} colony at the center of swarm culture agar and incubated at ٧٣°C for ٤٢ hours. A loop full of normal saline placed on a slide, with other loop growth from edge of motility zone on swarm culture was removed and mixed into the drop of normal saline until opaque suspension appeared, then specific subgroup of poly (H) antisera namely (HMA,HMB,HMC,HMD and HME) were used. Isolates gave agglutination to poly (H) specific subgroup were further serotyped. So the isolates gave agglutination to a specific antisera were subjected to further sub grouping using the following antisera (H:aH:b, H:c, H:d, H:i, H:z). Further more agglutinating isolates were sub grouped using using antisera namely, (salinatis \textasciitilde, \textasciitilde, \textasciitilde, \textasciitilde, annex, enz, Iw, z, H:w). Isolates gave positive reaction to HMC were subjected for further typing using specific subgroup (H:I, H:W, H: L) antisera figure (٧) illustrated poly (H) subgroups typing.
Poly “H” antisera

Figure 2: Poly (H) antisera Subgroups Typing
Antimicrobial Activity

Antibiotic susceptibility

From the identified *salmonella* isolates, 5 were selected for antimicrobial activity. Each isolate was cultured into tube containing 5 ml of nutrient broth, and incubated at 73°C for 42 hours until slight visible turbidity appeared. A sterile swab applicator stick was dipped into suspension of the bacterial growth then streaked over entire surface of nutrient agar plate, allowed to stand for 3-5 minutes then the antibiotic discs were placed onto the agar surface using sterile forceps. The agar was incubated at 73°C over night. The inhibition zones of the growth around the disk were measured in mm by a ruler. The isolate then reported as susceptible, moderate or resistant according to the size of inhibition zone (Monica, 1991).

Antimicrobial Activity of Some Natural Plants extracts

Plants

The plants used in this study were *Acacia nilotica* (cissus quandrangulorisi) locally named (Garad) and *Solenostomma largel (DEL) Hayne* locally named (Hargel).

Preparation of the Crude Plant Extracts

Preparation of the Crude Methanolic and Chloroformic Extract

Fifty grams of the air dried and coarsely powdered of fruit of *Acacia nilotica* and leaves of *Solenostomma largel (DEL) Hayne* were exhaustively extracted for 2 hours with 0.52 ml of Chloroform in soxhlet apparatus (figure 3). The aqueous extract of each plant was filtrated and evaporated under
reduced pressure. Then put in two weighted plates and left to dry by air. After
drying, the remedy of each plant material was completed to 0.5 g by adding
more powder of plant material and repacked in two soxhlets and exhaustively
extracted with 0.5 ml of methanol for 2 hours. Then filtrated, evaporated, and
put in two weighted plates and left to dry. The residue of the chloroform
extracts were redissolved in mixture of petroleum ether and methanol (1:2 v/v)
and methanolic extracts residue were redissolved or suspended in the methanol
and the final volume were adjusted to give the specific concentration and kept
in the refrigerator till used.

\subsection*{4.3.8.4. Preparation of The Test Organism.}

One ml aliquots of 24 hours broth cultures of the test organism were
aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24
hours. The bacterial growth was harvested and washed off with sterile normal
saline, to produce suspension containing about \(10^8–10^9\) Colony forming
units (CFU) per ml. The suspension was refrigeratorated at 4°C until used.
The average number viable organism per ml of the stock suspension was
determined by means of surface viable counting technique (Miles and Misra,
1938; Quinn et al., 2000).
٥٢٨.٣.٢. Testing of Extract for Antibacterial Activity.

The cup-plate agar diffusion method (Kavanagh, ٢٧٩١) was adopted with some minor modifications to assess the antibacterial activity of the prepared extracts. Two ml of the test organism stock suspension (٨٠١–٩٠١) colony-forming unit per ml were thoroughly mixed with ٠٠٢ ml of sterile molten nutrient agar, which was maintained at ٥٤°C. Twenty ml aliquots of inoculated agar were distributed into sterile Petri dishes. The agar was left to set and in each plate ٤ cups (١٠٠٠٠٠٠٠٠٠٠٠٠٠٠٠٠٠ mm in diameter) were cut using a sterile cork borer (No٤). The cups were filled with ؛١ ml sample of each of the extracts in different concentration (١:٠١، ١:٠٢، and ١:٠٤) using adjustable pipette, and allowed to diffuse at room temperature for two hours. The plates were incubated in the upright position at ٧٣°C for ٤٢ hours; two replicates were carried out for each extract against each of tested organism. After incubation, the diameter of the resultant growth, inhibition zone were measured. Average and the mean values were tabulated.
Figure 3: Soxhlets Used For Extractions
CHAPTER THREE

RESULTS

١٫٣ Isolation

One hundred and forty four isolates were recovered from ١٧١ samples from different farms. Sixty one were lactose fermenting bacteria, eighty three were non lactose fermenting bacteria. Twenty-six samples showed no growth of bacteria. Table (٢) showes the distribution of isolation of lactose fermenting and non-lactose fermenting bacteria from different samples.

