HAEMATOLOGICAL, SERUM BIOCHEMICAL AND PATHOLOGICAL CHANGES IN JAPANESE QUAIL (*COTURNIX COTURNIX JAPONICA*) EXPERIMENTALLY INFECTED WITH *SALMONELLA ENTERICA* SEROVAR GALLINARUM

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES AHMADU BELLO UNIVERSITY, ZARIA NIGERIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF MASTER OF SCIENCE IN VETERINARY PATHOLOGY

DEPARTMENT OF VETERINARY PATHOLOGY

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DECLARATION

I declare that the work presented in this thesis entitled **"Haematological, serum biochemical and pathological changes in Japanese quail (*Coturnix coturnix japonica*) experimentally infected with *Salmonella enterica* serovar Gallinarum"** was carried out in the Department of Veterinary Pathology, Ahmadu Bello University, Zaria and in Central Diagnostic Laboratory Division of the National Veterinary Research Institute, Vom. The information derived from the literature has been duly acknowledged. No part of this thesis has been previously submitted for another Degree or Diploma elsewhere.

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CERTIFICATION

This thesis entitled **"HAEMATOLOGICAL, SERUM BIOCHEMICAL AND PATHOLOGICAL CHANGES IN JAPANESE QUAIL (*COTURNIX COTURNIX JAPONICA*) EXPERIMENTALLY INFECTED WITH *SALMONELLA ENTERICA* SEROVAR GALLINARUM"** by Israel Joshua BARDE meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This thesis is passionately dedicated to

Almighty God,

for granting me the enablement to go through this programme successfully.

With special thanks to

my dear wife (Dorcas)

and

my two children (Isaiah and Israel Jnr.),

for their love and prayers.

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My gratitude goes to God Almighty and to my Supervisory Committee Members: Professors J. O. O. Bale, S.B. Oladele and M. Y. Fatihu. Also, the Executive Director, National Veterinary Research Institute (NVRI), Vom, Dr. M.S.Ahmed, for sponsoring this M.Sc. work, the Director Diagnostic and Extension, Dr. P. A. Okewole and my Head of Division, Dr. P. R. Kumbish, for their guidance, encouragement and support.

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Thank you all.

Israel Joshua BARDE

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ABSTRACT

A total of 160 (108 males and 52 females) Japanese quails (*Coturnix coturnix japonica*) were used for the experiment. The quails were obtained at the age of four weeks from the Poultry Unit of the National Veterinary Research Institute, Vom, Plateau State, Nigeria. They were randomly selected and assigned into four groups (A, B, C and D) of forty quails each; with 27 males and 13 females in group A , 23 males and 17 females in group B, 29 males and 11 females in group C and D respectively. Groups A, B and C were infected with *Salmonella enterica* serovar Gallinarum per os at the dose of 10^6,10^4 and 10^2, respectively, while group D served as the control. Blood and sera were used for the determination of haematological and biochemical parameters, respectively. The values were relatively unchanged ($P > 0.05$) between the groups before infection in the haematological and serum biochemical parameters. There were, however, significant changes ($P<0.05$) in haematological parameters like; red blood cells (RBC), haemoglobin (Hb) concencentrations, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC)**,** white blood cell (WBC), heterophil and lymphocyte counts between the infected groups when compared with the control group. There were also significant changes ($P<0.05$) in these haematological parameters post-infection compared to before infection within each infected group. Furthermore, there were also significant changes ($P<0.05$) in serum biochemical parameters like; glucose, total protein, albumin, activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and

urea concentrations between infected groups and the control group. Significant changes ($P<0.05$) in these biochemical parameters were also observed post-infection compared to pre-infection within each infected group. Clinical signs observed in the infected groups included; weakness, ruffled feathers, somnolence, greenish-yellow diarrhea, weight loss, increase body temperature, drop in egg production, decrease in feed and water consumption. Grossly, congestion of the liver, lung and ovarian follicle was generally noted; also hepato-splenomegaly, swollen and congested kidney, bronzed liver and ascites were observed. Mortality rate ranged from 30% to 35%. Histopathological lesions included; congestion and cellular infiltration of various organs, vacuolation of hepatocytes and haemorrhagic liver. The experiment lasted 120 days. The findings in this study indicated that Japanese quails *(Coturnix coturnix japonica*) are highly susceptible (high morbididty) to *Salmonella enterica* serovar Gallinarum. Further studies should be carried out in the areas of pathogenesis of fowl typhoid and virulence of the organism that caused fowl typhoid in quails.

TABLE OF CONTENTS

Content **Page**

Title Page i

Declaration ii

Certification iii

Dedication iv

Acknowledgments v

Abstract vi

Table of Contents viii

List of Tables xii

List of Figures xiii

List of Plates xv

List of Appendices xvi

List of Abbreviations, Definition and Symbols Used xvii

CHAPTER 1: INTRODUCTION 1

1.1 Background of the study 1

1.2 Statement of Research Problems 4

1.3 Justification for the Study 5

1.4 Aims of the Study 6

1.5 Specific Objectives of the Study 7

1.6 Research Hypothesis (Ho) 7

CHAPTER 2: LITERATURE REVIEW 8

2.1 Preamble 8

2.2 Historical Background of Fowl Typhoid 8

2.3 Pathogenicity of *Salmonella enterica* serovar Gallinarum 10

2.4 Incidence and Distribution of Fowl Typhoid 10

2.5 Age Commonly Affected by Fowl Typhoid 11

2.6 Transmission of Fowl Typhoid 11

2.7 Clinical Signs of Fowl Typhoid 12

2.8 Morbidity and Mortality of Associated with Fowl Typhoid 12

2.9 Gross Pathological Lesions of Fowl Typhoid 13

2.10 Histopathology of fowl typhoid 15

2.11 Pathogenesis of fowl typhoid 16

2.12 Diagnosis of Fowl Typhoid 17

2.13 Differential Diagnosis of Fowl Typhoid 18

2.14 Prevention and Control of Fowl Typhoid 19

2.15 Treatment of Fowl Typhoid 20

2.16 Quail 21

CHAPTER 3: MATERIALS AND METHODS 24

3.1 Area of Study 24

3.2 Housing 24

3.3 Experimental Birds 24

3.4 Feeds and Feeding 25
3.5 Determination of Body Parameters… ……25
3.6 Bacteriological Monitoring Before Infection 25
3.7 The *Salmonella* Isolate used for the Challenge…… 25
3.8 Culture and Determination of Bacterial Inoculum 26
3.9 Challenge of the Quails with *Salmonella enterica* serovar Gallinarum 26
3.10 Blood Sampling 27
3.11 Determination of Hematological Parameters.....27
3.12 Differential Leukocyte Count 28
3.13 Serum Biochemistry 28
3.14 Clinical and Pathological Examinations.....32
3.15 Bacteriological Isolation……33
3.16 Data Analysis.....33

CHAPTER 4: RESULTS 34
4.1 Bacteriological Monitoring Before Infection… …… 34
4.2 Effect on Haematological Values on Japanese Quails.....36
4.3 Effect on Serum Biochemistry on Japanese Quails.....47
4.4 Clinical Manifestation of Fowl Typhoid in Japanese Quails.....55
4.5 Mortality Rate of Fowl Typhoid on Japanese Quails 58
4.6 Effect on Temperature on Japanese Quails.....60
4.7 Effect on Body Weight on Japanese Quails……. …….63
4.8 Effect on Daily Egg Production on Japanese Quails…… ..... ….65

4.9 Infection-induced Postmortem Lesions on Japanese Quails 67

4.10 Infection-induced Histopathological Lesions on Japanese Quails……72

4.11 Isolation of *Salmonella enterica* serovar Gallinarum from Quail Organs..…. 76

CHAPTER 5: DISCUSSION.....77

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS 82

6.1 CONCLUSION.....82

6.2 RECOMMENDATIONS.....83

REFERENCES 84

APPENDICES 93

LIST OF TABLES

Table Page

1. Bacteria growth from the cloaca of Japanese Quails before they were infect with *Salmonella enterica* serovar Gallinarum 35
2. Mortality Rate of Japanese Quails after infection with *Salmonella enterica* serovar Gallinarum 59
3. Cummulative cloacal temperature (°C) of Japanese Quails before and after infection with *Salmonella enterica* serovar Gallinarum 61
4. Mean Daily Egg Production of Japanese Quails before and after infection with *Salmonella enterica* serovar Gallinarum 66
5. Gross Lesions of Japanese Quails in different groups, experimentally infected with *Salmonella enterica* serovar Gallinarum 68

LIST OF FIGURES

Figure Page

1. Mean haemoglobin concentration of the quails before and after infection with *Salmonella enterica* serovar Gallinarum 39

2. Mean red blood cell count of the quails before and after infection with *Salmonella enterica* serovar Gallinarum 40

3. Mean packed cell volume (PCV) of the quails before and after infection with *Salmonella enterica* serovar Gallinarum 41

4. Mean corpuscular volume of the quails before and after infection with *Salmonella enterica* serovar Gallinarum 42

5. Mean corpuscular haemoglobin of the quails before and after infection with *Salmonella enterica* serovar Gallinarum 43

6. Mean corpuscular haemoglobin concentration of the quails before and after infection with *Salmonella enterica* serovar Gallinarum 44

7. Mean heterophil count of the quails before and after infection with *Salmonella enterica* serovar Gallinarum 45

8. Mean lymphocyte count of the quails before and after infection with *Salmonella enterica* serovar Gallinarum 46

9. Mean serum glucose of the quails before and after infection with *Salmonella enterica* serovar Gallinarum 48

10. Mean serum total protein of the quails before and after infection with *Salmonella enterica* serovar Gallinarum 49

11. Mean serum albumin of the quails before after infection with *Salmonella enterica* serovar Gallinarum 50

12. Mean serum aspartate transferase (AST) of the quails before and after infection with *Salmonella enterica* serovar Gallinarum 51

LIST OF FIGURES CONTINUE

Figure Page

13. Mean serum alanine transferase (ALT) of the quails before and after infection with *Salmonella enterica* serovar Gallinarum 52

14. Mean serum alkaline phosphatase (ALP) of the quails before and after infection with *Salmonella enterica* serovar Gallinarum 53

15. Mean serum urea of the quails before and after infection with *Salmonella enterica* serovar Gallinarum 54

16. Mean daily cloacal temperature (^{0}C) of Japanese quails (*Coturnix coturnix japonica)* before and after infection with *Salmonella enterica* serovar Gallinarum 62

17. Mean weekly weight of the quails before and after infection with *Salmonella enterica* serovar Gallinarum 64

LIST OF PLATES

Plate Page

I. Quail experimentally infected with 10^2 of *Salmonella enterica* serovar Gallinarum. Note depession, somnolence and ruffled feathers 56

II. Quail experimentally infected with 10^6 of *Salmonella enterica* serovar Gallinarum. Note greenish-yellow pasting of the vent 57

III. Quail experimentally infected with 10^6 of *Salmonella enterica* serovar Gallinarum. Note enlarged and bronze liver.....69

IV. Quail experimentally infected with 10^6 of *Salmonella enterica* serovar Gallinarum. Note congested lung, egg yolk peritonitis and congested ovarian follicle 70

V. Quail experimentally infected with 10^6 of *Salmonella enterica* serovar Gallinarum. Note enlarged and congested kidneys 71

VI. Photomicrograph of the heart of Japanese quail (*Coturnix coturnix japonica)* experimentally infected with 10^6 of *Salmonella enterica* serovar Gallinarum. Note generalized cellular infiltration.....73

VII. Photomicrograph of the liver of Japanese quail (*Coturnix coturnix japonica)* experimentally infected with 10^6 of *Salmonella enterica* serovar Gallinarum. Note severe congestion and haemorrhages.....74

VIII. Photomicrograph of the liver of Japanese quail (*Coturnix coturnix japonica)* experimentally infected with 10^6 of *Salmonella enterica* serovar Gallinarum. Note generalized vacuolation and cellular infiltration 75

LIST OF APPENDICES

Appendix Page

1. Haematological parameters pre-infection 93
2. Haematological parameters post-infection 94
3. Daily egg production of quails pre-infection 95
4. Daily egg production of quails post-infection 98
5. *Salmonella enterica* serovar Gallinarum colony on MacConkey agar plate 100
6. *Salmonella enterica* serovar Gallinarum colonies on Blood agar plates from which colonies were scooped into nutrient broth and incubated pre-infection 101
7. Serum biochemistry pre and post-infection 102
8. *Salmonella enterica* serovar Gallinarum isolated from quail's Organs.....103
9. *Salmonella enterica* serovar Gallinarum Gram-stain (Gram-negative rods).....104
10. The Japanese Quails used for the experiment.....105
11. Japanese Quail Eggs produced by the control group.....106

LIST OF ABBREVIATIONS, DEFINITIONS AND SYMBOLS USED

FT	Fowl typhoid
FAO	Food and Agricultural Organization
NVRI	National Veterinary Research Institute
Ad libitum	Allowed unrestricted access
RBCs	Red blood cells
LPS	Lipopolysaccharide
PCR	Polymerase chain reaction
et al	And others
H&E	Hematoxyline and eosin
°C	Degree centigrade (degree Celcius)
pH	potential Hydrogen ions concentration
cfu	colony forming units
kb	kilobase
µl	microlitre
µm	micrometer
mm	millimetre
ml	milliliter
g	gram
%	per cent
SPI	Salmonella pathogenicity island
MPS	Mononuclear phagocytic system
CDC	Centre for Disease Control

USA	United States of America
&	And
EDTA	Ethylene diamine tetra acetic acid
Ltd	Limited
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
Std	Standard
BCG	Bromo-cresol green
dl	deciliter
ANOVA	Analysis of variance
MCV	Mean corpuscular volume
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
PCV	Packed cell volume
Spp	Species
ME	Metabolisable energy
MJ	Mega joules
Fl	fentolitre
Pg	picogram
g/dl	gram per deciliter
mg/dl	milligram per deciliter
F	female
M	male
L	Litre

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

The genus *Salmonella* is a member of the Family *Enterobacteriaceae* and consists of Gram-negative, non-spore forming bacilli. The bacteria constituting the genus have three different types of antigens: the somatic "O", flagella "H" and capsular "Vi". The antigens are used to differentiate more than 2,500 serologically distinct types of *Salmonella* (Popoff *et al*., 2003).

Salmonella enterica serovar Gallinarum (*Salmonella* Gallinarum) is a non-motile host adaptive *Salmonella* that causes fowl typhoid (FT), a severe systemic disease responsible for heavy economic losses to the commercial poultry industry through morbidity, mortality and reduced egg production (Berchieri *et al.,* 2001; Song *et al*., 2002; Parmer and Davies, 2007). *Salmonella enterica* serovar Gallinarum infection primarily causes disease in chickens and turkeys of all ages, whilst the disease has also been described in ducks, ring doves, pheasants, peacocks, peafowl and canaries (Berchieri *et al.,* 2001; Shivaprasad and Barrow, 2008).

The causative agent of FT has been consistently reported to cause acute and chronic infections with high morbidity and mortality rates in various young and adult avian species (Tunca *et al.,* 2012). Fowl typhoid has wide distribution around the world and transmission is either by horizontal or vertical route (Berchieri *et al.,* 2001). Infected breeding flocks are

mainly associated with vertical transmission of salmonellae to their progeny (Agbaje *et al*., 2010). In Europe, transmission by the poultry red mites is a very important part of the epidemiology. The fact that they feed by sucking blood means they can also theoretically transmit *Salmonella* through contaminated blood (Parmar and Davies, 2007). The impact of FT is hard to judge in developing countries due to lack of systematic surveillance, but its importance is clear, from both published and anecdotal evidence, with outbreaks in Mexico, Argentina, Nigeria and India ((Barrow *et al*., 2012). Endemic FT is still found in many countries in both commercial production and backyard flocks including countries with expanding poultry industries such as Brazil and South Korea where there has been considerable research activity in recent years (Barrow *et al*., 2012).

With the continuous expansion of poultry farming in Nigeria, FT has gained ground as a major disease of poultry which can cause heavy economic losses in poultry through mortality and reduced production (Khan *et al*., 1998; Agbaje *et al*., 2010). A research report put the prevalence of FT in sampled flocks in Kaduna state, northern Nigeria at 18.4% (Mbuko *et al.*, 2009).

Quail production has been on a large scale in many countries of the world. In Nigeria, for example, some research institutes have gone into commercial production and investigation into the nutrition and disease control in quails (Lombin, 2007). Quails are also ideally suited for avian research, because of their small size and require little cage space for rearing. They are easy to raise and are suitable for genetic studies since they rapidly attain sexual maturity (Haruna *et al*., 1997). Quails are hardy birds which thrive very well in

cages and are relatively inexpensive to maintain. They are birds that every household can keep without stress. They mature in about six weeks and are usually in full egg production by 50 days of age. If properly mated, quails have high fertility and good egg hatchability. The adult male bird weighs about 100 to 140grams, while the females are slightly heavier, weighing from 120 to 160grams (Huss *et al.,* 2008).

The female quails are characterized by light tan feathers with black speckling on the throat and upper breast while the males have rusty brown throat and breast feathers. Males also have a cloacal gland, a bulbous structure on the upper edge of the vent that secretes a white, foamy material which the females don't have (Haruna *et al*., 1997). Quails are reared for meat and eggs in different countries of America, Asia and Africa. Although quail farming contribute to the alleviation of protein deficiency in the diets of people in developing countries, they have largely been neglected as a livestock species due to their small size. Most farmers have focused mainly on chicken production. Thus, their actual contributions to food production have been greatly ignored and or underestimated by extension and other development workers, and policy makers in the agricultural sector in developing countries. On the contrary Nigerians are interested in quail production especially for medicinal reasons (rightly or wrongly) and so quail farming should be encouraged with a special focus on the traditional subsistence rearing system as practiced by limited- resource farmers (NVRI, 2008).

1.2 Statement of Research Problems

Salmonella spp have repeatedly caused outbreaks of salmonellosis in humans after consumption of uncooked eggs or contaminated meat (Duguid and North, 1999). *Salmonella* infections in poultry are distributed worldwide and result in severe economic losses when no effort is made to control them (Abdu *et al.*, 1985; Kwon *et al*., 2000; Mdegela *et al*., 2000; Hafez, 2005; Okwori *et al*., 2007; Mbuko *et al*., 2008). These economic losses are usually caused by high cost of treatment, reduction in egg production, poor chick quality and high cost of eradication and control measures (Oladele and Raji, 1997; Oladele *et al.*, 1999; Hafez, 2005).

There is scarcity of information on microscopic lesions of FT in chickens and quails, and most of the lesions described are from field cases which might have been complicated by other bacterial and or viral agents in chickens talk less about the quails (Doyle and Mathews, 1928; Wong *et al*., 1996; Chadfield *et al.*, 2003).

Japanese quails have received less attention relative to other poultry species by researchers (Agrey *et al*., 2003). The quail has been raised in the National Veterinary Research Institute (NVRI), Vom for over ten years, and so far, only of recent that diseases like fowl cholera, worm infestation, fungal infection and *Salmonella* infection among others were noticed (NVRI, 2008). However, devastating diseases like Newcastle and Gumboro diseases were yet to be encountered in natural infections. This fact might suggest that quails are resistant to some of the diseases of poultry (Lima *et al.*, 2004; NVRI, 2008).

The quails are hardy birds compared to other types of poultry; they can still be affected by common poultry diseases, such as ulcerative enteritis, botulism, colibacillosis, aspergillosis, candidiasis, coccidiosis and salmonellosis, therefore good knowledge of diseases affecting quails is essential for proper preventive measures to be taken (Naveen and Arun, 1992; NVRI, 2008).