٢٫٣ Microscopic examination and biochemical reactions

Out of the eighty three non lactose fermenting bacteria, thirty found to be belonged to genus salmonellae. All the ٢٧ isolates found to be Gram-negative coccibacilli with rounded ends. Biochemically they were amino acid breaking with H₂S production indicating to be motile, negative to indole and urease and fermenting glucose with H₂S production. The salmonella isolates distribution from different farms was as follow:

Farm ١: The total salmonella isolates were ٢١. Five (٢٧,٤٪) from faecal samples and ٢ (٢٧,٧٪) from water samples. No isolation from the manure occurred.

Farm ٢: The total salmonella isolates were ٧١. Ten (٧١،٤٪) from faecal samples and ٢ (٧١،٧٪) from water samples. No isolation from the manure occurred.
Farm ٣: The total *salmonella* isolates were ٣. One (٣٪٣) from faecal samples and ٣ (٣٪٣) from water samples. No isolation from the manure occurred.

Farm ٤: The total *salmonella* isolates were ٨. Seven (٨٪٥) from faecal samples and ١ (١٪٥) from water samples. No isolation from the manure occurred.

Generally from all the farms, ٣٢ (٪٧٦٧) out of ٠٣ *salmonella* isolates were isolated from faecal samples and ٧ (٪٣٣) isolates were from water samples.

Clinical signs shown by chickens in farm ١ and farm ٣ were drop in egg production and in farm ٢ and farm ٤ white to greenish diarrhea misshaped ovaries and dullness (Table ٣).
<table>
<thead>
<tr>
<th>Farm</th>
<th>No. of chickens</th>
<th>No. of samples</th>
<th>Type of sample</th>
<th>No of each sample</th>
<th>Lactose fermenting Bacteria growth</th>
<th>Non lactose fermenting Bacteria growth</th>
<th>No growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>100</td>
<td>Manure Facaeses Water</td>
<td>0 25 10</td>
<td>0 10 3 10</td>
<td>1 14 3 10</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>100</td>
<td>Manure Facaeses Water</td>
<td>0 25 10</td>
<td>0 9 6 19</td>
<td>1 16 3 20</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>100</td>
<td>Manure Facaeses Water</td>
<td>0 25 10</td>
<td>0 1 2 10</td>
<td>2 6 3 29</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>100</td>
<td>Manure Facaeses Water</td>
<td>0 25 10</td>
<td>0 3 3 10</td>
<td>1 6 7 29</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>170</td>
<td>550</td>
<td>Munure Fcaeses Water</td>
<td>0 160 40</td>
<td>8 36 17 22</td>
<td>7 62 16 38</td>
<td>4</td>
</tr>
</tbody>
</table>
Table (1) Distribution of *salmonella* isolates among samples from different farms

<table>
<thead>
<tr>
<th>Farms</th>
<th>+ve from faecal samples (%)</th>
<th>+ve from water samples (%)</th>
<th>+ve from manure samples (%)</th>
<th>Mortality rate /Week (%)</th>
<th>Clinical signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm ۱</td>
<td>(٧٧٨, ٧)</td>
<td>(٧٧٨, ٧)</td>
<td>(٧٧٨, ٧)</td>
<td>(٧٧٨, ٧)</td>
<td>Drop in egg production</td>
</tr>
<tr>
<td>Farm ۲</td>
<td>(٧٧٨, ٧)</td>
<td>(٧٧٨, ٧)</td>
<td>(٧٧٨, ٧)</td>
<td>(٧٧٨, ٧)</td>
<td>Drop in egg production</td>
</tr>
<tr>
<td>Farm ۳</td>
<td>(٧٧٨, ٧)</td>
<td>(٧٧٨, ٧)</td>
<td>(٧٧٨, ٧)</td>
<td>(٧٧٨, ٧)</td>
<td>Drop in egg production</td>
</tr>
<tr>
<td>Farm ۴</td>
<td>(٧٧٨, ٧)</td>
<td>(٧٧٨, ٧)</td>
<td>(٧٧٨, ٧)</td>
<td>(٧٧٨, ٧)</td>
<td>Drop in egg production</td>
</tr>
</tbody>
</table>

Clinical signs:
- White to greenish diarrhea - misshaped ova - dullness
Serological Reactions

All isolates identified as genus *salmonella* by biochemical reactions were confirmed serologically and gave agglutination with polyvalent (O) and (H) hyper-immune sera which are genus specific.

Serotyping of the Isolates

To classify the *salmonella* isolates to serotypes level, serotyping was applied adding more specific sub groups of "O" and "H" antisera (Figures 4, a,b). Sub groups of "O" antisera named (OMA, OMB). All isolates found to be positive to OMB antisera then, examined by subgroups of OMB antisera which included (O:6, O:7, O:8, O:41, O:02) these showed different results depending on serotypes of *salmonella* (Table 4) and (Figure 4, a). Then all isolates examined by sub group of "H" antisera and found to be positive to HMA except one which found to be positive to HMC (figure 4 b). The *salmonella* isolates found to be positive to HMA when examined by HMA sub groups of antisera and six isolates found to be positive to H:d and they were examined using salinatis and found to be positive to Enz51 confirming that serotypes are *salmonella gombe*. The positive to H:i antisera were examined using the salinatis, one isolate found to be positive to Enx proving that it is *salmonella bonariensis*. Four isolates were not fully serotyped because of lack of salinatis but, according to initial serological reactions, the four isolates proved to be belong to three different serotypes and they named NS, N5, N6 and S9.
The positive isolates to HMC were examined using its subgroups and found to be positive to H: L and to specific antisera named salinatis Enz. confirming that it is *salmonella potsdam*. 
<table>
<thead>
<tr>
<th>Isolates No</th>
<th>&quot;O&quot; typing</th>
<th>&quot;H&quot; typing</th>
<th>Proteolysis</th>
<th>Triphasic variant</th>
<th>Salmonella</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title</td>
<td>OMB</td>
<td>HMA</td>
<td>Antiseras</td>
<td>&quot;Salinatis&quot;</td>
<td>Sertypes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Antiseras</td>
<td>&quot;Vi&quot;</td>
<td></td>
</tr>
<tr>
<td>N₄</td>
<td>O₄</td>
<td>H : d</td>
<td></td>
<td>Enz₁₀</td>
<td>S.gombe</td>
</tr>
<tr>
<td>N₆</td>
<td>O⁻</td>
<td>H : I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N₈</td>
<td>O⁻, O₄</td>
<td>H : I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N₉</td>
<td>O₄, O⁻</td>
<td>H : d</td>
<td>Enz₁₀</td>
<td>S.gombe</td>
<td></td>
</tr>
<tr>
<td>N₁₀</td>
<td>O₄, O⁻</td>
<td>H : d</td>
<td>Enz₁₀</td>
<td>S.gombe</td>
<td></td>
</tr>
<tr>
<td>N₁₁</td>
<td>O₄, O⁻</td>
<td>H : d</td>
<td>Enz₁₀</td>
<td>S.gombe</td>
<td></td>
</tr>
<tr>
<td>S₁₂</td>
<td>O₄, O⁻</td>
<td>H : d</td>
<td>Enz₁₀</td>
<td>S.gombe</td>
<td></td>
</tr>
<tr>
<td>S₁₃</td>
<td>-</td>
<td>H : d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N₁₄</td>
<td>O₄, O⁻</td>
<td>H : I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N₁₅</td>
<td>O⁻</td>
<td>H : I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N₁₆</td>
<td>O₄, O⁻</td>
<td>H : L</td>
<td>Enz₁₀</td>
<td>S.potsdam</td>
<td></td>
</tr>
</tbody>
</table>
Figure 4a: Poly (O) antisera Subgroups Serotyping

Figure 4b: Poly (H) antisera Subgroups Serotyping
Antimicrobial activity

Antibiotic susceptibility

Different serotypes gave different inhibition zones to the same antimicrobial agents e.g. the inhibition zone diameters of *S. gombe* to tetracycline is \( 41 \) mm while inhibition zone diameters of *S. potsdam* to tetracycline is \( 9 \) mm, some serotypes were resistant to one while others were sensitive to it. Inhibition zones diameters were measured after \( 42 \) hours of incubation (Table 4).