1.3 Justification for the Study

Salmonella causes gastroenteritis in both man and animals; it is one of the major foodborne pathogens of significant public health concern in both developed and developing countries (Hafez *et al*., 2005; Philippe *et al*., 2005). With a population of over 160 million people, Nigeria has over the years not been able to meet the Food and Agricultural Organization (FAO) recommended minimum protein intake requirement of 65 g per person per day (NVRI, 2008). This is partly because the expansion of cattle population, cannot progress at a satisfactory rate to cope with the increasing demands for meat, which has given rise to the increase in commercial chicken production. However, chicken production has suffered some set-backs due to high cost of feed, inadequate supplies of day old chicks; high cost of drugs and biologicals and of recent the avian influenza scourge among others (NVRI, 2008). This led to search for alternative cheaper sources of protein and subsequent introduction of quails in Nigeria (NVRI, 2008). The Japanese quail has the potential of filling some of the gap in the protein needs of Nigerians, it is therefore important to investigate diseases that can interfere with the production such as FT in order to control them. Quail meat and eggs are known for high quality protein, high biological value and

low caloric value, thus making them good choices for hypertension prone individuals (Haruna *et al*., 1997; Chindo and Olowoniyan, 2006; NVRI, 2008). Because its lifespan is relatively short and its physiology is comparable to that of humans, the adult quail is useful for studies of aging and diseases (Huss *et al*., 2008). An increasing number and variety of quails are being kept for food, experimental use, released on hunting preserves, preservation of endangered species, zoological displays and as companion birds. This is because they are easy to raise and are suitable for genetic studies since they rapidly attain sexual maturity (Khare *et al*., 1975). To the best of our knowledge, no available information on the haematological, serum biochemical and pathological changes of Japanese quail (*Coturnix coturnix japonica*) experimentally infected with *Salmonella enterica* serovar Gallinarum in Zaria, Nigeria.

It is hoped that the results of this study will give insights into the pathology of FT in Japanese quails and hence define the basis for diagnosis and control.

1.4 Aim of the Study

The aim of the study is to determine the haematological, serum biochemical and pathological changes in Japanese quail (*Coturnix coturnix japonica*) experimentally infected with *Salmonella enterica* serovar Gallinarum.

1.5 Specific Objectives of the Study

The specific objectives of the study were to determine:

1. The haematological changes in Japanese quail (*Coturnix coturnix japonica*) experimentally infected with *Salmonella enterica* serovar *Gallinarum*.
2. The serum biochemical changes in Japanese quail (*Coturnix coturnix japonica*) experimentally infected with *Salmonella enterica* serovar *Gallinarum*.
3. The clinical signs, gross and histopathological changes in Japanese quail (*Coturnix coturnix japonica*) experimentally infected with *Salmonella enterica* serovar Gallinarum.

1.6 Research Hypothesis (Ho)

Japanese quail (*Coturnix coturnix japonica*) show no haematological, serum biochemical and pathological changes when experimentally infected with *Salmonella enterica* serovar Gallinarum.`

CHAPTER TWO

LITERATURE REVIEW

2.1 Preamble

The disease FT is a septicaemic disease affecting primarily chickens and turkeys, but other birds such as pheasants, ducks, peacocks and guinea fowl are also susceptible (Barrow *et al*., 1994). The genus *Salmonella* is a member of the family *Enterobacteriaceae* and consists of only two species: *Salmonella bongori* and *Salmonella enterica*, with the latter being divided into six subspecies: I-*entericae,* II-*salamae,* III-*orizonae,* IV-*diarizonae,* V-*houtenae* and VI-*indica.* Within *Salmonella enterica* subspecies I (*Salmonella enterica* subspecies *entericae*), the most common 'O' antigen serogroups are A, B, C1, C2, D and E. Strains within these serogroups cause approximately 99% of salmonella infections in human and warm blooded animals (Uzzau *et al*., 2000).

The infection and prevalence of salmonellosis in poultry depend on different factors, such as age of birds, infectious dose and route of infection, bad management practices and concurrent disease may contribute to the development of a systemic infection (Hafez, 2005).

2.2 Historical Background of Fowl Typhoid

The genus *Salmonella* was named after Daniel Elmer Salmon, an American veterinary pathologist. While Theobald Smith was the actual discoverer of the type bacterium ("*Salmonella enterica*" var. Choleraesuis) in 1885; Dr. Salmon was the administrator of the USDA research program, and thus the organism was named after him (Klein, 1889). Fowl

typhoid (*Salmonella enterica* subspecies *entericae* Gallinarum infection) was first recognized in 1888 by Klein. Initially, the causative agent was named *Bacillus gallinarum*, then changed to *Bacillus sanguinium* and later *Salmonella gallinarum* (Klein, 1889). The name FT was first applied in 1902 by Klein (Klein, 1889). The disease can be transmitted through transovarian infection or faecoral route (Barrow *et al*., 1994). The organism that causes FT is highly host adapted just like Salmonella Pullorum and seldom causes significant clinical signs, morbidity or mortality in hosts other than chickens and turkeys (Barrow *et al*., 1994). The basis of host adaptation is unknown (Chadfield *et al*., 2003), although it is likely that it is predominantly expressed at the level of macrophage monocyte series (Barrow *et al*., 1994). Salmonella Gallinarum and Salmonella Pullorum are antigenically identical; they are without flagella antigen and require biochemical testing to differentiate (Barrow *et al*., 1994).

Salmonella is a major issue for raw material suppliers, feed manufacturers, poultry and egg producers and food manufacturers worldwide, because they are microrganisms which survive in many feed materials and through feed manufacturing processes (Clifford, 1999).

Although *Salmonella* is primarily an intestinal pathogen of many species of animals, it is widely found in the environment such as, farm effluents, human sewage and many materials subject to faecal contamination (Geoffrey, 1999). The organism can also survive for long periods in poultry house dust, despite cleaning and disinfection (Geoffrey, 1999).

2.3 Pathogenicity of *Salmonella* Gallinarum

Salmonella Gallinarum, being a Gram negative bacterium has endotoxin. The nature of pathogenicity of *Salmonella enterica* serovar Gallinarum is multifactorial (Kokosharov, 2003); however, its endotoxin plays an essential role and it is directly linked to the virulence of this micro-organism (Kokosharov, 2002). Like most pathogenic microrganism, this organism may lose virulence rapidly during propagation and passage on artificial media; hence cultures should be passaged serially in their natural host, the chicken, before testing the pathogenicity of the organism. Pathogenicity of such cultures is best maintained in the lyophilized/frozen state (Mdegela *et al*., 2000). The molecular and cellular mechanisms of FT are relatively poorly understood. The 85-kb serovar Gallinarum plasmid is essential for virulence (Barrow *et al*., 1987), and several plasmid virulence genes have been identified (Rychlik *et al*., 1998). Two pathogenicity islands, *Salmonella* pathogenicity island 1 (SPI-1) and SPI-2, play key roles in mediating disease by *Salmonella enterica* through their respective type III secretion systems (TTSS) (Hueck, 1998). The TTSS mediate the translocation of various virulence-associated effector proteins from the bacteria into the host cells (Darwin and Miller, 1999; Hensel, 2000; Wallis and Galyov, 2000).

2.4 Incidence and Distribution of Fowl Typhoid

Fowl typhoid is worldwide in distribution (Mdegela *et al*., 2000; Kwon *et al*., 2000; Okwori *et al*., 2007 and Mbuko *et al*., 2009). Okwori *et al*., (2007) carried out a serological survey of the prevalence of the antibodies to *Salmonella enterica* serovar Gallinarum in two different management systems (exotic and local breed of chickens) around Jos, Plateau

state, Nigeria and reported 37.9% seropositivity. A FT outbreak was reported in commercial laying hens in Nigeria affecting 11,000 birds with the mortality rate reaching up to 25% (Ezema *et al*., 2009). In the same country, another 129 outbreaks of FT were diagnosed by the Avian Unit of Veterinary Teaching Hospital, Ahmadu Bello University, Zaria, between 2003 and 2007 (Mbuko *et al*., 2009). Canada and several European countries have a low incidence or absence of FT. Mexico, Central and South America, Africa and the Indian subcontinent continue to report FT in poultry flocks (Silva, 1984, Abdu *et al*., 1985, Salami *et al*., 1989b, Sato *et al*., 1997, Mdegela *et al*., 2000; Burros, 2006).

2.5 Age Commonly Affected by Fowl Typhoid

Although FT is frequently referred to as a disease of adult birds, there have been reports of high mortality in young chicks (Komarov, 1932; Wong *et al*., 1996). Fowl typhoid can cause mortality as high as 26% in chicks during the first month of life. Mortalities of 65% and 100% within 11days after inoculation of one day old broiler chickens with 10^4 colony forming units (CFU) and 10^8 CFU per ml of *Salmonella enterica* serovar Gallinarum respectively have been reported by Wong *et al*., (1996).

2.6 Transmission of Fowl Typhoid

Transmission is of FT either by horizontal or vertical route (Berchieri *et al.*, 2001). Infected breeding flocks are mainly associated with vertical transmission of salmonellae to their progeny (Shivaprasad, 2003). Chicks usually get exposed to the disease at a very young age without any morbidity or mortality, but can harbor infection until they come into lay and

can then produce infected eggs and or progeny (Agbaje *et al*., 2010). Horizontal transmission can occur following ingestion of food or water already contaminated with faeces of clinically infected birds or carriers cannibalism, infected wild birds; attendants (through hands, feet and clothes) as well as rodents and vehicles are common sources of infection (Jordan and Pattison,1992). Feed dealers, chicken buyers and visitors who move from house to house and from farm to farm may also spread this disease, unless precautions are taken to disinfect footwear, hands and clothing; similarly trucks, crates and feed sacks may also be contaminated (Mdegela *et al*., 2000).

2.7 Clinical Signs of Fowl Typhoid

Common clinical signs of FT are depression, weakness, ruffled feathers (Freitas Neto *et al.*, 2007), weight loss, 50-70% drop in egg production (Ezema *et al*., 2009), prostration, apathy, drooped wings, loss of appetite, dehydration and greenish-yellow to bloody diarrhea (Garcia *et al*., 2010).

2.8 Morbidity and Mortality Associated With Fowl Typhoid

Both morbidity and mortality are highly variable in chickens and turkeys and are influenced by age, strain of bird, nutrition, flock management, and mode of exposure (Shivaprasad, 1996). Mortality due to FT has been recorded as ranging from 10 to 93% (Shivaprasad, 1996). Morbidity is often higher than mortality with some of the affected birds recovering spontaneously (Wong *et al*., 1996).

2.9 Gross Pathological Lesions of Fowl Typhoid

Descriptions of gross lesions associated with FT are non-specific. Some of the earliest descriptions were those of Rettger (1909). Since then, there have been many reports in various species of birds primarily involving chickens and turkeys, but also pheasants, budgerigars and guinea fowls (Beach and Davis, 1927; Evans *et al*., 1955; Chishti *et al*., 1985; Wong *et al*., 1996; Kokosharov *et al*., 1997; Henderson *et al*., 1999; Shivaprasad, 2000; Beyaz *et al*., 2010).

In peracute cases of FT, birds that die in the early stages of brooding may show no gross lesions. However, in acute cases there may be enlarged and congested liver, spleen and kidney. Liver may have white foci of 2-4mm in diameter (Beyaz *et al*., 2010). The yolk sac and its contents may or may not reveal any abnormality, but in protracted cases, interference of yolk absorption may occur. In such cases, the yolk sac contents may be creamy/caseous in colour and consistency (Beyaz *et al*., 2010). Occasionally, white nodules resembling Marek's disease tumours, may be present in the myocardium. The pericardial sac may be thickened and may contain yellow sero-fibrinous exudate. Similar nodules may be present in the muscles of the ventriculus (gizzard) and occasionally in the wall of the caeca and rectum. The caeca may contain caseous cores known as caecal cores (Shivaprasad, 2000).

Other changes that can be seen include serous exudate in the peritoneal cavity and anterior chamber of the eye and thickening of the wall of the intestine (Evans *et al*., 1955; Pomeroy and Nagaraja, 1999). Splenomegaly with gray necrotic foci and petechial haemorrhages as

well as pale or discoloured liver may be observed (Kokosharov *et al*., 1997; Chishti *et al*., 1985). The lesions found most frequently in chickens that are chronic carriers of FT are a few misshapen, discoloured ovarian follicles or nodular ova among a few normally – appearing ovules. The affected ova may contain oily or caseous material enclosed in a thickened capsule (Shivaprasad and Barrow, 2008). These degenerative ovarian follicles may be closely attached to the ovary, but frequently they are pedunculated and may become detached from the ovarian mass. In such cases, they may become embedded in the inner lining of the peritoneal cavity causing peritonitis. The oviduct often contains caseous exudate in the lumen. Dysfunction of the ovary and oviduct may lead to peritoneal ovulation or oviduct impaction which may also bring about extensive peritonitis and adhesions of the abdominal viscera (Pomeroy and Nagaraja, 1991; Shivaprasad and Barrow, 2008).

Fibrinous peritonitis and perihepatitis with or without involvement of the reproductive tract may sometimes be seen (Beach and Davies, 1927; Pomeroy, 1984). Ascites may develop, especially in turkeys, and frequently pericarditis is observed. Changes in the pericardium, epicardium and pericardial fluid depend on the duration of the disease (Henderson *et al*., 1999). Occasionally small cysts containing amber caseous material may be found embedded in the abdominal fat or attached to the ventriculus and or intestine. Frequently the pancreas may have white foci or nodules. In the male, the testes may have white foci or nodules. Occasionally, caseous granulomas can be found in the lungs and air sac (Kokosharov *et al*., 1999).

Lesions due to FT in turkeys are similar to those observed in chickens; but enlarged, mahogany or brown – streaked livers, splenomegaly, areas of necrosis in the heart and grayish lungs are characteristic lesions of FT in turkeys and poults (Woolhouse and Gowtage, 2005). Ulceration throughout the small intestine and caeca is a common lesion in turkeys but uncommon in chickens (Waltman *et al*., 1998; Woolhouse and Gowtage, 2005). Also lesions due to FT in ducklings and adult ducks are those involving the respiratory tract and are cha`racterized by congested lungs and increased mucus in the nasal cleft and trachea (Kokosharov *et al*., 1997).

2.10 Histopathology of Fowl Typhoid

In peracute cases of FT only severe vascular congestion in various organs, especially liver, spleen and kidney can be identified. In acute to subacute cases, there is multifocal necrosis of hepatocytes with accumulation of fibrin and infiltration of heterophils mixed with a few lymphocytes and plasma cells can be seen in the liver (Kokosharov *et al*., 1997; Hossain *et al*., 2006). In chronic cases, especially in cases where there are large nodules in the heart, the liver will have congestion with interstitial fibrosis. The spleen may have severe congestion or fibrin deposits and severe hyperplasia (Chishti *et al*., 1985). In chicks or poults the caeca may have extensive necrosis of the mucosa and submucosa with an accumulation of necrotic debris mixed with fibrin and heterophils in the lumen (Barrow *et al*., 1999). However, the most characteristic microscopic lesions are in the heart, where they begin as necrosis of myofibers with infiltration by heterophils mixed with lymphocytes and plasma cells. In later stages these cells are replaced by large number of uniform histiocytes,

which are fairly large with irregular vesicular nuclei and faintly staining eosinophilic cytoplasm. They are arranged in solid sheets, forming nodules that often protrude from the epicardial surface. These nodules both grossly and histologically can be confused with lymphoid tumours caused by Marek's disease virus and possibly retroviruses (Gauger, 1934; Pomeroy and Nagaraja, 1991).

A similar lesion can be seen in the ventriculus and pancreas. The lesions in the pancreas can be so severe that the normal architecture is obliterated (Liu *et al*., 2002). Microscopic lesions in the ovary range from acute fibrinosuppurative inflammation to severe pyogranulomatous inflammation. The pyogranulomatous inflammation is characterized by infiltration of heterophils mixed with fibrin and bacterial colonies in the coagulated layers of multinucleated giant cells and mixed population of other inflammatory cells which may include macrophages, plasma cells and lymphocytes. In males, degeneration, necrosis and inflammation of the epithelial cells lining of the seminiferous tubules can be seen (Kokosharov *et al*., 1984). Other but less common changes are catarrhal bronchitis, catarrhal enteritis and interstitial pneumonia as well as nephritis. There are other non-specific changes involving the endocrine glands, such as hyperplasia of the thyroid gland and hypertrophy of adrenal and pituitary glands (Garren and Barber, 1955; Wigley *et al*., 2005).

2.11 Pathogenesis of Fowl Typhoid

Differences in the ability of various salmonellae including Salmonella Gallinarum to survive and multiply in various organs (especially in spleen and liver) have been due to

unknown mechanisms, but involve the mononuclear phagocytic system (MPS) of the host (Barrow *et al*., 1994). Ducks may be resistant to *Salmonella enterica* serovar Gallinarum due to the inherent inability of the bacteria to multiply in the MPS of ducks (Barrow *et al*., 1999).

Because of the ability of the bacteria to infect and multiply within the cells of the MPS of chickens and turkeys, it is probable that cell-mediated immunity to *Salmonella enterica* serovar Gallinarum may play a role in recovery or resistance to infection in them (Wigley *et al*., 2005). In a study with *Salmonella enterica* serovar Gallinarum in chickens, leucocytosis was observed on the third day of infection which was followed by leukopenia that lasted for 10 days (Kokosharov, 1998). Infection with this organism may cause anaemia (Christensen *et al*., 1996) and increase sialic acid content in serum (Kokosharov, 2000).

2.12 Diagnosis of Fowl Typhoid

A definitive diagnosis of FT requires the isolation and identification of Salmonella Gallinarum. A tentative diagnosis however, can be made based on flock history, clinical signs, mortality and lesions (Waltman *et al*., 1998). Positive serological findings can also be of significant value in detecting infection, however, negative results should not be considered adequate for a definite diagnosis because of the delay of 3-10 days in the appearance of agglutinating antibodies following infection. Similarly, positive results should be interpreted with caution because of cross-reactions with other serogroup, for example serogroup D Salmonellae, such as *Salmonella enteritidis* (Gast and Beard, 1990).

Further identification of the organism can be carried out by standard biochemical test and polymerase chain reaction (PCR), which is the most sensitive molecular technique (Barrow, 1994).

2.13 Differential Diagnosis of Fowl Typhoid

The clinical signs and lesions produced by FT are not pathognomic. Other Salmonella infections may produce similar lesions in the liver, spleen and intestine which cannot be distinguished grossly or microscopically from those produced by FT. Fowl typhoid is antigenically identical with pullorum disease (PD), but fowl typhoid has a different epidemiology and disease presentation to pullorum disease. FT is principally associated with an acute septicemic illness of high morbidity and mortality in birds during the later growing period and in adults. This contrasts with PD which manifests as severe, acute losses in chicks (Shivaprasad and Barrow, 2008).

Aspergillus or other fungi may produce similar lesions in the lungs, although the lung lesions in FT are rarely nodular as in fungal infection (Hafez, 2005). Salmonella Gallinarum can localize in major joints and tendon sheaths of chicks. Clinical signs and lesions from this may resemble those produced by organisms such as *Mycoplasma synoviae*, *Staphylococcus aureus*, *Pasteurella multocida* or *Erysipelothrix rhusiopathia*. Artritis, sinovitis and tendosynovitis are common with these organisms but not common in FT (Shivaprasad, 2003). Fowl typhoid is confused with infection due to *Pasteurella multocida*, *Erysipelothrix rhusiopathia, Staphylococcus aureus* and coliforms because of septicaemia that is noticed in them. Also local infections with Salmonella Gallinarum in

adult carriers particularly of the ovary may appear identical to those produced by other bacterial infections such as Coliforms, Staphylococci, Pasteurella, Streptococci and other salmonellae (Shivaprasad and Barrow, 2008).