Isolate NS inhibition zone diameters to *tetracycline* was \( 8 \) mm, to *gentamycin* was \( 0 \) mm, to neomycin was \( 1 \) mm and to *colistin* was \( 11 \) mm.

*S.gombe* inhibition zone diameters to *tetracycline* was \( 41 \) mm, to *gentamycin* was \( 0 \) to *neomycin* was \( 4 \) and to *colistin* was \( 5 \) mm.

*S.potsdam* inhibition zone diameters to *tetracycline* was \( 9 \) mm, to *gentamycin* was \( 0 \) mm, and to neomycin \( 9 \) mm and colistin was \( 1 \) mm.

*S.bonariensis* inhibition zone diameters to tetracycline was \( 9 \) mm, to gentamycin was \( 1 \) mm, to neomycin was \( 4 \) mm and to colistin was \( 11 \) mm.

Isolate S\( ^{91} \) inhibition zone diameters to tetracycline was \( 0 \) mm, to gentamycin was \( 2 \) mm, to neomycin was \( 6 \) mm and to colistin was \( 11 \) mm.

Most of *salmonella* serotypes found to be more sensitive to gentamycin and resistance to tetracycline Table (\( \gamma \)) and Figure (\( \gamma \), \( \gamma \))
Table (5) Five salmonella serotypes diameter of inhibition zones to four chosen antimicrobial agent

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Antimicrobial inhibition Diameter of inhibition zones to different serotypes(mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conc. ma/al</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>TE 30/0</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>CN 10/0</td>
</tr>
<tr>
<td>Neomycin</td>
<td>N 10/0</td>
</tr>
<tr>
<td>Colistin</td>
<td>C 10/0</td>
</tr>
</tbody>
</table>
Figure ٥: Effect of different antibiotics upon varied serotypes of *Salmonella*
Figure ٦: Different serotypes reactions to different antibiotics
Antimicrobial Activity of Some Natural Plants Extracts

Acacia nilotica and Solenostomma Largel (Del) Hayne extracts were tested against five serotypes namely S.gombe, S.potsdam, S.bonariensis and NS and N91.

Acacia nilotica showed significantly higher antibacterial activity in different concentration (proportions) against Salmonella serotypes vice versa Solenostemma Largel (Del) Hayne showed lower antibacterial activity against the same serotypes (Table 7).

The antibacterial activity of Acacia nilotica (Garad) extracts decreased significantly with decreasing of concentration e.g. in (1:1) concentration it has highly antibacterial activity which decreased gradually according to dilution in (1:2) dilution it has less activity compared with the first concentration and in (1:4) dilution has less antibacterial activity compared with the two concentrations while Solenostemma Largel (Del) Hayne antibacterial effect remain constant in different concentrations except rarely increased in inhibition zone of chloroformic extract in some serotypes according to increase of concentration. The inhibition zones of different extracts in different concentrations on serotypes was shown in Table(7) and Figure (7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18).
Table (*1) Antimicrobial activity of Acacia nilotica and Solenostemma Largel (Del) Hayne chloroformic and methanolic extracts in different concentrates against different serotypes of salmonella