2.14 Prevention and Control of Fowl Typhoid

Although no single measure will eradicate or work efficiently to eradicate *Salmonella* from poultry production systems, yet FT is still good example of disease that has decreased in incidence over the years by application of basic management procedures such as the following (Hafez, 2005; Berge and Kay, 2011):

1. Ensuring that the breeding stock is free of *Salmonella* through sero-surveillance (test and slaughter)

2. Thorough cleaning and disinfection, egg fumigation and monitoring of *Salmonella* in hatcheries.

3. Using all-in/ all-out system for raising birds and appropriate times between flocks.

4. Using pest control system to prevent access by wild rodents, birds or other animals to poultry houses.

5. Optimizing nutrition and immune status in the birds through vaccination in endemic areas.

6. Eliminating stressors such as thinning the flock and moulting layers. Providing at least 6 hours of darkness per day.

7. Taking appropriate action in cases of Salmonella outbreaks.

8. Initiating effective biosecurity measures. Installing dedicated equipment for poultry houses, do not share equipment; ensure that all visitors and workers have clean cloths and boots. Do not permit outside personnel into the poultry facility for loading of birds and eggs.

9. Ensuring that Salmonella-free stock is not mixed with other poultry or confined birds.
10. Ensuring that chicks and poults are placed in an environment that can be cleaned and sanitized to eliminate any residential *Salmonella* from previous flocks.
11. Ensuring the use of feed ingredient free of *Salmonella.*
12. Ensuring that introduction of Salmonella from outside sources is minimized by the use of sound biosecurity programme.

2.15 Treatment of Fowl Typhoid

There are good prophylactic and therapeutic drugs that have been developed against FT. In Canada and USA, every effort has been made to eradicate this disease where treatment is neither feasible nor desired. Various sulfonamides, nitrofurans, chloramphenicol, tertacyclines and aminoglycosides have been found to be effective in reducing mortality from FT, however, no drug or combination of drugs has been found capable of eliminating infection in treated flock. Hence microbial culture and sensitivity is advised to get the drug of choice (Aziz *et al*., 1997; Lee *et al*., 2004).

2.16 The Quail

Quails belong, along with chickens, pheasants and partridges to the family Phasianoidea, order Galliformes, class Aves of the animal Kingdom. The quail belongs to the genus Coturnix and has the following species (Khare *et al*., 1975; Aggrey *et al*., 2003):

i. *Coturnix coturnix coturnix* (European quail)
ii. *Coturnix coturnix japonica* (Japanese quail)
iii. *Coturnix pectoralis* (Stubble quail)
iv. *Coturnix yapsilophorus* (Australian Brown quail)
v. *Coturnix delegorguei* (African Harleguin quail)
vi. *Coturnix coromendelia* (Indian Rain quail)
vii. *Coturnix coturnix communis* (Eurasian/Pharaoh quail)
viii. *Coturnix novaezelandicae* (New Zealand quail).

Japanese quails are hardy birds that thrive in small cages and are inexpensive to keep. They are affected by some common poultry diseases but are fairly hardy (Huss *et al.,* 2008). They mature in about 6 weeks and are usually in full egg production by 50 days of age. With proper care, hens should lay 200 eggs in their first year of lay and their life expectancy is $2 - 2^{1}/_{2}$ years (Huss *et al*., 2008). Quail eggs are nearly identical in taste and nutritional quality to chicken eggs. Quail hens; however, need less than 2 pounds of feed to produce a pound of eggs, while chickens need almost 3 pounds of feed to make that same pound of eggs. Five quail eggs are equivalent to one chicken egg in size. Quail meat is delicious (Huss *et al.,* 2008).

The meat is tender and can be boiled, baked, roasted, stir – fried or stewed (Chindo and Olowoniyan, 2006). Quail can be fed with any available chicken feed at the rate of 2kg for every 50 quail layers per day (NVRI, 2008). Quail farming was introduced into Nigeria in 1992 to boost sources of animal protein for the populace (Haruna *et al*., 1997). The short generation interval, fast growth and lower feed requirement made the quail an ideal bird and an additional source of revenue for low and medium income poultry farmers (Naveen and Arun, 1992).

The females are characterized by light tan feathers with black speckling on the throat and upper breast while the males have rusty brown throat and breast feathers. Males also have a cloacal gland, a bulbous structure on the upper edge of the vent that secretes a white, foamy material. This unique gland can be used to assess the reproductive fitness of the males. Japanese quail eggs are mottled brown in colour and are often covered with a light blue, chalky material. Each hen appears to lay eggs with a characteristic shell pattern or colour. Some strains lay only white eggs. The average egg weighs about 10 g, about 8% of the bodyweight of the quail hen. Young chicks weigh 6 to7 g when hatched and are brownish with yellow stripes. The egg shells are very fragile (Huss *et al.,* 2008).

Research indicates that grouping a single male with two or three females will generally give high fertility. When quails are kept in colony pens, one male to three females is sufficient and reduces fighting among males. Pair mating in individual cages also gives good fertility. Fertility decreases markedly in older birds. Avoid mating closely related individuals, because inbreeding increases the incidence of abnormalities and can greatly reduce

reproductive performance (Lima *et al*., 2004). A standard ration for either growing or breeding quail may not be available commercially, therefore, good quality, fresh, commercial turkey or game bird diets are recommended. In Nigeria, quails are fed commercial chicken feed, preferably fed as crumbles to minimise feed wastage. For the first 6 weeks quails should be fed a diet containing approximately 25% protein, about 12.6 mega joules (MJ) of metabolisable energy (ME) per kilogram, and 1.0% calcium. A good quality commercial starter ration for game birds or turkeys contains about 25% to 28% protein. If this is not available, a chicken starter ration (20% to 22% protein) can be used, but the birds will grow more slowly (Huss *et al.,* 2008). The dietary requirements for birds nearing maturity are similar except that calcium and phosphorus levels must be increased. Shell grit or ground limestone can be added to the diets after 5 weeks of age, or it may be provided separately as free choice. Laying diets should contain about 24% protein, 11.7 MJ of metabolisable energy per kilogram, and 2.5% to 3.0% calcium. The latter may need to be increased to 3.5% in hot weather when the birds eat less food but still require calcium to maintain egg production. Adult Japanese quail eat between 14 g and 18 g of feed per day (Huss *et al.,* 2008). There is no documented report of FT outbreak in Nigeria yet to the best of our knowledge.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Area of Study

The study was carried out in Zaria, Kaduna State, which is located within the Northern Guinea Savannah Zone of Nigeria, between latitude 7^0 and 11^0 N, and longitude 7^0 and 44^0E; the average rainfall of this zone ranges from 1,000 to 1,250 mm, and the average temperature ranges from 17^0C to 33^0C (Saidu *et al*., 1994).

3.2 Housing

The quails were kept in mesh cages of 120 × 140 × 120 cm in size in an enclosed house with the litter changed every week throughout the experimental period.

3.3 Experimental Birds

A total of 160, four-week old Japanese quails (108 males and 52 females) were obtained from the Poultry Unit of National Veterinary Research Institute, Vom, and used for the experiment. They were randomly divided into four groups (A, B, C and D) of 40 birds each; with 27 males and 13 females in group A , 23 males and 17 females in group B, 29 males and 11 females in group C and D respectively. The quails were allowed to acclimatize for two weeks, during which they were observed for clinical signs of any infection. They were dewormed with levamisole at the dose of 5mg per kilogram body weight at the age of eight weeks and repeated at the age of ten weeks.

3.4 Feeds and Feeding

The quails were fed for two weeks on commercial chick mash, before they were fed commercial layer mash throughout the period of the research which lasted 120 days. They were fed and provided with water *ad libitum* during this period.

3.5 Determination of Body Parameters

The baseline values for temperature, weight, egg production, haematological and serum biochemical parameters were determined before the commencement of the experiment, according to standard procedures (Benjamin, 1985; Esievo and Saror, 1992; Oladele *et al.*, 2005; Freitas Neto *et al*., 2007; Beyaz *et al*., 2010).

3.6 Bacteriological Monitoring Before Infection

Before infection, cloacal swabs were collected from all the quails in order to confirm if they were free from *Salmonella* organism. This was done by pre-enrichment of the swab samples in buffered peptone water, followed by plating on MacConkey agar (MCA) and blood agar (BA) using standard laboratory methods (Wigley *et al.*, 2001; Parmer and Davies, 2007).

3.7 The *Salmonella* Isolate used for the Challenge

The bacterium, *Salmonella enterica* serovar Gallinarum, was obtained from the bacterial culture bank of the Central Diagnostic Laboratory, National Veterinary Research Institute, Vom. The *Salmonella* isolate was from day chick that died of Salmonella Gallinarum infection.

3.8 Culture and Determination of Bacterial Inoculum

The lyophilized bacterium from the culture bank was reactivated by sub-culturing on blood agar (BA) and MacConkey agar (MCA). The resulting colonies were examined for their features, colour and morphology and tested for Gram-reaction (Gram-negative). Three colonies were scooped and inoculated into 20 ml of nutrient broth and this was incubated for 24 hours at 37^{o}C after which a ten-fold dilution was carried out in test tubes. The colony counts from the test tubes were determined. To obtain the number of organisms that was inoculated into the quails, the number of organisms was multiplied by volume by the dilution factor (CFU= No. of colony × Volume × Reciprocal of Dilution factor) (Miles and Misra, 1938).

3.9 Challenge of the Quails with *Salmonella enterica* serovar Gallinarum

Challenge of the quails was done per orally. Group A quails received a dose of 1×10^6 organisms/0.2 ml of nutrient broth, group B quails received a dose of 1×10^4 organisms/0.2 ml of nutrient broth, while group C quails received a dose of 1×10^2 organisms/0.2 ml of nutrient broth. Group D quails served as control and were not challenged with the bacterium, but were given bacteria-free nutrient broth in accordance with the method of Miles and Misra (1938).

After challenging the quails with the bacterium, the weights of the quails (in grams) were measured weekly using a digital weighing balance (KERN 572), while the cloacal temperatures (^{0}C) were taken on daily basis using digital clinical thermometer.

3.10 Blood Sampling

Blood samples were collected via the wing vein, using 25 gauge sterile hypodermic needles and syringes. These were collected 5 days before challenge and 5 days after challenge, so as to reduce stress pre-infection and to get the blood picture within the incubation period of the disease respectively. Blood for haematological analysis were collected into 2ml labelled Bijou-bottles, containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant. The blood samples for serum chemistry analysis were collected in plain bottles without anticoagulant (Benjamin, 1985; Esievo and Saror, 1992; Oladele *et al*., 2008).

3.11 Determination of Haematological Parameters

Red blood cell count, packed cell volume and haemoglobin concentration were determine using Mindray auto haemo analyser, model BC-3000 PLUS, manufactured by Shenzhen Mindray Biomedical Electronics Ltd, China. The haemo analyser machine was switched on and it automatically flushed the fluidic lines, checked the background and entered the count screen. The menu key was pressed to select the sample mode, and whole blood was selected. The blood in the sample tubes were mixed well using Stuart roller mixer machine model SRT9D, after bringing it to room temperature. It was then presented to the analyser probe tip making sure it submerges well into the sample. The aspirate key was pressed to start the count. The result was printed out in a hard copy automatically from the machine, and the result recorded (Freitas Neto *et al*., 2007).

3.12 Differential Leukocyte Count

Samples were mixed well using Stuart roller mixer machine model SRT9D. A drop of the blood was placed one centimetre away from the edge of a clean, greased-free slide. A spreader was placed in front of the blood to allow the blood to spread across its edge. The spreader was moved forward quickly and smoothly. The smear was allowed to air dry, and the slide labelled. The air dried smear was then placed on a staining rack and flooded with filtered Leishman's stain and fixed for two minutes. The smeared slides were then flooded with equal volume of buffered distilled water (pH 6.8) and allowed to stain for 8 minutes. Excess stain was washed off and was allowed to differentiate to salmon pink colour. The slides were air dried and viewed using oil immersion objective, starting from the thin end, a systematic meander system of slow, careful and detailed leucocytes count was made, and the result recorded (Beyaz *et al*., 2010).

3.13 Serum Biochemistry

Two millilitres of serum from each quail sampled were used to measure the serum glucose, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea and total protein levels in the Clinical Chemistry Laboratory of National Veterinary Research Institute (N.V.R.I), Vom.

3.13.1 Assay for Serum Albumin

Three small labelled tubes 'A (reagent)' containing 0.01ml of distilled water and 3.0 ml of bromocresol green (BCG) reagent were mixed thoroughly. In the second tube labelled 'A (standard)', 0.01ml of standard and 3.0ml of BCG reagent were mixed thoroughly in the

third tube ‘A (serum) 0.01ml of the quail serum and 3.0 ml of BCG reagent. The samples were mixed thoroughly and incubated at 20^0C – 25^0C for 5 minutes. The absorbance of each tube was measured using a spectrophotometer (model 6400/6405, Jenway) at 600 – 650 nm against the reagent blank as follows:

$$\text{Albumin concentration (g/dl)} = \frac{A(\text{serum}) \times \text{concentration of standard}}{A\ (\text{standard})}$$

(Grant, 1987)

3.13.2 Assay for Serum Total Protein

Biuret method was used to determine total protein, as described by Benjamin (1985). A blank test tube, standard and the test sample tubes were labelled. 1,000 microlitre of biuret reagent was pipetted into each tube, and 200 microlitres of distilled water, standard and test samples were pipetted into the respective tubes. They were thoroughly mixed and incubated for 30 minutes at 25^0C. The spectrophotometer (model 6400/6405 manufactured by Jenway, Barloworld Scientific, England) was set at 546 nm and zero using blank test tube, the absorbance was read, and the total protein calculated using the formula:

$$\frac{A1 \times C}{A2}$$

Where:

A_1 = Absorbance of the test sample,
A_2 = Absorbance of the standard,
C= Standard concentration (Grant, 1987)

3.13.3 Assay for Serum Glucose

Ten microlitres (10µl) of serum sample and 1.0 ml of reagent were measured into a cuvette and labelled 'T (serum)' and were mixed thoroughly. A cuvette labelled 'T (standard)' containing 10 µl of standard and 1.0 ml of reagent were mixed thoroughly. The 10 µl of reagent pipetted into a cuvette was labelled 'T (reagent)'. The contents were mixed thoroughly and incubated at 20-25^{0}C for 10 minutes. The absorbance of 'T (serum)' and 'T (standard)' was measured against reagent blank within 60 minutes in a spectrophotometer (model 6400/6405, Jenway) at 500nm wave length.

$$\text{Gucose concentration (mg/dl)} = \frac{\text{T(serum)} \times \text{concentration of standard}}{\text{T (standard)}}$$

(Barham and Trinder, 1972)

3.13.4 Assay for Serum Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT)

Pippeted into tubes was 100 microlitres of sample and 500 microlitres of buffer added, mixed and incubated for 30 minutes at 37^{0}C. Then 500 microlitres of dye reagent was added and allowed to stand for 20 minutes at 25^{0}C; after which 5 ml of sodium hydroxide was added, mixed and the absorbance of the sample against sample blank was read after 5 minutes. Calculation for both AST and ALT were obtained from the activity table (Reitmans and Frankel, 1957).

3.13.5 Assay for Serum Alkaline Phosphatase (ALP)

This was done using the method of Buttery *el tal* (1980). 500 µl of blank; standard and sample was pipette into test tube and incubated at 37^0C for 10 minutes, after which 2500 µl of alkaline phosphatase colour developer was added into each of the test tubes, mixed and then the absorbance was read using a spectrophotometer at wavelength of 590 nm. The serum alkaline phosphatase was calculated using this formular:

$$\frac{\text{Absorbance of test} \times \text{Concentration of standard}}{\text{Absorbance of standard}}$$

3.13.6 Assay for Serum Urea

One ml of buffer was pippeted into 3 test tubes (blank, standard and sample) and 50 microlitres added to the 3 test tubes, mixed and incubated for 5 minutes at 37^0C. Then 200 microlitres of hydrochloride was added and incubated for further 5 minutes at 37^0C. The absorbance of the sample and standard was measured against reagent blank at 600 nm. The value was calculated using this formular:

$$\frac{\text{Absorbance of test} \times \text{Concentration of standard}}{\text{Absorbance of standard}}$$

(Patton and Crouch, 1977)

3.14 Clinical and Pathological Examinations

The quails in all the experimental groups were observed daily for clinical signs and recorded. The mortality was also recorded. Post-mortem examination was conducted on dead birds, and those not dead at the end of the experiment, were euthanized by cervical dislocation. Thereafter, gross lesions were observed, recorded and photographed. Samples of the liver, spleen, heart, intestine, lung, ovary, pancreas and caecal tonsil showing gross lesions were taken and fixed in10% buffered neutral formalin for at least 48 hours. The fixed tissues were dehydrated in graded concentrations of alcohol (70%, 80%, 90% and 100%) using an automatic tissue processor. The tissues were cleared with xylene, embedded in molten paraffin wax blocked and labelled appropriately (Baker *et al.*, 2000; Oladele *et al.,* 2008).

Tissue sections of 5 μm thick were made from the embedded tissues using a microtome knife attached to a microtome. The sectioned tissues were mounted on a grease-free, clean glass slide, dried at room temperature and stained with haematoxylin and eosin (H & E) stains. The slides were studied using light microscope at ×40 and ×100 magnifications in order to make microscopic morphological diagnoses of the lesions on the slides. Photomicrographs of the lesions on the slides were taken, transferred to a computer and labelled appropriately (Hossain *et al*., 2006).

3.15 Bacteriological Isolation

At post-mortem, tissues from the liver, lung, heart, spleen and intestine were aseptically taken for isolation of *Salmonella enterica* serovar Gallinarum using standard laboratory methods (Wigley *et al*., 2001; Parmer and Davies, 2007).

3.16 Data Analysis

Data obtained were expressed as mean ± S.E.M (standard error of the mean). They were subjected to repeated measure one way analysis of variance (ANOVA) to determine the difference in the parameters before infection and post-infection between the groups. This was followed by Tukey's post-hoc multiple comparison tests using Graph-pad prism version 4.0 for windows. Values of $P < 0.05$ were considered significant.

CHAPTER FOUR

RESULTS

4.1 Bacteriological Monitoring Before Infection

Table 1 shows bacteria growth from the cloacal swabs of the quails before they were infected with *Salmonella enterica* serovar Gallinarum. A total of 121 (**75.6%)** bacterial growths were lactose fermenters, 7 **(4.3%)** *Bacillus* species, 2 (**1.3%**) *Staphylococcus* species, 2 (**1.3%)** *Citrobacter* species and 28 (**17.5%)** had no bacteria growth. This implies that the quails were free from *Salmonella* spp since *Salmonella* is a none lactose fermenter and no none lactose fermenter was found from the cloacal swabs screening before infection with *Salmonella enterica* serovar Gallinarum.

Table 1: Bacterial growth from the cloacal swabs of quails before they were infected with *Salmonella enterica* serovaar Gallinarum.