<table>
<thead>
<tr>
<th>Plant</th>
<th>Part</th>
<th>Solvent</th>
<th>Yield</th>
<th>Conc.</th>
<th>S₁</th>
<th>S₂</th>
<th>S₃</th>
<th>S₄</th>
<th>S₅</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acacia nilotica</em></td>
<td>Fruit</td>
<td>Chloroform</td>
<td>٦٠١٫٢</td>
<td>١ : ١</td>
<td>١٧</td>
<td>١٧</td>
<td>١٧</td>
<td>١٨</td>
<td>١٩</td>
</tr>
<tr>
<td>(Garad)</td>
<td></td>
<td></td>
<td></td>
<td>١ : ٢</td>
<td>١٧</td>
<td>١٧</td>
<td>١٧</td>
<td>١٨</td>
<td>١٩</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>١ : ٤</td>
<td>١٥</td>
<td>١٥</td>
<td>١٥</td>
<td>١٨</td>
<td>١٨</td>
</tr>
<tr>
<td></td>
<td>Fruit</td>
<td>Methanol</td>
<td>٣٧٫٣٢</td>
<td>١ : ١</td>
<td>٢١</td>
<td>٢١</td>
<td>٤٠</td>
<td>٣٢</td>
<td>٢٧</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>١ : ٢</td>
<td>٢٢</td>
<td>٢٢</td>
<td>٣٢</td>
<td>٣٢</td>
<td>٢٧</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>١ : ٤</td>
<td>٣٠</td>
<td>٣٠</td>
<td>٣٠</td>
<td>٣٠</td>
<td>٣٠</td>
</tr>
<tr>
<td><em>Solenostemma Lagel</em></td>
<td>Leaf</td>
<td>Chloroform</td>
<td>٤٤٣٫٩</td>
<td>١ : ١</td>
<td>٣٠</td>
<td>٣٠</td>
<td>٣٠</td>
<td>٣٠</td>
<td>٣٠</td>
</tr>
<tr>
<td>(Del) Hayne</td>
<td></td>
<td></td>
<td></td>
<td>١ : ٥</td>
<td>٣٠</td>
<td>٣٠</td>
<td>٣٠</td>
<td>٣٠</td>
<td>٣٠</td>
</tr>
<tr>
<td>(Hargel)</td>
<td></td>
<td></td>
<td></td>
<td>١ : ٥٫٢</td>
<td>٢١</td>
<td>٢١</td>
<td>٢١</td>
<td>٢١</td>
<td>٢١</td>
</tr>
<tr>
<td></td>
<td>Leaf</td>
<td>Methanol</td>
<td>٤٩٫٥١</td>
<td>١ : ١</td>
<td>٣١</td>
<td>٣١</td>
<td>٣١</td>
<td>٣١</td>
<td>٣١</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>١ : ٥</td>
<td>٣٢</td>
<td>٣٢</td>
<td>٣٢</td>
<td>٣٢</td>
<td>٣٢</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>١ : ٦</td>
<td>٣٢</td>
<td>٣٢</td>
<td>٣٢</td>
<td>٣٢</td>
<td>٣٢</td>
</tr>
</tbody>
</table>

S₁ = S.gombe, S₂ = S₄₁, S₃ = S.potsdam, S₄ = S.bonariensis, S₅ = Ns

Concentration used ١٠٠ mg/ml
High activity inhibition zone diameter >١٠
Low activity inhibition zone diameter <١٠
Chloroformic extraction

Methanol extraction

Figure 7: antimicrobial activity of Acaia nilotia extract in concentration (1:1) upon S.gombe
Methanol extraction

Chloroformic extraction

Figure 8: antimicrobial activity of Acaia nilotia extract in concentration (\( \cdot \) upon S.gombe
Methanol extraction

Chloroformic extraction

Figure 4: antimicrobial activity of Acaia nilotia extract in concentration (1:4) upon S.gombe
Chloroformic extraction

Methanol extraction

Figure ٠١: antimicrobial activity of Solenostemma argel (Del) Hayne extract in concentration (١:١) upon S.gombe
Figure ١١: Effect of different concentration of Acaia nilotica chloroformic extract upon varied serotypes of Salmonella

$S_1 = S. gombe$, $S_2 = S., S_3 = S. potsdam$, $S_4 = S. bonariensis$, $S_5 = Ns$
Figure 21: Different serotypes reactions to different concentration of Acaia nilotica chloroformic extract

S1 = S.gombe, S2 = S.1.3, S3 = S.potsdam, S4 = S.bonariensis, S5 = Ns
Figure ٣١: Effect of different concentration of Acaia nilotica methanolic extract upon varied serotypes of Salmonella

S₁ = S.gombe , S₂ = S.₉₁, S₃ = S.potsdam , S₄ = S.bonariensis , S₅ = Ns
Figure 4: Different serotypes reactions to different concentration of Acaia nilotica methanolic extract

$S_1 = S.gombe$, $S_2 = S.\alpha$, $S_3 = S.potsdam$, $S_4 = S.bonariensis$, $S_5 = Ns$
Figure ٥١ : Different serotypes reactions to different concentration of Solenstoma laryel (Del) Hayne chloroformic extract

$S_1 = S.gombe$, $S_2 = S_{R}$, $S_3 = S.potsdam$, $S_4 = S.bonariensis$, $S_5 = Ns$
Figure ٦١: Effect of different concentration of Solenstoma laryel (Del) Hayne chloroformic extract upon varied serotypes of Salmonella
S₁ = S.gombe , S₂ = S., S₃ = S.potsdam , S₄ = S.bonariensis , S₅ = Ns
Figure ٧١: Effect of different concentration of Solenstoma laryel (Del) Hayne methanolic extract upon varied serotypes of Salmonella

$S_1 = S.gombe$, $S_2 = S_{11}$, $S_3 = S.potsdam$, $S_4 = S.bonariensis$, $S_5 = Ns$
Figure ٨١: Differencesalmonella serotypes reactions to different concentration of Solenstoma laryel (Del) Hayne methanolic extract

$S_1 = S.gombe$, $S_2 = S._{1..}, S_3 = S.potsdam$, $S_4 = S._{bonariensis}$, $S_5 = Ns$


\subsection*{Questionnaire}

From data obtained from the questionnaire: Outbreak of \textit{salmonella} in the laying farm using preventive dose is less than other not used. Mortality rate in open system laying farms with good hygiene is less than the poor one. Rate of drop in egg production in the chicken varied according to prompt treatment. The crowdness increase the rate of outbreak in farms in spite of good hygiene.
Table 7: Epidemiological data collected from different farms

<table>
<thead>
<tr>
<th>Farm</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Of chickens</td>
<td>1000</td>
<td>1000</td>
<td>1500</td>
<td>1000</td>
</tr>
<tr>
<td>Preventive dose</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Age of chickens</td>
<td>8.5</td>
<td>8.5</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Outbreak of salmonella/month</td>
<td>0.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1</td>
</tr>
<tr>
<td>Mortality rate/week</td>
<td>6</td>
<td>10</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>No. Of laborers</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Egg collection</td>
<td>7</td>
<td>5</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Veterian cleaning</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Distance of farm from other farms</td>
<td>1000</td>
<td>60</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>Treatment</td>
<td>4</td>
<td>3</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>Egg/day</td>
<td>700</td>
<td>230</td>
<td>1750</td>
<td>650</td>
</tr>
</tbody>
</table>
CHAPTER FOUR

DISCUSSION

Salmonellosis is one of important bacterial diseases that affect animals and human. The disease constitutes a major threaten to both human and animal health and to welfare. Food borne infections due to *salmonella* are considered as one of the main health problem in many countries. Over ٠٠٠٠٠ serotypes have been described within the *salmonella* group, of these more than ٠٠٠٠٠ have been isolated from infected persons and animals (Coma, ٠٠٠٠٠). Chicken salmonellosis is considered as one of the serious problems that threaten poultry industry in Sudan. The disease affects adversely poultry production and led to sever decreased in egg production (Imad, ٠٠٠٠٠). Hence great economical losses occur, moreover the costs associated with salmonellosis could be considerable. The present study was aimed to investigate the disease in open system laying farms with special emphasis on hygiene and the serotypes prevalent in these farms.

In the present study, *salmonella* seotypes namely *S. bonariensi S.gombe* and *S. Potsdam* and two unidentified salmonella serotypes were isolated. These isolates were obtained from chicken’s faecal and water samples. These serotypes were for the first time to be isolated in Sudan which signifies the importance of our finding. Previously in Sudan, isolation of *salmonella* was reported by Khan (٣٦٩١) who Isolated *S.Chicago, S.London, S.Omdurman S.*
Gaminara, S. newport and S. Uganda from fowls. S. typhimurium from pigeons and S. east bourne S. reading and S. typhimurium for wild bird. Out of ٥٠٥٩ sample collected from poultry were examined of salmonella serotypes, S. amersfoort, S. eneritidis S. heidel and other salmonella serotypes were isolated (Imad, ٣٠٠٢).

The isolation of these serotypes for the first time is very interesting because it may open new avenues of the epidemiology of chicken salmonellosis in the country. These serotypes specifically S. gombe have epidmlogical importance in chicken flock. On the other hand, these serotypes are zoonotic. This may give attention that chickens and their products may contribute seriously to the human health hazard created from animal origin. It is very interesting that accidental infection occurred to the author when she was handling these isolates. A sever abdominal disturbances including colic, diarrhea and vomiting were shown due to the infection. The diagnosis of the infection was confirmed by the isolation of Ns serotype from the faecal sample of the author. Although it was not desirable that the author to be infected, but good insight of the zoonotic importance of these isolates was appeared.