	Number and percentage of bacterial growth per group of infected quail			
Bacterial growth	**GROUP A**	**GROUP B**	**GROUP C**	**GROUP D**
Lactose fermenters	23 (57%)	36 (90%)	30 (75%)	32 (80%)
Bacillus spp	2 (5%)	1 (2%)	2 (5%)	2 (5%)
Staphylococcus spp	1 (2%)	-	-	1 (1%)
Citrobacter spp	2 (5%)	-	-	-
No growth	12 (30%)	3 (7%)	8 (20%)	5 (12%)

4.2 Effect of *Salmonella enterica* serovar Gallinarum Infection on Haematological Values of Japanese Quails *(Coturnix coturnix japonica)*

Figures 1 to 8 show mean haemoglobin concentration, mean red blood cell (RBC) count, mean packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean heterophil count and mean lymphocyte count, respectively before and after infection.

4.2.1 Mean haemoglobin concentration for groups A, B, C and D before infection were 186.6 ± 8.65 g/L, 194.3 ±5.11 g/L, 177.8± 5.56 g/L and 188.2± 6.92 g/L, respectively. While the mean haemoglobin concentration after infection for groups A, B, C and D were 172.4± 3.10 g/L,181.5 ± 6.77 g/L, 167.8 ± 4.64 g/L and 188.7.2 ± 4.23 g/L, respectively (Figure 1).

4.2.2 Mean RBC counts for groups A, B, C and D before infection were $3.611\pm0.16x10^{12}$/L, $3.618\pm0.10x10^{12}$/L, $3.464\pm0.10x10^{12}$/L and $3.610\pm0.11x10^{12}$/L, respectively. While after infection the mean RBC count for groups A, B, C and D were $3.2793\pm0.6x10^{12}$/L, $3.106\pm0.12x10^{12}$/L, $3.209\pm0.09x10^{12}$/L and $3.664\pm0.07x10^{12}$/L, respectively (Figure 2 and Appendices 1 and 2)

4.2.3 Mean packed cell volume (PCV) for groups A, B, C, and D before infection were 55.22 ±2.31 %, 56.76 ± 1.38 %, and 52.78 ± 1.44 % and 54.62 ± 1.66 %, respectively. While mean PCV for groups A, B, C, and D after infection were 50.80 ± 0.83 %,

47.48 ± 1.73 %, 50.15± 1.24 % and 55.16 ± 0.94%, respectively (Figure 3 and Appendices 1 and 2).

4.2.4 Mean corpuscular volume (MCV) for groups A, B, C and D before infection were 153.5 ± 1.20 fl, 155.1 ± 1.90 fl, 152.7 ± 1.51 fl and 151.5 ± 1.46 fl, respectively. While MCV for groups A, B, C and D after infection were 148.2 ± 1.02 fl, 152.9 ± 1.63 fl,169.4 ± 16.33 fl and 151.0 ± 1.36 fl , respectively (Figure 4 and Appendices 1 and 2)

4.2.5 Mean corpuscular haemoglobin (MCH) for groups A, B, C and D before infection were 51.52 ± 0.63 pg, 52.33 ± 0.85 pg, 50.83 ± 0.65 pg and 52.01± 0.67 pg, respectively. While the MCH for groups A, B, C and D after infection were 56.76 ± 0.67 pg, 56.33 ± 0.76 pg, 56.98 ± 0.55 pg and 52.45 ± 0.54 pg, respectively (Figure 5 and Appendices 1 and 2).

4.2.6 Mean corpuscular heamoglobin concentration (MCHC) for groups A, B, C and D before infection were 335.7 ± 3.31 g/dl, 341.6± 2.61 g/dl, 339.5± 4.36 g/dl and 335.7± 7.70 g/dl, respectively. While the MCHC for groups A, B, C and D after infection were 383.0 ± 2.69 g/dl, 368.7 ± 2.44 g/dl, 373.4 ± 2.47 g/dl and 338.1 ± 1.80 g/dl, respectively (Figure 6 and Appendices 1 and 2).

4.2.7 Mean heterophil counts for groups A, B, C and D before infection were 54.88 ± 4.47%, 62.33 ± 2.85 %, 60.41 ± 3.90 % and 64.33± 4.42 %, respectively. While the mean heterophil counts for groups A, B, C and D after infection were 58.21 ± 3.06 %,

65.31 ± 2.11 %, 56.26 ± 4.30 % and 64.25 ± 4.04 %, respectively (Figure 7 and Appendices 1 and 2).

4.2.8 Mean lymphocyte counts for groups A, B, C and D before infection were 49.63 ± 5.02%, 37.67 ± 2.85%, 40.18 ± 3.64% and 35.67 ± 4.42 %, respectively. While the mean lymphocyte counts for groups A, B, C and D after infection were 41.74 ± 3.07%, 37.82 ± 4.07 %, 42.94 ± 4.33 % and 35.50± 3.97 %, respectively (Figure 8 and Appendices 1 and 2).

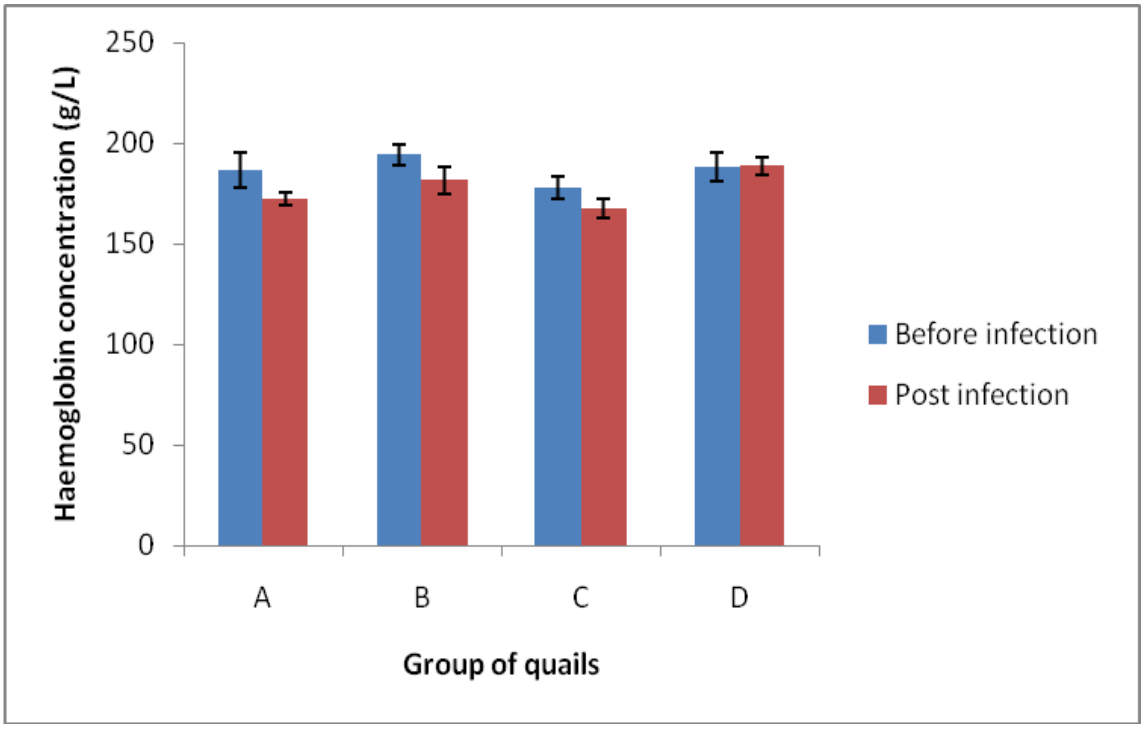


Figure 1: Mean haemoglobin concentration before and after infection of quails with *Salmonella enterica* serovar Gallinarum.

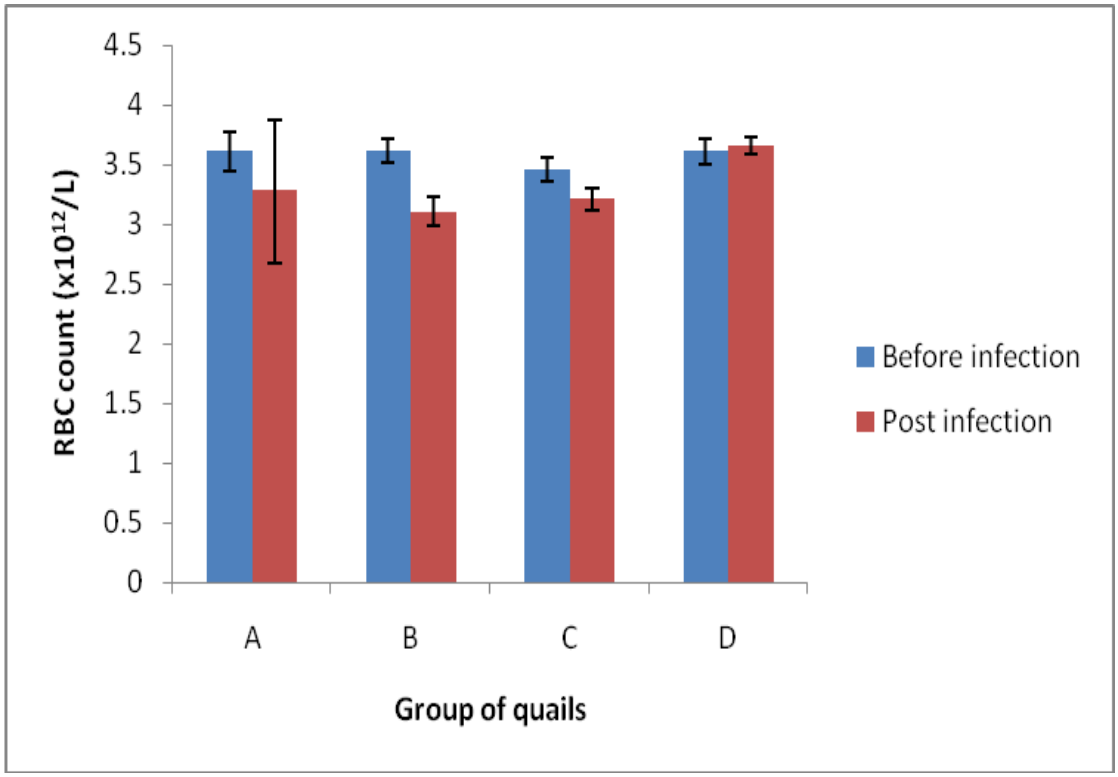


Figure 2: **Mean red blood cell count before and after infection of quails with *Salmonella enterica* serovar Gallinarum.**

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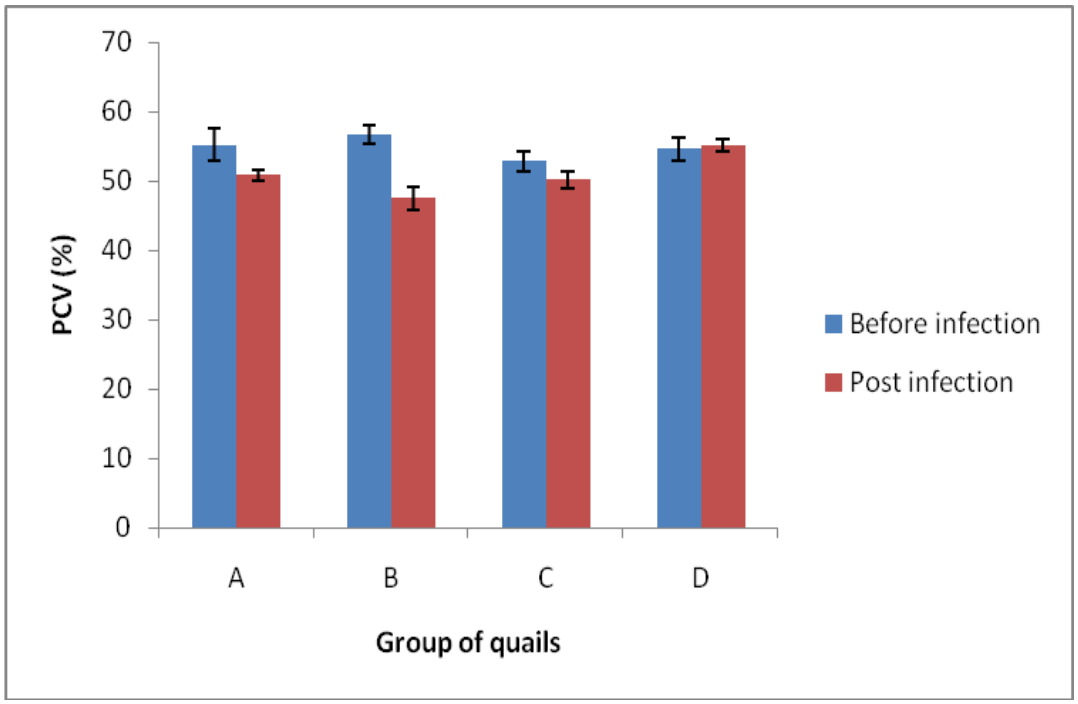


Figure 3: **Mean packed cell volume (PCV) before and after infection of quails with *Salmonella enterica* serovar Gallinarum.**

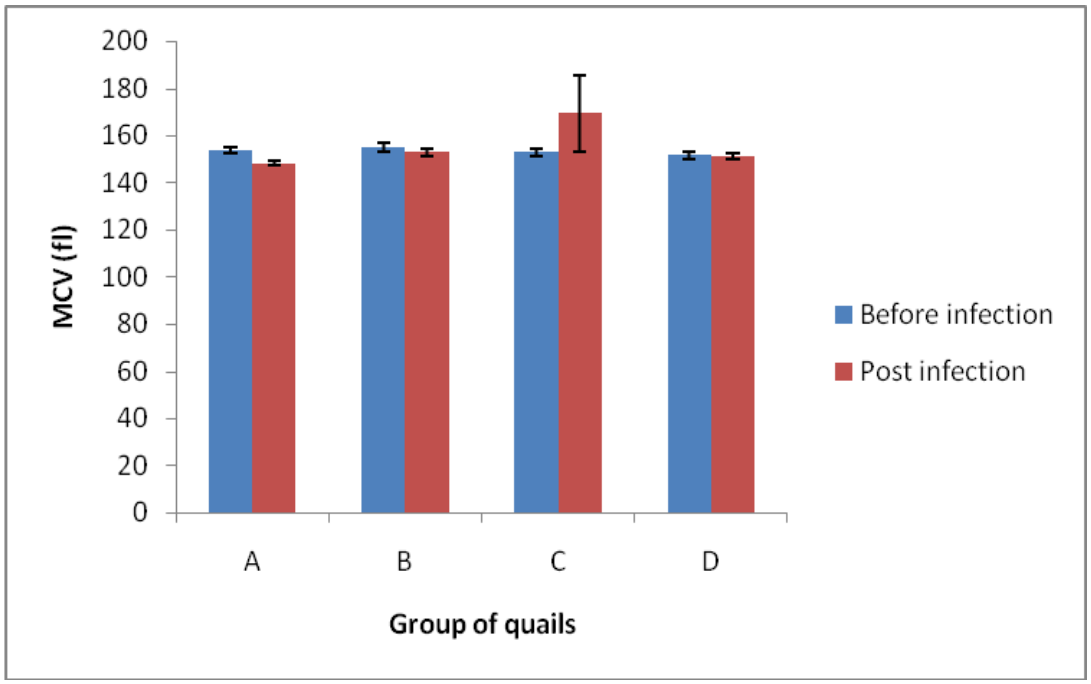


Figure 4: **Mean corpuscular volume before and after infection of quails with *Salmonella enterica* serovar Gallinarum.**

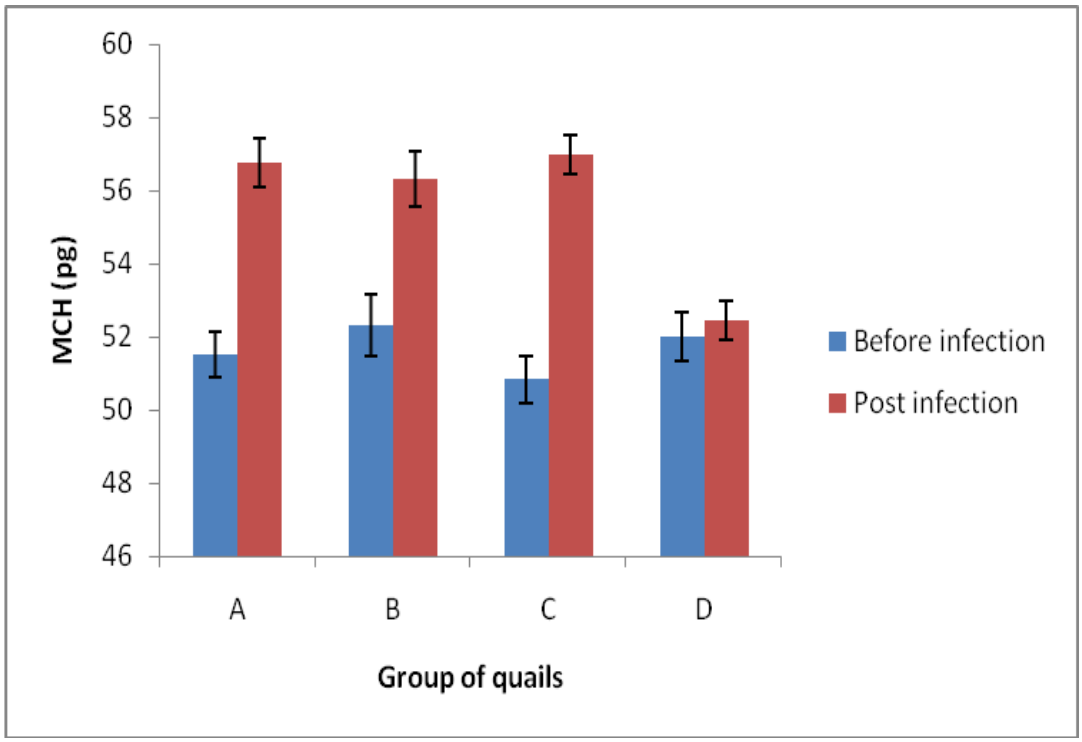


Figure 5: **Mean corpuscular haemoglobin before and after infection of quails with *Salmonella enterica* serovar Gallinarum.**