Varied culture media were tested for isolation of salmonella serotypes from faecal, manure and water samples of poultry to fulfill instant detection. Primary subculture was done into selenite broth which is toxic for great deal of many bacteria and hence it considered as selective media for isolation of salmonella. Accordingly, use of this medium for primary isolation is useful and recommended. Not only that but also primary identification of our isolates
based on morphologic, cultural characteristic and biochemical tests. This system of detection produced a good result and with high isolation sensitivity (Clanek et al., 1991). Confirmation of the identity of salmonella isolates was based on serological reaction ("O" and "H" antisera) which detect presence of genus specific antigen namely somatic {O} and flagellar {H} antigens. Identification to level of serotypes was rely on serotyping method using specific subgroups of {O} and {H} antisera. That showed highly sensitivity for identification (Popoff, 2002). Serotyping is a proper method for identification of salmonella to serotypes level specify in epidemiological cases because the sequence of typing is so sensitive and not confused. (Monica, 2002). Serotyping is a critical component of a public health response to the global challenge of salmonellosis. Support for serotyping as part of national Salmonella surveillance, and for rapid international communication of the results will help target future prevention strategies (WHO, 1995).

This study is the first one that applied serotyping procedures of salmonella in Sudan. In previous researchers serotyping was done by sending the isolates to references laboratories abroad for serotyping of salmonella (Yagoup, 1987; Imad, 2009). During this survey, the disease was found to be prevalent in poultry farms examined and threaten the welfare of the farms production and challenge to veterinarian. The disease reported different rates of prevalence in varied farms according to level of hygiene which observed to be rely on the management of farms. In the farm with good management, eleven out of 50 samples showed
no growth of the bacteria from the samples and vice versa, the farms with poor hygiene all samples showed growth except two. This showed significant relationship between the hygiene and the bacterial load in the environment (Clanek et al., 1991; Estrada et al., 2002). The salmonella was isolated from water provided for birds drinking from all farms (suspected or randomly). This may indicated that water represent important factor for reinfection with salmonella and other bacteria. The water containers were open ones, so its contamination will be easier. Hence waterier must changed to a close one so as to reduce the possibility of contamination of drinking water because drinking water is one of the potential source of infections in chickens farms (Ingrid et al., 2002) proved water as source of salmonella infection in swine farms.

The samples during this study were taken during variant seasons. salmonella was isolated during all seasons indicating that salmonellosis was chronic in the examined farms and not affected by the seasons although generally bacterial infections are known to be increased during summer as the ambient temperature is high which promote the bacterial growth. salmonellosis outbreaks are more common at bird feeders during the late winter and early spring, and also during hot periods in the summer when birds are stressed and must congregate for feed and water (Mörner, 2001; M. Friend, 1999).

Prevalence rate in flocks varied from moderate in farm(1) to high in farm(2). These variations in prevalence rates can be attributed to earlier treatment with antibiotics and some time the use of prophylactic dose, which is practice in some chicken farms.
In this study, in vitro antibiotic sensitivity using standard disk diffusion test was applied to determine the susceptibility of the isolated serotypes to some antibiotics that routinely used by farmers to treat salmonellosis. These findings showed that *Salmonella* serotypes were varied in their sensitivities to the antibiotics used (tetracycline, gentamycin, colistin and neomycin). The antibiotics were selected because of their availability as anti-*salmonella* agents in the field. Some serotypes were highly sensitive to a certain drug while others were lower sensitive. *S.gombe* was found considerably sensitive to gentamycin then colistin, neomycin and tetracycline frequently, *S.bonariensis* was found sensitive to gentamycin then neomycin, colistin and tetracycline frequently. So gentamycin was the most effective drug against *S.gombe* and *S.bonariensis* and it is recommended for successful treatment of affected birds with *S.gombe* and *S.bonariensis*. Neomycin was the most effective drug against serotypes NS N, so it is recommended for successful treatment of affected birds with them.

On the other hand, *S.potsdam* was less sensitive to the mentioned antibiotics. Generally, it is fortunately that all isolated serotypes were sensitive to most antibiotics, which help greatly in the treatment when infections due to these serotypes occur.

In this study, we used natural plants extracts to test their antimicrobial activity against isolated serotypes. These natural plants are *Acacia nilotica* (Garad) and *Solenostemma Largel (Del) Hayne* (Hargel). These two plants are widely cultivated in Sudan hence they are available to the procedures so their
use as antimicrobial agents could be useful from cost wise. It was obvious that *Acacia nilotica* extracts showed highly antimicrobial activity against *Salmonella* serotypes. Methanolic extract of *Acacia nilotica* reported significantly higher antimicrobial activity against *salmonella* serotypes when compared with chloroformic extract and antibiotics, the inhibition zone of methanolic extract in minimum concentration was \(32\) mm, while the inhibition zone of the high effective antibiotic (gentamycin) was \(0.2\) mm and chloroformic inhibition zone was \(51\) mm. So *Acacia nilotica* may be suggested as a potent anti *salmonella* agent. This effectiveness of Methanolic extract of *Acacia nilotica* may suggested that these extracts could be formulated in chicken ration so it could give prophylactic dose which may prevent the chickens from salmonellosis or other bacterial infections. *Solenosemma Largel (Del) Hayne* extracts showed low antibacterial activity against *Salmonella* serotypes when compared with *Acacia nilotica*, so it is not recommended to be used against *salmonella* infection.
CONCLUSION