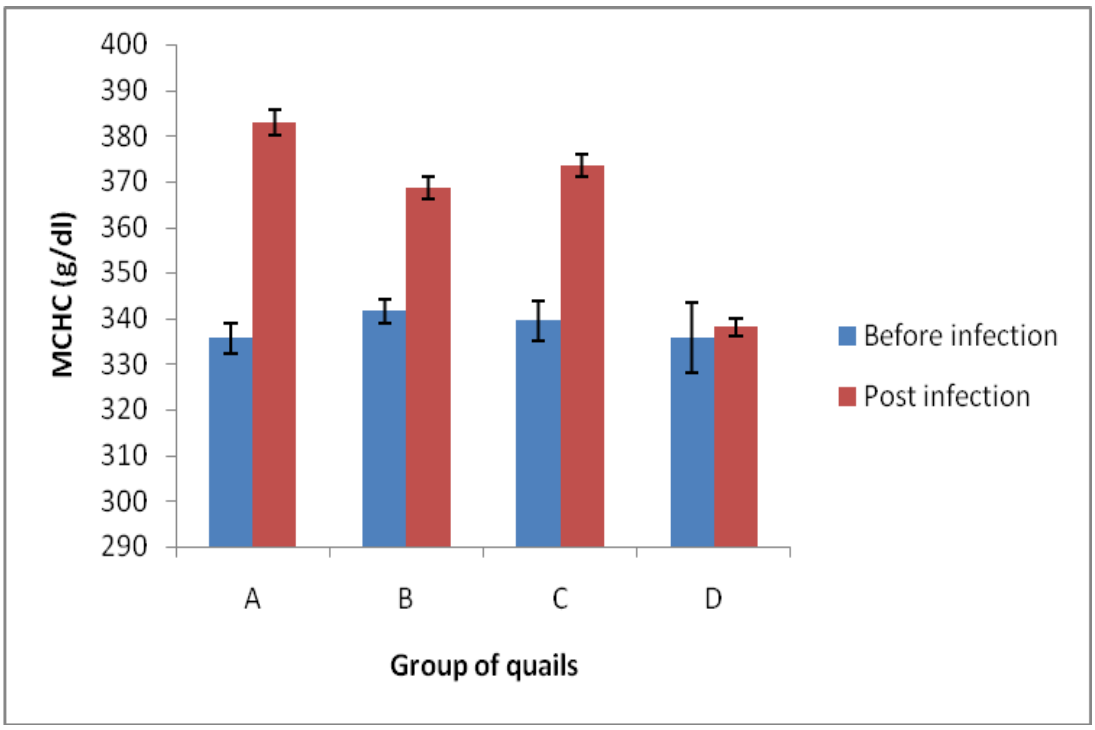


Figure 6: **Mean corpuscular haemoglobin concentration before and after infection of quails with *Salmonella enterica* serovar Gallinarum.**

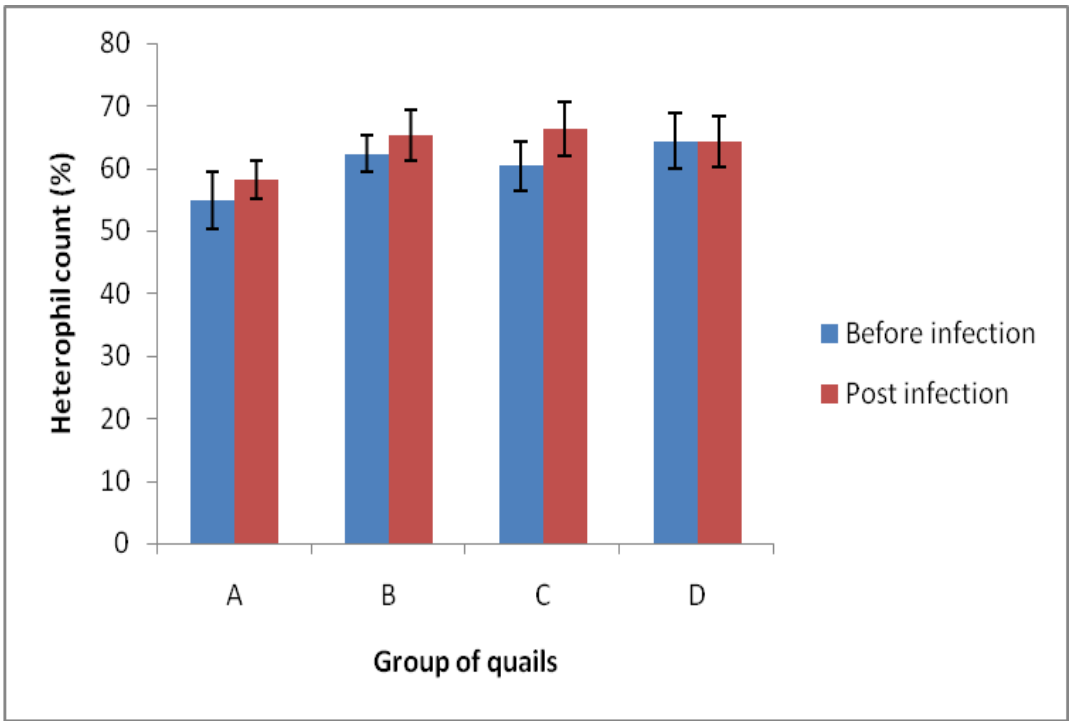


Figure 7: **Mean heterophil count before and after infection of quails with *Salmonella enterica* serovar Gallinarum.**

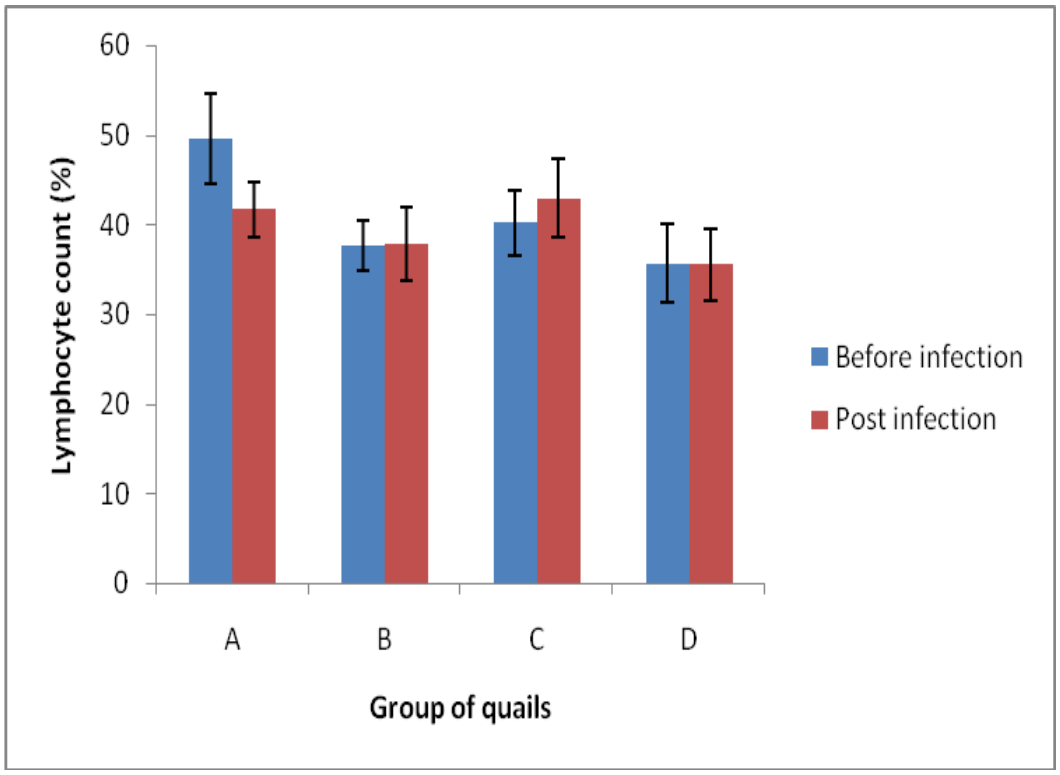


Figure 8: **Mean lymphocyte count before and after infection of quails with *Salmonella enterica* serovar Gallinarum.**

4.3 Effect of *Salmonella enterica* serovar Gallinarum Infection on Serum Biochemistry Values of Japanese Quails *(Coturnix coturnix japonica)*

Figures 9 to 15 show the mean serum biochemistry values of the quails before and after infection with *Salmonella enterica* serovar Gallinarum. There was a marked increase ($P<0.05$) in the mean serum glucose after infection in all infected groups with values almost doubled what they were before infection and no change in the control group (Figure 9). There was significant decrease ($P<0.05$) in the mean serum total protein and albumin in all the infected groups post-infection, with no change in the control group (Figures 10 and 11). There was significant increase ($P<0.05$) in the mean aspartate transferase (AST), alanine transferase (ALT) and alkaline phosphatase (ALP), after infection in all the infected groups but no change ($p>0.05$) was noticed in the control group (Figures 12 to 14). There was a marked increase ($P<0.05$) in the mean serum urea values in all infected groups, post infection and no significant change ($P>0.05$) in the control group (Figure 15).

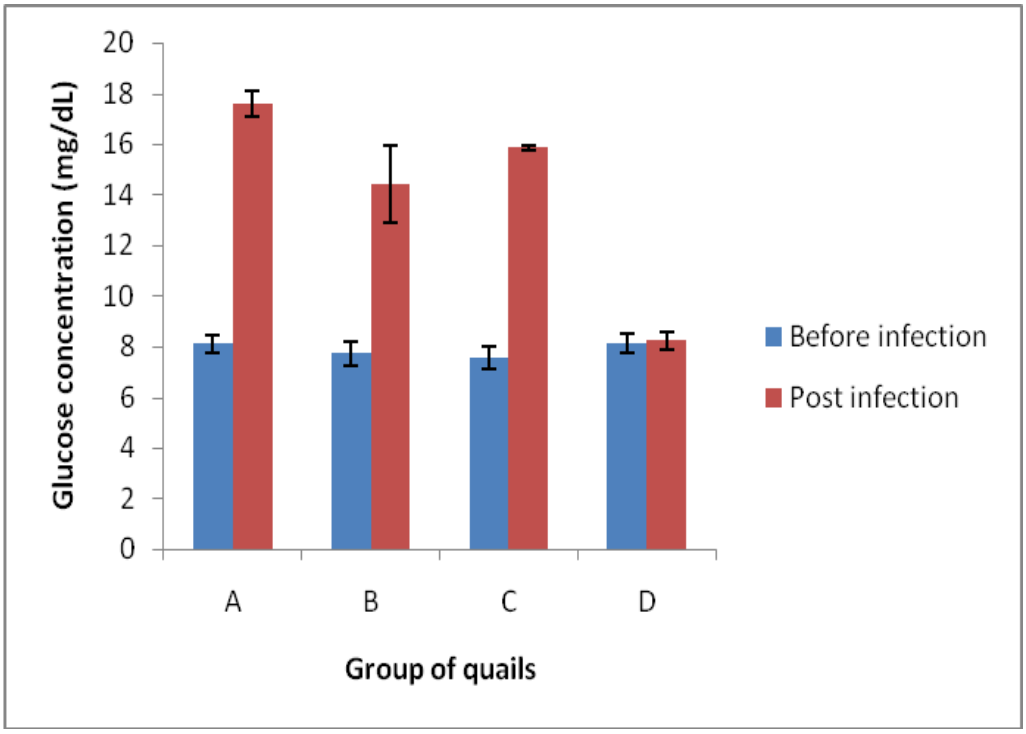


Figure 9: **Mean serum glucose before and after infection of quails with *Salmonella enterica* serovar Gallinarum.**

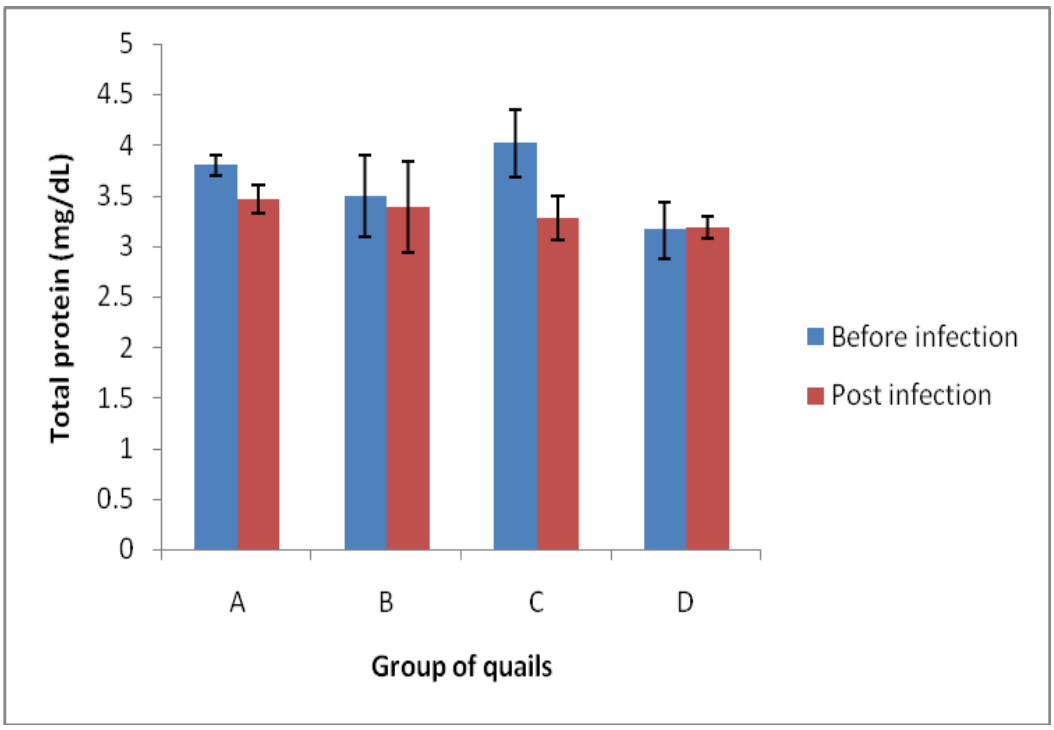


Figure 10: **Mean serum total protein before and after infection of quails with *Salmonella enterica* serovar Gallinarum.**

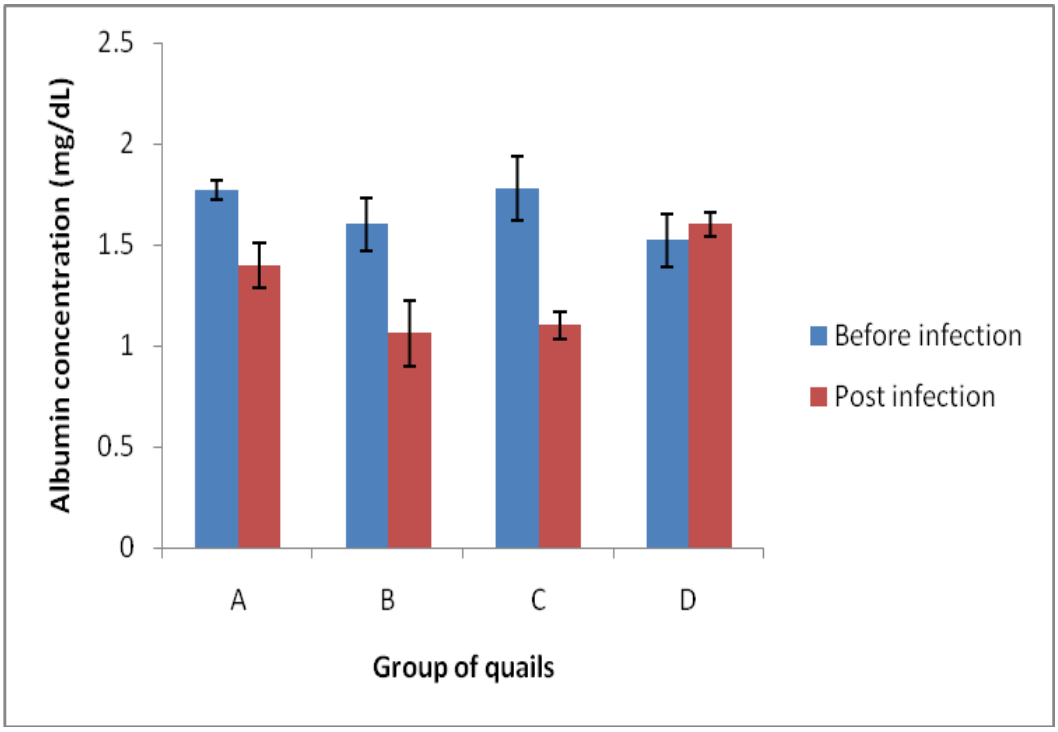


Figure 11: Mean serum albumin before and after infection of quails with *Salmonella enterica* serovar Gallinarum.

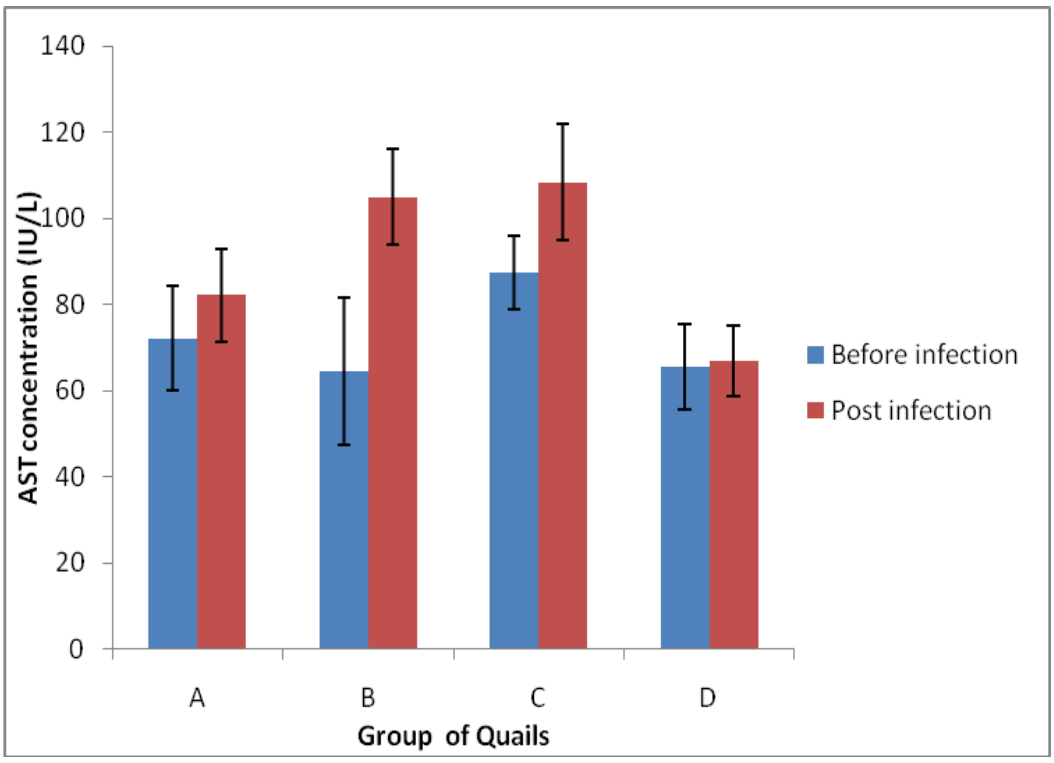


Figure 12: **Mean serum aspartate transferase (AST) before and after infection of quails with *Salmonella enterica* serovar Gallinarum.**

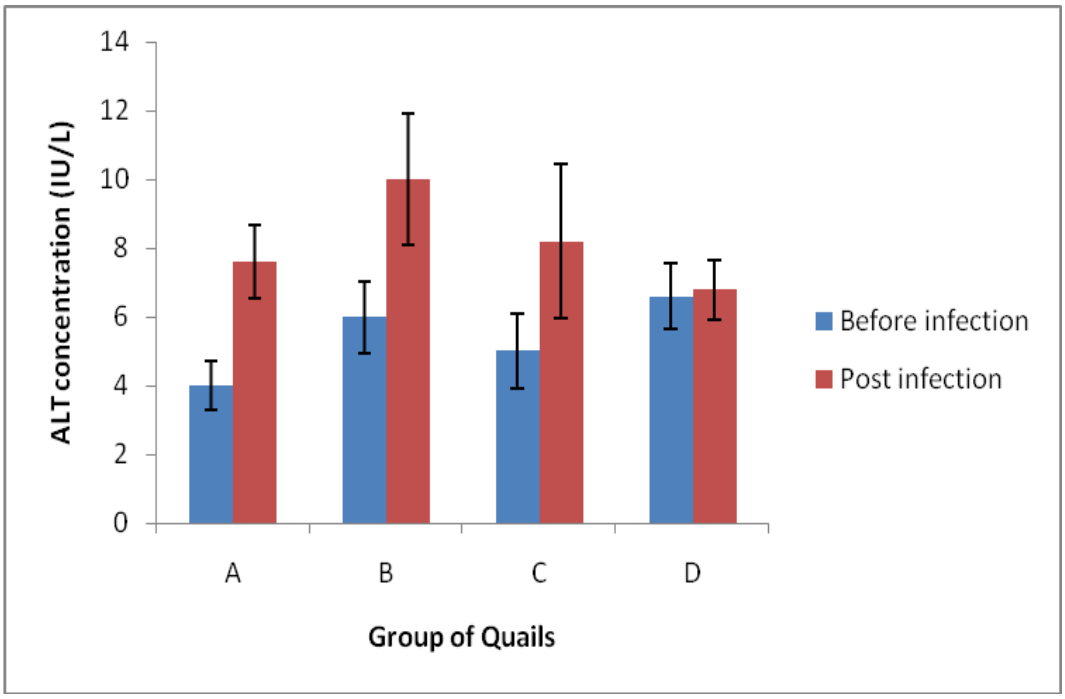


Figure 13: **Mean serum alanine transferase (ALT) before and after infection of quails with *Salmonella enterica* serovar Gallinarum.**

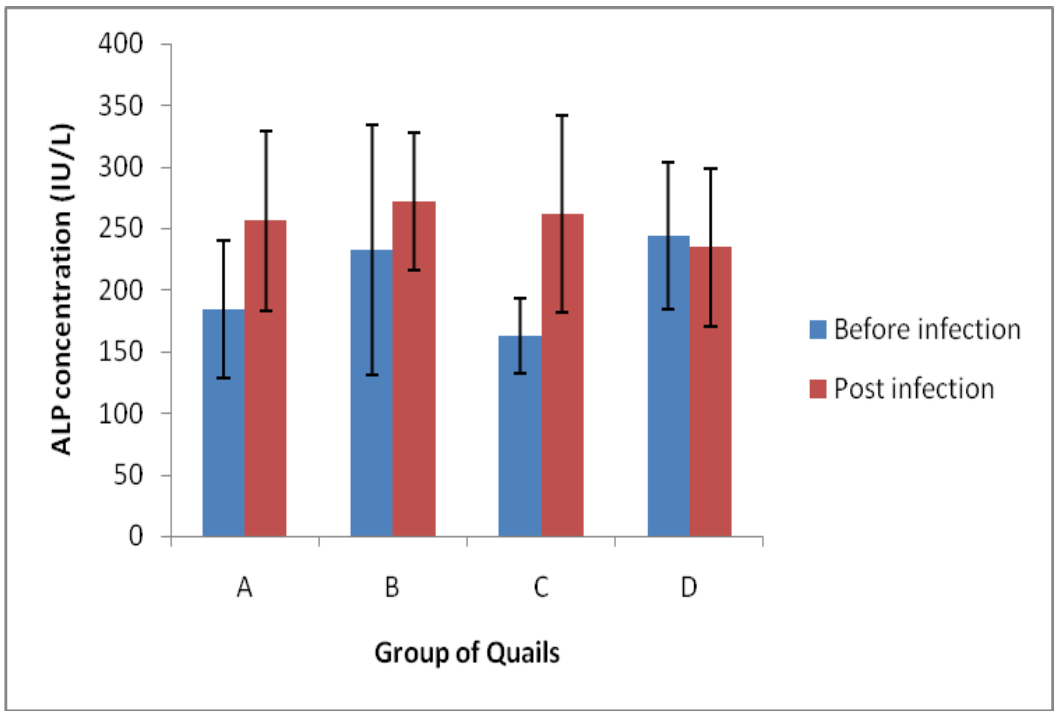


Figure 14: **Mean serum alkaline phosphatase (ALP) before and after infection of quails with *Salmonella enterica* serovar Gallinarum.**

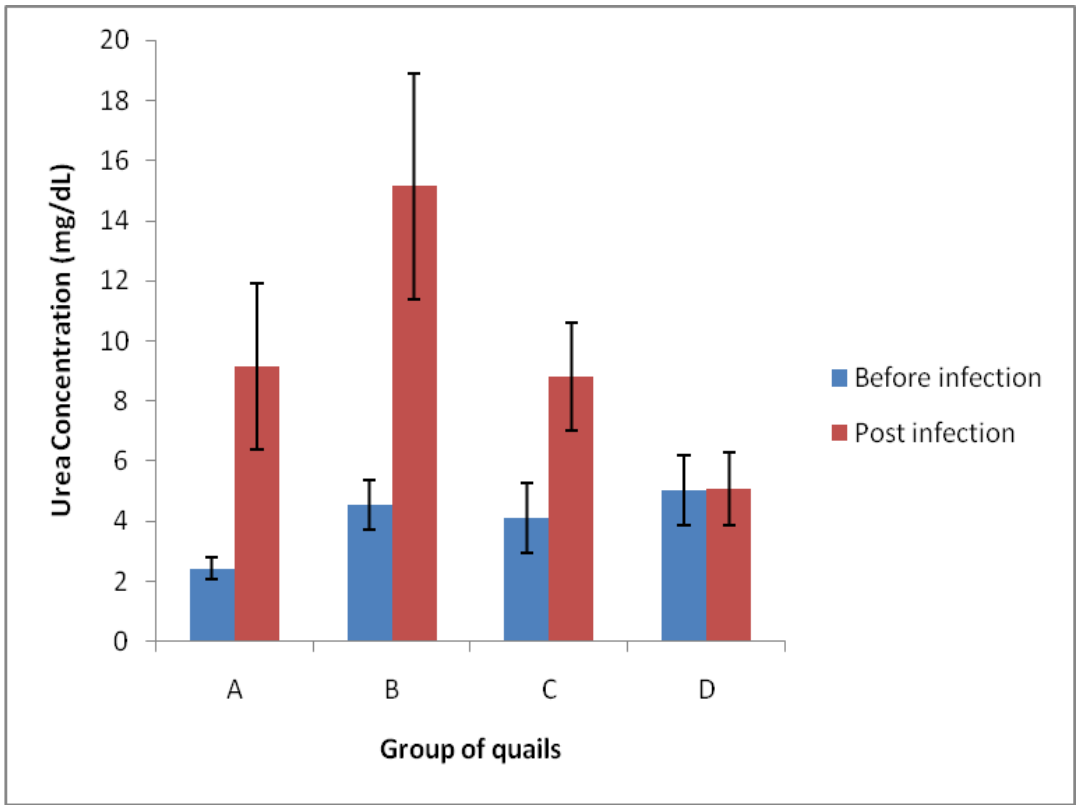


Figure 15: **Mean serum urea before and after infection of quails with *Salmonella enterica* serovar Gallinarum.**

4.4 Clinical Manifestations of Fowl Typhoid in Japanese Quails (*Coturnix coturnix japonica)*

All the infected groups (A, B and C) showed clinical signs which included: sudden death, depression, ruffled feathers, somnolence, greenish-yellow diarrhea, loss of weight, increased body temperature, a decrease in feed and water consumption and decreased egg production(Plates I and II). Group D (control) showed no clinical signs.



Plate I: **Japanese quail (*Coturnix coturnix japonica*) experimentally infected with 10^2 of *Salmonella enterica* serovar Gallinarum. Note depression, somnolence and ruffled feathers**



Plate II: **Japanese quail (*Coturnix coturnix japonica*) experimentally infected with 10^6 of *Salmonella enterica* serovar Gallinarum. Note greenish-yellow pasting of the vent (a)**

4.5 Mortality Rate of FT on Japanese Quails

There was mortality in all the infected groups (A, B and C), with mortality rates of 35%, 30% and 30%, respectively (Table 2). No mortality in group D (control).

Table 2: **Mortality rate of Japanese quails after infection with *Salmonella enterica* serovar Gallinarum**

Day after infection	Mortality rate per group			
	Group A (10^6)	**Group B (10^4)**	**Group C (10^2)**	**Group D (control)**
5	3	-	-	-
7	4	3	3	-
8	3	3	3	-
9	2	1	2	-
10	1	2	1	-
11	1	1	1	-
15	-	1	-	-
18	-	1	1	-
20	-	-	1	-
Total	**14 (35%)**	**12(30%)**	**12(30%)**	-

4.6 Effect of *Salmonella enterica* serovar Gallinarum Infection on Temperatures of Japanese Quails *(Coturnix coturnix japonica)*

Table 3 shows mean daily cloacal temperatures of the quails before and after infection. The mean temperatures for groups A, B, C and D before infection were 41.837 ± 0.76^0C, 42.125 ± 0.41^0C, 41.783 ± 0.51^0C and 41.815 ± 0.76^0C, respectively. While the temperatures after infection were 42.316 ± 0.29^0C, 42.283 ± 0.20^0C, 42.225 ± 0.21^0C and 41.837 ± 0.16^0C for groups A, B, C and D, respectively.

Table 3: Cummulative cloacal temperature (^{0}C) of Japanese quails *(Coturnix coturnix japonica*) before and after infection with *Salmonella enterica* serovar Gallinarum

Mean rectal temperature (^{0}C)	Group A (10^6)	Group B (10^4)	Group C (10^2)	Group D (control)
Before infection	41.837±0.76	42.125±0.41	41.783±0.51	41.815±0.76
After infection	42.316±0.29	42.283±0.20	42.225±0.21	41.837±0.16

$P<0.05$

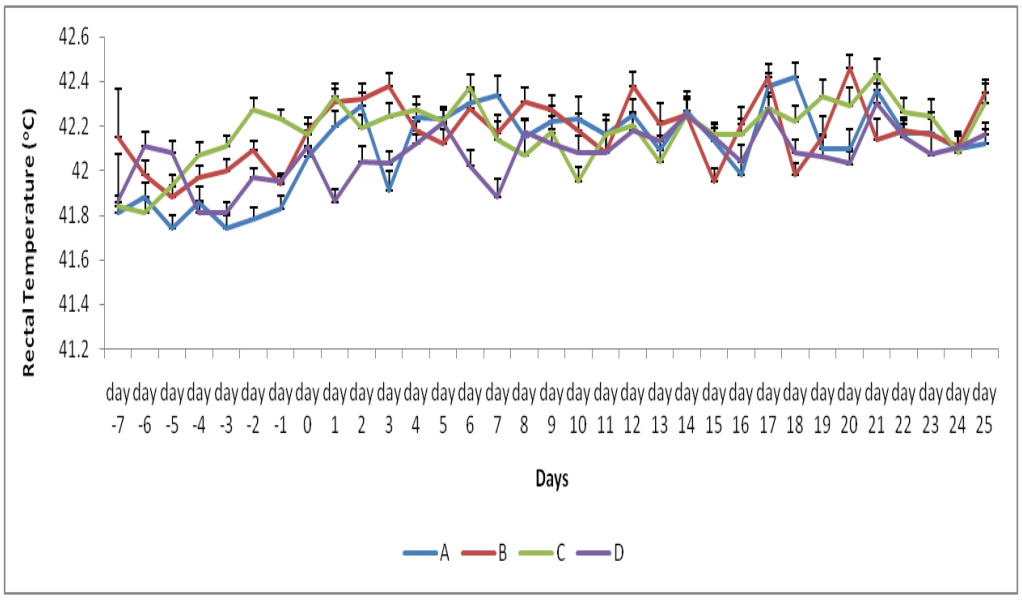


Figure 16: Mean daily cloacal temperature (^{0}C) of Japanese quails *(Coturnix coturnix japonica*) before and after infection with *Salmonella enterica* serovar Gallinarum

4.7 Effect of *Salmonella enterica* serovar Gallinarum Infection on Body Weight (G) of Japanese Quails (*Coturnix coturnix japonica*)

Figure 17 shows a steady rise in the mean weights of the quails from four weeks before infection to one week before infection in all the groups and a drop in the mean weights one week after infection in groups A,B and C, with group D (control) maintaining a steady rise all through. The mean weights of the quails rose from two week post-infection in all the groups, but dropped at four weeks post-infection in group B.

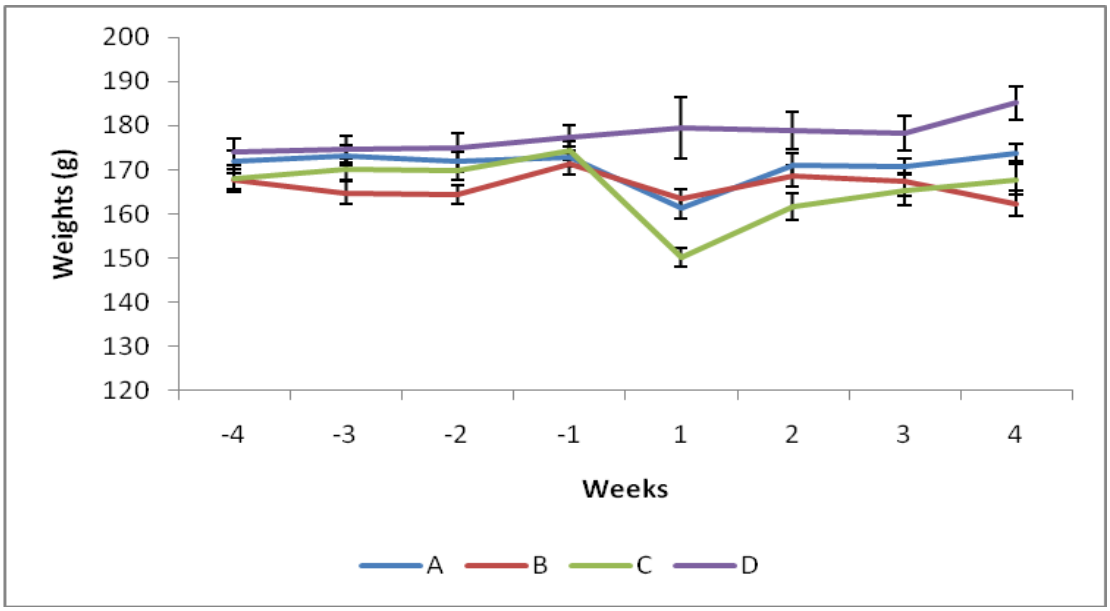


Figure 17: **Mean weekly weights of the quails before and after infection with *Salmonella enterica* serovar Gallinarum.**

4.8 Effect of *Salmonella enterica* serovar Gallinarum Infection on Mean Daily Egg Production of Japanese Quails (*Coturnix coturnix japonica*)

Table 4 shows mean daily egg production of the quails before and after infection. The mean daily egg productions for groups A, B, C and D before infection were 10.61±0.165, 14.00±0.147, 9.29±0.148 and 9.06±0.139, respectively. While the mean daily egg productions after infection for groups A, B, C and D were 4.04±0.464, 6.00±0.705, 3.75±0.47 and 8.91±0.134, respectively. This implies a percentage drop in egg production of 61%, 57% and 60% in the infected groups A, B and C, respectively with no significant drop in the control group D.

Table 4: Mean daily egg production of Japanese quails (*Coturnix coturnix japonica*) before and after infection with *Salmonella enterica* serovar Gallinarum

Egg Production	Group A (10^6)13F	Group B (10^4)17F	Group C (10^2)11F	Group D (control)11F
Before inoculation	10.61 ± 0.165^{acd}	14.00 ± 0.147^{bce}	9.29 ± 0.148^{dc}	9.06 ± 0.139^{ab}
After inoculation	4.04 ± 0.464^{ad}	6.00 ± 0.705^{bde}	3.75 ± 0.47^{ce}	8.91 ± 0.134^{abc}
Percentage (%) drop	**61.92%**	**57.14%**	**59.63%**	**1.63%**

Superscripts with the same alphabet are significantly different abc $p< 0.05$

4.9 *Salmonella enterica* serovar Gallinarum Infection-induced Postmortem Lesions in Japanese Quails *(Coturnix coturnix japonica)*

Table 5 shows that all the infected groups (A, B and C) had severely to slightly enlarged liver and spleen, severely to slightly congested liver, ovarian follicle and lung, bronze liver and cloudy pericardial sac, while group A had greenish-yellow diarrhea and hydroperitoneum (ascites); group C had friable liver. Congestion of the liver was observed in 11 (27.5%), 7 (17.5%) and 6 (15%) of the quails in groups A, B and C, respectively.While congestion was observed in 12 (30%), 11 (27.5%) and 11 (27.5%) of the quails in groups A, B and C, respectively. Matted vent with greenish-yellow faeces and hydroperitonuem (ascites) were only observed in group a (10^6). Friable liver was only observed in group C (10^2), while swollen kidney was only seen in group B (10^4).

Plate III shows enlarged and bronze liver. Plate IV shows congested lung, egg yolk peritonitis and congested ovarian follicles. Plate V shows enlarged and congested kidneys. Plate VI shows enlarged and congested liver. Plate VII shows swollen kidney.