From our findings, it could be concluded that :-

١- The serotypes *S. gombe*, *S. bonariensis* and *S. Potsdam* serotypes N_s and N_7 have been isolated from farms examined.

٢- Gentamysin found to be effective against *salmonella* in vitro.

٣- *Acacia nilotica* (Garad) extracts have high in vitro antimicrobial activity against serotypes isolated *salmonella* serotypes; vice versa, *Solenstemma largel* (Del) hayne (Hargel) extracts have low antimicrobial activity.

٤- Serotyping is the best method for identification of *salmonella* and gave a confirmatory results

٥- Good hygiene and management represent the main factor for reducing infection of *salmonella* in chickens kept in open system farms.

٦- Drinking water may be considered as an important factor of reinfection
RECOMMENDATIONS

In the light of these results, the following recommendations could be drawn:

١- Molecular characterization of the new serotypes would be useful for the epidemiology of the disease.

٢- Further study must be applied on Acaia nilotica in vivo antibacterial activity against salmonella in the future.

٣- Good management and hygiene should be highly considered in open system farms to reduce salmonellosis.

٤- Early treatment of infected flock with specific antibiotics e.g. gentamycin, reduce mortality rate significantly.
A.del Cerro; S.M.soto ; E.Landers ; M.A. Gonzalez ;Hevia ; J.A.Guijarro and M.C. Mendoza (٢٠٠٢)., PCR – based Procedures in detection and DNA-Fingerprinting of Salmonella from samples of animal origin. Food Microbiology. £6: ٥٧٥-٥٧٥.

Ahmed., A. and Ansari N.O.(٢٠٠٢) Study of Multi-drug resistant Salmonellosis at LNH-A ٤٢ years Experience. $٩٠$th international symposium on typhoid Fever and other Salmonellosis, workshop on Molecular Method in the epidom. And diag . of typhoid . Karachi. Pakistan, the Aga Khan Univ.PP.

Annual report, Sudan veterinary service, (١٩٨٩) Cited by Abdul Quddus khan ١٩٧٩ Animal Salmonellosis in the Sudan University of Khartoum.


Baudart J; Lemarchand K; Brisabois A and Lebaran P.(٢٠٠٣). Diversity of salmonella strains as determned by serotyping and amplification of ribosomal DNA spacer region .


Hayat Ahmed Salih (1991), The economic importance of poultry industry in Sudan University of Khartoum Faculty of Agriculture Department of animal production.


Herrera-Lean S; McQuiston J R; Usera MA; Fields PI; Garaizar J and Echeita MA. (2002). Multiplex PCR for distinguishing the most common phase-1 flagellar antigens of Salmonella spp National Library of Medicine I J Clin Microbiol Jun:185-785.


Ingrid Feder; Jerome C. Nietfeld; John Galland; Teresa Yeary; Jan M; Sargeant; Richard Oberst; Mark L; Tamplin, and John B. Luchansky. (2002).

Ivanoff B. (٢٠٠٣). Global status of typhoid fever and other Salmonellosis Workshop on Molecular Methods in epidemiology and diagnosis of typhoid Karachi, Pakistan, the Agakan Univ., in collaboration with ICGEB, NIBGE, and welcome trust. PP١٠٠.


Journal of Bacteriology AEM. (٦٨٩٢). American Society for Microbiology. Production of Monoclonal Antibodies specific for I and ٢٠ Flagellar Antigens of *Salmonella typhimurium* and characterization of their Respective Epitopes.


Khan (٦٨٩١) Animal Salmonellosis In Sudan A. PHD, University of Khartoum, Sudan.


Palaniswamy-S and Raggini-JK. (1991). Influeny of certain plant extract on mite polyphagotarsonemus latus (Bank) on chillies. Department of Agriculture Entomology, Tamil Nadu Agricultural University, Coimbatapuram 641 004, India.