Table 5: Gross lesions of quails in different groups experimentally infected with *Salmonella enterica* serovar Gallinarum

Organ	Gross lesions	Number and percentage of birds with lesions in each infected group		
		GroupA (10^6)	**Group B (10^4)**	**Group C (10^2)**
Vent	Matted with greenish-yellow faeces	2**(5%)**	-	-
Liver	Hepatomegaly	5**(**12.5**%)**	5**(**12.5**%)**	4(10**%)**
Liver	Congested	19(47.5%)	26 (65**%)**	17(42.5%)
Liver	Bronze colouration	2(5%)	1(2.5%)	1(2.5%)
Liver	Friable	-	-	2(5%)
Lung	Congested	19**(**47.5%)	26**(**65%**)**	17**(**42.5%)
Lung	Edematous	3(7.5%)	6**(**15%)	5**(**12.5%)
Spleen	Splenomegaly	6(15%)	4(10%)	3(7.5%)
Kidney	Swollen	-	1(2.5%)	-
Ovarian follicle	Egg yolk peritonitis	1(2.5%)	1(2.5%)	-
Ovarian follicle	Congested	1(2.5%)	2(5%)	1(2.5%)
Peritoneum	Hydroperitoneum (Ascites)	1(2.5%)	-	-

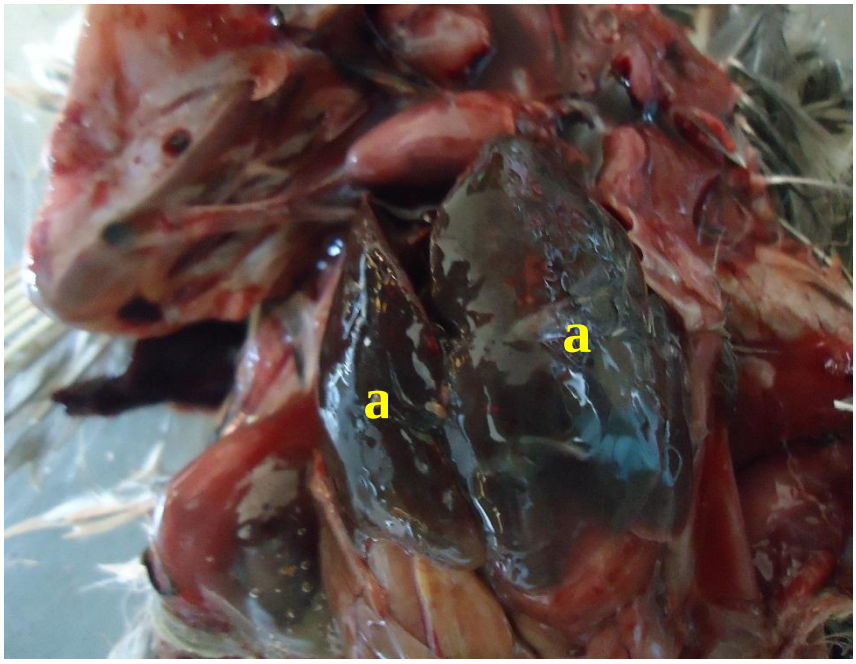


Plate III: **Japanese quail (*Coturnix coturnix japonica*) experimentally infected with 10^4 of *Salmonella enterica* serovar Gallinarum. Note hepatomegaly and bronze liver (a)**

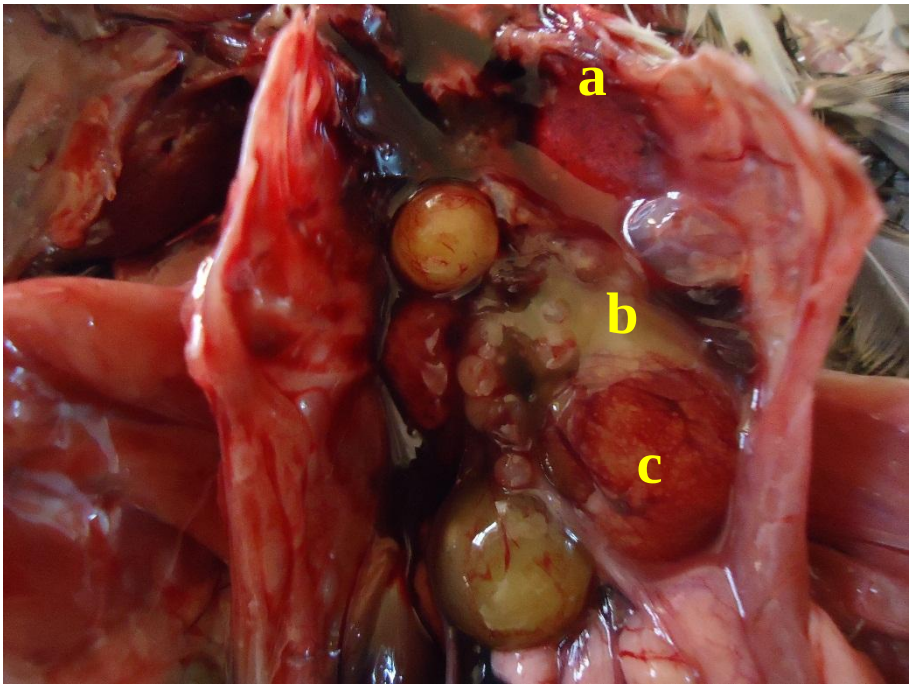


Plate IV: **Japanese quail *(Coturnix coturnix japonica*) experimentally infected with 10^6 of *Salmonella enterica* serovar Gallinarum. Note congested lung (a), egg yolk peritonitis (b) and congested ovarian follicle (c)**

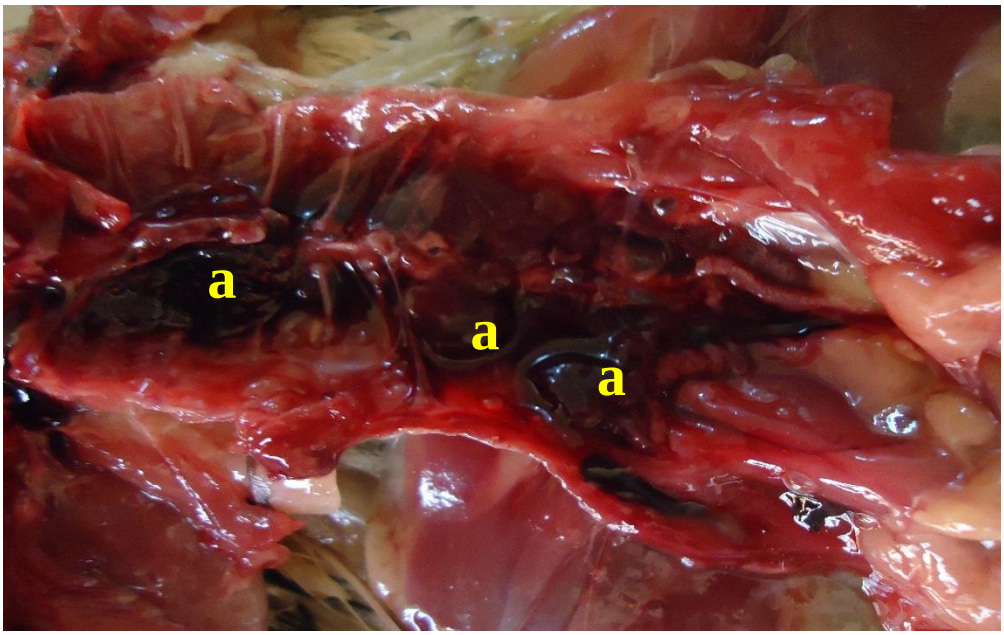


Plate V: **Japanese quail (*Coturnix coturnix japonica*) experimentally infected with 10^6 of *Salmonella enterica* serovar Gallinarum. Note enlarged and congested kidneys (a)**

4.10 Histopatho-logical Lesions of *Salmonella enterica* serovar Gallinarum Infection-induced in Japanese Quails (*Coturnix coturnix japonica*)

Quails in group A which were infected with 10^6 of *Salmonella enterica* serovar Gallinarum each had generalized cellular infiltration of the heart (Plate VIII), severe hepatic congestion and haemorrhages (Plate IX) and generalized hepatic vacuolation and cellular infiltration (Plate X). Quails in group B which were infected with 10^4 of *Salmonella enterica* serovar Gallinarum each and quails in group C which were infected with 10^2 of *Salmonella enterica* serovar Gallinarum each, had severe cellular infiltration within the myocardium (Plate XI).

Quails in group D which were the control had no visible histopathological lesions in all the organs.

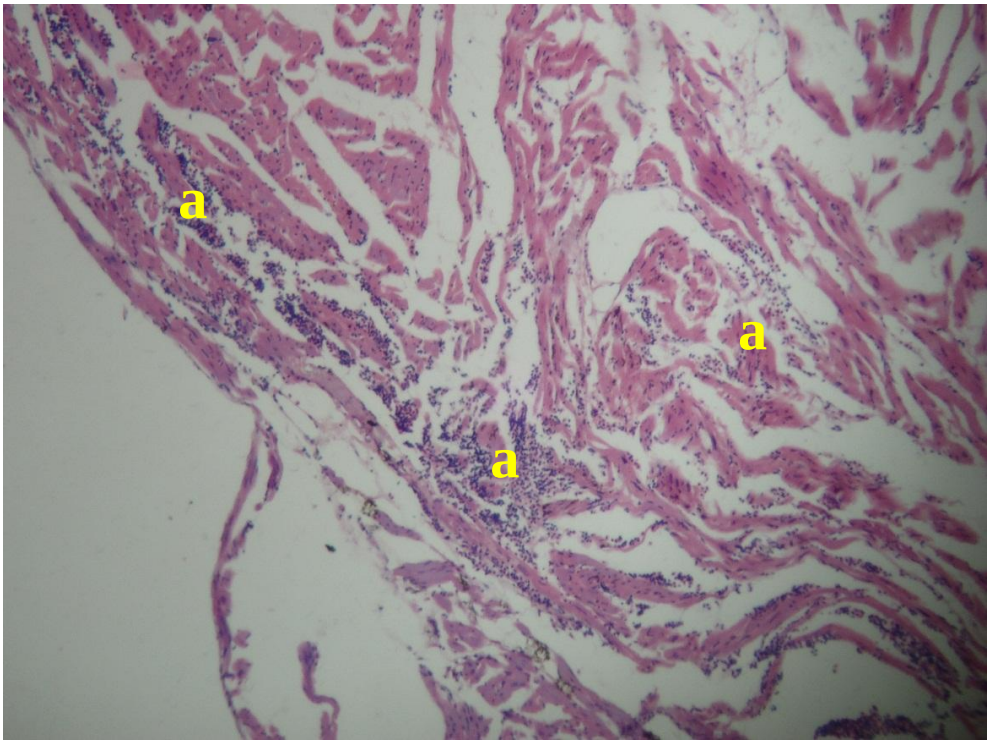


Plate VI: **Photomicrograph of the heart of Japanese quail *(Coturnix coturnix japonica)* experimentally infected with 10^6 of *Salmonella enterica* serovar Gallinarum. Note generalized cellular infiltration of the myocardium (a). H&E.×100**

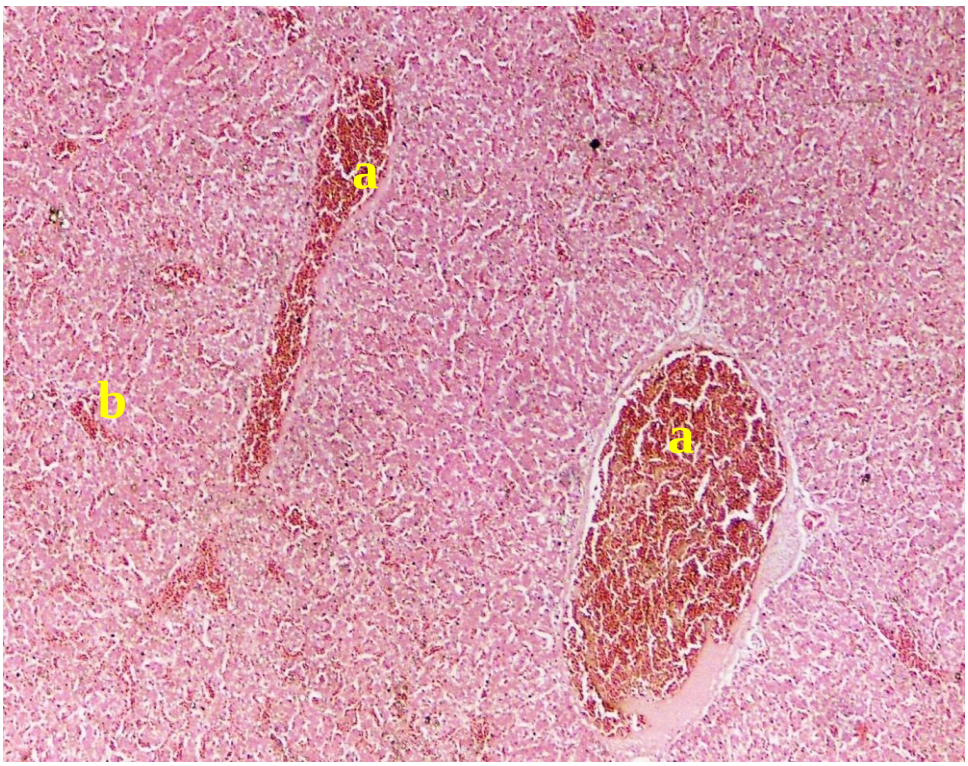


Plate VII: **Photomicrograph of the liver of Japanese quail *(Coturnix coturnix japonica)* experimentally infected with 10^6 of *Salmonella enterica* serovar Gallinarum. Note severe congestion (a) and haemorrhages (b). H &E. ×40**

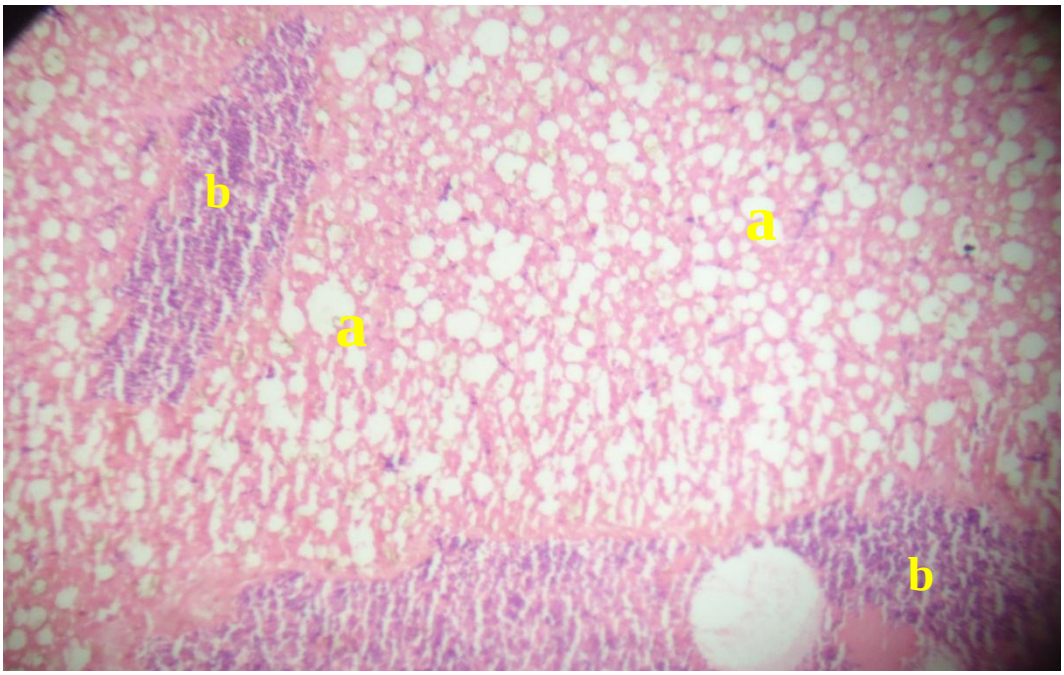


Plate VIII: **Photomicrograph of the liver of Japanese quail *(Coturnix coturnix japonica)* experimentally infected with 10^6 of *Salmonella enterica* serovar Gallinarum. Note generalised vacuolation due to fatty degeneration (a) and cellular infilteration (b). H & E.×100**

4.11: Isolation of *Salmonella enterica* serovar Gallinarum from quail organs

The organism was isolated from the liver, spleen, heart and intestine of the quails at the end of the experiment. Appendices 14 and 15 show the *Salmonella enterica* serovar Gallinarum growth on blood agar and Gram-stain-(Gram-negative rods (Wigley *et al.*,2001; Parmer and Davies,2007)

CHAPTER FIVE

DISCUSSION

The haematological changes in the quails presented slight reduction in mean haemoglobin concentration, mean red blood cell (RBC) count and mean packed cell volume (PCV) in all the infected groups. The reduction observed in this study corresponded with the acute phase of fowl typhoid in which anemia has been reported in chickens (Assoku and Penhale, 1978; Prasanna and Paliwal, 2002). Assoku and Penhale (1978) suggested that the anemia associated with acute fowl typhoid in chicken may be a direct result of the increased ability of the reticuloendothelial cells to take up erythrocytes. Christensen *et al*. (1996) indicated that the modification of the erythrocytes is associated directly (lipopolysaccharide/ outer membrane proteins) or indirectly (by induction of antibodies) or both, to the number of bacteria present in the tissues. There was no significant change ($p>0.05$) in the mean corpuscular volume (MCV) post-infection, but there were significant changes ($p<0.05$) in the mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) in the infected groups, when compared with the corresponding mean values obtained in the control group. This haematological result could be due to haemorrhages and congestion observed in some organs in this study, which is in agreement with findings of Shah *et al.,* (2013) in broiler chickens.

The differential leukocyte counts revealed a slight increase ($p<0.05$) in percentage of heterophils in all the infected groups and significant decrease ($p<0.05$) in the relative percentage of lymphocytes in infected group A, but slight increase ($P>0.05$) in infected

group C and no changes observed in infected group B and control group D ($p>0.05$). This result is slightly different from the report of Shah *et al.,* (2013) who reported that leukocytosis in broiler chicken infected with *Salmonella* Gallinarum was due to increase in both the number of heterophils and lymphocytes. This difference could be due to species variation.

Significantly elevated glucose levels are found most commonly in endocrine disorders, pancreatic, liver or kidney disease, while decrease levels may be due to fasting, drugs or toxins but can also be indicative of various physiological disorders, such as excess insulin production (Barham and Trinder, 1972). The elevation of the mean serum glucose level observed in this study could be due to the lesions, such as manifested in the liver and kidney. Albumin is the most abundant serum protein in all species of animals, representing 55-65% of total protein. It is synthesized in the liver and the main biological function of albumin is to maintain water balance in serum and plasma and to transport and store substances with low plasma solubility such as fatty acids, calcium, bilirubin and hormones. Hypoalbuminaemia also reflected as ascites as seen in one of the infected groups, is associated with impaired albumin production in the liver (analbuminaemia) due to liver disease and over excretion in kidney disease and intestinal diseases (Grant *et al.,* 1987). The hypoalbuminaemia observed in this study could also be due to the hepatic and renal lesions manifested and the ascites seen. Elevated levels of AST as seen in the infected groups can signal myocardial infarction, hepatic disease, muscular dystrophy and organ damage. The ALT levels are increased during various hepatic disease state including

hepatitis. Elevation of alkaline phosphatase (ALP) may be attributed to liver or bone disease, congestive heart failure and abdominal bacterial infections (Reitmans and Frankel, 1957). The elevation levels in the mean AST, ALT and ALP observed in this study could be due to the lesions, such as manifested in the liver and heart. Elevated urea concentrations can be indicative of high protein diet, congested heart failure, dehydration and increased protein catabolism as a result of stress or renal disease (Patton and Crouch, 1977). In this study, stress due to infection and the kidney lesions could be the cause of the significant elevated urea concentrations observed .

The clinical signs, gross lesions and histopathological findings observed in Japanese quails (*Coturnix coturnix japonica*) in this study were consistent with previous studies in chickens (Shivaprasad, 2000; Freitas Neto *et al.*, 2007; Garcia *et al*., 2010), except that in this study bloody diarrhea was not noticed.

Starting on day seven after infection, quails in all the infected groups showed typical clinical signs of fowl typhoid, such as depression, ruffled feathers, somnolence and greenish-yellow diarrhea. This clinical picture is similar to the one observed by Garcia *et al*., (2010) in commercial layers, except that in their study the onset of the clinical signs started three days post-infection. This supported the claim that quails are hardy birds compared to other types of poultry (Naveen and Arun, 1992; NVRI, 2008).

The percentage drop in egg production in this study (57- 61%) is similar to that reported by Ezema *et al*.,(2009), who reported 50-70% drop in egg production in commercial laying birds. The drop in egg production could be due to decreased feed and water consumption

ovarian lesions and mortality involving the laying quails in the infected groups, observed in this study. There was weight loss in all the infected groups which agrees with the report of Ezema *et al*., (2009), who reported weight loss due to fowl typhoid in commercial laying birds. The loss of body weights of quails infected with *Salmonella enterica* serovar Gallinarum in this study may be caused by anorexia and diarrhea, which agreed with the report of Shah *et al.* (2013) in broiler chickens.

Mortality rate of 30-35% was recorded in this study, which is in agreement with the mortality range of 10- 93% reported by Shivaprasad (1996) in chickens. The mortality in this study was mostly that of sudden death, implying that the infection was peracute to acute. The mortality started five days post-infection and persisted till twenty days post-infection. The high mortality recorded in this experiment indicated that Japanese quails are also susceptible to *Salmonella enterica* serovar Gallinarum like the chickens.

There was an increase in temperature in all the infected groups at day one post-infection to day eight post-infection, and started fluctuating thereafter all through the experimental period. This is probably due to the multiplication of the organism in the body systems of the quails. This is evidence by the cellular infiltration in the various organs (liver and heart). It is believed that body temperature is elevated by the action of the organism's toxin on the thermoregulatory centre in chickens (Garcia *et al.,* 2010).

The gross lesions of greenish-yellow diarrhea, hepatomegaly, splenomegaly, swollen kidneys, congested liver, lung and ovarian follicle, bronze liver, egg yolk peritonitis, edematous lung and hydroperitonuem (ascites),observed in this study were similar to those reported by previous researchers in chickens (Freitas Neto *et al*., 2007; Shivaprasad and

Barrow, 2008; Beyaz *et al*., 2010; Garcia *et al.*, 2010). The histopathogical findings (cellular infiltration of the liver and heart, vacuolation of hepatocytes and congested liver and lung) in this study were also similar to previous works in chickens (Wigley *et al.*, 2005; Hossain *et al*., 2006; Nwiyi and Omadamiro, 2012).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

i. This study has shown that *Salmonella* Gallinarum caused decrease in haematological parameters, such as packed cell volume (10% decrease) and red blood cell count (10% decrease) and haemoglobin concentration (5% decrease) and increase in heamatological indices, such as mean corpuscular volume (5% increase), mean corpuscular haemoglobin (30% increase), and mean corpuscular haemoglobin concentration (30% increase) above control group values in Japanese quails (*Coturnix coturnix japonica*).

ii. This study showed that *Salmonella* Gallinarum caused increase in serum glucose (65%), urea (50% increase), aspartate transferase (25% increase), alanine transferase (20% increase) and alkaline phosphatase (10% increase) above the control group values in Japanese quails (*Coturnix coturnix japonica*); while a decrease was noticed in the mean serum albumin (30% decrease) below the control group values in Japanese quails (*Coturnix coturnix japonica*).

iii. This study has shown that *Salmonella* Gallinarum caused a septicaemic disease with distribution of the organism in major organs and characterized by high mortality, congestion and enlargement of various organs, and marked drop in egg production in Japanese quails (*Coturnix coturnix japonica*).

iv. The clinical and pathological findings, such as increase temperature, bronze liver greenish-yellow diarrhoea, drop in egg production, hepatomegaly, splenomegaly and congested liver, lung and ovarian follicle in this study indicated that *Salmonella* Gallinarum produces a disease similar to that in chickens.

6.2 Recommendations

1. Further studies should be carried out in the areas of pathogenesis and virulence of other organisms that were previously thought not to affect quails.

2. Those keeping quails should adhere to strict biosecurity measures as means of prevention and control of fowl typhoid in the quail farm.

3. Further works should be carried out by studying the disease condition at different ages of the quail to see whether age plays a role in the spread of fowl typhoid.

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APPENDICES

Appendix 1: Haematological parameters (Pre-infection) with *Salmonella enterica* serovar Gallinarum

Parameters	Group	A	B	C	D
PCV (%)		55.22 ±2.31	56.76 ± 1.38	52.78 ± 1.44	54.62 ± 1.66
Hb (g/dl)		186.6 ± 8.65	194.3 ± 5.11	177.8± 5.56	188.2± 6.92
RBC		3.611 ± 0.16	3.618 ± 0.10	3.464 ± 10	3.610 ± 0.11
MCV		153.5 ± 1.20	155.1 ± 1.90	152.7 ± 1.51	151.5 ± 1.46
MCH		51.52 ± 0.63	52.33 ± 0.85	50.83 ± 0.65	52.01± 0.67
MCHC		335.7 ± 3.31	341.6± 2.61	339.5± 4.36	335.7± 7.70
Heterophil		54.88 ± 4.47	62.33 ± 2.85	60.41 ± 3.90	64.33± 4.42
Lymphocyte		49.63 ± 5.02	37.67 ± 2.85	40.18 ± 3.64	35.67 ± 4.42

Appendix 2: Haematological parameters (Post-infection) with *Salmonella enterica* serovar Gallinarum

Parameters	Group	A	B	C	D
PCV		50.80 ± 0.83	47.48 ± 1.73	50.15 ± 1.24	55.16 ± 0.94
Hb		172.4± 3.10[a]	181.5 ± 6.77[abc]	167.8 ± 4.64b	188.7 ± 4.23[c]
RBC		3.279 ± 0.6[a]	3.106 ± 0.12[abc]	3.209 ± 0.09b	3.664 ± 0.07[c]
MCV		148.2 ± 1.02	152.9 ± 1.63	169.4 ± 16.33	151.0 ± 1.36
MCH		56.76 ± 0.67	56.33 ± 0.76	56.98 ± 0.55	52.45 ± 0.54
MCHC		383.0± 2.69[abc]	368.7 ± 2.44[a]	373.4 ± 2.47b	338.1 ± 1.80[c]
Heterophil		58.21 ± 3.06	61.65 ± 4.11	56.75 ± 4.30	64.25 ± 4.04
Lymphocyte		41.74 ± 3.07	65.31 ± 2.11	56.26 ± 4.33	35.50± 3.97

Superscripts with the same alphabets are significantly different ($P < 0.05$).

Appendix 3: Daily Egg production of the quails pre-infection with *Salmonella enterica* serovar Gallinarum

Days	Date	Group A (10^6) 13F	Group B (10^4) 17F	Group C (10^2) 11F	Group D Control) 11F
1	04-05-12	12	16	10	9
2	05-05-12	12	15	10	10
3	06-05-12	11	14	9	9
4	07-05-12	10	15	10	9
5	08-05-12	11	14	11	9
6	09-05-12	11	14	10	8
7	10-05-12	10	14	9	9
8	11-05-12	10	16	10	8
9	12-05-12	12	15	9	8
10	13-05-12	10	14	9	10
11	14-05-12	11	14	8	9
12	15-05-12	10	13	9	9
13	16-05-12	10	14	8	9

Appendix 3 Continue

Days	Date	Group A (10^6)13F	Group B (10^4)17F	Group C (10^2)11F	Group D (Control)11F
14	17-05-12	9	14	8	8
15	18-05-12	12	14	9	8
16	19-05-12	11	14	11	11
17	20-05-12	12	14	10	10
18	21-05-12	11	15	10	10
19	22-05-12	11	13	9	9
20	23-05-12	10	14	10	10
21	24-05-12	12	13	9	10
22	25-05-12	10	14	9	9
23	26-05-12	11	13	10	9
24	27-05-12	11	14	8	9
25	28-05-12	10	13	10	10
26	29-05-12	10	14	9	9

Appendix 3 Continue

Days	Date	Group A (10^6)13F	Group B (10^4)17F	Group C (10^2)11F	Group D (Control)11F
27	30-05-12	11	13	9	9
28	01-06-12	9	13	9	8
29	02-06-12	9	13	8	9
30	03-06-12	10	14	9	9
31	04-06-12	10	14	9	9

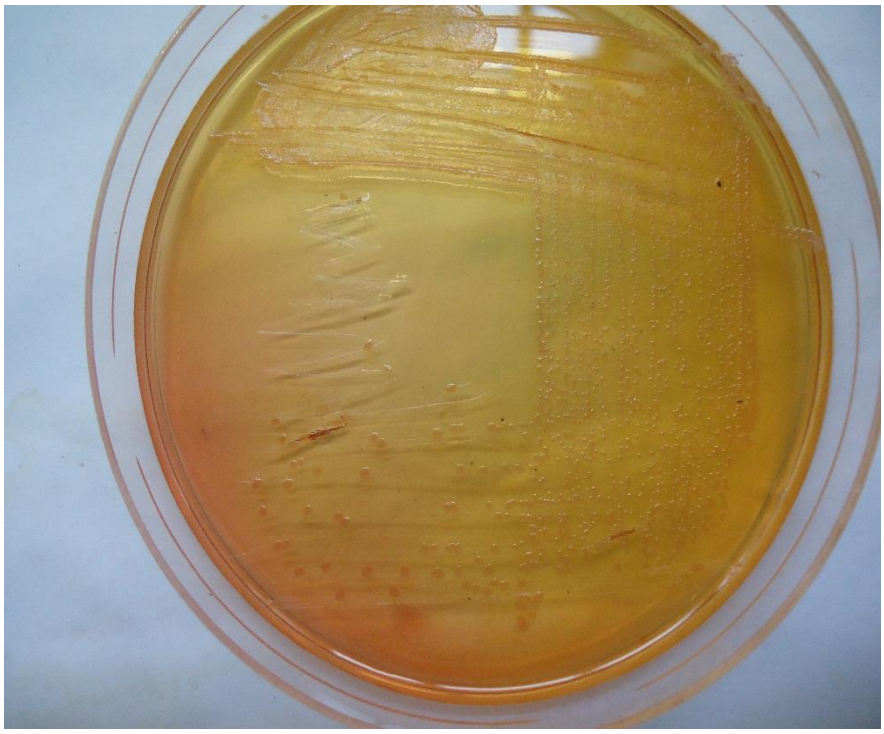
Appendix 4: Daily egg production of the quails post-infection with *Salmonella enterica* serovar Gallinarum

Days	Date	Group A(10^6)-13F	Group B(10^4)-17F	Group C(10^2)-11F	Control groupD-11F
32	05-06-12	9	13	9	9
33	06-06-12	8	12	7	10
34	07-06-12	9	11	7	9
35	08-06-12	7	12	7	9
36	09-06-12	6	10	6	10
37	10-06-12	6	9	6	9
33	11-06-12	5	8	6	8
34	12-06-12	3	8	5	9
35	13-06-12	3	7	5	8
36	14-06-12	2	5	4	9
37	15-06-12	2	4	3	9
38	16-06-12	5	3	2	8

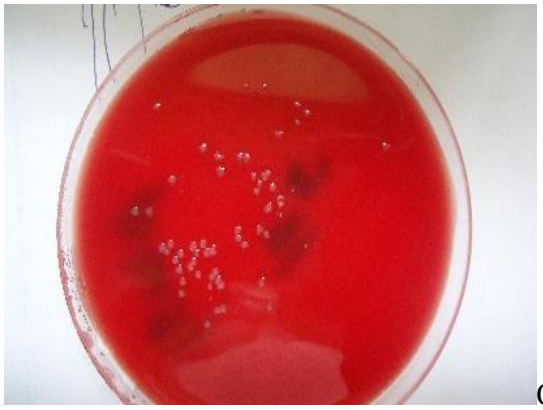
Appendix 4 Continue

Days	Date	Group A(10^6)-13F	Group B(10^4)-17F	Group C(10^2)-11F	Control groupD-11F
39	17-06-12	3	3	2	9
40	18-06-12	2	2	1	10
41	19-06-12	3	4	2	9
42	20-06-12	3	4	2	8
43	21-06-12	3	4	2	9
44	22-06-12	2	3	2	9
45	23-06-12	2	4	2	9
46	24-06-12	2	4	2	8
47	25-06-12	3	4	2	10
48	26-06-12	3	4	2	8
49	27-06-12	3	3	2	9
50	28-06-12	3	3	2	9

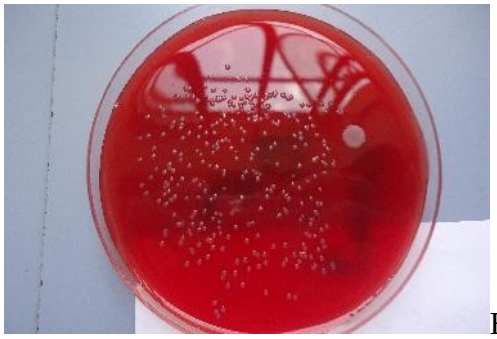
Appendix 5: *Salmonella enterica* serovar Gallinarum colony on MacConkey agar plate



Appendix 6: *Salmonella gallinarum* colonies on Blood agar plates from which 3 colonies were scooped into nutrient broth and incubated for 24 hours pre-infection



C



B



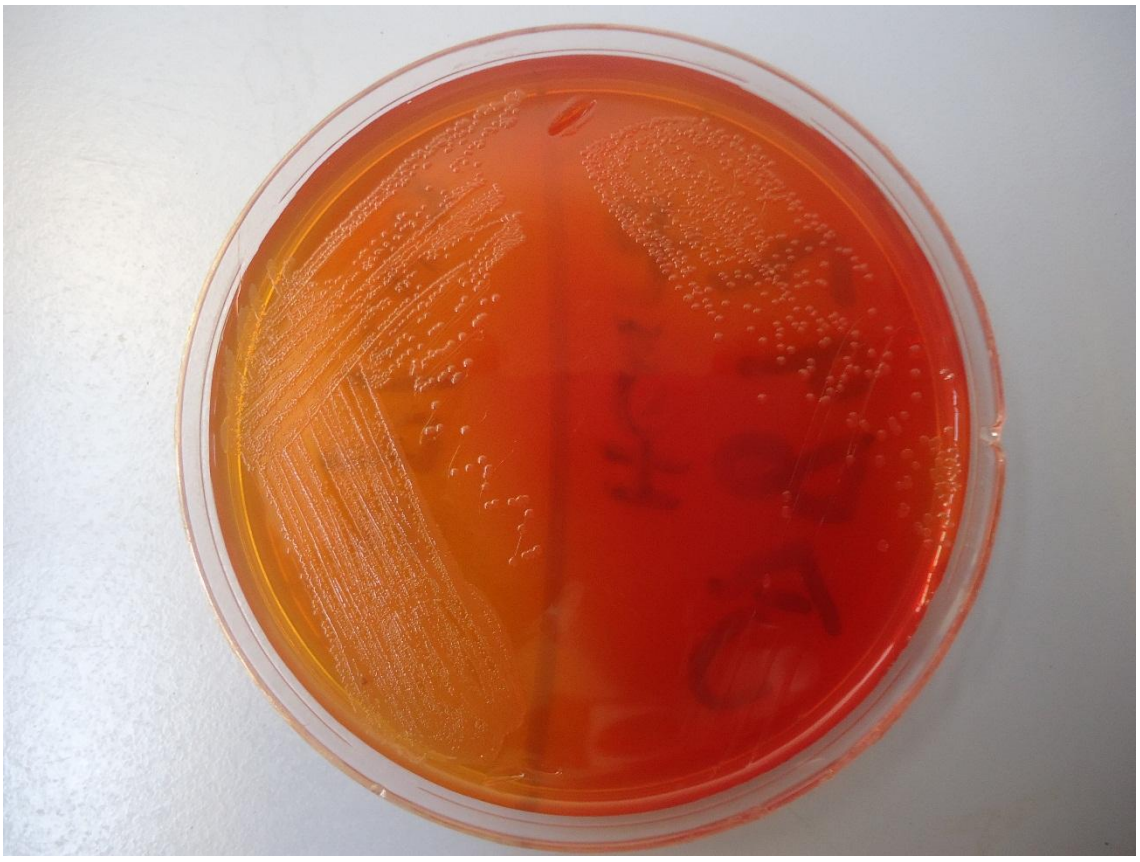
A

Appendix 7: Serum biochemical parameters (pre and post-infection) with *Salmonella enterica* serovar Gallinarum

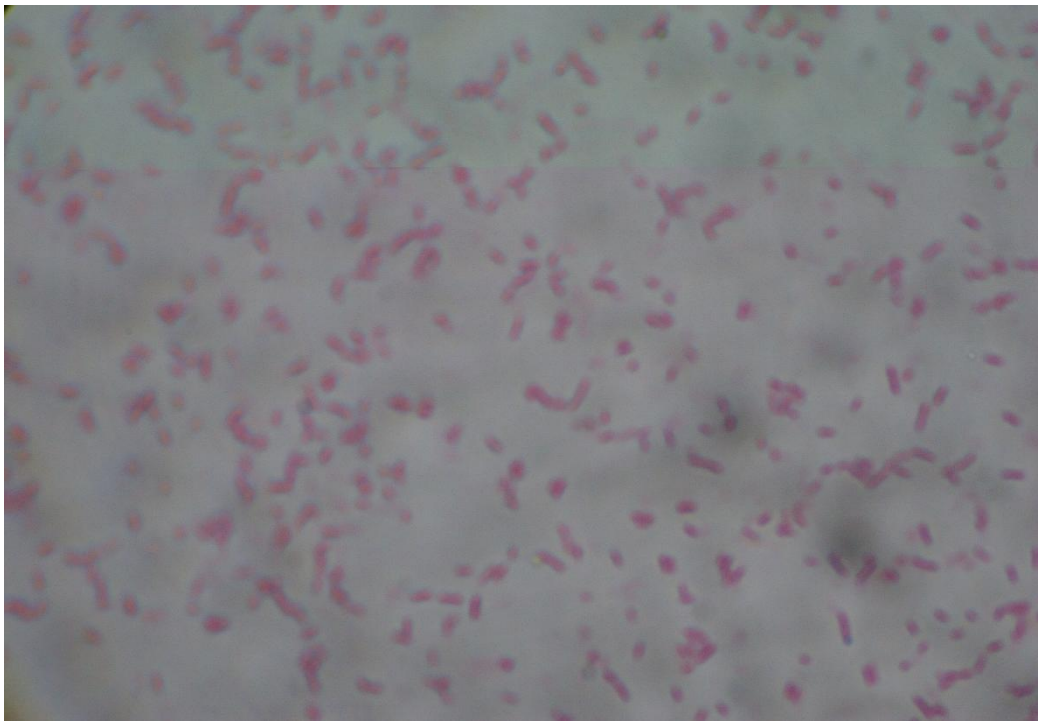
Parameters Group	A	B	C	D
Glucose (Pre)	8.10 ± 0.34	7.74 ± 0.48	7.56 ± 0.42	8.14 ± 0.37
Glucose (Post)	17.58 ± 0.52^{a}	14.42 ± 1.53^{b}	15.86 ± 0.11^{c}	9.44 ± 0.29^{abc}
Total protein (Pre)	3.80 ± 0.10	3.50 ± 0.40	4.02 ± 0.33	3.16 ± 0.28
Total protein (Post)	3.46 ± 0.14	3.38 ± 0.45	3.28 ± 0.22	3.18 ± 0.11
Albumin (Pre)	1.70 ± 0.05	1.66 ± 0.13	1.78 ± 0.16	1.52 ± 0.13
Albumin (Post)	1.40 ± 0.11	1.06 ± 0.16	1.10 ± 0.07	1.60 ± 0.06
AST (Pre)	72.20 ± 12.24	64.60 ± 17.12	87.40 ± 8.56	65.60 ± 9.94
AST (Post)	82.0±10.75	105.0±10.99	108.4± 13.45	67.0 ± 8.23
ALT (Pre)	4.00 ± 0.71	6.00 ± 1.05	5.00 ± 1.09	6.600 ± 0.93
ALT (Post)	7.60±1.08	10.0±1.92	8.20±2.25	6.80±1.87
ALP (Pre)	184.2 ± 55.84	233.0 ± 101.4	162.8 ± 30.54	244.0 ± 59.62
ALP (Post)	256.2 ± 72.87	271.8 ± 55.54	261.8±80.33	234.5 ± 64.29
Urea (Pre)	2.44 ± 0.35	4.54 ± 0.81	4.10 ± 1.15	5.02 ± 1.16
Urea (Post)	9.16 ± 2.73	15.14 ± 3.75	8.820 ± 1.80	5.54 ± 2.51

Superscripts with the same alphabets are significantly different ($P < 0.05$).

Appendix 8: *Salmonella enterica* serovar Gallinarum isolated from quail organs on Blood agar plate



Appendix 9: ***Salmonella enterica*** **serovar Gallinarum Gram-stain (Gram-negative short rods) from lypholized culture.**



Appendix 10: The Japanese Quails (*Coturnix coturnix japonica*) used for the experiment



Appendix 11: Japanese Quail Eggs produced by the control group

